
R G U H S · M O D E L A N S W E R B A N K

RGUHS MD/MS Anatomy — Paper III



M O D E L A N S W E R B A N K

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883 recurring topics · Textbook-grounded model answers · cited Recent Advances

G R O U N D E D I N :

Gray's Anatomy (Standring) 41e — spine text
BD Chaurasia · AK Datta · Moore's Clinically Oriented Anatomy — gross anatomy
Langman · Moore (Developing Human) · Larsen — embryology
Thompson & Thompson · Gangane — genetics
Junqueira · Ross · IB Singh — histology
Bancroft · Pranab Dey — microscopy & histotechniques
Halder · Govt Museum Handbook — organ donation & body preservation
Snell · Vishram Singh · IB Singh — neuroanatomy



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Cell biology



Endoplasmic reticulum

Asked in: Paper III 12-Jul-2023 · Paper III May 2009 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The endoplasmic reticulum (ER) is a cytomembrane system consisting of an anastomosing network of interconnecting, membrane-lined channels — cisternae (flattened sacs), tubules and vesicles — that extends from the outer nuclear membrane throughout the cytoplasm. Its membrane divides the cytoplasm into two compartments: the cytosol and the intramembranous (cisternal) space, the latter being continuous with the perinuclear space. With a membrane surface area up to 30 times that of the plasma membrane, the ER is the principal site of protein and lipid biosynthesis in the cell. Structurally and functionally it is divided into rough (granular) ER, studded with ribosomes, and smooth (agranular) ER, which lacks them.

Structure

Feature	Rough ER (RER)	Smooth ER (SER)
Ribosomes	Present on cytosolic surface	Absent
Shape of cisternae	Flattened sacs / parallel stacks	Tubular, anastomosing network
Staining (light microscopy)	Basophilic (ergastoplasm) — RNA of attached ribosomes binds basic dyes	Eosinophilic; not visualised well by light microscopy, best seen by TEM
Continuity	Continuous with outer nuclear membrane and perinuclear space	Continuous with RER
Prominent in	Pancreatic acinar cells, plasma cells (immunoglobulin), fibroblasts (collagen), goblet cells, ameloblasts, odontoblasts	Hepatocytes, steroid-secreting cells (adrenal cortex, Leydig cells), striated/smooth muscle (as sarcoplasmic reticulum)

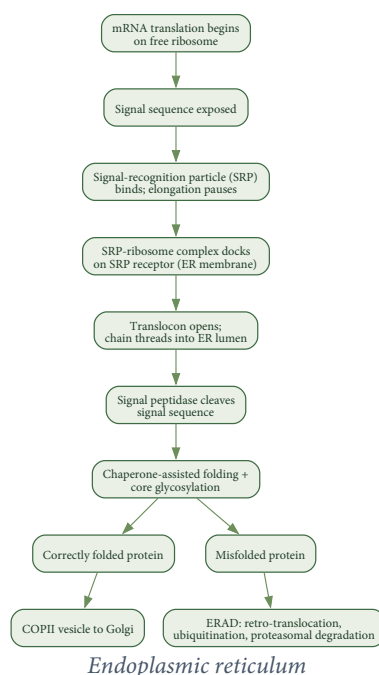
- ▶ Both compartments are part of the same continuous membrane system; a given cell has variable proportions of each depending on its principal secretory activity.
- ▶ On transmission electron microscopy, RER cisternae appear as flattened, ribosome-studded sacs; SER cisternae are more tubular and vesicular.

Rough endoplasmic reticulum — protein synthesis pathway

- ▶ **Initiation** — translation of secretory/membrane-protein mRNAs begins on free cytosolic ribosomes; the nascent chain's N-terminal **signal sequence** (15–40 hydrophobic amino acids) is exposed.
- ▶ **Recognition** — a **signal-recognition particle (SRP)** binds the signal sequence and halts further elongation.
- ▶ **Docking** — the SRP–ribosome–nascent-chain complex binds an **SRP receptor** on the ER membrane; a second receptor binds the large (60S) ribosomal subunit, firmly attaching the ribosome to the ER surface (giving the "rough" appearance).

- **Translocation** — SRP is released; the signal peptide inserts into a membrane pore, the **translocon**, and elongation resumes, threading the growing polypeptide into the cisternal lumen.
- **Processing** — the signal peptide is cleaved by **signal peptidase**; within the cisterna, resident **chaperones** guide folding and prevent aggregation, and core **glycosylation** (N-linked) and multichain protein assembly occur.
- **Quality control** — misfolded proteins are retro-translocated to the cytosol, ubiquitinated and degraded by proteasomes — **ER-associated degradation (ERAD)**.
- **Export** — correctly folded/assembled proteins bud off in COPII-coated transport vesicles for delivery to the Golgi apparatus; inappropriate/escaped ER-resident proteins are retrieved from the Golgi back to the ER in COPI-coated vesicles, maintaining bidirectional ER–Golgi traffic.

Ribosomes of the RER — the ribosomes studding the cytosolic surface are macromolecular ribonucleoprotein granules about 25 nm in diameter, composed of a large **60S** and a small **40S** subunit (sedimentation coefficients), built from rRNA (28S, 5.8S and 5S in the large subunit; 18S in the small) complexed with roughly 73 proteins. The 40S subunit binds and translates the mRNA; the 60S subunit releases the finished polypeptide and, where a signal sequence is present, docks onto the ER membrane via an intermediate docking protein so that the nascent chain is threaded directly into the cisternal space. Groups of ribosomes translating a single mRNA strand form **polyribosomes (polysomes)** — free polysomes make cytosolic/cytoskeletal proteins, while RER-bound polysomes make secretory and membrane proteins.



Smooth endoplasmic reticulum — functions

- **Lipid biosynthesis** — synthesis of phospholipids, cholesterol and steroid hormones; new membrane lipids move on to other organelles by lateral diffusion, transfer proteins, or SER-derived vesicles. Prominent in adrenal cortical cells and Leydig cells that secrete steroid hormones.

- ▶ **Detoxification** — cytochrome P450 enzyme system on the SER membrane detoxifies lipophilic exogenous compounds (drugs, alcohol, barbiturates) and processes endogenous molecules (e.g., bilirubin conjugation, bile component handling) in hepatocytes.
- ▶ **Carbohydrate metabolism** — hepatocyte SER contains glucose-6-phosphatase, converting glucose-6-phosphate to glucose in gluconeogenesis and glycogenolysis.
- ▶ **Calcium sequestration and release** — SER stores Ca^{2+} within its cisternal space and releases it in response to stimuli; in striated muscle this specialised SER is termed the **sarcoplasmic reticulum**, central to excitation–contraction coupling.
- ▶ **Membrane biogenesis** — SER cooperates with RER and the Golgi apparatus to synthesize new membrane, adding protein, carbohydrate and lipid components in distinct compartments.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the ER as a continuous system arising from the outer nuclear membrane:

- ▶ **Nucleus with outer/inner nuclear membrane and perinuclear space** (label continuity with ER cisternal space).
- ▶ **Rough ER**: flattened, stacked cisternae with **ribosomes** studding the cytosolic (outer) surface; label the **cisternal lumen**.
- ▶ **Smooth ER**: tubular, anastomosing network continuous with RER, without ribosomes.
- ▶ Show a **budding transport vesicle** leaving the RER, directed toward the **Golgi apparatus** (cis-face).
- ▶ Inset: a single ribosome docked on the RER membrane via a **translocon**, with the nascent polypeptide threading into the cisterna and the **signal peptide** being cleaved.

□ Fig 2.10 — Rough and smooth endoplasmic reticulum. (Junqueira's Basic Histology — Text & Atlas — 17e, p.92)

Applied / Clinical anatomy

- ▶ **Basophilia as a diagnostic clue** — cells with abundant RER (plasma cells, active fibroblasts, pancreatic acinar cells) show strong cytoplasmic basophilia on H&E owing to ribosomal RNA; this "ergastoplasm" is a routine light-microscopic marker of active protein-secreting cells.
- ▶ **Osteogenesis imperfecta** — defective procollagen molecules synthesised in the RER of osteoblasts fail to assemble properly, illustrating RER quality-control (chaperone/ERAD) failure and producing structurally weak bone.
- ▶ **Neonatal jaundice** — an underdeveloped SER in hepatocytes of the newborn liver reduces bilirubin conjugation, so unconjugated bilirubin accumulates, producing physiological jaundice of the newborn.
- ▶ **Drug metabolism and induction** — hepatocyte SER (cytochrome P450) hypertrophies with chronic alcohol or barbiturate use, altering drug metabolism and tolerance — the structural basis of enzyme induction.
- ▶ **Sarcoplasmic reticulum pathology** — abnormal Ca^{2+} handling by this SER derivative underlies conditions such as malignant hyperthermia in skeletal muscle.
- ▶ **Electron-microscopic technique** — ER cisternae and ribosome studding are preserved for study only by proper ultrastructural fixation: primary fixation in buffered **glutaraldehyde** followed by

post-fixation in **osmium tetroxide**, which stabilises membranous organelles (ER, Golgi, mitochondria) for thin-section TEM; inadequate or delayed fixation causes swelling/vesiculation of the ER and loss of the fine reticular pattern.

- ▶ **Radiologic/immunofluorescence correlate** — the ER can be visualised in cultured cells by immunofluorescence using antibodies against an ER-resident enzyme, appearing as a lace-like network continuous with the nuclear envelope — used in cell-biology research and diagnostic immunocytochemistry.

High-yield exam pointers

- ▶ ER = system of interconnecting membrane-lined cisternae, tubules and vesicles; cisternal space continuous with perinuclear space.
- ▶ Two types: **RER** (ribosome-studded, basophilic, protein synthesis) and **SER** (ribosome-free, eosinophilic, lipid/steroid synthesis, detoxification, Ca^{2+} storage).
- ▶ RER protein-targeting sequence: signal sequence → SRP → SRP receptor → translocon → signal peptidase cleavage → chaperone folding/glycosylation → ERAD if misfolded.
- ▶ SER's three principal roles: lipid/steroid biosynthesis, cytochrome-P450 detoxification, Ca^{2+} sequestration (sarcoplasmic reticulum in muscle).
- ▶ Cell examples: RER-rich — plasma cells, fibroblasts, pancreatic acinar cells; SER-rich — hepatocytes, adrenal cortical cells, Leydig cells, muscle cells.
- ▶ Clinical correlates: osteogenesis imperfecta (RER/ERAD failure), neonatal jaundice (immature hepatocyte SER), drug-induced SER hypertrophy, malignant hyperthermia (sarcoplasmic reticulum Ca^{2+} dysregulation).
- ▶ EM fixation for ER preservation: glutaraldehyde primary fixation + osmium tetroxide post-fixation.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Vishram Singh — General Anatomy 4e; Bancroft's Theory & Practice of Histological Techniques (for EM fixation of membranous organelles).



Cell junctions

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction Cell junctions are specialized, multiprotein complexes at the plasma membrane that mediate mechanical attachment and/or intercellular communication between adjacent cells, or between a cell and the extracellular matrix (basal lamina). They are most numerous and best developed in epithelia, where they maintain tissue integrity, establish a paracellular permeability barrier, and create distinct apical and basolateral membrane domains. Functionally they are grouped as **occluding**, **anchoring**, and **communicating** junctions; in most simple epithelia the first three types are stacked in a fixed apico-basal sequence forming the **junctional complex**.

Classification of cell junctions

Functional class	Junction	Also called	Principal role
Occluding	Tight junction	Zonula occludens (ZO)	Seals paracellular space; barrier + fence function
Anchoring (cell–cell)	Adherens junction	Zonula adherens (belt) / Fascia adherens (strip)	Encircling adhesion; anchors actin
Anchoring (cell–cell)	Desmosome	Macula adherens	Spot-weld adhesion; anchors intermediate filaments
Anchoring (cell–matrix)	Hemidesmosome	—	Anchors basal cell to basal lamina (integrin-laminin)
Anchoring (cell–matrix)	Focal adhesion	Focal contact	Transient integrin–actin anchorage; signalling
Communicating	Gap junction	Nexus / macula communicans	Direct cytoplasmic continuity for ions/small molecules

1. Tight junction (zonula occludens)

- Most apical member of the junctional complex; forms a continuous, belt-like band completely encircling each epithelial cell (hence "zonula").
- On transmission EM the outer leaflets of the two adjacent plasma membranes appear fused at focal points ("kissing points"); on freeze-fracture it appears as an anastomosing network of branching ridges/strands on the P-face with complementary grooves on the E-face.
- Molecular architecture: transmembrane proteins **claudin** (backbone of the strands, 20–27 kDa, ~24 isoforms, confer strand-specific permeability) and **occludin** (~60 kDa, present in most but not all tight junctions) interact homotypically with corresponding proteins of the neighbouring cell to seal the strand; **junctional adhesion molecule (JAM)** stabilizes the assembly. Cytoplasmically, occludin/claudin bind **ZO-1, ZO-2, ZO-3** (PDZ-domain plaque proteins) which link the junction to the actin cytoskeleton.

- ▶ Number of fused strands determines "tightness" — few strands (e.g., proximal renal tubule) = leaky epithelium, permeable to water/solutes; many strands (e.g., urinary bladder urothelium) = tight epithelium.
- ▶ Two functions: (i) **barrier/gate function** — forces molecules crossing the epithelium to take a transcellular rather than paracellular route, and prevents back-diffusion of actively transported solutes; (ii) **fence function** — prevents lateral diffusion of apical membrane lipids/proteins into the basolateral domain, thereby maintaining epithelial cell polarity.

2. Adherens junction (zonula adherens / fascia adherens)

- ▶ Lies immediately below the tight junction; like the tight junction it forms a continuous adhesion belt (zonula adherens) around the apex of columnar/cuboidal epithelial cells — together the tight and adherens junctions function like the plastic rings binding a six-pack of cans.
- ▶ Adhesion is mediated by **cadherins** (classically **E-cadherin**), Ca^{2+} -dependent transmembrane glycoproteins that form homotypic links with cadherins of the neighbouring cell.
- ▶ Cytoplasmic tails of cadherin bind **catenins** (α , β , γ /plakoglobin), which link the complex to the **actin** filament bundle of the apical terminal web — i.e., adherens junctions anchor the actin cytoskeleton (in contrast to desmosomes, which anchor intermediate filaments).
- ▶ A linear, non-encircling variant — the **fascia adherens** — is seen at intercalated discs of cardiac muscle and between smooth-muscle cells/glia-neuronal contacts.

3. Desmosome (macula adherens)

- ▶ A discrete, spot-like ("rivet" or button-like) plaque rather than an encircling band; typically located below the zonula adherens, and paired symmetrically on the facing membranes of two adjacent cells.
- ▶ The intercellular space (~25–30 nm) contains an electron-dense midline rich in glycoprotein, spanned by cadherin-family adhesion molecules — **desmoglein** and **desmocollin**.
- ▶ Cytoplasmic tails of desmoglein/desmocollin bind **plakoglobin** and **desmoplakin**, forming a dense cytoplasmic plaque into which **intermediate filaments** (**keratin tonofilaments in epithelium**) insert, looping back into the cytoplasm — giving desmosomes great tensile strength and distributing mechanical stress across the epithelial sheet.
- ▶ Abundant in tissues subject to mechanical stress — epidermis, cardiac muscle intercalated discs, myometrium.
- ▶ Clinical relevance: autoantibodies against **desmoglein-3** (and desmoglein-1) cause **pemphigus vulgaris**, with acantholysis and intraepidermal blistering.

4. Hemidesmosome and focal adhesion (cell-matrix anchoring)

- ▶ **Hemidesmosome**: structurally resembles "half a desmosome" but the transmembrane adhesion molecules are **integrins** ($\alpha 6 \beta 4$), not cadherins; integrins bind **laminin** in the basal lamina, and cytoplasmically link (via plectin/BP230) to keratin intermediate filaments. Anchors basal epithelial cells (e.g., basal keratinocytes) to the underlying basal lamina.
- ▶ **Focal adhesion (focal contact)**: smaller, more numerous, dynamic integrin-based contacts linking (via talin/vinculin/paxillin) to actin filaments rather than intermediate filaments; paxillin

also recruits focal adhesion kinase (FAK), triggering signalling cascades that control cell adhesion, migration and gene expression — prominent in migrating cells (fibroblasts, leukocytes, epithelial cells during wound repair).

5. Gap junction (nexus / macula communicans)

- ▶ A communicating rather than adhesive junction; the normal ~20 nm intercellular gap is reduced to a narrow ~2–3 nm space traversed by aligned channels.
- ▶ Each channel ("connexon") is a hexamer of the transmembrane protein **connexin**; a connexon in one cell membrane docks precisely with a connexon in the adjacent cell to form a continuous, gated aqueous channel (~1.5–2 nm pore) that directly connects the cytoplasms of the two cells.
- ▶ Permits passage of ions, second messengers (cAMP, cGMP, IP₃), and small metabolites, allowing electrical and metabolic coupling — hence gap junctions are also called **low-resistance junctions** and mediate **electrical synapses** in cardiac and smooth muscle (rhythmic, coordinated contraction) and in some neurons.
- ▶ Channel opening/closing (gating) is regulated by conformational change in connexin subunits, triggered by rising intracellular Ca²⁺ or falling pH — a protective "uncoupling" mechanism that isolates injured cells from healthy neighbours.
- ▶ About 21 connexin isoforms are known; mutation of **connexin-26 (Cx26/GJB2)**, expressed in cochlear gap junctions responsible for K⁺ recycling in the sensory epithelium, is a major cause of **congenital (non-syndromic) hereditary deafness**; mutations of Cx46/Cx50 are associated with congenital cataract, reflecting the role of lens gap junctions in nutrient delivery to the avascular lens fibres.

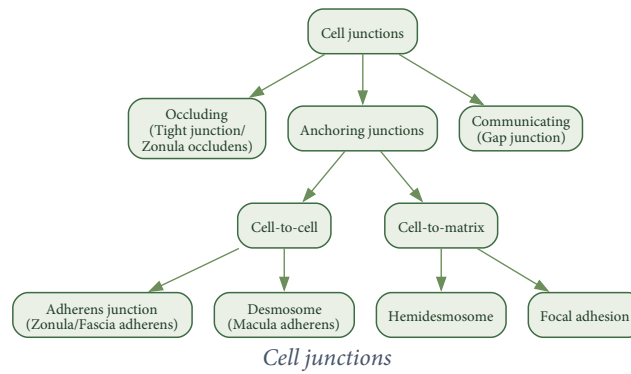
 Fig 4.5 — Epithelial cell junctional complex. (Junqueira's Basic Histology — Text & Atlas — 17e, p.203)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label, from apex to base of a columnar epithelial cell, on a vertical section through the lateral membrane:

- ▶ Microvilli (apical surface)
- ▶ **Zonula occludens** (tight junction) — fused membrane strands
- ▶ **Zonula adherens** (adherens junction) — cadherin–catenin–actin link
- ▶ **Macula adherens** (desmosome) — desmoglein/desmocollin–desmoplakin–keratin filament link (draw the intermediate filaments looping into the plaque)
- ▶ **Gap junctions** — small circular patches lower on the lateral membrane, connexon channels shown bridging the narrowed intercellular gap
- ▶ Basal lamina with **hemidesmosomes** anchoring the basal cell membrane (integrin–laminin link)

Add a small inset table alongside listing: junction → adhesion molecule → cytoskeletal link → function, for quick recall.



Applied / Clinical anatomy

- ▶ **Pemphigus vulgaris:** autoantibodies to desmoglein-3 destroy desmosomes → intraepidermal (suprabasal) blistering, positive Nikolsky sign; bullous pemphigoid targets hemidesmosomal antigens (BP180/BP230) → subepidermal blistering.
- ▶ **Bacterial exploitation of tight junctions:** *Clostridium perfringens* enterotoxin binds claudin, preventing tight-junction assembly and causing paracellular fluid loss (food-poisoning diarrhoea); *Helicobacter pylori* CagA protein disrupts ZO-1 signalling, contributing to gastric mucosal barrier breakdown and ulcerogenesis.
- ▶ **Connexin-26 (GJB2) mutations:** commonest genetic cause of congenital non-syndromic sensorineural deafness (loss of cochlear endolymph K⁺ recycling); Cx46/Cx50 mutations cause congenital cataract.
- ▶ **Cardiac/smooth muscle physiology:** gap junctions at intercalated discs (alongside fascia adherens and desmosomes) allow the myocardium to behave as a functional syncytium — basis of coordinated cardiac contraction; disruption (e.g., ischaemia-induced connexin dephosphorylation) predisposes to arrhythmia.
- ▶ **Epithelial permeability grading:** "leaky" (proximal renal tubule, gallbladder) vs "tight" (urinary bladder, distal nephron, blood–brain-barrier endothelium) epithelia are graded by the number of tight-junction strands — clinically relevant to reabsorption/secretion capacity and to barrier pathologies (e.g., BBB breakdown in inflammation).
- ▶ **Tumour biology:** loss of E-cadherin-mediated adherens junctions is a key step in epithelial–mesenchymal transition and invasive carcinoma spread.

High-yield exam pointers

- ▶ Three functional classes: **occluding, anchoring, communicating**; anchoring further split into cell–cell (adherens junction, desmosome) and cell–matrix (hemidesmosome, focal adhesion).
- ▶ **Junctional complex** (apex to base): zonula occludens → zonula adherens → macula adherens (desmosome) — classic TEM triad.
- ▶ Adhesion molecules: tight junction = **claudin + occludin** (+JAM); adherens junction & desmosome = **cadherin family** (E-cadherin; desmoglein/desmocollin); hemidesmosome/focal adhesion = **integrins**.
- ▶ Cytoskeletal link distinguishes desmosome (**intermediate filaments/keratin**) from adherens junction and focal adhesion (**actin**).

- ▶ Gap junction = connexon (hexamer of **connexin**) forming a ~1.5–2 nm pore; low-resistance/electrical synapse; permits ion and second-messenger flux.
- ▶ Tight junction has dual "gate" (barrier) and "fence" (polarity-maintaining) functions.
- ▶ Clinical triad to remember: pemphigus vulgaris (desmoglein), Cx26/GJB2 (deafness), *C. perfringens*/*H. pylori* (claudin/ZO-1 disruption).

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Gray's Anatomy (Standring) 41e.



Cilia

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction — Cilia are hair-like, motile or non-motile evaginations of the apical plasma membrane of a cell, built around a core cytoskeletal rod called the **axoneme**, which is anchored to the apical cytoplasm by a centriole-derived **basal body**. Together the axoneme, basal body and its accessory structures constitute the **ciliary apparatus**. Cilia are present on almost every cell of the body (as a single non-motile primary cilium) and, in large numbers, on the ciliated epithelia of the respiratory and female reproductive tracts, efferent ductules of the testis, and the ependyma — where their coordinated beating moves fluid or particulate matter across the epithelial surface.

1. Classification of cilia

Type	Axonemal pattern	Motility	Location	Function
Motile cilia	9 + 2 (9 peripheral doublets + 2 central singlets)	Motile — planar, asymmetric beat	Respiratory tract, oviduct, efferent ductules of testis, ependyma	Move mucus/fluid/ova/CSF across the epithelial surface
Primary cilia (monocilia)	9 + 0 (central pair absent)	Non-motile; passively bend with flow	Solitary — one per cell, on almost all eukaryotic cells (renal tubular cells, fibroblasts, chondrocytes, neurons)	Chemo-, osmo- and mechanosensation; Hedgehog signalling; light/odorant/sound sensation in modified forms
Nodal cilia	9 + 0, but motile (rotational)	Motile — rotational (clockwise) movement	Primitive node of the bilaminar embryonic disc (gastrulation)	Generate leftward "nodal flow" that establishes left-right visceral asymmetry

2. Ultrastructure of a motile cilium

- ▶ **Dimensions:** shaft ~0.25 µm in diameter, 5–10 µm in length (up to 10 µm), covered throughout by plasma membrane except at its base.
- ▶ **Axoneme** — the core cytoskeleton, a cylinder of **nine peripheral microtubule doublets surrounding a central pair of single microtubules** ("9 + 2" arrangement).
 - Each doublet has a complete **A-subfibre** (13 tubulin protofilaments) and an incomplete **B-subfibre** (10–11 protofilaments) that shares part of the A wall.
 - **Dynein arms** — two rows of ATPase-bearing "arms" project from the A-subfibre of one doublet toward the B-subfibre of the adjacent doublet (spaced at ~24-nm intervals); these are the motor proteins of ciliary movement.
 - **Nexin links** — elastic protein bridges (165 kDa) permanently tether adjacent doublets (~86-nm intervals), limiting the sliding produced by dynein.
 - **Radial spokes** — extend inward from each doublet toward the central pair (~29-nm intervals), which is itself enclosed by an **inner (central) sheath**.
 - **Tektins** are scaffolding filaments running the length of the axonemal microtubules.
- ▶ Tubulin of the axoneme is stabilised by post-translational **acetylation and polyglutamylation**, resisting depolymerisation.
- ▶ Ciliary length is maintained by **intraflagellar transport (IFT)**: cargo (tubulin dimers, axonemal/membrane proteins) is carried anterograde to the tip by **kinesin-2** and retrograde back to the base by **cytoplasmic dynein-2**.

□ Fig 5.14 — *Ultrastructure of cilium.* (Sontakke — *Textbook of Human Histology* — 2e, p.64)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw (i) a **longitudinal section** of a cilium from tip to base showing the shaft, tapering tip, plasma membrane covering, and the basal body embedded in the apical cytoplasm; and (ii) a **transverse (cross) section through the shaft at two levels** — label:

- ▶ Plasma membrane
- ▶ 9 peripheral microtubule doublets (label one doublet "A subfibre" and "B subfibre")
- ▶ 2 central single microtubules + central/inner sheath
- ▶ Dynein arms (on the A subfibre, pointing to B subfibre of next doublet)
- ▶ Radial spokes (doublet → central sheath)
- ▶ Nexin links (between adjacent doublets)
- ▶ A second cross-section at the level of the **basal body** showing 9 microtubule **triplets** arranged in a ring with **no central pair** (as in a centriole)
- ▶ Striated rootlet arising from the base of the basal body

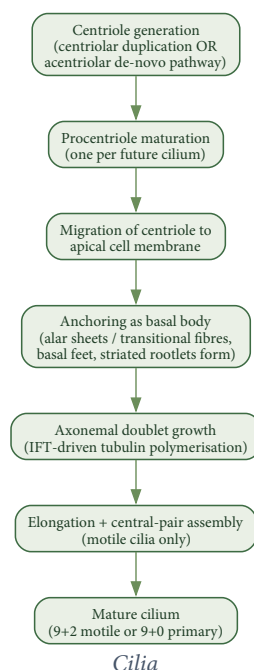
3. Basal body and its accessory structures

- ▶ The **basal body** is a modified centriole: nine short microtubule **triplets** (A, B and an incomplete C tubule) arranged in a ring, with **no central pair** — structurally continuous with the axoneme

(the A and B tubules of the axonemal doublet are direct continuations of the A and B tubules of the basal body triplet).

- ▶ Accessory structures anchoring the basal body in the apical cytoplasm:
 - **Alar sheet (transitional fibres)** — a collar-like extension linking the basal body's transition zone to the plasma membrane.
 - **Basal foot** — a single asymmetric projection from the basal body, oriented in the direction of the effective stroke; orientation of basal feet in the same direction across a cell coordinates ciliary beat.
 - **Striated rootlet** — a cross-banded fibrous structure anchoring the basal body deep into the apical cytoplasm.
- ▶ In light microscopy, the row of basal bodies at the ciliary base stains as a continuous dark band, giving ciliated epithelium its characteristic "**crew-cut**" appearance.

4. Ciliogenesis



- ▶ Ciliogenesis begins with **centriole generation** — either by duplication of existing centrioles (centriolar pathway) or, more commonly in differentiating ciliated epithelium, by **de novo (acentriolar) formation** of multiple procentrioles.
- ▶ Procentrioles mature into centrioles, migrate to the apical surface, align perpendicularly and anchor via alar sheets to become **basal bodies**; basal feet and striated rootlets then form.
- ▶ A microtubule doublet grows upward from each of the nine basal-body triplets; the growing axoneme pushes the apical membrane outward as the mature cilium.
- ▶ In non-dividing cells generally, the centriole-derived basal body instead gives rise to a single **non-motile primary cilium** with mechanosensory/chemosensory function.

5. Mechanism of ciliary movement

- ▶ Movement is generated by **ATP-dependent sliding of the outer doublet microtubules**, not by any change in microtubule length.
- ▶ **Dynein arms on the A-subfibre form transient cross-bridges with the B-subfibre of the adjacent doublet**; ATP hydrolysis drives the bridge to "walk" along the B-tubule, producing a shearing/sliding force directed toward the ciliary tip.
- ▶ **Effective stroke** — the cilium remains stiff except at its base, producing a rapid, oar-like forward stroke; energy from dynein sliding is used directly.
- ▶ **Recovery stroke** — the elastic recoil of nexin links and radial spokes (which accumulate strain during the effective stroke) returns the cilium to its start position by a slower bend that passes from base to tip.
- ▶ Sliding is prevented from being uniform (which would simply elongate the cilium) by **nexin cross-links restraining the doublets**, and by the **central microtubule pair**, which is thought to rotate (possibly kinesin-driven) and sequentially "distribute" dynein activation around the nine doublets to generate the bend.
- ▶ **Metachronal rhythm** — successive rows of cilia beat with a fixed phase lag, creating a travelling wave across the epithelium in the direction of the effective stroke; this coordination depends on basal feet of neighbouring basal bodies all being oriented in the same direction.

6. Distribution and functions in the body

Site	Ciliary type	Function
Nasal cavity, paranasal sinuses, trachea, bronchi	Motile (9+2)	Mucociliary clearance of trapped particles/pathogens toward the oropharynx
Uterine tube (oviduct)	Motile (9+2)	Propels the ovum and tubal fluid toward the uterus
Efferent ductules of the testis	Motile (9+2)	Assists transport of spermatozoa (non-motile at this stage) toward the epididymis
Ependyma lining brain ventricles/central canal	Motile (9+2)	Circulation of cerebrospinal fluid
Primitive node (embryonic disc)	Nodal (9+0, rotational)	Generates leftward nodal flow → determines left–right visceral asymmetry
Renal tubular and collecting-duct cells, fibroblasts, chondrocytes, most somatic cells	Primary (9+0, monocilium)	Mechanosensor of luminal fluid flow (Ca^{2+} influx via polycystin channels); Hedgehog signalling; tissue morphogenesis
Vestibular and cochlear hair cells	Modified cilium (kinocilium)	Mechanotransduction for balance and hearing

Site	Ciliary type	Function
Photoreceptors (rods/cones)	Modified connecting cilium	Links inner and outer segments; transport of opsin/phototransduction proteins
Olfactory receptor neurons	Modified non-motile cilia	Chemosensation (odorant reception)

Applied / Clinical anatomy

- ▶ **Primary ciliary dyskinesia (PCD)** ("immotile cilia syndrome") — a group of autosomal recessive disorders (≈ 1 in 20,000 births) in which structural defects of the axoneme (classically **absent or deficient dynein arms**) impair ciliary motility.
 - **Kartagener's syndrome** — a variant of PCD with the triad of **situs inversus** (nodal cilia dysfunction — random, hence $\sim 50\%$ mirror-image organ placement), **chronic sinusitis/bronchiectasis** (failed mucociliary clearance in the respiratory tract), and **male infertility** (immotile sperm flagella, which share the 9+2 architecture).
 - **Young's syndrome** — ciliary dysfunction from malformed radial spokes and dynein arms, also causing respiratory disease and infertility.
 - Diagnosis is by **electron microscopy of a nasal or bronchial mucosal biopsy**, examining basal body orientation and axonemal ultrastructure.
 - Females with PCD may retain reduced but adequate tubal ciliary activity for ovum transport (increased risk of ectopic pregnancy), and PCD may also present with hydrocephalus (ependymal ciliary dysfunction) or chronic otitis media.
- ▶ **Polycystic kidney disease (PKD)** — mutations in *PKD1/PKD2* (encoding **polycystin-1 and polycystin-2**) disrupt the mechanosensory primary cilium of renal tubular cells; loss of flow-induced Ca^{2+} signalling leads to uncontrolled tubular epithelial proliferation and cyst formation, with associated hepatic/pancreatic cysts, retinitis pigmentosa and sensorineural hearing loss (ciliopathy spectrum).
- ▶ **Surface marking/histology correlation** — ciliated pseudostratified columnar epithelium (respiratory type) is destroyed and replaced by **squamous metaplasia** in chronic smokers/pollution exposure, abolishing mucociliary clearance and predisposing to infection.
- ▶ Cilia are essential for **Hedgehog signalling** during organogenesis; ciliary dysfunction is implicated in developmental anomalies collectively termed **ciliopathies**.

□ Fig 4.10 — Cilia. (Junqueira's Basic Histology — Text & Atlas — 17e, p.212)

High-yield exam pointers

- ▶ Axoneme = **9 + 2 microtubule arrangement** (9 peripheral doublets + 2 central singlets); basal body = **9 triplets, no central pair** (like a centriole).
- ▶ Motor protein = **ciliary dynein** (ATPase); elastic links = **nexin**; radial spokes connect doublets to the central sheath.
- ▶ Movement = **effective stroke** (rapid, dynein-powered) + **recovery stroke** (slow, elastic recoil); coordinated as **metachronal waves**.

- ▶ Three functional types: **motile** (9+2), **primary/monocilium** (9+0, sensory), **nodal** (9+0, motile, rotational — L-R asymmetry).
- ▶ **Kartagener's syndrome** = PCD + situs inversus + bronchiectasis/sinusitis + infertility, due to defective dynein arms.
- ▶ **Polycystic kidney disease** = ciliopathy of the renal primary cilium (polycystin-1/2).
- ▶ Sites of motile cilia: respiratory tract, oviduct, efferent ductules, ependyma.
- ▶ IFT: **kinesin-2** (anterograde) and **dynein-2** (retrograde) maintain ciliary length.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e; Sontakke — Textbook of Human Histology 2e.



Golgi apparatus

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction — The Golgi apparatus (Golgi complex or Golgi body) is a membrane-bound cytoplasmic organelle composed of a polarized stack of flattened, curved cisternae, situated close to the nucleus and centrosome. It is the principal site of post-translational modification, sorting, and packaging of proteins and lipids synthesized in the rough endoplasmic reticulum (rER), and forms the central relay station of the cell's secretory pathway. It is best developed in actively secreting cells (plasma cells, goblet cells, pancreatic acinar cells, osteoblasts, epididymal epithelium, neurons).

Historical background and identification

- ▶ First described in 1898 by **Camillo Golgi (1843–1926)**, an Italian histologist, while studying **osmium-impregnated** (metallic/silver impregnation, "black reaction") owl and cat nerve cells; he named it the "**internal reticular apparatus**" — a network surrounding the nucleus.
- ▶ Its structure and secretory-state-dependent changes in shape/location were described decades **before the electron microscope** confirmed it and before its functional relationship to the rER was established.
- ▶ **Does not stain with haematoxylin & eosin (H&E)** — because of its high lipid/membrane content it appears in routine paraffin sections as a **clear, unstained perinuclear zone** (sometimes called a "Golgi ghost" or negative-image area), typically partly surrounded by basophilic ergastoplasm (rER). This clear zone is well seen in **plasma cells, osteoblasts, and epididymal cells**.
- ▶ Classically demonstrated by **heavy-metal (silver/osmium) impregnation** methods; today visualised by transmission/scanning EM and immunofluorescence against Golgi-specific proteins (e.g. GRASP — Golgi reassembly-stacking protein).

Location and light-microscopic features

- ▶ Lies **close to the nucleus and centrosome**, usually on the **secretory (apical) pole** of polarised secretory cells, positioned near the microtubule-organising centre.

- ▶ In light microscopy of appropriately stained/oriented cells (e.g. toluidine-blue plastic sections of plasma cells), it appears as a **clear cytoplasmic area near the nucleus**, corresponding to the extensive membranous cisternae; the surrounding cytoplasm stains deeply because of RER-bound ribosomes.
- ▶ Number and size of Golgi stacks vary with cell type and secretory activity — cells may contain **multiple Golgi stacks**, or a single continuous ribbon-like Golgi complex.

Ultrastructure (electron-microscopic organisation)

Component	Description
Cisternae	3–20 (typically 4–8 in most mammalian cells; up to ~60 stacked cisternae reported in some unicellular organisms) flattened, curved, membrane-limited sacs stacked like a pile of plates, forming a shallow cup-shaped structure; cisternae differ from one another in enzyme content/activity
cis-Golgi network (CGN)	Convex, forming/entry face , directed toward the rER and nucleus; complex tubular network that receives COPII-coated transport vesicles from the rER; selects correctly-folded proteins for onward transport, shuttles escaped rER-resident proteins back retrogradely
Medial cisternae	Central stack between CGN and TGN; site of remodelling of N-linked oligosaccharides, addition/removal of sugar residues
trans-Golgi network (TGN)	Concave, maturing/exit (shipping) face , directed toward the plasma membrane; final protein sorting station — packages modified proteins into vesicles addressed to lysosomes, secretory granules, or specific plasma-membrane domains; site of clathrin-coat assembly
Golgins	Long coiled-coil proteins on the cytoplasmic cisternal surface forming a fibrillar matrix that stabilises the stack and participates in vesicle tethering
Associated vesicles	Transport vesicles (rER → cis-Golgi, COPII-coated, anterograde); COPI-coated vesicles (intra-Golgi and Golgi → rER, retrograde); clathrin-coated vesicles budding from the TGN toward lysosomes/plasma membrane
Support	The whole stack is supported by cytoplasmic microtubules radiating from the microtubule-organising centre — hence microtubule-disrupting drugs fragment/disperse the Golgi

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 3.7 — Golgi apparatus. (Sontakke — Textbook of Human Histology — 2e, p.36)

Reproduce as a stack of curved, flattened cisternae with the following labels:

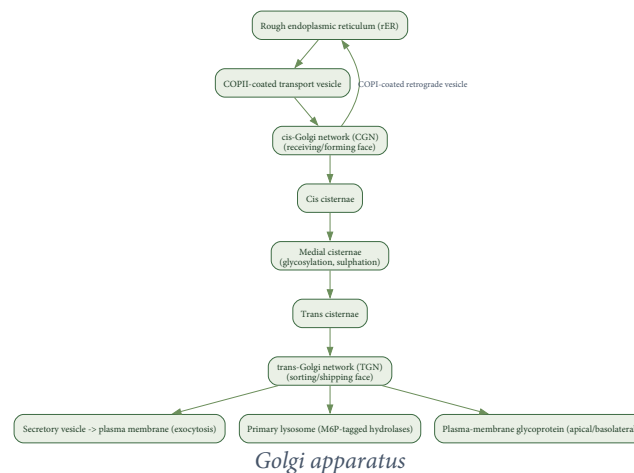
- ▶ **cis-face (receiving/forming end)** — facing the rER; label incoming **vesicles carrying proteins from rER**
- ▶ Stack of **cisternae** (cis → medial → trans), each drawn as a flattened curved sac
- ▶ **trans-face (shipping/maturing end)** — facing the plasma membrane; label budding **secretory vesicles containing modified proteins**
- ▶ Small dots within the trans-face vesicles labelled "**modified protein molecules**"
- ▶ Adjacent **rER with ribosomes** and **protein molecules** entering via transport vesicles

Functions

- ▶ **Post-translational modification** of proteins and lipids synthesised in the rER:
 - **Glycosylation** — trimming of mannose residues and addition of N-acetylglucosamine, galactose and sialic acid to N-linked oligosaccharides (begun in rER, completed/remodelled across the Golgi stack)
 - **Sulphation** — addition of sulphate groups to glycosaminoglycans (proteoglycan synthesis) and to some tyrosine residues
 - **Phosphorylation** — including addition of **mannose-6-phosphate (M6P)** to N-linked oligosaccharides of lysosomal hydrolases in the cis-Golgi — the key tag for lysosomal targeting
 - **Limited proteolysis** — converts some inactive proproteins to their active form (e.g. proteolytic activation of secretory proproteins/zymogens)
- ▶ **Sorting and packaging** — the TGN reads amino-acid/oligosaccharide signal sequences on proteins and dispatches them, enclosed in appropriately-composed vesicles, to their correct destination (lysosomes, secretory granules, apical or basolateral plasma-membrane domains in polarised epithelial cells).
- ▶ **Formation of secretory vesicles/granules** — condensing vacuoles at the trans-face concentrate (by extracting water) and mature into secretory granules.
- ▶ **Formation of primary lysosomes** — M6P-tagged acid hydrolases are recognised by M6P receptors in the TGN and packaged into vesicles that bud off as primary lysosomes (Golgi hydrolase vesicles).
- ▶ **Membrane biogenesis and recycling** — contributes new membrane and membrane-associated glycoproteins to the plasma membrane; participates in membrane-recycling pathways together with endosomes.
- ▶ Provides a **membrane platform** for signalling and sorting proteins (golgins, coat-protein complexes) to bind.

Secretory pathway and vesicular traffic

The passage of a newly synthesised secretory protein through the Golgi follows a fixed sequence of vesicle budding, transport and fusion steps:



- **Constitutive secretory pathway** — transport and secretion occur continuously without storage, immediately after synthesis and exit from the Golgi; e.g. immunoglobulins from plasma cells, procollagen from fibroblasts; present to some degree in all cells.
- **Regulated secretory pathway** — the secretory product is concentrated and stored temporarily in membrane-bound secretory granules and released only on an external hormonal or neural signal; e.g. pancreatic acinar cell zymogen granules, salivary secretion under autonomic stimulation.
- Coat-protein complexes govern directionality: **COPII** mediates anterograde transport (rER → cis-Golgi); **COPI** mediates retrograde transport (Golgi → rER, and intra-Golgi cisterna-to-cisterna); **clathrin** coats vesicles budding from the TGN destined for lysosomes or the plasma membrane.

Applied / Clinical anatomy

- **I-cell disease (Inclusion-cell disease, Mucopolipidosis II)** — deficiency of the Golgi enzyme **GlcNAc-1-phosphotransferase** prevents mannose-6-phosphate tagging of lysosomal hydrolases in the cis-Golgi; the untagged enzymes are not recognised by the TGN's M6P receptor and are **defaultly secreted out of the cell** instead of being packaged into lysosomes. The lysosomes, deprived of their digestive enzymes, accumulate undigested substrate as coarse inclusions (hence "I-cell"), while the enzymes appear in raised concentration in body fluids; clinically manifests with skeletal deformity, coarse facies and psychomotor regression.
- **α 1-antitrypsin (A1AT) deficiency** — a mutant A1AT protein fails to fold correctly and cannot be exported from the rER to the Golgi; it accumulates within hepatocyte rER as PAS-positive, diastase-resistant globules, contributing to hepatocyte injury/cirrhosis, while the lack of circulating A1AT predisposes to **panacinar emphysema**.
- Because the Golgi is supported by, and positioned via, the **microtubule cytoskeleton**, microtubule-disrupting agents (e.g. **colchicine**, **vinca alkaloids** used in cancer chemotherapy) fragment and disperse the Golgi ribbon, disrupting secretory traffic — one basis of their cytotoxic action.

- ▶ Its absence of H&E staining and characteristic **clear perinuclear zone** is a useful light-microscopic clue to identifying highly secretory cells (plasma cells, osteoblasts, epididymal principal cells) in routine histology.
- ▶ Golgi-mediated **sulphation of proteoglycans** underlies its importance in cartilage- and connective-tissue-forming cells (chondroblasts, fibroblasts) and in mucus-secreting goblet cells.

High-yield exam pointers

- ▶ Discovered 1898 by **Camillo Golgi**, using osmium/silver impregnation of nerve cells; originally called the "internal reticular apparatus."
- ▶ Composed of 3–20 (usually 4–8) **stacked, flattened cisternae** with three functional zones: **cis-Golgi network** (forming face, toward rER) → **medial cisternae** → **trans-Golgi network** (maturing/shipping face, toward plasma membrane).
- ▶ **Does not stain with H&E**; appears as a clear perinuclear zone ("Golgi ghost") in secretory cells.
- ▶ Key functions: **glycosylation, sulphation, phosphorylation (mannose-6-phosphate tagging), limited proteolysis, sorting and packaging** of proteins/lipids.
- ▶ Vesicle traffic directionality: **COPII = anterograde (rER→Golgi)**; **COPI = retrograde**; **clathrin = TGN→lysosomes/plasma membrane**.
- ▶ Gives rise to **primary lysosomes**, secretory vesicles/granules, and contributes new plasma membrane.
- ▶ Two secretory pathways: **constitutive** (continuous, e.g. plasma cells) vs **regulated** (stimulus-dependent storage/release, e.g. pancreatic acinar cells).
- ▶ Clinical correlations: **I-cell disease** (failure of M6P tagging) and **α 1-antitrypsin deficiency** (rER export block) — both classic viva/theory links.
- ▶ Best developed in cells with high secretory or membrane-synthetic activity: plasma cells, goblet cells, pancreatic acinar cells, osteoblasts, epididymal cells, neurons.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — Text & Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



RNA

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Ribonucleic acid (RNA) is a single-stranded polynucleotide molecule, located chiefly in the nucleolus and cytoplasm of the cell, that is transcribed from a DNA template and mediates the flow of genetic information from DNA to protein. Unlike DNA, RNA contains the pentose sugar ribose and the pyrimidine base uracil in place of thymine, and it does not replicate itself. Three classical functional species — messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) — cooperate at the ribosome to translate the genetic code into polypeptide chains, while additional regulatory RNAs (hnRNA, snRNA, snoRNA, miRNA) govern gene expression at several levels. Because its phosphate backbone is strongly basophilic, RNA is also of direct histological importance, forming the basis of a dedicated special-staining technique.

Chemical structure of RNA

- ▶ Basic building block: the **nucleotide**, made up of (i) a nitrogenous base, (ii) the pentose sugar **ribose**, and (iii) a phosphate group; base + sugar without phosphate constitutes a **nucleoside**.
- ▶ Bases: two purines — **adenine (A)** and **guanine (G)** — and two pyrimidines — **cytosine (C)** and **uracil (U)**. Uracil replaces the thymine of DNA and pairs only with adenine.
- ▶ Adjacent nucleotides are linked by 3'-5' **phosphodiester bonds** to form a single polynucleotide strand; the molecule is single-stranded but folds back on itself through intramolecular (complementary) base pairing to generate secondary structure (stem-loops), rather than forming a double helix like DNA.
- ▶ RNA is synthesised in the nucleus by transcription of one strand of DNA and is transported into the cytoplasm through **nuclear pore complexes**.

Feature	DNA	RNA
Location	Predominantly nucleus (also mitochondria)	Nucleolus and cytoplasm
Strands	Double-stranded, antiparallel helix	Single-stranded
Sugar	Deoxyribose	Ribose
Pyrimidine base	Thymine	Uracil
Function	Stores genetic information; replicates	Expresses genetic information; does not replicate
Amount in cell	Constant, stable	Variable, turned over

Types of RNA and their functions

Type	Site of synthesis	Approx. share of cell RNA	Principal function
mRNA (messenger)	Nucleus, from DNA	~5%	Carries the coded nucleotide sequence (codons) from DNA to the ribosome; template for translation
tRNA (transfer)	Nucleus, from DNA	~15%	Adaptor molecule; matches a specific activated amino acid to its complementary codon on mRNA via the anticodon
rRNA (ribosomal)	Nucleolus, from DNA	~80%	Combines with ribosomal proteins to form the ribosome; some rRNA is catalytically active (ribozyme)

- **mRNA** is synthesised in the nucleus, copies the nucleotide sequence of the coding DNA strand, and moves into the cytoplasm to dictate the amino-acid sequence of the protein; the genetic code is carried in triplet groups of nucleotides (**codons**), and the code is degenerate (more than one codon may specify the same amino acid).
- **tRNA** links the codon sequence of mRNA to the amino-acid sequence of the growing polypeptide; each tRNA is charged with (activated to carry) one specific amino acid before translation.
- **rRNA** constitutes the great majority of total cellular RNA and, with ribosomal proteins, assembles into the **small (40S)** and **large (60S)** ribosomal subunits in the nucleolus; these are exported separately and unite in the cytoplasm during translation.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

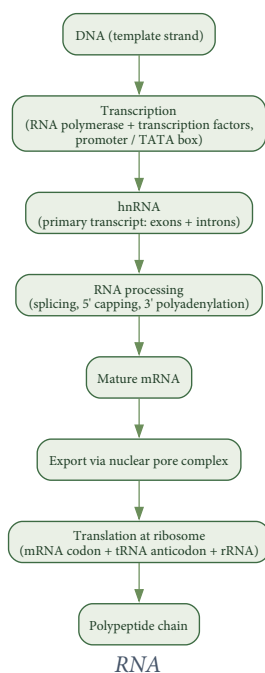
□ Fig 30.2 — Clover leaf model of tRNA (IB Singh — *Textbook of Anatomy Vol 3 — 6e, p.480*)

Reproduce the two-dimensional clover-leaf fold of a single tRNA strand, labelling in sequence:

- ▶ **Acceptor stem** — base-paired 5' and 3' ends of the strand; the free 3'-CCA terminus is the site of amino-acid attachment
- ▶ **D-arm (D-loop)** — one of the side loops of the clover leaf
- ▶ **Anticodon arm (loop)** — projects furthest from the acceptor stem; carries the **anticodon triplet** at its tip, antiparallel and complementary to an mRNA codon
- ▶ **T Ψ C arm (loop)** — the remaining side loop
- ▶ **Variable loop** — connects the anticodon and T Ψ C arms; differs in length between different tRNA species

Biosynthesis of RNA — transcription, processing and translation

- ▶ Transcription is catalysed by **RNA polymerase**, which binds the gene's promoter region (typically containing a **TATA box**) together with **transcription factors**, and reads the DNA template strand in the 3'→5' direction while synthesising RNA 5'→3'.
- ▶ RNA polymerase II transcribes protein-coding genes into a **primary transcript**, or **heterogeneous nuclear RNA (hnRNA)**, containing both coding (**exon**) and non-coding (**intron**) sequences; other classes of RNA polymerase transcribe the rRNA and tRNA genes.
- ▶ **RNA processing**: introns are excised and exons spliced together, with addition of a 5' cap and a 3' poly-A tail, to yield mature mRNA, which then exits the nucleus through the nuclear pore complex.
- ▶ **Translation**: mRNA is read at cytoplasmic ribosomes — commonly several ribosomes attached simultaneously to one mRNA strand, forming **polyribosomes (polysomes)**; tRNA delivers the correct activated amino acid at each codon by codon–anticodon base pairing, and successive amino acids are joined by peptide bonds until a stop codon is reached.



Other (non-classical) RNA species

- ▶ **hnRNA (heterogeneous nuclear RNA)** — the unprocessed primary transcript; precursor of mature mRNA.
- ▶ **snRNA (small nuclear RNA)** — a component of the spliceosome, the complex responsible for intron removal during RNA splicing.
- ▶ **snoRNA (small nucleolar RNA)** — guides chemical modification of rRNA within the nucleolus.
- ▶ **miRNA / siRNA (micro- and small interfering RNA)** — short non-coding RNAs that regulate gene expression post-transcriptionally by promoting degradation of, or blocking translation from, a target mRNA (the molecular basis of RNA interference).
- ▶ **lncRNA (long non-coding RNA)** — longer regulatory transcripts implicated in gene silencing and epigenetic regulation.

Histochemical and molecular demonstration of RNA

- ▶ The phosphate backbone of RNA is **polyanionic**, giving strong **basophilia** with haematoxylin and other basic dyes; this underlies the deep basophilia of the nucleolus and of ribosome-rich cytoplasm (**ergastoplasm**), seen for example in the **Nissl substance** of neurons and in actively secretory acinar cells of the pancreas and salivary glands.
- ▶ The **Feulgen reaction** stains DNA specifically (magenta/red-purple), leaving RNA unstained, and so serves as a DNA-specific comparator.
- ▶ The **methyl green–pyronin technique** is the method of choice for demonstrating RNA: methyl green binds DNA (chromatin stains green) while pyronin Y binds RNA (nucleolus and RNA-rich cytoplasm stain red); Carnoy's fixative is preferred as it preserves RNA well.
- ▶ Specificity of RNA staining is confirmed by a **ribonuclease (RNase) digestion control** — pre-treating a parallel section with RNase abolishes pyroninophilia/basophilia in RNA-containing regions, while a corresponding DNase control abolishes Feulgen positivity.

- ▶ Molecular techniques that exploit RNA include **reverse-transcriptase PCR (RT-PCR)**, which converts an RNA target to complementary DNA before amplification, and **in-situ hybridisation/FISH** with labelled complementary nucleotide probes, used in diagnostic molecular pathology.

Applied / Clinical anatomy

- ▶ **Chromatolysis**: after axonal injury, the RNA-rich Nissl substance (rough endoplasmic reticulum) of the neuronal cell body disperses and loses its basophilic staining — a classical histological marker used to time and assess axonal degeneration/regeneration.
- ▶ **α 1-Antitrypsin deficiency**: illustrates the clinical consequence of disordered handling of an rER (ribosome/RNA-dependent)-synthesised protein — the enzyme fails to be delivered from rER to the Golgi apparatus for secretion.
- ▶ **RNA viruses and retroviruses**: e.g. HIV, whose reverse transcriptase converts the viral RNA genome into proviral DNA — relevant to understanding retroviral replication.
- ▶ **RNA-interference (RNAi) therapeutics**: synthetic siRNA agents are used clinically to silence a disease-causing mRNA (e.g. in the treatment of hereditary transthyretin amyloidosis), a direct translational application of small regulatory RNA biology.
- ▶ **Diagnostic molecular pathology**: FISH and RT-PCR are used to detect specific fusion transcripts (e.g. gene rearrangements in Ewing sarcoma), extending RNA/DNA hybridisation principles into oncological diagnosis.
- ▶ **Practical/viva relevance**: the methyl green–pyronin stain with its RNase control is a standard special-stain demonstration in the histology/microscopy curriculum, directly testing understanding of RNA histochemistry.

High-yield exam pointers

- ▶ RNA is single-stranded, contains ribose sugar and the bases A, G, C, U (uracil replaces thymine of DNA); synthesised by transcription from a DNA template.
- ▶ Three classical types: **mRNA** (template for translation, ~5% of cell RNA), **tRNA** (adaptor molecule, clover-leaf structure, ~15%), **rRNA** (structural/catalytic component of ribosomes, ~80%).
- ▶ The tRNA clover-leaf model has an acceptor stem (3'-CCA + amino acid) and three loops — D-arm, anticodon arm (with anticodon triplet), and T ψ C arm — plus a variable loop.
- ▶ The **nucleolus** is the site of rRNA transcription and assembly of the 40S and 60S ribosomal subunits.
- ▶ Central dogma of information flow: DNA $\rightarrow$ hnRNA (transcription) $\rightarrow$ mature mRNA (splicing/processing) $\rightarrow$ protein (translation, using tRNA and ribosomal rRNA).
- ▶ The genetic code is a triplet, non-overlapping, and degenerate code.
- ▶ Strong basophilia of RNA-rich structures (ergastoplasm, Nissl bodies, nucleolus) is explained by the polyanionic phosphate backbone of RNA.
- ▶ **Methyl green–pyronin** stains RNA red/pink and DNA green; an **RNase digestion control** abolishes RNA-specific staining — a classic practical/viva question.

- ▶ Newer non-coding RNA species (miRNA, siRNA, lncRNA) regulate gene expression and underlie RNA-interference-based therapeutics.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Anatomy Vol 3, 6e (chapter: DNA and RNA); Vishram Singh — General Anatomy, 4e; Gangane — Human Genetics, 5e; Thompson & Thompson — Genetics in Medicine, 8e; Junqueira's Basic Histology, 17e; Ross Histology — A Text and Atlas, 7e; Sontakke — Textbook of Human Histology, 2e; Bancroft's Theory and Practice of Histological Techniques; Langman's Medical Embryology (Sadler), 14e.

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

RNA is primarily a molecular/cell-biology topic; the dedicated gross-anatomy texts cover it only briefly as a single combined "DNA and RNA" chapter. This answer therefore draws its structural and functional core from the genetics and histology texts in the library (as anticipated in the topic note), supplemented only by universally accepted, standard molecular biology.



Stem cells

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction — Stem cells are undifferentiated cells defined by two cardinal properties: **multilineage differentiation** (the potential to give rise to cells of one or more of the three germ layers — ectoderm, mesoderm, endoderm) and **self-renewal** (the ability to divide and generate further stem cells, thereby propagating themselves indefinitely). They are broadly categorised into **embryonic** and **adult (somatic)** stem cells, with a third, laboratory-derived category — **induced pluripotent stem cells (iPSCs)** — now recognised. Stem cells underlie normal tissue renewal throughout life and form the biological basis of regenerative medicine.

1. Properties and behaviour of stem cells

- ▶ **Self-renewal:** each division can regenerate an identical stem cell, maintaining the stem-cell pool.
- ▶ **Asymmetric division:** many stem cells divide infrequently and asymmetrically — one daughter remains an undifferentiated stem cell, the other becomes a **progenitor** ("transit-amplifying") cell that divides several times more rapidly before terminally differentiating.
- ▶ **Stem cell niche:** stem cells of most renewing tissues occupy specific anatomical locations (niches) where the local microenvironment (neighbouring cells, extracellular matrix, paracrine/juxtacrine signals) maintains their undifferentiated state; niche cells are typically rare and inconspicuous on routine histology.
- ▶ Best studied in rapidly renewing populations — **blood cells, epidermis, and gut epithelium**; in stable tissues (most connective tissue, smooth muscle, vascular endothelium) stem cells are not readily identifiable and turnover is slow/episodic.

□ Fig 3.17 — Stem cells. (Junqueira's Basic Histology — Text & Atlas — 17e, p.177)

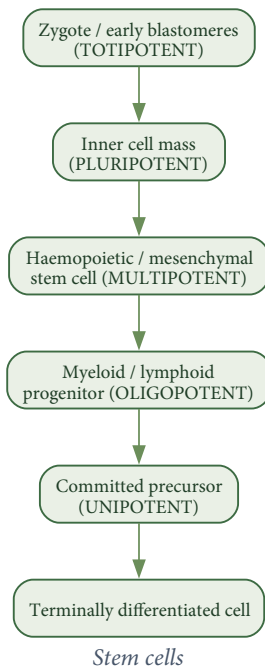
DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple asymmetric-division/niche schematic with: **Niche** (basal lamina/stromal cells) → **Stem cell** (attached in niche) dividing asymmetrically into (a) **new stem cell** (remains in niche) and (b) **progenitor/transit-amplifying cell** (leaves niche, divides rapidly) → several **differentiating (committed) cells** → **fully differentiated, terminal cell**.

2. Classification of stem cells

(A) By developmental potency

Type	Differentiation potential	Example
Totipotent	Can form the entire embryo AND extra-embryonic tissues (placenta)	Zygote, cells of the early blastomere (up to ~8-cell stage)
Pluripotent	Can form all cell types of the embryo proper (all 3 germ layers) but not extra-embryonic tissues	Inner cell mass (ICM) cells / embryonic stem cells
Multipotent	Can form several related cell types within one lineage	Haemopoietic stem cell (blood cell lineages); mesenchymal stem cell
Oligopotent	Can form a few related cell types	Myeloid or lymphoid progenitor cell
Unipotent	Can form only one cell type, but retains self-renewal	Hepatocyte progenitor; spermatogonial stem cell



(B) **By source/origin** — embryonic stem cells (ESCs), adult/somatic stem cells (mesenchymal, haemopoietic, epithelial), amniotic-fluid stem cells, umbilical-cord stem cells, and induced pluripotent stem cells (iPSCs).

(C) **Immunological classification** (relevant to transplantation) — **syngeneic** (from an identical twin), **autologous** (from the same individual), **allogeneic** (from another member of the same species), **xenogeneic** (from a different species). Syngeneic and autologous cells carry the least risk of immune rejection.

3. Embryonic stem cells (ESCs)

- ▶ Derived from the **inner cell mass of the blastocyst**; the archetypal **pluripotent** stem cell, capable of differentiating into any cell type of the body.
- ▶ Can be propagated indefinitely in culture while remaining undifferentiated.
- ▶ **Limitations**: risk of immunological rejection; safety concern of **teratoma (tumour) formation**; ethical dilemmas over the source material; requirement for donor oocytes.
- ▶ Somatic cell nuclear transfer (cloning) has been used experimentally to generate patient-matched ESC lines, potentially avoiding rejection.

4. Adult (somatic) stem cells and their niches

Adult stem cells are tissue-specific, multipotent (or unipotent) cells that persist throughout life to maintain and repair their tissue of residence.

Haemopoietic stem cells (HSCs) — the best-characterised adult stem cell:

- ▶ All blood cells derive from a single **pluripotent haemopoietic stem cell** in the bone marrow, which divides slowly and gives rise to two committed progenitor lineages: **myeloid** (erythrocytes, granulocytes, monocytes, megakaryocytes) and **lymphoid** (B cells, T cells, NK cells).
- ▶ Progenitor/precursor cells are called **colony-forming units (CFUs)** — erythroid, thrombocytic (megakaryocyte), granulocyte-monocyte, and lymphoid CFUs — because each produces a colony of one cell type in culture or in an irradiated spleen.
- ▶ HSCs reside in a **bone marrow niche** with endocrine, paracrine and juxtacrine signalling; **haemopoietic growth factors/cytokines** (erythropoietin, colony-stimulating factors, other cytokines) drive proliferation and lineage-specific differentiation.
- ▶ Normal output is enormous — approximately 3×10^9 erythrocytes and 0.85×10^9 granulocytes per kg body weight per day.
- ▶ Can be isolated by fluorescence-activated cell sorting (FACS) using surface antigen markers; studied by transplanting irradiated animals' marrow (basis of clinical bone-marrow transplantation).

□ Fig 10.19 — Hemopoiesis. Chart based on current concepts of hemopoiesis. (Ross Histology — A Text and Atlas — 7e, p.310)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the haemopoietic tree with labels: **Pluripotent haemopoietic stem cell** → branching into **Myeloid stem cell** and **Lymphoid stem cell**; from the myeloid stem cell show **CFU-erythroid** → **erythrocyte**, **CFU-granulocyte/monocyte** → **granulocytes & monocytes**, **CFU-megakaryocyte** → **platelets**; from the lymphoid stem cell show **B lymphocyte**, **T lymphocyte**, **NK cell**. Mark the **bone marrow niche** around the stem cell compartment.

Mesenchymal stem cells (MSCs):

- ▶ **Multipotent**, restricted largely to the **mesodermal** lineage — differentiate into chondrocytes (cartilage), osteoblasts (bone), and adipocytes (fat), depending on the local microenvironment.
- ▶ Derived mainly from **bone marrow stroma**, but also enriched from adipose tissue, synovium, skeletal muscle and placental tissue.
- ▶ Ultrastructurally, adult stem-cell/MSC niches correspond in part to **pericytes** (perivascular/adventitial cells) around capillaries and venules.
- ▶ Easily expanded in culture in large numbers; carry minimal ethical concern (comparable to established bone-marrow transplantation).

Other epithelial niches (general principle, exemplified across the body):

- ▶ **Gastric gland stem cells** — undifferentiated cells near the neck of gastric glands multiply to replace surface and glandular cells and increase in number during mucosal injury (e.g. peptic ulcer healing).
- ▶ Analogous renewing niches exist in the **intestinal crypts** and the **basal layer of the epidermis**, where stem cells generate transit-amplifying/progenitor cells that migrate, divide, and terminally differentiate (villus enterocytes; keratinocytes).

5. Other clinically important stem-cell types

Type	Advantages	Disadvantages
Embryonic (ESC)	Pluripotent	Immune rejection; teratoma risk; ethical concerns; oocyte requirement
Amniotic-fluid (AFSC)	Pluripotent; non-tumorigenic; can be harvested by amniocentesis and used to correct defects in utero or at birth	Low yield (~1% of amniotic fluid cells are c-kit ⁺ /CD117 ⁺); needs further characterisation
Umbilical-cord blood	Easy, low-morbidity procurement; cryopreservable/bankable; minimal ethical concern	Low cell yield, finite quantity; storage/quality-control issues

Type	Advantages	Disadvantages
Mesenchymal (MSC)	Multipotent; large numbers harvestable from marrow, fat, etc.; autologous; immunomodulatory; minimal ethical concern	Differentiation depends on microenvironment; limited long-term potential; optimal delivery route unclear
Induced pluripotent (iPSC)	Pluripotent; derivable from any somatic cell; autologous, probably non-immunogenic	Low reprogramming efficiency (<1%); viral-vector safety concerns; tumour risk

- **Induced pluripotent stem cells (iPSCs)**: generated by Takahashi and Yamanaka (2006), who showed that introducing four transcription factors — **Oct3/4, Sox2, Klf4, c-Myc** (the "Yamanaka"/stemness factors) — into a differentiated somatic cell (e.g. a fibroblast) reverts it to a pluripotent state; Yamanaka received the **2012 Nobel Prize in Physiology or Medicine** for this work. Such cells have even been reprogrammed toward **totipotency**. Current research aims to generate iPSCs without viral vectors (using small molecules) to reduce mutagenic/tumorigenic risk.

Applied / Clinical anatomy

- **Bone-marrow (haemopoietic stem cell) transplantation** — autologous or allogeneic HSC infusion reconstitutes marrow after ablative chemo-/radiotherapy; used for leukaemias, aplastic anaemia, and inherited haemopoietic/immunodeficiency disorders.
- **Haemopoietic growth factors** (erythropoietin, G-CSF/GM-CSF and other cytokines) are used clinically to boost blood counts in severe anaemia and chemotherapy-/radiotherapy-induced leukopenia, and to enhance marrow-transplant efficiency.
- **Tissue engineering**: autologous stem cells (e.g. bone-marrow and respiratory epithelial stem cells) are seeded onto natural (decellularised) or synthetic scaffolds in a bioreactor to fashion replacement tissue; the trachea was the **first stem-cell-based tissue-engineered organ successfully transplanted in humans**.
- **MSC-based therapy** is in clinical trials for several diseases, including post-myocardial-infarction repair, with encouraging early results.
- **Blastocyst complementation** — an experimental approach in which iPSCs are injected into blastocysts of animals (e.g. pigs) genetically engineered to lack a specific organ; the iPSCs "home" to the developmentally deficient niche and generate that organ, raising the future possibility of growing transplantable human organs in livestock (with attendant immunological and ethical concerns, including theoretical human–animal hybridisation if cells home to brain or germline).
- **Cancer/teratoma risk**: uncontrolled proliferation of pluripotent cells (ESC, iPSC) can produce teratomas — a key safety barrier to clinical translation.
- Gastric-mucosal stem-cell proliferation underlies epithelial healing after peptic ulceration — a routine example of adult stem-cell physiology with direct clinical relevance.

High-yield exam pointers

- **Definition**: multilineage differentiation potential + self-renewal (Thomson et al 1998).

- ▶ **Potency hierarchy:** Totipotent (zygote) → Pluripotent (ICM/ESC) → Multipotent (HSC/MSC) → Oligopotent (myeloid/lymphoid progenitor) → Unipotent (single lineage, e.g. hepatocyte progenitor).
- ▶ **Source classes:** Embryonic vs Adult (somatic) vs iPSC; also amniotic-fluid and umbilical-cord stem cells.
- ▶ **Immunological classes:** syngeneic, autologous, allogeneic, xenogeneic.
- ▶ **ESC** = pluripotent, from blastocyst ICM; main drawbacks — rejection, teratoma, ethics.
- ▶ **iPSC** = Takahashi–Yamanaka 2006; factors **Oct3/4, Sox2, Klf4, c-Myc**; Nobel Prize 2012.
- ▶ **HSC:** single pluripotent marrow stem cell → myeloid + lymphoid lineages; CFUs; driven by EPO/CSFs; niche concept.
- ▶ **MSC:** multipotent, mesodermal (bone/cartilage/fat), bone-marrow/adipose/synovium, linked to pericytes.
- ▶ Asymmetric division → progenitor ("transit-amplifying") cell is the mechanistic basis of tissue renewal in skin, gut and blood.
- ▶ Clinical translation: bone-marrow transplant, tissue-engineered trachea, MSC trials in myocardial infarction, blastocyst complementation.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e — Commentary 1.2, "Stem cells in regenerative medicine"; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e.



Genetics & cytogenetics



Anaphase lag

Asked in: Paper III Nov 2017 · Paper III May 2010 · Paper III Sep 2007 (3×) — RGUHS MD/MS Anatomy

Definition / Introduction Anaphase lag is a mechanism of post-mitotic (occasionally post-meiotic) **chromosome loss** in which a chromosome or chromatid separates normally from its partner at anaphase but then fails to migrate to its pole in time, so that it is left out of both reforming daughter nuclei and is subsequently degraded. Unlike non-disjunction, where the two chromatids/homologues fail to separate at all and both pass to the same pole, anaphase lag involves *normal disjunction with delayed, incomplete transport* of one member. It is one of the two classic cytogenetic mechanisms (the other being mitotic non-disjunction) that generate **chromosomal mosaicism**, and it is the principal explanation for monosomic mosaic states such as the 45,X/46,XX Turner mosaic.

1. Cytological basis — what actually happens at anaphase

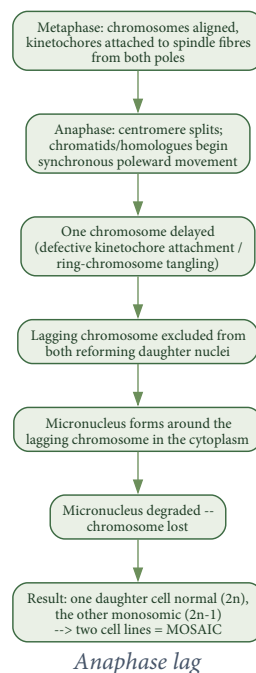
- ▶ Normally, at metaphase every chromosome's kinetochore is correctly attached to spindle fibres from both poles (amphitelic attachment), so that at anaphase the centromere splits and sister

chromatids (mitosis/meiosis II) or homologous chromosomes (meiosis I) move synchronously and completely to opposite poles.

- ▶ In anaphase lag, disjunction itself is **normal**, but one chromosome is delayed in its poleward movement because of:
 - a defective or unstable **kinetochore–spindle fibre attachment** to the migrating chromosome;
 - a structurally abnormal chromosome (a **ring chromosome** or dicentric/marker chromosome) whose two chromatids become **tangled while attempting to disjoin at anaphase**, causing breakage-fusion cycles and variable-sized derivatives;
 - increased cytoplasmic viscosity or a mechanically hindered chromosome that cannot keep pace with its homologue/sister.
- ▶ The lagging chromosome is left in the cytoplasm outside the newly forming nuclear envelope. It is walled off as a **micronucleus**, which typically fails to replicate in step with the main nucleus and is progressively degraded, so the chromosome is effectively **lost**.

2. Consequence — one normal cell + one monosomic cell

- ▶ Because only the lagging element is lost (nothing is gained by the sister nucleus), anaphase lag produces **one euploid daughter cell and one monosomic daughter cell** — in contrast to non-disjunction, which produces one trisomic and one reciprocal monosomic daughter cell.
- ▶ If this occurs in an **early post-zygotic (cleavage-stage) mitotic division**, both resulting cell lines are propagated through subsequent divisions, giving rise to a **mosaic individual** with two (or more) karyotypically distinct cell populations derived from a single zygote.
- ▶ If it occurs later, or is confined to a particular tissue lineage (e.g., the cytotrophoblast), only that tissue is mosaic — the basis of **confined placental mosaicism**.



3. Anaphase lag vs non-disjunction — the key exam distinction

Feature	Non-disjunction	Anaphase lag
Primary defect	Chromatids/homologues fail to separate at anaphase	Chromosomes separate normally ; one is simply delayed in migration
Fate of the involved chromosome	Both copies pass to the same pole	The delayed copy is excluded, forms a micronucleus, and is degraded/lost
Daughter-cell outcome	One cell trisomic (gain), the reciprocal cell monosomic (loss) — two abnormal lines	One cell normal (euploid) , the other monosomic — only one abnormal line
Stage at which it occurs	Meiosis I, meiosis II, or mitosis (including early post-zygotic cleavage)	Chiefly mitosis (early post-zygotic cleavage); rarely meiosis
Typical mosaic karyotype produced	e.g., 46/47,+21 (normal + trisomic)	e.g., 45,X/46,XX (normal + monosomic)
Relative phenotypic effect	Trisomic line often carries the more marked imbalance	Generally milder — loss of one chromosome copy in a minority clone is comparatively better tolerated

4. Timing and the patterns of mosaicism it produces

- ▶ **True mosaicism:** anaphase lag in an early cleavage division of the zygote generates two genetically distinct, heritable cell lines present in the individual — confirmed when *all* parallel cell cultures from a sample show the same abnormal line.
- ▶ **Pseudomosaicism:** an artefactual abnormal clone arising only in tissue culture (or from maternal cell contamination in prenatal samples), present in only one culture vessel — must be distinguished from true mosaicism before counselling a family.
- ▶ **Confined placental mosaicism (CPM):** anaphase lag/chromosome loss restricted to a cytotrophoblast progenitor produces a mosaic placenta with a euploid fetus (or vice versa); it explains why chorionic villus sampling (CVS) karyotypes can be discordant with the true fetal karyotype in 1–2% of pregnancies.
- ▶ **"Trisomy rescue":** when a trisomic zygote loses the supernumerary chromosome post-zygotically (by anaphase lag or non-disjunction), a diploid cell line is restored and the pregnancy may become viable; if the two retained homologues happen to be from the same parent, this yields **uniparental disomy (UPD)** — clinically important for imprinted chromosomes (e.g., maternal UPD 15 → Prader-Willi syndrome; paternal UPD 15 → Angelman syndrome).
- ▶ **Ring/marker chromosome instability:** a ring chromosome's two sister chromatids can become tangled while attempting to disjoin at anaphase, so the ring may break and re-fuse or simply be lost — this is why ring and small supernumerary marker chromosomes are so often found in **only a proportion of cells** (a mosaic state).

5. Cytogenetic and clinical examples

Karyotype / setting	Mechanism	Comment
45,X/46,XX (mosaic Turner syndrome)	Anaphase lag with loss of one X in an early cleavage division	Classic example; features of Turner syndrome are less evident than in non-mosaic 45,X
45,X/46,XY	Anaphase lag involving the Y (or X)	May present anywhere from a Turner phenotype to ambiguous genitalia
Mosaic Down syndrome (46/47,+21)	Post-zygotic chromosome-number change in a trisomy-21 conceptus	Phenotype and cognitive impact are often milder and variable, correlating with the proportion of the trisomic clone
Ring/marker chromosome mosaics	Anaphase bridging/tangling of a ring chromosome	Risk of associated abnormality ranges from negligible to very high depending on chromosomal origin/content
Uniparental disomy (e.g., UPD 15)	"Trisomy rescue" — postzygotic loss of the extra chromosome	Produces imprinting disorders even with a normal chromosome number/karyotype

6. Laboratory detection

- ▶ Conventional **karyotyping of multiple cells/cultures** (typically ~50 cells) remains the most sensitive method for detecting mosaicism; chromosomal microarray (CMA) analyses pooled DNA and is far less sensitive, generally missing mosaic clones below ~10–20% of cells.
- ▶ A finding is accepted as **true mosaicism** only if the abnormal line recurs in independent culture vessels/tissues; a clone confined to a single vessel is treated as **pseudomosaicism** (culture artefact) until proven otherwise.
- ▶ Because interpretation of low-level or tissue-limited mosaicism (lymphocyte vs CVS vs amniocyte) is often uncertain, confirmatory testing (e.g., amniocentesis after an abnormal CVS result) is frequently required.

Applied / Clinical anatomy

- ▶ **Turner syndrome mosaicism** is the single most tested clinical correlate — 45,X/46,XX individuals usually have a **milder, more variable phenotype** (taller stature, better-preserved ovarian function, fewer somatic stigmata) than classic non-mosaic 45,X, directly because a proportion of tissues retain a normal karyotype.
- ▶ **Mosaic Down syndrome** likewise tends to show milder intellectual and phenotypic involvement, correlating with the proportion and tissue distribution of the trisomic clone.
- ▶ **Prenatal diagnosis:** confined placental mosaicism arising by anaphase lag/postzygotic chromosome loss in the cytotrophoblast is a recognised cause of a CVS result that does not reflect the true fetal karyotype, necessitating amniocentesis for confirmation; it also raises the possibility of trisomy rescue and consequent uniparental disomy for imprinted chromosomes (15, 11, 7, 14).

- ▶ **Genetic counselling:** because the proportion and tissue distribution of a mosaic clone cannot be fully predicted from a peripheral blood or single-tissue karyotype, counselling for any mosaic karyotype carries inherent uncertainty about severity of expression.
- ▶ **Cancer cytogenetics:** anaphase lag/lagging chromosomes at mitosis (visualised as micronuclei) are also a recognised mechanism of aneuploidy generation in malignant and unstable cell lines.

High-yield exam pointers

- ▶ Anaphase lag = **normal disjunction + delayed migration + loss** of one chromosome as a degraded micronucleus — NOT a failure of separation (that is non-disjunction).
- ▶ Produces **one normal + one monosomic** daughter cell (mosaic), whereas non-disjunction produces **one trisomic + one monosomic** daughter cell.
- ▶ Classic result: 45,X/46,XX **mosaic Turner syndrome** — milder phenotype than pure 45,X.
- ▶ Chiefly a **mitotic**, early post-zygotic (cleavage-stage) event; occurs occasionally in meiosis too.
- ▶ Underlies "**trisomy rescue**" (postzygotic loss of the supernumerary chromosome), which can generate **uniparental disomy** when the retained pair is from one parent.
- ▶ Ring and small marker/supernumerary chromosomes are prone to anaphase tangling → breakage/loss → mosaicism.
- ▶ Distinguish **true mosaicism** (reproducible across cultures/tissues) from **pseudomosaicism** (culture artefact) and from **confined placental mosaicism** (placenta-only).
- ▶ Karyotyping multiple cells is more sensitive than microarray for detecting low-level mosaicism.

SOURCE & COVERAGE

Moore's The Developing Human 9e; Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e. Coverage note: Gray's Anatomy (Standring) 41e has no equivalent content (anaphase lag is a cytogenetic mechanism, not a gross-anatomical topic); grounding was therefore drawn from the genetics/embryology texts in the library, per this topic's specialty designation.



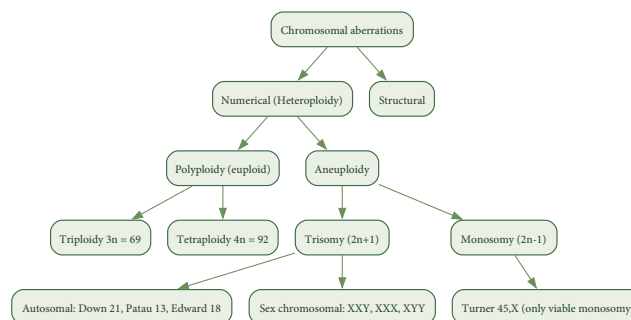
Classify and describe numerical chromosomal aberrations

Asked in: Paper III 16-Nov-2019 · Paper III Nov 2018 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction Chromosomal aberrations are deviations from the normal human chromosome complement of 46 chromosomes (22 autosome pairs + one pair of sex chromosomes, XX or XY) and are broadly classified as **numerical** (change in chromosome number) or **structural** (change in chromosome architecture — deletion, translocation, inversion, etc.). Numerical aberrations, also called **heteroploidy**, are the commonest and clinically most important class of chromosome disorder: they are present in about 1% of live births, in roughly 2% of pregnancies of women older than 35 years undergoing prenatal diagnosis, and in fully half of all first-trimester spontaneous abortions. They arise chiefly from an error of chromosome segregation called **non-disjunction** occurring during gametogenesis (or, less often, after fertilisation) and are subdivided into **polyploidy** (whole extra haploid sets) and **aneuploidy** (gain or loss of individual chromosomes).

Classification of numerical chromosomal aberrations

- ▶ **On the basis of relation to the haploid number ($n = 23$):**
 - **Euploid** — an exact multiple of the haploid number, e.g. diploid ($2n = 46$, normal), **triploid** ($3n = 69$), **tetraploid** ($4n = 92$).
 - **Aneuploid** — not an exact multiple of n ; loss or gain of one (or a few) single chromosomes, e.g. **trisomy** ($2n+1$), **monosomy** ($2n-1$).
- ▶ **On the basis of the chromosomes involved:**
 - **Autosomal numerical aberrations** — e.g. Down syndrome (trisomy 21), Patau syndrome (trisomy 13), Edward syndrome (trisomy 18).
 - **Sex-chromosomal numerical aberrations** — e.g. Klinefelter syndrome ($47,XXY$), Turner syndrome ($45,X$), triple-X syndrome ($47,XXX$), $47,XYY$.
- ▶ **On the basis of cell-line distribution:**
 - **Complete (non-mosaic)** — the abnormal karyotype is present in every cell, arising from non-disjunction during meiosis.
 - **Mosaic** — two (or more) chromosomally distinct cell lines coexist in one individual, arising from non-disjunction in an early post-zygotic mitotic division; the phenotype is generally milder than in the non-mosaic form.



Classify and describe numerical chromosomal aberrations

Mechanism — non-disjunction

- ▶ **Non-disjunction** is the failure of a pair of homologous chromosomes (in meiosis I) or of sister chromatids (in meiosis II, or in a post-zygotic mitosis) to separate properly at anaphase, so that both members pass into the same daughter cell.
- ▶ Normal meiosis in both primary spermatocytes and primary oocytes yields four daughter cells each with 23 chromosomes; fusion of two haploid (23-chromosome) gametes at fertilisation restores the diploid (46) zygote.
- ▶ If non-disjunction occurs in **meiosis I**, the resulting 24-chromosome gamete carries both the paternal and the maternal member of the pair; if it occurs in **meiosis II**, the 24-chromosome gamete carries two copies of the same (paternal or maternal) chromosome.
- ▶ Consequently, one gamete receives 24 chromosomes and the reciprocal gamete 22. At fertilisation, a normal 23-chromosome gamete fusing with a 24-chromosome gamete produces a **47-chromosome (trisomic)** zygote; fusion with a 22-chromosome gamete produces a **45-chromosome (monosomic)** zygote.
- ▶ **Mitotic non-disjunction** occurring after zygote formation produces **mosaicism** — e.g. a zygote that is 47,+21 may lose the extra chromosome 21 during an early cleavage division and continue to develop as a 46/47,+21 mosaic, usually with a less severe phenotype than complete trisomy 21.
- ▶ A less common mechanism is premature separation of sister chromatids during meiosis I (instead of at meiosis II), which can likewise generate an unbalanced gamete.
- ▶ The propensity to non-disjoin is increased by too few (or abnormally placed) meiotic recombination events and rises sharply with **advancing maternal age**.

□ Fig 1.8 — Chromosomal Non-Disjunction in Meiosis (Larsen — Human Embryology — 6e, p.97)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Non-disjunction during meiosis (label exactly):

- ▶ Primary oocyte/spermatocyte entering **Meiosis I**
- ▶ A homologous chromosome pair that **fails to separate** at anaphase I (non-disjunction site marked with an arrow)
- ▶ Products of Meiosis II → two secondary-derived gametes: one with **24 chromosomes**, one with **22 chromosomes** (contrast with the normal 23/23 outcome)
- ▶ **Fertilisation** of each abnormal gamete by a normal gamete (23 chromosomes)
- ▶ Resulting zygotes labelled: **47 chromosomes — trisomy** and **45 chromosomes — monosomy**

Polyploidy

Type	Chromosome number	Mechanism	Outcome
Triploidy	69 (3n); 69,XXX or 69,XXY	Dispermy (fertilisation of one ovum by two sperm), or fertilisation involving a diploid gamete from failure of a meiotic division	Extra paternal haploid set → abnormal degenerative placenta (partial hydatidiform mole) with a small fetus; extra maternal set → early spontaneous abortion. Rare live-born infants do not survive long
Tetraploidy	92 (4n); always 92,XXXX or 92,XXYY	Failure of the first cleavage division of the zygote	Always ends in spontaneous abortion

Aneuploidy — autosomal numerical aberrations

Syndrome	Karyotype	Key mechanism	Incidence	Salient features
Down syndrome (trisomy 21, "mongolism")	47,XX/XY,+21 ($\approx$ 95–97%, "Triplo-21," from meiotic non-disjunction, non-familial); translocation ("familial") form has 46 chromosomes with the extra chromosome-21 material translocated onto another autosome ($\sim$ 3%); mosaic form also occurs	Non-disjunction (usually maternal meiosis I); risk rises with maternal age	1 in 700 births — the commonest numerical chromosomal abnormality in man	Round mongoloid facies with oblique palpebral fissures and epicanthic folds, flat nasal bridge, low-set square ears, single transverse palmar (simian) crease, open mouth with protruding tongue, hyperflexible joints, short stature, mental retardation (IQ 25–50), congenital heart disease, low maternal serum/amniotic α -fetoprotein
Patau syndrome (trisomy 13)	47,+13	Meiotic non-disjunction	Rare	Profound mental retardation, cleft lip/palate, omphalocele, polydactyly; usually die soon after birth
Edward syndrome (trisomy 18)	47,+18	Meiotic non-disjunction	Rare	Elongated skull with prominent occiput, low-set ears, micrognathia, overlapping fingers, rocker-bottom heels, congenital heart defects; usually die within a few weeks of birth

Trisomy for a whole chromosome is compatible with life for only a handful of chromosomes — 13, 18 and 21 (the autosomes with the lowest gene content) plus the sex chromosomes; trisomy for gene-richer autosomes is almost always lethal in utero. Monosomy for an entire autosome is essentially never viable.

Aneuploidy — sex-chromosomal numerical aberrations

Feature	Klinefelter syndrome	Turner syndrome
Type	Trisomic, found only in males	Monosomic, found only in females
Karyotype	47,XXY	45,X (45,XO)
Mechanism	Non-disjunction of the XX (or XY) pair during gametogenesis	Loss of one X chromosome by non-disjunction during meiosis
Phenotype	Male, eunuchoid habitus	Female
Stature	Tall (long arms and legs)	Short
Key features	Hypogonadism, azoospermia/sterility, gynaecomastia, sparse axillary/pubes hair, reduced chest hair, some mental retardation, raised gonadotropins	Webbed neck, shield chest with widely spaced/pinpoint nipples, cubitus valgus, low-set ears, infantile external genitalia, gonadal dysgenesis with primary amenorrhoea, coarctation of aorta
Incidence	~1:500 male births; increases with maternal age	2:3000 female births; only viable human monosomy; in ~75% the retained X is maternal

- ▶ **Triple-X (super-female) syndrome**, 47,XXX — arises from fertilisation of an XX-bearing oocyte by a normal X-bearing sperm; phenotypically female with two Barr bodies; some mental retardation, scanty menses, infantile personality.
- ▶ 47,YYY is a further recognised numerical sex-chromosomal aneuploidy seen in liveborn males.
- ▶ **Sex chromatin (Barr body) correlation (Lyon's "n – 1" rule** — number of Barr bodies = total X chromosomes minus one): normal male XY = 0; **Turner XO = 0**; normal female XX = 1; **Klinefelter XXY = 1**; triple-X XXX = 2.

Applied / Clinical anatomy

- ▶ **Karyotyping and banding** (Q-, G-, R- and C-banding) remains the reference method for demonstrating numerical (and structural) chromosome abnormality; chromosomes are conventionally arranged into 7 groups by the Denver classification (1960) on the basis of size and centromere position.
- ▶ **Buccal-smear sex chromatin (Barr body) analysis** offers a rapid presumptive screen for X-chromosome aneuploidy in the newborn, based on Lyon's hypothesis.
- ▶ **FISH and chromosomal microarray/genome sequencing** allow rapid interphase detection of aneuploidy (e.g. trisomy 21) in prenatal or postnatal samples, supplementing conventional karyotyping.
- ▶ **Maternal age effect** — the risk of meiotic non-disjunction, especially of chromosome 21, rises sharply after 35 years of age; this underlies the offer of prenatal chromosome/genome analysis to women of advanced maternal age.
- ▶ **Mosaicism and genetic counselling** — because the proportion of the abnormal cell line varies with the tissue sampled and the timing of the mitotic non-disjunction event, counselling a

mosaic patient is far less certain than counselling a complete aneuploid; mosaic Down syndrome and mosaic Turner syndrome are generally milder than their non-mosaic counterparts.

- ▶ **Robertsonian translocation carriers** (e.g. a phenotypically normal 45-chromosome parent carrying a der(14;21) translocation) are at specific risk of transmitting "translocation Down syndrome" to their offspring — an important distinction from ordinary trisomy 21 in genetic counselling of recurrent cases.
- ▶ **Turner syndrome** is the commonest cause of primary amenorrhoea and requires evaluation for coarctation of the aorta and renal anomalies; **Klinefelter syndrome** is an important treatable cause of male infertility and hypogonadism.

High-yield exam pointers

- ▶ Numerical aberrations = **heteroploidy**, subdivided into **polyploidy** (exact multiple of n) and **aneuploidy** (not a multiple of n).
- ▶ **Non-disjunction** during gametogenesis is the principal mechanism; meiosis-I vs meiosis-II non-disjunction differ in whether the extra chromosome copy is of mixed (paternal + maternal) or single-parent origin.
- ▶ **Down syndrome (trisomy 21)** is the commonest numerical chromosomal abnormality (1/700 births): ~95–97% simple trisomy, ~3% translocation (Robertsonian, familial), remainder mosaic.
- ▶ **Turner syndrome (45,X)** is the **only monosomy compatible with life** in humans.
- ▶ **Barr body count** = **total X chromosomes** – 1 (Lyon's $n - 1$ rule).
- ▶ **Triploidy (69)** typically follows dispermy; **tetraploidy (92)** follows failure of the first zygotic cleavage — both are almost always lost as spontaneous abortions.
- ▶ Live-born trisomies are essentially restricted to chromosomes **13, 18 and 21** (lowest gene content) plus the sex chromosomes.
- ▶ Risk of aneuploidy, particularly trisomy 21, increases with **advancing maternal age (>35 years)**.
- ▶ **Mosaic** aneuploidy is usually phenotypically milder than the corresponding complete (non-mosaic) aneuploidy.

SOURCE & COVERAGE

Vishram Singh — General Anatomy (4e); Thompson & Thompson — Genetics in Medicine (8e); Larsen — Human Embryology (6e) (figure). **Coverage note:** Gray's Anatomy (Standring, 41e) has no dedicated cytogenetics/medical-genetics chapter, so this answer is grounded in the paper's designated genetics specialty texts (Thompson & Thompson; Vishram Singh's General Anatomy genetics chapter) rather than the spine text.



Genetic counseling

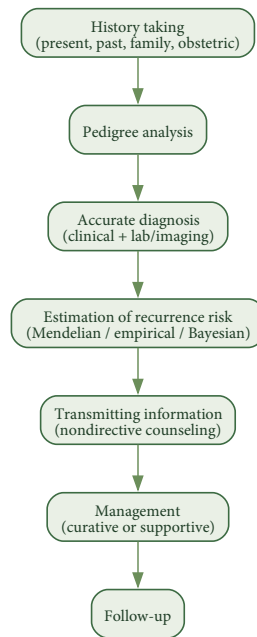
Asked in: Paper III May 2010 · Paper III Oct 2008 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction Genetic counseling is defined as a process by which patients or their relatives at risk of a genetic disorder are made aware of the consequences of the disorder, its mode of transmission, and the ways by which it can be prevented or mitigated. It is the applied, patient-facing arm of human genetics — it integrates an accurate diagnosis, pedigree/probability-based risk estimation, and psychosocial communication so that the family can make an informed, autonomous reproductive or management decision. It is delivered by physicians, clinical geneticists, or, in the West, by specially trained genetic counselors/nurse geneticists working with the clinical team.

Indications for genetic counseling

Category	Examples
Congenital anomaly/disability	Previous child with multiple congenital anomalies, intellectual disability, or an isolated defect (neural tube defect, cleft lip/palate)
Family/personal history	Cystic fibrosis, fragile X syndrome, congenital heart defect, hereditary cancer, Huntington disease, diabetes
Pregnancy-related	Pregnancy at risk of a chromosomal/hereditary disorder; advanced maternal age (>35 years); repeated pregnancy loss or infertility
Consanguinity	Increases risk especially of autosomal recessive disorders
Teratogen exposure	Occupational chemicals, medications, alcohol
Screening-related	Follow-up of an abnormal newborn test (e.g., PKU), a positive carrier screen (e.g., Tay-Sachs), an abnormal maternal serum/NIPT screen, or an abnormal fetal ultrasound (e.g., nuchal translucency ≥ 3 mm at 12 weeks 5 days)
Pre/post genetic testing	Before and after testing for late-onset disorders such as hereditary cancer syndromes

Steps in the process of genetic counseling



Genetic counseling

- ▶ **History:** present and past history; family history of sibs/relatives with a similar problem; obstetric history (teratogen exposure, abortion/stillbirth); enquiry about **consanguinity** (raises risk chiefly of autosomal recessive disease).
- ▶ **Pedigree analysis:** constructs, in shorthand, the pattern of the disorder across the family using standard symbols (see diagram below); an indispensable, if time-consuming, step towards counseling. The proband/index case is marked with an arrow.
- ▶ **Diagnosis:** must be "accurate" — confirmed by survey of similarly affected relatives (or otherwise-normal relatives for carrier detection), supported by pathological, hormonal, and imaging investigations.
- ▶ **Estimation of risk ("recurrence risk"):** the most important step, using the mode of inheritance, pedigree analysis, and results of tests such as linkage studies (detailed below).
- ▶ **Transmitting information:** communicating risk and options, respecting the patient's psychology, emotional stress, family attitudes, and socio-educational background.
- ▶ **Management:** curative where possible, otherwise supportive/preventive (early detection to limit damage, e.g., dietary control in PKU).
- ▶ **Follow-up:** essential and repeated, aimed at preventing recurrence through reproductive planning and prenatal diagnosis.

□ Fig 17.23 — Symbols used in pedigree chart. (Vishram Singh — *General Anatomy* — 4e, p.734)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Reproduce a labelled key with these exact elements:

- ▶ **Square** = normal male; **circle** = normal female; **diamond** = sex unspecified
- ▶ **Filled (black) square/circle** = affected male/female
- ▶ **Square or circle with a number inside** = number of children of that sex
- ▶ **Diagonal line through square/circle** = deceased; **diamond with a cross** = stillbirth
- ▶ **Square/circle in square brackets []** = adopted into family; **circle in round brackets ()** = adopted out of family
- ▶ **Diamond with "P"** = pregnancy, sex unspecified
- ▶ **Horizontal line joining square–circle** = marriage; with a single oblique stroke = divorced; **double horizontal line** = consanguineous marriage
- ▶ **Lines dropping from a couple to an asymmetric bracket** = **dizygotic twins**; to a symmetric bracket = **monozygotic twins**
- ▶ **Triangle** = miscarriage; triangle with a line through it = termination of pregnancy
- ▶ **Couple line with no vertical drop** = **no offspring**; one partner joined to two others = **multiple unions**
- ▶ **Arrow** = proband (index case)

Estimation of recurrence risk — the probability laws

Method	Principle	Worked illustration
Law of addition	For mutually exclusive events, probability that either occurs = sum of individual probabilities	—
Law of multiplication	For independent events, probability that both occur = product of individual probabilities	Probability dizygotic twins are both boys = $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$
Binomial distribution	Expansion of $(p + q)^n$ gives probabilities of all combinations of two alternative outcomes across n events; introduced into genetics by Jacob Bernoulli (17th c.) , based on the binomial theorem of Sir Isaac Newton (1676)	For 3-child families: $p^3 + 3p^2q + 3pq^2 + q^3$ gives probabilities of 3, 2, 1, or 0 boys
Bayes' theorem (Bayesian analysis)	First published 1763; combines a prior probability with a conditional probability to give a joint probability , from which the posterior probability is derived	See below

Bayesian worked example (X-linked recessive, Duchenne muscular dystrophy): a woman (II_3), daughter of an obligate DMD carrier, has three normal sons. Prior probability she is a carrier = $1/2$;

conditional probability of three normal sons if she is a carrier = $(1/2)^3 = 1/8$ (≈ 1 if she is not a carrier). Joint probability as carrier = $1/2 \times 1/8 = 1/16$; as non-carrier = $1/2 \times 1 = 1/2$. **Posterior probability she is a carrier** = $(1/16)/(1/16 + 1/2) = 1/9$; risk her next child is an affected male = $1/9 \times 1/4 = 1/36$ — markedly lower than the naïve prior risk of $1/8$.

Autosomal recessive example (cystic fibrosis): if II2 and II3 (a couple) each have one affected sib, probability each is a carrier = $2/3$; combined with three normal children already born, Bayesian analysis reduces the risk that their next child is affected from the naïve $1/4 \times 4/9 = 1/9$ down to $1/16$.

Genetic counseling providers and the principle of nondirectiveness

- ▶ In many countries counseling is provided by physicians; in the USA, Canada, and the UK it is often delivered by dedicated **genetic counselors/nurse geneticists**, a self-regulating profession with its own certifying boards.
- ▶ The counselor's role is to help consultands reach the decision most appropriate to them — **not** to direct it. This approach is termed **nondirective counseling**, rooted in respect for the couple's reproductive **autonomy**.
- ▶ Common case-management sequence: collection of information (family/medical history, tests) → assessment (examination, laboratory/radiological testing, diagnosis) → counseling (nature/consequences, recurrence risk, options) → decision-making and referral → continuing psychosocial support.

Screening programmes

Criteria for a genetic screening programme:

- ▶ The disorder must be clearly defined.
- ▶ It should have a reasonable population frequency to warrant screening.
- ▶ It should preferably be treatable.
- ▶ The test should be quick.
- ▶ The test should be inexpensive enough for large-scale use.
- ▶ The test should be reliable — minimal false positives, ideally no false negatives.

Applications: **newborn screening** (e.g., phenylketonuria — early diagnosis and phenylalanine-restricted diet prevent mental retardation) and **maternal serum screening** — elevated alpha-fetoprotein suggests a neural tube defect, while an unusually low value suggests fetal Down syndrome.

Applied / Clinical anatomy

- ▶ **Prenatal diagnosis** is central to preventive genetics: karyotyping/chromosome analysis, biochemical carrier testing, ultrasonography (increased nuchal translucency, ≥ 3 mm at 12 weeks 5 days, flags chromosomal risk), amniocentesis, chorionic villus sampling, and fetal blood sampling; **preimplantation genetic diagnosis** (blastocyst/blastomere biopsy) allows selection before implantation, avoiding pregnancy termination but requiring IVF.
- ▶ **Down syndrome follow-up:** after acceptance and referral for developmental training, parents are offered carrier detection (Robertsonian translocation) and future prenatal diagnosis to prevent recurrence.

- ▶ **Medico-legal precedent:** a couple screened negative for Tay–Sachs disease (deficient hexosaminidase A) yet had an affected child; the court recognised the birth as a case of "wrongful life," underscoring the medico-legal weight of accurate counseling.
- ▶ **Ethical controversies:** selective termination after prenatal diagnosis of disability; a reported UK case where deaf parents deliberately selected a deaf embryo (autosomal recessive deafness); preimplantation HLA-matching of an embryo to provide compatible marrow/cord cells for a sibling with Fanconi anaemia; childhood predictive testing is justified when clinical intervention exists (e.g., colonoscopy from age 12 in familial adenomatous polyposis/APC carriers) but is avoided in conditions without preventive benefit (e.g., Huntington disease).
- ▶ Alternatives offered when a couple cannot have an unaffected child by natural conception: **adoption** (with pre-adoption clinical screening of the child), **artificial insemination by donor** (indicated if the father carries an autosomal dominant/X-linked defect, or both partners carry an autosomal recessive gene — not indicated if the mother is the carrier), and **donor-egg IVF** (if the mother carries an autosomal dominant or X-linked defect).

High-yield exam pointers

- ▶ **Definition:** process making at-risk patients/relatives aware of nature, transmission risk, and prevention/mitigation of a genetic disorder.
- ▶ Three components: **accurate diagnosis** → **risk estimation** → **management** (curative or supportive).
- ▶ Diagnostic work-up = **history** + **pedigree** + **investigations**; consanguinity specifically raises autosomal recessive risk.
- ▶ Recurrence risk is estimated via **Mendelian ratios**, **empirical risk data**, and **Bayesian analysis** (prior × conditional = joint → posterior probability).
- ▶ Counseling is **nondirective** — information and support, not directed decisions — respecting reproductive autonomy.
- ▶ **Screening criteria:** defined disorder, adequate frequency, treatable, quick, cheap, reliable (PKU newborn screening; maternal AFP for NTD/Down syndrome).
- ▶ Prenatal diagnostic tools: USG (nuchal translucency), amniocentesis, CVS, fetal blood sampling, karyotyping, preimplantation diagnosis.
- ▶ Key ethico-legal issues: wrongful life (Tay–Sachs), selective termination, childhood testing only when intervention exists, HLA-matched "saviour sibling" and deliberate disability-selection controversies.

SOURCE & COVERAGE

Gangane — Human Genetics 5e (Chapter "Genetic Counselling," pp 232–243); Thompson & Thompson — Genetics in Medicine 8e (Chapter 16, "Risk Assessment and Genetic Counseling," pp 333–350); Vishram Singh — General Anatomy 4e (Genetic counseling section, p.734, incl. Fig 17.23).



Lyon's hypothesis

Asked in: Paper III 18-Jul-2020 · Paper III Nov 2018 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — Lyon's hypothesis (proposed by the English geneticist Mary F. Lyon in 1961) explains **dosage compensation** of X-linked genes between the two sexes: in every somatic cell of a normal female, only **one X chromosome remains genetically active**; the other is randomly and permanently inactivated early in embryonic life and is seen cytologically as the condensed **sex chromatin (Barr body)**. This process is called **Lyonisation**, and it renders normal females functional **mosaics** for X-linked gene expression.

1. Historical background

- ▶ **Barr and Bertram (1949)** first described a small, deeply stained chromatin mass in the interphase nucleus of neurons of a female cat — later named the **Barr body** (after Murray Barr, a Canadian geneticist) or **sex chromatin**.
- ▶ **Mary F. Lyon (1961)** proposed that this body represents an inactivated X chromosome and formulated the hypothesis that now bears her name.

2. Postulates of Lyon's hypothesis

- ▶ **a.** In a normal female somatic cell, only **one X chromosome is genetically active**; the second X is inactive and condensed to form the sex chromatin.
- ▶ **b.** X-inactivation occurs **early in embryonic life** — around the 100-cell (blastocyst) stage, roughly the 15th–16th day of gestation; before this, both X chromosomes are active.
- ▶ **c.** The choice of which X (paternally or maternally derived) is inactivated in a given cell is **random**.
- ▶ **d.** Once an X is inactivated in a cell, the **same X remains inactive in all its mitotic descendants** — the inactivation is clonally propagated and permanent (fixed).
- ▶ **e.** This rule applies to **all X chromosomes in excess of one**: whatever the total X/Y complement, exactly one X remains active per cell (e.g., in 47,XXY or 47,XXX).
- ▶ **Net result:** a normal female is a clonal mosaic — some cells express the paternal-X alleles, others the maternal-X alleles, for X-linked genes.

3. Molecular basis of X-inactivation

- ▶ Inactivation is controlled by a locus on the **proximal long arm of the X chromosome** — the **X-inactivation centre (XIC)**, mapped to an ~800-kb region at Xq13.2.
- ▶ The XIC harbours the **XIST gene** ("inactive-X-specific transcript"), an unusual **non-coding RNA gene** transcribed *only* from the allele destined to become the inactive X (Xi); it is silent on the active X in both sexes.
- ▶ The **XIST long ncRNA spreads in cis along the length of that X**, coating it and recruiting the silencing machinery; X-inactivation cannot occur without XIST.
- ▶ Silencing is achieved chiefly through **DNA methylation** of the X, together with characteristic chromatin changes: the inactive X becomes **facultative heterochromatin**, is enriched for heterochromatin-associated histone marks and the histone variant **macroH2A**, and is deficient

in euchromatin marks — converting an originally euchromatic, transcriptionally active chromosome into a silenced one.

- ▶ The inactive X is also **late-replicating** in S phase (the active X replicates synchronously with the autosomes).
- ▶ A minority of genes (more on distal Xp than on Xq) "**escape**" **inactivation** and remain biallelically expressed — this is clinically relevant when assessing partial X-chromosome aneuploidies.
- ▶ The XIC must be physically present (in *cis*) for a chromosome to be inactivated; small structurally abnormal X fragments that lack the XIC cannot be inactivated and are instead expressed biallelically.

4. Sex chromatin (Barr body): structure, sites, and the $n - 1$ rule

embed: ![Fig 17.9 — Formation of sex chromatin in a female. (Vishram Singh — General Anatomy — 4e, p.698)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/a77ddd762d6bc4af.jpg)

embed: ![Fig 17.10 — Location of Barr body as seen in (A) buccal smear; (B) neuron and (C) neutrophil. (Vishram Singh — General Anatomy — 4e, p.698)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/367794be1908793f.jpg)

- ▶ **Structure:** a small, dark-staining (basophilic), planoconvex/wedge-shaped or rectangular condensed chromatin mass, about **1 μm** , situated just inside (subjacent to) the nuclear membrane of the interphase nucleus.
- ▶ **Distribution:** present in the nuclei of most somatic cells of female mammals (buccal, skin, vaginal, urethral, and corneal epithelium; also placenta, dental pulp, skin fibroblasts) — **absent in the germ cells**.
- ▶ **In neurons:** appears as a small dark body opposite the nucleolus (a "nucleolar satellite").
- ▶ **In neutrophils:** appears as a **drumstick (Davidson body)** — a knob about **1.5 μm** in diameter attached to a nuclear lobe by a thin filament; seen in only about 2–3% (roughly 6/500 cells) of neutrophils in normal females.
- ▶ **The $n - 1$ rule:** the maximum number of Barr bodies per cell equals the total number of X chromosomes (n) minus one.

Individual	Sex-chromosome constitution	No. of Barr bodies
Normal male	XY	0
Turner syndrome	45,XO	0
Normal female	46,XX	1
Klinefelter syndrome	47,XXY	1
Triple-X ("super female")	47,XXX	2
Tetra-X	48,XXXX	3

- ▶ **Side-note:** on the Y chromosome, a distal-Yq heterochromatic segment stains as a brightly fluorescent "F-body" with quinacrine (fluorochrome) dye under the fluorescence microscope — used together with Barr-body counting for rapid sex-chromosome screening.

5. Dosage compensation

- ▶ The X chromosome carries far more genes than the Y; without correction, XX females would have double the gene product of XY males for every X-linked locus.
- ▶ **Dosage compensation** equalises this by ensuring that, regardless of the total number of X (or Y) chromosomes present, **exactly one X chromosome remains transcriptionally active** in every somatic cell — achieved by random Lyonisation of all additional X chromosomes.

Applied / Clinical anatomy

- ▶ **Rapid screening test:** a buccal smear stained and examined for sex chromatin (Barr body count) — together with the Y-linked F-body — provides a quick first-line test for suspected numerical sex-chromosome disorders (Barr-body-negative female → Turner syndrome; Barr-body-positive male → Klinefelter syndrome) and in the work-up of ambiguous genitalia, pending confirmatory karyotyping.
- ▶ **Manifesting heterozygotes:** because the choice of which X is inactivated is random and occurs among a small number of embryonic cells, the ratio of the two cell populations can be **unbalanced ("skewed") by chance**. A female carrier of an X-linked recessive disorder (e.g., Duchenne muscular dystrophy, haemophilia A, red-green colour blindness, G6PD deficiency) may then show clinical features herself — a **manifesting heterozygote**. In DMD carriers, immunostaining of muscle for dystrophin shows a characteristic **mosaic pattern of positive and negative fibres**, corresponding to fibres in which the normal or the mutant allele lies on the active X.
- ▶ **Protective non-random inactivation:** in females with a structurally abnormal X chromosome, the abnormal X is preferentially inactivated; in balanced X;autosome translocations, the normal X is preferentially inactivated (leaving the translocated segments active). This preferential ("skewed") inactivation minimises — though does not always eliminate — the phenotypic impact of the chromosomal defect, and is one reason X-chromosome numerical/structural abnormalities are generally better tolerated clinically than comparable autosomal imbalances.
- ▶ **Genetic counselling:** assessment of the X-inactivation pattern is used in evaluating carriers of X;autosome translocations and in explaining variable clinical severity among heterozygous relatives in X-linked pedigrees.

High-yield exam pointers

- ▶ Barr & Bertram (1949) first described the body (female cat neuron); **Mary F. Lyon (1961)** proposed the hypothesis — hence "Lyonisation."
- ▶ Core postulates: only **one active X** per cell; inactivation is **random, early (~100-cell stage), and permanently fixed (clonal)**; every X beyond one is inactivated.
- ▶ Molecular control: **X-inactivation centre (Xq13.2) → XIST ncRNA** acts in *cis*, spreading along the chosen X → DNA methylation + heterochromatinisation → late replication.
- ▶ Result: normal females are **functional mosaics** for X-linked genes.

- ▶ Sex chromatin (Barr body) = cytological marker of the inactive X; **drumstick** appendage in neutrophils; located just inside the nuclear envelope.
- ▶ **n – 1 rule**: XY = 0, XO = 0, XX = 1, XXY = 1, XXX = 2, XXXX = 3.
- ▶ Clinical uses: rapid buccal-smear screening for sex-chromosome-number disorders; explains **manifesting heterozygotes/skewed Lyonisation** in X-linked recessive disease carriers (e.g., DMD); preferential inactivation of abnormal/translocated X limits phenotypic severity.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e; Vishram Singh General Anatomy 4e; IB Singh Textbook of Human Embryology (CBME 2021); IB Singh Textbook of Anatomy Vol 3 6e.



Mutation

Asked in: Paper III Nov 2016 · Paper III May 2009 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction A **mutation** is a permanent, heritable change in the nucleotide sequence or arrangement of DNA, producing a **variant (mutant) allele** that differs from the prevailing **wild-type/reference allele** at that locus. The terms *mutation* and *mutant* describe a change in DNA sequence and carry no inherent connotation of harm — many mutations are functionally silent. A variant allele that reaches a population frequency of >1% is, by convention, reclassified as a **polymorphism** rather than a rare mutation. Mutations arise during the fundamental processes of DNA replication, repair, recombination, and chromosome segregation, and may be **spontaneous** or **induced** by mutagens; they form the molecular basis of virtually all monogenic and many multifactorial (including neoplastic) diseases.

1. Classification by level of the genome affected

Level	What changes	Representative example
Genome (numerical/chromosome) mutations	Chromosomes remain structurally intact, but the number of chromosomes in the cell changes (aneuploidy)	Trisomy 21 (Down syndrome)
Regional / subchromosomal mutations	A portion of a chromosome changes — copy-number change or structural rearrangement (duplication, deletion, inversion, translocation) of part of one or more chromosomes	Cri-du-chat (5p deletion), Charcot-Marie-Tooth 1A (PMP22 duplication)
Gene / DNA mutations	Substitution, deletion, or insertion of DNA sequence, ranging from a single nucleotide up to ~100 kb	Sickle cell mutation (β-globin, single base substitution)

Chromosome and regional mutations are covered separately (karyotype anomalies); the remainder of this answer focuses on **gene/DNA mutations**, which are the classical exam focus.

2. Types of gene mutation, by molecular mechanism

Type	Mechanism	Approx. share of disease-causing mutations
Missense	Single base substitution (point mutation) changes a codon to specify a different amino acid (nonsynonymous)	~50%
Nonsense	Substitution converts a sense codon to a premature stop codon ; truncated mRNA/protein is usually degraded (nonsense-mediated decay)	~10%
Silent (synonymous)	Substitution does not change the encoded amino acid (codon degeneracy) — usually functionally neutral	—
RNA-processing (splice-site) mutations	Destroy or create donor/acceptor splice sites, cap sites, or polyadenylation signals; may cause exon skipping or intron retention, often producing a frameshift and premature stop	~10% (splice-site) + ~10% (other RNA-processing)
Long-range regulatory mutations	Alter promoter/enhancer or other regulatory elements, changing the amount, timing, or tissue of gene expression	Rare
Small insertions/deletions	Addition or loss of a small number of bases; if not a multiple of three , produces a frameshift (altered reading frame downstream, usually a premature stop); if a multiple of three, produces an in-frame insertion/deletion of amino acids	~25%
Larger deletions/duplications/inversions/fusions	Homologous recombination between repeat sequences, or non-homologous end-joining after double-strand breaks	~5%
Mobile-element insertion	Insertion of a LINE or Alu retroelement into an exon disrupts the coding sequence (insertional mutagenesis)	Rare

Type	Mechanism	Approx. share of disease-causing mutations
Dynamic mutations	Meiotic expansion of an unstable trinucleotide/tetranucleotide repeat (e.g., CAG, CGG, CCTG) beyond a threshold	Rare

3. Germline versus somatic mutation

- ▶ A **germline mutation** arises in a germ cell (or its precursor); if transmitted, it becomes present as a **constitutional mutation** in every cell of the offspring and is heritable.
- ▶ A **somatic mutation** arises in any non-germline cell after fertilisation; it is confined to the clonal descendants of that cell (**somatic mosaicism**) and is **not transmitted** to offspring.
- ▶ Clinical significance — **Knudson's two-hit model**, illustrated by **retinoblastoma**: hereditary cases carry one **germline** RB1 mutation and require only a single additional **somatic** "second hit" in a retinal cell, giving early, often bilateral tumours; sporadic cases require **two independent somatic hits** in the same cell, giving later-onset, unilateral disease.
- ▶ Cancer in general is driven by the **stepwise accumulation of somatic mutations** in oncogenes and tumour-suppressor genes within a single clone (e.g., activating point mutations in the *RAS* gene family, found in ~30% of human cancers overall — up to 90% of pancreatic carcinomas).

4. Origin of mutations — spontaneous vs induced

Spontaneous mutations arise from the inherent imperfection of normal cellular processes:

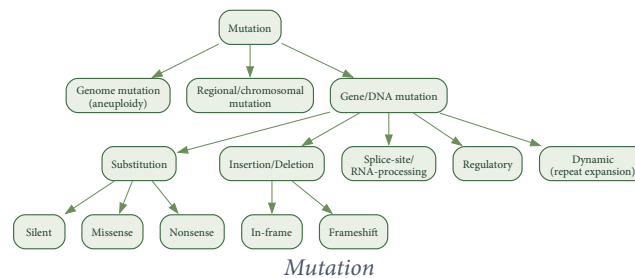
- ▶ **Replication errors** — DNA polymerase misincorporates a base at a rate of about 1 in 10 million bp; proofreading exonuclease activity corrects >99.9% of these, but the small residual fraction persists as new mutations.
- ▶ **Spontaneous deamination** of 5-methylcytosine to thymine at **CpG dinucleotides** produces C→T (or G→A) transitions; CpG doublets are consequently a true mutational "**hot spot**," accounting for over 30% of all point substitutions and mutating at ~25 times the rate of other single-nucleotide changes.
- ▶ Mutation rates vary widely by locus (10^{-4} to 10^{-7} per locus per generation); the median gene mutation rate is $\approx 1 \times 10^{-6}$, and each individual acquires roughly 75 new base-pair mutations genome-wide per generation. Some loci (e.g., *FGFR3* in achondroplasia, rate $\approx 1.4 \times 10^{-5}$) show an exceptionally high, largely **paternal-age-related**, site-specific mutation rate.

Induced mutations result from exposure to exogenous **mutagens**:

- ▶ **Physical** — ionising radiation and ultraviolet light causing DNA strand breaks and pyrimidine dimers.
- ▶ **Chemical** — base analogues, alkylating agents, and intercalating agents that damage or misalign the DNA template.
- ▶ **Biological** — insertion of mobile elements or retroviral sequences (**insertional mutagenesis**); e.g., integration of a retroviral long-terminal-repeat adjacent to *c-myc* drives its constitutive expression and is implicated in Epstein-Barr-virus-associated Burkitt lymphoma.

5. Molecular mechanisms by which mutation produces disease

Mechanism	Effect	Example
Loss of function (commonest)	Reduced/absent normal protein activity — from substitutions, deletions, insertions, or rearrangements	α - and β -thalassemias
Gain of function — increased dosage	Extra copies of a normal gene/chromosome	Trisomy 21; CMT type 1A (PMP22 duplication)
Gain of function — enhanced activity	Coding mutation makes the protein "over-perform" one normal function	Hb Kempsey (locked high- O_2 -affinity state); achondroplasia (FGFR3)
Novel property	Mutant protein acquires an abnormal property unrelated to its normal function	Sickle haemoglobin (HbS) polymerisation on deoxygenation
Ectopic/heterochronic expression	Gene expressed at the wrong time or place, usually via regulatory-region mutation	Hereditary persistence of fetal haemoglobin; oncogene activation



Applied / Clinical anatomy

- ▶ **Sickle cell disease** — single missense mutation (Glu→Val, β -globin) causing a *novel property* mutation (HbS polymerisation); classic example of allelic heterogeneity's opposite — one recurrent mutation, uniform phenotype.
- ▶ **β -thalassemias** — heterogeneous mixture of nonsense, frameshift, splice-site, and regulatory mutations, all converging on loss of β -globin function; **α -thalassemias** are typically gene-deletion (loss-of-function by reduced dosage).
- ▶ **Duchenne/Becker muscular dystrophy** — frameshift dystrophin deletions give the severe Duchenne phenotype, whereas in-frame deletions preserving the reading frame give the milder Becker phenotype — a key frameshift-vs-in-frame teaching example.
- ▶ **Haemophilia A** — nearly half of severe cases arise from an **intron-22 inversion** (recombination between inverted homologous repeats); others from LINE-element insertional mutagenesis into the factor VIII gene.
- ▶ **Achondroplasia** — virtually all cases due to an **identical recurrent gain-of-function point mutation** in *FGFR3*, almost always of paternal origin and increasing with paternal age — a model for new-mutation-rate calculation.

- ▶ **Retinoblastoma / BRCA1-breast cancer** — germline-plus-somatic "two-hit" tumour-suppressor inactivation; underlies genetic counselling and cancer-risk prediction.
- ▶ **Trinucleotide-repeat (dynamic mutation) disorders** — Huntington disease (CAG repeat; normal 9–35, disease 36 to >100 repeats), fragile X syndrome (CGG repeat expansion; commonest inherited cause of intellectual disability), and myotonic dystrophy all show **anticipation** — worsening severity/earlier onset in successive generations as the repeat expands further at meiosis.
- ▶ **Forensic/diagnostic relevance** — because most polymorphisms (SNPs, microsatellites) are functionally neutral variants rather than disease mutations, they are exploited for DNA fingerprinting, paternity testing, and linkage-based prenatal diagnosis, while disease-causing gene mutations remain the target of mutation-specific diagnostic testing and genetic counselling.

High-yield exam pointers

- ▶ Mutation = permanent DNA sequence change; polymorphism = allele frequency >1% in the population — same underlying event, different frequency threshold.
- ▶ Three levels: **genome** (number), **chromosomal/regional** (structure), **gene/DNA** (nucleotide-level) mutations.
- ▶ Substitution mutations: **silent, missense, nonsense**; indels: **in-frame vs frameshift** (multiple-of-3 rule).
- ▶ **Dynamic mutations** = trinucleotide/tetranucleotide repeat expansion → **anticipation** (Huntington disease, fragile X, myotonic dystrophy).
- ▶ **Germline** mutation → constitutional, heritable; **somatic** mutation → mosaic, non-heritable, but the driver of sporadic cancer (Knudson two-hit model).
- ▶ Spontaneous mutation hot spot = **CpG dinucleotide** (5-methylcytosine deamination); induced mutation = physical/chemical/biological **mutagens**.
- ▶ Four molecular mechanisms of disease: **loss of function** (commonest), **gain of function**, **novel property**, **ectopic/heterochronic expression**.
- ▶ Achondroplasia and retinoblastoma are the classic teaching examples for new-mutation-rate calculation and the two-hit hypothesis respectively.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine (8e) — Chapters "The Human Genome: Gene Structure and Function," "Human Genetic Diversity: Mutation and Polymorphism," "The Molecular Basis of Genetic Disease," "The Molecular, Biochemical, and Cellular Basis of Genetic Disease," and "Cancer Genetics and Genomics"; Gangane Human Genetics (5e) — chapter on Cancer Genetics (RAS activation, insertional mutagenesis).



Sex Chromatin

Asked in: Paper III Nov 2017 · Paper III Nov 2016 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction Sex chromatin (the **Barr body**) is a small, deeply-staining mass of condensed chromatin seen in the interphase nucleus of somatic cells of normal females but not normal males. It was first observed by **Murray Barr and Ewart Bertram in 1949** in the neurons of female cats, and represents a single, transcriptionally inactivated X chromosome — the cytological expression of **Mary Lyon's X-inactivation hypothesis (1961)**. Because the number of Barr bodies per nucleus follows the simple "**n – 1**" rule (one less than the total number of X chromosomes), a buccal smear examined for sex chromatin remains a quick, inexpensive screening test for numerical sex-chromosome disorders and for the sex of a tissue sample.

1. Discovery and nomenclature

- ▶ **Murray Barr and Ewart Bertram (1949)** observed a small, deeply-stained body in the interphase nuclei of female cat neurons; it was named the **Barr body**, or **sex chromatin**, in his honour.
- ▶ The same structure is seen in most somatic cells of the human female, although it is most conveniently studied in a **buccal (oral) mucosal smear**.
- ▶ In **polymorphonuclear neutrophils** it projects from a nuclear lobe as a small drumstick-shaped appendage — the **drumstick** or **Davidson body**.
- ▶ "Sex chromatin" is therefore an umbrella term for **two morphologically distinct expressions of the same inactivated X chromosome**: (1) the Barr body of epithelial/other tissue-cell nuclei, and (2) the drumstick (Davidson body) of neutrophils.

2. Structure and cytological features

- ▶ The Barr body represents **one inactivated X chromosome** rendered heterochromatic, late-replicating and hyper-methylated — i.e., **facultative heterochromatin** that is genetically inert (in contrast to the other, active, euchromatic X).
- ▶ **Location**: applied to the inner surface of the nuclear membrane in most cells (buccal, skin, vaginal, urethral, corneal epithelium; also placenta, dental pulp, skin fibroblasts); in **neurons** it lies as a small dark body adjacent to the nucleolus (a "nucleolar satellite").
- ▶ **Shape**: planoconvex, wedge-shaped or rectangular.
- ▶ **Size**: approximately **1 µm**.
- ▶ **Positivity**: because not every cell shows an optimally oriented, demonstrable Barr body, a normal female buccal smear is chromatin-positive in roughly **20–80% of cells**, against **0–2% in normal males** — hence many cells must be screened before scoring a smear as positive or negative.
- ▶ **Drumstick (Davidson) body**: a deeply-stained appendage attached to a nuclear lobe of the neutrophil by a thin filament; the head measures about **1.5 µm** in diameter; it is found in only a small proportion of neutrophils in a normal female film (about **2–3%**, or **~6 per 500 cells counted**).

□ Fig 17.10 — Location of Barr body as seen in (A) buccal smear; (B) neuron and (C) neutrophil (Vishram Singh — *General Anatomy — 4e*, p.698)

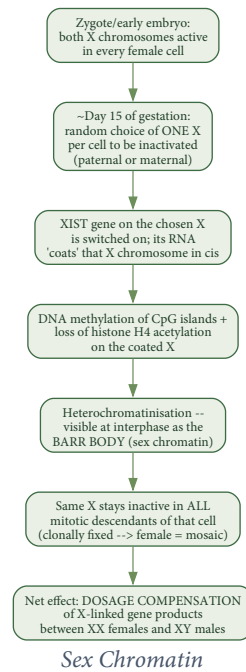
DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A three-panel schematic (buccal smear / neuron / neutrophil), labelling:

- ▶ Nuclear membrane / nuclear envelope
- ▶ **Barr body** — planoconvex mass applied to the inner surface of the nuclear membrane (buccal smear panel)
- ▶ Nucleolus, with the **Barr body** as a small dark "satellite" body immediately adjacent to it (neuron panel)
- ▶ Multilobed neutrophil nucleus with the **drumstick (Davidson) body** attached to one lobe by a fine chromatin filament (neutrophil panel)
- ▶ Label the active, euchromatic X chromosome vs the inactive, heterochromatic (Barr-body-forming) X chromosome

3. Lyon hypothesis — mechanism of X-inactivation

- ▶ Proposed by the geneticist **Mary F. Lyon** (1961); its main postulates are:
- ▶ In the somatic cells of female mammals, **only one X chromosome remains genetically active**; the other is condensed and inactivated.
- ▶ Inactivation of the **paternal or maternal X is random** in any given cell.
- ▶ Once an X chromosome is inactivated in a cell, the **same X remains inactive in all its mitotic descendants** — i.e., the choice is random but clonally fixed.
- ▶ Inactivation ("**lyonisation**") occurs **early in embryonic life** (around the 15th day of gestation).
- ▶ **Molecular control**: governed by the **X-inactivation centre (XIC)**, located in the proximal part of the long arm of the X chromosome close to the centromere. The master regulatory locus within the XIC is the **XIST gene** ("X-inactive specific transcript"), a non-protein-coding gene whose RNA product "coats" the chosen X chromosome in cis and triggers **DNA methylation of CpG islands** and loss of histone (H4) acetylation, producing stable heterochromatinisation.
- ▶ **Functional consequence — dosage compensation**: X-inactivation equalises the effective dose of X-linked gene products between 46,XX females (two X chromosomes, one active) and 46,XY males (one active X), so that both sexes express a functionally single dose of most X-linked genes.
- ▶ A practical corollary: heterozygous **female carriers of an X-linked recessive disorder are functional mosaics** of two cell populations (one expressing the normal allele, one the mutant allele on the active X), which explains variable/patchy expression in manifesting carriers.



4. The Barr-body ("n – 1") rule in health and chromosomal disorders

- ▶ The number of Barr bodies visible per interphase nucleus is always **one less than the total number of X chromosomes present** — the "**n – 1**" rule, where n = number of X chromosomes (for tetraploid cells the rule becomes n – 2, and for octaploid cells n – 4).

Individual / condition	Sex-chromosome constitution	No. of Barr bodies
Normal male	46,XY	0
Turner syndrome	45,XO	0
Normal female	46,XX	1
Klinefelter syndrome	47,XXY	1
Triple-X ("super-female")	47,XXX	2
Tetra-X female	48,XXXX	3

5. Y chromatin (F body)

- ▶ During interphase, the **heterochromatic distal long arm of the Y chromosome** shows up as an intensely fluorescent mass — the **F body** — when nuclei are stained with a **fluorochrome dye** and examined under a **fluorescence microscope**.
- ▶ The **number of F bodies equals the number of Y chromosomes** present in the cell (a normal male shows one F body; 47,XYY shows two).
- ▶ F-body demonstration is a useful **complementary test to Barr-body counting** — together the two tests give a rapid cytological estimate of both X- and Y-chromosome number without full karyotyping.

6. Demonstration of sex chromatin — technique

- ▶ The commonest and simplest source is a **buccal (oral) mucosal smear**: cells are scraped from the inner cheek, smeared on a glass slide, fixed, and stained with a basic nuclear stain, then examined under the light microscope.
- ▶ Because Barr-body positivity is seen in only a fraction of cells in any smear (see above), a **large number of cells must be screened** before a smear can be confidently read as chromatin-positive or -negative, and orientation of the nucleus must be favourable to display the body clearly.
- ▶ For the drumstick (Davidson) body, a **peripheral blood smear** is examined and several hundred neutrophils counted, since it is present in only a small percentage of cells even in a normal female.
- ▶ Other tissue sources historically used include skin/hair-root, vaginal, urethral and corneal epithelial smears, placental tissue, dental pulp, and amniotic-fluid or chorionic-villus cells for prenatal work.

Applied / Clinical anatomy

- ▶ **First-line screen for numerical sex-chromosome disorders**: a chromatin-negative buccal smear in a phenotypic female raises suspicion of **Turner syndrome (45,XO)**, while a chromatin-positive smear in a phenotypic male raises suspicion of **Klinefelter syndrome (47,XXY)** — both are then confirmed by formal karyotyping.
- ▶ **Disorders of sex development / intersex evaluation**: in **true hermaphroditism** the chromosomal sex — and hence the Barr-body pattern — may be either male or female (or mosaic), so sex chromatin alone cannot resolve the diagnosis and full karyotyping/gonadal histology is required. In **androgen insensitivity (testicular feminisation) syndrome**, the individual is genetically **46,XY** with intra-abdominal testes but a female external phenotype (due to mutation of the androgen-receptor gene); a sex-chromatin study in such a patient shows a **male (chromatin-negative) pattern despite unambiguous female external genitalia**, an important teaching point on discordance between phenotype, gonad and genetic sex.
- ▶ **Prenatal and forensic sexing**: sex chromatin study of amniotic-fluid or chorionic-villus cells has historically served as a rapid adjunct to karyotyping for fetal sex determination, and chromatin/drumstick study of tissue, blood or hair-root smears has been used in **forensic medicine** to determine the sex of a body or tissue sample when external genitalia cannot be examined.
- ▶ **Gender verification in competitive sport**: buccal-smear sex-chromatin testing was historically used as a simple screening method for gender verification of female athletes before molecular (SRY-based) methods became standard.
- ▶ **X-linked disease expression**: because X-inactivation is random and clonally fixed, female carriers of X-linked recessive conditions are cellular mosaics, which underlies the variable clinical severity occasionally seen in "manifesting carriers."

High-yield exam pointers

- ▶ Barr body = **inactivated X chromosome**; discovered by **Barr and Bertram (1949)** in female cat neurons.

- ▶ **Lyon hypothesis (1961)**: random, single, clonally-fixed X-inactivation in every female somatic cell around the **15th day** of gestation; controlled by the **XIC/XIST locus** on the proximal long arm of X.
- ▶ **"n – 1" rule**: number of Barr bodies = total X chromosomes – 1 (Turner 45,XO → 0; normal female 46,XX → 1; Klinefelter 47,XXY → 1; triple-X 47,XXX → 2).
- ▶ Barr body: ~1 µm, planoconvex, applied to inner nuclear membrane (buccal smear/epithelium) or adjacent to the nucleolus (neurons).
- ▶ Drumstick/Davidson body: neutrophil nuclear-lobe appendage, ~1.5 µm head, present in only ~2–3% of neutrophils in normal females.
- ▶ **F body** = fluorescent Y-chromosome heterochromatin seen with fluorochrome staining under fluorescence microscopy; number of F bodies = number of Y chromosomes.
- ▶ Functional significance = **dosage compensation** of X-linked genes between males and females.
- ▶ Clinical uses: rapid screen for Turner/Klinefelter syndromes, prenatal/forensic sex determination, historical gender verification in sport, and explaining phenotype–genotype discordance (e.g., androgen insensitivity syndrome).

SOURCE & COVERAGE

Vishram Singh General Anatomy 4e; IB Singh Textbook of Anatomy Vol 3 6e; IB Singh Human Embryology (CBME 2021); Vishram Singh Textbook of Clinical Embryology; Junqueira's Basic Histology 17e; Ross Histology Text & Atlas 7e; Gangane Human Genetics 5e; Thompson & Thompson Genetics in Medicine 8e. Coverage note: Gray's Anatomy (Standring) 41e treats chromatin/nuclear structure only in general terms and has no dedicated "sex chromatin" section; this is a genetics–cytology crossover topic, so grounding was drawn chiefly from the histology and genetics/embryology specialty texts, as flagged in this topic's spec.

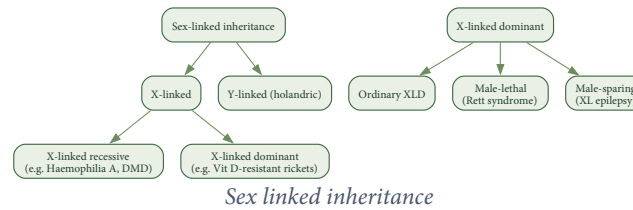


Sex linked inheritance

Asked in: Paper III 30-May-2025 · Paper III May 2019 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction

Sex-linked inheritance is the mode of transmission of genes carried on the sex chromosomes (X or Y) rather than the 22 pairs of autosomes. The Y chromosome is much shorter than the X and, apart from a small pseudoautosomal segment, carries very few genes with no X counterpart; hence the overwhelming majority of clinically important sex-linked disorders are X-linked, and "sex-linked" is practically synonymous with "X-linked," Y-linked (holandric) inheritance being a small, distinct category. A male, having only one X, is **hemizygous** for every X-linked gene — a single mutant allele always produces the phenotype — whereas a female may be homozygous normal, a heterozygous carrier, or (rarely) homozygous affected. This asymmetry generates three characteristic pedigree patterns — X-linked recessive, X-linked dominant, and Y-linked — that must be distinguished from autosomal and from sex-limited/sex-influenced inheritance for correct genetic counselling.



1. Basis of sex-linked inheritance and dosage compensation

- ▶ Humans have 22 pairs of autosomes + 1 pair of sex chromosomes: **XX** in the female, **XY** in the male.
- ▶ Genes on the non-homologous (differential) segment of the X have no allelic partner on the Y — hence **hemizygous** expression in the male; a single copy, normal or mutant, is always phenotypically expressed.
- ▶ Small **pseudoautosomal regions** at the tips of Xp/Yp and Xq/Yq are homologous and recombine during male meiosis; genes here (e.g., **SHOX**, mutated in **dyschondrosteosis**) can show male-to-male transmission and mimic autosomal dominant inheritance rather than strict X-linkage.
- ▶ **Dosage compensation — Lyon hypothesis (X-inactivation)**: in every somatic cell of a normal female, one X chromosome is randomly inactivated early in embryonic life and stays inactive in all clonal descendants, becoming facultative heterochromatin (**Barr body**) coated by **XIST** RNA; any X in excess of one, in either sex, is likewise inactivated — equalising X-linked gene expression between the sexes.
- ▶ Since inactivation is random and clonally fixed, every female is a **mosaic** for X-linked gene expression; unfavourable ("skewed") inactivation can make a carrier symptomatic — a **manifesting heterozygote** (e.g., a DMD carrier with mild myopathy/cardiomyopathy).

2. X-linked recessive inheritance

□ Fig 6.10 — Pedigree of X-linked recessive (Gangane — Human Genetics — 5e, p.129)

The mutant allele is expressed phenotypically in every hemizygous male who inherits it but manifests in a female only when present in double (homozygous) dose; a heterozygous female is usually an unaffected **carrier**. The disorder is therefore far commoner in males.

Features of X-linked recessive inheritance:

- ▶ Incidence of the trait is much higher in males than in females.
- ▶ An affected male transmits the gene through **all** his daughters (obligate carriers) but to **none** of his sons (a father gives his sons only the Y chromosome).
- ▶ **No male-to-male transmission** occurs — the single most useful rule for excluding autosomal inheritance.
- ▶ A carrier (heterozygous) female × normal male mating gives, on average, 50% of sons affected and 50% of daughters carriers.
- ▶ In a kindred, affected males are related to one another **through carrier females**, so the trait may be transmitted silently through a series of carrier women before re-appearing in a male descendant.
- ▶ Heterozygous females are usually unaffected, but skewed X-inactivation may produce a manifesting heterozygote.

- An affected female is possible only if she is homozygous — practically seen only when an affected father mates a carrier/affected mother (e.g., an affected father × carrier mother in colour blindness).

Disorder	Defect / locus	Key clinical points
Haemophilia A	Deficiency of factor VIII (antihaemophilic globulin)	Incidence ≈ 1 in 10,000 male births; profuse bleeding after trivial injury, haemarthrosis; classic example — Queen Victoria was a carrier
Duchenne muscular dystrophy (DMD)	Dystrophin gene, locus Xp21; largest human gene (~2300 kb, 79 exons)	Incidence ≈ 1 in 3500 male births; onset 3–5 yrs, waddling gait, Gower sign , wheelchair-bound by ~11 yrs; serum creatine kinase raised (1000–20,000 IU vs <200 IU normal); milder allelic form = Becker muscular dystrophy (≈ 1 in 20,000)
G6PD deficiency	Glucose-6-phosphate dehydrogenase deficiency	Haemolytic episodes precipitated by oxidant drugs (primaquine, sulphonamides, aspirin)
Red-green colour blindness	Gene for colour vision on X	Affected female genotype X ^c X ^c requires a carrier mother and a colour-blind father

3. X-linked dominant inheritance

The mutant allele is expressed in heterozygous females as well as in hemizygous males, though expression in females is usually milder and more variable because only a proportion of their cells have the mutant allele on the active X.

Features of X-linked dominant inheritance:

- The trait is roughly **twice as common in females** as in males, but affected females show milder, variable expression.
- An **affected male** transmits the trait to **all** his daughters and to **none** of his sons — the same "no male-to-male transmission" rule as for X-linked recessive.
- An affected **heterozygous female**, mated to a normal male, transmits the trait to 50% of children of **either** sex — this mimics autosomal dominant inheritance, so only the progeny of an *affected male* distinguishes the two.
- A homozygous affected female transmits the trait to all her children.

Classic example: **X-linked hypophosphataemic (vitamin D-resistant) rickets** — impaired renal tubular reabsorption of phosphate, with less severe rickets and less depressed serum phosphate in heterozygous females than in hemizygous males. The **Xg blood group** is another example.

Two special variants:

- ▶ **Male-lethal X-linked dominant:** lethal in hemizygous males in utero, so pedigrees show only affected females, who produce affected daughters, normal daughters, and normal sons in equal (1:1:1) proportion, with **no affected males** — e.g., **Rett syndrome** (MECP2 gene).
- ▶ **Male-sparing X-linked dominant:** hemizygous males are largely spared and the disease manifests mainly in carrier females — e.g., female-limited epilepsy with cognitive impairment.
- ▶ **Pseudoautosomal exception:** dyschondrosteosis (short stature, forearm deformity; **SHOX** gene) is commoner in females like an X-linked dominant trait but shows male-to-male transmission, since SHOX lies in the pseudoautosomal region — proof it is not strictly X-linked.

4. Y-linked (holandric) inheritance

Since a male has only one Y chromosome, any gene on it is necessarily unpaired — the question of dominance versus recessiveness does not arise; if present, it is expressed. The gene passes directly from an affected father to **all** his sons and to **none** of his daughters (never affected, never carriers), and it never skips a generation. There is therefore **no carrier state** for a Y-linked trait.

- ▶ Confirmed functional Y-linked genes: **H-Y antigen gene**; **SRY** (sex-determining region Y/testis-determining factor, Yp), which initiates male sexual differentiation; and the **AZF_a**, **AZF_b**, **AZF_c** (**azoospermia factor**) regions on Yq (e.g., the **DAZ** gene family) essential for spermatogenesis — microdeletion here causes oligospermia/azoospermia in otherwise normal males.
- ▶ The trait classically cited in teaching as a visible holandric character is **hypertrichosis of the pinna** ("hairy ears/hairy pinna").

5. Fragile X syndrome — an atypical sex-linked pattern

Fragile X syndrome is the most common heritable cause of moderate intellectual disability and an autism-spectrum disorder. It is a combined cytogenetic-and-molecular disorder rather than a classic dominant/recessive trait:

- ▶ A fragile site is demonstrable cytogenetically at **Xq27–28** (Xq27.3) when cells are cultured in **folate- and thymidine-deficient** medium.
- ▶ Molecular basis: expansion of a **CGG trinucleotide repeat** in the 5' UTR of **FMR1** — normal allele up to ~55 repeats; **premutation** ~56–200 repeats (clinically silent/mild, unstable, prone to further expansion through a female); **full mutation** >200 (up to several thousand) repeats causes hypermethylation and silencing of FMR1.
- ▶ Penetrance in carrier females is only ~50–60% (unlike classic X-linked recessive); frequency ≈ 1 in 3000–4000 male births.
- ▶ Clinical features: intellectual disability/autism-spectrum features, large prominent ears, macro-orchidism (enlarged testes) after puberty.
- ▶ Risk of maternal premutation expanding to a full mutation rises steeply with increasing repeat number.

6. Distinguishing sex-linked from sex-limited and sex-influenced traits

Category	Gene location	Expression	Example
Sex-linked	X or Y chromosome	Unequal frequency/pattern in the sexes because the gene itself is on a sex chromosome	Haemophilia, DMD, hairy pinna
Sex-limited	Autosome	Transmitted by both sexes, expressed in one sex only	Precocious puberty in males (autosomal dominant, expressed only in males)
Sex-influenced	Autosome	Expressed in both sexes but at different frequency/severity	Pattern baldness (males); congenital adrenal hyperplasia (more evident in females)

Applied / Clinical anatomy

- **Pedigree analysis** is the essential first step in any suspected sex-linked disorder: absence of male-to-male transmission plus demonstration of hemizygoty/carrier status distinguishes X-linked from autosomal patterns and guides recurrence-risk figures given during genetic counselling.
- **Carrier detection:** elevated serum creatine kinase helps identify DMD carrier mothers; factor VIII assay identifies haemophilia carriers; buccal-smear sex-chromatin (Barr body) studies reflect the X-inactivation pattern; a **manifesting heterozygote** may show mild myopathy/cardiomyopathy (DMD) or a mild bleeding tendency (haemophilia) from skewed X-inactivation — relevant to pre-operative and obstetric risk assessment.
- **Prenatal diagnosis:** chorionic villus sampling/amniocentesis with fetal sexing and molecular analysis is offered to known carriers of serious X-linked recessive disorders (DMD, haemophilia).
- **Drug safety:** hemizygous males with G6PD deficiency must avoid oxidant drugs (primaquine, sulphonamides, aspirin) to prevent a haemolytic crisis.
- **Work-up of intellectual disability:** fragile-X testing (folate-deficient culture medium/FMR1 CGG-repeat analysis) is routine in evaluating undiagnosed developmental delay or autism, especially with a positive family history.
- **Historical/forensic relevance:** the pedigree of haemophilia in the European royal families (descended from carrier Queen Victoria) remains the classic teaching illustration of X-linked recessive transmission.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Reproduce pedigree conventions alongside the classic X-linked recessive pattern shown in the plate above:

- ▶ **Square** = male; **Circle** = female; **solid/filled** symbol = affected; **circle with a central dot** = carrier female.
- ▶ **Horizontal line** joining a square and circle = mating; **vertical line** to a **horizontal sibship line** = their offspring; label generations **I, II, III** top to bottom.
- ▶ Show: Gen I — unaffected male × carrier female; Gen II — one affected son + one carrier daughter among unaffected sibs; Gen III — the carrier daughter's own son affected — illustrating **no male-to-male transmission** and **affected males linked through carrier females**.
- ▶ For contrast, add a small second pedigree for **X-linked dominant**: affected male × normal female → **all daughters affected**, **no sons affected**.

High-yield exam pointers

- ▶ Sex-linked inheritance is, for practical purposes, synonymous with **X-linked** inheritance; the Y chromosome carries very few genes (H-Y antigen, SRY, AZFa/b/c).
- ▶ Males are **hemizygous** for X-linked genes — a single mutant allele is always expressed.
- ▶ **No male-to-male transmission** is the key rule distinguishing both X-linked recessive and X-linked dominant inheritance from autosomal dominant inheritance.
- ▶ X-linked recessive: affected males vastly outnumber females; carrier daughters of an affected male; classic examples — haemophilia A, DMD, G6PD deficiency, red-green colour blindness.
- ▶ X-linked dominant: about twice as common in females (milder expression); example — vitamin D-resistant rickets; special forms are male-lethal (Rett syndrome) and male-sparing (X-linked epilepsy).
- ▶ **Lyon hypothesis**: random X-inactivation in each female somatic cell → Barr body, dosage compensation, female mosaicism, and occasional manifesting heterozygotes.
- ▶ Y-linked (holandric) inheritance: only males affected, direct father-to-son transmission, never through daughters, no carrier state — classic teaching trait is hairy pinna.
- ▶ **Fragile X syndrome** (FMR1, CGG-repeat expansion at Xq27.3) is the commonest heritable cause of intellectual disability and does not follow a strict dominant/recessive rule.
- ▶ Distinguish true sex-linked traits from **sex-limited** (autosomal gene, one-sex expression, e.g., precocious puberty) and **sex-influenced** traits (autosomal gene, both sexes but unequal frequency, e.g., baldness).

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e; Vishram Singh — General Anatomy 4e; IB Singh — Textbook of Anatomy Vol 3 6e (Inheritance of Genetic Disorders). Coverage note: this is a classic Mendelian-genetics topic and is not covered in Gray's Anatomy (Standring) 41e, which is a gross-anatomy spine text; grounding above is drawn entirely from the dedicated genetics/anatomy texts listed.



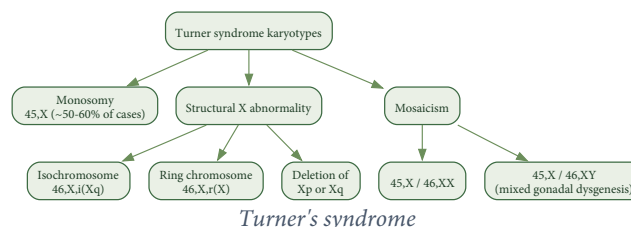
Turner's syndrome

Asked in: Paper III May 2010 · Paper III Oct 2008 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — Turner's syndrome is a sex-chromosomal disorder of genetic females caused by complete or partial monosomy of one X chromosome (classic karyotype **45,X**), first described clinically by Henry Turner in 1938; the 45,X karyotype itself was demonstrated by Ford and colleagues in 1959 at Harwell. It is the **only monosomy compatible with post-natal life** in humans, and produces a distinctive phenotype of short stature, gonadal dysgenesis (streak ovaries) with primary amenorrhoea, and a cluster of somatic (webbed neck, shield chest, cardiac and renal) anomalies in a phenotypically female individual.

Etiology, incidence & cytogenetics

- Results from **non-disjunction** of a sex chromosome during parental gametogenesis (or post-zygotic mitotic loss), leading to absence or structural abnormality of one X chromosome in somatic cells.
- **Incidence:** ~1 in 2500–4000 live female births (Thompson & Thompson); Indian texts cite similar orders (roughly 1 in 2000–3000 to 1 in 5000–10,000). It is genuinely common at conception — monosomy X accounts for a large fraction of all chromosomally abnormal spontaneous abortions — but over 95–99% of 45,X conceptuses are lost as early miscarriages, so the live-born rate is much lower than the conception rate.
- **Parental origin:** in the majority of cases (~75%) the single retained X is of **maternal** origin, i.e., the paternal sex chromosome (X or Y) is the one lost.
- Cytogenetic subtypes (karyotype spectrum):



- About **60%** of patients show the pure **45,X** karyotype; the remainder show a structural X abnormality (the isochromosome **46,X,i(Xq)** is the commonest structural variant; a ring chromosome **Xr** is also seen) or **mosaicism** (45,X/46,XX being most frequent). Mosaic and structural-variant patients tend to have a milder phenotype than pure 45,X individuals.

- ▶ A mosaic 45,X/46,XY line produces **mixed gonadal dysgenesis** (an asymmetric dysgenetic testis and a streak gonad) with a variable phenotype and a definite risk of **gonadoblastoma** in the dysgenetic gonad — this mosaic subgroup needs prophylactic gonadectomy.

Molecular and developmental basis of the phenotype

- ▶ Normal females inactivate one X chromosome in every somatic cell (Lyon hypothesis), controlled by the **X-inactivation centre (XIC)/XIST** locus, forming the **Barr body**. With only one X present, Turner patients have **no Barr body** (buccal smear is chromatin-negative) — the mirror image of the extra Barr body seen in Klinefelter syndrome.
- ▶ The **SHOX** (short-stature homeobox) gene lies in the **pseudoautosomal region** of Xp/Yp, escapes X-inactivation, and therefore needs two active copies for normal linear growth; **SHOX haploinsufficiency** in 45,X cells (only one functional copy) is the accepted major contributor to the short stature of Turner syndrome (the same gene's heterozygous mutation independently causes Léri–Weill dyschondrosteosis).
- ▶ **Gonadal dysgenesis**: ovaries develop normally in early fetal life, but in the absence of a second X chromosome, oocytes undergo accelerated atresia/apoptosis before or shortly after birth; by the time of birth the gonads are reduced to **fibrous "streak" gonads** composed only of connective tissue stroma, with no oocytes or follicles — hence primary amenorrhoea and infertility rather than a primary failure of gonadal formation.
- ▶ The **webbed neck** (pterygium colli) and peripheral lymphoedema of the hands/feet at birth are the residue of an in-utero **jugular lymphatic obstruction sequence** (resolving fetal cystic hygroma), which is why prenatal ultrasound in a 45,X fetus classically shows nuchal cystic hygroma with or without non-immune hydrops.

Clinical features

System	Features
General/growth	Phenotypically female; short stature (usual presenting complaint); intelligence usually normal, occasionally mildly reduced
Head & neck	Webbed neck , low posterior hairline, low-set ears, high-arched palate, epicanthic folds
Trunk	Shield/broad chest with widely-spaced ("pin-point") nipples
Limbs	Bilateral cubitus valgus , lymphoedema of hands and feet (often the clue at birth), hyperconvex/upturned nails
Skin	Multiple pigmented (brown) naevi
Reproductive	Streak gonads/gonadal dysgenesis , infantile external genitalia, small uterus, absent secondary sexual characters, primary amenorrhoea , sterility — the commonest cause of primary amenorrhoea
Cardiovascular	Coarctation of aorta , bicuspid aortic valve, ventricular septal defect

System	Features
Renal	Horseshoe kidney, renal hypoplasia/aplasia, duplication of ureters

□ Fig 4.13 — Karyotype of Turner syndrome showing only one X chromosome. (Gangane — Human Genetics — 5e, p.97)

□ Fig 4.11 — Turner syndrome case showing short stature and webbed neck. (Gangane — Human Genetics — 5e, p.96)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled outline sketch of a Turner-syndrome patient (frontal view), with arrows to:

- ▶ Low-set ears + low posterior hairline
- ▶ Webbed neck
- ▶ Shield/broad chest with widely-spaced nipples
- ▶ Bilateral cubitus valgus at the elbows
- ▶ Infantile external genitalia
- ▶ Lymphoedema of dorsum of hands and feet
- ▶ Short stature (compare to an age-matched normal outline)

Alongside, draw a simplified **karyotype strip**: 44 autosomes + a single X, no Y, labelled "45,X".

Diagnosis / investigations

- ▶ **Buccal smear (Barr body study)**: chromatin-negative (absent Barr body) — screening test.
- ▶ **Dermatoglyphics**: high total finger ridge count and a distally placed axial triradius.
- ▶ **Karyotyping** (peripheral blood lymphocyte culture, GTG-banding): the definitive/gold-standard test; identifies 45,X, structural X variants, or mosaicism.
- ▶ **Prenatal**: ultrasound shows nuchal cystic hygroma ± non-immune hydrops fetalis; confirmed by karyotyping on chorionic villus sampling/amniocentesis or by non-invasive prenatal testing.

Management

- ▶ **Growth hormone** (or, historically, anabolic steroid) therapy started around 10–12 years of age to improve final adult height.
- ▶ **Oestrogen replacement** at the expected age of puberty to induce secondary sexual characters, followed by cyclical oestrogen–progesterone for uterine health.
- ▶ **Assisted reproduction** (donor-oocyte in vitro fertilisation) — patients are sterile but the uterus can usually support a pregnancy.
- ▶ Surveillance and treatment of associated cardiac, renal, and (in mosaic 45,X/46,XY patients) prophylactic gonadectomy for gonadoblastoma risk.

Turner syndrome vs Klinefelter syndrome

Feature	Klinefelter syndrome	Turner syndrome
Nature	Trisomic condition, males only	Monosomic condition, females only

Feature	Klinefelter syndrome	Turner syndrome
Karyotype	47,XXY	45,X (45,XO)
Phenotype	Male	Female
Stature	Tall	Short
Gonads	Small, fibrosed testes; azoospermia	Streak (fibrous) gonads
Secondary sexual characters	Poorly developed; gynaecomastia	Poorly developed; absent breast development
Barr body	One (chromatin-positive)	None (chromatin-negative)

Applied / clinical anatomy

- ▶ **Webbed neck** is a direct anatomical sequel of failed/delayed connection between the jugular lymph sac and the internal jugular vein in fetal life (jugular lymphatic obstruction sequence) — the same mechanism that produces fetal nuchal cystic hygroma seen on antenatal ultrasound.
- ▶ **Coarctation of the aorta/bicuspid aortic valve**: the commonest cardiovascular anomalies in Turner syndrome; clinically correlate with radiofemoral delay and are relevant to surface/cross-sectional anatomy of the aortic arch.
- ▶ **Horseshoe kidney**: a fusion anomaly from abnormal medial fusion of the lower poles of the metanephric masses with arrested renal ascent (arrest below the inferior mesenteric artery) — an embryological correlate frequently paired with Turner syndrome in viva questions.
- ▶ **Streak gonad**: a fibrous cord occupying the normal anatomical position of the ovary in the broad ligament/pelvic side wall, devoid of germ cells and follicles — distinguishes gonadal dysgenesis from simple ovarian hypoplasia.
- ▶ **Differential**: Noonan syndrome produces a very similar somatic phenotype (webbed neck, short stature, cardiac defects) but has a **normal karyotype**, is autosomal (commonly *PTPN11*-related), and affects either sex — a classical "Turner-phenotype-with-normal-karyotype" differential.
- ▶ A **45,X/46,XY mosaic** with a dysgenetic testis is a disorder-of-sex-development variant and carries a definite gonadoblastoma risk, so gonadal histology/imaging is part of anatomical work-up in such mosaics.

High-yield exam pointers

- ▶ Turner syndrome = **only viable monosomy** in humans; classic karyotype **45,X**.
- ▶ Buccal smear is **Barr-body/chromatin-negative** (absent second X).
- ▶ Commonest cause of **primary amenorrhoea** with a female phenotype.
- ▶ Triad to remember: **short stature + webbed neck + streak gonads**.
- ▶ Cardiac lesion of choice to remember = **coarctation of aorta**; renal lesion = **horseshoe kidney**.
- ▶ ~60% pure 45,X; commonest structural variant = **isochromosome i(Xq)**; mosaics commonly 45,X/46,XX.
- ▶ In ~75% of cases the surviving X is **maternal** in origin.

- ▶ 45,X/46,XY mosaicism → mixed gonadal dysgenesis → **gonadoblastoma risk**, needs gonadectomy.
- ▶ Management pillars: growth hormone for height, oestrogen for puberty, donor-oocyte IVF for fertility.

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e; Vishram Singh — Textbook of Clinical Embryology; Vishram Singh — General Anatomy 4e; IB Singh — Human Embryology (CBME 2021); Sontakke — Textbook of Human Embryology.



Amniocentesis

Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction Amniocentesis is an invasive prenatal diagnostic procedure in which a sample of amniotic fluid is aspirated transabdominally from the amniotic cavity, under real-time ultrasound guidance, for cytogenetic, biochemical and molecular analysis of the fetus. First used clinically for Rh-disease assessment and later extended to genetic diagnosis, it remains the most widely practised invasive method of prenatal diagnosis, forming — together with chorionic villus sampling (CVS), cordocentesis and non-invasive screening — the backbone of antenatal genetic counselling. The amniotic fluid it yields contains desquamated fetal cells (skin, urinary tract, amnion) that can be cultured for karyotyping and dissolved fetal proteins (notably alpha-fetoprotein, AFP) that can be assayed directly.

1. Indications

- ▶ **Advanced maternal age** (≥ 35 years) — the single commonest indication, because the risk of trisomies rises steeply with maternal age.
- ▶ **Previous child with a chromosomal abnormality or a neural tube defect (NTD).**
- ▶ **Either parent a known carrier of a balanced chromosomal translocation** or other structural rearrangement.
- ▶ **Couple at risk of a mendelian (autosomal or X-linked) recessive metabolic disorder** that is severe and accurately detectable prenatally (e.g., by enzyme or DNA analysis of cultured amniocytes).
- ▶ **Abnormal maternal serum screening** (raised/lowered MSAFP, abnormal quadruple screen) or **an abnormal detailed ultrasound/nuchal translucency scan.**
- ▶ **Maternal disease**, e.g. diabetes mellitus, associated with an increased risk of fetal anomaly.
- ▶ General principles governing the decision to offer prenatal diagnosis: the disorder must be one for which treatment is absent/unsatisfactory, an accurate prenatal test exists, the risk to the pregnancy/fetus is significant, the disorder is severe enough to justify termination if positive, and termination must be acceptable to the couple.

2. Timing

- ▶ Classically taught (Gangane) as ideal at **14–16 weeks** of gestation — by this time enough amniotic fluid has accumulated for safe tapping, and any decision to terminate can still be taken before quickening (fetal movements), which alters parental acceptance of termination.
- ▶ Standard genetics texts (Thompson & Thompson; Langman) describe the routine window as **15/16–20 weeks**, terming this "**midtrimester amniocentesis**."
- ▶ **Early amniocentesis (10–14 weeks)** was tried to allow earlier diagnosis but is **no longer recommended**: it carries a roughly **threefold increase in spontaneous abortion** and a **sixfold-to-sevenfold increase in talipes equinovarus (clubfoot)** compared with the 0.1–0.3% background population risk, because too little fluid is available at this age. Early amniocentesis has effectively been replaced by CVS.
- ▶ **Late amniocentesis**, closer to term, is occasionally used to assess fetal lung maturity or the severity of Rh-isoinmunisation, though this is a secondary, non-genetic obstetric application.

3. Pre-procedure work-up

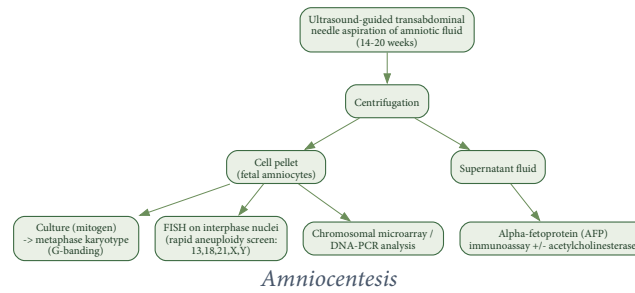
Real-time ultrasonography is mandatory before (and during) the tap, to assess:

- ▶ fetal viability and number of fetuses;
- ▶ gestational age (biparietal diameter, head/abdominal circumference, femur length);
- ▶ volume of amniotic fluid and normality of fetal anatomy;
- ▶ **placental localisation** — so the needle path can be planned to avoid the placenta.

4. Technique

□ Fig 9.9 — A. Ultrasound image showing position of the fetal skull and placement of the needle into the amniotic cavity (arrow) during amniocentesis. B. Twins — ultrasound showing two gestational sacs. (Langman's Medical Embryology — Sadler — 14e, p.159)

- ▶ Written informed consent; the maternal urinary bladder is emptied.
- ▶ Under **continuous real-time ultrasound guidance**, a needle-entry point on the anterior abdominal wall is chosen that avoids the placenta, the fetus, the bladder and the bowel.
- ▶ The layers traversed by the needle are the standard planes of the anterior abdominal wall — **skin** → **superficial fascia** → **external oblique aponeurosis/rectus sheath** → **rectus abdominis** (or its **lateral border**) → **transversalis fascia** → **extraperitoneal fat** → **parietal peritoneum** — and then the **myometrium and decidua of the gravid uterus** before the needle tip enters the **amniotic cavity**.
- ▶ Under local anaesthesia, a spinal needle is passed transabdominally into the amniotic cavity, and **10–30 mL (commonly ~20 mL)** of fluid is withdrawn by syringe.
- ▶ A **clear, amber-coloured (not blood-stained) tap** must be ensured; a blood-tinged sample suggests placental or fetal injury and risks maternal-cell/fetal-blood contamination of the sample.
- ▶ The aspirate is centrifuged to separate the **cell pellet** (fetal amniocytes) from the **supernatant fluid**, each sent for different tests.



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Sagittal schematic of the gravid uterus with the needle in place — label: (1) Anterior abdominal wall layers (skin, superficial fascia, rectus sheath/muscle, transversalis fascia, parietal peritoneum); (2) Urinary bladder (emptied, displaced); (3) Ultrasound transducer on maternal abdomen with beam directed to the needle path; (4) Myometrium; (5) Placenta (needle path kept clear of it); (6) Decidua/chorioamniotic membrane; (7) Amniotic cavity with amniotic fluid; (8) Fetus; (9) Spinal needle tip within the amniotic cavity; (10) Syringe aspirating fluid.

5. Laboratory analysis of the aspirate

Component	Test	Purpose / turnaround
Fetal amniocytes (cell pellet)	Culture with mitogen → metaphase arrest → G-banded karyotype	Detects numerical/structural chromosome abnormalities; full result in about 1–3 weeks (culture and chromosome preparation together take roughly 7–10 days at minimum)
Fetal amniocytes	FISH on interphase nuclei for chromosomes 13, 18, 21, X, Y	Rapid aneuploidy screen, result in 1–2 days, without waiting for culture
Fetal amniocytes	Chromosomal microarray (CMA) / targeted DNA-PCR	Detects copy-number variants and specific mutations; increasingly supplements/replaces conventional karyotyping for selected indications
Supernatant fluid	Alpha-fetoprotein (AFP) immunoassay (± acetylcholinesterase)	Screens for open neural tube defects and ventral wall defects; combined with detailed ultrasonography, detects ~99% of open spina bifida and virtually all anencephaly
Supernatant fluid	Biochemical/enzyme assay	Diagnoses specific inborn errors of metabolism when a family is known to be at risk

6. Complications and risks

Complication	Notes
Procedure-related miscarriage	Genetics texts (Thompson & Thompson) quote 1 in 300 to 1 in 500 additional risk over the baseline ~1–2% pregnancy-loss risk at this gestation for midtrimester (16–20 week) amniocentesis; older teaching (Gangane) quotes an overall 1–1.5% abortion risk, rising to ~9% after a repeat tap
Amniotic fluid leakage	Uncommon; more frequent after early amniocentesis
Infection	Rare, with strict asepsis
Fetal injury by direct needle puncture	Rare with continuous ultrasound guidance
Neonatal respiratory distress	Reported to increase roughly threefold
Postural orthopaedic problems	Talipes equinovarus (clubfoot) and congenital dislocation of the hip are increased, especially after early amniocentesis (6- to 7-fold for clubfoot)
Rh (D) sensitisation	Needle passage can cause feto-maternal haemorrhage; Rh-negative, unsensitised mothers should receive anti-D immunoglobulin prophylaxis after the procedure
Perinatal mortality / antepartum haemorrhage	Slightly increased

Applied / Clinical anatomy

- ▶ The needle traverses the same anterior-abdominal-wall planes used for any midline/paramedian abdominal puncture (as in ascitic/peritoneal tap), which is why an empty bladder and ultrasound-confirmed placental position are essential safety steps before the tap.
- ▶ **CVS (chorionic villus sampling)** is the principal alternative: villous tissue is aspirated transcervically or transabdominally at **8–10 weeks (up to 12 weeks)**; because villous cells are already dividing, results are available in 2–3 days rather than 1–3 weeks, and first-trimester termination (if indicated) is technically simpler than the second-trimester termination that follows a positive amniocentesis — but CVS carries a slightly higher procedure-related loss rate and a small added risk of limb-reduction defects.
- ▶ **Cordocentesis (percutaneous umbilical blood sampling, PUBS)**, performed from about 14 weeks under ultrasound guidance, obtains fetal blood directly from the umbilical cord for rapid cytogenetic or haematological diagnosis when a faster result is needed than amniocentesis allows, at a marginally higher fetal-loss rate.
- ▶ In India, prenatal diagnostic procedures including amniocentesis are governed by the **PCPNDT Act**, which permits their use strictly for detecting genetic/chromosomal disorders and prohibits their use for fetal sex determination and sex-selective abortion.
- ▶ Late-pregnancy amniocentesis retains a secondary obstetric role in assessing **fetal lung maturity** and the **severity of Rh-isoimmunisation**, though these are non-genetic, non-anatomical applications outside the core embryology/genetics syllabus.

- ▶ If the first culture attempt fails, or when speed is paramount, fetal blood obtained by fetoscopy/cordocentesis is an accepted fallback for chromosome analysis.

High-yield exam pointers

- ▶ **Definition:** ultrasound-guided transabdominal aspiration of amniotic fluid for fetal cytogenetic/biochemical diagnosis.
- ▶ **Timing:** ideal 14–16 weeks (classic teaching); standard "midtrimester" window 15/16–20 weeks; early amniocentesis (10–14 weeks) abandoned due to higher miscarriage and clubfoot risk.
- ▶ **Volume withdrawn:** ~10–30 mL (commonly ~20 mL), under continuous ultrasound guidance; a clear amber tap must be ensured.
- ▶ **Two arms of testing:** cell pellet → culture → karyotype (± FISH for rapid aneuploidy screen, ± CMA); supernatant fluid → AFP (± acetylcholinesterase) for open NTDs.
- ▶ **Turnaround:** karyotype in roughly 1–3 weeks; FISH in 1–2 days.
- ▶ **Main risk:** procedure-related miscarriage, quoted as 1 in 300–500 (midtrimester) or 1–1.5% (classic teaching); rises further with a repeat tap.
- ▶ **Compare with CVS** (8–10, up to 12 weeks; faster karyotype but slightly higher loss rate and limb-defect risk) and **cordocentesis/PUBS** (~14 weeks onward; fetal blood, fastest cytogenetics).
- ▶ Rh-negative mothers require **anti-D prophylaxis** after the procedure.
- ▶ Regulated in India under the **PCPNDT Act** to prevent sex-selective misuse.

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e; Langman's Medical Embryology (Sadler) 14e; IB Singh — Human Embryology (CBME 2021); Larsen — Human Embryology 6e. Coverage note: Gray's Anatomy (Standring) 41e and the assigned microscopy/histotechnique texts (Bancroft's; Pranab Dey) carry only passing mentions of amniocentesis with no procedural detail, so the technique, timing and complication data above are drawn from the genetics/embryology corpus, which covers this lab-technique topic in depth.



Barr body

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction The **Barr body** is a small, deeply-staining, planoconvex mass of chromatin seen just inside the nuclear membrane of the interphase nucleus of normal female somatic cells; it is the cytological expression of the single, genetically inactive X chromosome. It was first observed by **Murray Barr and Ewart Bertram in 1949** in the interphase nuclei of female cat neurons, and its formation is explained by the **Lyon hypothesis (Mary F. Lyon, 1962)** — the postulate that only one X chromosome remains active in every female somatic cell, the other being randomly and permanently inactivated (**Lyonisation**) as the mechanism of **dosage compensation** between 46,XX females and 46,XY males. Together with its neutrophil counterpart, the drumstick (Davidson body), the Barr body constitutes **sex chromatin**, and its presence, absence, or number is of direct diagnostic value in disorders of sexual differentiation.

1. Discovery and terminology

- ▶ **Barr and Bertram (1949)** described a small, deeply-stained body in the interphase nuclei of female cat neurons; it was named the **Barr body** after Murray Llewellyn Barr, Professor of Anatomy, University of Western Ontario, Canada, and is also called **sex chromatin**.
- ▶ "Sex chromatin" is an umbrella term covering **two morphologically distinct expressions of the same inactivated X chromosome**:
- ▶ **Barr body** — seen in epithelial and most other tissue cells (buccal mucosal, skin, vaginal, urethral and corneal epithelium; also placenta, dental pulp, hair-root sheath and amniotic-fluid cells).
- ▶ **Drumstick (Davidson body)** — seen only in the neutrophil among the polymorphonuclear leukocytes.
- ▶ Study of sex chromatin gives a relatively simple, rapid diagnostic test for certain chromosomal/genetic abnormalities, historically preceding routine karyotyping.

2. Lyon hypothesis — X-inactivation

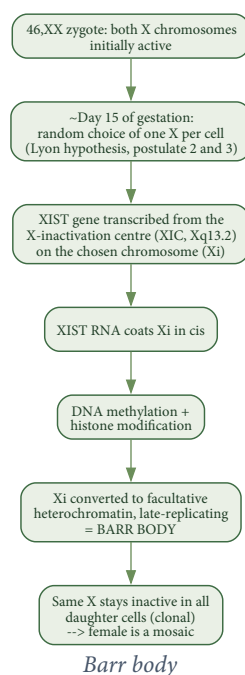
Mary F. Lyon's hypothesis (1962) rests on three postulates:

- ▶ In every female somatic cell, **only one X chromosome is genetically active**; the second is inactivated and condensed to form sex chromatin.
- ▶ The **inactivation occurs early in embryonic life** — around the 15th day of gestation.
- ▶ The inactivation is **random but fixed (clonal)** — either the paternally- or the maternally-derived X may be inactivated in any given cell, but once chosen, the *same* X remains inactive in that cell and in all its mitotic daughter cells.

Because the choice is made independently, cell-by-cell, in a small population of embryonic cells, every normal female is a **mosaic** of two clonal cell populations — one expressing the paternal X, the other the maternal X — with the ratio of the two populations normally distributed around 50:50 (range roughly 25:75 to 75:25). This mosaicism explains why heterozygous female carriers of X-linked disorders can show patchy expression of the trait (e.g., the retinal mosaic pattern in carriers of X-linked colour blindness).

3. Mechanism and molecular basis

- Inactivation is initiated from the **X-inactivation centre (XIC)**, located in the proximal part of the long arm of the X chromosome (**Xq13.2**).
- The XIC contains the non-coding RNA gene **XIST**, which is transcribed *only* from the chromosome destined to become inactive (**Xi**); XIST RNA then **coats the Xi in cis** along nearly its entire length.
- This is followed by **DNA methylation** and characteristic histone modifications, converting the Xi into **facultative heterochromatin** — visible microscopically as the Barr body.
- The inactive X thereafter becomes **late-replicating** in S phase, in contrast to the active X and the autosomes, which replicate synchronously.
- Net effect: **dosage compensation** — equalising the expression of X-linked genes between the two sexes, since both a 46,XX female and a 46,XY male end up with only one transcriptionally active X per cell. Notably, not all X-linked genes are silenced — around 15% continue to be expressed from the inactive X (chiefly pseudoautosomal-region genes), which is why complete monosomy for the X (Turner syndrome) is still phenotypically abnormal.



Feature	Active X	Inactive X (Xi)
Gene expression	Full — similar to the single male X	Most genes silenced; ~15% still expressed to some degree
Chromatin state	Euchromatin	Facultative heterochromatin — the Barr body
XIST	Silenced	Expressed only from Xi; XIST RNA associates with the Barr body
DNA replication	Synchronous with autosomes	Late-replicating in S phase

Feature	Active X	Inactive X (Xi)
Histones	As autosomes/male X	Enriched for macroH2A and heterochromatin marks

4. Morphology and identification of the Barr body

- **Site:** usually located subjacent to (just inside) the nuclear membrane of the interphase nucleus; in neurons it instead appears as a small dark body opposite the nucleolus.
- **Shape:** ovoid, planoconvex, wedge-shaped, or rectangular.
- **Size:** approximately 1 μm .
- **Best studied in:** a **buccal mucosal smear** (also skin, vaginal smear, hair-root sheath and amniotic-fluid cells).
- **Frequency of chromatin-positive nuclei:** normal females show the Barr body in roughly 20–80% of nuclei; normal males show it in only 1–2% (background/artefact), so a clear positive/negative distinction is diagnostically usable.
- **n – 1 rule (Lyon's rule):** the maximum number of Barr bodies per nucleus = number of X chromosomes – 1 (in tetraploid cells, two less than the number of X chromosomes; in octaploid cells, four less).

□ Fig 17.10 — ■ Location of Barr body as seen in (A) buccal smear; (B) neuron and (C) (Vishram Singh — General Anatomy — 4e, p.698)

5. Barr body vs drumstick (Davidson body)

Feature	Barr body	Drumstick (Davidson body)
Cell type	Buccal, skin, vaginal, urethral, corneal epithelium; placenta, dental pulp, hair root, amniocytes	Neutrophilic polymorphonuclear leukocyte only
Location	Subjacent to nuclear membrane (opposite nucleolus in neurons)	Small appendage attached to a nuclear lobe by a thin filament of variable length
Shape/size	Planoconvex/wedge-shaped, ~1 μm	Drumstick-shaped head, ~1.5 μm diameter
Incidence in normal females	20–80% of nuclei	~2–3% of neutrophils (about 6 per 500 cells)
Nature	Facultative heterochromatin of the inactive X, subjacent to envelope	Same inactive X chromosome, extruded from the main nuclear mass

6. Number of Barr bodies in different karyotypes

Individual	Karyotype	No. of X chromosomes	Barr bodies (n – 1)
Normal male	46,XY	1	0
Turner syndrome	45,X	1	0

Individual	Karyotype	No. of X chromosomes	Barr bodies (n - 1)
Normal female	46,XX	2	1
Klinefelter syndrome	47,XXY	2	1
Triple-X ("super-female")	47,XXX	3	2
Tetrasomy X	48,XXXX	4	3

7. Y chromatin (F body) — the complementary test

- ▶ In males, the distal heterochromatic segment of the long arm of the Y chromosome fluoresces brightly with quinacrine/fluorochrome staining under fluorescence microscopy — the **F body** (Y chromatin).
- ▶ The number of F bodies equals the number of Y chromosomes present, making it a useful complementary test to Barr-body counting when a full karyotype is not immediately available (e.g., 47,XXY shows two F bodies).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw an interphase nucleus in three cell types side by side (as in the figure above), with these exact labels:

- ▶ (A) **Buccal epithelial cell** — nuclear membrane; **Barr body** as a small planoconvex/wedge-shaped, deeply-staining body applied to the inner surface of the nuclear membrane.
- ▶ (B) **Neuron** — nucleus with prominent nucleolus; Barr body shown as a small dark body opposite the nucleolus.
- ▶ (C) **Neutrophil (polymorphonuclear leukocyte)** — multilobed nucleus; **drumstick (Davidson body)** shown as a small, densely-stained, drumstick-shaped head attached to one nuclear lobe by a thin filament.
- ▶ Caption: "Sex chromatin — Barr body (A, B) and drumstick (C)."

Applied / Clinical anatomy

- ▶ **Buccal smear (sex chromatin) test:** a rapid, minimally invasive screening test historically used in the work-up of ambiguous genitalia and disorders of sexual differentiation, before karyotyping became routinely available.
 - A **Barr-body-negative** phenotypic female suggests **Turner syndrome (45,X)**.
 - A **Barr-body-positive** phenotypic male suggests **Klinefelter syndrome (47,XXY)**.
- ▶ **Antenatal sex chromatin study:** amniotic-fluid cells were historically examined for Barr bodies to indicate fetal sex/aneuploidy screening; this has now been **superseded by karyotyping, FISH and molecular (QF-PCR/NIPT) methods**, which are more sensitive and specific.
- ▶ **Forensic medicine:** sex chromatin study of tissue remnants, hair roots or bone marrow can help establish the sex of unidentified biological material.

- ▶ **Sports/gender-verification testing:** buccal smear sex-chromatin analysis was historically used at international athletic events for female-eligibility screening before molecular genetic methods replaced it.
- ▶ **Skewed X-inactivation:** extreme (non-50:50) skewing of X-inactivation can unmask X-linked recessive disease in an otherwise "carrier" female if the X carrying the normal allele is preferentially inactivated (a manifesting carrier), e.g., in Duchenne muscular dystrophy or haemophilia carriers.
- ▶ **Structurally abnormal X chromosomes:** an X lacking the XIC cannot be inactivated; in X;autosome translocations and structural X rearrangements, the **structurally abnormal X is always the one inactivated** (when it retains an XIC), minimising the effective genetic imbalance — a principle used in interpreting such karyotypes clinically.

High-yield exam pointers

- ▶ Barr body = 1949, Barr & Bertram, in female cat neurons; Lyon hypothesis = Mary F. Lyon, 1962.
- ▶ Sex chromatin = **Barr body** (epithelial/tissue cells) + **drumstick/Davidson body** (neutrophils only).
- ▶ **n – 1 rule:** number of Barr bodies = number of X chromosomes – 1.
- ▶ Turner (45,X) = 0 Barr bodies; normal female (46,XX)/Klinefelter (47,XXY) = 1; triple-X (47,XXX) = 2; tetrasomy X (48,XXXX) = 3.
- ▶ Molecular basis: XIC (Xq13.2) → XIST ncRNA coats Xi in cis → **methylation/heterochromatinisation** → late replication.
- ▶ Timing: random choice around **day 15 of gestation**, thereafter **clonally fixed** → females are X-inactivation mosaics.
- ▶ Morphometry: Barr body ~1 µm, planoconvex, subjacent to nuclear membrane; drumstick head ~1.5 µm, seen in ~2–3% of neutrophils.
- ▶ **Y chromatin (F body):** fluorescent Yq heterochromatin in males; number of F bodies = number of Y chromosomes.
- ▶ Clinical use: rapid screening test in disorders of sexual differentiation (Turner/Klinefelter); now largely superseded by karyotyping/FISH/molecular testing.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh Human Embryology (CBME 2021); Vishram Singh General Anatomy 4e; Vishram Singh Textbook of Clinical Embryology; IB Singh Textbook of Anatomy Vol 3 6e; Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e. Coverage note: Gray's Anatomy (Standring) 41e mentions the drumstick/Barr body only briefly (in the neutrophil-morphology section and its eponym glossary) and does not carry a dedicated genetics chapter, consistent with this being a genetics topic; the principal grounding was therefore drawn from the embryology/genetics specialty texts, as intended for this topic's specialty designation.



Chorionic villi biopsy

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction Chorionic villi are finger-like projections of the trophoblast (the extraembryonic covering of the blastocyst) that anchor the conceptus to the decidua and mediate materno-fetal exchange; because they arise from the same zygote as the embryo, they are genetically fetal tissue. Chorionic villus sampling (CVS), also called chorion villous biopsy, is an invasive first-trimester prenatal diagnostic procedure in which a small sample of these villi is aspirated — transcervically or transabdominally, under ultrasound guidance — for karyotyping, DNA analysis or enzyme assay of the fetus. It is one of the standard techniques of prenatal diagnosis, complementary to amniocentesis, ultrasonography, foetoscopy, foetal blood sampling and preimplantation genetic diagnosis.

1. Chorionic villi — embryological origin and structure

The fetal component of the placenta (chorionic villi + chorionic plate) is derived from the trophoblast and extraembryonic mesoderm; the maternal component is derived from the endometrium. Villus development passes through three stages:

Stage	Timing	Structure
Primary villi	Begin ~day 11–13 (early 3rd week)	Solid columns of cytotrophoblast covered by a syncytiotrophoblastic shell; no mesodermal core
Secondary villi	End of the 3rd week	Extraembryonic mesoderm penetrates the cytotrophoblastic core of each primary villus
Tertiary (definitive) villi	~Day 21 onward	Mesodermal core becomes vascularised; capillaries within the villus link with capillaries of the chorionic plate and connecting stalk, establishing continuity with the embryonic circulation

- **Anchoring (stem) villi** extend from the chorionic plate to the cytotrophoblastic shell and fix the placenta to the decidua; **floating (free) villi** branch off the stem villi into the intervillous space and are the principal site of exchange.
- One pole of the chorion bears numerous villi — the **chorion frondosum** (villous chorion), which becomes the fetal placenta; the opposite pole becomes villous-poor and atrophies as the **chorion laeve** (smooth chorion).
- The **placental membrane** separating maternal and fetal blood is initially four-layered (syncytiotrophoblast, cytotrophoblast, connective-tissue core, fetal endothelium); from the 4th month the cytotrophoblastic layer disappears in most villi, thinning the barrier to syncytium + endothelium and improving exchange. Because maternal blood bathes a chorionic-derived syncytial surface, the human placenta is of the **haemochorial** type.

- ▶ Villi (particularly the actively dividing cytotrophoblastic cells) are a ready, accessible source of fetal genetic material — this is the anatomical rationale for chorionic villus biopsy.

2. Indications for prenatal diagnosis

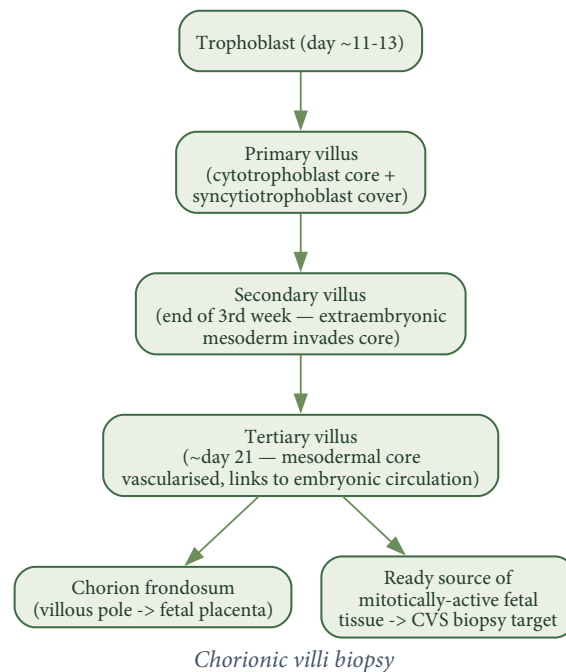
- ▶ A genetic disorder for which treatment is absent or unsatisfactory.
- ▶ A disorder for which an accurate prenatal test is available.
- ▶ Pregnancy at sufficiently high risk (abnormal pedigree/family history).
- ▶ A disorder severe enough to justify termination of pregnancy, acceptable to the couple.

Prenatal diagnosis is considered a must when:

- ▶ Maternal age is above 35 years.
- ▶ One parent is a balanced translocation carrier.
- ▶ The pregnancy carries a severe, prenatally-detectable autosomal/X-linked recessive metabolic disorder.
- ▶ The couple already has a child with a chromosomal disorder or a neural tube defect.

3. Procedure of chorionic villus sampling

- ▶ **Timing** — a first-trimester procedure; classically 8–10 weeks (extendable to ~12 weeks) of gestation, and generally recommended after 10 completed weeks by experienced operators (see complications, below).
- ▶ **Guidance** — continuous real-time ultrasonography localises the placenta and confirms fetal viability before and during sampling.
- ▶ **Route** — two alternative approaches:
 - *Transcervical*: a flexible cannula is passed through the vagina and cervix uteri into the chorion frondosum, and villi are aspirated under vacuum/suction.
 - *Transabdominal*: a spinal needle is introduced through the anterior abdominal wall and uterine wall directly into the placental villous tissue.
- ▶ **Sampling** — a few milligrams of chorionic villi are withdrawn, washed free of maternal decidual contaminants, and processed either (i) directly, exploiting the actively dividing cytotrophoblastic cells for a rapid chromosome preparation without culture, or (ii) after short-term culture of mesenchymal core cells, to confirm the direct result.
- ▶ **Downstream tests** — karyotype/chromosomal microarray for aneuploidy and structural rearrangements; molecular (DNA) analysis for single-gene disorders (e.g., thalassaemia, Duchenne muscular dystrophy, cystic fibrosis); biochemical/enzyme assay for inborn errors of metabolism.



□ Fig 13.2 — Chorion villous sampling for prenatal diagnosis in the first trimester of pregnancy (Gangane — Human Genetics — 5e, p.226)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A single labelled sketch showing both approaches to CVS (as in the figure above), with these exact labels:

- ▶ Vagina, cervix uteri (external os), endocervical canal
- ▶ Anterior abdominal wall
- ▶ Uterine wall, uterine cavity
- ▶ Placenta / **chorion frondosum** (villous chorion)
- ▶ Chorionic cavity, amniotic cavity
- ▶ **Transcervical route:** flexible cannula passed via cervix into the villous chorion
- ▶ **Transabdominal route:** spinal needle passed through the abdominal wall into the placental villi
- ▶ Ultrasound transducer/scanner (shown guiding both routes)

4. Advantages of CVS over amniocentesis

- ▶ Performed much earlier in gestation (first trimester) than amniocentesis (second trimester), so results are available earlier and the period of parental uncertainty is shortened.
- ▶ Because chorionic villi contain cytotrophoblastic cells already in active mitosis, a chromosome preparation can be obtained directly, without the 2–3 week culture that amniotic fluid cells require — a faster result.
- ▶ If an abnormality is found, termination of pregnancy in the first trimester is technically simpler and medically safer than a mid-trimester termination after amniocentesis.

- ▶ Diagnostic accuracy for chromosome analysis is comparable to amniocentesis (success rate >99%).

5. Complications and limitations

- ▶ **Procedure-related pregnancy loss:** in experienced centres, the fetal-loss rate attributable to CVS is only slightly above the baseline spontaneous loss of 2–5% for any 7–12-week pregnancy, and approximates the 1-in-300 to 1-in-500 risk quoted for amniocentesis; Gangane notes CVS still carries a somewhat greater risk of abortion than amniocentesis overall, though it is safe in experienced hands.
- ▶ **Limb-reduction defects:** early reports linked CVS with an increased frequency of limb-reduction and oromandibular defects; this association has **not** been confirmed in large series when CVS is performed after 10 completed weeks by experienced operators — hence the recommendation not to perform CVS before 10 weeks.
- ▶ **No amniotic-fluid alpha-fetoprotein (AFAFP) assay is possible** on villous tissue, so CVS cannot screen for open neural tube defects; this must be done separately by maternal serum AFP, later amniocentesis, or detailed ultrasonography.
- ▶ **Ambiguous/mosaic results:** about 1% of CVS samples show chromosomal mosaicism on culture, requiring follow-up amniocentesis to resolve whether it is a true fetal mosaicism or **confined placental mosaicism** (an abnormal line restricted to the extraembryonic trophoblast/mesoderm lineage, not the embryo proper).

6. Pitfalls in interpreting chorionic villus chromosome analysis

- ▶ **Culture failure** — the first attempt at culturing CVS (or amniotic fluid) may fail, requiring a repeat sample or an alternative source such as fetal blood by cordocentesis/foetoscopy.
- ▶ **Maternal cell contamination** — decidual (maternal) cells adherent to the villi may be co-cultured, occasionally producing a misleading result (e.g., a male fetus misread as female, or an apparent 46,XY/46,XX mosaic).
- ▶ **True vs pseudomosaicism** — pseudomosaicism can arise as a culture artefact or from maternal cell contamination. Dividing the sample into multiple culture vessels helps distinguish them: an abnormal line present in *all* vessels favours true mosaicism, while one confined to a *single* vessel favours pseudomosaicism or confined placental mosaicism.

7. CVS vs amniocentesis — comparison

Feature	Chorionic villus sampling	Amniocentesis
Timing	First trimester, ~8–13 weeks	Second trimester, ~14–20 weeks
Tissue sampled	Chorionic villi (trophoblast)	Amniotic fluid + suspended fetal cells
Route	Transcervical cannula or transabdominal needle into placenta	Transabdominal needle into amniotic sac
Turnaround	Rapid — direct prep from mitotic cytotrophoblast possible	Slower — 2–3 weeks of cell culture usually needed

Feature	Chorionic villus sampling	Amniocentesis
AFP/NTD screening	Not possible on villous tissue	AFAFP directly assayable
Procedure-related loss	≈1 in 300–500 (experienced centres)	≈1 in 300–500
Diagnostic pitfall	Confined placental mosaicism (~1%)	Lower mosaicism rate
If abnormal	Safer, simpler first-trimester termination	Second-trimester termination

Applied / Clinical anatomy

- ▶ **Clinical use:** CVS is the invasive test of choice for early diagnosis of aneuploidy (trisomy 21/18/13, sex-chromosome disorders), parental balanced-translocation carrier status, and known single-gene disorders (thalassaemia, DMD, cystic fibrosis) where DNA/enzyme diagnosis is required in the first trimester.
- ▶ **Anatomical corridor of the transcervical route:** vagina → external os → endocervical canal → lower uterine segment → chorion frondosum, avoiding the amniotic sac — hence the mandatory ultrasound guidance.
- ▶ **Confined placental mosaicism** is clinically important precisely because chorionic villi, though derived from the same zygote, belong to the extraembryonic trophoblast/mesoderm lineage; a post-zygotic mitotic error confined to this lineage can make the placental karyotype differ from the true fetal karyotype — the reason discordant CVS mosaicism is always confirmed by amniocentesis.
- ▶ **Legal/regulatory note:** prenatal diagnostic procedures including CVS are regulated under the PCPNDT Act (Preconception and Prenatal Diagnostic Techniques Act), which permits their use strictly for detecting genetic/chromosomal abnormalities and prohibits use for fetal sex determination/selection.
- ▶ CVS complements, and does not replace, ultrasonography (structural anomaly screening/placental localisation), maternal serum screening (AFP, β -hCG, etc.), foetal blood sampling/cordocentesis (haematological and rapid karyotype confirmation), and preimplantation genetic diagnosis (pre-conceptional selection in IVF).

High-yield exam pointers

- ▶ CVS = biopsy of **chorionic villi** (trophoblast-derived, hence fetal tissue), obtained **transcervically** (cannula) or **transabdominally** (needle), always under ultrasound guidance.
- ▶ Performed in the **first trimester**, ~8–13 weeks; recommended **after 10 weeks** by experienced hands to avoid the (unconfirmed but historically reported) limb-reduction-defect association.
- ▶ Villi are in active mitosis → allows **direct karyotyping without culture** → main advantage over amniocentesis (faster + earlier, first-trimester termination if needed).
- ▶ Cannot assay AFAFP → cannot screen NTDs from CVS tissue alone.
- ▶ Procedure-related loss risk ≈ 1 in 300–500, comparable to amniocentesis; ~1% give ambiguous mosaic results (true vs confined placental mosaicism, resolved by follow-up amniocentesis).

- ▶ Villus development: **primary** (cytotrophoblast + syncytium) → **secondary** (mesodermal core added) → **tertiary** (vascularised, links to embryonic circulation) villi.
- ▶ Chorion frondosum (villous pole → placenta) vs chorion laeve (smooth pole); placenta is **haemochorial**.
- ▶ Governed by the PCPNDT Act — sex determination is prohibited.

SOURCE & COVERAGE

Gangane — Human Genetics, 5e (Chapter 13, Prenatal Diagnosis — chorion villous biopsy technique, indications, merits, risks, mosaicism interpretation); Thompson & Thompson — Genetics in Medicine, 8e (Chapter 17, Prenatal Diagnosis and Screening — CVS timing/route, complications, limb-reduction-defect data, confined placental mosaicism); Langman's Medical Embryology (Sadler), 14e (chorionic villus development — primary/secondary/tertiary villi, chorion frondosum/laeve, placental membrane, haemochorial placenta); Gray's Anatomy (Standring), 41e (brief confirmatory note on chorionic villus tissue as a karyotyping source). Coverage note: Gray's Anatomy carries only a passing line on this procedural topic, as expected for a purely diagnostic/genetics technique; the depth above is drawn from the genetics and embryology specialty texts, consistent with house-style targeted grounding.

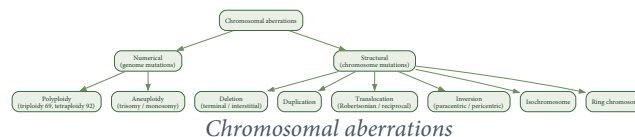
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Chromosomal aberrations

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Chromosomal aberrations (chromosomal abnormalities) are deviations from the normal human chromosomal complement — 46 chromosomes arranged as 22 pairs of autosomes and 1 pair of sex chromosomes (44 + XX or 44 + XY) — arising from errors during gametogenesis, fertilisation or early cleavage. They are detected by **karyotyping** of metaphase chromosome spreads and are of major clinical importance because they underlie a large group of congenital malformation syndromes, pregnancy loss, infertility, intellectual disability and malignancy. Chromosomal abnormalities are classified on two bases: (i) the **type** of abnormality — numerical or structural, and (ii) the **chromosomes involved** — autosomal or sex-chromosomal.

Classification of chromosomal aberrations



On the basis of chromosomes involved: **autosomal** (e.g. Down syndrome) or **sex-chromosomal** (e.g. Turner, Klinefelter syndromes).

Karyotyping and cytogenetic nomenclature

A **karyotype** is the complete chromosomal complement of a cell/individual, arranged in homologous pairs from longest to shortest. Chromosomes are classified by centromere position into **metacentric**

(centromere central), **submetacentric** (centromere off-centre), **acrocentric** (centromere near one end, short arm carries a satellite) and **telocentric** (centromere at the very end — not seen in normal humans). By the **Denver classification (1960)**, the 46 human chromosomes (including the sex chromosomes) are arranged into seven groups, A–G, on the basis of (1) size, (2) position of the centromere, (3) arm-length ratio and (4) presence of satellites.

□ Fig 17.7 — Karyotype of a normal male. (Vishram Singh — General Anatomy — 4e, p.695)

Standard cytogenetic symbols used in reporting a karyotype:

Symbol	Meaning
p	short arm
q	long arm
t	translocation
i	isochromosome
inv	inversion
r	ring chromosome
+ / – before a number	extra / missing whole chromosome
+ / – after a symbol	increase / decrease in arm length

Example: 47,XX,+21 = female, trisomy 21; 45,X = Turner syndrome.

Numerical aberrations

Result from failure of normal disjunction during gametogenesis or from errors of fertilisation/cleavage.

Type	Mechanism	Result	Example
Polyplidy — multiple of the whole haploid set (23) added to the diploid set			
Triploidy	Dispermy (2 sperm fertilise 1 ovum) or fertilisation of a diploid ovum by a haploid sperm	69 chromosomes (23×3)	Spontaneous abortion / brief survival
Tetraploidy	Failure of the first cleavage division	92 chromosomes (23×4)	Spontaneous abortion
Aneuploidy — chromosome number not an exact multiple of 23	Non-disjunction (failure of paired chromosomes/sister chromatids to separate) at meiosis I or meiosis II, or anaphase lag	Trisomy (2n+1) or monosomy (2n–1)	Down (trisomy 21), Turner (monosomy X)

Mechanism of non-disjunction: normally each primary spermatocyte/oocyte gives four (or one functional) gametes with 23 chromosomes. If the two members of a pair fail to separate — at meiosis I

both homologues move to one pole; at meiosis II both sister chromatids move to one pole — one gamete gets 24 chromosomes and the other 22. Fertilisation of such a gamete by a normal (23-chromosome) gamete produces a **47-chromosome (trisomic)** or **45-chromosome (monosomic)** zygote. Trisomy generally causes spontaneous abortion, but trisomy 13, 18, 21 and XXY are compatible with live birth; monosomy is almost always lethal in utero — monosomy X (Turner syndrome) is the only monosomy compatible with life. **Mosaicism** — two or more chromosomally distinct cell lines in one individual (e.g. mosaic Down or mosaic Turner syndrome) — results from non-disjunction occurring after fertilisation, in early mitotic divisions of the zygote.

Structural aberrations

Result from one or more chromosome breaks followed by loss or abnormal reunion of the fragments (caused by ionising radiation, chemical mutagens or viruses).

□ Fig 4.2 — Chromosomal aberration – deletion: (A) Terminal deletion of the short arm of a chromosome, (B) Interstitial deletion. (Gangane — Human Genetics — 5e, p.85)

Type	Description	Example
Deletion	Loss of a chromosome segment — terminal (from the end) or interstitial (from within an arm, two breaks)	Wolf–Hirschhorn syndrome (4p–), Cri-du-chat syndrome (5p–)
Microdeletion	Submicroscopic deletion, needs high-resolution banding/FISH	Prader–Willi & Angelman syndromes (15q11–13), DiGeorge syndrome (22q11.2), Miller–Dieker syndrome (17p13.3)
Duplication	A segment is present in excess (partial trisomy)	Generally better tolerated than deletion
Ring chromosome	Deletion at both ends of a chromosome with fusion of the two broken ends	Commonly involves chromosome 14
Isochromosome	Centromere splits transversely instead of longitudinally, so one arm is duplicated and the other lost	i(Xq) in some Turner syndrome variants
Inversion	A segment is excised, flipped 180° and reinserted — pericentric (includes the centromere) or paracentric (does not include the centromere)	Usually balanced in the carrier; risk of unbalanced gametes in offspring

Type	Description	Example
Translocation	Exchange of segments between two non-homologous chromosomes — Robertsonian (centric fusion of two acrocentric chromosomes at the centromere, with loss of the short arms; e.g. rob(13;14), the single commonest chromosome rearrangement in humans, ~1 in 1300) or Reciprocal (breaks and exchange of segments distal to the break in two chromosomes; balanced, no loss of material, carrier phenotypically normal)	Robertsonian → 3–4% of Down syndrome (familial/translocation Down); Reciprocal → Philadelphia chromosome t(9;22) in CML, t(15;17) in acute promyelocytic leukaemia
Insertion	A segment removed from one chromosome is inserted into another (needs 3 breaks) — rare	—
Fragile site	Localised, heritable gap/constriction that predisposes to breakage	Fragile X (Martin–Bell) syndrome

Balanced rearrangements (reciprocal translocation, pericentric/paracentric inversion) carry no loss of genetic material, so the carrier is usually phenotypically normal, but **abnormal (unbalanced) gametes** at meiosis carry a high risk of miscarriage or a malformed, unbalanced offspring. **Chimaerism** — two or more genetically distinct cell populations derived from **more than one zygote** (e.g. dispermic chimaera, blood-group chimaera) — must be distinguished from mosaicism, which arises from a **single zygote**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- **Karyotype grouping (Denver classification)** — draw 7 boxes labelled Group A–G with representative pairs: A (1–3, large metacentric), B (4–5, large submetacentric), C (6–12 + X, medium submetacentric), D (13–15, medium acrocentric with satellites), E (16–18, small metacentric/submetacentric), F (19–20, small metacentric), G (21–22 + Y, small acrocentric with satellites).
- **Structural aberrations schematic** — draw a normal chromosome pair alongside each abnormal type, labelling: Normal → Terminal deletion → Interstitial deletion → Duplication → Paracentric inversion → Pericentric inversion → Ring chromosome → Isochromosome → Robertsonian translocation → Reciprocal translocation. Mark the centromere and breakpoints on each.

Clinically important chromosomal syndromes

Syndrome	Karyotype	Type	Key features
Down syndrome	47,XX/XY,+21 (trisomy; 46 with translocation in ~3–4%; rarely mosaic)	Numerical (autosomal)	Mongoloid facies, epicanthic folds, simian crease, mental retardation, congenital heart disease; incidence 1/700; risk rises with maternal age
Patau syndrome	47,+13	Numerical (autosomal)	Cleft lip/palate, polydactyly, omphalocele, profound mental retardation; early death
Edward syndrome	47,+18	Numerical (autosomal)	Prominent occiput, micrognathia, overlapping fingers, rocker-bottom feet, cardiac defects; death within weeks
Klinefelter syndrome	47,XXY	Numerical (sex chromosome)	Phenotypic male, eunuchoid habitus, hypogonadism, gynaecomastia, infertility, Barr-body positive; incidence ~1/500 male births
Turner syndrome	45,X	Numerical (sex chromosome, only viable monosomy)	Phenotypic female, short stature, webbed neck, shield chest, cubitus valgus, gonadal dysgenesis/amenorrhoea, coarctation of aorta

Syndrome	Karyotype	Type	Key features
Triple X (super female)	47,XXX	Numerical (sex chromosome)	Female phenotype, mild mental retardation, scanty menses
Cri-du-chat syndrome	46,del(5p)	Structural (deletion)	Cat-like cry, microcephaly, hypertelorism, mental retardation
Wolf-Hirschhorn syndrome	46,del(4p)	Structural (deletion)	Severe growth/mental retardation, "Greek-warrior-helmet" facies
Prader-Willi / Angelman syndrome	del(15q11-13), paternal / maternal	Structural (microdeletion, imprinting)	Hyperphagia-obesity-hypotonia / happy-puppet ataxic phenotype
DiGeorge syndrome	del(22q11.2)	Structural (microdeletion)	Thymic aplasia (immunodeficiency), hypoparathyroidism (hypocalcaemia), cardiac outflow defects
Miller-Dieker syndrome	del(17p13.3)	Structural (microdeletion)	Lissencephaly, microcephaly
CML	t(9;22) Philadelphia chromosome	Structural (reciprocal translocation)	BCR-ABL fusion oncogene
Acute promyelocytic leukaemia	t(15;17)	Structural (reciprocal translocation)	PML-RARA fusion

Applied / Clinical anatomy

- **Prenatal diagnosis:** karyotyping/FISH on amniocentesis or chorionic villus sampling cells; maternal serum screening (Down syndrome associated with **low α -fetoprotein**); non-invasive prenatal testing on cell-free foetal DNA.
- **Advanced maternal age** is the leading recognised risk factor for meiotic non-disjunction and trisomies (Down, Klinefelter), reflecting the prolonged arrest of the oocyte in meiosis I.
- **Genetic counselling:** parents of a translocation-Down child must be karyotyped, since a parental Robertsonian translocation carrier has a substantially raised recurrence risk in future pregnancies, whereas free trisomy 21 does not.
- **Barr body (sex chromatin) analysis** on a buccal smear is a simple screening test — absent in Turner syndrome (45,X), present (single) in normal females and Klinefelter syndrome, double in triple-X.
- **Cancer cytogenetics:** acquired somatic translocations (Philadelphia chromosome in CML, t(15;17) in APL) are used both diagnostically and as targets for molecular therapy (e.g. imatinib in Philadelphia-positive CML).
- **Radiological/clinical correlation:** microdeletion syndromes (22q11.2, 15q11-13) require FISH/microarray for diagnosis as they are below the resolution of conventional karyotyping.

- ▶ Recurrent pregnancy loss, unexplained infertility, ambiguous genitalia and multiple congenital anomalies are standard clinical indications for karyotyping.

High-yield exam pointers

- ▶ Chromosomal aberrations = **numerical** (genome mutation) or **structural** (chromosome mutation); each may be **autosomal** or **sex-chromosomal**.
- ▶ Numerical: **polyploidy** (69/92 chromosomes) vs **aneuploidy** (trisomy/monosomy) — mechanism is **non-disjunction** at meiosis I or II (or anaphase lag).
- ▶ Turner syndrome (45,X) is the **only monosomy compatible with life**.
- ▶ Structural types (7): **deletion, microdeletion, duplication, ring, isochromosome, inversion (paracentric/pericentric), translocation (Robertsonian/reciprocal)** — plus insertion and fragile sites.
- ▶ **Robertsonian translocation** = centric fusion of acrocentric chromosomes, causes ~3–4% of Down syndrome (familial); **reciprocal translocation** = balanced exchange, carrier phenotypically normal but at reproductive risk.
- ▶ Karyotype nomenclature: **p** = short arm, **q** = long arm, **t** = translocation, **i** = isochromosome, **inv** = inversion, **r** = ring.
- ▶ Denver (1960) classification groups human chromosomes A–G by size, centromere position, arm ratio and satellites.
- ▶ Distinguish **mosaicism** (one zygote, post-zygotic error) from **chimaerism** (two zygotes fuse).
- ▶ Know the karyotype and one key clinical feature for Down, Patau, Edward, Klinefelter, Turner, Cri-du-chat, DiGeorge, Philadelphia chromosome.

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e; Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e.

Coverage note: Gray's Anatomy (Standring 41e) has no dedicated cytogenetics chapter — chromosomal aberrations are core clinical-genetics content rather than gross-anatomy content, so this answer is grounded primarily in the genetics texts, cross-checked against Vishram Singh's General Anatomy medical-genetics chapter.



Chromosomal anomalies

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction A normal human somatic cell has a **diploid karyotype of 46 chromosomes** — 22 homologous pairs of **autosomes** plus one pair of **sex chromosomes** (46,XX in the female; 46,XY in the male); germ cells are **haploid** with 23 chromosomes. A **chromosomal anomaly** is any deviation from this normal karyotype in **number** (numerical) or in **structure** (structural), arising chiefly from errors of chromosome segregation at meiosis/mitosis or from chromosome breakage–reunion. Chromosomal anomalies are detected by **karyotyping** (banding techniques — Q-, G-, R-, C-banding) and by molecular cytogenetics (FISH, chromosomal microarray), and they account for a substantial proportion of spontaneous abortions, stillbirths and congenital malformation syndromes.

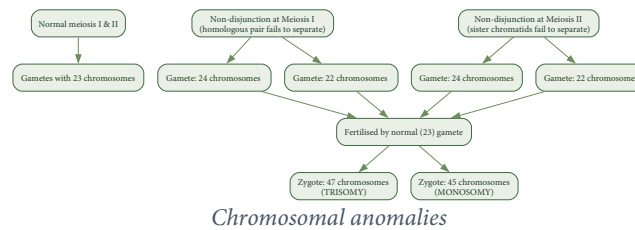
1. Classification of chromosomal anomalies

Basis	Type	Examples
Type of abnormality	Numerical — change in chromosome number (polyploidy, aneuploidy)	Down, Patau, Edward, Turner, Klinefelter syndromes
	Structural — change in chromosome structure (deletion, translocation, inversion, isochromosome, ring, fragile site)	Cri-du-chat, Philadelphia chromosome (CML)
Chromosomes involved	Autosomal	Down syndrome (trisomy 21)
	Sex chromosomal	Turner syndrome, Klinefelter syndrome

2. Mechanisms producing numerical anomalies

- ▶ Normal gametogenesis: both primary spermatocytes and primary oocytes give rise to gametes with **23 chromosomes** each; fusion of haploid sperm and ovum restores the diploid (46) zygote.
- ▶ **Non-disjunction** — failure of paired homologous chromosomes (at meiosis I) or paired sister chromatids (at meiosis II) to separate at anaphase, so both members move into one daughter cell. One resulting gamete then has **24 chromosomes**, the other **22**.
- ▶ Fertilisation of a normal (23-chromosome) gamete by a 24-chromosome gamete gives a **47-chromosome zygote (trisomy)**; by a 22-chromosome gamete gives a **45-chromosome zygote (monosomy)**.
- ▶ **Mitotic (post-zygotic) non-disjunction** in early cleavage produces two or more cell lines with different chromosome numbers in the same individual — **mosaicism** (e.g. mosaic Down syndrome, mosaic Turner syndrome); mosaic individuals are generally less severely affected than non-mosaic ones.
- ▶ **Polyploidy** — addition of a whole extra haploid set: **triploidy** (69 chromosomes; e.g. dispermy, or fertilisation of a diploid ovum by a haploid sperm) and **tetraploidy** (92 chromosomes; failure of the first cleavage division) — both are incompatible with survival and result in spontaneous abortion.

- **Aneuploidy** — gain (trisomy) or loss (monosomy) of a single chromosome due to non-disjunction; most trisomies/monosomies abort spontaneously, but **trisomy 13, 18, 21** and **47,XXY** are compatible with live birth, as is **monosomy X (45,X)**.



3. Numerical anomalies affecting autosomes

□ Fig 4.9 — Karyotype of Down syndrome patient showing trisomy 21 (arrow). (Gangane — Human Genetics — 5e, p.92)

Syndrome	Karyotype	Mechanism	Key clinical features	Prognosis
Down syndrome (trisomy 21)	47,XX/XY,+21 (~95–96%, "triplo-21", non-disjunction); ~3% translocation (familial, extra chromosome 21 attached to another acrocentric — Robertsonian type); small proportion mosaic	Non-disjunction of chromosome 21 pair; risk rises with advancing maternal age	Mongoloid facies (round face, oblique palpebral fissures, epicanthic folds), flat nasal bridge, low-set ears, single palmar (simian) crease, protruding tongue, hypotonia, mental retardation (IQ 25–50) , congenital heart defects, low maternal serum/amniotic α-fetoprotein	Commonest liveborn chromosomal disorder (~1 in 700 births); many survive to adulthood
Patau syndrome (trisomy 13)	47,XX/XY,+13	Non-disjunction	Profound mental retardation, cleft lip/palate, omphalocele, polydactyly	Usually die soon after birth
Edward syndrome (trisomy 18)	47,XX/XY,+18	Non-disjunction	Elongated skull with prominent occiput, low-set ears, micrognathia, overlapping fingers, rocker-bottom heels, congenital heart defects	Usually die within weeks of birth

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a karyotype chart (chromosomes arranged in decreasing size, Denver A–G groups) and label:

- ▶ Groups A (1–3, long metacentric), B (4–5, submetacentric), C (6–12 + X, medium submetacentric), D (13–15, acrocentric with satellites), E (16–18, short submetacentric), F (19–20, short metacentric), G (21–22 + Y, short acrocentric with satellites)
- ▶ The extra chromosome 21 (three copies) in the G group for trisomy 21
- ▶ Sex-chromosome pair labelled XX/XY
- ▶ Caption: *Karyotype of Down syndrome — 47,XX/XY,+21*

4. Numerical anomalies affecting sex chromosomes

Feature	Klinefelter syndrome	Turner syndrome	Triple-X ("super female")
Karyotype	47,XXY (trisomic)	45,X (monosomic)	47,XXX
Sex	Male (phenotypically)	Female (phenotypically)	Female
Mechanism	Non-disjunction of XX during gametogenesis	Non-disjunction/loss of an X chromosome at meiosis	Non-disjunction of XX
Barr bodies	1	0	2
Key features	Eunuchoid habitus, tall with long limbs, gynaecomastia, small testes, hypogonadism, azoospermia/infertility, sparse axillary/pubic/chest hair, mild intellectual impairment, ↑gonadotropins	Short stature, webbed neck (delayed lymphatic maturation), shield chest with widely spaced nipples, cubitus valgus, low-set ears, infantile genitalia, gonadal dysgenesis with primary amenorrhoea, coarctation of the aorta	Usually near-normal phenotype; mild intellectual impairment, scanty menses in some
Incidence	~1 in 500 male births	~2 in 3000 female births (only viable human monosomy)	Uncommon, variable
Note	Increases with advancing maternal age	In ~75% the retained X is of maternal origin	—

Barr body / Lyon's hypothesis: the number of Barr bodies (inactivated, heterochromatic X chromosome seen on the inner nuclear membrane) equals (number of X chromosomes – 1) — the "**n – 1**" rule. Buccal smear/neutrophil "drumstick" screening exploits this for rapid bedside sexing and screening for sex-chromosome aneuploidy.

5. Structural chromosomal anomalies

Mechanism	Description	Clinical condition(s)
Deletion	Loss of a chromosomal segment	Wolf–Hirschhorn syndrome (del 4p); Cri-du-chat syndrome (del 5p) — cat-like cry, microcephaly, hypertelorism, mental retardation
Ring chromosome	Both ends of a chromosome are lost and the broken ends fuse to form a ring	Described classically for chromosome 14; variable phenotype
Microdeletion (submicroscopic; detected by high-resolution banding/FISH)	Loss of a small segment	Prader–Willi syndrome (del 15q11–q13, paternal chromosome — hyperphagia/obesity, hypotonia, hypogonadism); Angelman syndrome (del 15q11–q13, maternal chromosome — happy disposition, severe mental retardation, ataxic gait, seizures); DiGeorge syndrome (del 22q11.2 — thymic aplasia/immunodeficiency, hypoparathyroidism/hypocalcaemia, cardiac outflow defects); Miller–Dieker syndrome (del 17p13.3 — lissencephaly, microcephaly)
Translocation — Robertsonian	Fusion at the centromeres of two acrocentric chromosomes with loss of the short arms; carriers are phenotypically balanced	Most common structural rearrangement in humans (t 13;14 ≈ 1 in 1300 persons); a carrier is at risk of producing a child with translocation Down syndrome
Translocation — reciprocal	Exchange of segments between two non-homologous chromosomes	t(15;17) → acute promyelocytic leukaemia ; t(9;22) → Philadelphia chromosome — <i>BCR–ABL</i> fusion gene → chronic myeloid leukaemia
Isochromosome	Centromere divides transversely instead of longitudinally, duplicating one arm and losing the other	An isochromosome of Xq is a recognised structural variant of Turner syndrome
Inversion	A segment is excised and reinserted in reverse order	Pericentric (includes centromere) or paracentric (does not); carriers are usually phenotypically normal but at risk of unbalanced gametes

Mechanism	Description	Clinical condition(s)
Fragile site	Non-staining gap/constriction at a specific locus, exacerbated in folate-deficient culture medium	Fragile X (Martin–Bell) syndrome — fragile site near Xq27.3; large ears, prominent jaw, macro-orchidism, intellectual disability; a common cause of inherited mental retardation
Breakage (chromosome-instability syndromes)	Increased chromosome breakage from defective DNA-repair	Xeroderma pigmentosum (UV sensitivity), Ataxia–telangiectasia (radiosensitivity), Fanconi anaemia (DNA cross-link sensitivity)

Applied / Clinical anatomy

- **Indications for karyotyping:** recurrent pregnancy loss, stillbirth, ambiguous genitalia, primary amenorrhoea/infertility, dysmorphic newborn, suspected leukaemia (t9;22, t15;17).
- **Prenatal diagnosis:** maternal serum/amniotic **α-fetoprotein is low in Down syndrome** (part of the "double/triple/quadruple marker" screen); definitive diagnosis by **chorionic villus sampling** or **amniocentesis** with karyotyping/FISH/microarray; non-invasive prenatal testing (cell-free fetal DNA) is now widely used to screen for trisomy 21/18/13.
- **Genetic counselling:** parents of a child with translocation Down syndrome or a balanced Robertsonian/reciprocal translocation carrier need karyotyping and recurrence-risk counselling, since carriers are phenotypically normal but at high risk of unbalanced offspring.
- **Surface/clinical correlates:** buccal smear/neutrophil-drumstick Barr-body screening; **webbed neck and coarctation of aorta** in Turner syndrome guide cardiovascular work-up; **gynaecomastia and small testes** in Klinefelter syndrome prompt hormonal evaluation; **simian crease** is a classical bedside sign of Down syndrome.
- **Radiological/haematological correlation:** Philadelphia chromosome (t9;22) positivity guides diagnosis and tyrosine-kinase-inhibitor therapy in chronic myeloid leukaemia; a Philadelphia-negative CML has a poorer chemotherapy response.

High-yield exam pointers

- Normal karyotype: **46 chromosomes** (44 autosomes + XX/XY); gametes are haploid (23).
- Non-disjunction (meiosis I or II, or post-zygotic mitosis → mosaicism) is the chief mechanism of numerical anomalies.
- **Down syndrome (trisomy 21)** — commonest liveborn chromosomal disorder, ~1 in 700 births; risk rises with maternal age; ~3% are translocation type.
- **Turner (45,X)** is the only viable human monosomy; **Klinefelter (47,XXY)** and **triple-X (47,XXX)** are the classic sex-chromosome trisomies.
- **Barr body rule:** number of Barr bodies = number of X chromosomes – 1.
- **Robertsonian translocation** (t13;14 commonest, ~1/1300 persons) is the single most common structural chromosome rearrangement in humans.

- ▶ Genomic imprinting example: **Prader–Willi (paternal 15q11-q13 deletion) vs Angelman (maternal 15q11-q13 deletion)**.
- ▶ **Philadelphia chromosome t(9;22), BCR–ABL fusion** → chronic myeloid leukaemia; **t(15;17)** → acute promyelocytic leukaemia.
- ▶ Deletion syndromes to remember: Cri-du-chat (5p–), Wolf–Hirschhorn (4p–), DiGeorge (22q11.2–).

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e (Medical Genetics chapter); Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e (figures). Coverage note: Gangane's dedicated chromosomal-disorders chapter text was not available in the split corpus (only its figures were), so narrative grounding for this topic drew chiefly on Vishram Singh's General Anatomy genetics chapter, cross-verified against Thompson & Thompson (incidence figures, translocation mechanisms, 22q11.2/15q11-q13 loci).



Chromosomal studies in foetus

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Chromosomal (cytogenetic) studies in the foetus refer to the laboratory analysis of foetal chromosome number and structure — the foetal **karyotype** — performed in utero to detect aneuploidies, structural rearrangements and other genomic imbalances before birth. Foetal material for such studies is obtained either by an **invasive procedure** (amniocentesis, chorionic villus sampling, foetal blood sampling, pre-implantation biopsy) or inferred **non-invasively** (ultrasonography, maternal serum analytes, cell-free foetal DNA). This forms the core investigative step of **prenatal diagnosis**, an integral part of genetic counselling of couples at increased reproductive risk.

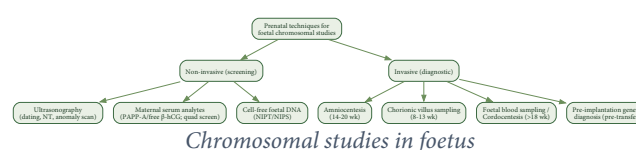
Indications for foetal chromosomal studies

- ▶ **Advanced maternal age** (conventionally >35 years) — rising non-disjunction risk for trisomy 21/18/13.
- ▶ **Previous child with a de novo chromosomal aneuploidy** or other genomic imbalance (recurrence risk higher than age-matched population risk).
- ▶ **Parent is a carrier of a structural chromosomal rearrangement** — e.g. a balanced (21q21q) Robertsonian translocation carries up to 100% recurrence risk for Down syndrome.
- ▶ **Family history of a genetic/chromosomal disorder** diagnosable by biochemical or DNA analysis, including X-linked disorders with no specific prenatal test (fetal sexing to guide further testing).
- ▶ **Couple already has a child with a neural tube defect (NTD)**, or a severe autosomal/X-linked recessive metabolic disorder detectable prenatally.
- ▶ **Increased risk on maternal serum screening, ultrasonographic soft markers/structural anomaly, or a positive non-invasive prenatal screen (NIPT/NIPS) of cell-free DNA.**

- Couple/parents specifically request invasive testing, after counselling, regardless of the above.

Methods of obtaining foetal material for chromosome analysis

Method	Ideal gestational window	Technique	Foetal-loss risk	Chief use
Amniocentesis	14–20 wk (commonly 16–18 wk)	Transabdominal needle aspiration of 10–20 mL amniotic fluid under USG guidance	≈1–1.5% (rises to ≈9% on a repeat tap); contemporary series quote ≈1 in 300–500 over baseline	Karyotype/CMA + amniotic-fluid AFP (AFAFP) for open NTD
Chorionic villus sampling (CVS)	8–13 wk (commonly 8–10 wk, up to 12 wk)	Transcervical (cannula) or transabdominal (spinal needle) aspiration of chorionic villi under USG	Slightly higher than amniocentesis in inexperienced hands; comparable in expert hands	Early, first-trimester karyotype/CMA; villi in active mitosis permit a rapid direct preparation
Foetal blood sampling / cordocentesis	≈18–22 wk (via foetoscope) or later (USG-guided)	Placental (indirect) aspiration or direct visualisation with a foetoscope	≈10%	Rapid karyotype confirmation; DNA/haematological analysis for sickle cell disease, thalassaemia, haemophilia A, Duchenne muscular dystrophy, immune deficiency
Pre-implantation genetic diagnosis (PGD)	Pre-transfer (8–16-cell or blastocyst stage, after IVF)	Single blastomere or ~5 trophoblast cells biopsied; PCR/FISH/CMA	Minimal to embryo; 10–20% assay failure with single-cell blastomere biopsy	Avoids termination altogether; used when a specific mendelian/aneuploidy risk is already known
Non-invasive (cell-free foetal DNA / NIPT-NIPS)	From ≈7 wk (clinically from ~10 wk)	Maternal venous blood; next-generation sequencing of cell-free DNA	None (non-invasive)	Screening , not diagnosis: sensitivity >99%, false-positive <1% for trisomy 21



Amniocentesis

- ▶ **Timing:** 14–20 weeks (ideally 16–18 weeks) — enough amniotic fluid is present without harming the conceptus, and termination (if indicated) still falls in the acceptable second-trimester window. Early amniocentesis (10–14 wk) is **no longer recommended**: it carries a ~3-fold increase in spontaneous abortion and a 6–7-fold increase in talipes equinovarus, and has been superseded by CVS.
- ▶ **Procedure:** USG localises the placenta; under local anaesthesia a needle is passed transabdominally, avoiding the placenta; 10–20 mL of clear (never blood-stained) fluid is withdrawn.
- ▶ **Processing:** centrifugation separates **cells** (cultured 7–10 days for karyotype/CMA) from the **supernatant** (assayed for alpha-fetoprotein, AFAFP, to screen for open NTD).
- ▶ **Complications:** neonatal respiratory distress (~3-fold increase), postural orthopaedic problems (talipes equinovarus, congenital hip dislocation), Rh sensitisation, antepartum haemorrhage, and a small perinatal-mortality increment.

Chorionic villus sampling (CVS)

- ▶ **Timing:** 8–13 weeks — the major advantage over amniocentesis, allowing first-trimester diagnosis and, if needed, a safer and simpler first-trimester termination.
- ▶ **Procedure:** chorionic villi (trophoblast-derived) are aspirated via a cannula passed transcervically, or a spinal needle transabdominally, both under continuous USG guidance.
- ▶ **Merits:** villi are already in active mitosis, permitting a rapid direct chromosome preparation without full culture; karyotype/CMA success rate exceeds 99%, equalling amniocentesis.
- ▶ **Demerits:** AFAFP cannot be assayed at this gestation (NTD screening must rely on maternal serum AFP and ultrasonography instead); ~1% of samples give ambiguous results from chromosomal mosaicism, requiring a follow-up amniocentesis.

Non-invasive & adjunctive screening

- ▶ **Ultrasonography:** essential before every invasive procedure (placental localisation, gestational dating, exclusion of multiple pregnancy); also directly detects structural anomalies (anencephaly, spina bifida, cystic hygroma, limb/facial defects) and measures **nuchal translucency (NT)** at 11–13 weeks — increased NT accompanies trisomy 21, 18, 13 and 45,X.
- ▶ **Maternal serum screening:**
 - **First trimester** (11–13 wk): PAPP-A (↓ in all trisomies) + free β-hCG (↑ in trisomy 21, ↓ in 18/13) combined with NT.
 - **Second trimester quadruple screen** (16–18 wk): MSAFP, hCG, unconjugated estriol, inhibin A — MSAFP is also the principal screening test for open NTD (elevated when present).
 - Combined (integrated) first- + second-trimester screening raises Down-syndrome detection to ~95% at a 5% false-positive rate.
- ▶ **Non-invasive prenatal screening (NIPT/NIPS):** sequencing of cell-free foetal DNA circulating in maternal serum (2–10% of total cell-free DNA is placental/foetal in origin, detectable from ~7 weeks); achieves >99% sensitivity and <1% false-positive rate for trisomy 21, and can also assess

foetal Rh status and sex. It remains a **screening**, not a diagnostic, test — a positive result still requires invasive confirmation.

- ▶ **Foetoscopy**: direct visualisation of the foetus (18–22 wk) for limb, facial or genital malformations, and to obtain foetal skin biopsy/blood; carries a 3–5% loss risk and is now largely superseded by USG plus targeted invasive sampling.

Laboratory analysis of foetal chromosomes

- ▶ Cultured amniotic-fluid cells or chorionic villi (7–10 days, long-term culture of the mesenchymal core) are arrested in metaphase, harvested and G-banded for conventional **karyotyping**.
- ▶ **Short-term/direct villous preparation** (cytotrophoblast in active mitosis) gives a faster result but poorer-quality banding and a higher mosaicism rate; long-term culture remains the technique of choice where only one method is used.
- ▶ **Fluorescence in-situ hybridisation (FISH)** on interphase nuclei screens rapidly (1–2 days) for the common aneuploidies of chromosomes 13, 18, 21, X and Y when urgent results are needed.
- ▶ **Chromosomal microarray (CMA)** detects copy-number variants at far higher resolution than karyotyping and is now recommended as the **first-line test** whenever a structural anomaly is seen on ultrasonography; it is progressively replacing conventional karyotyping.
- ▶ The same amniotic-fluid/villus/foetal-blood samples also permit **biochemical and DNA-probe analysis** (e.g., recombinant-DNA probes for the haemoglobinopathies).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Chorionic villus sampling (CVS) — labelled sketch (transabdominal & transcervical routes):

- ▶ Maternal anterior abdominal wall and pubic bone
- ▶ Urinary bladder
- ▶ Uterine wall and uterine cavity
- ▶ Chorionic cavity with villous chorion at the implantation site
- ▶ Amniotic cavity (embryo/foetus)
- ▶ USG transducer/scanner on the abdomen
- ▶ Transabdominal spinal needle path (one approach)
- ▶ Transcervical flexible cannula path through the cervix and vagina (alternative approach)

□ Fig 13.1 — Amniocentesis, a prenatal diagnostic technique used in second trimester of pregnancy (Gangane — *Human Genetics* — 5e, p.225)

Problems in interpreting foetal chromosome analysis

- ▶ **Culture failure** on first attempt (amniotic fluid or CVS) — may require a repeat sample, or an alternative foetal blood sample by foetoscopy.
- ▶ **Maternal cell contamination** — can wrongly report a male foetus as female, or produce an apparent 46,XY/46,XX mosaic.
- ▶ **Mosaicism** — **true vs pseudomosaicism**: the sample is divided into multiple culture vessels; an abnormal line in *all* vessels favours true mosaicism, whereas one showing it in only a single vessel

favours pseudomosaicism (a culture artefact or, in CVS, confinement of the abnormal line to extra-embryonic tissue — confined placental mosaicism).

- ▶ **Ambiguous CVS results** (~1%) mandate a confirmatory amniocentesis.
- ▶ **PGD single-cell analysis** carries a false-positive rate of ~6% and false-negative rate of ~1%, appreciably higher than analysis of CVS/amniotic samples.

Applied / Clinical anatomy

- ▶ The **transabdominal amniocentesis needle** traverses anterior abdominal wall, myometrium and (avoiding the placenta) enters the amniotic cavity — USG guidance is mandatory to keep clear of placenta, foetus and maternal bladder.
- ▶ **Transcervical CVS** passes the cannula through the vagina and cervical canal into the chorionic space at the implantation site; the **transabdominal** alternative uses a spinal needle, again under real-time USG.
- ▶ Karyotypes obtained by these techniques are the diagnostic basis for **trisomy 21 (Down syndrome)**, **trisomy 18 (Edward syndrome)**, **trisomy 13 (Patau syndrome)**, **45,X (Turner syndrome)** and **47,XXY (Klinefelter syndrome)** — each carrying a characteristic ultrasound/soft-marker association (e.g., cystic hygroma with 45,X; duodenal atresia with trisomy 21).
- ▶ In India, invasive/imaging techniques used for foetal diagnosis are regulated by the **PCPNDT Act (Pre-Conception and Pre-Natal Diagnostic Techniques Act, 1994)**, which prohibits their use for sex determination/sex selection and permits them only for detecting genetic, chromosomal or structural disorders at registered, licensed centres.
- ▶ Genetic counselling — pre-test (informed choice, risk explanation) and post-test (result interpretation, reproductive options including termination) — must accompany every foetal chromosomal study.

High-yield exam pointers

- ▶ Amniocentesis: 14–20 wk (ideal 16–18 wk); 10–20 mL fluid; karyotype from cultured cells + AFAFP for NTD; risk ≈1–1.5% (early amniocentesis abandoned).
- ▶ CVS: 8–13 wk; villi already mitotic → rapid direct prep; earlier, safer termination if abnormal; no AFAFP possible; ~1% mosaic/ambiguous results need amnio follow-up.
- ▶ Cordocentesis/FBS: ~18–22 wk; ~10% loss risk; used for haemoglobinopathies, DMD, immunodeficiencies.
- ▶ PGD: 8–16-cell/blastocyst biopsy before IVF transfer; avoids termination; highest false-positive/negative rate of all techniques.
- ▶ NIPT/NIPS: cell-free foetal DNA, from ~7 wk; >99% sensitivity, <1% false-positive for trisomy 21; screening only, not diagnostic.
- ▶ True vs pseudomosaicism distinguished by single- vs multi-vessel culture positivity.
- ▶ CMA is replacing karyotyping as first-line when a structural anomaly is seen on USG.
- ▶ PCPNDT Act, 1994 governs legal use of these techniques in India (prohibits sex selection).

SOURCE & COVERAGE

Gangane — Human Genetics — 5e (Chapter, Prenatal Diagnosis — primary source for indications, amniocentesis, CVS, foetoscopy, foetal blood sampling, maternal serum AFP, pre-implantation diagnosis, and problems of foetal chromosome analysis); Thompson & Thompson — Genetics in Medicine — 8e (Chapter 17, Prenatal Diagnosis and Screening — timing/complication data, first- and second-trimester serum screening, NIPT/NIPS, FISH, CMA); Vishram Singh — General Anatomy — 4e (karyotype figure reference). Coverage note: this is a clinical-cytogenetics topic not addressed in Gray's Anatomy (Standring) — the spine text has no prenatal-diagnosis content — so the answer is grounded entirely in the genetics specialty texts above.



Chromosome analysis techniques

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction Chromosome analysis (clinical cytogenetics) is the laboratory process of culturing dividing cells, arresting them in metaphase, harvesting and staining their chromosomes, and then microscopically identifying, counting and arranging them to detect abnormalities of number or structure. The ordered photographic/digital display of an individual's complete chromosome complement is called a **karyotype**. Chromosome analysis is indicated in developmental delay/dysmorphism, recurrent miscarriage and infertility, stillbirth, a positive family history, malignancy, and advanced maternal-age pregnancy, and remains an essential diagnostic tool over 50 years after its introduction. Techniques span a spectrum of resolution — from conventional light-microscopic **banding** (the long-standing gold standard) to molecular-cytogenetic methods such as **FISH** and **chromosomal microarray**, which extend detection down to the sub-microscopic and nucleotide level.

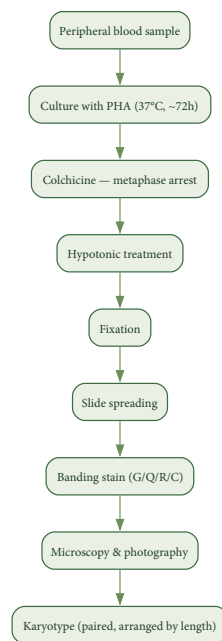
SOURCE & COVERAGE

Source	Cells obtained	Culture requirement	Chief use
Peripheral blood	T lymphocytes	Short-term culture, 3–4 days; short-lived	Commonest — routine constitutional karyotype
Bone marrow	Cells with a naturally high mitotic index	Little/no culturing needed (invasive marrow-biopsy sample)	Suspected haematological malignancy
Skin biopsy	Fibroblasts	Long-term culture; suitable for permanent storage	Biochemical/molecular studies, mosaicism work-up
Amniotic fluid	Amniocytes	Culture required, ~2–3 weeks	Prenatal diagnosis (amniocentesis, 14–16 weeks)
Chorionic villi	Trophoblast cells	Can be analysed directly , without culture	Earlier prenatal diagnosis (CVS, 8–10 up to 12 weeks)
Tumour tissue	Neoplastic cells	Direct or short-term culture	Cancer cytogenetics (constitutional vs acquired abnormality)

Conventional cytogenetic technique — karyotyping

- ▶ **Sample collection** — e.g., ~5 mL venous blood for a peripheral-blood karyotype.
- ▶ **Culture** — white blood cells (lymphocytes) are placed in tissue-culture medium and stimulated to divide with **phytohaemagglutinin (PHA)**; cultured at 37 °C for about 3 days (72 hours).
- ▶ **Metaphase arrest** — **colchicine** (or a derivative) is added to inhibit mitotic-spindle-microtubule formation, arresting the dividing cells in metaphase, when chromosomes are maximally condensed and visible.

- **Hypotonic treatment** — cells are treated with a hypotonic solution (hypotonic saline/KCl) which swells them and disperses the chromosomes.
- **Fixation** — the cell suspension is fixed.
- **Slide preparation** — the fixed suspension is dropped onto a glass slide from a height to spread the metaphase chromosomes.
- **Staining** — the spread is stained by one of several banding techniques (see below), depending on the diagnostic question.
- **Microscopy and photography** — a well-spread metaphase is photographed/microphotographed.
- **Karyotype construction** — individual chromosomes are cut out of the photomicrograph (or, with modern software, sorted digitally), homologues are paired, and all 46 are arranged in descending order of length into the standard group layout to yield the karyotype.



Chromosome analysis techniques

Chromosome banding techniques

Technique	Method	Result	Principal use
Q-banding	Quinacrine (quinacrine mustard/dihydrochloride) staining + UV fluorescence microscopy	Alternating bright and dark fluorescent bands	First banding method described; identifies fluorescent Y chromosome
G-banding (GTG)	Trypsin pre-treatment followed by Giemsa stain	Alternating dark and light bands along each chromosome	Gold-standard routine banding ; ~400–550 bands/haploid karyotype at standard metaphase resolution
R-banding	Reverse of the Giemsa procedure (developed in France)	Band pattern is the reverse of G-banding (dark where G is light)	Useful for terminal/telomeric chromosome regions

Technique	Method	Result	Principal use
C-banding	Reveals constitutive heterochromatin	Dark bands at the centromere and secondary constrictions, notably of chromosomes 1, 9, 16 and the long arm of Y	Demonstrates heterochromatic variants
High-resolution (prometaphase) banding	Chromosomes arrested and stained at an earlier, less-condensed prophase/prometaphase stage	Up to ~850 bands /haploid set (vs 400–550 at routine metaphase)	Detects subtler structural abnormalities and microdeletions

- The acrocentric chromosomes (13, 14, 15, 21, 22) carry satellite bodies on stalks (secondary constrictions) rich in ribosomal-RNA gene copies — the **nucleolar organizer regions (NOR)**, classically demonstrated by silver (Ag-NOR) staining; high-resolution banding is now often superseded by chromosomal microarray for detecting subtle rearrangements.

Chromosome classification and nomenclature

Denver classification (1960; accepted at London, 1963) — human chromosomes are arranged, in descending order of length, into **seven groups (A–G)** on the basis of size, centromere position and presence of satellites:

Group	Chromosomes	Features
A	1, 2, 3	Large, metacentric
B	4, 5	Large, submetacentric
C	6–12 + X	Medium-sized, submetacentric (X resembles chromosome 6)
D	13–15	Medium-sized, acrocentric, satellites on short arms
E	16–18	Fairly short, submetacentric (16 is metacentric)
F	19, 20	Short, metacentric
G	21, 22 + Y	Very short, acrocentric; 21 and 22 carry satellites (Y resembles chromosome 21)

- **Paris nomenclature (1971)** — with the advent of banding, each chromosome arm is divided into numbered **regions** (1, 2, 3...) counted outward from the centromere, each further subdivided into numbered **bands** (e.g., band 5q13), allowing precise, reproducible localisation of an abnormality.
- **ISCN** — the *International System for Human Cytogenetic Nomenclature*, first published in 1978 (incorporating the Denver, London and Paris conventions) and periodically revised, is the standard reference for writing a karyotype result.

- ▶ **Karyotype-writing convention** (chromosome number, sex chromosomes, then abnormality): e.g. **46,XX** (normal female), **46,XY** (normal male), **47,XX,+21** (female with trisomy 21), **46,XX,del(5)(p13)** (terminal deletion), **46,XY,t(2;8)(q22;p21)** (balanced translocation), **45,XY,rob(14;21)(q10;q10)** (Robertsonian translocation), **46,XX/47,XX,+21** (mosaicism, "/" denotes different cell lines).
- ▶ Common abbreviations: **p** = short arm, **q** = long arm, **cen** = centromere, **del** = deletion, **dup** = duplication, **inv** = inversion, **t** = translocation, **rob** = Robertsonian translocation, **r** = ring chromosome, **mar** = marker chromosome, **+/-** = gain/loss of a chromosome, **mat/pat** = maternal/paternal origin.

Molecular cytogenetic techniques

- ▶ **Fluorescence in situ hybridisation (FISH)** — also called interphase cytogenetics. Double-stranded target DNA is denatured to single strands; a fluorescently tagged, complementary single-stranded DNA probe is hybridised to it and visualised by fluorescence microscopy.
 - **Probe types:** **centromeric/chromosome-enumeration probes (CEP)**, directed at α/β -satellite centromeric repeats, used to count chromosome copy number (aneuploidy/monosomy); **locus-specific identifier probes (LSI)**, used to demonstrate deletion, translocation, amplification or precise gene localisation; **whole-chromosome paint probes (WCP)**, multiple overlapping probes that "paint" an entire chromosome a single fluorochrome colour, used to characterise structural rearrangements.
 - **Advantages:** bypasses the tedious cell-culture step; can be performed on interphase nuclei and on archival paraffin-embedded/formalin-fixed tissue; correlates cytogenetic abnormality directly with cell morphology; rapid, high-resolution, slides can be stored long-term.
 - **Limitations:** only detects the specific, known abnormality targeted by the probe used — not a genome-wide screen; gives no allele-specific information.
 - **Clinical examples:** rapid detection of trisomy 21/13/18 in prenatal or postnatal samples; **trisomy 12** in chronic lymphocytic leukaemia; **t(11;22)(q24;q12)** in Ewing sarcoma/PNET; **HER-2 gene amplification** in breast carcinoma; **9p21 deletion** in urothelial carcinoma; disease monitoring for minimal residual disease.
- ▶ **Chromosomal microarray analysis (CMA)** — extends the concept of targeted FISH to the whole genome. In **array comparative genomic hybridisation (array-CGH)**, patient and control genomic DNA (differently labelled) are co-hybridised to a microarray of ordered genomic segments, and relative signal intensity reveals copy-number gains/losses genome-wide. **SNP arrays** similarly assess relative allele dosage across the genome. CMA detects submicroscopic deletions/duplications (in the 2–3 Mb range and smaller) beyond the resolution of routine or even high-resolution banding, and has increasingly replaced the G-banded karyotype as the frontline test for developmental delay/dysmorphism. Its key limitation: it measures only relative copy number, **not** balanced rearrangements (translocations, inversions) — these require confirmatory banding/FISH.
- ▶ Other techniques: **somatic cell hybridisation** (fusing cells of two different species in culture, historically used for gene mapping) and **fluorescence-activated cell sorting (FACS)** of chromosomes for specialised chromosome analysis.

Prenatal chromosome analysis

Procedure	Timing	Sample/technique	Notes
Amniocentesis	14–16 weeks of gestation	10–20 mL amniotic fluid aspirated per abdomen under ultrasound guidance; cells cultured (~2–3 weeks) for karyotype, fluid assayed for α -fetoprotein	Risk of abortion $\approx$ 1–1.5% (rises to $\sim$ 9% on repeat); allows second-trimester termination if abnormal
Chorionic villus sampling (CVS)	8–10 weeks (up to $\sim$ 12 weeks)	Villi aspirated via a cannula through the cervix under ultrasound guidance	Villus cells under active mitosis permit analysis without culture (faster result); earlier, first-trimester termination possible if abnormal; carries a somewhat greater abortion risk than amniocentesis
Fetal blood sampling (fetoscopy/cordocentesis)	Later gestation	Fetal blood cultured	Reserve option if amniotic-fluid/CVS culture fails

Applied / Clinical anatomy

- **Indications for chromosome/genome analysis:** failure to thrive, developmental delay, dysmorphic facies, multiple malformations or ambiguous genitalia; stillbirth and neonatal death; infertility or recurrent miscarriage; a known/suspected chromosome abnormality in a first-degree relative; neoplasia (constitutional or acquired); and pregnancy in women of advanced maternal age (>35 years).
- **Diagnostic yield across common syndromes:** **Down syndrome** (47,XX/XY,+21), **Klinefelter syndrome** (47,XXY), **Turner syndrome** (45,X), and translocation/mosaic variants of each, are all confirmed by conventional karyotyping; FISH offers a rapid same-day screen for the same aneuploidies prenatally.
- **Cancer cytogenetics:** karyotyping/FISH of bone marrow or tumour tissue detects acquired abnormalities of major diagnostic/prognostic value, e.g. the **Philadelphia chromosome** t(9;22) in chronic myeloid leukaemia, t(15;17) in acute promyelocytic leukaemia, and trisomy 12 in chronic lymphocytic leukaemia.
- **Sex-chromatin (Barr body) screening** of a buccal smear is a rapid, low-cost adjunct for suspected X-aneuploidy, though full karyotyping is required for confirmation.
- **Genetic counselling:** precise ISCN description of a karyotype (e.g., distinguishing a balanced Robertsonian translocation carrier-parent from a child with an unbalanced karyotype) is essential for calculating recurrence risk.

High-yield exam pointers

- ▶ Karyotype = the ordered chromosomal complement of a cell; conventional technique = **culture** → **colchicine metaphase arrest** → **hypotonic treatment** → **fixation** → **spreading** → **banding stain** → **photography** → **arrangement**.
- ▶ **Denver classification (1960)** groups the 46 human chromosomes into 7 groups, A–G, by length and centromere position.
- ▶ **Q-banding** = quinacrine + UV fluorescence; **G-banding** = trypsin + Giemsa (routine gold standard, 400–550 bands); **R-banding** = reverse-Giemsa; **C-banding** = constitutive heterochromatin (chromosomes 1, 9, 16, Yq); **high-resolution/prometaphase banding** = up to ~850 bands.
- ▶ **ISCN (1978, periodically revised)** standardises karyotype nomenclature, integrating the Denver, London and Paris conventions.
- ▶ **FISH** = interphase cytogenetics using fluorescent DNA probes (CEP/LSI/WCP); needs no culture, works on paraffin sections, but only detects the targeted, known abnormality.
- ▶ **Chromosomal microarray (array-CGH/SNP array)** gives genome-wide, sub-microscopic resolution of copy-number changes but cannot detect balanced rearrangements.
- ▶ **Amniocentesis (14–16 weeks)** vs **CVS (8–10, up to 12 weeks)** — CVS is earlier and needs no culture but carries a somewhat higher miscarriage risk.
- ▶ Chromosome analysis is indicated in developmental delay/dysmorphism, recurrent pregnancy loss/infertility, stillbirth, malignancy, positive family history, and advanced maternal-age pregnancy.

□ Fig 2.11 — A human male karyotype with Giemsa banding (G banding) (Thompson & Thompson — *Genetics in Medicine* — 8e, p.21)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A G-banded karyotype (male), labelled:

- ▶ The **seven Denver groups A–G**, chromosomes arranged left-to-right/top-to-bottom in descending order of length within each group
- ▶ Representative chromosome from each group with its **centromere position** marked (metacentric in A/B/F, submetacentric in B/C/E, acrocentric with **satellites** in D/G)
- ▶ The **X chromosome** placed at the end of group C and the **Y chromosome** at the end of group G
- ▶ Alternating dark and light **G-bands** drawn along at least one representative chromosome pair
- ▶ Label **p arm** (short) above and **q arm** (long) below the centromere on one chromosome

SOURCE & COVERAGE

Thompson & Thompson — Genetics in Medicine (8e) (text and figure); IB Singh — Textbook of Anatomy Vol 3 (6e); Vishram Singh — General Anatomy (4e); Gangane — Human Genetics (5e); Pranab Dey — Lab Techniques in Histopathology & Cytology (2e). **Coverage note:** Gray's Anatomy (Standring, 41e) has no dedicated cytogenetic-technique chapter, and Gangane's own Chapter 2 ("Cytogenetics") is missing from this library's chapter split (the extracted text jumps from front matter directly to Chapter 6). This answer is therefore grounded chiefly in Thompson & Thompson's cytogenetics chapter, the dedicated "Chromosomes"/"Medical genetics" chapters of IB Singh's and Vishram Singh's anatomy texts, Pranab Dey's FISH chapter, and Gangane's prenatal-diagnosis chapter (plus its MCQ-answer appendix, which retains some Chapter 2 content).



Chromosomes

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction — A **chromosome** (Gk. *chroma* = colour, *soma* = body; term coined by Waldeyer-Hartz in 1888 from Flemming's "chromatin", 1880s) is a deeply-staining, rod/V/J-shaped structure seen in the nucleus of a dividing cell, formed by progressive condensation of chromatin. It is composed of **DNA** (the genetic material, arranged in linear sequence) complexed with **proteins**, and is the physical vehicle of heredity (Sutton-Boveri chromosomal theory of inheritance, 1902). Chromosomes are best defined and studied at the **metaphase** stage of cell division, when they are maximally condensed. Their number, morphology, and behaviour form the basis of cytogenetics and of clinical karyotyping.

1. Chemical composition and molecular organisation

- ▶ A chromosome consists of **DNA** (the key, most stable constituent), a small amount of **RNA**, **proteins** — basic **histones** (H1, H2A, H2B, H3, H4) and acidic **non-histone proteins** (mostly enzymes) — and metallic ions.
- ▶ **Histones** aggregate as spheroidal particles around which the DNA strand is coiled to form a **nucleosome** ("beads-on-a-string"); further coiling produces a **solenoid**, and looping of the solenoid fibre produces **chromatin loops**, which condense further to form the visible metaphase chromosome.
- ▶ Depending on the state of coiling and staining, chromatin is **euchromatic** (lightly stained, uncoiled, extended, genetically active) or **heterochromatic** (darkly stained, tightly coiled, genetically inactive).

2. Structure of a metaphase chromosome

A metaphase chromosome consists of two identical, parallel filaments called **chromatids**, held together at a narrow region:

- ▶ **Centromere (primary constriction)** — divides each chromatid into a **short arm (p, French petite)* and a *long arm (q)**; the mitotic spindle attaches here.
- ▶ **Kinetochores** — a specialised protein structure located at the centromere to which the **spindle fibres** actually attach, mediating chromosome movement during division.
- ▶ **Secondary constriction** — present on some chromosomes; the segment of the arm distal to it is called the **satellite**, and such chromosomes are termed **SAT chromosomes** (contain the ribosomal-RNA genes).
- ▶ **Telomere** — the free end of each arm, made of a repetitive nucleotide sequence (TTAGGG in humans, repeated ~2500 times) that confers polarity and prevents end-to-end fusion or degradation.

□ Fig 29.1 — Structure of a chromosome (IB Singh — Textbook of Anatomy Vol 3 — 6e, p.473)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A metaphase chromosome (two chromatids joined at the centromere):

- ▶ Two **sister chromatids**, side by side.
- ▶ **Primary constriction (centromere)** joining the chromatids, with the **kinetochore** marked on it.
- ▶ **Short arm (p)** above and **long arm (q)** below the centromere on both chromatids.
- ▶ A **secondary constriction** near one end of an arm, with the distal segment labelled **satellite**.
- ▶ Both free ends labelled **telomere**.

3. Morphological classification (by position of the centromere)

Type	Centromere position	Arms
Metacentric	Middle of the chromosome	Both arms $\approx$ equal
Submetacentric	Between the middle and the end	One arm short, one long
Acrocentric	Close to one end	One arm very short, one very long
Telocentric	At the very end	Single arm only — not seen in normal human chromosomes

4. Chromosome number and ploidy

- ▶ The chromosome number is **constant for a species**. Each human **somatic cell** has **46 chromosomes**, arranged as **23 pairs** — a **diploid (2n)** cell; each **gamete (sperm/ovum)** has **23 chromosomes**, a single set — a **haploid (n)** cell. Fertilisation restores the diploid (2n) zygote, one member of each pair from each parent (**homologous chromosomes**).
- ▶ Of the 46, **44 are autosomes** (22 homologous pairs, numbered 1–22) and **2 are sex chromosomes (allosomes/gonosomes)**: a normal female is **44 + XX**, a normal male **44 + XY**; the presence of a Y chromosome determines the male sex regardless of the number of X chromosomes.

- **Historical note:** Painter (1923), using early light-microscopy, wrongly counted 24 pairs (48); Tjio and Levan (1956), using improved hypotonic-saline technique, established the correct diploid number as **46 (23 pairs)** — the founding observation of human cytogenetics.
- **Comparative note:** chromosome number varies across species (cat 38, mouse 40, dog 78, chimpanzee/great apes 48). Human chromosome 2 is believed to have arisen from **end-to-end fusion of two ancestral ape chromosomes**, accounting for the reduction from 48 to 46 during hominid evolution.

5. Classification of the human karyotype — the Denver system and banding nomenclature

A **karyotype** is the chromosomal constitution of a cell, arranged, paired, and numbered in a standard format; **karyotyping** is the process of preparing it (commonly from cultured, PHA-stimulated, colchicine-arrested peripheral blood lymphocytes, stained and photomicrographed at metaphase).

- The **Denver system (1960)**, accepted at London (1963), arranges the 46 chromosomes into **seven groups, A–G**, in descending order of length, using **size** and **centromere position**:

Group	Chromosomes	Features
A	1, 2, 3	Large, metacentric
B	4, 5	Large, submetacentric
C	6–12 + X	Medium, submetacentric (X resembles chromosome 6)
D	13–15	Medium, acrocentric, satellited
E	16–18	Small, submetacentric (16 nearly metacentric)
F	19, 20	Short, metacentric
G	21, 22 + Y	Very short, acrocentric; 21/22 satellited, Y resembles 21 but has no satellite

- The Denver system alone could not resolve subtle structural change, so **banding** techniques followed: **Q-banding** (quinacrine dye, UV fluorescence), **G-banding** (trypsin + Giemsa — the routine method), **R-banding** (reverse of G-pattern), and **C-banding** (stains centromeric heterochromatin). The **Paris Convention (1971)** numbered each arm's bands as **regions and sub-bands** counted outward from the centromere, and these agreements were consolidated in the **International System for human Cytogenetic Nomenclature (ISCN, 1978)**, periodically updated, the current universal reporting standard.

□ Fig 2.11 — A human male karyotype with Giemsa banding (G banding). The chromosome (Thompson & Thompson — *Genetics in Medicine* — 8e, p.21)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

The karyogram of a normal karyotype:

- ▶ Chromosomes 1–22 arranged in descending length, in **Groups A–G**, plus the **sex chromosome pair** (XX or XY) at the end.
- ▶ On one chromosome of each group, mark the **centromere position** (metacentric/submetacentric/acrocentric) that defines that group.
- ▶ Shade a few **alternating dark/light bands** on one chromosome to depict G-banding.

6. Sex chromatin (Barr body) and the Lyon hypothesis

- ▶ **Barr and Bertram (1949)** first described a small, deeply-stained, ovoid body applied to the inner surface of the nuclear membrane in the interphase nucleus of a female cat neuron — the **Barr body / sex chromatin**; in neutrophils it appears as a **drumstick-shaped** nuclear appendage.
- ▶ The Barr body represents an **inactivated, heterochromatic X chromosome**. The **Lyon hypothesis** (Mary F. Lyon) states that in somatic cells of female mammals only **one X chromosome remains genetically active**; the other is condensed and inactivated (**lyonisation**), appearing as sex chromatin.
- ▶ The number of Barr bodies is always **one less than the total number of X chromosomes** (the "**n – 1 rule**"):

Constitution	Example	Barr bodies
46, XX (normal female)	—	1
46, XY (normal male)	—	0
45, X (Turner syndrome)	—	0
47, XXY (Klinefelter syndrome)	—	1
47, XXX (triple-X syndrome)	—	2

Applied / Clinical anatomy

Chromosomal abnormalities are classified as **numerical** or **structural**, and by the chromosomes involved (autosomal or sex):

- ▶ **Numerical** — **polyploidy** (whole extra haploid sets, from meiotic failure/dispermy): **triploidy** (69 chromosomes) and **tetraploidy** (92 chromosomes); both usually cause spontaneous abortion.
- ▶ **Numerical** — **aneuploidy** (gain/loss of a single chromosome, from **non-disjunction**): **trisomy** — **Down syndrome** (trisomy 21), **Edwards syndrome** (trisomy 18), **Patau syndrome** (trisomy 13), **Klinefelter syndrome** (47, XXY); **monosomy** — **Turner syndrome** (45, X); also XYY and XXX syndromes.
- ▶ **Structural** — **deletion**: loss of a chromosome segment, e.g. **Cri-du-chat syndrome** (5p– — deletion of the short arm of chromosome 5) and **Wolf–Hirschhorn syndrome** (4p– deletion).
- ▶ **Structural** — **microdeletion** (detectable only by high-resolution banding/FISH): **Prader–Willi syndrome** (15q deletion, paternal chromosome) vs **Angelman syndrome** (same 15q region,

maternal chromosome); **DiGeorge syndrome** (22q11.2 microdeletion); **Miller–Dieker syndrome** (17p13.3 microdeletion).

- ▶ **Structural — translocation:** exchange of segments between chromosomes; **Robertsonian translocation** (centromeric fusion, e.g. 13;14 — the commonest human translocation) and **reciprocal translocations** with malignant correlates — t(15;17) in acute promyelocytic leukaemia and t(9;22) (**Philadelphia chromosome**) in chronic myeloid leukaemia.
- ▶ **Structural — other: ring chromosome** (deletion at both ends with fusion of the broken ends), **isochromosome** (transverse instead of longitudinal centromeric division, duplicating one arm and losing the other), **pericentric/paracentric inversion**, and **fragile sites** (e.g. Fragile X syndrome, fragile site at Xq27–28).
- ▶ **Clinical utility:** karyotyping/banding and FISH are used for prenatal diagnosis (amniocentesis/CVS), work-up of developmental delay/dysmorphism, recurrent miscarriage/infertility, and cytogenetic diagnosis/prognosis in leukaemias and other malignancies.

High-yield exam pointers

- ▶ Chromosome = condensed chromatin + DNA + histone/non-histone protein; best seen at **metaphase**.
- ▶ Parts: chromatid, centromere (primary constriction), kinetochore, p/q arms, secondary constriction, satellite, telomere (TTAGGG × ~2500).
- ▶ Four morphological types by centromere position: metacentric, submetacentric, acrocentric, telocentric (absent in humans).
- ▶ Human somatic cell: **46 = 23 pairs (diploid)**; gamete: **23 (haploid)**; 44 autosomes + 2 sex chromosomes (XX/XY). Number fixed as 46 by **Tjio and Levan (1956)**.
- ▶ **Denver classification (1960):** seven groups A–G by size and centromere position; banding sequence Q → G (routine) → R → C; **Paris Convention (1971) → ISCN (1978)**.
- ▶ **Barr body ("n – 1" rule):** XX = 1, XY = 0, XXY = 1, XXX = 2, XO = 0.
- ▶ Numerical disorders: Down (21), Edwards (18), Patau (13), Klinefelter (XXY), Turner (X0). Structural disorders: Cri-du-chat (5p–), Robertsonian 13;14, Philadelphia t(9;22).

SOURCE & COVERAGE

IB Singh — Textbook of Anatomy Vol 3 6e (dedicated "Chromosomes" chapter — structure, morphological types, Denver classification, Paris/ISCN nomenclature, karyotyping, sex chromatin, chromosome time-line); Vishram Singh — General Anatomy 4e (chemical composition, packaging, karyotyping method, Barr body/Lyon hypothesis, chromosomal abnormality classification and eponymous syndromes); Thompson & Thompson — Genetics in Medicine 8e (karyotype/cytogenetics figure, chromosome number history); Gangane — Human Genetics 5e (cross-reference for chromosomal-disorder categories). Gray's Anatomy (Standing) 41e does not carry a dedicated chromosome/cytogenetics section — this is basic-science content drawn from the genetics specialty texts above.



Classify chromosomal disorders with examples

Asked in: Paper III Nov 2017 — RGUHS MD/MS Anatomy

Definition / Introduction Chromosomal disorders are clinical syndromes caused by a change in the **number** or **structure** of chromosomes, detected by karyotyping (G-banding) or molecular cytogenetics (FISH, chromosomal microarray). They arise chiefly from **non-disjunction** during meiosis/mitosis or from chromosome breakage-and-reunion. Chromosomal abnormalities affect about 1 in 154 live births ($\approx 0.65\%$) and are found in at least 40–50% of spontaneous first-trimester abortions. They are classified on two axes: the **type of abnormality** (numerical or structural) and the **chromosomes involved** (autosomal or sex-chromosomal).

1. Basis of classification

Basis	Category	Examples
Type of abnormality	Numerical — change in chromosome number	Polyploidy (triploidy, tetraploidy); Aneuploidy (trisomy, monosomy)
	Structural — change in chromosome architecture	Deletion, microdeletion, translocation, inversion, isochromosome, ring chromosome, fragile site, breakage syndromes
Chromosomes involved	Involving autosomes	Down syndrome (trisomy 21), Cri-du-chat syndrome (5p–)
	Involving sex chromosomes	Klinefelter syndrome (47,XXY), Turner syndrome (45,X)



Classify chromosomal disorders with examples

2. Numerical chromosomal abnormalities

- ▶ **Mechanism** — non-disjunction: failure of homologous chromosomes to separate at meiosis I, or of sister chromatids to separate at meiosis II/mitosis, so one daughter cell gains and the other loses a chromosome.
- ▶ **Polyplody** — an extra complete haploid (23-chromosome) set added to the diploid complement:
 - *Triploidy* (69 chromosomes) — from dispermy (two sperm fertilising one ovum) or fertilisation of a diploid gamete; ends in spontaneous abortion or brief neonatal survival.
 - *Tetraploidy* (92 chromosomes) — from failure of the first cleavage division; ends in spontaneous abortion.
- ▶ **Aneuploidy** — gain or loss of a single chromosome:
 - *Trisomy* ($2n+1$, 47 chromosomes) — e.g., trisomy 21 (Down), trisomy 18 (Edward), trisomy 13 (Patau), 47,XXY (Klinefelter).

- **Monosomy** ($2n-1$, 45 chromosomes) — almost always lethal in utero; the **only autosomal-or-sex-chromosome monosomy compatible with live birth is 45,X (Turner syndrome)**.
- **Mosaicism** — two or more cytogenetically distinct cell lines in one individual, from post-zygotic mitotic non-disjunction (e.g., mosaic Down syndrome, mosaic Turner syndrome); mosaic individuals are generally less severely affected than non-mosaic ones.

3. Structural chromosomal abnormalities

Type	Mechanism	Example(s)
Deletion	Loss of a chromosome segment	Cri-du-chat syndrome (5p-); Wolf-Hirschhorn syndrome (4p-)
Microdeletion	Sub-microscopic loss, seen only on high-resolution banding/FISH	Prader-Willi syndrome (paternal 15q11-q13 deletion); Angelman syndrome (maternal 15q11-q13 deletion); DiGeorge syndrome (22q11.2 deletion); Miller-Dieker syndrome (17p13 deletion)
Ring chromosome	Deletion at both ends with fusion of the broken ends	Ring chromosome 14
Isochromosome	Centromere divides transversely instead of longitudinally — one arm is lost, the other duplicated	i(Xq) — the commonest isochromosome, seen as a Turner-syndrome variant
Translocation — Robertsonian	Two acrocentric chromosomes fuse at the centromere with loss of the short arms	der(13;14) — commonest human translocation, usually a harmless balanced-carrier state; der(14;21)/der(21;22) — familial (translocation) Down syndrome
Translocation — Reciprocal	Exchange of segments between two non-homologous chromosomes	t(9;22) Philadelphia chromosome — chronic myeloid leukaemia; t(15;17) — acute promyelocytic leukaemia; t(8;14) — Burkitt lymphoma
Inversion	A segment reverses orientation within the same chromosome	Pericentric (includes centromere) and paracentric (excludes centromere) inversion
Fragile site	Non-staining gap/constriction at a specific locus	Fragile X (Martin-Bell) syndrome
Chromosome-breakage syndrome	Excess spontaneous breakage from defective DNA-repair	Xeroderma pigmentosum (UV-sensitive); Ataxia-telangiectasia (radiation-sensitive); Fanconi anaemia (cross-linker-sensitive); Bloom syndrome

4. Disorders involving autosomes — with examples

(a) Numerical (trisomies)

Disorder	Karyotype	Incidence	Key features
Down syndrome (trisomy 21)	47,XX/XY,+21 — ~95% free (non-disjunction) trisomy, ~4% Robertsonian translocation, ~1% mosaic	1 in 700 live births; risk rises with maternal age	Mongoloid facies with oblique palpebral fissures and epicanthic folds, flat nasal bridge, low-set ears, single palmar (simian) crease, hypotonia, IQ 25–50, congenital heart disease, low maternal-serum/amniotic-fluid AFP
Patau syndrome (trisomy 13)	47,XX/XY,+13	Rare	Cleft lip/palate, polydactyly, omphalocele, profound mental retardation; usually fatal within days of birth
Edward syndrome (trisomy 18)	47,XX/XY,+18	Rare	Elongated skull with prominent occiput, micrognathia, overlapping fingers, rocker-bottom feet, congenital heart defects; usually fatal within weeks

□ Fig 4.9 — Karyotype of Down syndrome patient showing trisomy 21 (arrow). (Gangane — Human Genetics — 5e, p.92)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A schematic karyotype chart (Denver classification), 22 autosome pairs + sex chromosomes arranged in groups A–G by size/centromere position:

- ▶ **Group A** (1–3), **B** (4–5), **C** (6–12 + X), **D** (13–15, acrocentric), **E** (16–18), **F** (19–20), **G** (21–22 + Y, acrocentric)
- ▶ Label and circle: **three copies of chromosome 21** (Group G) → Down syndrome; **single X, no second sex chromosome** → Turner syndrome (45,X); **XXY** in the sex-chromosome pair → Klinefelter syndrome
- ▶ Show the centromere, short (p) and long (q) arm on one representative chromosome, and mark a deleted short arm (5p–) for Cri-du-chat as a second labelled example

(b) Structural (see Section 3 table for full list and mechanism)

- ▶ Cri-du-chat syndrome (5p– deletion) — round face, cat-like cry, hypertelorism, congenital heart defects, microcephaly, mental retardation.
- ▶ Prader-Willi/Angelman syndromes — same 15q11–q13 microdeletion; paternal deletion → Prader-Willi (hyperphagia/obesity, hypotonia, hypogonadism); maternal deletion → Angelman (happy disposition, severe mental retardation, ataxic gait, seizures).
- ▶ DiGeorge syndrome (22q11.2 microdeletion) — thymic aplasia (immunodeficiency), parathyroid aplasia (hypocalcaemia), hypertelorism, micrognathia.
- ▶ Miller-Dieker syndrome (17p13 deletion) — lissencephaly, microcephaly.
- ▶ Translocation-associated neoplasia — Philadelphia chromosome t(9;22)(BCR-ABL fusion) in chronic myeloid leukaemia; t(15;17) in acute promyelocytic leukaemia; t(8;14)(MYC-IGH) in Burkitt lymphoma.

5. Disorders involving sex chromosomes — with examples

Feature	Klinefelter syndrome	Turner syndrome	Triple X ("super female")
Karyotype	47,XXY	45,X	47,XXX
Nature	Trisomic; males only	Monosomic; females only — the only viable human monosomy	Trisomic; females only
Approx. incidence	1 in 500–600 live male births	1 in 2500–4000 live female births	1 in 1000 live female births
Barr bodies	1	0	2
Phenotype	Tall, eunuchoid habitus, gynaecomastia, sparse axillary/pubes hair, small firm testes	Short stature, webbed neck, shield chest with pinpoint (widely spaced) nipples, bilateral cubitus valgus, low-set ears, infantile external genitalia	Essentially normal female phenotype

Feature	Klinefelter syndrome	Turner syndrome	Triple X ("super female")
Gonads / fertility	Hypogonadism, azoospermia, infertility; ↑ gonadotropins	Streak gonads, gonadal dysgenesis, primary amenorrhoea	Usually fertile; mild ↓ IQ, scanty menses in some
Other associations	—	Coarctation of aorta; horseshoe kidney; 75% of the retained X is maternal in origin	—

Applied / Clinical anatomy

- **Karyotyping** (G-banded metaphase spread from cultured lymphocytes/amniocytes/chorionic villi) is the gold-standard diagnostic test; **FISH** and **chromosomal microarray** are needed for microdeletions/microduplications below light-microscopic resolution.
- **Antenatal screening** — combined first-trimester test (nuchal translucency + PAPP-A + free β-hCG) and second-trimester triple/quadruple test flag high-risk pregnancies for Down/Edward/Patau syndromes; confirmed by chorionic villus sampling or amniocentesis karyotyping.
- **Maternal age** is the principal risk factor for non-disjunction trisomies (Down, Edward, Patau); recurrence risk in **translocation** (familial) Down syndrome instead depends on which parent carries the balanced Robertsonian translocation and its type.
- **Turner syndrome** is the commonest cause of primary amenorrhoea in girls and mandates screening for coarctation of the aorta and renal anomalies; **Klinefelter syndrome** is the commonest genetic cause of male hypogonadism/infertility, often first diagnosed during infertility work-up.
- **Chromosome-breakage syndromes** (xeroderma pigmentosum, ataxia-telangiectasia, Fanconi anaemia, Bloom syndrome) carry an increased malignancy risk and require genetic counselling and cancer surveillance.
- **Somatic translocations** (Philadelphia chromosome, t(15;17), t(8;14)) are diagnostic/prognostic markers in haematological malignancy and molecular-targeted-therapy decisions (e.g., imatinib for BCR-ABL-positive CML).

High-yield exam pointers

- Two-axis classification to always state first: **numerical vs structural**, and **autosomal vs sex-chromosomal**.
- **Non-disjunction** is the mechanism common to all aneuploidies; its timing (meiosis I, meiosis II, or post-zygotic mitosis) decides non-mosaic vs mosaic outcome.
- No **autosomal monosomy** is compatible with live birth; **45,X (Turner)** is the only viable human monosomy.
- **Down syndrome** = commonest live-born autosomal trisomy (1/700); **Klinefelter** = commonest cause of male hypogonadism/infertility; **Turner** = commonest cause of primary amenorrhoea.
- **der(13;14)** is the commonest Robertsonian translocation in humans (usually a harmless balanced-carrier state); translocation Down syndrome results from **der(14;21)** or **der(21;22)**.

- ▶ **Prader-Willi vs Angelman** — classic genomic-imprinting pair: identical 15q11–q13 deletion, phenotype dictated by parent of origin (paternal → Prader-Willi; maternal → Angelman).
- ▶ **Philadelphia chromosome t(9;22)** → CML is the classic disease-associated reciprocal translocation.
- ▶ **i(Xq)** is the commonest isochromosome, occurring as a structural variant of Turner syndrome.

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e; Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e.

♦ ♦ ♦

Classify genetic disorders, describe the mechanism of disorders & give examples

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction A **genetic disorder** is a disease caused wholly or partly by an abnormality in the genome — a numerical or structural change in chromosomes, a mutation in a single gene, a mutation in mitochondrial DNA, or an interaction of multiple genes with environmental factors. Based on the underlying mechanism, genetic disorders are classified into four major categories: **chromosomal disorders**, **single-gene (Mendelian) disorders**, **mitochondrial (cytoplasmic) disorders**, and **multifactorial (polygenic) disorders**. Each category has a distinct mechanism of origin, a characteristic pattern of transmission, and representative clinical examples, discussed below.

1. Classification of genetic disorders

Category	Basic defect	Mechanism	Transmission pattern
Chromosomal	Numerical or structural change in whole chromosomes	Non-disjunction, deletion, translocation, inversion	Sporadic or familial (unbalanced karyotype)
Single-gene (Mendelian)	Mutation in one gene locus	Altered gene product (enzyme/structural protein)	Autosomal or X-linked, dominant or recessive
Mitochondrial	Mutation in mitochondrial DNA (mtDNA)	Defective oxidative-phosphorylation proteins/tRNAs	Strict maternal (cytoplasmic) inheritance
Multifactorial	Additive effect of several genes + environment	Polygenic predisposition crossing a liability threshold	No single Mendelian pattern; familial clustering



Classify genetic disorders, describe the mechanism of disorders & give examples

2. Chromosomal disorders — mechanism and examples

Chromosomal disorders result from an error during **gametogenesis (meiosis)** or early **mitosis**, producing a numerical or structural chromosomal imbalance.

(a) Numerical abnormalities (aneuploidy)

- ▶ Mechanism: **non-disjunction** — failure of homologous chromosomes (meiosis I) or sister chromatids (meiosis II) to separate, so one gamete gets an extra chromosome and the other lacks it; fertilisation of the extra-chromosome gamete gives a **trisomy**, and of the deficient gamete a **monosomy**.
- ▶ Autosomal trisomies:
 - **Down syndrome (trisomy 21)** — karyotype 47,XX/XY,+21; incidence ~1 in 700 live births; risk rises with maternal age. Three cytogenetic types: **free/primary trisomy 21** (~95%, non-disjunction, 47 chromosomes), **translocation Down syndrome** (~3–4%, extra chromosome 21 fused to another acrocentric, 46 chromosomes, may be familial via a parental Robertsonian translocation), and **mosaic Down syndrome** (post-zygotic non-disjunction, two cell lines, milder phenotype). Features: mongoloid facies with oblique palpebral fissures and epicanthic folds, flat nasal bridge, simian crease, hypotonia, IQ 25–50, congenital heart disease, low maternal-serum/amniotic α -fetoprotein.
 - **Patau syndrome (trisomy 13)** — profound mental retardation, cleft lip/palate, omphalocele, polydactyly; usually fatal in infancy.
 - **Edward syndrome (trisomy 18)** — prominent occiput, low-set ears, micrognathia, overlapping fingers, rocker-bottom heels, congenital heart defects; usually fatal within weeks.
- ▶ Sex-chromosomal aneuploidies:
 - **Klinefelter syndrome (47,XXY)** — non-disjunction of XX; phenotypic male, Barr-body positive; eunuchoid habitus, hypogonadism, azoospermia, gynaecomastia, mild mental retardation; incidence ~1:500 male births.
 - **Turner syndrome (45,X)** — only viable human monosomy; phenotypic female; short stature, webbed neck, shield chest, cubitus valgus, gonadal dysgenesis with amenorrhoea, coarctation of aorta; incidence ~1:2500–3000 female births.
 - **Triple-X (super female, 47,XXX)** — two Barr bodies; female phenotype, usually fertile, mild learning difficulty.

(b) Structural abnormalities — arise from chromosome breakage with abnormal reunion.

- ▶ **Deletion:** loss of a chromosomal segment.
 - **Cri-du-chat syndrome** — deletion of short arm of chromosome 5 [del(5p)]; characteristic cat-like cry, hypertelorism, microcephaly, mental retardation.
 - **Prader-Willi syndrome** — microdeletion of 15q11–q13 on the **paternally**-derived chromosome (loss of paternal imprinting); hyperphagia/obesity, hypotonia, hypogonadism, short stature.
 - **Angelman syndrome** — microdeletion of the **same** 15q11–q13 region on the **maternally**-derived chromosome; happy disposition with inappropriate laughter, severe mental

retardation, ataxic gait, seizures. (Prader-Willi/Angelman together illustrate **genomic imprinting** — identical deletion, opposite phenotype depending on parental origin.)

- **DiGeorge syndrome** — microdeletion of 22q11.2; thymic aplasia (immunodeficiency), parathyroid aplasia (hypocalcaemia), conotruncal cardiac defects, hypertelorism, micrognathia.
- **Miller-Dieker syndrome** — microdeletion of 17p13.3; lissencephaly, microcephaly.
- ▶ **Translocation**: exchange of segments between non-homologous chromosomes.
 - **Robertsonian translocation** — fusion of two acrocentric chromosomes at the centromere with loss of the short arms; balanced carriers have 45 chromosomes and are phenotypically normal but at risk of unbalanced gametes (basis of familial translocation Down syndrome).
 - **Philadelphia chromosome, t(9;22)** — reciprocal translocation forming the *BCR-ABL* fusion oncogene; causes **chronic myeloid leukaemia**.

Diagram to draw

- ▶ Draw and label a **karyotype of trisomy 21**: 46 pairs of chromosomes arranged by group (A–G), showing three copies of chromosome 21 (group G); mark the sex-chromosome pair (XX/XY), the centromere position, and label "47,XX,+21" beneath.
- ▶ A real captioned plate of this karyotype is embedded below for reference before hand-reproduction.

□ Fig 4.9 — Karyotype of Down syndrome patient showing trisomy 21 (arrow). (Gangane — Human Genetics — 5e, p.92)

3. Single-gene (Mendelian) disorders — mechanism and examples

A mutation in **one gene locus** alters the corresponding protein (enzyme, receptor, structural or transport protein), producing disease that follows classic Mendelian segregation.

Pattern	Mechanism	Pedigree pattern	Examples
Autosomal dominant	One mutant allele (from either parent) suffices to produce the phenotype	Vertical transmission; 50% of children of an affected parent affected	Huntington disease (HD gene, chromosome 4p — progressive caudate/putamen neuronal loss, chorea, dementia), Marfan syndrome, achondroplasia, neurofibromatosis, osteogenesis imperfecta, brachydactyly
Autosomal recessive	Both alleles must be mutant (homozygous); heterozygous carriers unaffected	Horizontal transmission; siblings affected, parents normal (often consanguineous); 25% recurrence risk	Cystic fibrosis (CFTR gene, chromosome 7q — thick mucus obstructing respiratory/GI epithelium), sickle-cell anaemia, thalassemia, phenylketonuria, other inborn errors of metabolism

Pattern	Mechanism	Pedigree pattern	Examples
X-linked recessive	Mutant allele on X; hemizygous males affected, females usually carriers	No male-to-male transmission; carrier mother → half of sons affected	Duchenne muscular dystrophy (dystrophin gene, Xp21 — progressive muscle weakness, cardiorespiratory failure), haemophilia (factor VIII deficiency), red-green colour blindness
X-linked dominant	One mutant X-linked allele produces phenotype in either sex; more common/severe in females (2:1)	Affected male → all daughters, no sons affected	Vitamin D-resistant (hypophosphataemic) rickets, Xg blood group
Y-linked (holandric)	Gene present only on Y, obligatorily expressed, no dominance/recessiveness	Father → all sons	Hairy pinna of the ear

Diagram to draw — a pedigree chart for each pattern using standard symbols (square = male, circle = female, filled = affected, double line = consanguinity), showing the characteristic vertical (autosomal dominant), horizontal (autosomal recessive), or "knight's-move" (X-linked recessive) transmission.

4. Mitochondrial (cytoplasmic) disorders — mechanism and examples

- ▶ **Mechanism:** mitochondria (and their circular mtDNA, coding for tRNAs/rRNA and oxidative-phosphorylation subunits) are contributed almost entirely by the ovum; sperm mitochondria are excluded at fertilisation. Hence mtDNA mutations show **strict maternal inheritance** — an affected mother transmits the trait to all her children, but an affected father transmits it to none.
- ▶ Each cell contains hundreds of mitochondria/thousands of mtDNA copies; a mutation may affect all copies (**homoplasmy**) or a mixture of mutant and normal copies (**heteroplasmy**). During cell division, mutant and normal mitochondria assort randomly among daughter cells (**replicative segregation**), so severity varies between siblings and even between tissues of the same individual — tissues with the highest energy demand (muscle, brain, retina) are worst affected.
- ▶ **Examples:**
 - **Leber hereditary optic neuropathy (LHON)** — sudden-onset adult blindness from optic-nerve degeneration.
 - **MERRF** (myoclonic epilepsy with ragged red fibres) — muscle weakness, ataxia, seizures; muscle biopsy shows "ragged red fibres" from subsarcolemmal mitochondrial aggregates.
 - **MELAS** (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes).
 - **Kearns-Sayre syndrome** — chronic progressive external ophthalmoplegia, retinopathy, cardiac conduction defects.
 - **Pearson marrow-pancreas syndrome** — bone-marrow failure in infancy, often fatal.

5. Multifactorial (polygenic) disorders — mechanism and examples

- ▶ **Mechanism:** results from the additive/interactive effect of **multiple genes at different loci plus environmental factors**; liability is continuously distributed in the population, and disease appears only once a **threshold** of combined genetic + environmental liability is crossed. There is no single Mendelian ratio; recurrence risk is empirical (usually 2–5% for first-degree relatives) and rises with the number of affected relatives and with severity of the defect.
- ▶ **Examples of congenital malformations:** neural tube defects, cleft lip/palate, clubfoot, congenital dislocation of the hip, congenital heart disease.
- ▶ **Contributing environmental (teratogenic) factors:** drugs (thalidomide, anticonvulsants), infections (rubella), ionising radiation.
- ▶ Common adult multifactorial diseases (same principle, later onset): hypertension, diabetes mellitus, coronary artery disease, many cancers.

6. Mutation — the basic molecular mechanism common to genetic disease

- ▶ A **mutation** is a permanent, heritable change in genomic DNA sequence, produced by substitution of a single base or by deletion/insertion/rearrangement of larger DNA segments; it may occur at the level of a single **gene** or of a whole **chromosome**.
- ▶ **Mutagens:** ultraviolet light/X-rays, chemicals, viruses, transposons ("jumping genes"). About 70% of mutations are harmful; the rest are silent or occasionally beneficial.
- ▶ Mutations are classified by the cell type involved:

Feature	Somatic mutation	Germline mutation
Site	Somatic cells	Germ cells
Transmission	Not passed to offspring	Passed to offspring
Consequence	Can cause cancer (e.g., in a single tissue)	Can cause heritable cancer predisposition (e.g., retinoblastoma, Wilms tumour) and all classes of inherited disease above
Individual	Becomes a genetic mosaic	Not mosaic — mutation present in every cell

Applied / Clinical anatomy

- ▶ **Pedigree analysis** is the essential clinical tool to determine mode of inheritance and calculate recurrence risk before genetic counselling.
- ▶ **Genetic counselling** is offered when relatives are at risk of transmitting a genetic disorder — explains nature, transmission risk and options (prenatal diagnosis, prevention); indicated for known carrier couples, a child already born with a malformation (spina bifida, cleft lip/palate) or metabolic disease (PKU, galactosaemia), late-onset dominant disease in the family (Huntington disease, hereditary cancer), and maternal age >35 years.
- ▶ **Prenatal screening/diagnosis:** karyotyping/FISH on amniotic fluid or chorionic villi for suspected trisomies/translocations; maternal serum/amniotic α -fetoprotein is characteristically

low in Down syndrome and raised in open neural tube defects; increased **nuchal translucency** (≥ 3 mm at 12 weeks 5 days) on ultrasound screens for trisomy 21.

- **Clinical correlations:** congenital heart disease is common to Down, Patau, Edward and DiGeorge syndromes; coarctation of the aorta is a hallmark of Turner syndrome; thymic/parathyroid agenesis in DiGeorge syndrome predisposes to infection and hypocalcaemic tetany; the Philadelphia chromosome is both a diagnostic marker and, via BCR-ABL tyrosine kinase, a therapeutic target (imatinib) in chronic myeloid leukaemia.

High-yield exam pointers

- Four categories: **chromosomal**, **single-gene (Mendelian)**, **mitochondrial**, **multifactorial** — state the mechanism, then the example, for each.
- Down syndrome is the commonest chromosomal disorder (47,XX/XY,+21); know its three cytogenetic subtypes (free trisomy, translocation, mosaic) and the maternal-age association.
- Turner (45,X) is the only viable human monosomy; Klinefelter (47,XXY) is the commonest cause of male hypogonadism from a chromosomal cause.
- Prader-Willi/Angelman pair = same 15q11-q13 deletion, opposite phenotype by parent of origin = classic exam example of **genomic imprinting**.
- Mitochondrial disease = **strict maternal transmission** + heteroplasmy/homoplasmy + replicative segregation; LHON/MERRF/MELAS/Kearns-Sayre are the standard named examples.
- Multifactorial = polygenic + environment crossing a liability threshold; empirical (not Mendelian) recurrence risk; NTDs/cleft lip-palate/CHD/CDH are classic examples.
- Somatic mutation is not heritable; germline mutation is heritable and is the basis of all transmissible single-gene, chromosomal, and mitochondrial disease.
- Philadelphia chromosome t(9;22) → BCR-ABL fusion → chronic myeloid leukaemia is the classic translocation-mutation link to malignancy.

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e; Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e; Ross Histology — A Text and Atlas 7e (mitochondrial myopathy nomenclature). Note: DiGeorge syndrome's deletion locus is stated here as chromosome 22 (22q11.2) per Thompson & Thompson (confirmed also by standard nomenclature "22q11.2 deletion syndrome"), correcting an apparent chromosome-number misprint in the Vishram Singh source text.



Deletion

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction A deletion is a structural chromosomal aberration in which a segment of a chromosome — together with every gene it carries — is lost, leaving a shorter, structurally altered homologue. Because the other homologue remains normal, a deletion always produces **partial monosomy** for the missing segment, and the resulting phenotype reflects **haploinsufficiency** (a single copy being unable to perform the function normally requiring two). Deletions range from large, cytogenetically visible losses detectable on a routine karyotype to submicroscopic "microdeletions" detectable only by FISH or chromosomal microarray (CMA); several produce well-defined, recurrent clinical syndromes such as cri-du-chat syndrome and the 22q11.2 (DiGeorge/velocardiofacial) deletion syndrome.

1. Types of Deletion — mechanism and structure

Type	Breaks	Mechanism	Structural result	Example
Terminal deletion	Single break near one telomere	Break occurs along an arm; the distal, acentric (centromere-less) fragment cannot attach to the spindle and is lost at the next division	Loss of the distal part of an arm; derivative chromosome is shorter	5p– (cri-du-chat syndrome)
Interstitial deletion	Two breaks on the same arm	The intercalary (in-between) acentric segment is excised; the proximal and distal fragments reunite	Loss of the intervening genes only; both telomeres retained	22q11.2 deletion (DiGeorge/VCFS); 15q11–q13 deletion (Prader-Willi/Angelman)
Ring chromosome	Two breaks, one near each telomere	Both telomeric fragments are lost; the two "sticky" broken ends of the remaining chromosome fuse together	Partial monosomy for both deleted ends, plus mitotic instability (sister-chromatid tangling at anaphase; variable ring size in different cell lines)	Ring chromosome 4, ring 18

Not to be confused with an **isochromosome** (one arm duplicated in mirror-image while the other arm is lost), which combines partial monosomy of one arm with partial trisomy of the other — e.g., i(X)(q10) in Turner syndrome — a distinct mechanism from a pure deletion.

2. Mechanism of Origin

- ▶ **Simple chromosome breakage** with loss of the resulting acentric fragment — the mechanism for most "idiopathic" deletion syndromes, which show highly variable breakpoints between patients (e.g., cri-du-chat syndrome; the chromosome 1p36 deletion syndrome, ~95% de novo).
- ▶ **Non-allelic homologous recombination (NAHR)/unequal crossing-over** between misaligned **segmental duplications (low-copy repeats)** that flank a chromosomal region during meiosis — the mechanism underlying the recurrent "**genomic disorders**"/**contiguous gene syndromes**, which produce a reciprocal deletion product on one homologue and a duplication product on the other (e.g., 22q11.2, 15q11–q13, 7q11.23, 17p11.2, 1q21.1, 16p11.2 regions).
- ▶ **Malsegregation of a parental balanced translocation** — accounts for roughly 10–15% of cri-du-chat cases; the remainder are sporadic, de novo terminal or interstitial deletions.
- ▶ The event may be constitutional (present in every cell, having occurred in a parental germ cell or the zygote) or **mosaic** (post-zygotic, present in only a proportion of cells).

3. Cytogenetic Consequences

- ▶ The carrier is **monosomic** for the corresponding segment of the normal homologue; clinical severity generally correlates with the **size of the deleted segment and the number/function of the genes lost**.
- ▶ In some contiguous gene syndromes the phenotype results from the combined loss of several genes; in others a single gene within a larger deleted region is chiefly responsible (e.g., **TBX1** in 22q11.2 deletion syndrome, **UBE3A** in Angelman syndrome).
- ▶ **Incidence:** cytogenetically visible autosomal deletions occur in approximately **1 in 7000 live births**; smaller, submicroscopic deletions detected by microarray are considerably more common, though the clinical significance of many such variants is not always established.
- ▶ **Genomic imprinting** can make the phenotype of an identical deletion depend on its parental origin — the classic example being the **15q11–q13 deletion**: a **paternally-derived** deletion (~70% of cases) causes **Prader-Willi syndrome** (neonatal hypotonia, later hyperphagia/obesity, hypogonadism, short stature, intellectual disability), whereas the same deletion of **maternal** origin (~70% of cases) causes **Angelman syndrome** (unusual facial appearance, short stature, severe intellectual disability, spasticity/ataxic gait, seizures). The remaining cases of each arise from uniparental disomy or imprinting-centre/gene mutations rather than a visible deletion.

4. Nomenclature (ISCN) and Detection

ISCN shorthand for deletion uses **del**, e.g. **46,XX,del(5)(q13)** = a female with a terminal deletion of chromosome 5 distal to band q13.

Technique	Approximate resolution	Role
Routine G-banded karyotype (400–550-band stage)	≥ 5–10 Mb	First-line test; detects large deletions/duplications
High-resolution (prometaphase) banding	Up to 850 bands	Improves detection of subtler structural deletions

Technique	Approximate resolution	Role
FISH with a locus-specific probe	~50 kb–few Mb	Confirms/screens for a specific known microdeletion (e.g., a 22q11.2 probe for DiGeorge syndrome)
Chromosomal microarray (CMA/array-CGH)	Genome-wide, kb-level	First-tier test for unexplained developmental delay, intellectual disability, dysmorphism, or multiple congenital anomalies; detects submicroscopic deletions
Whole-genome sequencing	Single base-pair	Precisely maps breakpoints; highest resolution

5. Clinically Important Deletion Syndromes

Syndrome	Deleted region	Size / incidence	Key clinical features	Gene(s)/ mechanism
Cri-du-chat syndrome	5p (terminal or interstitial); critical region 5p15	≈1 in 15,000 live births	Cat-like cry in infancy, microcephaly, hypertelorism, epicanthal folds, low-set ears ± preauricular tags, micrognathia, severe intellectual disability	Haploinsufficiency for gene(s) in 5p14–p15; ~10–15% inherited from a parental balanced translocation
Wolf-Hirschhorn syndrome	4p16.3	Rare	Severe growth failure, "Greek warrior helmet" facies, seizures, severe intellectual disability, midline defects	Wolf-Hirschhorn critical region
1p36 deletion syndrome	Terminal 1p36 (variable breakpoints within terminal 10 Mb)	≈1 in 5000; ~95% de novo	Intellectual disability, hypotonia, seizures, cardiac defects; often not visible on routine banding, needs CMA	Contiguous gene loss
Williams syndrome	7q11.23	≈1.6 Mb	"Elfin" facies, supravalvular aortic stenosis, infantile hypercalcemia, intellectual disability with a sociable personality	ELN (elastin) haploinsufficiency; NAHR between flanking segmental duplications

Syndrome	Deleted region	Size / incidence	Key clinical features	Gene(s)/ mechanism
Prader-Willi / Angelman syndrome	15q11–q13	≈3.5–6 Mb	See Section 3 above	Imprinted gene cluster: paternally-expressed genes lost in PWS; maternal UBE3A lost in AS
Smith-Magenis syndrome	17p11.2	≈3.7 Mb	Intellectual disability, sleep disturbance, self-injurious behaviour, brachycephaly	Reciprocal duplication is dup(17)(p11.2p11.2)
22q11.2 deletion syndrome (DiGeorge/velocardiofacial syndrome)	22q11.2	≈3 Mb (nested ≈1.5 Mb in ~10%); ≈1 in 4000 live births	Conotruncal cardiac defects, thymic hypoplasia, immunodeficiency, hypocalcemia, characteristic facies, cleft palate	TBX1 haploinsufficiency implicated in up to 5% of all congenital heart defects; NAHR between flanking segmental duplications

Other recurrent NAHR-mediated microdeletion/duplication regions include **1q21.1** (≈0.8 Mb) and **16p11.2** (≈0.6 Mb), each associated with variable developmental delay and dysmorphism.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 4.2 — Chromosomal aberration – deletion: (A) Terminal deletion of P arm of chromosome. (B) Int (Gangane — Human Genetics — 5e, p.85)

Real plate above: (A) terminal deletion of the short arm of chromosome 5 producing cri-du-chat syndrome (5p–); (B) interstitial ("2-part") deletion within an arm.

For hand reproduction in the exam, draw:

- ▶ A normal chromosome 5 (metacentric; short **p arm**, long **q arm**, **centromere**) beside its terminal-deletion derivative — label **break point** (arrow), **acentric fragment lost**, **derivative 5p– chromosome**.
- ▶ Alongside it, an interstitial deletion of the same arm — label **break 1**, **break 2**, **excised intercalary segment**, **rejoined derivative chromosome**.
- ▶ A small linear ideogram of chromosome 22 marking band **22q11.2**, flanked by **segmental duplications (LCRs)**, with the deleted ≈3-Mb interval bracketed and **TBX1** labelled within it, to illustrate the NAHR mechanism of a genomic-disorder microdeletion.

Applied / Clinical anatomy

- ▶ **Embryological basis of 22q11.2 deletion syndrome:** the deletion disrupts cardiac neural-crest cells that populate the 3rd and 4th pharyngeal arches/pouches and the cardiac outflow tract, explaining the classic clinical picture — conotruncal cardiac defects, thymic hypoplasia (3rd

pouch) with T-cell immunodeficiency, and parathyroid hypoplasia (3rd/4th pouch) causing neonatal hypocalcaemic tetany — together with cleft/palatal abnormalities and a characteristic facies.

- ▶ **Indications for karyotype/FISH/CMA testing:** unexplained developmental delay or intellectual disability, dysmorphic facies, multiple congenital malformations, ambiguous genitalia, short stature, and recurrent first-trimester pregnancy loss.
- ▶ **Prenatal diagnosis:** chorionic villus sampling or amniocentesis followed by targeted FISH (e.g., a 22q11.2 probe) or CMA when a familial deletion or a parental balanced translocation is already known.
- ▶ **Genetic counselling:** most deletion syndromes (cri-du-chat, 1p36, Williams, non-UPD Prader-Willi/Angelman) are de novo events with a low recurrence risk; when a parent carries a balanced translocation (as in a minority of cri-du-chat families), recurrence risk is substantially higher, so parental karyotyping is essential.
- ▶ **Imprinting counselling:** because the 15q11–q13 deletion phenotype (Prader-Willi vs Angelman) depends strictly on parental origin, methylation/parental-origin studies are required before recurrence risk can be given.

High-yield exam pointers

- ▶ Deletion = loss of a chromosomal segment → **partial monosomy** → phenotype driven by **haploinsufficiency**.
- ▶ Three structural types: **terminal** (1 break), **interstitial** (2 breaks, same arm), **ring** (2 breaks, both telomeres lost, ends fuse).
- ▶ ISCN shorthand: **del**, e.g. **46,XX,del(5)(q13)**.
- ▶ NAHR between flanking segmental duplications generates recurrent "genomic disorder"/contiguous-gene microdeletions (22q11.2, 15q11–q13, 7q11.23, 17p11.2).
- ▶ **Cri-du-chat** = 5p– (critical region 5p15); **Wolf-Hirschhorn** = 4p16.3; **DiGeorge/VCFS** = 22q11.2 (TBX1); **Williams** = 7q11.23 (ELN); **Smith-Magenis** = 17p11.2 (RAI1).
- ▶ **15q11–q13 deletion:** paternal origin → Prader-Willi syndrome; maternal origin → Angelman syndrome — the classic genomic-imprinting example.
- ▶ Detection hierarchy: karyotype (>5–10 Mb) → high-resolution banding (up to 850 bands) → FISH (targeted) → chromosomal microarray (genome-wide, submicroscopic) → whole-genome sequencing.
- ▶ Incidence: cytogenetically visible autosomal deletions ≈ 1 in 7000 live births; 22q11.2 deletion alone ≈ 1 in 4000 live births.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e; Langman's Medical Embryology (Sadler) 14e; Larsen Human Embryology 6e; IB Singh Human Embryology (CBME 2021); Vishram Singh General Anatomy 4e.



Describe in detail about Karyotyping

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction A **karyotype** is the complete chromosomal constitution of a cell — the number, size, shape and banding pattern of its chromosomes arranged in a standard, size-descending order into homologous pairs. **Karyotyping** is the laboratory process by which dividing cells are cultured, arrested in metaphase, stained and photographed so that the chromosomes can be cut out (or computer-sorted) and arranged in this standard format — the resulting picture is called a **karyogram**. Karyotyping is the basic tool of clinical cytogenetics, used to detect numerical and structural chromosomal abnormalities in constitutional (postnatal and prenatal) as well as acquired (malignant) disease.

Structure of a chromosome — the basis of classification

- ▶ A metaphase chromosome consists of two identical sister **chromatids** joined at a primary constriction, the **centromere**; the **kinetochore**, to which spindle fibres attach, lies at the centromere.
- ▶ Each chromatid has a short arm (**p**, French *petit*) and a long arm (**q**) separated by the centromere.
- ▶ A **secondary constriction** may separate a small terminal segment of the arm, called the **satellite**; the stalk of the secondary constriction carries the nucleolar organiser (ribosomal RNA genes).
- ▶ The **telomere** caps each arm — a zone of repetitive nucleotide sequence (TTAGGG in humans, repeated ~2500 times) that prevents end-to-end fusion.
- ▶ Based on the position of the centromere, four morphological types are described: **metacentric** (centromere central, arms equal), **submetacentric** (centromere off-centre, one arm longer), **acrocentric** (centromere near one end, one arm very short) and **telocentric** (centromere at the very end, single arm — not seen in the normal human karyotype).
- ▶ The five human acrocentric pairs (13, 14, 15, 21, 22) carry satellites on their short arms.

Procedure of karyotyping

□ Fig 2.8 — Procedure of karyotyping. (Gangane — Human Genetics — 5e, p.36)

- ▶ **Sample collection** — the commonest source is peripheral blood **lymphocytes** (5 mL venous blood); other sources include bone marrow, amniotic-fluid cells, chorionic villi and skin fibroblasts.
- ▶ **Culture** — cells are cultured in a growth medium with **phytohaemagglutinin (PHA)**, a mitogen that stimulates lymphocytes to divide, and incubated at 37 °C for about 3 days.
- ▶ **Metaphase arrest** — **colchicine** (a spindle poison) is added to arrest dividing cells at metaphase, when chromosomes are maximally condensed and individually visible.
- ▶ **Hypotonic treatment** — cells are treated with hypotonic saline, which swells them and disperses the chromosomes for better spreading.
- ▶ **Fixation** — cells are fixed in a 1:3 mixture of glacial acetic acid and methanol.
- ▶ **Spreading and staining** — the fixed cell suspension is dropped onto a glass slide from a height to spread the metaphase chromosomes, which are then stained (commonly Giemsa).

- **Photomicrography and arrangement** — the metaphase spread is photographed; individual chromosomes are cut out (or, increasingly, sorted by computerised image analysis) and arranged in descending order of length into homologous pairs to produce the karyogram.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Flowchart with these exact labels, in sequence: Collection of blood → Culture/incubation at 37 °C, 3 days → Add colchicine → White cells separated → Hypotonic treatment → Fixative (acetic acid : methanol, 1:3) → Cells fixed on slide → Staining → Chromosome spread → Photographed and arranged in standard manner.

Classification of human chromosomes — the Denver system

The **Denver Classification** (Denver Conference, 1960; ratified at London, 1963) arranges the 46 human chromosomes into 23 pairs and groups them into **seven groups, A to G**, in descending order of length and by centromere position.

Group	Chromosome pairs	Features
A	1, 2, 3	Largest, metacentric
B	4, 5	Large, submetacentric
C	6–12 + X	Medium-sized, mostly submetacentric; X resembles chromosome 6
D	13, 14, 15	Medium-sized, acrocentric, with satellites
E	16, 17, 18	Short, submetacentric (16 is metacentric)
F	19, 20	Short, metacentric
G	21, 22 + Y	Shortest, acrocentric; 21 and 22 carry satellites; Y resembles chromosome 21 but has no satellite

□ Fig 17.7 — Karyotype of a normal male. (Vishram Singh — General Anatomy — 4e, p.695)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A karyotype grid of 22 autosomal pairs + XY, labelled by group: A (1–3) — B (4,5) — C (6–12, X) — D (13–15) — E (16–18) — F (19,20) — G (21,22, Y), with each pair drawn to show relative length and centromere position (metacentric/submetacentric/acrocentric).

Chromosomal banding techniques and nomenclature

The Denver system alone could identify only gross length/shape groups; it could not detect deletions, translocations or other subtle rearrangements. **Banding techniques**, introduced through the 1970s, produce a reproducible pattern of alternating stained and unstained bands unique to each chromosome pair, allowing every chromosome — and even sub-regions of it — to be identified unambiguously.

Banding	Method	Chief use
Q-banding	Quinacrine dye + UV fluorescence	First banding method; fluorescent bands (Q bands)
G-banding	Trypsin digestion + Giemsa stain	Commonest/gold-standard method for routine karyotyping; G-dark/G-light bands
R-banding	"Reverse" Giemsa technique	Bands reversed relative to G-banding; useful for terminal regions that stain poorly with G-banding
C-banding	Demonstrates constitutive heterochromatin	Highlights centromeres and secondary constrictions (e.g., chromosomes 1, 9, 16 and the long arm of Y)
T-banding	Stains telomeric regions	Identifies telomeres

- ▶ The **Paris Conference (1971)** standardised banding-based nomenclature: each chromosome arm is divided into numbered **regions** (1, 2, 3...) counting outward from the centromere, and each region is subdivided into numbered **bands** — allowing any band to be described precisely (e.g., 9q34).
- ▶ The **International System for Human Cytogenetic Nomenclature (ISCN, 1978)**, periodically updated, consolidated the Denver, London and Paris agreements into the single standard notation still used today, e.g. **46,XY** (normal male), **47,XX,+21** (trisomy 21/Down syndrome), **46,XY,t(9;22)(q34;q11)** (a balanced translocation).
- ▶ **Resolution**: a routine metaphase G-banded karyotype shows roughly 400–550 bands per haploid set and detects deletions/duplications above ~5–10 Mb. **High-resolution (prometaphase) banding**, obtained by staining less-condensed prophase/prometaphase chromosomes, resolves up to ~850 bands and detects smaller (2–3 Mb) rearrangements.

Complementary cytogenetic techniques

- ▶ **Fluorescence in situ hybridisation (FISH)** — fluorescent DNA probes specific to a chromosome, region or gene are hybridised directly to metaphase spreads or interphase nuclei; it rapidly confirms aneuploidy, marker chromosomes and microdeletions, and can be combined with conventional karyotyping.
- ▶ **Chromosomal microarray / comparative genomic hybridisation and whole-genome sequencing** give still higher (submicroscopic) resolution and increasingly supplement conventional karyotyping, especially for non-invasive prenatal screening.

Indications for karyotyping

Category	Indications
Growth/developmental	Failure to thrive, developmental delay, dysmorphic facies, multiple malformations, short stature, ambiguous genitalia, intellectual disability

Category	Indications
Perinatal	Stillbirth and neonatal death (chromosome abnormality found in ~10% of stillbirths/neonatal deaths vs ~0.7% of live births)
Reproductive	Infertility, amenorrhoea, or recurrent miscarriage (a parental chromosome abnormality is found in 3–6% of such couples)
Family history	A known/suspected chromosomal abnormality in a first-degree relative
Neoplasia	Diagnostic/prognostic evaluation of leukaemias and other cancers (e.g. Philadelphia chromosome in chronic myeloid leukaemia)
Pregnancy	Maternal age >35 years, a parent carrying a balanced translocation, a previous child with a chromosomal/neural-tube defect, or an abnormal antenatal screen

Applied / Clinical anatomy

- **Prenatal karyotyping** is obtained from fetal cells sampled by **amniocentesis** (ideal 14–16 weeks; ~1–1.5% risk of abortion) or **chorionic villus sampling** (8–10, up to 12, weeks; faster result as villous cells are already dividing, and permits earlier termination if indicated).
- **Numerical abnormalities** detected on karyotype: trisomy 21/Down syndrome (47,XX/XY,+21), trisomy 18/Edwards syndrome, trisomy 13/Patau syndrome, Turner syndrome (45,X), Klinefelter syndrome (47,XXY), and 47,XYY/47,XXX.
- **Structural abnormalities**: deletion of 5p (Cri-du-chat syndrome); the reciprocal translocation t(9;22)(q34;q11) producing the shortened chromosome 22 (**Philadelphia chromosome**, BCR-ABL fusion) in chronic myeloid leukaemia — of major diagnostic and prognostic value.
- **Sex chromatin (Barr body)** — an interphase condensed inactive X chromosome (Lyon hypothesis) seen on a buccal smear — is a rapid adjunct screening test for X-chromosome number but does not replace full karyotyping.
- In India, prenatal karyotyping is governed by the **PCPNDT Act**, which regulates its use to prevent sex-selective abortion.

High-yield exam pointers

- Karyotype = chromosomal constitution; karyotyping = culture → colchicine metaphase arrest → hypotonic → fix → stain → arrange.
- Lymphocytes cultured with PHA at 37 °C for 3 days is the standard technique.
- Denver Classification (1960/London 1963): 7 groups A–G by length and centromere position.
- Four centromere-based types: metacentric, submetacentric, acrocentric, telocentric (telocentric absent in humans).
- Banding types: Q (quinacrine), G (trypsin-Giemsa, routine), R (reverse Giemsa), C (centromeric heterochromatin), T (telomeric).

- ▶ Paris Conference (1971) → regions/bands nomenclature; ISCN (1978) is the current standard notation.
- ▶ Routine G-banding resolves 400–550 bands; high-resolution (prometaphase) banding resolves up to ~850 bands.
- ▶ FISH complements karyotyping for rapid aneuploidy/microdeletion detection.
- ▶ Key applications: prenatal diagnosis (amniocentesis/CVS), infertility work-up, dysmorphism evaluation, and cancer cytogenetics (Philadelphia chromosome in CML).

SOURCE & COVERAGE

Gangane — Human Genetics 5e; IB Singh — Textbook of Anatomy Vol 3 6e (Chromosomes); Vishram Singh — General Anatomy 4e and Textbook of Clinical Embryology; Thompson & Thompson — Genetics in Medicine 8e; Junqueira's Basic Histology 17e.



Describe the indications, methodology of prenatal diagnosis of genetic disorders

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction

Prenatal diagnosis is the application of imaging and laboratory techniques to a pregnancy already known (or suspected) to be at increased risk, in order to determine — with a definitive "yes/no" answer — whether the fetus is affected by a specific genetic disorder or structural malformation. It is distinguished from prenatal **screening**, which is offered to all pregnant women regardless of risk and only estimates a probability that requires diagnostic confirmation. Prenatal diagnosis forms the final, preventive step of genetic counselling: because most genetic disorders have no cure, it allows an at-risk couple to plan a pregnancy, confirm the fetal status, and exercise an informed reproductive choice — continuing the pregnancy with appropriate management, or, where acceptable to the couple, terminating an affected pregnancy under the Medical Termination of Pregnancy (MTP) Act.

Indications for Prenatal Diagnosis

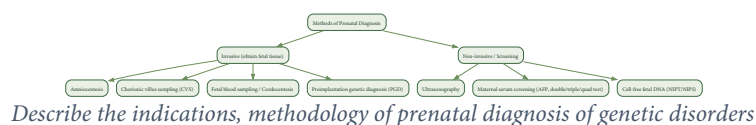
General criteria that justify offering prenatal diagnosis (all should ideally be satisfied):

- ▶ The genetic disorder in question has **no satisfactory treatment**.
- ▶ An **accurate prenatal diagnostic test** is available for that disorder.
- ▶ The **risk to the pregnancy is sufficiently high** to justify an invasive procedure.
- ▶ The disorder is **severe enough** to warrant consideration of termination.
- ▶ **Termination of pregnancy is acceptable** to the couple concerned — the decision always remains theirs, and counselling must be non-directive.

Specific ("definite") indications:

Category	Indication
Maternal age	Advanced maternal age (>35 years) — increasing incidence of trisomy 21, 18, 13 with maternal non-disjunction
Previous affected child	Earlier child with a de novo chromosomal aneuploidy/other genomic imbalance, or with a neural tube defect (NTD)
Parental chromosomal abnormality	Either parent is a balanced structural translocation/inversion carrier — e.g., a parental 21q21q Robertsonian translocation carries a 100% recurrence risk of Down syndrome
Family history — single-gene disorder	An autosomal or X-linked recessive/dominant metabolic or structural disorder that is severe and diagnosable prenatally by biochemical or DNA analysis
Family history — X-linked disorder with no specific test	Fetal sex determination (± preimplantation diagnosis) to guide continuation/termination when no direct test exists, e.g. Duchenne muscular dystrophy (DMD), haemophilia A/B
Abnormal screening result	Increased risk identified by maternal serum screening, soft markers on ultrasonography, or noninvasive prenatal screening (NIPT/NIPS)
Consanguinity	Consanguineous marriage raises risk, especially of autosomal recessive disorders
Couple's request	Some guidelines (e.g., ACOG) support offering invasive testing to any pregnant woman on request, regardless of age or risk

Overview — Classification of Methods



Invasive Methods of Prenatal Diagnosis

Procedure	Timing	Technique	Sample / Tests	Risk of pregnancy loss	Key indications
Amniocentesis	14–16 weeks classically (up to 20 weeks); early amniocentesis (10–14 wk) is no longer recommended	Under real-time USG guidance, a needle is passed transabdominally into the amniotic cavity avoiding the placenta; 10–20 mL clear (not blood-stained) fluid withdrawn	Cells cultured for karyotype/CMA; fluid assayed for amniotic fluid AFP (AFAFP) — screens for open NTD	~1–1.5% (repeat tap raises this); contemporary data ~1 in 300–500	Karyotype/CMA for aneuploidy, biochemical/DNA analysis, AFAFP for NTD
Chorionic villus sampling (CVS)	8–10 weeks (up to 12–13 weeks); transcervical (flexible cannula through the cervix) or transabdominal (spinal needle)	Chorionic villi aspirated from the trophoblast under USG control	Direct/short-term karyotype or CMA — result available faster than amniocentesis (villi are already mitotically active)	Slightly higher than amniocentesis; approximates 1 in 300–500 in experienced hands	Earlier, first-trimester diagnosis when termination (if needed) is safer
Fetal blood sampling (cordocentesis)	~18–22 weeks	Placental (indirect) aspiration or direct puncture of the umbilical vessel, guided by fetoscope/USG	Fetal karyotype and DNA/haematological analysis	~10%	Sickle cell disease, thalassaemias, haemophilia A, DMD, immune deficiency disorders; also when CVS/amniocentesis culture fails

Procedure	Timing	Technique	Sample / Tests	Risk of pregnancy loss	Key indications
Preimplantation genetic diagnosis (PGD)	During IVF, before implantation	Blastomere biopsy (single cell at the 8–16-cell, day-3 stage) or blastocyst biopsy (~5 trophoblast cells, day 5–6); DNA amplified by PCR, or FISH/CMA for chromosomes	Selects unaffected embryos for transfer	Failure rate 10–20% (single-cell PCR); false-positive ~6%, false-negative ~1%	X-linked disorders (e.g., DMD — only male embryos affected), known single-gene mutations, couples opposed to pregnancy termination

Real library plates for the two most-tested invasive procedures:

☐ Fig 13.1 — Amniocentesis, a prenatal diagnostic technique used in second trimester (Gangane — Human Genetics — 5e, p.225)

☐ Fig 13.2 — Chorion villous sampling for prenatal diagnosis in the first trimester (Gangane — Human Genetics — 5e, p.226)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label on a mid-sagittal schematic of the gravid uterus under ultrasound guidance:

- **Amniocentesis:** maternal anterior abdominal wall → myometrium (uterine wall) → amniotic cavity/amniotic fluid; spinal needle tip; placenta (avoided); fetus; ultrasound transducer/scanner; urinary bladder.
- **CVS (adjacent panel):** vagina → cervix uteri → cervical canal → chorionic cavity; flexible transcervical cannula within the villous chorion; alternative transabdominal spinal-needle route; decidua; uterine cavity.

Non-invasive Methods & Prenatal Screening

Method	Timing	Principle	What it detects	Sensitivity / limitation
Ultrasonography	Any trimester; detailed anomaly scan ~18–20 wk	B-mode/real-time reflected echoes	Gestational age, number of fetuses, placental localisation (before amniocentesis/CVS), structural anomalies — anencephaly, spina bifida, cystic hygroma, nuchal translucency (NT), limb/facial defects, cardiac defects	Adjunct to invasive tests; a normal scan is reassuring but not exhaustive; abnormal karyotype rates rise steeply when a marker is one of <i>multiple</i> findings rather than isolated

Method	Timing	Principle	What it detects	Sensitivity / limitation
Maternal serum AFP (MSAFP)	16–18 weeks	AFP crosses from fetal circulation into maternal serum	Elevated in open NTD (and multiple pregnancy, abdominal wall defects); low in Down syndrome	~20% of open NTDs missed at usual cut-off (2 multiples of median)
First-trimester screen	11–13 weeks	Serum PAPP-A (↓ in all trisomies) + free β-hCG (↑ in T21, ↓ in T18/T13) + nuchal translucency (↑ in T21, T18, T13, 45,X)	Trisomy 21/18/13 risk	~85% sensitivity for T21 at 5% false-positive rate
Second-trimester quadruple screen	15–18 weeks	MSAFP + hCG + unconjugated estriol (uE3) + inhibin A	T21 (AFP↓, hCG↑, uE3↓, inhibin A↑) and other trisomies (all analytes ↓); NTD (AFP↑↑)	~81% sensitivity for T21; combined first + second trimester sequential screening raises sensitivity to ~95%
NIPT / NIPS (cell-free fetal DNA)	From ~10 weeks (placental cfDNA present in maternal serum from ~7 weeks)	Next-generation sequencing of cell-free DNA (2–10% fetal fraction) counts reads per chromosome; excess reads mapping to chr 13/18/21/X indicate trisomy/monosomy	Common autosomal trisomies, sex-chromosome aneuploidy, fetal sex, fetal RhD	Sensitivity >99% for T21, false-positive <1% — but it remains a screening , not diagnostic, test; positive results require invasive confirmation

Problems & Limitations in Interpretation

- **Mosaicism:** two or more cell lines in cultured cells — must be distinguished as *true* mosaicism (present in several independent primary cultures), *pseudomosaicism* (artefact confined to one culture/one cell), or *confined placental mosaicism* (present in CVS-derived placental tissue but not the fetus itself).
- **Maternal cell contamination:** more frequent with CVS than amniocentesis; may wrongly suggest a 46,XY/46,XX mosaic or mask the true fetal sex; resolved by genotyping polymorphisms or repeat amniocentesis.
- **Culture failure:** uncommon but time-critical; managed by repeating with amniocentesis (if CVS failed) or cordocentesis.

- ▶ **Screening tests are not diagnostic:** any positive maternal serum/USG/NIPT screen must be followed by a confirmatory invasive test (amniocentesis/CVS) before a couple is counselled about termination.

Applied / Clinical anatomy

- ▶ **Needle-path anatomy:** amniocentesis traverses anterior abdominal wall → parietal peritoneum → myometrium → amniotic cavity, with the needle path chosen by real-time ultrasound to avoid the placenta and fetal parts. CVS is performed either transcervically (through the external and internal os into the chorionic cavity) or transabdominally with a spinal needle, again under continuous ultrasound guidance.
- ▶ **PCPNDT Act** — in India, all prenatal diagnostic procedures are regulated by the Pre-Conception and Pre-Natal Diagnostic Techniques (Prohibition of Sex Selection) Act, 1994 (amended 2003), which permits amniocentesis/CVS/USG strictly for detection of genetic, chromosomal, or structural fetal abnormalities and explicitly **prohibits their use for sex determination or sex-selective termination**; registered centres must maintain records and display statutory notices, and violation is a criminal offence.
- ▶ **MTP Act correlation:** if an invasive/noninvasive test confirms a serious fetal anomaly, termination of the affected pregnancy is carried out under the Medical Termination of Pregnancy Act, and is more straightforward, medically and legally, when the diagnosis is reached in the **first trimester** (favouring CVS/PGD/NIPT) than in the second trimester (classical amniocentesis window).
- ▶ **Perinatal complications** to counsel about: after amniocentesis — neonatal respiratory distress (~3-fold increase), postural orthopaedic anomalies (talipes equinovarus, congenital hip dislocation), Rh sensitisation, antepartum haemorrhage; after CVS performed before 10 weeks — an early reported (though since largely unconfirmed in later series) association with limb-reduction defects.
- ▶ **Radiological correlation:** soft markers seen on the anomaly scan (increased nuchal translucency/cystic hygroma, duodenal atresia, choroid-plexus cysts, echogenic bowel, ventriculomegaly, cardiac defects) correlate with a substantially higher rate of an abnormal karyotype when they occur as *multiple* findings rather than in isolation — this stratifies which pregnancies proceed to invasive testing.

High-yield exam pointers

- ▶ Prenatal **diagnosis** (known high-risk pregnancy, definitive answer) vs prenatal **screening** (all pregnancies, risk estimate only) — a favourite distinction to define first.
- ▶ Five general pre-requisites before offering prenatal diagnosis: no treatment / accurate test available / high risk / severe disorder / termination acceptable to couple.
- ▶ Amniocentesis: 14–16 (up to 20) weeks; CVS: 8–10 (up to 12–13) weeks — CVS gives an earlier, first-trimester answer but carries marginally higher risk.
- ▶ AFP: raised in amniotic fluid/maternal serum in open NTD; lowered in maternal serum in Down syndrome.

- First-trimester screen = PAPP-A + free β -hCG + nuchal translucency (11–13 wk); second-trimester quadruple screen = AFP + hCG + uE3 + inhibin A.
- NIPT (cell-free fetal DNA) — highly sensitive (>99% for T21) screening test, not a substitute for diagnostic karyotype/CMA.
- PGD uses blastomere (8–16 cell, day 3) or blastocyst (trophectoderm, day 5–6) biopsy — key application: X-linked disorders like DMD.
- Fetal blood sampling (cordocentesis) is reserved for haematological disorders (sickle cell, thalassaemia, haemophilia, DMD) or when culture from CVS/amniocentesis fails.
- PCPNDT Act, 1994 — Indian legal safeguard: prenatal techniques permitted only for genetic/anomaly detection, never for sex selection.

SOURCE & COVERAGE

Gangane — Human Genetics 5e (primary — Ch. "Prenatal Diagnosis": indications, amniocentesis/CVS/fetoscopy/fetal blood sampling/preimplantation diagnosis technique, timing, and risk figures; PCPNDT Act); Thompson & Thompson — Genetics in Medicine 8e (Ch. "Prenatal Diagnosis and Screening": diagnosis-vs-screening framework, principal indications box, CVS/amniocentesis complication rates, first-/second-trimester analyte screening, cell-free fetal DNA/NIPS, mosaicism and interpretation issues).

♦ ♦ ♦

Describe the numerical and structural aberrations of chromosomes in brief

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction A **chromosomal aberration (chromosomal mutation)** is any deviation from the normal diploid human karyotype (46 chromosomes, 23 pairs) in either chromosome **number** or chromosome **structure**, arising from errors of cell division or from chromosome breakage. Aberrations are broadly divided into **numerical (genomic) aberrations** — a change in the total chromosome count (polyploidy or aneuploidy) — and **structural aberrations** — a change in the architecture of one or more individual chromosomes (deletion, duplication, inversion, translocation, ring, isochromosome, etc.) following breakage and abnormal reunion. Together they are the single largest identifiable cause of pregnancy loss, congenital malformation, and intellectual disability in humans; overall, some form of chromosome abnormality is found in about **1 in 154 newborns (0.65%)**.

1. Classification of chromosomal aberrations



Describe the numerical and structural aberrations of chromosomes in brief

- Both categories can further be present in **all** cells of the individual (**constitutional**) or in only a proportion of cells (**mosaicism**), arising from post-zygotic mitotic non-disjunction or anaphase lag.

- Structural rearrangements are also classed as **stable** (deletion, inversion, translocation, isochromosome — pass through cell division intact) or **unstable** (a true dicentric with two active centromeres pulled to opposite poles, and the ring chromosome — both prone to anaphase bridging/breakage-fusion cycles). A dicentric in which one centromere is epigenetically inactivated behaves as a functionally monocentric, mitotically **stable** "pseudodicentric" — the common clinical form.

2. Numerical aberrations

Numerical aberrations affect the **total chromosome count**. They are of two kinds:

(a) **Euploid variation** — a change by a whole haploid (n) set:

- **Triploidy ($3n = 69$)**: the commonest euploid abnormality, seen in 1–3% of recognised conceptions; most often from **dispermy** (fertilisation of one ovum by two sperm), occasionally from failure of a meiotic division producing a diploid gamete. An extra **paternal** haploid set gives a degenerative, cystic placenta (**partial hydatidiform mole**) with a small fetus; an extra **maternal** set is usually aborted very early. Rare liveborns do not survive long.
- **Tetraploidy ($4n = 92$)**: karyotype **92,XXXX** or **92,XXYY**; results from failure of completion of an early cleavage division of the zygote; seen only in early abortuses.

(b) **Aneuploidy** — gain or loss of one (or a few) individual chromosomes, not a whole set. It is the **most common and clinically most significant** type of chromosome disorder, found in at least 5% of all clinically recognised pregnancies.

Type	Definition	Mechanism	Example / incidence
Trisomy ($2n+1$)	Three copies of one chromosome	Meiotic non-disjunction	Trisomy 21 (Down) 1 in 850; Trisomy 18 (Edward) 1 in 6000–8000; Trisomy 13 (Patau) 1 in 12,000–20,000
Monosomy ($2n-1$)	One copy only of a chromosome	Non-disjunction / anaphase lag; almost always lethal for autosomes	45,X — Turner syndrome , 1 in 2500–4000 female births (an important exception to lethality)
Sex-chromosome aneuploidy	Extra/missing X or Y	Non-disjunction	47,XXY Klinefelter 1 in 600 male births; 47,XYY 1 in 1000 male births; 47,XXX 1 in 1000 female births

Mechanism of aneuploidy — non-disjunction:

- **Meiotic non-disjunction**: a pair of homologous chromosomes (meiosis I) or a pair of sister chromatids (meiosis II) fails to disjoin (separate) properly at anaphase; the resulting gametes have either 24 or 22 chromosomes instead of 23.

- ▶ Meiosis-I non-disjunction → the extra chromosome carries **both** parental homologues; meiosis-II non-disjunction → the extra chromosome is **two copies of one** parental homologue.
- ▶ A chromosome pair with **too few, or mis-positioned (too close to centromere/telomere), recombination events** in meiosis I is more prone to non-disjunction.
- ▶ **Mitotic non-disjunction** occurring after zygote formation produces two different cell lines in one individual — **mosaicism** — rather than a uniform aneuploid state.
- ▶ Autosomal trisomy is only occasionally compatible with life; the three autosomes seen as trisomies in liveborns (13, 18, 21) are, notably, those with the **fewest genes**. Whole-chromosome **monosomy is almost always lethal**, the X chromosome (Turner syndrome) being the important exception.
- ▶ Incidence context: overall newborn chromosome-abnormality rate **1 in 154**; unbalanced structural rearrangements **1 in 1600**; balanced structural rearrangements **1 in 500**.

3. Structural aberrations

Structural aberrations arise from **chromosome breakage** (caused by ionising radiation, mutagenic chemicals, or viruses) followed by **abnormal reunion/reconstitution** of the broken ends, rather than by a change in chromosome number. They are classed as **balanced** (full genomic complement present, just rearranged — usually no phenotypic effect in the carrier) or **unbalanced** (extra or missing material — usually phenotypically abnormal, from gene-dosage imbalance/haploinsufficiency).

embed: ![\[Fig 4.5 — Structural aberrations in chromosomes: \(A\) Insertion, \(B\) Pericentric inversion, \(C\) Paracentric inversion. X, Y, Z in \(A\) indicate sites of break. \(Gangane — Human Genetics — 5e, p.88\)\]](#)
(C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/ff947fef97f79224.jpg)

Type	Mechanism	Consequence	Example / note
Deletion (terminal / interstitial)	Loss of a chromosome segment	Partial monosomy; haploinsufficiency	Cri-du-chat (5p-; ~1 in 15,000); autosomal deletions overall ~1 in 7000 live births
Duplication	Gain of a chromosome segment	Partial trisomy; usually less harmful than deletion, but breakpoint may disrupt a gene	Reciprocal of a deletion elsewhere in the family
Inversion — paracentric	Two breaks in the same arm ; centromere excluded from inverted segment	Balanced in carrier; unbalanced meiotic recombinants are acentric/dicentric → usually inviable , so risk of an abnormal liveborn is very low	

Type	Mechanism	Consequence	Example / note
Inversion — pericentric	One break in each arm ; centromere included	Balanced in carrier; recombination within the pairing loop → gametes with duplication + deficiency → 5–10% risk of unbalanced child	
Reciprocal translocation	Breakage and reciprocal exchange of segments between two non-homologous chromosomes	Balanced in carrier (forms a meiotic quadrivalent); alternate segregation → balanced gametes; adjacent segregation → unbalanced gametes	Philadelphia chromosome t(9;22) — BCR-ABL fusion, chronic myeloid leukaemia
Robertsonian translocation	Fusion of two acrocentric chromosomes near the centromere with loss of the (redundant, satellite-DNA-rich) short arms	Balanced despite only 45 chromosomes ; commonest human chromosome rearrangement	rob(13;14) ~1 in 1300; rob(14;21) carriers risk translocation Down syndrome
Insertion	Three breaks — a segment is excised and inserted into a non-homologous chromosome	Rare (needs 3 breaks); abnormal segregation risk up to 50%	
Ring chromosome	Two breaks with fusion of the broken (telomere-less) ends into a ring	Mitotically unstable — sister chromatids tangle at anaphase → frequent mosaicism	
Isochromosome	Transverse (rather than longitudinal) centromere division — one arm lost, the other duplicated in mirror image	Partial monosomy of one arm + partial trisomy of the other	i(Xq) — a recognised variant karyotype in Turner syndrome
Dicentric chromosome	Fusion, end to end, of two centromere-bearing fragments	Usually mitotically stable (pseudodicentric — one centromere inactivated)	Often arises via Robertsonian translocation or involves sex chromosomes
Marker / supernumerary chromosome	Small, unidentified extra chromosome, often mosaic	Risk ranges from negligible to very high depending on origin	Prenatal frequency ≈ 1 in 2500; commonly derived from chromosome 15 or the sex chromosomes

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Reproduce a single labelled schematic of a **normal chromosome** (A–B–C–D, centromere marked •) alongside the structural derivatives, each clearly labelled:

- ▶ **Normal chromosome** — bands A-B-C-D, centromere shown between B and C
- ▶ **Terminal deletion** — loss of segment D (acentric fragment lost)
- ▶ **Interstitial deletion** — loss of segment C only, ends re-joined
- ▶ **Duplication** — segment B repeated (A-B-B-C-D)
- ▶ **Paracentric inversion** — segment B-C inverted, centromere outside the inverted segment
- ▶ **Pericentric inversion** — segment inverted across the centromere
- ▶ **Reciprocal translocation** — segments exchanged between two labelled non-homologous chromosomes ("chromosome 1" / "chromosome 2")
- ▶ **Robertsonian translocation** — two acrocentric chromosomes (e.g. 14 and 21) fused at the centromere, short arms lost
- ▶ **Ring chromosome** — both telomeric ends lost, ends fused into a ring
- ▶ **Isochromosome** — one arm duplicated as a mirror image, other arm absent

5. Cytogenetic (ISCN) nomenclature — brief

Symbol	Meaning	Example
p / q	Short arm / long arm	5p–, 21q
+ / –	Gain of / loss of a chromosome	47,XX,+21 (trisomy 21); 45,XY,–22
del	Deletion	46,XX,del(5)(q13)
dup	Duplication	
inv	Inversion	inv(3)(p25q21) — pericentric
t	Translocation	46,XX,t(2;8)(q22;p21)
rob	Robertsonian translocation	rob(14;21)(q10;q10)
r	Ring chromosome	46,X,r(X)
dic	Dicentric chromosome	dic(X;Y)
mar	Marker chromosome	47,XX,+mar
mos / "/"	Mosaicism	46,XX/47,XX,+21

Applied / Clinical anatomy

- ▶ **Down syndrome** is the paradigm of aneuploidy and shows all three mechanisms of trisomy: ~95% free trisomy 21 (meiotic non-disjunction — ~90% maternal, most often meiosis I, with risk rising sharply after maternal age 30; ~10% paternal); ~4% a Robertsonian translocation, usually rob(14;21) or rob(21;22), unrelated to maternal age but carrying a **high recurrence risk** if a parent (especially the mother) is a balanced carrier; ~2% mosaic trisomy 21, often with a

milder, more variable phenotype. Features: hypotonia, flat occiput/brachycephaly, epicanthal folds, Brushfield spots, single palmar crease, clinodactyly, congenital heart disease (~1/3 of patients), duodenal atresia, a **15-fold increase in leukaemia risk**, and premature Alzheimer-type dementia; life expectancy today is over 50 years.

- ▶ **Turner syndrome (45,X)**: commonest monosomy compatible with life; short stature, webbed neck, lymphoedema of hands/feet, coarctation of the aorta; an **isochromosome i(Xq)** is a recognised structural variant. 45,X is also the single commonest karyotype in **spontaneous abortions**.
- ▶ **Klinefelter syndrome (47,XXY)**: tall stature, hypogonadism, gynaecomastia — the classic example of sex-chromosome aneuploidy in males.
- ▶ **Cri-du-chat syndrome**: a **terminal/interstitial deletion of chromosome 5p**, giving a characteristic cat-like cry in infancy, microcephaly, hypertelorism, and intellectual disability — the classic teaching example of a deletion syndrome.
- ▶ **Philadelphia chromosome**: a **reciprocal translocation t(9;22)** producing a shortened chromosome 22 (BCR–ABL fusion gene) — the classic example linking a structural rearrangement to a malignancy, **chronic myeloid leukaemia**.
- ▶ **Prader-Willi / Angelman syndromes**: both can result from the same 15q11–q13 microdeletion; the phenotype produced depends on the **parent of origin** of the deleted chromosome (genomic imprinting) — paternal deletion → Prader-Willi, maternal deletion → Angelman.
- ▶ **Genetic counselling and prenatal diagnosis**: karyotyping, FISH, and chromosomal microarray are used to distinguish free trisomy from translocation trisomy (critical for recurrence-risk counselling), and to identify balanced-translocation or inversion carriers among couples with recurrent pregnancy loss or infertility.

High-yield exam pointers

- ▶ Numerical = change in chromosome **number** (euploid — triploidy/tetraploidy; or aneuploid — trisomy/monosomy); Structural = change in chromosome **architecture** after breakage (deletion, duplication, inversion, translocation, ring, isochromosome, dicentric).
- ▶ **Non-disjunction** (meiosis I, meiosis II, or mitosis) is the chief mechanism of aneuploidy; mitotic non-disjunction after zygote formation gives **mosaicism**.
- ▶ Trisomy 21 (1/850) > Trisomy 18 (1/6000–8000) > Trisomy 13 (1/12,000–20,000) in live-birth frequency; all three trisomic autosomes have the fewest genes among autosomes.
- ▶ **Paracentric** inversion → inviable recombinants → very low risk to offspring; **pericentric** inversion → duplication-deficiency gametes → 5–10% risk.
- ▶ **Reciprocal** translocation = exchange between two non-homologous chromosomes, no change in chromosome number; **Robertsonian** translocation = fusion of two acrocentric chromosomes with loss of short arms, **45 chromosomes**, balanced.
- ▶ Down syndrome mechanism split: **95% trisomy 21** : **4% Robertsonian translocation** : **1–2% mosaic** — translocation form alone shows no maternal-age link but a high familial recurrence risk.
- ▶ Isochromosome = one arm lost, other duplicated as a mirror image — classic example i(Xq) in Turner syndrome.

- Cri-du-chat (deletion) and the Philadelphia chromosome (reciprocal translocation → CML) are the standard clinical examples of structural aberrations.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e. Coverage note: this is a cytogenetics topic within Paper III's Genetics component; Gray's Anatomy (Standring) 41e has no equivalent content, so grounding was drawn entirely from the specialty genetics texts per this topic's specialty designation.

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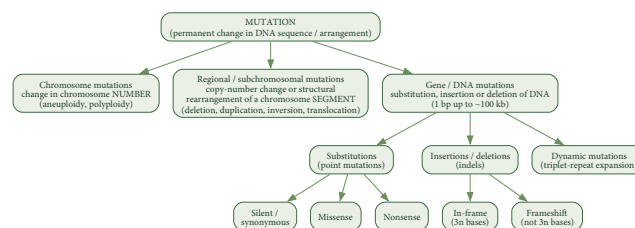
Discuss in detail about mutations

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction A **mutation** is a permanent, heritable change in the nucleotide sequence or arrangement of DNA, ranging from a single base substitution to an alteration of an entire chromosome. The term refers strictly to the change in the DNA — not to the individual carrying it — and carries no built-in connotation of harm: most mutations are functionally silent, and only a minority cause disease. A DNA locus is called **polymorphic** when a variant allele is present at a frequency greater than 1% in a population; rarer variants remain simply "mutations." Mutations are the raw material of both human genetic diversity and inherited disease, and understanding their origin, classification and molecular consequences underlies all of clinical and medical genetics.

1. Classification of mutations by level (size-based)

Mutations are conventionally divided into three levels, depending on how much of the genome is altered:



Discuss in detail about mutations

- **Chromosome mutations** — leave individual chromosomes intact but change the total chromosome *number* (numerical aberrations: aneuploidy, polyploidy).
- **Regional (subchromosomal) mutations** — alter only part of a chromosome: a copy-number change of a segment, or a structural rearrangement (deletion, duplication, inversion, translocation) between one or more chromosomes.
- **Gene/DNA mutations** — alterations of the DNA sequence itself (substitution, deletion, insertion), from a single nucleotide up to an arbitrary limit of ~100 kb; this is the principal focus of "mutation" in the molecular sense.

2. Origin of mutations — mechanisms and cell of origin

A. Mechanism of origin

- ▶ **DNA replication errors** — DNA polymerase mis-incorporates a non-complementary base roughly once every 10 million bp; proofreading corrects >99.9% of these, so the net replication-error rate is only $\approx 1 \times 10^{-10}$ per base per cell division.
- ▶ **Failure to repair DNA damage** — 10,000 to 1,000,000 nucleotides are damaged per cell per day by spontaneous processes (depurination, demethylation, deamination), by environmental chemical mutagens, and by ultraviolet or ionising radiation. Damage that escapes or is mis-repaired becomes a permanent mutation.
- ▶ **CpG "hot spots"** — spontaneous deamination of 5-methylcytosine to thymine at CpG dinucleotides produces C→T (or G→A) changes at a rate 25 times higher than other single-nucleotide changes; more than 30% of all point mutations arise this way.
- ▶ **Insertional mutagenesis by mobile elements** — retrotransposition of *Alu*/LINE repeat sequences can insert into an exon and inactivate a gene (e.g. LINE insertion into the factor VIII gene causing haemophilia A).

B. Germline vs somatic mutations

- ▶ A **germline (constitutional) mutation** arises in a germ cell; once transmitted, it is present in every cell of the offspring's body and is heritable.
- ▶ A **somatic mutation** arises after fertilisation in any body cell, is confined to the clone of cells derived from it, and is *not* transmitted to offspring. Because somatic mutations are usually diluted among millions of unaffected cells they are normally undetectable — the major exception being **cancer**, where 1,000–10,000 (sometimes many more) somatic mutations accumulate clonally in a tumour genome, with mutation frequency and pattern specific to the tumour type.

3. Molecular types of gene mutations and their functional consequences

Type	Mechanism	% of disease-causing mutations
Silent (synonymous) substitution	Base change does not alter the encoded amino acid (degeneracy of the genetic code)	—
Missense substitution	Changes the codon to specify a <i>different</i> amino acid	$\approx 50\%$
Nonsense substitution	Changes a sense codon to a premature stop (termination) codon → truncated, usually degraded protein (nonsense-mediated mRNA decay)	$\approx 10\%$
RNA-processing mutations	Destroy/alter consensus splice, cap, or polyadenylation sites, or create cryptic sites	$\approx 10\%$
Splice-site mutations	Abolish or shift a donor/acceptor splice site → frameshift + premature stop	$\approx 10\%$

Type	Mechanism	% of disease-causing mutations
Long-range regulatory mutations	Affect promoter/enhancer sequences → altered gene expression	Rare
Small indels	Insertion/deletion of a few bases; frameshift if not a multiple of 3 (alters all downstream codons until a new stop is reached); in-frame if a multiple of 3	≈25%
Larger deletions/inversions/duplications/fusions	Homologous recombination between repeat sequences, or non-homologous end-joining after double-strand breaks	≈5%
Mobile-element insertion	<i>Alu</i> /LINE retrotransposition into a gene	Rare
Dynamic mutations	Expansion of a trinucleotide/tetranucleotide repeat during gametogenesis, beyond the normal range seen in microsatellite polymorphism	Rare

4. Mutation rates and the factors influencing them

- ▶ The overall new-mutation rate, measured directly by whole-genome sequencing of parent–child trios, is $\approx 1.2 \times 10^{-8}$ mutations per base pair per generation — so every person inherits **≈75 new point mutations** not present in either parent.
- ▶ **Disease-locus mutation rates** vary over a 1000-fold range (10^{-4} to 10^{-7} per locus per generation); the **median gene mutation rate** is $\approx 1 \times 10^{-6}$. With ≈ 5000 genes currently known to cause disease when mutated, roughly **1 in 200 people** receives a new disease-causing mutation in a known gene from a parent.
- ▶ Copy-number variants (CNVs) arise by recombination, not replication error, at a far higher rate ($\approx 1.2 \times 10^{-2}$ per locus per generation) than point mutations.
- ▶ **Achondroplasia** illustrates a classic mutation-rate calculation: 7 achondroplastic children born to normal-stature parents among 242,257 births gives a rate of $\approx 1.4 \times 10^{-5}$ mutations/locus/generation — one of the highest known — because virtually all cases share the identical **FGFR3** mutation 1138G>A (Gly380Arg, codon 380), a CpG deamination hot spot.
- ▶ **Sex and age effects:** sperm DNA has undergone far more replication cycles than ova, so many gene mutations (e.g. achondroplasia) arise almost exclusively in the **paternal germline**, and their frequency rises with **increasing paternal age**.

5. Clinical examples correlating mutation type with disease

Disease	Gene/locus	Type/mechanism of mutation	Rate (per locus/generation)
Achondroplasia	<i>FGFR3</i>	Missense (1138G>A, Gly380Arg), CpG hot spot	1.4×10^{-5}
Sickle cell disease	<i>HBB</i> (β -globin)	Missense, GAG→GTG (Glu6Val)	—
Haemophilia A	<i>F8</i>	~45% of severe cases: intron-22 inversion by intrachromosomal homologous recombination; also LINE insertional mutagenesis	$3.2\text{--}5.7 \times 10^{-5}$
Duchenne muscular dystrophy	<i>DMD</i>	Large deletions → frameshift (loss of dystrophin)	$3.5\text{--}10.5 \times 10^{-5}$
Neurofibromatosis type 1	<i>NF1</i>	Heterogeneous point and structural mutations	$4\text{--}10 \times 10^{-5}$
Retinoblastoma	<i>RB1</i>	Point mutations/deletions (two-hit)	$5\text{--}12 \times 10^{-6}$
Aniridia	<i>PAX6</i>	Point mutations	$2.9\text{--}5 \times 10^{-6}$
Hereditary non-polyposis colon cancer	DNA mismatch-repair genes	Loss-of-function mutation in a genome-integrity gene	—

The **sickle cell** example is the classic exam illustration of a missense point mutation with a visible cellular phenotype:

embed: ![Fig 11.5 — Scanning electron micrographs of red cells from a patient with sickle (Thompson & Thompson — Genetics in Medicine — 8e, p.205)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/a11c74323dcd646d.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A three-tier schematic of the sickle-cell missense mutation (DNA → protein → phenotype), labelled:

- ▶ **Normal:** β -globin codon 6 = GAG → glutamic acid (Glu) → normal HbA → biconcave discoid RBC (oxygenated, round)
- ▶ **Mutant:** β -globin codon 6 = GTG (single A→T substitution) → valine (Val) → HbS → on deoxygenation, HbS polymerises → RBC deforms to the classic **sickle shape**
- ▶ Arrows connecting the three tiers (gene → protein → cell) to show how one base change propagates to a visible structural change

Applied / Clinical anatomy

- ▶ **Prenatal and carrier diagnosis:** known disease mutations (e.g. *HBB*, *F8*, *DMD*, *FGFR3*) are targeted directly by DNA sequencing/PCR for carrier testing and prenatal diagnosis.
- ▶ **Genome-integrity gene mutations predispose to further mutation and cancer:** loss-of-function mutations in DNA mismatch-repair genes cause hereditary non-polyposis colon cancer (Lynch syndrome); *BRCA1/BRCA2* mutations (also genome-integrity genes) predispose to breast and ovarian cancer — illustrating that a mutation in a repair gene compounds itself by allowing further mutations to accumulate.
- ▶ **Somatic mutation burden in cancer:** 1,000–10,000+ somatic mutations typically accumulate in an adult tumour genome; sequencing this somatic mutation profile increasingly guides targeted cancer therapy (e.g. anti-BRAF drugs are effective only in BRAF-mutated tumours of certain tissue types).
- ▶ **Forensic/identity use of non-disease polymorphisms:** although not mutations causing disease, the same mutational processes that generate microsatellite/indel polymorphisms underlie DNA fingerprinting (e.g. the FBI CODIS 13-locus panel) — a useful contrast point between "mutation" (any change) and "polymorphism" (a common, usually neutral, variant).
- ▶ **Genetic counselling:** paternal-age-related mutation risk (e.g. achondroplasia, some autism-associated CNVs) is now a recognised counselling point in couples with an older father.

High-yield exam pointers

- ▶ **Mutation** = permanent change in DNA sequence/arrangement; a variant is a **polymorphism** only once its frequency exceeds 1% in a population.
- ▶ Three structural levels: **chromosome** (number) → **regional/subchromosomal** (segment rearrangement) → **gene/DNA** (sequence change).
- ▶ Point-mutation types: **silent, missense, nonsense**; indel types: **in-frame vs frameshift** (frameshift = not a multiple of 3 bp).
- ▶ **Germline** mutations are heritable (present in all body cells); **somatic** mutations are not transmitted but are the basis of cancer's clonal mutation burden.
- ▶ **CpG dinucleotides are mutational hot spots** (5-methylcytosine deamination) — responsible for >30% of point mutations, and for the recurrent *FGFR3* mutation in achondroplasia.

- ▶ Classic quantitative facts: overall new-mutation rate $\approx 1.2 \times 10^{-8}$ /bp/generation (~ 75 new mutations/genome); median disease-gene mutation rate $\approx 1 \times 10^{-6}$ /locus/generation; CNV formation rate is $\sim 10^6$ -fold higher than point-mutation rate.
- ▶ Achondroplasia (FGFR3, Gly380Arg) and sickle cell disease (HBB, Glu6Val) are the two standard worked examples of missense point mutations.
- ▶ New gene mutations are usually **paternal in origin** and increase with **paternal age**.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e. Coverage note: Gray's Anatomy (Standing) 41e has no equivalent content — mutation is a molecular/classical genetics topic, not gross anatomy — so grounding was drawn entirely from the genetics texts in the library, per this topic's GENETICS specialty designation.



Discuss the role and significance of blood groups in genetics

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction A **blood group** is a genetically determined pattern of antigens (agglutinogens) carried on the red-cell membrane (and, in some systems, in secretions and other tissues), each system being the phenotypic expression of one or more alleles at a single gene locus. Because blood-group antigens are fixed at birth, inherited in strict Mendelian ratios, unaffected by age or environment, and vary in frequency between populations, they were the earliest reliable **genetic markers** available to human geneticists — long before DNA-based markers existed. The ABO and Rh systems remain the standard textbook illustrations of **multiple allelism** and **codominance**, while the blood groups collectively have shaped linkage analysis, population/anthropological genetics, twin-zygosity determination, forensic identity testing, and transfusion/transplantation medicine.

1. Blood groups as genetic markers — why they qualify

A red-cell antigen system is useful as a genetic marker only if it satisfies four criteria:

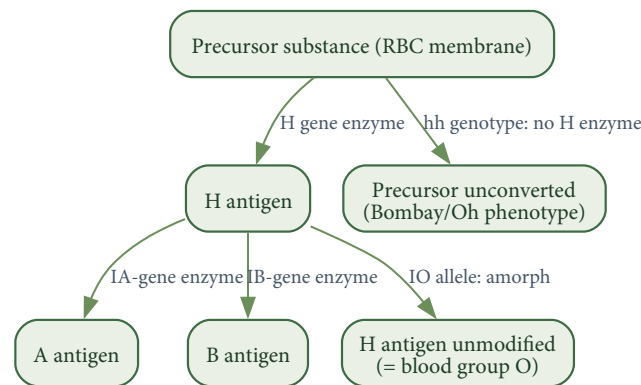
- ▶ It produces **distinct, discontinuous phenotypes** (not a continuously variable trait).
- ▶ It follows a **simple (Mendelian) pattern of inheritance**.
- ▶ Its **gene frequency differs between populations**, making it useful for population and migration studies.
- ▶ It is **not influenced by age, sex, diet, or environment** — the genotype is read directly and permanently from the phenotype.

Clinically, blood-group polymorphisms (chiefly ABO, Rh and HLA) matter in blood **transfusion**, **tissue/organ transplantation**, and **haemolytic disease of the newborn (HDN)**.

2. Genetics of the ABO blood-group system — the classic example of multiple allelism and codominance

- ▶ The ABO locus lies on the **long arm of chromosome 9**, with **three alleles** — **IA, IB and IO** — at the same locus (**multiple allelism**).

- ▶ **IA and IB are codominant:** when both are present, both A and B antigens are expressed together (blood group AB). **IO is an amorph** (recessive) — it produces no detectable antigen.
- ▶ The three genotypes IAIA/IAIO, IBIB/IBIO, IAIB and IOIO give the **four phenotypes A, B, AB and O**, with corresponding serum antibodies (a person never has an antibody against his own antigen): group A → anti-B; group B → anti-A; group AB → no antibody; group O → both anti-A and anti-B.
- ▶ **Biosynthetic basis:** the A and B antigens are built on a common **H-antigen precursor** on the red-cell membrane. The **H gene** converts the precursor to H substance; the **IA-gene enzyme** adds N-acetylgalactosamine to H substance to form the A antigen, and the **IB-gene enzyme** adds D-galactose to form the B antigen. The **IO allele has no enzymatic activity**, so H substance remains unmodified — this unmodified H substance is what is detected as blood group O.



Discuss the role and significance of blood groups in genetics

- ▶ **Subtypes:** A further divides into **A1 and A2** (A1 ≈ 85% of group-A individuals); AB similarly subdivides into A1B and A2B.
- ▶ **Bombay (Oh) phenotype:** individuals homozygous for a rare recessive allele **h** (genotype **hh**) cannot synthesise H substance at all, so the IA/IB enzymes have no substrate to act on — red cells and secretions test negative for A, B **and** H antigens even though IA/IB genes may be present, and the serum contains anti-A, anti-B **and** anti-H. This is a classic exam example of **epistasis** (one locus masking the expression of another) and a transfusion trap — an Oh individual can be transfused safely only with Oh blood.
- ▶ **Secretor status:** the ability to secrete soluble A, B and H antigens into saliva and other body fluids is governed by a separate **secretor (Se) gene on chromosome 19**; genotype SeSe/Sese = secretor (~78% of people), sese = non-secretor (antigens present on red cells but absent from secretions).

□ Fig 34.1 — ABO blood group system showing antigens and antibodies (IB Singh — Textbook of Anatomy Vol 3 — 6e, p.492)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the **ABO antigen–antibody scheme**: four columns (A, B, AB, O); in each column show the RBC with its surface antigen(s) (A antigen, B antigen, both, or neither) and the corresponding serum antibody (anti-B, anti-A, none, or both anti-A + anti-B). Label the ABO locus on chromosome 9 and the three alleles I^A , I^B , I^O alongside.

3. Genetics of the Rh blood-group system

- ▶ Discovered by **Landsteiner and Wiener in 1940** using Rhesus-monkey red cells (hence the name); central to its genetics is the **D antigen** — **Rh-positive** persons are homozygous or heterozygous for the gene specifying antigen D, **Rh-negative** persons lack it.
- ▶ The Rh locus is on **chromosome 1**. **Fisher and Race** described **five antigens** — D, C, E, c, e — encoded by closely linked genes, giving **eight common haplotypes (alleles)**:

Allele (Fisher-Race)	Antigens carried
R1	D, C, e
R2	D, c, E
R0	D, c, e
Rz	D, C, E
r	c, e
r'	C, e
r''	c, E
ry	C, E

- ▶ **Rh-null phenotype**: homozygosity for a modifier gene results in complete absence of all Rh antigens (and of the related LW antigen) from the red cell; because Rh antigens are an integral structural component of the red-cell membrane, Rh-null individuals develop a chronic **haemolytic anaemia** — first described in Australian aborigines.
- ▶ **Clinical significance — haemolytic disease of the newborn (HDN)**: in a Rh-negative mother carrying a Rh-positive foetus, foetal red cells crossing placental breaks (increasing towards term) evoke maternal anti-D antibody formation; these IgG antibodies cross the placenta and destroy foetal red cells, provoking compensatory release of immature erythroblasts (**erythroblastosis foetalis**) and, after birth, hyperbilirubinaemia that risks kernicterus. Sensitisation is prevented by giving **Rh immunoglobulin (anti-D)** to the Rh-negative mother during pregnancy and within hours of delivery, destroying foetal Rh-positive cells before they can immunise her.

□ Fig 8.2 — *Genesis of haemolytic disease of newborn (HDN)*. (Gangane — *Human Genetics* — 5e, p.159)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a **flow scheme of HDN pathogenesis**: Rh-negative mother + Rh-positive foetus → foetal RBCs cross placental breaks (late pregnancy/delivery) → maternal anti-D antibody formation → antibody crosses placenta → foetal RBC destruction → compensatory erythroblastosis + postnatal hyperbilirubinaemia → kernicterus if untreated. Add a side box for the anti-D immunoglobulin prophylaxis step.

4. Other blood-group systems and what they illustrate genetically

System	Locus	Alleles / antigens	Genetic principle illustrated
MN	Chromosome 4	LM, LN (M and N antigens)	Second classic example of codominance — LMLN individuals express both M and N antigens
Kell	Precursor gene <i>Xk</i> on short arm of X chromosome	K-positive / K-negative	X-linked precursor locus; its mutation causes phagocytic dysfunction in some cases of chronic granulomatous disease
Duffy	Chromosome 1	Fya, Fyb, Fy0	The Fy(a–b–) genotype is resistant to <i>Plasmodium vivax</i> — a textbook example of a blood-group allele under natural selection by an infectious disease
Lutheran	Chromosome 19	Lua, Lub	First example of proven autosomal linkage and crossing-over in humans — Mohr (1951) showed linkage between the Lutheran locus and the secretor locus
Xg	X chromosome (short arm)	Xg(a+) / Xg(a–)	Used as an X-linked genetic marker ; also an example of X-linked dominant inheritance ; one of the few X-loci that escapes X-inactivation

5. Significance of blood groups in genetics — the union of applications

- Teaching models of Mendelian principles: ABO = standard example of **multiple allelism**; AB and MN genotypes = standard examples of **codominance**; Xg blood group = standard example of **X-linked dominant inheritance**.

- ▶ **Linkage analysis and gene mapping:** blood-group loci gave the first proof of linkage in humans — the Lutheran–secretor linkage was used to build early human gene maps before DNA markers existed.
- ▶ **Population and anthropological genetics:** ABO, Rh and other blood-group allele frequencies differ characteristically between populations and are unaffected by environment, so they have long been used to study racial admixture and migration.
- ▶ **Natural selection:** the Duffy-null genotype's resistance to *P. vivax* malaria shows a blood-group allele under selection in malaria-endemic regions, paralleling the sickle-cell/malaria relationship.
- ▶ **Twin zygosity determination:** monozygotic twins, sharing identical DNA, show strong concordance of blood groups; typing was historically used (before DNA fingerprinting) to help distinguish monozygotic from dizygotic twins.
- ▶ **Medico-legal/forensic genetics:** codominant blood-group alleles gave the earliest genetic test for **disputed paternity**, by excluding a putative parent whose group could not have contributed the child's antigens — a role now largely taken over by DNA (STR) profiling, though blood grouping remains its historical foundation.
- ▶ **A related polymorphic marker system — HLA:** the chromosome-6 HLA complex is genetically analogous (multiple closely-linked, highly polymorphic loci inherited en bloc, showing linkage disequilibrium) and is used similarly for transplant tissue-typing and disease-association studies (e.g., HLA-B27 with ankylosing spondylitis).

Applied / Clinical anatomy

- ▶ **Blood transfusion:** ABO/Rh compatibility is confirmed by grouping and cross-matching; group O is the theoretical universal donor, AB the universal recipient, but same-group transfusion is preferred in practice.
- ▶ **Bombay phenotype pitfall:** an Oh individual's serum contains anti-A, anti-B and anti-H, so even "compatible" O blood is haemolysed — transfusion is possible only with Oh blood.
- ▶ **Organ transplantation:** graft survival depends on donor–recipient histocompatibility — ABO compatibility is checked alongside HLA typing to minimise rejection.
- ▶ **Haemolytic disease of the newborn:** the key clinical consequence of Rh incompatibility, preventable by anti-D immunoglobulin prophylaxis.
- ▶ **Disease associations:** excess of group A among gastric-carcinoma patients, and of group O among duodenal-ulcer patients; group A also shows increased thrombotic tendency, relevant in women on oral contraceptives.

High-yield exam pointers

- ▶ ABO locus: chromosome 9; alleles IA, IB (codominant), IO (amorph/recessive); four phenotypes A, B, AB, O.
- ▶ H-antigen precursor pathway: H gene → H antigen → IA/IB enzymes add sugars to form A/B antigens; IO leaves H antigen unmodified.
- ▶ Bombay phenotype = genotype hh, no H substance, classic exam example of epistasis; anti-A, anti-B and anti-H all present.
- ▶ Secretor gene (Se) on chromosome 19 governs secretion of ABH antigens into saliva/secretions.

- ▶ Rh locus: chromosome 1; Fisher-Race D, C, E, c, e antigens, 8 haplotypes; Rh-negative mother + Rh-positive foetus → HDN; prevent with anti-D immunoglobulin.
- ▶ Codominance classic examples: AB blood group, MN blood group. X-linked dominant classic example: Xg blood group.
- ▶ Lutheran–secretor linkage = first proven human autosomal linkage (Mohr, 1951) — historic basis of human gene mapping.
- ▶ Duffy-null resistance to *P. vivax* = classic example of natural selection acting on a blood-group allele.
- ▶ Disease associations: gastric carcinoma with group A; duodenal ulcer with group O.

SOURCE & COVERAGE

Gangane — Human Genetics 5e (Genetics of Blood Groups chapter; Modes of Inheritance chapter); IB Singh — Textbook of Anatomy Vol 3 6e (Codominance chapter; Alleles chapter; Twinning chapter); Thompson & Thompson — Genetics in Medicine 8e (forensic/population-genetics applications of genetic markers).



Down's syndrome

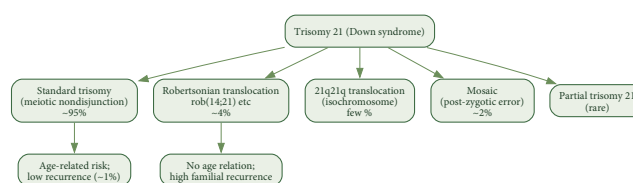
Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Down's syndrome (trisomy 21, historically "mongolism") is a chromosomal disorder in which all or a critical part of chromosome 21 is present in three copies instead of two. It is the single most common chromosomal abnormality in live-born humans and the commonest genetic cause of moderate intellectual disability, first described clinically by John Langdon Down. The population incidence in live births is approximately **1 in 850**, and the disorder is diagnosed by its distinctive dysmorphic facies plus confirmatory karyotyping, which is also essential for genetic-counselling because the recurrence risk differs sharply between the cytogenetic subtypes.

Cytogenetic types of trisomy 21

Type	Karyotype	Mechanism /origin	Proportion of Down cases	Relation to maternal age	Recurrence risk
Standard (regular) trisomy 21	47,XX or XY, +21	Meiotic nondisjunction of the chromosome-21 pair; ~90% of errors are maternal (chiefly meiosis I), ~10% paternal (chiefly meiosis II)	≥95%	Strongly age-dependent; risk rises sharply after age 30, approaching 1 in 10 births in the oldest maternal age-group	~1% overall after one affected child; ~1.4% if mother <30 yr; same as the age-related risk if mother is older

Type	Karyotype	Mechanism /origin	Proportion of Down cases	Relation to maternal age	Recurrence risk
Robertsonian translocation	46,XX/XY,rob(14;21)(q10;q10),+21 (chromosome 21q attached to another acrocentric, usually 14, occasionally 22)	Parent is a balanced carrier (45 chromosomes) of a Robertsonian translocation	~4%	No relation to maternal age	High — carrier mother : ~10–15%; carrier father : only a few percent
21q21q (isochromosome) translocation	46,XX/XY,rob(21;21)	Isochromosome of 21q in a parent	A few percent of translocation cases	No relation to maternal age	Carrier parent's gametes are inevitably unbalanced — surviving offspring have either Down syndrome or (non-viable) monosomy 21 ; recurrence effectively very high
Mosaic Down syndrome	46,XX/47,XX,+21 (two cell lines)	Post-zygotic mitotic nondisjunction	~2%	—	Phenotype milder and variable, reflecting the proportion of trisomic cells
Partial trisomy 21	Triplication of only part of 21q	Rare structural rearrangement	Very rare	—	A <2 Mb "Down-syndrome critical region" on 21q has been mapped to the cardiac-defect component of the phenotype



Down's syndrome

Etiology and embryological basis

- ▶ **Nondisjunction** of the chromosome-21 bivalent during gametogenesis (usually maternal meiosis I) is the commonest cause; the resulting gamete carries two copies of chromosome 21, and fertilisation by a normal gamete yields a 47,+21 zygote.
- ▶ Only about **20–25% of trisomy-21 conceptuses survive to birth** — the majority are lost as early spontaneous abortions, reflecting the severity of gene-dosage imbalance during embryogenesis.
- ▶ A **Robertsonian translocation carrier parent** has only 45 chromosomes (one chromosome 14 and one chromosome 21 are replaced by the fused translocation chromosome); of the theoretically possible gamete types, only a minority combine to give a liveborn translocation-Down child — hence carrier mothers transmit at ~10–15% and carrier fathers at only a few percent.
- ▶ **Paternal age has no influence** on the risk of trisomy 21.

Clinical (dysmorphic) features

- ▶ **Craniofacial**: brachycephaly with a flat occiput; short neck with loose skin over the nape; flat nasal bridge; upslanting palpebral fissures with inner epicanthic folds (the classical "mongoloid facies"); **Brushfield spots** at the margin of the iris; small, low-set, folded ears; open mouth with a protruding tongue.
- ▶ **Neuromuscular**: generalised **hypotonia** — often the first abnormality noted in the newborn; short stature; hyperflexibility (laxity) of joints.
- ▶ **Limbs/hands**: short, broad hands with a single transverse palmar (**simian**) crease and **clinodactyly** of the incurved fifth finger; a wide gap between the first and second toes ("sandal gap").
- ▶ **Dermatoglyphics** (supportive diagnostic sign): the **axial triradius is displaced distally** on the palm (an abnormal finding, seen in Down syndrome and trisomy 13); a **simian crease** is present in about 50% of Down syndrome patients (versus ~1% of normal individuals); a **Sydney line** (an additional proximal palmar crease) may be seen; a **tibial-arch pattern in the hallucal (great-toe) area** of the sole is present in about 50% of patients.
- ▶ **Intellectual disability**: moderate to mild; developmental delay usually becomes obvious by the end of the first year, though many patients become interactive and even self-reliant.

Associated systemic anomalies

- ▶ **Congenital heart disease** in at least **one-third** of liveborn infants — the commonest single cause of early mortality in Down syndrome, and a major determinant of survival.
- ▶ **Duodenal atresia and tracheo-oesophageal fistula** are much more frequent than in the general population.
- ▶ A **fifteen-fold increase** in the risk of leukaemia among patients who survive the neonatal period.
- ▶ **Premature Alzheimer-type neurodegeneration** — cortical atrophy, ventricular dilatation and neurofibrillary tangles appear in nearly all patients, several decades earlier than typical-population Alzheimer disease onset (attributed to the extra dosage of chromosome-21 genes, including *APP*, the amyloid-precursor-protein gene).
- ▶ **Life expectancy** is around 55 years with modern care.

Diagnosis

- ▶ **Clinical diagnosis** at birth is usually straightforward from the dysmorphic phenotype; **karyotyping is mandatory** to confirm the diagnosis and, crucially, to identify which cytogenetic subtype is present, since this determines the recurrence risk given to the family.
- ▶ **Prenatal:** karyotyping, FISH, and chromosomal microarray/genome sequencing on chorionic villus or amniotic-fluid cells; increasingly, **non-invasive prenatal screening (NIPS)** of cell-free fetal DNA in maternal plasma is used to screen pregnancies before deciding on an invasive (and miscarriage-risk-bearing) diagnostic test.
- ▶ If a translocation is found in the proband, **both parents (and sometimes other relatives) must be karyotyped** before accurate genetic counselling can be given.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Real captioned plates (embed verbatim):

- Fig 4.8 — A boy with Down syndrome (trisomy 21). Note the epicanthal folds, depressed nasal bridge (Gangane — Human Genetics — 5e, p.92)
- Fig 4.9 — Karyotype of Down syndrome patient showing trisomy 21 (arrow). (Gangane — Human Genetics — 5e, p.92)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a facial/hand sketch of Down syndrome with:

- ▶ Flat occiput (brachycephaly); short neck with loose nuchal skin
- ▶ Flat/depressed nasal bridge
- ▶ Upslanting palpebral fissures with epicanthic fold
- ▶ Brushfield spots on iris margin
- ▶ Low-set, small, folded ear
- ▶ Open mouth, protruding tongue
- ▶ Single transverse (simian) palmar crease; clinodactyly of 5th finger
- ▶ Wide gap between 1st and 2nd toes (sandal gap)
- ▶ Karyotype box: 47,XX/XY,+21 (or 46,XX/XY,rob(14;21),+21 for the translocation type)

Applied / Clinical anatomy

- ▶ **Surface markers used at bedside:** hypotonia, simian crease, epicanthic folds and Brushfield spots are the quickest clinical pointers prompting a karyotype request in a newborn.
- ▶ **Cardiac screening:** because congenital heart disease affects $\geq 1/3$ of livebirths, a screening echocardiogram is indicated in every neonate diagnosed with Down syndrome.
- ▶ **GI anomaly work-up:** the strong association with duodenal atresia (classic "double-bubble" sign) and tracheo-oesophageal fistula means these must be actively excluded.
- ▶ **Genetic-counselling anatomy of risk:** distinguishing standard trisomy (age-related, low recurrence) from Robertsonian/21q21q translocation (age-independent, high familial

recurrence) on karyotype is the single most important applied step, since it changes counselling from "~1%" to "10–15%" or effectively "obligate" recurrence.

- ▶ **Antenatal correlation:** relevant to combined first-trimester screening and NIPS counselling offered in obstetric/radiology postings, linking this genetics topic to the applied antenatal-diagnosis curriculum.
- ▶ **Museum/viva relevance:** karyotype charts and dermatoglyphic palm prints of Down syndrome are standard viva specimens in the genetics/embryology section of the practical examination.

High-yield exam pointers

- ▶ Population incidence ≈ **1 in 850** live births; commonest chromosomal disorder in humans.
- ▶ ≥95% are standard trisomy 21 from meiotic **nondisjunction**; ~4% are Robertsonian translocations; ~2% are mosaics.
- ▶ Standard trisomy 21 is **maternal-age-dependent**; translocation types are **not**.
- ▶ Recurrence risk: standard trisomy ≈1% (≈1.4% if mother <30 yr); Robertsonian carrier mother **10–15%**, carrier father a few %; 21q21q carrier parent — obligate recurrence.
- ▶ Hallmark clinical triad to write first: **hypotonia + epicanthic folds/Brushfield spots + simian crease with clinodactyly**.
- ▶ Key associated anomalies: **congenital heart disease** (≥1/3), **duodenal atresia**, **15-fold increased leukaemia risk**, premature Alzheimer-type change.
- ▶ Only **20–25%** of trisomy-21 conceptuses survive to term; karyotyping is mandatory for accurate recurrence counselling.
- ▶ Life expectancy today ≈ **55 years**.

SOURCE & COVERAGE

Thompson & Thompson — Genetics in Medicine, 8e (chromosomal basis of disease chapter — incidence, cytogenetic subtypes, mechanism, recurrence risk, clinical features and associated anomalies); Gangane — Human Genetics, 5e (dermatoglyphic features and figures); Vishram Singh — Textbook of Clinical Embryology (historical/eponymous description, classical clinical-feature summary). Coverage note: Down's syndrome is a cytogenetics/medical-genetics topic and is **not** covered in Gray's Anatomy (Standring) 41e, the spine text for this bank; grounding is drawn entirely from the dedicated genetics specialty texts as directed by the topic spec.



Ethical issues involved in Medical Genetics

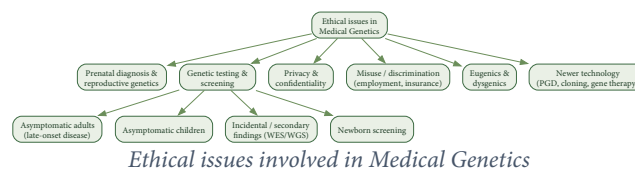
Asked in: Paper III 02-Aug-2021 — RGUHS MD/MS Anatomy

Definition / Introduction — Medical genetics raises ethical, legal and social issues in a wider spectrum of clinical contexts (fetus, neonate, child, adult and even future, as-yet-unborn generations) than perhaps any other branch of medicine, because a single genetic test result is never about the patient alone — it carries implications for the whole biological family. All ethical dilemmas in genetics are analysed against four cardinal principles of biomedical ethics: respect for individual **autonomy**, **beneficence**, avoidance of **maleficence** ("primum non nocere"), and **justice**. The role of the geneticist/counsellor is to weigh and balance these principles, which frequently conflict with one another, across the domains of reproductive genetics, testing/screening, privacy, and misuse of genetic data.

1. Cardinal principles of biomedical ethics in genetics

Principle	Core meaning	Application in medical genetics
Autonomy	Right to control one's own medical care and information, free of coercion	Basis of non-directive counselling — the couple/patient, not the counsellor, decides on testing, disclosure and reproductive choices
Beneficence	Doing good	Justifies population/newborn screening only when early detection changes outcome (e.g. PKU, congenital hypothyroidism)
Non-maleficence	First, do no harm	Argues against testing that only produces anxiety, stigma or unmodifiable knowledge without benefit (e.g. low-yield incidental findings)
Justice	Equal, fair treatment of all individuals	Demands equitable access to genetic services and freedom from discrimination in employment/insurance

2. Classification of ethical issues in medical genetics



3. Ethical issues in prenatal diagnosis and reproductive genetics

- **Termination for non-fatal disability:** couples commonly seek prenatal diagnosis (PND) intending termination for a disorder that is serious but not fatal in infancy, or for a treatable

adult-onset condition — pitting parental **reproductive autonomy** against fairness to the fetus and to the wider community of persons with that disability.

- ▶ **Deafness/achondroplasia dilemma:** couples in which both parents are deaf or have achondroplasia may see their phenotype as valued family identity rather than disability; surveys show most would still not terminate to avoid an affected child, though the conflict is debated.
- ▶ **Sex selection:** PND/ultrasonography or IVF with blastomere biopsy used purely to choose fetal sex (not to avoid an X-linked disorder) has measurably skewed the female-to-male infant ratio (from ~0.95 to <0.85) in some populations, and is regarded as an unethical use of prenatal testing.
- ▶ **Non-disease trait selection/"enhancement":** seeking PND for cosmetic or performance traits is criticised as treating children as "commodities"; there is no consensus on where a serious disorder ends and a non-disease trait begins.
- ▶ In India, sex determination/selection is barred by the **Pre-Conception and Pre-Natal Diagnostic Techniques Act**, and termination for fetal abnormality is governed by the **Medical Termination of Pregnancy Act** — both requiring the geneticist to weigh legal alongside ethical obligations.

4. Ethical issues in genetic testing and screening across the lifespan

- ▶ **Asymptomatic adults, late-onset disease** (e.g. Huntington disease): a personal decision after counselling — some choose not to know; those testing positive report transient depression but also benefit for life-planning, while true-negatives can feel guilt toward affected relatives.
- ▶ **Predisposition testing with an available intervention** (e.g. BRCA1/2, 50–90% lifetime breast/ovarian cancer risk) favours testing, since surveillance/prophylactic surgery can reduce risk — at the cost of possible distress, stigma and discrimination.
- ▶ **Asymptomatic children:** predictive testing for adult-onset disease or carrier status should be deferred until the child can decide for himself/herself, *unless* there is clear childhood benefit — contrast familial adenomatous polyposis (colonoscopy from ~12 years is life-saving) with Huntington disease (no preventive benefit, only anxiety/stigma).
- ▶ **Incidental/secondary findings on WES/WGS:** laboratories may find deleterious mutations unrelated to the test indication; a defined panel of actionable genes (mainly hereditary cancer/cardiovascular) is reported only after counselling and patient consent.
- ▶ **Newborn screening:** an accepted public-health triumph, yet raises consent (active vs "opt-out"), and secondary-use dilemmas — one US state was sued and ordered to destroy millions of retained blood spots used without parental consent; a sound programme also needs a well-defined, treatable disorder and a cheap, reliable, sensitive test.

5. Privacy, confidentiality and the "duty to warn"

- ▶ Genetic information is unique because it also implicates **blood relatives who are not the patient**, yet standard privacy rules protect only the tested individual's own record.
- ▶ A dilemma arises when a patient with a hereditary disorder **refuses to let at-risk relatives know**, even though disclosure could allow presymptomatic surveillance. This pits confidentiality against the practitioner's duty to prevent foreseeable harm (analogous to the psychiatric "duty to warn" precedent, *Tarasoff v Regents of the University of California*, 1976). In genetics, *Safer v Estate of*

Pack (1996, USA) held a physician had a duty to warn a patient's daughter of her risk for familial adenomatous polyposis, ruling a genetic threat is "no different" from a contagious one, and that telling the patient alone does not discharge the duty.

- Counselling itself carries **medico-legal liability**: a couple wrongly reassured they were both Tay-Sachs non-carriers subsequently had an affected child; the court recognised the birth as a "wrongful life," underscoring the need for accurate diagnosis and honest risk communication.

6. Misuse of genetic information: discrimination in employment and insurance

- This is the **justice** principle in action — the concern that a healthy person could be denied a job or insurance for a genetic predisposition.
- The US **Genetic Information Nondiscrimination Act (GINA)**, 2008 bars employers (≥15 employees) from using genetic information, including family history, in employment decisions, and bars most group health insurers from using it to deny cover or adjust premiums.
- GINA does **not** cover life, disability or long-term-care insurance — insurers cite the risk of "adverse selection" (those testing positive for APOE ε4 were nearly six times more likely to buy extra long-term-care cover than those not knowing their genotype). Elsewhere, comprehensive legislation is largely absent; some countries rely instead on voluntary insurer moratoria.

7. Eugenics, dysgenics and non-directive counselling

- **Eugenics** (Francis Galton, Darwin's cousin, 1883) is the deliberate "improvement" of a population by selective breeding of its "best" specimens. Its "ideal" traits were defined by social/ethnic/racial prejudice (poverty as "shiftlessness," poor education as "feeble-mindedness"), culminating in Nazi racial-hygiene and mass murder; involuntary sterilisation of the "mentally incompetent" persisted in parts of North America/Europe even after World War II.
- Modern counselling is distinguished from eugenics by **non-directive counselling** — helping families reach free, informed reproductive decisions without coercion, autonomy paramount, never a societal goal of "improving the gene pool" — though true non-directiveness is hard to fully achieve.
- **Dysgenics**, the converse, is a rise in deleterious-allele frequency when treatment removes selection against a genotype: e.g. surviving, reproducing Duchenne muscular dystrophy males would pass the allele to all daughters; the effect is far smaller for recessive disorders such as cystic fibrosis, whose alleles are mostly silent in heterozygotes.

8. Newer technology: preimplantation diagnosis, "saviour siblings," cloning and gene therapy

- **Preimplantation genetic diagnosis (PGD)** selects an embryo before implantation and can reveal fetal sex, raising the same sex-selection concern as PND. In one cited case, deaf parents used PGD to select an embryo homozygous for autosomal recessive deafness — parental preference conflicting with the future child's interest. PGD has also selected an **HLA-matched "saviour sibling"** embryo whose cord blood/marrow could treat an existing sick child (e.g. Fanconi anaemia).

- ▶ **Reproductive cloning** of humans is technically feasible in mammals but unanimously opposed for humans; **therapeutic cloning** for patient-matched stem cells is more widely accepted.
- ▶ **Gene therapy: germ-line gene therapy must never be undertaken**; only **somatic-cell gene therapy** is sanctioned, requiring regulatory/ethics-committee approval (national bodies and hospital ethics committees) before any human trial.

Applied / Clinical anatomy

- ▶ **Statutory framework relevant to India:** the **Pre-Conception and Pre-Natal Diagnostic Techniques (Prohibition of Sex Selection) Act** bans prenatal sex determination/disclosure and sex-selective termination and mandates registration of genetic/ultrasound clinics; the **Medical Termination of Pregnancy Act** governs lawful termination for a fetus with substantial abnormality, requiring due medical-board process before advice to terminate — both statutes require the geneticist to weigh legal alongside ethical obligations.
- ▶ **Clinical vignette (non-directive counselling in practice):** a family with a child with Down syndrome should first be helped to accept the diagnosis and referred for developmental support, then informed — without direction — of options for prenatal detection and parental carrier/translocation testing in a future pregnancy; this same non-directive template applies to the *Tarasoff/Safer v Estate of Pack* duty-to-warn dilemma and to the Tay–Sachs wrongful-life scenario discussed above.

High-yield exam pointers

- ▶ Four cardinal principles: **autonomy, beneficence, non-maleficence, justice** — the framework for every genetics ethics question.
- ▶ **Non-directive counselling** is the ethical principle that separates genetic counselling from eugenics.
- ▶ Major domains of ethical debate: genetic testing (prenatal/adult/child/incidental findings), privacy/duty to warn, misuse/discrimination, and screening — this maps directly to typical long-answer sub-points.
- ▶ **Eugenics** (Galton, 1883) vs **dysgenics** — define both and give one example each (Nazi racial hygiene / Duchenne muscular dystrophy survival raising carrier frequency).
- ▶ **Duty to warn:** *Tarasoff* (1976) and *Safer v Estate of Pack* (1996) — confidentiality can be overridden to prevent serious, preventable harm to an identifiable relative.
- ▶ **GINA (2008, USA)** bars genetic discrimination in employment and group health insurance but **not** in life/disability/long-term-care insurance.
- ▶ Predictive testing of **asymptomatic children** should be deferred unless there is a clear, immediate medical benefit (contrast FAP vs Huntington disease).
- ▶ India-specific statutes to name: **PC-PNDT Act** (sex-selection ban) and **MTP Act** (lawful termination for fetal abnormality).
- ▶ **PGD** raises the sharpest modern dilemmas: sex selection, selecting for a disease trait (deafness case), and "saviour siblings" (HLA-matched embryo selection).
- ▶ **Wrongful life/wrongful birth** — know this term with the Tay–Sachs example as the classic case illustrating counselling liability.

SOURCE & COVERAGE

Thompson & Thompson — Genetics in Medicine, 8e (Chapter: *Ethical and Social Issues in Genetics and Genomics* — principles of biomedical ethics, ethical dilemmas in prenatal/predictive/childhood testing, incidental findings, privacy and duty to warn, GINA and insurance discrimination, eugenics and dysgenics; adjoining chapter on newborn screening/population screening criteria). Gangane — Human Genetics, 5e (Genetic Counselling chapter — non-directive counselling, screening-programme criteria, the Tay–Sachs "wrongful life" case, and its dedicated "Ethical and Legal Issues in Genetics" section covering the Human Genome Project's ELSI focus, preimplantation diagnosis/saviour-sibling and cloning debates, and childhood testing in FAP vs Huntington disease; Prenatal Diagnosis chapter — PC-PNDT Act; Gene Therapy chapter — germ-line vs somatic gene therapy and regulatory bodies).

Coverage note: This is a bioethics/social-issues topic that lies outside Gray's Anatomy (the spine text) and is not itself gross/microscopic anatomy; per the topic spec it is grounded entirely in the Genetics specialty texts (Thompson & Thompson; Gangane) rather than the anatomy spine. Indian statutory names (PC-PNDT Act, MTP Act) are supplied as standard, universally-known legal facts to contextualise the corpus material for the Indian PG-exam audience, since the corpus references them only by abbreviation.



Gene

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction A **gene** is the fundamental unit of heredity — a sequence of DNA that specifies the production of a functional product, either a polypeptide or a functional RNA molecule, and includes not only the coding sequence itself but also the adjacent regulatory sequences required for its correct expression in the right amount, place and time. The term was coined by the Danish botanist **Wilhelm Johannsen (1909)**. The human genome contains an estimated **20,000 protein-coding genes** together with roughly **20,000–25,000 non-coding RNA (ncRNA) genes**; older, pre-Human-Genome-Project teaching estimates of 50,000–100,000 total genes have since been revised downward. Genes occupy a fixed **locus** on a chromosome, are transmitted from parents to offspring, and their alteration (**mutation**) underlies the great majority of inherited and acquired genetic disease.

1. Structure of a typical human gene

Every protein-coding gene has, at minimum, the following elements, arranged 5'→3' along the coding strand:

Element	Location / feature
Promoter	Immediately upstream (5') of the transcription start site; contains the TATA box ($\approx 25\text{--}30$ bp upstream, AT-rich, fixes the start of transcription) and the CAT/CCAAT box (a little further upstream) in most tissue-specific genes; binds RNA polymerase and transcription factors to initiate transcription
CpG islands	GC-rich promoters of many housekeeping genes , which characteristically lack TATA/CAT boxes
5' untranslated region (5' UTR)	Transcribed but not translated; begins at the transcription start site
Translation initiation (start) codon	AUG, coding for methionine; establishes the reading frame
Exons	Coding segments; ultimately determine the amino acid sequence of the protein
Introns (intervening sequences)	Non-coding segments interspersed between exons; transcribed into the primary RNA but spliced out during RNA maturation; bounded by conserved 5' donor and 3' acceptor splice sites
Translation termination (stop) codon	UAA, UAG or UGA; ends translation
3' untranslated region (3' UTR)	Transcribed, untranslated; carries the polyadenylation signal
Polyadenylation signal (AATAAA)	Directs addition of the polyA tail to the mature RNA, increasing its stability
Enhancers, silencers, locus-control regions	Additional regulatory elements, which may lie 5', 3', or within introns, sometimes far from the coding region; enhancers act independent of position/orientation

A gene's overall size is highly variable: most genes contain at least one, usually several, introns whose cumulative length commonly exceeds that of the exons, though a few genes are intronless. The classic teaching example is the **β -globin gene** (chromosome 11, ~ 1.6 kb, **3 exons and 2 introns**, promoter ~ 200 bp upstream of the transcription start), whereas the **dystrophin (DMD) gene** on Xp21 is the largest human gene — it spans roughly **2.3 Mb (2300 kb)** of genomic DNA and contains **79 exons**, yet less than 1% of it is coding sequence.

□ Fig 3.9 — Schematic representation of the structural gene in humans. (Gangane — Human Genetics — 5e, p.59)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a horizontal linear map of a gene, 5' end on the left to 3' end on the right, with these exact labels (as in the figure above):

- ▶ 5' end → CAT box → TATA box → arrow marked "Initiation of transcription"
- ▶ Translation initiation codon (ATG) sitting at the start of Exon 1
- ▶ Exon 1 — Intron 1 — Exon 2 — Intron 2 — Exon 3 (shade exons distinctly from introns)
- ▶ Translation termination codon (TAA) near the end of Exon 3, followed by Polyadenylation signal, then an arrow marked "Termination of transcription", ending at 3' end
- ▶ Caption: "Structure of a typical human gene — promoter, exons, introns and 3' regulatory elements."

2. Types of genes

Type	Feature
Structural genes	Code for a specific amino acid sequence of a protein
Operator/regulatory genes	Regulatory DNA sequences that permit or control transcription of adjacent structural genes
Dominant genes	Express their trait whether present on one or both homologous chromosomes
Recessive genes	Express their trait only in the homozygous state
Sex-linked genes	Located on the X or Y chromosome
Jumping genes (transposons)	Mobile DNA elements that can relocate within a chromosome or to another one
Gene families	Clusters of genes with closely related sequence and function, arising by duplication of a common ancestral gene — e.g. the β -globin cluster (chromosome 11) and α -globin cluster (chromosome 16), and the olfactory-receptor (OR) gene family (~1000 members, over half on chromosome 11)
Pseudogenes	Non-functional DNA sequences closely resembling a known gene; nonprocessed pseudogenes are vestigial genes inactivated by mutation (retain introns); processed pseudogenes arise by retrotransposition of a reverse-transcribed mRNA copy (lack introns, often on a different chromosome)
Non-coding RNA (ncRNA) genes	Encode functional RNA rather than protein — tRNA, rRNA, snoRNA, long ncRNA, and microRNA (miRNA) , ~22-base regulatory RNAs that suppress translation of target mRNAs

3. Gene expression — from gene to protein

Information flows DNA → RNA → **protein** (the central dogma): the gene is **transcribed** into a primary RNA transcript, which is processed (5' capping, splicing out of introns, polyadenylation) into mature mRNA, which is then **translated** into protein on ribosomes. Key features of the **genetic code**:

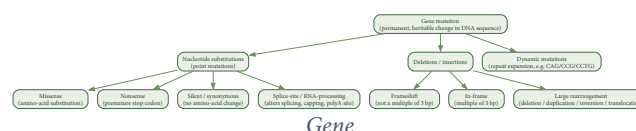
- ▶ Four bases taken three at a time give **64 possible codons** (the triplet code).
- ▶ The code is **degenerate** — most of the 20 amino acids are specified by more than one codon (only methionine and tryptophan have a single unique codon each).
- ▶ Translation always begins at the **AUG (methionine) initiator codon**, which fixes the reading frame; each subsequent codon is read in a **non-overlapping**, fixed frame.
- ▶ **Three stop (nonsense) codons** — UAA, UAG, UGA — terminate translation.
- ▶ **tRNA anticodons** pair with mRNA codons to bring the correct amino acid to the ribosome; both transcription and translation proceed 5' → 3' (amino- to carboxy-terminus for the protein).

4. Properties of a gene: locus, alleles and expression

- ▶ **Locus**: the fixed position a gene occupies on a chromosome; both members of a homologous chromosome pair carry genes for the same trait at corresponding loci.
- ▶ **Alleles**: alternative forms of a gene at the same locus. Identical alleles = **homozygous**; different alleles = **heterozygous**.
- ▶ **Dominant vs recessive**: a dominant gene produces its effect whether present on one or both chromosomes of the pair; a recessive gene produces its effect only when present on both (homozygous state).
- ▶ **Penetrance**: whether the gene is expressed at all — an all-or-none phenomenon; a mutant-gene carrier who shows no phenotype exhibits **reduced penetrance**.
- ▶ **Expressivity**: the degree/severity of expression in those who do manifest the trait; different severity among individuals with the same genotype = **variable expressivity**.
- ▶ **Pleiotropy**: a single gene producing multiple, apparently unrelated phenotypic effects — e.g. **phenylketonuria** (phenylalanine hydroxylase deficiency causing mental retardation, hypopigmentation and excretion of phenyl ketones) and **galactosaemia** (galactose-1-phosphate uridyl transferase deficiency causing cirrhosis, cataract, mental retardation and galactosuria).

5. Gene mutations

A **mutation** is a permanent, heritable change in the genomic sequence, produced by alteration of a single base or by deletion, insertion, or rearrangement of larger DNA segments. Common **mutagens** include UV/X-ray radiation, chemicals, viruses, and transposons. Roughly 70% of mutations are harmful; the rest are neutral or occasionally beneficial. Mutation can occur at the level of the **gene** (point/molecular mutation) or the **chromosome** (structural/numerical mutation); it can also arise in **somatic cells** (not transmitted to offspring, may cause cancers such as retinoblastoma or Wilms tumour, produces a genetic mosaic) or in **germ cells** (transmitted to offspring, individual not mosaic).



Category (Thompson & Thompson)	Approx. % of disease-causing mutations
Missense substitution	50%
Nonsense substitution	10%
RNA-processing mutation (cap/splice/polyA site)	10%
Splice-site mutation → frameshift/premature stop	10%
Small in/del (few bases)	25%
Large deletion/inversion/duplication/fusion	5%
Mobile-element (LINE/Alu) insertion	Rare
Dynamic mutation (repeat expansion)	Rare

Nonsense-mediated decay: mRNA carrying a premature stop codon is often rapidly degraded, or — if translated — yields an unstable, rapidly degraded truncated protein. **Frameshift** occurs when an insertion/deletion is not a multiple of 3 base pairs, altering every downstream codon and usually generating a premature stop; an in-frame in/del (multiple of 3 bp) instead simply adds or removes amino acids.

Applied / Clinical anatomy

- **Duchenne muscular dystrophy:** the **dystrophin (DMD)** gene, mapped to **Xp21**, is the largest human gene (~2.3 Mb, 79 exons); two-thirds of cases result from gene deletions, with well-recognised deletion "**hot spots**" in the first 20 exons and between exons 45–53 (detected by Southern blotting with cDNA probes). X-linked recessive inheritance; milder allelic disorder = Becker muscular dystrophy.
- **β-thalassaemia and haemoglobinopathies:** point mutations in the promoter (TATA/CAT box), splice sites, or coding exons of the **β-globin gene** reduce or abolish normal globin synthesis; nearly half of haemophilia A cases arise from an **intragenic inversion** disrupting the factor VIII gene.
- **BRCA1** (24 exons) mutations cause hereditary breast/ovarian cancer; **MYH7** (β-myosin heavy chain, 40 exons) mutations cause familial hypertrophic cardiomyopathy — both illustrate how mutations scattered across a multi-exon gene produce a single recognisable clinical phenotype.
- **Achondroplasia:** caused almost always by the same **FGFR3 missense mutation**, arising nearly exclusively as a new mutation in the paternal germline, with increasing incidence with paternal age.
- **Genetic counselling:** indicated when parents are known carriers, when a child is born with a birth defect, unexplained intellectual disability, or a metabolic disorder (e.g. PKU, galactosaemia), or in adult-onset genetic disease (e.g. Huntington disease, hereditary cancers) — all ultimately traceable to identification of the underlying gene mutation.
- **Molecular diagnosis:** identification of the causal gene mutation underlies prenatal diagnosis, carrier detection, and gene-therapy strategies for single-gene disorders.

High-yield exam pointers

- ▶ Term "gene" coined by **Wilhelm Johannsen (1909)**; molecular definition = a DNA sequence coding for a functional product (polypeptide or RNA) plus its regulatory flanking sequences.
- ▶ Structure (5'→3'): **promoter (TATA + CAT box, or CpG island in housekeeping genes)** → 5' UTR → **exon–intron–exon...** → 3' UTR → **polyadenylation signal (AATAAA)**.
- ▶ Human genome ≈ **20,000 protein-coding genes + 20,000–25,000 ncRNA genes** (older estimate 50,000–100,000, now revised).
- ▶ Genetic code: **64 codons**, degenerate, **AUG initiator**, **3 stop codons (UAA/UAG/UGA)**, non-overlapping, read 5'→3'.
- ▶ Types of genes: structural, regulator/operator, dominant, recessive, sex-linked, jumping genes (transposons); plus gene families, pseudogenes (processed vs nonprocessed), ncRNA genes.
- ▶ Locus/allele, homozygous/heterozygous; **penetrance** (all-or-none) vs **expressivity** (degree); **pleiotropy** (PKU, galactosaemia).
- ▶ Mutation = permanent heritable DNA change; **somatic** (not transmitted, mosaic, may cause cancer) vs **germline** (transmitted, not mosaic).
- ▶ Molecular mutation classes: substitutions (missense/nonsense/silent/splice-site) vs indels (frameshift vs in-frame) vs dynamic (repeat expansion).
- ▶ Largest human gene = **dystrophin (DMD, Xp21, ~2.3 Mb, 79 exons)**.

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e; Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e; Langman's Medical Embryology (Sadler) 14e. Coverage note: Gray's Anatomy (Standring) 41e carries no dedicated molecular-genetics chapter, consistent with this being a pure molecular-genetics topic outside the gross-anatomy spine text; grounding was therefore drawn entirely from the genetics/general-anatomy specialty texts, as intended for this topic's GENETICS specialty designation.



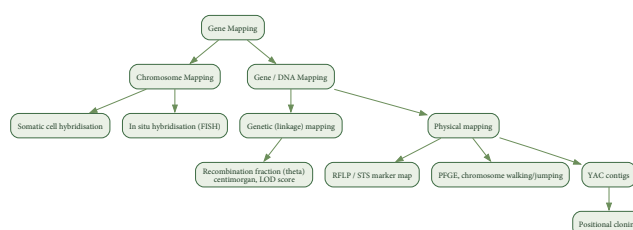
Gene map

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction Gene mapping (genome mapping) is the process of determining the location of a gene or a DNA marker on a chromosome and establishing its linear order and distance relative to other genes/markers on the same chromosome. It is broadly of two types — **chromosome mapping** (assigning a gene or DNA sequence to a specific chromosome) and **gene/DNA mapping**, a finer analysis that gives the physical relationship of a locus to flanking DNA-sequence polymorphisms and the detailed internal structure of the gene. Gene-mapping methods generated the **genetic (linkage) map** and the **physical map** — two of the three deliverables of the Human Genome Project (HGP) — and remain the foundation of modern disease-gene identification, prenatal diagnosis, forensic medicine and genetic counselling.

1. Classification of gene-mapping methods

Category	What it establishes	Key methods
Chromosome mapping	Assigns a gene/DNA sequence to a specific chromosome	Somatic cell hybridisation; in situ hybridisation (FISH)
Genetic (linkage) mapping	Relative order/distance of markers, measured via recombination	Family-based linkage analysis; recombination fraction (θ); LOD score; centimorgan (cM)
Physical mapping	Actual physical distance (base pairs) between markers	RFLP/STR/SNP marker maps; STS map; pulsed field gel electrophoresis (PFGE); chromosome walking/jumping; YAC contigs; positional cloning
Association mapping	Population-based marker–disease correlation (not family-based)	Linkage disequilibrium (LD) analysis
Direct mapping	Base-by-base localisation	Genome/exome sequencing



Gene map

2. Chromosome mapping techniques

- **Somatic cell hybridisation** — somatic cells from two different species (e.g. human + mouse/hamster) are fused under favourable culture conditions; the hybrid cell progressively and randomly loses human chromosomes over successive divisions. A gene product/marker is correlated with the *retained* human chromosome, thereby assigning that gene to a specific chromosome — historically the first technique to establish **genetic linkage** and localise genes on chromosomes.

□ Fig 2.11 — Schematic representation of somatic cell hybridisation. Note progressi (Gangane — Human Genetics — 5e, p.39)

- **Fluorescence in situ hybridisation (FISH)** — exploits the ability of a single-stranded, fluorescently-labelled DNA **probe** to anneal (hybridise) with its complementary target sequence in the genome, visualised under UV light. Probes may be locus-specific, centromeric, or cover an entire chromosome (**whole chromosome paint**, WCP), the last being valuable for characterising complex rearrangements (deletions, insertions, translocations, ring chromosomes). FISH can be applied even at **interphase**, enabling rapid prenatal diagnosis (e.g. trisomy 21 on interphase nuclei from chorionic villus sampling) and detection of **submicroscopic microdeletions** missed on routine banding.

3. Genetic (linkage) mapping

- **Linkage analysis is family-based**: it uses pedigrees to test for consistent, repeated co-inheritance of a disease with a particular genomic region or marker allele, exploiting the fact that loci close together on a chromosome tend to be inherited together (recombination between them is infrequent).
- **Recombination fraction (θ)** is the proportion of offspring showing a crossover between two loci; $\theta = 0.5$ means the loci are unlinked (assort independently), while $\theta < 0.5$ indicates linkage — the smaller the θ , the closer the loci.
- **Centimorgan (cM)** — the unit of genetic map distance, named after **Thomas Hunt Morgan**; 1 cM is the genetic distance over which, on average, one crossover occurs in 1% of meioses ($\theta = 0.01 \approx 1$ cM). Genetic map distance and physical distance correlate only approximately — on average ~ 1 cM per megabase (Mb) of DNA across the genome, but this varies widely (recombination "hot spots" account for a disproportionate share of crossovers).
- **LOD score (Z)** — "Logarithm of the ODDs" in favour of linkage versus no linkage, calculated from family pedigree data at a given θ . By convention, a **LOD score of +3 or greater** is accepted as firm statistical evidence that two loci are linked; LOD scores from independent families can be summed (logarithms) to combine evidence.
- **Historic example** — **Huntington disease** was the **first human disease gene mapped to a specific chromosome using an RFLP marker** (Gusella and colleagues, 1983), localising the gene to the **short arm of chromosome 4**; the gene itself was cloned about a decade later. Similarly, the **Duchenne muscular dystrophy (DMD)** locus was mapped to **Xp21** by linkage analysis before the *dystrophin* gene was cloned.

4. Physical (DNA) mapping

- **Marker types used for mapping**: **RFLPs** (restriction fragment length polymorphisms), **STRPs** (short tandem repeat polymorphisms), **VNTRs** (variable number of tandem repeats), and **SNPs** (single nucleotide polymorphisms — several million identified across the genome, with a lower mutation rate and amenability to automated computerised analysis).
- **STS (sequence-tagged sites)** — short, unique sequences of known location placed at roughly 100-kb intervals across the genome; the **physical map** built from STS markers is especially useful for **positional cloning**.
- **Pulsed field gel electrophoresis (PFGE)** — routine agarose gel electrophoresis resolves DNA fragments up to $\sim 20,000$ bp; PFGE, using rare-cutting restriction enzymes (e.g. *NotI*, *PvuI*) that

recognise 6–8 bp sequences, resolves much larger fragments (up to ~2,000,000 bp), permitting maps of large stretches of DNA.

- ▶ **Chromosome jumping/linking** — circularised DNA fragments (cut with a restriction enzyme in the presence of a plasmid) are recut with a second enzyme that does not cut the plasmid; the plasmid sequence "tags" and allows cloning of the ends of the original fragment, letting distant markers (many kilobases apart) be linked/mapped.
- ▶ **YAC contigs** — **yeast artificial chromosomes** permit cloning of very large genomic DNA segments (2–3 million base pairs), essential for mapping genes with large flanking regions, e.g. the genes for **cystic fibrosis**, **neurofibromatosis** and **dystrophin**; overlapping YAC clones (contigs) allow long-range physical mapping.
- ▶ **Positional cloning** — once a disease locus is localised (by linkage/physical mapping) to a chromosomal region, the physical map (STS/YAC contigs) is used to clone and identify the actual gene without prior knowledge of its protein product — the strategy by which most mendelian disease genes were identified after completion of the HGP.

5. The Human Genome Project — the gene map realised

- ▶ Initiated **October 1990**, with **three major goals**: (1) a **genetic marker map**, (2) a **physical map**, and (3) the complete **3-billion-base-pair sequence** of the human genome.
- ▶ The marker map (RFLP/STRP/VNTR/SNP markers at ~1 cM intervals) and the STS-based physical map (markers at ~100-kb intervals) were completed first, providing the scaffold for positional cloning.
- ▶ A "rough draft" (~90% sequence, high accuracy) was announced in **February 2001**; the **complete, highly accurate sequence** was announced in **2003** — exactly **50 years after Watson and Crick** described the double-helical structure of DNA (1953).
- ▶ Completion of the HGP made positional cloning of disease genes routine, has improved genetic diagnosis, and underlies modern recombinant-DNA-based diagnostics and gene therapy.

Applied / Clinical anatomy

- ▶ **Positional cloning of disease genes** (fruits of the gene map): **CFTR** gene for cystic fibrosis at **7q31**; **dystrophin gene for Duchenne muscular dystrophy at Xp21 — the largest human gene identified (~2300 kb, 79 exons), with two deletion "hot spots" detectable on Southern blotting; Huntington disease gene on the short arm of chromosome 4 (4p16.3), the first disease gene ever mapped by an RFLP marker; PAX3 gene for Waardenburg syndrome at 2q35**.
- ▶ **Prenatal and rapid cytogenetic diagnosis**: interphase FISH on chorionic villus/amniotic fluid samples permits same-day detection of numerical abnormalities such as **trisomy 21**, without waiting for metaphase culture.
- ▶ **Genetic counselling**: mapping and cloning of a disease gene enables predictive/carrier testing, but its clinical use is tempered by the natural history of the disease — e.g. childhood testing for the **APC** gene (familial adenomatous polyposis) is recommended because early colonoscopy is life-saving, whereas childhood testing for the **Huntington disease** gene is generally avoided since no preventive or therapeutic intervention exists.

- ▶ **Gene therapy:** a mapped and cloned disease locus is the prerequisite for vector-based correction, e.g. ADA-gene transfer into T-lymphocytes for ADA-deficiency SCID, and direct/myoblast-mediated gene transfer strategies explored for DMD.
- ▶ **Forensic and identity applications:** the same polymorphic markers (RFLP, STR/VNTR) used for gene mapping underpin **DNA fingerprinting** for paternity testing and forensic identification.

High-yield exam pointers

- ▶ Gene mapping = locating a gene/marker on a chromosome + establishing its order/distance relative to others; two broad types — **chromosome mapping** and **gene/DNA mapping**.
- ▶ Chromosome mapping tools: **somatic cell hybridisation**, **in situ hybridisation (FISH)**; FISH also gives **whole chromosome paints** and detects **microdeletions**.
- ▶ Genetic (linkage) mapping is **family-based**; key quantities are **recombination fraction (θ)**, the **centimorgan** (named after T.H. Morgan; 1 cM $\approx$ 1% recombination), and the **LOD score** ($\geq +3$ = accepted linkage).
- ▶ Physical mapping tools: **STS map** (~ 100 kb intervals), **PFGE** (resolves up to 2 million bp), **chromosome walking/jumping**, **YAC contigs** (2–3 Mb inserts) $\rightarrow$ **positional cloning**.
- ▶ Marker polymorphisms used: **RFLP**, **STRP**, **VNTR**, **SNP**.
- ▶ HGP (started Oct 1990): three goals — genetic marker map, physical map, 3-billion-bp sequence; rough draft **2001**, complete sequence **2003** (50 years after Watson & Crick).
- ▶ Classic examples: **Huntington disease** — first disease gene mapped by RFLP (Gusella, 1983), chromosome **4p**; **DMD/dystrophin** — **Xp21**, largest human gene; **CFTR** — **7q31**.

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e. Coverage note: Gene mapping/Human Genome Project is not carried as a dedicated chapter in Gray's Anatomy (Standring) 41e; this answer is grounded entirely in the specialty genetics texts, per the topic's grounding note.



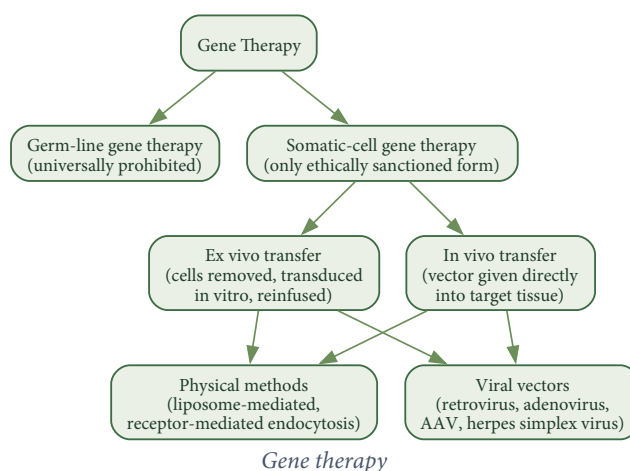
Gene therapy

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction — **Gene therapy** is the deliberate introduction of a biologically active, functional gene (or its complementary DNA) into the cells of a patient in order to correct or compensate for a genetic defect and achieve a therapeutic benefit. The technical basis of gene therapy is **gene delivery** — introducing the desired DNA sequence into appropriate target cells with stable integration, adequate expression, and physiological regulation. Gene delivery may be **ex vivo** (cells are removed, engineered outside the body, and reinfused) or **in vivo** (the vector is introduced directly into the target tissue), and transfer itself is achieved by either **physical methods** or **viral vectors**. Only **somatic-cell** gene therapy is ethically and legally practised; **germ-line gene therapy** is universally prohibited.

1. Strategies of gene delivery — ex vivo vs in vivo

Feature	Ex vivo transfer	In vivo transfer
Procedure	Target cells removed from the patient → gene introduced in culture → genetically engineered cells transplanted back	Vector/gene construct injected or instilled directly into the target tissue or extracellular fluid
Control over transduction	High — transduced cells can be selected/expanded before reinfusion	Lower — depends on vector tropism reaching the correct cells in situ
Typical target cells	HSCs, T-lymphocytes, hepatocytes, myoblasts, fibroblasts, endothelial cells	Muscle, airway epithelium (CFTR), CNS, tumour tissue
Examples	ADA-SCID (T-lymphocytes), X-linked SCID (HSCs), familial hypercholesterolaemia (hepatocytes), haemophilia B (fibroblasts)	Duchenne dystrophy (muscle), cystic fibrosis (airway epithelium), Huntington disease (caudate implant)



2. Methods of gene transfer

(A) Physical transfection methods — free of viral risk:

- **Liposome-mediated DNA transfer** — plasmid DNA carrying the foreign gene is complexed with liposomes and delivered into the target cell; can carry very large DNA (even an artificially constructed minichromosome with its own regulatory elements). Expression is **transient**, so treatment must be repeated. Actively tested for CFTR delivery in cystic fibrosis.
- **Receptor-mediated endocytosis** — plasmid DNA is complexed to a specific ligand (e.g. a galactose-terminated glycoprotein) recognised by a cell-surface receptor (e.g. the hepatocyte asialoglycoprotein receptor); the complex is internalised by endocytosis. Escape from the endosome/lysosome before degradation can be enhanced by co-administering adenovirus or fusogenic influenza proteins.

(B) Viral vectors (biological transfection)

Vector	Genome/ insert capacity	Target cells	Advantages	Disadvantages
Retrovirus (needs a packaging cell line + a helper virus/vector stripped of >90% of its viral genome)	RNA virus; insert ≤7–8 kb	Dividing cells only — hepatocytes, HSCs, fibroblasts, myoblasts, endothelial cells	Integrates stably into host genome via reverse transcriptase → long-term expression	Small cargo; cannot transduce non-dividing cells (e.g. neurons); usable only in vitro; unstable; risk of contamination with replication-competent virus; insertional mutagenesis can activate an oncogene
Lentivirus (a retroviral subclass, e.g. HIV-derived vectors)	Similar capacity to retrovirus	Dividing and non-dividing cells (including neurons)	Integrates in non-dividing cells; no strong preference for a specific integration site, lowering oncogene-activation risk	Derived from HIV — biosafety handling concerns
Adenovirus	DNA virus; large capacity (up to ~36 kb)	Non-dividing cells especially (e.g. CNS, respiratory epithelium)	Stable, easily purified, high titre, infects dividing or non-dividing cells, large cloning capacity	Does not integrate into host genome → transient expression; strong immune/inflammatory response; contains sequences with oncogenic potential

Vector	Genome/ insert capacity	Target cells	Advantages	Disadvantages
Herpes simplex virus (HSV)	DNA virus; neurotropic	Neurons; also hepatocytes	Natural affinity for non-dividing cells — well suited to CNS gene transfer	Toxic to neurons; provokes immune response; non-integrating, so expression is unstable
Adeno-associated virus (AAV) (parvovirus-derived)	Small capacity (~5 kb)	Dividing and non-dividing cells	Forms a stable episomal preparation, non-pathogenic, high efficiency of integration, low immunogenicity → durable expression	Small insert size limits genes that can be carried
Parvovirus / Simian virus 40 (SV-40)	—	CNS (parvovirus); smooth muscle (SV-40)	Under evaluation for CNS and vascular smooth-muscle gene transfer	Limited clinical experience

3. Essential requirements ("prerequisites") for gene therapy of an inherited disorder

- ▶ The **molecular defect must be identified** — the affected gene known and cloned.
- ▶ A **functional copy of the gene** (or a truncated but functional version, if the native gene/cDNA is too large for current vectors) must be available.
- ▶ An **appropriate vector** capable of efficient, targeted delivery.
- ▶ **Knowledge of the pathophysiological mechanism** — so gene transfer is predicted to correct the disease process (replacement for loss-of-function mutations; inactivation for dominant-negative mutations).
- ▶ A **favourable risk-to-benefit ratio** in a disease of substantial burden.
- ▶ **Appropriate regulatory elements** governing level, tissue-specificity and timing of expression (critical in some diseases, e.g. thalassaemia; less so in others).
- ▶ An **appropriate target cell** — long-lived/good replicative potential, accessible, and able to be engrafted if manipulated ex vivo.
- ▶ **Strong evidence of efficacy and safety** in cultured-cell and (ideally large-) animal models.
- ▶ **Regulatory approval** — IRB/ethics-committee review and, in most countries, a governmental regulatory body.

Gangane's candidate-disease criteria add: ideally a **genetically recessive** disorder, transduced cells with a **selective survival advantage**, and **no strict requirement for precise dose-regulation** of the gene product.

4. Target tissues in gene therapy

- ▶ **Liver** — hepatocytes removed after partial hepatectomy, transduced ex vivo with a retroviral vector, and returned via the hepatic artery/portal vein (risk: portal-vein thrombosis). Used for **familial hypercholesterolaemia** (LDL-receptor gene); considered for haemophilia A, α 1-antitrypsin deficiency, phenylketonuria.
- ▶ **Muscle** — direct DNA injection or transduced myoblasts; also used to insert genes unrelated to muscle function (e.g. factor VIII, growth hormone).
- ▶ **CNS** — E1-deleted neurotropic adenoviral vectors, or ex vivo-modified cells stereotactically implanted (e.g. caudate nucleus in Huntington disease).
- ▶ **Bone marrow/HSCs** — the ideal target (self-renewing, engraftable, CD34⁺); used for ADA-deficiency and X-linked SCID. Other targets: fibroblasts, endothelial cells, airway epithelial cells, glial cells.

5. Diseases amenable to gene therapy

(A) Monogenic (single-gene) disorders

- ▶ **ADA-deficiency SCID** — T-lymphocytes transduced ex vivo with a retroviral vector carrying the normal ADA gene.
- ▶ **X-linked SCID** — bone marrow HSCs transduced ex vivo with a retroviral vector carrying the γ c-cytokine-receptor-subunit (IL2RG) gene.
- ▶ **Familial hypercholesterolaemia** — hepatocytes transduced with the LDL-receptor gene.
- ▶ **Haemophilia B** — retroviral transfer of the factor IX gene into skin fibroblasts.
- ▶ **Cystic fibrosis** — CFTR gene (or α 1-antitrypsin gene) delivered to airway epithelium by liposome-mediated transfer or by adenoviral vector (lung epithelium proliferates slowly, favouring non-integrating adenoviral delivery).
- ▶ **Duchenne muscular dystrophy** — a "mini-dystrophin" gene (introns removed to fit vector capacity) delivered to muscle.

(B) Cancer — several distinct strategies:

- ▶ **Tumour-suppressor gene replacement** — e.g. wild-type p53, or the gene involved in Wilms tumour.
- ▶ **Blocking an oncogene** — e.g. antisense K-RAS message.
- ▶ **Suicide gene therapy** — insertion of a sensitising gene, e.g. **herpes simplex virus thymidine kinase** (HSV-TK), rendering tumour cells selectively sensitive to a prodrug.
- ▶ **Promoting tumour immunogenicity** — introducing genes for foreign antigens.
- ▶ **Cytokine gene transfer** — enhancing anti-tumour immune-cell activity.
- ▶ **Chemoprotection of stem cells** — introducing the **MDR-1 (multidrug-resistance)** gene to protect marrow stem cells from chemotherapy toxicity.

(C) Cardiovascular disease

- ▶ **Peripheral vascular disease** — vascular grafts resurfaced with endothelial/smooth-muscle cells engineered to carry anti-clotting genes.

- ▶ **Coronary artery disease** — LDL-receptor gene transfer in familial early coronary disease; adenoviral delivery of mutant tissue-plasminogen-activator (t-PA) genes, or t-PA-secreting endothelial cells, to prevent post-angioplasty reocclusion.

(D) **AIDS** — transduction/transplantation of totipotent haemopoietic stem cells, or strategies to inhibit viral replication.

6. Risks and limitations of gene therapy

- ▶ **Adverse reaction to the vector or vector–disease combination** — anticipated (where possible) by prior animal and preliminary human studies.
- ▶ **Insertional mutagenesis causing malignancy** — the transferred gene may integrate near and activate a proto-oncogene, or disrupt a tumour-suppressor gene. This is the chief demonstrated risk of retroviral vectors (see Applied/Clinical anatomy below).
- ▶ **Germ-line mutation risk** — an insertion into a germ cell could, in principle, create a heritable mutation in the patient's offspring; considered rare but is the underlying reason germ-line gene therapy itself is prohibited.
- ▶ **Vector-specific limitations** — retroviruses transduce only dividing cells and are usable only in vitro; adenoviruses and HSV do not integrate, giving only transient expression, and provoke immune responses; AAV vectors have very limited cargo capacity.
- ▶ **Difficulty regulating expression level** of the introduced gene to match physiological need (e.g. an inserted insulin gene must respond appropriately to blood glucose).
- ▶ **Logistic/scale problems** — some diseases (e.g. muscular dystrophies) require gene transfer into a very large number of target cells for clinical benefit.

7. Regulatory and ethical framework

- ▶ **Germ-line gene therapy is not permitted anywhere**; all approved protocols are restricted to somatic-cell gene therapy.
- ▶ **USA** — the **National Institutes of Health (NIH)** lays down guidelines for human gene-therapy trials; a protocol requires approval from both the **Food and Drug Administration (FDA)** and the **Recombinant DNA Advisory Committee (RAC)**.
- ▶ **UK** — the **Committee on the Ethics of Gene Therapy** requires that every gene-therapy protocol be approved by the hospital's research ethics committee.
- ▶ Institutional review board/ethics-committee approval, informed consent, and ongoing safety monitoring are mandatory prerequisites everywhere.

Applied / Clinical anatomy

- ▶ **First human gene therapy** — 14 September 1990: a 4-year-old girl with **ADA-deficiency SCID** received autologous T-lymphocytes transduced ex vivo with a retroviral ADA-gene vector; over 11 transfusions across 2 years her intracellular ADA activity rose from undetectable to 20–30% of normal with normalisation of T-lymphocyte count. A second ADA-SCID patient (9 years old) was treated similarly from January 1991.
- ▶ **X-linked SCID trial** — HSCs transduced ex vivo with a retroviral γ c-cytokine-receptor-subunit vector produced the **first gene-therapy cure of an inherited disease (2000)**. This success was

tempered by a serious complication: **5 of 20 treated patients developed a leukaemia-like disorder from retroviral insertional mutagenesis**, the clinical demonstration of the theoretical safety risk described above.

- ▶ **Familial hypercholesterolaemia** — ex vivo hepatocyte gene therapy with the LDL-receptor gene gave ~30% transduction efficiency and only short-term LDL reduction; carries the risk of portal-vein thrombosis.
- ▶ **Haemophilia B** — first trial, Shanghai, December 1991: retroviral factor IX gene transfer into skin fibroblasts.
- ▶ **Cystic fibrosis** — first NIH trial (17 April 1993) delivered CFTR to airway epithelium; corrects only the pulmonary component of the disease.
- ▶ **Huntington disease** — genetically modified cells transplanted into the caudate nucleus as an experimental CNS approach.

High-yield exam pointers

- ▶ Gene therapy = introduction of a functional gene into a patient's cells for therapeutic benefit; delivery is **ex vivo** or **in vivo**.
- ▶ Transfer methods: **physical** (liposome-mediated, receptor-mediated endocytosis) and **biological/viral** (retrovirus, lentivirus, adenovirus, AAV, HSV).
- ▶ Retrovirus = integrates, dividing cells only, small insert ($\leq 7-8$ kb); Adenovirus = large insert (~36 kb), non-dividing cells, non-integrating/transient; AAV = small insert (~5 kb), stable, low immunogenicity.
- ▶ Only **somatic-cell** gene therapy is ethically permitted; **germ-line gene therapy is prohibited** worldwide.
- ▶ Governing bodies: **NIH, FDA, RAC (USA)**; **Committee on the Ethics of Gene Therapy + hospital ethics committees (UK)**.
- ▶ Essential prerequisites: identified/cloned gene, appropriate vector, favourable risk–benefit ratio, appropriate regulatory elements, accessible/long-lived target cell, regulatory approval.
- ▶ Target tissues: liver (familial hypercholesterolaemia, haemophilia, $\alpha 1$ -antitrypsin deficiency), muscle (DMD), CNS (Huntington disease), bone marrow/HSC (SCID).
- ▶ First human gene therapy: **ADA-deficiency SCID, 14 September 1990**.
- ▶ Chief safety concern: **insertional mutagenesis** — demonstrated clinically by leukaemia developing in patients treated in the X-linked SCID retroviral gene-therapy trial.

SOURCE & COVERAGE

Gangane — Human Genetics 5e (Gene Therapy chapter — gene delivery strategies, transfection methods, viral vectors, target tissues, candidate-disease criteria, governing bodies, landmark trials); Thompson & Thompson — Genetics in Medicine 8e (The Treatment of Genetic Disease — essential requirements for gene therapy, vector biology, risks of gene therapy, X-linked SCID trial outcome).

Coverage note: Gray's Anatomy (Standring) has no dedicated content on gene therapy, a molecular/genetics technique rather than a gross-anatomical topic; grounding is drawn entirely from the Genetics specialty texts as directed by the topic spec.

**Genetic counselling**

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction — Genetic counselling is a communicative process by which patients and/or their relatives who are at risk of a hereditary disorder are made aware of the nature and consequences of the disorder, the probability of transmitting or developing it, and the options available to prevent, avoid, or mitigate its impact. It is nondirective in principle — the counsellor supplies accurate information and psychosocial support but leaves reproductive and management decisions to the family. Because genetic disease is unique in its tendency to recur within a family, counselling addresses not only the index patient (the **proband**) but also present and future relatives (the **consultands**).

Definition, aims, and providers

Genetic counselling advises consultands about three things:

- ▶ **Nature and consequences** of the genetic disorder.
- ▶ **Chances of transmitting** the disorder (recurrence risk).
- ▶ **Options to prevent, avoid, or improve the outcome** of the disorder.

Its broader aims are to help the individual/family:

- ▶ Comprehend the medical facts — diagnosis, natural history, and management.
- ▶ Understand how heredity contributes to the disorder and the recurrence risk for themselves and relatives.
- ▶ Understand the options available for dealing with that risk (testing, prenatal diagnosis, reproductive alternatives).
- ▶ Choose the course of action that fits their own values, beliefs, and family goals — **nondirective counselling**, a principle rooted in respect for reproductive autonomy.
- ▶ Make the best possible psychological adjustment to the disorder or its risk of recurrence.

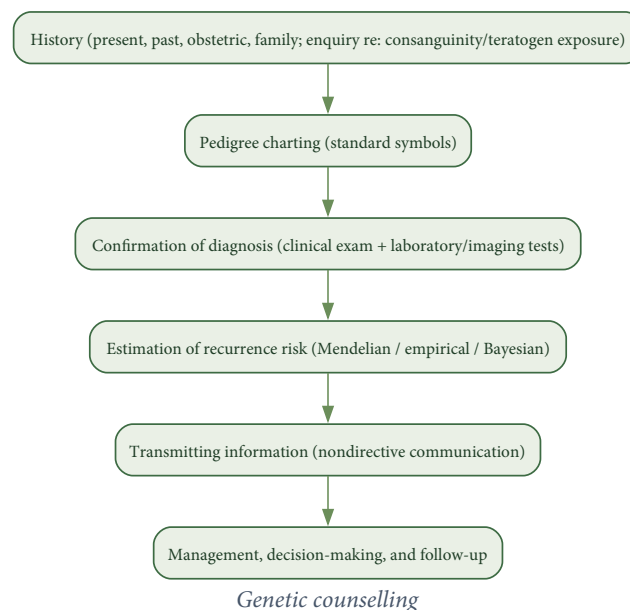
Providers — In many countries genetic counselling is delivered by physicians/clinical geneticists; in the USA, Canada, and the UK it is largely delivered by certified **genetic counsellors** or nurse geneticists (a

self-regulating allied profession with its own accrediting boards), working as part of a team with the clinical geneticist and diagnostic laboratory.

Indications for genetic counselling

Category	Examples
Family/personal history of a genetic disorder	Cystic fibrosis, fragile X syndrome, congenital heart defect, hereditary cancer, haemoglobinopathy
Previous child with anomaly	Multiple congenital anomalies, intellectual disability, isolated birth defect (neural tube defect, cleft lip/palate)
Known carrier parent(s)	Autosomal recessive/X-linked trait carriers
Pregnancy at risk	Advanced maternal age (>35 years), abnormal maternal serum screen, abnormal fetal ultrasound (e.g., increased nuchal translucency ≥ 3 mm at 12 weeks 5 days)
Consanguinity	Parents who are blood relatives (raises autosomal recessive risk)
Teratogen exposure	Occupational chemicals, medications, alcohol, radiation
Reproductive history	Repeated pregnancy loss or infertility
Testing-related	Before/after genetic testing, especially for late-onset disorders (hereditary cancer syndromes); positive newborn-screen follow-up (e.g., phenylketonuria); positive carrier or prenatal screen

The process of genetic counselling



- ▶ **History** — present and past illness, family history (sibs, relatives), obstetric history (teratogen exposure, abortion/stillbirth), and specific enquiry about **consanguinity**, which raises the risk of autosomal recessive disorders.
- ▶ **Pedigree charting** — a concise graphic record of the disorder's distribution in the family, drawn with standardised symbols; time-consuming to construct accurately but an indispensable first step toward risk estimation.

! [Fig 17.23 — ■ Symbols used in pedigree chart. (Vishram Singh — General Anatomy — 4e, p.734)]
(C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/32394585e6d07d6c.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a standard pedigree symbol key and label:

- ▶ Square — male; circle — female; diamond — unknown/unspecified sex
- ▶ Filled (shaded) symbol — affected individual; half-filled symbol — carrier/heterozygote (or obligate carrier dot within an unshaded symbol)
- ▶ Horizontal line joining a square and circle — mating/union; double horizontal line — consanguineous mating
- ▶ Vertical line descending from a union — line of descent to offspring
- ▶ Horizontal "sibship" line joining siblings, in birth order left to right
- ▶ Arrow pointing to a symbol — **proband/index case** (the person through whom the family was ascertained)
- ▶ Diagonal line through a symbol — deceased individual
- ▶ Roman numerals down the left margin — successive generations (I, II, III...)

Pedigree analysis and estimation of recurrence risk

Estimation of recurrence risk is the pivotal step of counselling and depends on the mode of inheritance, pedigree analysis, and (where available) laboratory/linkage test results.

Method	When used	Example
Mendelian calculation	Single-gene disorder with genotypes fully known	Two known heterozygous carriers of an autosomal recessive condition → 1 in 4 risk with each pregnancy, unaffected by the outcome of previous pregnancies
Empirical risk	Multifactorial or chromosomal disorders, mechanism not simply mendelian	Isolated neural tube defect: ≈4% recurrence; Down syndrome (trisomy 21, non-disjunction): ≈1% recurrence — substantially higher if a parent carries a Robertsonian translocation

Method	When used	Example
Bayesian analysis (conditional probability)	Genotype of a key individual is uncertain (e.g., a woman's carrier status for an X-linked recessive trait)	Combines <i>prior</i> probability, <i>conditional</i> probability (from pedigree observations, e.g., unaffected sons), and <i>joint</i> probability to yield a refined <i>posterior</i> probability

Worked Bayesian example (X-linked recessive, e.g., Duchenne muscular dystrophy): A woman is the daughter of an obligate carrier and has three unaffected sons.

	She is a carrier	She is not a carrier
Prior probability	1/2	1/2
Conditional probability (3 normal sons)	1/8	≈ 1
Joint probability	1/16	1/2
Posterior probability	1/9	8/9

Her risk of a future affected son thus falls from the naive prior of 1/8 to $1/9 \times 1/4 = 1/36$ — considerably lower once the pedigree information (three unaffected sons) is incorporated. The same Bayesian logic (prior $\times$ conditional $\rightarrow$ joint $\rightarrow$ posterior) is applied to autosomal recessive pedigrees where sibs of a proband have an a-priori 2/3 chance of being carriers.

Two laws underlie all such calculations: the **law of addition** (mutually exclusive events — probabilities add) and the **law of multiplication** (independent events — probabilities multiply), together with the **binomial distribution** for predicting sibship patterns.

Preventive and management options

Once risk is established, options discussed with the family (never imposed, in keeping with nondirective counselling) include:

- ▶ **Carrier testing** (karyotyping, biochemical, or molecular/genome analysis) — may reassure a couple or confirm increased risk.
- ▶ **Prenatal diagnosis** (ultrasound, maternal serum screening, amniocentesis, chorionic villus sampling, fetal blood sampling, non-invasive cell-free DNA screening) with the option of pregnancy termination if the couple finds this acceptable.
- ▶ **Preimplantation genetic diagnosis (PGD)** on a blastomere/blastocyst biopsy after IVF — avoids the issue of pregnancy termination but requires assisted reproduction.
- ▶ **Reproductive alternatives** — contraception/sterilisation if no further children are desired; adoption (with pre-adoption counselling); artificial insemination by donor (indicated when the father carries an autosomal dominant/X-linked defect, or when both partners carry an autosomal recessive trait — not indicated if the mother carries the defect); donor-egg IVF if the mother carries an autosomal dominant or X-linked defect.
- ▶ **Early detection and treatment** where a treatable disorder exists (e.g., a phenylalanine-restricted diet in phenylketonuria detected on newborn screening, preventing intellectual disability).

- ▶ **Referral** to specialists, genetic clinics, and disease-specific patient/family support groups, with **periodic follow-up** — essential in genetic clinics to reinforce understanding, support decision-making in future pregnancies, and arrange carrier/prenatal testing for at-risk relatives (e.g., translocation-carrier testing in relatives of a Down syndrome child).

Applied / Clinical anatomy

- ▶ **Antenatal screening thresholds** — increased nuchal translucency (≥ 3 mm) on ultrasound at 12 weeks + 5 days, and advanced maternal age (>35 years), are standard triggers for referral to genetic counselling and further prenatal testing.
- ▶ **Maternal serum screening** — raised alpha-fetoprotein suggests a neural tube defect; unusually low alpha-fetoprotein suggests fetal Down syndrome.
- ▶ **Newborn/carrier screening programmes** — must satisfy set criteria: the disorder should be clearly defined, of reasonable population frequency, preferably treatable, and the test should be quick, inexpensive, and reliable (minimal false positives/negatives). Examples: phenylketonuria (newborn screen), Tay–Sachs disease (heterozygote/preconception screen in Ashkenazi Jewish populations — hexosaminidase A deficiency).
- ▶ **Medico-legal precedent** — a "wrongful life" suit was upheld after a couple, wrongly reassured of non-carrier status for Tay–Sachs disease, had an affected child, underscoring the medico-legal weight of accurate counselling.
- ▶ **Childhood genetic testing controversy** — presymptomatic testing is justified when clinical benefit follows (e.g., APC gene carriers begin colonoscopy surveillance by 12 years of age) but is discouraged when no preventive/therapeutic benefit exists and only anxiety/stigma results (e.g., childhood testing for Huntington disease).
- ▶ **Preimplantation diagnosis ethics** — beyond avoiding an affected embryo, PGD has been used controversially for HLA-matched embryo selection to provide a stem-cell/bone-marrow donor sibling (e.g., Fanconi anaemia), and even, in one reported case, to deliberately select for an autosomal recessive trait (deafness) — illustrating the ethical tension between parental choice and the future child's interests.
- ▶ **Follow-up model** — Down syndrome (trisomy 21): counselling includes acceptance and referral for developmental support, plus future-pregnancy counselling covering recurrence risk (higher if a parent carries a Robertsonian translocation) and prenatal/carrier testing options.

High-yield exam pointers

- ▶ Genetic counselling = communication process covering **nature, transmission risk, and prevention/management** of a genetic disorder — always **nondirective**.
- ▶ Five-step process: **history** → **pedigree charting** → **diagnosis confirmation** → **risk estimation** → **transmitting information/management/follow-up**.
- ▶ Standard pedigree symbols: square = male, circle = female, filled = affected, line of descent, horizontal sibship line, arrow = proband, double line = consanguineous union.
- ▶ Recurrence risk methods: **Mendelian** (single-gene, genotype known) vs **empirical** (multifactorial/chromosomal) vs **Bayesian/conditional probability** (uncertain genotype).

- ▶ Bayes' theorem combines prior $\times$ conditional $\rightarrow$ joint probability $\rightarrow$ posterior probability; classic worked example is X-linked recessive carrier risk refinement using unaffected sons.
- ▶ Key indications: previous child with anomaly, family history of a hereditary disorder, consanguinity, teratogen exposure, advanced maternal age, abnormal prenatal screen/scan, recurrent pregnancy loss.
- ▶ Preventive/management options: carrier testing, prenatal diagnosis, PGD, contraception/sterilisation, adoption, donor gametes, early treatment (e.g., PKU diet), and follow-up.
- ▶ Screening programme criteria: clearly defined disorder, adequate frequency, treatable, test quick/cheap/reliable.
- ▶ Ethical-legal issues: nondirectiveness, confidentiality, "wrongful life" litigation, childhood testing only if clinically beneficial, PGD controversies (HLA matching, selecting for a trait).

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e; Vishram Singh — General Anatomy 4e.

Coverage note: Genetic counselling is a clinical-genetics topic with no dedicated chapter in the spine text, Gray's Anatomy (Standring) 41e; the answer is grounded instead in the genetics specialty texts (Gangane; Thompson & Thompson) and the genetics chapter of Vishram Singh's General Anatomy, which are the standard sources used for this topic in the RGUHS anatomy curriculum.



Genetic research and engineering

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction — **Genetic engineering** is the deliberate, in-vitro manipulation of DNA — isolating, cutting, modifying, and transferring genes across organisms — to produce a **recombinant DNA molecule** with new hereditary information. **Genetic research** encompasses the wider set of molecular tools (gene cloning, PCR, blotting, sequencing, gene mapping) built on this technology that have transformed classical cytogenetics into modern molecular medical genetics. Together they underlie the Human Genome Project, molecular diagnosis of disease genes, recombinant therapeutic proteins, and gene therapy, and are therefore core "recent advances" content for the PG anatomy/genetics curriculum.

1. Basic tools of genetic engineering

Tool	Key features
Restriction endonucleases	Bacterial enzymes that cleave DNA at specific recognition sites; discovered by Hamilton Smith and colleagues (1970); over 200 are now known; form the "molecular scissors" of genetic engineering

Tool	Key features
DNA ligase	Enzyme that joins ("glues") the cut DNA fragment into the vector, sealing the phosphodiester backbone
Vectors (carrier molecules)	Plasmids — circular extra-chromosomal bacterial DNA, self-replicating; e.g. pBR322 (4362 bp), carries up to ~8 kb insert and antibiotic-resistance markers for selection. Phages (bacteriophages) — carry larger inserts, up to ~15 kb. Cosmids — phage-plasmid hybrids, carry 35–45 kb. Yeast artificial chromosomes (YACs) — contain centromere + telomere sequences, carry inserts up to 1000 kb; used for large genomic fragments (e.g. cystic fibrosis, neurofibromatosis, dystrophin genes)
Host organism	Usually <i>E. coli</i> , rendered permeable to the recombinant plasmid by exposure to calcium salts

2. Recombinant DNA technology — procedure

Constructing a recombinant DNA molecule involves five sequential steps:

- ▶ **Restriction digestion** — DNA is cut into fragments using a restriction endonuclease.
- ▶ **Ligation** — the fragment is incorporated into a suitable vector (plasmid/phage/cosmid/YAC) using DNA ligase.
- ▶ **Transformation** — the recombinant vector is reinserted into a host cell (e.g. *E. coli*) by exposing the bacterial cell to calcium salts, rendering it permeable to plasmid uptake.
- ▶ **Cloning** — host cells carrying the vector are grown in culture medium, producing clones that give multiple copies of the foreign DNA fragment.
- ▶ **Selection** — clones bearing the correct/desired DNA fragment are identified and selected (commonly via antibiotic-resistance markers on the vector).

□ Fig 3.11 — Constructing recombinant plasmid and transformation of host cell. (Gangane — Human Genetics — 5e, p.67)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A simple boxed schematic of recombinant plasmid construction, labelled:

- ▶ Donor (foreign) DNA + bacterial plasmid, each cut by the **same restriction endonuclease** (showing complementary "sticky ends")
- ▶ Foreign DNA fragment ligated into the plasmid using **DNA ligase** → recombinant plasmid
- ▶ Recombinant plasmid taken up by a host bacterium (*E. coli*) rendered permeable by **calcium salts** — labelled "transformation"
- ▶ Host cell cultured to form **clones**, each carrying multiple copies of the recombinant plasmid
- ▶ Arrow to "**selection of clones**" bearing the desired DNA fragment (antibiotic-resistance marker)

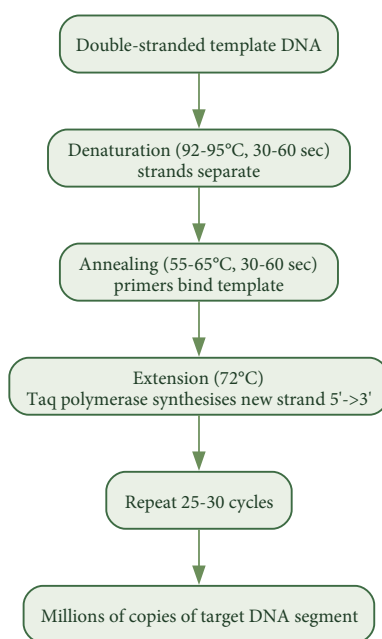
3. Gene cloning, gene bank and DNA probes

- ▶ **Gene cloning:** the desired gene is inserted into a vector, which carries it across the host-cell membrane; once accepted, the host cell replicates and expresses the cloned gene, producing multiple identical copies ("clones") of the gene/protein.
- ▶ **Gene bank:** a collection of recombinant DNA molecules that together represent the complete genetic information of an organism, preserved as plasmids in bacterial/bacteriophage cultures; can be re-isolated on demand — useful also for conserving genetic material of endangered species.
- ▶ **DNA probe:** reverse transcriptase (from RNA tumour viruses) synthesises a complementary DNA (cDNA) from a known mRNA; the radiolabelled cDNA (the "probe") hybridises specifically with its target gene/mRNA and is the basis of Southern/Northern blotting and FISH.

4. Polymerase chain reaction (PCR)

PCR was discovered by **Kary Mullis** and developed by Saiki and colleagues in **1985**; it is an *in-vitro* method that rapidly amplifies a specific DNA segment without replicating the rest of the genome.

Requirements: template DNA, a pair of oligonucleotide primers (20–30 nucleotides, complementary to the two ends of the target sequence), dNTPs, Mg^{2+} , buffer, and a thermostable DNA polymerase (**Taq polymerase**), run in a programmable thermal cycler.



Genetic research and engineering

Applications: molecular diagnosis, criminology/forensics, prenatal diagnosis, and as a template-amplification step preceding Southern blot RFLP/DNA-fingerprint analysis of degraded/limited samples. **Advantages:** rapid, highly sensitive, works on impure/minute samples (blood, bone, single sperm, hair follicle, paraffin-embedded tissue). **Limitations:** false-positive results from contamination; false-negative results from low yield.

5. Molecular hybridisation and typing techniques

Technique	Principle	Use
Southern blot (Edwin Southern, 1975)	DNA cut by restriction enzymes → separated by agarose gel electrophoresis → denatured → blotted onto nitrocellulose → hybridised with radiolabelled DNA probe → autoradiography	Detects a specific DNA fragment/RFLP
Northern blot	Same principle applied to isolated mRNA	Determines size and quantity of an mRNA transcript
DNA fingerprinting (Sir Alec Jeffreys, 1985)	Exploits hypervariable regions (variable number of tandem repeats, VNTRs) unique to each individual; digested DNA typed with single-locus/multilocus probes	Forensic identification, paternity/tissue matching, mass-disaster/exhumed-body identification

6. Human Genome Project (HGP) and newer genomic tools

- Initiated **October 1990** with three goals: (i) genetic marker map (RFLPs, STRPs, VNTRs, SNPs), (ii) physical map (sequence-tagged sites), (iii) complete 3-billion-base-pair genome sequence.
- A "rough draft" (90% sequenced) was announced in **February 2001**; the complete, highly accurate sequence was announced in **2003** — 50 years after Watson and Crick's DNA structure.

- Completion enabled **positional cloning** of disease genes, improving genetic diagnosis and drug/gene-therapy design.
- **Gene chips/microarrays**: postage-stamp-sized devices holding DNA from thousands of genes, used for large-scale genetic screening and drug-target discovery.
- **Genome (gene) editing** — a newer engineered-nuclease technology that creates a site-specific double-stranded break, repaired by homology-directed repair to correct the mutant sequence in situ; the **CRISPR/Cas9** system is the most widely used current platform (Thompson & Thompson, 8e), offering an alternative to viral-vector gene therapy without the risk of semi-random vector integration.

7. Applications of genetic engineering — Gene therapy

Gene therapy = replacement/correction of a defective gene in a patient's cells. Only **somatic-cell** gene therapy is ethically sanctioned worldwide; germ-line gene therapy is prohibited. Regulatory oversight (e.g. NIH, FDA, Recombinant DNA Advisory Committee in the USA) and institutional ethics-committee approval are mandatory.

Delivery strategy: *ex vivo* (cells removed, engineered, then re-transplanted) or *in vivo* (gene introduced directly into target tissue).

Vector/method	Advantage	Disadvantage
Liposome-mediated transfer	Free of viral risk; can carry large DNA (mini-chromosomes)	Transient expression, needs repeated dosing
Receptor-mediated endocytosis	Cell/tissue-targeted (e.g. hepatocyte galactose receptors)	Escape from lysosomal degradation is inefficient
Retrovirus	Stably integrates into host genome	Small insert only (<7 kb); transduces dividing cells only; risk of insertional oncogenesis
Adenovirus	Stable, easily purified, infects non-dividing cells, carries up to 36 kb	Does not integrate — transient expression; immunogenic
Herpes virus	Naturally neurotropic — suited to CNS/neurons	Toxic to neurons; provokes immune response
Adeno-associated virus (AAV)	Stable, non-pathogenic, high integration efficiency	Possible immune response

Candidate-disease prerequisites: genetically recessive disorder; defective gene identified and cloned; precise dose-regulation of the product not essential; corrected cells have a selective survival advantage.

Illustrative applications:

- **ADA-deficiency SCID** — the world's first human gene therapy (14 September 1990): autologous T-lymphocytes transduced *ex vivo* with a retroviral vector carrying the normal ADA gene.
- **Familial hypercholesterolaemia** — hepatocytes transduced with the LDL-receptor gene via retroviral vector.
- **Haemophilia B** — retroviral transfer of the Factor IX gene into skin fibroblasts (first trial, Shanghai, 1991).

- ▶ **Cystic fibrosis** — CFTR gene delivered to airway epithelium by adenoviral or liposomal vectors (first NIH trial, 1993).
- ▶ **Cancer** — insertion of tumour-suppressor genes (p53), anti-oncogene antisense constructs, suicide genes (HSV-thymidine kinase), and MDR-1 to protect marrow stem cells from chemotoxicity.

Other medical applications of recombinant DNA technology: production of therapeutic human proteins (insulin, growth hormone), recombinant vaccine antigens (hepatitis B), diagnostic reagents (HIV/AIDS test kits), and molecular understanding of disease mechanisms (sickle cell disease, thalassaemias, cystic fibrosis, Huntington disease).

Applied / Clinical anatomy

- ▶ **Prenatal diagnosis:** PCR/DNA analysis of chorionic villus samples and amniotic fluid cells detects chromosomal/single-gene disorders in utero.
- ▶ **Forensic medicine:** DNA fingerprinting (VNTR/STR typing) from blood, hair, saliva, or skeletal remains — used in paternity disputes, criminal identification, and mass-disaster/exhumed-body identification (e.g. identification of Romanov remains).
- ▶ **Transplantation:** DNA-based tissue typing improves donor–recipient matching.
- ▶ **Genetic counselling:** recombinant-DNA-based carrier/mutation testing (e.g. cystic fibrosis, Duchenne muscular dystrophy) informs risk counselling.
- ▶ **Regulatory/ethical anatomy of the field:** strict prohibition of germ-line manipulation preserves the integrity of the human gene pool across generations.

High-yield exam pointers

- ▶ Recombinant DNA = restriction enzyme cut → vector ligation → transformation (calcium salts) → cloning → selection (5 steps).
- ▶ Vectors in order of increasing insert capacity: plasmid (~8 kb) → phage (~15 kb) → cosmid (35–45 kb) → YAC (up to 1000 kb).
- ▶ PCR: denaturation (92–95°C) → annealing (55–65°C) → extension (72°C, Taq polymerase); 25–30 cycles; discovered by Kary Mullis, 1985.
- ▶ Southern blot = DNA; Northern blot = RNA; DNA fingerprinting (Alec Jeffreys, 1985) uses VNTRs.
- ▶ Human Genome Project: initiated 1990, draft 2001, complete 2003.
- ▶ First human gene therapy: ADA-deficiency SCID, 14 September 1990.
- ▶ Only somatic-cell gene therapy is permitted; germ-line gene therapy is banned worldwide.
- ▶ Common viral vectors: retrovirus (small insert, integrates, dividing cells only), adenovirus (large insert, non-integrating, non-dividing cells), AAV (stable, non-pathogenic).
- ▶ CRISPR/Cas9 is the current leading genome-editing platform, offering site-specific correction without random vector integration.

SOURCE & COVERAGE

Gangane — Human Genetics 5e (Molecular Genetics; Gene Therapy chapters); Thompson & Thompson — Genetics in Medicine 8e (Treatment of Genetic Disease — gene therapy trials, gene editing/CRISPR-Cas9).

Coverage note: This topic has no dedicated chapter in Gray's Anatomy (Standring); grounding for genetic-engineering technique detail is drawn from the genetics specialty texts (Gangane, Thompson & Thompson) as directed by the topic spec.



Give an account of karyotyping of human chromosomes

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction

A **karyotype** is the complete chromosomal constitution of a somatic cell of an individual — a standard photomicrographic display of its metaphase chromosomes, cut out, paired by homology and arranged in descending order of size. **Karyotyping** is the laboratory process by which such a display is prepared and analysed; it is the foundational technique of clinical cytogenetics, exposing numerical and structural chromosomal abnormalities. The modern era of human cytogenetics began when **Joe Hin Tjio and Albert Levan (1956)**, using an improved hypotonic pre-treatment, established the correct human diploid number as **46 (23 pairs)**, replacing the earlier accepted figure of 48. Because individual chromosomes are distinguishable only when maximally condensed, every karyotyping protocol is built around capturing dividing cells at **metaphase**.

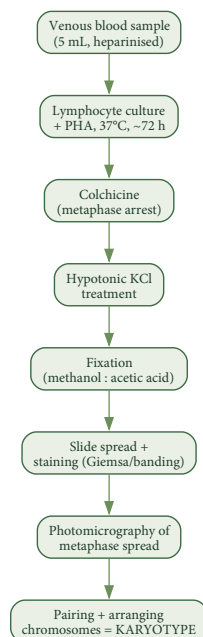
1. Sources of cells for karyotyping

Source	Feature
Peripheral blood lymphocytes (T-cells)	Commonest, routine method; short-term culture (3–4 days); rapid clinical turnaround
Bone marrow	High mitotic index, little/no culturing needed; used for suspected haematological malignancy
Skin fibroblasts (skin biopsy)	Long-term culture; useful for mosaicism and permanent storage
Amniocytes (amniotic fluid) / chorionic villus sample	Prenatal diagnosis; CVS can also be examined directly without culture
Lymphoblastoid cell lines	Transformed, potentially immortal lines for repeated/future study

2. Step-by-step procedure

- ▶ **Sample collection:** about 5 mL of venous blood is collected in a heparinised tube.

- **Culture:** lymphocytes are cultured in tissue-culture medium with **phytohaemagglutinin (PHA)**, a mitogen that stimulates T-lymphocytes to divide, and incubated at **37°C for about 3 days (66–72 hours)**.
- **Metaphase arrest:** **colchicine** (or colcemid) is added a few hours before harvest — it inhibits mitotic-spindle microtubule formation, arresting dividing cells at metaphase, when chromosomes are best visualised.
- **Hypotonic treatment:** cells are swollen in a hypotonic solution (e.g. dilute KCl), causing the chromosomes to disperse and separate from each other.
- **Fixation:** the cell suspension is gently fixed (chilled methanol : glacial acetic acid).
- **Spreading:** the suspension is dropped onto a chilled glass slide from a height, mechanically rupturing the cells and spreading the metaphase chromosomes flat.
- **Staining:** the spread is stained — routinely with Giemsa, or by one of the banding methods (below) depending on the diagnostic question.
- **Photomicrography:** well-spread metaphase plates are photographed under oil immersion.
- **Karyotype assembly:** individual chromosomes are cut out of the photograph (or digital image), paired by homology, and arranged in the standard sequence — this arranged picture is the karyotype. Computerised image-analysis systems now automate the cutting, pairing and arranging.



Give an account of karyotyping of human chromosomes

3. Morphological classification of a chromosome

A metaphase chromosome has two sister chromatids joined at the **centromere** (**primary constriction**), which bears the **kinetochore** (site of spindle attachment) and divides each chromatid into a **short arm** (**p**) and a **long arm** (**q**). Some chromosomes show a **secondary constriction**, distal to which lies a **satellite**. Each arm ends in a **telomere** (repetitive TTAGGG sequence).

Based on the position of the centromere, four morphological types are recognised:

Type	Centromere position	Arms
Metacentric	Central	Two arms of about equal length
Submetacentric	Between centre and end	One short (p), one long (q) arm
Acrocentric	Close to one end	One very short arm (often bearing a satellite), one very long arm
Telocentric	At the very end	Single arm only — not seen in the normal human karyotype

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Structure of a metaphase chromosome and the four morphological types. Labels required: sister chromatids; centromere (primary constriction)/kinetochore; short arm (p) and long arm (q); secondary constriction; satellite; telomere — then, side by side, the four types: metacentric (centromere central, arms equal), submetacentric (centromere off-centre, arms unequal), acrocentric (centromere near one end, satellite on short arm), telocentric (centromere at the extreme end, single arm).

4. Denver classification (1960) and the normal human karyotype

The Denver Conference (Denver, Colorado, 1960), ratified at London in 1963, grouped the 46 human chromosomes (including the sex chromosomes) into **seven groups, A to G**, based on (a) overall length, (b) position of the centromere, (c) arm-length ratio, and (d) presence of satellites. Chromosomes are numbered in order of decreasing length; the X chromosome resembles chromosome 6 and is placed in group C, while the Y chromosome resembles chromosome 21 and is placed in group G.

Group	Chromosomes	Features
A	1–3	Large, metacentric/submetacentric — longest
B	4–5	Large, submetacentric
C	6–12 + X	Medium-sized, submetacentric; X resembles no. 6
D	13–15	Medium-sized, acrocentric; satellites present
E	16–18	Short, submetacentric (16 nearly metacentric)
F	19–20	Shortest, metacentric
G	21–22 + Y	Shortest, acrocentric; 21 and 22 bear satellites, Y does not

A normal karyotype is written as **46,XX** (female) or **46,XY** (male) — total chromosome number, comma, sex-chromosome constitution.

□ Fig 2.11 — A human male karyotype with Giemsa banding (G banding). The chromosome (Thompson & Thompson — *Genetics in Medicine* — 8e, p.21)

5. Chromosome banding techniques

The Denver system alone could not detect subtle structural rearrangements. Banding techniques, introduced from the early 1970s, stain each chromosome with a reproducible pattern of light and dark bands, permitting unambiguous identification of every chromosome and precise localisation of breakpoints.

Technique	Method	Bands produced
Q-banding	Quinacrine mustard/fluorochrome stain + UV fluorescence	Bright/dull fluorescent Q bands — the earliest banding method
G-banding (GTG)	Trypsin pre-treatment + Giemsa stain	Alternating dark/light G bands — the routine, gold-standard method today
R-banding	Heat pre-treatment + reverse Giemsa	Reverse of the G-band pattern; useful for telomeric regions
C-banding	Special stain for constitutive heterochromatin	Centromeric heterochromatin and secondary constrictions (chromosomes 1, 9, 16 and the long arm of Y)
T-banding	Selective stain for chromosome ends	Telomeric regions
High-resolution (prometaphase) banding	Chromosomes harvested at prophase/prometaphase, less condensed	Up to ~850 bands per haploid set (versus ~400–550 at routine metaphase) — reveals subtler deletions/duplications

The Paris Conference (1971) used banding patterns to divide each chromosome arm into numbered regions (1, 2, 3... from centromere to telomere), each further subdivided into numbered bands, allowing any locus to be described precisely (e.g., band 5q13).

6. Karyotype nomenclature (ISCN)

The Denver, London and Paris agreements were consolidated in 1978 into "An International System for Human Cytogenetic Nomenclature" (ISCN), which is periodically revised and remains the current standard for describing a karyotype. A karyotype is written as: **total chromosome number, sex-chromosome constitution, description of any abnormality.**

Symbol	Meaning	Example
p / q	Short / long arm	—
cen	Centromere	—
del	Deletion	46,XX,del(5)(q13)
dup	Duplication	—

Symbol	Meaning	Example
inv	Inversion	inv(3)(p25q21)
t	Translocation	46,XX,t(2;8)(q22;p21)
rob	Robertsonian translocation	rob(14;21)(q10;q10)
r	Ring chromosome	46,X,r(X)
mar	Marker chromosome	47,XX,+mar
+ / –	Gain / loss of a whole chromosome	47,XX,+21 (trisomy 21); 45,XY,–22 (monosomy 22)
/	Mosaicism	46,XX/47,XX,+21
mat / pat	Maternal / paternal origin	47,XY,+der(1)mat

Applied / Clinical anatomy

- **Indications for karyotyping:** problems of early growth and development (developmental delay, dysmorphic facies, multiple congenital malformations, ambiguous genitalia, intellectual disability); stillbirth and neonatal death (chromosome abnormality found in up to ~10% of stillbirths versus ~0.7% of live births); infertility or two or more miscarriages (a parental chromosomal abnormality is found in 3–6% of such couples, usually a balanced translocation); a known chromosomal/genomic abnormality in a first-degree relative; neoplasia, especially haematological malignancy (bone-marrow karyotyping for diagnosis and prognosis — e.g., the Philadelphia chromosome translocation, t(9;22), in chronic myeloid leukaemia); and pregnancy in women of advanced maternal age (>35 years) or with an abnormal antenatal screen (prenatal karyotyping on amniocytes/chorionic villi).
- **Uses:** confirming a suspected clinical syndrome, gene mapping, prognostic evaluation of malignancy, and prenatal diagnosis.
- **Common disorders detected — numerical:** trisomy 21/Down syndrome, trisomy 18/Edwards syndrome, trisomy 13/Patau syndrome, 45,X/Turner syndrome, 47,XXY/Klinefelter syndrome, 47,XYY, 47,XXX.
- **Common disorders detected — structural:** 5p deletion (cri-du-chat syndrome), Robertsonian translocation (translocation Down syndrome), balanced reciprocal translocations (recurrent-miscarriage carriers), and acquired translocations in leukaemia (e.g., Philadelphia chromosome in CML).
- **Beyond conventional karyotyping:** fluorescence in situ hybridisation (FISH) allows rapid, targeted detection of a specific abnormality on metaphase or interphase cells; chromosomal microarrays detect genome-wide submicroscopic gains/losses beyond banding resolution; and non-invasive prenatal testing (cell-free fetal DNA) is used as a screening adjunct. Conventional karyotyping, however, remains the only routine technique that reliably detects **balanced structural rearrangements and mosaicism** across the whole genome.

High-yield exam pointers

- ▶ Karyotype = arranged photomicrograph of the metaphase chromosomes of a cell; karyotyping = the process of preparing it.
- ▶ **Tjio and Levan (1956)** established the human diploid chromosome number as **46**.
- ▶ Standard technique: peripheral blood lymphocyte culture with PHA (37°C, ~72 h) → colchicine metaphase arrest → hypotonic KCl → fixation → spread → Giemsa/banding stain → photomicrography → pairing/arrangement.
- ▶ **Denver system (1960)**: 7 groups, A–G, by size, centromere position, arm ratio and satellites; X in group C, Y in group G.
- ▶ **G-banding** (trypsin + Giemsa) is today's routine gold-standard stain; Q- (quinacrine/UV), R- (reverse Giemsa), C- (centromeric heterochromatin) and T- (telomeric) banding are the other named methods.
- ▶ **High-resolution/prometaphase banding** shows up to ~850 bands per haploid set versus ~400–550 at routine metaphase, detecting smaller structural changes.
- ▶ **ISCN (1978, periodically revised)** is the standard nomenclature — e.g., 47,XY,+21 for a male with Down syndrome.
- ▶ Four morphological types by centromere position: metacentric, submetacentric, acrocentric, telocentric (telocentric absent in the normal human karyotype).
- ▶ Karyotyping alone reliably detects balanced rearrangements and mosaicism; FISH, microarray and sequencing complement but do not replace it.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Thompson & Thompson — Genetics in Medicine 8e; IB Singh — Textbook of Anatomy Vol 3, 6e (Chromosomes); Vishram Singh — General Anatomy 4e and Textbook of Clinical Embryology; Gangane — Human Genetics 5e.



Karyotype

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — A **karyotype** is the complete chromosomal constitution of a somatic cell of an individual (or species) arranged, paired, and numbered in a standard format according to size and centromere position; the term also denotes the photomicrograph of this arrangement. **Karyotyping** is the laboratory process by which metaphase chromosomes are cultured, arrested, stained, and analysed to produce this arrangement. It is the single most important cytogenetic test for detecting numerical and structural chromosomal abnormalities and remains the frontline investigation in clinical, prenatal, and oncological genetics.

1. Chromosome morphology — the basis of classification

- ▶ Each human somatic cell is **diploid** ($2n = 46$): 44 autosomes (22 pairs) + 2 sex chromosomes (XX in the female, XY in the male); gametes are haploid ($n = 23$).

- ▶ A metaphase chromosome has two sister **chromatids** joined at the **centromere** (primary constriction), which divides each chromatid into a **short arm (p, French petite)* and a *long arm (q)**.
- ▶ Based on the **position of the centromere**, chromosomes are classified into four morphological types:

Type	Centromere position	Arm lengths
Metacentric	Central	$p \approx q$
Submetacentric	Between centre and end	one arm short, one long
Acrocentric	Near one end	p very short, q very long
Telocentric	At the very end (single arm)	not seen in normal human chromosomes

- ▶ Some chromosome arms show a **secondary constriction**, distal to which lies a small mass of chromatin called the **satellite**; satellited (acrocentric) chromosomes — **13, 14, 15, 21, and 22** — carry hundreds of ribosomal-RNA gene copies on these stalks. The **Y chromosome**, although also small and acrocentric, characteristically **lacks a satellite**, which helps distinguish it from chromosomes 21/22 within the same group.
- ▶ Each arm terminates in a **telomere**, a repetitive nucleotide sequence (TTAGGG in humans, repeated ~2500 times) that maintains chromosomal stability.

2. Denver classification of human chromosomes (1960)

Proposed at the Denver (Colorado) cytogenetics convention of 1960 and accepted at London in 1963, this system arranges the 46 chromosomes into **seven groups (A–G)** in descending order of length, using **size** and **centromere position** as the two classifying parameters:

Group	Chromosome numbers	Size	Centromere	Special feature
A	1, 2, 3	Large	Metacentric	Longest chromosomes
B	4, 5	Large	Submetacentric	—
C	6–12 + X	Medium	Submetacentric	X resembles chromosome 6
D	13–15	Medium	Acrocentric	Satellites present
E	16–18	Small	Submetacentric (16 nearly metacentric)	—
F	19, 20	Short	Metacentric	—
G	21, 22 + Y	Very short	Acrocentric	21 & 22 satellited; Y non-satellited, resembles chromosome 21

3. Chromosome banding and the Paris/ISCN nomenclature

The Denver system alone could not identify subtle structural rearrangements, deletions, or translocations, so **banding techniques** were developed:

- ▶ **Q-banding** — quinacrine dye, fluorescent bands under UV light (first banding method, early identification tool).
- ▶ **G-banding** — trypsin digestion followed by Giemsa stain; the routine, cost-effective, gold-standard method giving 400–550 bands per haploid set at metaphase; **high-resolution (prometaphase) banding** of early-division chromosomes can resolve up to 850 bands, improving detection of subtle deletions/duplications.
- ▶ **R-banding** — reverse of the G-banding pattern (light and dark bands interchanged), developed in France.
- ▶ **C-banding** — stains constitutive heterochromatin, particularly at the centromere and secondary constrictions.

The **Paris Convention (1971)** standardised numbering of these bands: each chromosome arm is divided into numbered **regions** (1, 2, 3...) from the centromere outward, and each region into numbered **bands**, allowing any locus to be designated unambiguously (e.g., 5q13 = chromosome 5, long arm, region 1, band 3). In 1978 these Denver–London–Paris agreements were consolidated into the **International System for Human Cytogenetic Nomenclature (ISCN)**, which is periodically revised (e.g., ISCN 2013) and is now the universal reporting standard. A karyotype is written as [total chromosome number], [sex chromosomes], [abnormality if any]:

Symbol	Meaning	Example	Interpretation
46,XX / 46,XY	Normal karyotype	46,XX	Normal female
+ / –	Gain / loss of a chromosome	47,XX,+21	Female, trisomy 21
del	Deletion	46,XX,del(5)(q13)	Terminal deletion, chromosome 5 long arm
t	Translocation	46,XX,t(2;8)(q22;p21)	Balanced translocation, chromosomes 2 & 8
rob	Robertsonian translocation	rob(14;21)(q10;q10)	Fusion at centromeric bands of 14 & 21
inv	Inversion	inv(3)(p25q21)	Pericentric inversion, chromosome 3
r	Ring chromosome	46,X,r(X)	Female, ring X
mar	Marker chromosome	47,XX,+mar	Extra unidentified chromosome
/	Mosaicism	46,XX/47,XX,+21	Two cell lines — normal and trisomy 21

4. Technique of karyotyping

- ▶ **Cell source** — peripheral blood **T lymphocytes** are the commonest and most convenient source; other sources include skin fibroblasts (biopsy, long-term culture), bone marrow (rapidly dividing, useful in haematological malignancy), and fetal cells from **amniotic fluid (amniocytes)** or **chorionic villus biopsy** for prenatal diagnosis.
- ▶ **Culture** — ~5 mL venous blood is treated with **phytohaemagglutinin (PHA)** to stimulate lymphocyte division and cultured at 37 °C for about 72 hours (3–4 days).
- ▶ **Metaphase arrest** — **colchicine** (a mitotic spindle poison) is added to arrest dividing cells in metaphase, the stage at which chromosomes are maximally condensed and individually distinguishable.
- ▶ **Hypotonic treatment** — cells are swollen in hypotonic saline to spread the chromosomes apart.
- ▶ **Fixation and spreading** — cells are fixed and dropped onto a slide from a height to spread the metaphase chromosomes.
- ▶ **Staining** — most commonly by **G-banding**; other stains (Q, R, C) are used for specific indications.
- ▶ **Photomicrography and karyogram construction** — the metaphase spread is photographed/digitally imaged, and individual chromosomes are identified, paired, and arranged in descending order of length into the standard karyogram (largely automated today).
- ▶ **Adjunct/complementary techniques** — **fluorescence in situ hybridisation (FISH)** uses fluorochrome-labelled DNA probes to detect specific chromosomal regions or numerical abnormalities rapidly, including in interphase nuclei; **chromosomal microarrays** and **whole-genome sequencing** now extend resolution well beyond conventional banding for submicroscopic deletions/duplications, though the G-banded karyotype remains the frontline test for whole-genome structural and numerical screening.

5. Diagram — the human karyotype

□ Fig 17.7 — Karyotype of a normal male (Vishram Singh — General Anatomy — 4e, p.695)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the standard karyogram layout (chromosomes arranged in two rows, descending length, paired):

- ▶ Row labels: **Groups A to G** placed above their respective chromosome pairs.
- ▶ Chromosome numbers 1–22 in sequence, followed by the **sex chromosome pair (XX or XY)** at the end.
- ▶ On one representative chromosome of each morphological type, label the **centromere**, **short arm (p)**, and **long arm (q)**; mark metacentric (Group A), submetacentric (Group C), and acrocentric (Group D/G) examples.
- ▶ Mark **satellites** on the short arms of chromosomes 13, 14, 15, 21, and 22 (Groups D and G); indicate that the Y chromosome (Group G) has no satellite.
- ▶ Optionally shade alternating **G-bands** on one chromosome pair to indicate banding pattern.

Applied / Clinical anatomy

- ▶ **Indications for chromosome/karyotype analysis:**
 - Problems of early growth and development — failure to thrive, developmental delay, dysmorphic facies, multiple malformations, ambiguous genitalia, intellectual disability.
 - **Stillbirth and neonatal death** — chromosome abnormality incidence is markedly higher among stillbirths and neonatal deaths (up to ~10%) than live births (~0.7%).
 - **Fertility problems** — amenorrhea, infertility, or recurrent miscarriage (a parental chromosome abnormality is found in 3–6% of such couples).
 - **Family history** of a known/suspected chromosome or genome abnormality in a first-degree relative.
 - **Neoplasia** — virtually all cancers show acquired chromosomal abnormalities of diagnostic/prognostic value (tumour tissue or bone marrow in haematological malignancy).
 - **Pregnancy** in women of advanced maternal age (>35 years) — prenatal karyotyping via amniocentesis or chorionic villus sampling.
- ▶ **Disorders diagnosed by karyotyping (numerical/structural):**
 - **Down syndrome** — trisomy 21 (47,XX/XY,+21).
 - **Edwards syndrome** — trisomy 18 (47,+18).
 - **Patau syndrome** — trisomy 13 (47,+13).
 - **Klinefelter syndrome** — 47,XXY.
 - **Turner syndrome** — 45,X.
 - **Cri-du-chat syndrome** — deletion of short arm of chromosome 5 (5p–).
 - **XXY and XXX syndromes** — extra Y or X chromosome respectively.
- ▶ Karyotyping (with G-banding) can detect **numerical abnormalities (aneuploidy)** and **structural abnormalities** (deletion, duplication, inversion, translocation, ring, isochromosome) down to a

resolution of ~5–10 Mb (standard banding) or ~2–3 Mb (high-resolution banding); smaller submicroscopic changes need FISH, microarray, or sequencing.

High-yield exam pointers

- ▶ Karyotype = complete chromosomal constitution/photomicrograph of a cell, arranged by size and centromere position.
- ▶ Normal human karyotype: 46 chromosomes — 44 autosomes + XX (female) or XY (male).
- ▶ Four morphological types by centromere position: metacentric, submetacentric, acrocentric, telocentric (telocentric absent in humans).
- ▶ **Denver Classification (1960)** — 7 groups, A to G; A = longest metacentric, G = shortest acrocentric (includes Y).
- ▶ Satellited chromosomes: 13, 14, 15, 21, 22 (Y has none, despite being in group G).
- ▶ Banding sequence to remember: **Q-banding** → **G-banding (routine, 400–550 bands)** → **high-resolution/prometaphase banding (up to 850 bands)** → FISH → microarray/whole-genome sequencing.
- ▶ **Paris Convention (1971)** standardised band numbering; **ISCN (1978, periodically revised)** is the current universal nomenclature (e.g., 47,XX,+21).
- ▶ Karyotyping technique: peripheral blood lymphocytes → PHA stimulation → 72-hour culture → colchicine arrest → hypotonic treatment → fixation/spreading → G-banding → photomicrograph → karyogram.
- ▶ Prime clinical indications: developmental delay/dysmorphism, stillbirth/neonatal death, infertility/recurrent miscarriage, family history, malignancy, advanced maternal-age pregnancy.

SOURCE & COVERAGE

Vishram Singh — General Anatomy 4e (karyotype figure); IB Singh — Textbook of Anatomy Vol 3 6e (chromosome structure, Denver classification, Paris nomenclature, ISCN, karyotyping technique); Thompson & Thompson — Genetics in Medicine 8e (chromosome identification, banding, ISCN abbreviation table, clinical indications for chromosome analysis). Gray's Anatomy (Standring) 41e does not carry a dedicated karyotyping section — content for this topic was drawn from the specialty genetics/anatomy texts above.



Karyotype method

Asked in: Paper III 02-Aug-2021 — RGUHS MD/MS Anatomy

Definition / Introduction — A **karyotype** (Gk. *karyon* = nucleus) is the complete chromosomal complement of a single cell, systematically arranged in homologous pairs according to decreasing length and centromere position, and displayed as a photomicrograph or digital image. **Karyotyping** is the cytogenetic laboratory technique used to culture dividing cells, arrest them in metaphase, stain the condensed chromosomes, and arrange them in this standard format so that numerical and structural chromosomal abnormalities can be identified. It remains the routine frontline diagnostic test in clinical cytogenetics, most commonly performed on peripheral-blood lymphocytes.

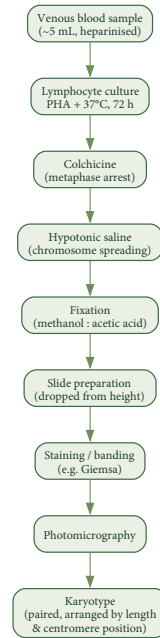
Cells / clinical material used for karyotyping

Source	Culture required	Typical use
Peripheral blood (T lymphocytes)	Short-term culture, 3–4 days	Fastest, most accessible — routine constitutional karyotyping
Bone marrow	Little or none (high mitotic index)	Suspected haematological malignancy (rapid turnaround)
Skin biopsy (fibroblasts)	Long-term culture	Mosaicism; further biochemical/molecular study
Amniotic fluid (amniocytes)	Culture required	Prenatal diagnosis after amniocentesis (~16 weeks)
Chorionic villi	Can be analysed directly or cultured	Prenatal diagnosis, earlier in pregnancy

Step-by-step method (chromosome preparation and karyotyping)

- ▶ **Sample collection** — about 5 mL of venous blood is drawn into a sterile, heparinised tube.
- ▶ **Culture** — the white blood cells are placed in tissue-culture medium and stimulated to divide with **phytohaemagglutinin (PHA)**, a mitogen that selectively stimulates T lymphocytes; the culture is incubated at 37 °C for about 72 hours (3 days).
- ▶ **Metaphase arrest** — **colchicine** (or colcemid) is added; this spindle poison inhibits microtubule polymerisation, arresting the dividing cells in metaphase, when chromosomes are maximally condensed and most easily analysed.
- ▶ **Hypotonic treatment** — the cells are treated with **hypotonic saline**, causing them to swell so that the chromosomes spread apart and separate from one another.
- ▶ **Fixation** — the swollen cells are fixed (typically methanol : acetic acid).
- ▶ **Slide preparation** — the fixed cell suspension is **dropped from a height onto a chilled glass slide**, which spreads the metaphase chromosomes flat for microscopy.
- ▶ **Staining / banding** — the slide is stained by one of several banding techniques (see below), depending on the diagnostic detail required.
- ▶ **Microscopy and photomicrography** — a well-spread metaphase is examined and photographed (or captured digitally).

- **Karyotype construction** — the individual chromosome images are cut out (or digitally sorted) and arranged into homologous pairs in descending order of length, according to an internationally accepted classification, to produce the final karyotype. Computerised image-analysis systems now automate most of these manual cutting/arranging steps.



Karyotype method

□ Fig 2.11 — A human male karyotype with Giemsa banding (G banding) (Thompson & Thompson — *Genetics in Medicine* — 8e, p.21)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled schematic karyotype layout (hand-reproducible), showing:

- 22 pairs of **autosomes** arranged left to right in **groups A–G** by decreasing length
- The **sex chromosome pair** (XX or XY) placed after group G
- Each chromosome numbered 1–22 beneath its pair, with group letters (A–G) bracketed above
- **Centromere** marked on 2–3 representative chromosomes, labelling **p** (short) arm above and **q** (long) arm below it
- One metacentric, one submetacentric, and one acrocentric chromosome labelled by type, with a satellite shown on an acrocentric (e.g. group D or G)
- Alternating dark/light **G-bands** sketched on 1–2 representative chromosomes

Chromosome banding techniques

Banding	Method	Appearance / significance
Q-banding	Quinacrine dye + UV fluorescence	Fluorescent bands (Q bands); the earliest banding method

Banding	Method	Appearance / significance
G-banding	Trypsin pre-treatment + Giemsa stain	Alternating dark/light bands (G bands); developed early 1970s; the gold-standard, routine banding method
R-banding	Reverse of G-banding	Reverse light/dark pattern to G-bands (French method)
C-banding	Special stain for constitutive heterochromatin	Highlights centromeric heterochromatin
High-resolution (prometaphase) banding	Chromosomes stained at an earlier, less-condensed pro-/prometaphase stage	Up to ~850 bands/haploid set (vs 400–550 at metaphase); detects subtler structural abnormalities

Classification and nomenclature of chromosomes

- By **centromere position**: **metacentric** (centromere central, arms equal), **submetacentric** (centromere off-centre, one arm short, one long), **acrocentric** (centromere near one end), **telocentric** (centromere at the very end — not seen in normal human chromosomes).
- **Denver classification (1960)** — proposed at a Denver, Colorado convention and accepted at London in 1963; groups all 46 human chromosomes into **7 groups (A–G)** by decreasing length and centromere position:

Group	Chromosome numbers	Description
A	1, 2, 3	Large, metacentric
B	4, 5	Large, submetacentric
C	6–12 + X	Medium, mostly submetacentric; X resembles chromosome 6
D	13, 14, 15	Medium, acrocentric, satellites on short arms
E	16, 17, 18	Small, submetacentric (16 is metacentric)
F	19, 20	Short, metacentric
G	21, 22 + Y	Very short, acrocentric, satellites; Y resembles chromosome 21

- **Paris nomenclature (1971)** — once banding techniques became available, each chromosome arm was further divided into numbered **regions** (1, 2, 3...) counted from the centromere outward, each region subdivided into numbered **bands**, allowing precise localisation of any point on a chromosome.
- **ISCN (International System for Human Cytogenetic Nomenclature, 1978)** — consolidated the Denver, London and Paris conventions into a single, periodically updated standard nomenclature (e.g. a karyotype is written as **47,XX,+21** for trisomy 21), which remains the current reporting standard.

Newer / molecular cytogenetic methods

- ▶ **Fluorescence in situ hybridisation (FISH)** — fluorochrome-labelled DNA probes are hybridised to metaphase chromosomes or interphase nuclei to rapidly detect a specific sequence, chromosome number, or rearrangement; it complements conventional banding for targeted questions.
- ▶ **Chromosomal microarray ("molecular karyotyping")** — detects submicroscopic copy-number gains/losses below the resolution of conventional G-banding, though it cannot detect balanced rearrangements (translocations/inversions).
- ▶ Despite these advances, the **conventional G-banded karyotype remains the routine frontline test**, resolving deletions/duplications of roughly 5–10 Mb or larger across the whole genome in a single preparation.

Applied / Clinical anatomy

- ▶ **Indications for chromosome/genome analysis:** (1) problems of early growth and development — failure to thrive, developmental delay, dysmorphic facies, multiple malformations, ambiguous genitalia, intellectual disability; (2) stillbirth and neonatal death — chromosome abnormality incidence is much higher among stillbirths (~10%) and neonatal deaths (~10%) than live births (~0.7%); (3) fertility problems — amenorrhoea, infertility, or recurrent miscarriage, in which a parental chromosome abnormality is found in 3–6% of couples; (4) a known chromosome/genome abnormality in a first-degree relative; (5) neoplasia — virtually all cancers show chromosome abnormalities, and marrow/tumour karyotyping gives diagnostic and prognostic information (e.g. leukaemias); (6) pregnancy in women of advanced maternal age (>35 years), for whom prenatal karyotyping is offered routinely.
- ▶ **Prenatal karyotyping:** by **amniocentesis** (~16 weeks' gestation, 10–20 mL of amniotic fluid tapped under ultrasound guidance, amniocytes cultured for fetal karyotype) or by **chorionic villus biopsy** (villi analysed directly or cultured, performed earlier in pregnancy).
- ▶ **Postnatal applications:** confirmation of clinically suspected trisomies (Down syndrome, 47,XX/XY,+21), sex-chromosome disorders (Turner 45,X; Klinefelter 47,XXY), and structural rearrangements — e.g. a balanced parental translocation transmitting an unbalanced karyotype to offspring.

High-yield exam pointers

- ▶ Karyotype = the complete chromosomal complement of a cell arranged and photographed in a standard format; karyotyping = the laboratory technique to obtain it.
- ▶ Cells of choice for routine karyotyping = peripheral blood T lymphocytes; PHA stimulates division; colchicine arrests metaphase; hypotonic saline spreads chromosomes.
- ▶ G-banding (Giemsa) is the gold-standard routine banding method; Q = quinacrine/UV, R = reverse Giemsa, C = centromeric heterochromatin.
- ▶ Denver system (1960) groups chromosomes A–G by length and centromere position; Paris convention (1971) added region/band-level nomenclature; ISCN (1978, periodically updated) is the current reporting standard.

- ▶ Conventional karyotyping resolves changes of ~5–10 Mb or larger; high-resolution banding gives up to ~850 bands/haploid set; FISH and chromosomal microarray detect targeted or submicroscopic changes below this resolution.
- ▶ Key indications: developmental delay/dysmorphism, stillbirth/neonatal death, infertility/recurrent miscarriage, family history of a chromosomal disorder, malignancy, advanced maternal age.
- ▶ Prenatal karyotyping is obtained via amniocentesis (~16 weeks) or chorionic villus biopsy (earlier in pregnancy).

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e; IB Singh Textbook of Anatomy Vol 3 6e (Chromosomes); Vishram Singh General Anatomy 4e.



Klinefelter Syndrome

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction Klinefelter syndrome is the commonest sex-chromosome disorder causing male hypogonadism and infertility, characterised cytogenetically by an extra X chromosome in an otherwise male (XY) individual. The classic karyotype is 47,XXY — a **trisomic** condition found only in phenotypic males (in contrast to Turner syndrome, a **monosomic** condition found only in females). The clinical syndrome (small testes, gynaecomastia, tall build, infertility) was first described by **Harry Klinefelter in 1942**, and its chromosomal basis (47,XXY) was demonstrated by **Jacobs and Strong in 1959**. It typically presents around puberty or later with hypogonadism, infertility, or mental/behavioural concerns, since affected boys are usually phenotypically normal before puberty.

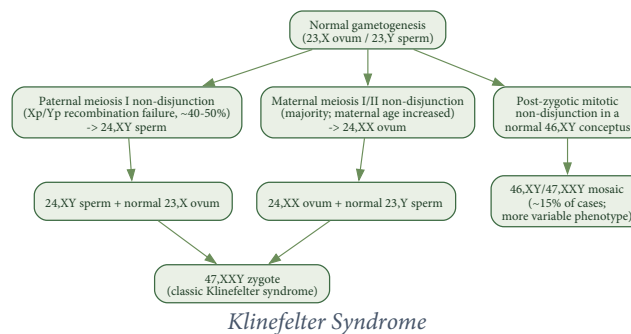
1. Incidence and karyotype

- ▶ Incidence is quoted as approximately **1 in 600 male live births** (Thompson & Thompson); other standard texts cite figures of about **1 in 500** (Vishram Singh) to **1 in 1000** (IB Singh) male births — i.e., one of the commonest chromosomal disorders in man, and the single commonest chromosomal cause of male hypogonadism/infertility.
- ▶ **Karyotype**: 47,XXY in the great majority of patients.
- ▶ **Mosaicism**: about 15% of patients show a mosaic karyotype, most commonly 46,XY/47,XXY. Mosaic patients have a more variable phenotype, and testicular development and mental status may be normal in some.
- ▶ **Variant (polysomy) karyotypes**: 48,XXXY, 48,XXYY, and 49,XXXXY — the additional X and/or Y chromosomes are associated with progressively more severe physical dysmorphism and greater intellectual disability than the classic 47,XXY form.
- ▶ Sex chromatin (**Barr body**) study of buccal smear cells is positive — a paradox, since the individual is phenotypically male; each additional X chromosome adds one further Barr body.

2. Cytogenetics — mechanism of nondisjunction

The extra X chromosome arises from **non-disjunction of the sex chromosomes during gametogenesis**, producing a disomic (24,XX or 24,XY) gamete that fertilises a normal gamete to give a 47,XXY zygote.

- ▶ **Paternal origin** (roughly 40–50% of cases): non-disjunction at **paternal meiosis I**, attributed to failure of normal Xp/Yp pairing and recombination in the pseudoautosomal region.
- ▶ **Maternal origin** (the remaining majority): errors chiefly at **maternal meiosis I** (also meiosis II or post-zygotic mitotic non-disjunction); maternal-origin cases show an association with **increased maternal age**.
- ▶ A minority of the mosaic (46,XY/47,XXY) cases arise by **post-zygotic mitotic non-disjunction** in an initially normal conceptus, rather than from a meiotic error in either parent.



3. Clinical features

Affected boys usually appear physically normal before puberty; the phenotype emerges as hypogonadism becomes evident from adolescence onward.

□ Fig 4.18 — Karyotype of Klinefelter syndrome showing 47,XXY complement. (Gangane — Human Genetics — 5e, p.100)

- ▶ **Habitus:** tall, thin, **eunuchoid** build with disproportionately long legs and arms; narrow shoulders and chest.
- ▶ **Gonads:** small, firm testes — the most consistent physical finding — due to progressive **hyalinisation of the seminiferous tubules**; the scrotum and penis are usually of normal size, though mild hypoplasia may occur in some patients.
- ▶ **Fertility:** spermatogenesis is absent or severely reduced, giving **azoospermia/oligospermia** and infertility — the commonest presenting complaint in adulthood.
- ▶ **Secondary sexual characters:** poorly developed; pubic, axillary, chest and facial hair are absent or scanty.
- ▶ **Gynaecomastia** in a proportion of patients.
- ▶ **Sex chromatin:** buccal smear is X-chromatin (Barr body) positive — unusual in a phenotypic male.
- ▶ **Neurocognitive/behavioural:** verbal comprehension and ability average somewhat below chromosomally normal (46,XY) males, with a several-fold increased risk of learning disability; however, there is wide individual variability, and many patients have normal intelligence — the overall impression, especially in adulthood, is one of a high degree of normalcy. More marked mental retardation is seen with the rarer polysomy variants (48,XXXY, 49,XXXXY, etc.).

- **Adulthood:** persistent, untreated androgen deficiency may cause decreased muscle tone, loss of libido, and decreased bone mineral density.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Karyotype/idiogram of Klinefelter syndrome Labels: 22 pairs of autosomes (1–22, arranged by group); sex chromosomes — one X, a second X, and one Y (total 47 chromosomes); annotate "extra X chromosome" against the sex-chromosome pair; caption "47,XXY".

Clinical phenotype (labelled line diagram of an affected male) Labels: tall stature with long legs/arms; narrow shoulders and chest; gynaecomastia; sparse facial/axillary/pubes hair; small, firm testes; normal-sized penis and scrotum.

5. Hormonal profile and laboratory diagnosis

Investigation	Finding in Klinefelter syndrome
Serum testosterone	Low
Serum FSH and LH	Raised (hypergonadotropic hypogonadism)
Buccal smear (sex chromatin/Barr body study)	Positive (X-chromatin positive) in a phenotypic male
Karyotyping (peripheral lymphocyte culture)	47,XXY — definitive diagnosis; may show 46,XY/47,XXY mosaicism or a polysomy variant
Gonadal (testicular) biopsy, when done	Hyalinised seminiferous tubules; spermatogenesis absent
Antenatal diagnosis	Karyotyping of chorionic villus/amniotic fluid cells; increasingly detected incidentally on non-invasive prenatal testing

6. Klinefelter syndrome vs Turner syndrome

Feature	Klinefelter syndrome	Turner syndrome
Type of aberration	Trisomic condition, males only	Monosomic condition, females only
Chromosomal complement	47, XXY	45, XO
Phenotype	Male	Female
Stature	Tall, long legs	Short stature
Sex chromatin (Barr body)	Positive (paradoxical in a male)	Absent (paradoxical in a female)
Gonads	Small, firm, hyalinised testes; azoospermia	Streak/rudimentary ovaries; gonadal dysgenesis
Other features	Eunuchoid habitus, gynaecomastia, sparse body hair	Webbing of neck, shield chest, cubitus valgus, low-set ears, coarctation of aorta
Approximate incidence	~1 in 500–600 male births	~1 in 1500–4000 female births

7. Management

- ▶ **Testosterone replacement therapy**, usually initiated from the time of diagnosis/puberty, improves secondary sexual characters, muscle bulk, bone mineral density, mood and libido — it does **not** restore fertility.
- ▶ **Gynaecomastia**: observation if mild; surgical correction if it is severe or a source of psychological distress.
- ▶ **Fertility**: the classic (non-mosaic) 47,XXY patient is almost always infertile; assisted-reproduction techniques (testicular sperm extraction with intracytoplasmic sperm injection) may occasionally succeed, particularly in mosaic individuals.
- ▶ **Educational/psychosocial support** for associated learning difficulties.
- ▶ **Genetic counselling** — the disorder is chromosomal and typically sporadic, arising from a fresh non-disjunction event; recurrence risk for the family is not significantly increased above the general population, except for the modest maternal-age effect on maternal-origin cases.

Applied / Clinical anatomy

- ▶ **Small, firm testes** are the single most consistent physical sign and should prompt karyotyping in any male presenting with infertility, gynaecomastia, or delayed/incomplete puberty.
- ▶ **Embryological basis**: because a Y chromosome (and SRY) is present, testis determination and differentiation proceed normally in utero, so internal and external genitalia are male; the supernumerary X chromosome instead impairs germ-cell survival and spermatogonial proliferation, producing progressive post-natal seminiferous tubule hyalinisation and fibrosis, most marked from puberty onward under gonadotropin drive.
- ▶ **Recognised clinical associations** include an increased risk of extragonadal (mediastinal) germ-cell tumours, male breast carcinoma, and osteoporosis secondary to chronic androgen deficiency — features that make Klinefelter syndrome relevant well beyond reproductive medicine.
- ▶ **Differential diagnosis of small testes with infertility** includes other causes of primary (hypergonadotropic) hypogonadism, e.g., bilateral cryptorchidism, mumps orchitis, and testicular irradiation/chemotherapy — Klinefelter syndrome is distinguished by the characteristic tall eunuchoid habitus and the 47,XXY karyotype.
- ▶ **Imaging/laboratory correlate**: DEXA scanning for bone mineral density and periodic hormonal monitoring guide long-term testosterone replacement.

High-yield exam pointers

- ▶ Klinefelter syndrome = commonest chromosomal cause of male hypogonadism/infertility; karyotype 47,XXY; incidence ~1 in 500–600 male births.
- ▶ First described by **Harry Klinefelter (1942)**; karyotype demonstrated by **Jacobs and Strong (1959)**.
- ▶ Arises from **non-disjunction** of sex chromosomes in gametogenesis — paternal meiosis I (Xp/Yp recombination failure) or maternal meiosis I/II (linked to advanced maternal age); ~15% are 46,XY/47,XXY **mosaics** with a milder, variable phenotype.
- ▶ Clinical triad: **small, firm testes + azoospermia/infertility + tall eunuchoid habitus**, ± gynaecomastia and sparse body hair.

- ▶ Hormonal pattern: **low testosterone with raised FSH/LH** (hypergonadotropic hypogonadism); buccal smear is **Barr-body positive** in a phenotypic male.
- ▶ Gonadal biopsy: **hyalinised seminiferous tubules** with absent spermatogenesis.
- ▶ Variants **48,XXXY / 48,XXYY / 49,XXXXY** — more sex chromosomes correlate with worse dysmorphism and intellectual disability.
- ▶ Contrast with Turner syndrome: **trisomic vs monosomic, male vs female, tall vs short, Barr-body positive vs negative**.
- ▶ Treatment is **testosterone replacement** (does not restore fertility); ICSI/TESE may help selected (often mosaic) infertile patients.

SOURCE & COVERAGE

Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e; Vishram Singh — General Anatomy 4e; Vishram Singh — Textbook of Clinical Embryology; IB Singh — Human Embryology (CBME 2021). Coverage note: Gray's Anatomy (Standing) 41e has no equivalent content — Klinefelter syndrome is a clinical cytogenetics/genetics topic rather than a gross-anatomical one; grounding was therefore drawn from the genetics specialty texts (per this topic's GENETICS designation), supplemented by the medical-genetics chapters of the general-anatomy and embryology texts in the library.



Lymphocyte culture

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction Lymphocyte culture is the standard short-term tissue-culture technique by which peripheral blood **T lymphocytes**, normally quiescent in the G₀ phase, are stimulated in vitro by a mitogen (**phytohemagglutinin**) to re-enter the cell cycle and undergo mitosis, so that their **metaphase chromosomes** can be arrested, harvested, stained and analysed. Because venous blood is the most accessible source of dividing human cells, this technique — completed in about 72 hours — is the routine, frontline method used for clinical **karyotyping** and chromosome/genome analysis. It underlies the diagnosis of numerical and structural chromosomal disorders, gene mapping, cancer cytogenetics and genetic counselling.

1. Principle

- ▶ Circulating T lymphocytes are mitotically inactive (arrested in **G₀**) but retain the capacity to divide.
- ▶ **Phytohemagglutinin (PHA)**, a plant lectin/mitogen, binds T-cell surface receptors and induces blast transformation, driving the cells through G₁ → S → G₂ → M.
- ▶ **Colchicine**, added toward the end of culture, inhibits spindle-microtubule polymerisation and so arrests dividing cells in **metaphase** — the stage at which chromosomes are maximally condensed, individually distinct, and therefore best suited for study.
- ▶ Hypotonic treatment then swells the cells and disperses the chromosomes for a well-spread metaphase preparation.

2. Requirements

Reagent / equipment	Function
Heparinised venous blood (1–2 mL)	Source of T lymphocytes; heparin prevents clotting without harming cell viability
Culture medium (RPMI-1640)	Basic nutrient medium
Calf/AB serum	Provides nutrition/growth factors
L-glutamine	Amino-acid supplement
Gentamicin (antibiotic)	Prevents bacterial contamination
Phytohemagglutinin-M (PHA-M)	Mitogen — stimulates lymphocytes to divide
Colchicine	Spindle poison — arrests division at metaphase
Hypotonic sodium citrate/saline	Lyses red cells, swells white cells, spreads chromosomes
Fixative (methanol : glacial acetic acid, 3 : 1)	Fixes cells for slide preparation
Trypsin + Giemsa stain	Produces G-banded chromosome preparation

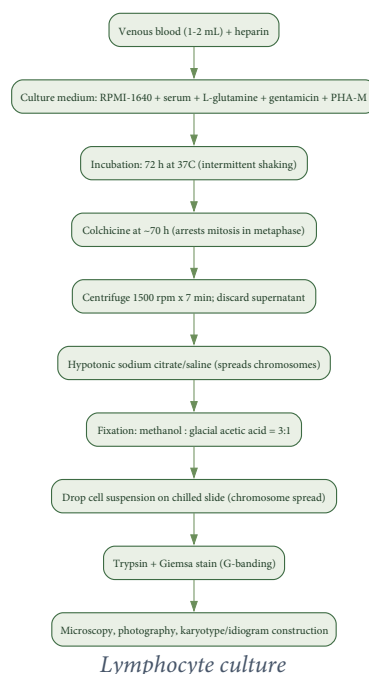
3. Procedure (stepwise)

- ▶ **Collection** — 1–2 mL venous blood is collected by venipuncture into a sterile, heparinised syringe.
- ▶ **Planting** — the heparinised blood is added to a 10 mL tube of karyotyping medium (RPMI-1640 + serum + L-glutamine + gentamicin + PHA-M).
- ▶ **Incubation** — 72 hours at 37 °C, with intermittent shaking of the tube.
- ▶ **Harvesting** — at ~70 hours, colchicine is added to arrest cells in metaphase.
- ▶ **Centrifugation** — 1500 rpm for 7 minutes; the supernatant is discarded and the cell pellet retained.
- ▶ **Hypotonic treatment** — hypotonic sodium citrate/saline lyses residual red cells, swells the white cells and separates the chromatids for a clean spread.
- ▶ **Fixation** — cells are fixed in methanol : glacial acetic acid (3 : 1).
- ▶ **Slide preparation** — the fixed cell suspension is dropped from a height onto a chilled slide, spreading the chromosomes without overlap.
- ▶ **Staining** — the slide is treated with trypsin (partial digestion) and then Giemsa stain to produce G-banding.
- ▶ **Microscopy and analysis** — metaphase spreads are examined, photographed, and the chromosome images are cut out and arranged in a standard sequence to construct the **karyotype (idiogram)**.

□ Fig 2.10 — A chromosome spread prepared from a lymphocyte culture that has been s (Thompson & Thompson — *Genetics in Medicine* — 8e, p.20)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the flowchart of the technique with these exact labelled boxes, top to bottom:



4. Karyotype analysis and chromosome classification

- ▶ **Karyotype** — the standard arrangement of the photographed metaphase chromosomes of a cell, in decreasing order of size, to study the complete chromosomal complement of an individual; also called an **idiogram**.
- ▶ Chromosomes are classified by **centromere position** into metacentric (arms roughly equal), submetacentric (arms unequal), acrocentric (centromere near one end) and telocentric (centromere at the very end — not seen in normal human chromosomes).
- ▶ **Denver system of classification (1960)** groups the 46 human chromosomes into seven groups (A–G) by size, centromere position and presence of satellites:

Group	Chromosome pairs	Features
A	1–3	Long, metacentric
B	4, 5	Long, submetacentric
C	6–12 + X	Medium, submetacentric
D	13–15	Medium, acrocentric, satellites present
E	16–18	Short, submetacentric
F	19, 20	Short, metacentric
G	21, 22 + Y	Short, acrocentric, satellites present

- ▶ **Banding techniques: G-banding** (Giemsa banding, after trypsin pretreatment) is the gold-standard, routinely used stain — it resolves a standard karyotype to about 400–550 bands per haploid set; staining chromosomes harvested at an earlier, less-condensed (prophase/prometaphase) stage gives **high-resolution (prometaphase) banding**, resolving up to ~850 bands and revealing subtler structural rearrangements. **C-banding** stains constitutive heterochromatin around the centromere and is positive at the secondary constrictions of chromosomes 1, 9, 16 and the long arm of the Y.

5. Applications / Indications

- ▶ Confirming a clinical diagnosis of a suspected numerical or structural chromosome disorder.
- ▶ Work-up of problems of early growth and development — developmental delay, dysmorphic facies, multiple congenital malformations, ambiguous genitalia, short stature.
- ▶ Investigation of unexplained **stillbirth or neonatal death**.
- ▶ **Infertility, recurrent miscarriage or amenorrhoea** — parental blood karyotyping detects balanced translocations/inversions carried by a parent.
- ▶ A known or suspected chromosome/genome abnormality in a **first-degree relative**.
- ▶ **Neoplasia** — blood/marrow chromosome analysis gives diagnostic and prognostic information (e.g., prognosis in chronic myelogenous leukaemia).
- ▶ **Gene mapping** and genetic counselling.
- ▶ A related but distinct application is the **mixed lymphocyte culture (MLC) test**, used for pre-transplant histocompatibility testing — donor and recipient lymphocytes are co-cultured, one set acting as the stimulator (mitogenic) and the other as the responder (proliferating) cell population, giving an index of HLA-D antigenic disparity between donor and host. This is not to be confused with the routine PHA-stimulated lymphocyte culture used for karyotyping.

6. Advantages and limitations

Advantages	Limitations
Peripheral blood obtained by simple venipuncture — minimally invasive	Short-lived culture (3–4 days) — cannot be maintained as a permanent cell line (unlike EBV-transformed lymphoblastoid lines or skin-fibroblast cultures)
T lymphocytes respond reliably and synchronously to PHA, giving a good metaphase yield	Reflects only the karyotype of circulating T lymphocytes — tissue-restricted (e.g., gonadal) mosaicism may be missed
Rapid turnaround (~3–5 days total) — suited to routine clinical diagnosis	Requires viable, actively dividing cells; cannot be used on non-dividing or fixed tissue
Simple, reproducible and inexpensive compared with marrow or skin biopsy	Routine G-banding cannot resolve submicroscopic (<5–10 Mb) imbalances — now supplemented by FISH, microarray or whole-genome sequencing

Applied / Clinical anatomy

- ▶ Frontline diagnostic test for the common autosomal and sex-chromosome disorders — Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), Turner syndrome (45,X) and Klinefelter syndrome (47,XXY).
- ▶ Provides prognostic information in haematological malignancy — e.g., detection of the Philadelphia chromosome in chronic myelogenous leukaemia on blood/marrow karyotype.
- ▶ Despite newer molecular techniques (FISH, chromosomal microarray, whole-genome sequencing), the G-banded lymphocyte karyotype remains the routine frontline test in most cytogenetics laboratories because it surveys the whole genome for both numerical and structural abnormality in a single preparation.
- ▶ Distinct from fetal cytogenetic sampling: fetal chromosomes for prenatal diagnosis are obtained from cultured amniotic-fluid cells or chorionic villi (amniocentesis/CVS), not lymphocytes — parental lymphocyte culture is used only to detect a carrier parent, not the fetus itself.
- ▶ The PHA-stimulated (karyotyping) lymphocyte culture must be distinguished in the exam from the mixed lymphocyte culture (MLC) test used for HLA/histocompatibility assessment before organ or tissue transplantation.

High-yield exam pointers

- ▶ Cells cultured: peripheral blood **T lymphocytes** (accessible, PHA-responsive, normally in G0).
- ▶ Mitogen used: **phytohemagglutinin (PHA-M)**; incubation **72 hours at 37 °C**.
- ▶ Metaphase arrest: **colchicine**, added at ~70 hours, inhibits the spindle.
- ▶ Key steps in order: collection → planting → incubation → colchicine (harvest) → centrifuge (1500 rpm/7 min) → hypotonic treatment → fixation (methanol : acetic acid 3:1) → slide spread → trypsin-Giemsa (G-banding) stain → microscopy/karyotype.
- ▶ **Denver system (1960)**: 7 groups, A–G, by size/centromere position/satellites.
- ▶ **G-banding**: gold standard; ~400–550 bands routine, up to ~850 bands with high-resolution (prometaphase) banding.
- ▶ Main limitation: short-lived culture (3–4 days) — cannot be banked long-term.
- ▶ Do not confuse with the mixed lymphocyte culture (MLC) test used for histocompatibility/HLA-D typing.

SOURCE & COVERAGE

IB Singh — Human Embryology (CBME 2021) [stepwise lymphocyte-culture protocol and Denver classification table]; Thompson & Thompson — Genetics in Medicine 8e [principle, cell type used, culture duration/limitations, G-banding resolution, clinical indications for chromosome analysis]; Gangane — Human Genetics 5e [indications of karyotyping, C-banding, mixed lymphocyte culture test]; Vishram Singh — Textbook of Clinical Embryology [corroborates Denver System of Classification, 1960].



Meiosis

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction — Meiosis is a specialised type of nuclear division, restricted to germ cells of the gonads, by which a diploid ($2n = 46$ chromosomes) primary spermatocyte or primary oocyte gives rise to four haploid ($n = 23$ chromosomes) gametes. It consists of **one round of DNA replication (S-phase)** followed by **two successive rounds of chromosome segregation** — meiosis I (reductional division, in which homologous chromosomes separate) and meiosis II (equational division, similar to mitosis, in which sister chromatids separate). Meiosis is the cytological basis of gametogenesis: it halves the chromosome number so that fertilisation restores the diploid number, and — through synapsis and crossing over — it generates genetic variation between siblings.

Meiosis I — the reductional division

Prophase I is long and complex, and is conventionally divided into five substages:

Substage	Key events
Leptotene	Homologous chromosomes (already replicated, each with two sister chromatids joined at the centromere) begin to condense as individual threads, attached by their telomeres to the nuclear envelope; genetic recombination is initiated.
Zygotene	Homologous chromosomes begin synapsis (pairing), often starting near the telomeres, which cluster to one side of the nucleus (the "bouquet" stage). Paired homologues (bivalents) are held by the tripartite synaptonemal complex (two lateral dense elements + a central element). The sex chromosomes also synapse, via the shared pseudoautosomal region, forming the condensed sex vesicle .
Pachytene	Synapsis is complete for all chromosomes; each bivalent appears as a single thick structure (two pairs of sister chromatids held by the synaptonemal complex). Recombination between non-sister chromatids is completed, marked by recombination nodules (usually one per chromosome arm).
Diplotene	The synaptonemal complex disassembles; homologous chromosomes shorten and separate except at points where crossing over occurred — these points are called chiasmata (at least one per bivalent, up to five observed), and their formation is termed disjunction . In the ovary, primary oocytes reach diplotene by the 5th fetal month and remain arrested here until just before ovulation — for up to 50 years.
Diakinesis	The prometaphase of meiosis I; bivalents condense further and attach to the spindle. In oocytes the spindle assembles without centrosomes.

- ▶ **Metaphase I** — bivalents (not single chromosomes) align at the equatorial plate; each pair of sister centromeres behaves as a single unit facing one spindle pole; homologues are held together at the spindle midzone by chiasmata.
- ▶ **Anaphase I** — cohesion between sister-chromatid arms is released; whole bivalents disjoin, and homologous chromosomes (each still consisting of two chromatids) move to opposite poles — sister centromeres do **not** split. Random orientation of maternal/paternal bivalents produces **independent assortment**.
- ▶ **Telophase I / cytokinesis** — cytoplasmic division is markedly asymmetric in the female (one large secondary oocyte + one small first polar body) and near-equal in the male (two secondary spermatocytes joined by cytoplasmic bridges).

□ Fig 2.5 — Chromosomal behaviour in meiosis. Only two pairs of chromosomes are (Gangane — Human Genetics — 5e, p.30)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw two homologous chromosome pairs (one maternal, one paternal) through the full cycle, labelling: **Interphase** — **premeiotic S-phase** → **Prophase I** (Leptotene → Zygotene: synapsis/synaptonemal complex → Pachytene: crossing over → Diplotene: chiasmata → Diakinesis) → **Metaphase I** (bivalents at the equator) → **Anaphase I** (homologues, each with 2 chromatids, move apart) → **Telophase I** → **Interkinesis** (no S-phase) → **Meiosis II**: Prophase II → Metaphase II → **Anaphase II** (sister chromatids finally separate) → Telophase II → **four haploid daughter cells**. Mark one chiasma explicitly to show recombinant chromatids in the final gametes.

Meiosis II — the equational division

- ▶ Begins after a brief interval with **no intervening S-phase** — DNA is not replicated again.
- ▶ Resembles an ordinary mitosis: sister-chromatid centromeres rotate to face opposite poles; onset of anaphase II follows loss of centromeric cohesion, and chromatids (now genetically non-identical because of crossing over in meiosis I) separate.
- ▶ Cytoplasmic division again occurs; in the male this yields **four haploid spermatids** (interconnected by cytoplasmic bridges); in the female it yields **one mature ovum + a second polar body**, and only proceeds to completion if **fertilisation occurs** — the secondary oocyte is otherwise arrested at metaphase II.

Meiosis vs mitosis

Feature	Mitosis	Meiosis
Occurs in	All somatic cells	Germ cells only
Number of divisions	One	Two (meiosis I + II), one S-phase
Daughter cells	2, diploid, genetically identical	4, haploid, genetically distinct
Synapsis / chiasmata	Absent	Present (prophase I only)
Crossing over / recombination	Absent (rare in somatic cells; disease-associated)	Essential feature of meiosis I

Feature	Mitosis	Meiosis
Centromere splitting	Every division (anaphase)	Not in anaphase I; occurs in anaphase II
Genetic outcome	No new combinations	New combinations via recombination + independent assortment

Significance of meiosis

- ▶ **Formation of gametes** — the prime biological purpose of meiosis.
- ▶ **Maintains constant chromosome number** across generations: halving at gametogenesis is restored at fertilisation ($23 + 23 \rightarrow 46$).
- ▶ **Genetic recombination** — crossing over exchanges maternal and paternal segments between non-sister chromatids, so no two gametes are identical.
- ▶ **Independent assortment** of the 23 bivalents at metaphase I multiplies the number of possible chromosomal combinations (2^{23} per parent), the cytological basis of Mendel's law of independent assortment.
- ▶ Together, recombination and independent assortment are the principal source of genetic variation within a species, distinct from mutation.

Meiosis in human gametogenesis

Both spermatogenesis and oogenesis require meiosis, but timing differs substantially between the sexes:

	Spermatogenesis	Oogenesis
Onset	Puberty	Meiosis I begins in fetal life ; most primary oocytes have already entered prophase I before birth
Continuity	Continuous, from a self-renewing stem-cell population, throughout adult life	Initiated once, in a fixed (non-renewing) pool of cells
Arrest point	None — proceeds promptly	Arrested at diplotene of prophase I from the fetal period until puberty; resumed just before each ovulation
Second arrest	None	Oocyte completes meiosis I at ovulation, then arrests again at metaphase II ; completed only on fertilisation
Cytokinesis	Symmetric — one primary spermatocyte $\rightarrow$ 2 secondary spermatocytes $\rightarrow$ 4 spermatids (equal cytoplasm)	Grossly asymmetric — one primary oocyte $\rightarrow$ 1 secondary oocyte + 1st polar body; meiosis II $\rightarrow$ 1 mature ovum + 2nd polar body

	Spermatogenesis	Oogenesis
Output per germ cell	4 functional sperm (~200 million/ejaculate; $\sim 10^{12}$ over a lifetime, needing several hundred successive mitoses of the stem line)	1 functional ovum + up to 3 polar bodies (which degenerate)

Applied / Clinical anatomy

- **Non-disjunction** — failure of a homologous pair (meiosis I) or of sister chromatids (meiosis II) to separate, so both members pass into one gamete. A gamete with 24 chromosomes fertilised by a normal (23-chromosome) gamete produces a **trisomic** zygote (47 chromosomes); a gamete with 22 chromosomes produces a **monosomic** zygote (45 chromosomes).

□ Fig 17.12 — Non-disjunction occurring during meiosis: (A) non-disjunction in me (Vishram Singh — General Anatomy — 4e, p.702)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a normal meiotic division (one bivalent $\rightarrow$ 4 gametes, each 23 chromosomes) alongside two non-disjunction variants: (A) **non-disjunction at meiosis I** — both homologues pass to one secondary cell, giving gametes with 24 and 22 chromosomes; (B) **non-disjunction at meiosis II** — sister chromatids fail to separate, giving one gamete with 24, one with 22, and two normal gametes with 23. Label the fertilisation outcomes: $24 + 23 \rightarrow 47$ (**trisomy**); $22 + 23 \rightarrow 45$ (**monosomy**).

- ▶ **Clinically important aneuploidies** (all due to meiotic non-disjunction):
 - **Trisomy 21 — Down's syndrome** (commonest viable trisomy; incidence rises steeply with maternal age; ~95% due to non-disjunction, usually of maternal meiosis I).
 - **Trisomy 13 — Patau's syndrome; Trisomy 18 — Edward's syndrome** (usually fatal in infancy).
 - **47,XXY — Klinefelter's syndrome and 45,X — Turner's syndrome** (non-disjunction of a sex chromosome).
- ▶ **Polyploidy** — an extra full haploid set: **triploidy** (69 chromosomes; e.g. dispermy, or fertilisation of a diploid gamete resulting from failed meiosis) causes early spontaneous abortion or brief neonatal survival; **tetraploidy** (92 chromosomes) follows failure of the first cleavage division and is uniformly lethal in utero.
- ▶ **Aneuploidy is the single most common mutational mechanism in humans** — chromosomally abnormal conceptions account for a large proportion of first-trimester spontaneous abortions; among pregnancies reaching term, chromosome abnormalities are a leading cause of developmental defects and intellectual disability.
- ▶ **Mosaicism** — non-disjunction occurring mitotically soon after fertilisation (rather than meiotically) produces a mixed chromosomal population, seen in a proportion of Down's syndrome cases and in tumour cytogenetics.
- ▶ **Premature ovarian failure**, a significant cause of female infertility, can result from mutation in a meiosis-specific gene required for correct sister-chromatid cohesion — reflecting the decades-long meiotic arrest unique to the human oocyte.
- ▶ **Diagnostic relevance:** karyotyping/FISH on amniotic-fluid or chorionic-villus cells detects these numerical abnormalities antenatally; the cytogenetic principles above underpin prenatal screening and genetic counselling.

High-yield exam pointers

- ▶ Meiosis = one S-phase + two divisions $\rightarrow$ four haploid cells; meiosis I is reductional, meiosis II is equational (mitosis-like).
- ▶ Prophase I substages, in order — Leptotene, Zygotene, Pachytene, Diplotene, Diakinesis.
- ▶ Synapsis occurs at zygotene via the synaptonemal complex; crossing over is completed at pachytene; chiasmata (the cytological sign of crossing over) appear at diplotene.
- ▶ Centromeres split at anaphase II, NOT anaphase I — this is the key mitosis-vs-meiosis-I distinction.

- ▶ Female meiosis I is arrested at diplotene from fetal life to puberty (up to ~50 years); meiosis II is arrested at metaphase II until fertilisation.
- ▶ Non-disjunction (meiosis I or II) → aneuploidy (trisomy/monosomy); the same error occurring mitotically post-zygotically → mosaicism.
- ▶ Down's (trisomy 21), Patau's (trisomy 13), Edward's (trisomy 18), Klinefelter's (47,XXY) and Turner's (45,X) are the classic non-disjunction syndromes to name with numbers.
- ▶ Significance of meiosis: gamete formation, constancy of chromosome number across generations, and generation of genetic variation (crossing over + independent assortment).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Thompson & Thompson — Genetics in Medicine 8e; Gangane — Human Genetics 5e; Vishram Singh — General Anatomy 4e; Junqueira's Basic Histology 17e.

Coverage note: the topic's tagged specialty texts (Bancroft's Histological Techniques; Pranab Dey — Lab Techniques in Histopathology & Cytology) are histotechnique manuals and carry no content on meiosis; grounding was instead drawn from the Gray's spine (which covers meiosis in unusual depth in its cell-biology chapter) plus the corpus's genetics texts, which are the standard home for this cytogenetics topic.



Mendel's laws

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction Johann Gregor Mendel (1822–1884), an Austrian monk who bred garden pea plants (*Pisum sativum*) at Brno, published his results in 1866 and is regarded as the **Father of Modern Genetics**. His work laid down the rules by which characters (traits) are transmitted from one generation to the next — a field he founded before the nucleus, chromosomes or genes were even known, so that Mendel spoke only of "factors," a term we now equate with **alleles of a gene**. From his monohybrid and dihybrid breeding experiments he derived **two basic laws of inheritance** — the **Law of Segregation** and the **Law of Independent Assortment** — together with the corollary **Principle of Dominance and Recessiveness**. These three principles remain the foundation of transmission genetics and of the analysis of single-gene ("Mendelian") disorders in man.

1. The monohybrid cross and the Law of Segregation

A **monohybrid cross** is a cross between individuals differing in a single pair of contrasting characters (Greek *mono* = single; Latin *hybrida* = offspring of parents with differing qualities). Mendel's classic experiment crossed a pure-bred **tall** pea plant (genotype TT) with a pure-bred **dwarf** plant (genotype tt):

- ▶ **F1 generation** — every offspring was **tall**, genotype Tt (all gametes of the tall parent carried T, all gametes of the dwarf parent carried t, so every zygote was Tt). Tallness is therefore the **dominant** trait, dwarfness the **recessive** trait.
- ▶ **F2 generation** — F1 plants were allowed to self-pollinate. The dwarf character, completely hidden in F1, **reappeared** in F2 in a fixed ratio.

Gametes of F1 (Tt)	T	t
T	TT	Tt
t	Tt	tt

Genotype TT : Tt : tt = 1 : 2 : 1; Phenotype tall : dwarf = 3 : 1.

- **F3 generation** — obtained by self-pollinating F2 plants. All dwarf (tt) F2 plants bred true (100% dwarf offspring); of the tall F2 plants, one-third (the TT homozygotes) bred true for tallness, while the remaining two-thirds (the Tt heterozygotes) again threw a 3:1 tall:dwarf ratio. This confirmed that the "hidden" factor had not blended away but was retained unaltered and could resurface.

From these results Mendel proposed his **first law** — the **Law of Segregation**: *the two members of a pair of factors (alleles of a gene) responsible for a character separate (segregate) from one another during the formation of gametes, so that each gamete receives only one member of the pair, in an unaltered state.*

Chromosomal/cytological basis: each pair of alleles lies on a **homologous pair of chromosomes**; segregation of the alleles corresponds physically to the separation of the two homologous chromosomes at **anaphase of meiosis I**, so that every gamete receives one — and only one — member of each homologous pair, one from the paternal and one from the maternal chromosome set. Failure of this separation is **non-disjunction**, the cytological "exception" that produces aneuploid gametes.

□ Fig 1.3 — Law of segregation. (Gangane — Human Genetics — 5e, p.20)

2. The dihybrid cross and the Law of Independent Assortment

A **dihybrid cross** studies inheritance of **two pairs of contrasting characters** together. Mendel crossed a pure-bred plant with **yellow, round** seeds (YYRR) with one having **green, wrinkled** seeds (yyrr):

- **F1 generation** — all offspring were **yellow, round**, genotype YyRr.
- **F2 generation** — F1 plants self-pollinated to give **four phenotypic classes** in the ratio 9 (yellow round) : 3 (yellow wrinkled) : 3 (green round) : 1 (green wrinkled), arising from **nine distinct genotype combinations**.

×	YR	Yr	yR	yr
YR	YYRR	YYRr	YyRR	YyRr
Yr	YYRr	YYrr	YyRr	Yyrr
yR	YyRR	YyRr	yyRR	yyRr
yr	YyRr	Yyrr	yyRr	yyrr

Two important observations followed: (i) as in the monohybrid cross, characters masked in F1 reappeared in F2; and (ii) F2 also showed **new combinations of characters never seen in either parent** (e.g., yellow-wrinkled, green-round) — proof that the two gene pairs had assorted into the gametes **independently** of each other rather than staying linked as they were inherited from the parents.

This led to Mendel's **second law** — the **Law of Independent Assortment**: *the members of different gene pairs assort independently of one another during gamete formation, so that new combinations of characters can appear in the offspring.*

Chromosomal/cytological basis: genes on **different (non-homologous) chromosome pairs** orient themselves randomly and independently of one another when the bivalents line up at **metaphase of meiosis I**, so alleles of unlinked genes assort into gametes in every possible combination — this parallel between the behaviour of "factors" and of chromosomes is the **chromosomal theory of inheritance**, which gave Mendel's mathematical ratios a concrete cytological basis. (The law strictly applies only to genes on different chromosome pairs — genes on the **same** chromosome show **linkage** and do not assort independently, a refinement added after Mendel by T.H. Morgan.)

□ Fig 1.4 — Law of independent assortment. (Gangane — Human Genetics — 5e, p.20)

Mendel additionally performed **trihybrid crosses**, which confirmed dominance, segregation and independent assortment as fundamental rules for all traits studied.

3. Principle of dominance and recessiveness

This is the direct corollary of the first law. When a heterozygote (Tt) shows only the "tall" feature and never a blend, the factor that is expressed is termed **dominant** (T, expressed by upper-case letter) and the masked factor **recessive** (t, lower-case letter).

- ▶ A **dominant allele** is expressed whether present in a single dose (heterozygous) or double dose (homozygous).
- ▶ A **recessive allele** is expressed only when present in double dose (homozygous), i.e., in the absence of the dominant allele.
- ▶ Strictly, genes themselves are neither dominant nor recessive — it is the **trait/phenotype** produced that is dominant or recessive; this distinction, though made for the convenience of pedigree analysis, is exam-important.

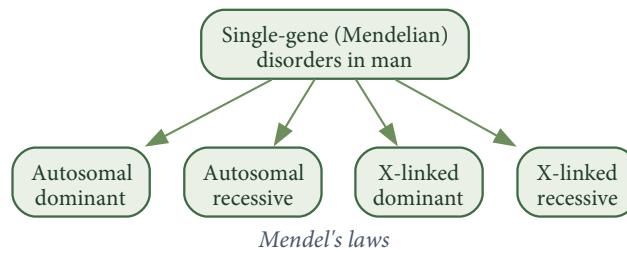
4. Punnett square, back cross and test cross

- ▶ **Punnett square** — a grid method of working out all possible gametic combinations, devised by the British geneticist **Reginald Crundall Punnett**. Gametes of one parent are listed along one axis and those of the other parent along the perpendicular axis; the genotype at the junction of a row and column gives one possible offspring. It is used for monohybrid, dihybrid and higher-order crosses (shown in the tables above).
- ▶ **Back cross** — a cross between an F1 individual and **either** of its parents; done to check the allelic make-up of the heterozygote and confirm segregation.
- ▶ **Test cross** — a special back cross in which the F1 (or any individual showing the dominant phenotype) is crossed with the **homozygous recessive** parent/individual; because the recessive parent contributes only recessive gametes, the phenotypic ratio of the offspring directly reveals whether the tested individual is homozygous dominant (all offspring show the dominant trait) or heterozygous (offspring segregate 1:1 dominant:recessive) — the basis of "testing" the unknown genotype.

Applied / Clinical anatomy

- ▶ **Human single-gene ("Mendelian") disorders** are caused by a single mutant gene and, like Mendel's pea characters, follow one of **four patterns of inheritance**: autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive (the Y chromosome carries

virtually no Mendelian genes other than the H-Y antigen locus, so "sex-linked" in practice means X-linked).



- ▶ **Pedigree analysis** is applied Mendelian analysis: the **proband/propositus** is identified, first-degree relatives are charted with standard symbols, and the pattern of affected relatives is matched against the four patterns above to estimate **recurrence risk** — e.g., 1 in 4 for an autosomal-recessive mating of two carriers, the same ratio the monohybrid Punnett square gives. **Consanguinity** raises autosomal-recessive risk by raising the chance both parents carry the same rare allele.
- ▶ The **ABO blood group** shows segregation acting on **multiple alleles** at one locus (I^A , I^B , I^O) — I^A / I^B co-dominant, both dominant over I^O — a single locus with more than two alleles, though any one gamete still carries only one.
- ▶ **Modifications of simple ratios**, built on the same two laws, are high-yield exceptions: **incomplete dominance/codominance**, **multiple alleles**, **epistasis** (one gene's allele masking another's phenotype), **lethal alleles**, **pleiotropy**, and **incomplete penetrance/variable expressivity**. A genuinely **non-Mendelian** exception is **genomic imprinting with uniparental disomy**, where parent-of-origin (not just dominance) determines expression — the basis of Prader-Willi and Angelman syndromes.
- ▶ Genetic counselling uses this same test-cross/Punnett-square logic to compute recurrence risk before offering prenatal diagnosis.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ **Monohybrid Punnett square** — 2×2 grid, gametes T, t on top and side, boxes TT, Tt, Tt, tt; mark ratio 1:2:1 genotype / 3:1 phenotype.
- ▶ **Dihybrid Punnett square** — 4×4 grid, gametes YR, Yr, yR, yr on top and side of YyRr × YyRr; group the 16 boxes into the 9:3:3:1 phenotype classes.
- ▶ **Chromosomal schematic** — a cell with a labelled **homologous chromosome pair** (mark **Gene**, **Gamete**) giving two normal daughter gametes versus a **non-disjunction** pair (one gamete with both chromosomes, one with none), for segregation; and a **bivalent** of maternal (M) and paternal (P) chromosome pairs assorting into four gamete types MM, MP, PM, PP, for independent assortment.

High-yield exam pointers

- ▶ Mendel (1822–1884), Austrian monk, *Pisum sativum*, published 1866 — "Father of Modern Genetics."

- ▶ **Monohybrid cross** $TT \times tt \rightarrow F_1$ all Tt (tall); F_1 selfed $\rightarrow F_2$ genotype 1:2:1, phenotype 3:1 (tall:dwarf).
- ▶ **Law of Segregation**: the two alleles of a gene separate from each other during gamete formation; each gamete gets only one, unaltered.
- ▶ **Dihybrid cross** $YYRR \times yyrr \rightarrow F_1$ all $YyRr$ (yellow round); F_1 selfed $\rightarrow F_2$ phenotype 9:3:3:1, 9 genotype classes.
- ▶ **Law of Independent Assortment**: alleles of different gene pairs assort independently during gamete formation, producing new character combinations.
- ▶ **Principle of dominance**: dominant allele expressed in single or double dose; recessive allele only in double (homozygous) dose — traits, not genes, are dominant/recessive.
- ▶ **Punnett square** (R.C. Punnett), **back cross** ($F_1 \times$ either parent), **test cross** (F_1 /dominant phenotype $\times$ homozygous recessive, to reveal unknown genotype).
- ▶ Cytological basis: segregation = homologous chromosome separation at meiosis I anaphase; independent assortment = random orientation of different bivalents at meiosis I metaphase (chromosomal theory of inheritance).
- ▶ Human single-gene disorders follow 4 Mendelian patterns: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive.

SOURCE & COVERAGE

IB Singh — Textbook of Anatomy Vol 3 — 6e (Chapter: Mendelian Laws of Inheritance; Chapter: Alleles); Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e. Coverage note: Gray's Anatomy (Standring) 41e does not cover classical Mendelian genetics at all (confirmed absent, per the topic's grounding note), so this answer is drawn entirely from the dedicated anatomy-genetics and human-genetics texts above rather than the spine.



PAX genes

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction

PAX (Paired box) genes are a family of developmental transcription-factor genes, the vertebrate orthologues of the *Drosophila* segmentation gene *paired*. Each PAX protein carries a highly conserved 128-amino-acid **paired-domain**, a bipartite helix-turn-helix DNA-binding motif that, together with an accessory **homeodomain** and an **octapeptide** repressor motif (present in some members), allows the protein to bind promoter/enhancer DNA and switch on or off batteries of downstream genes during organogenesis. Nine PAX genes (**PAX1–PAX9**) have been identified in humans and mice; each is expressed in a spatially and temporally restricted pattern and acts as a **master regulatory (control) gene** for a specific organ primordium — the eye, kidney, ear, axial skeleton, skeletal muscle, thyroid, pancreas, tooth and craniofacial structures. Because they sit high in developmental gene-regulatory cascades, mutations in a single PAX gene produce well-characterised, often dominantly-inherited human

malformation syndromes, and abnormal PAX activation (via chromosomal translocation) drives a specific childhood malignancy.

1. Molecular architecture and classification

- **Paired domain (PD):** ~128-aa, two helix-turn-helix subdomains (N- and C-terminal); binds DNA in the major and minor grooves; the signature feature of every PAX gene.
- **Octapeptide motif:** a short conserved sequence that recruits co-repressors (e.g., Groucho/TLE family); present in most but not all members.
- **Paired-type homeodomain:** a 60-aa helix-turn-helix DNA-binding domain shared with *Hox*/homeobox genes; may be **complete**, **partial (truncated)**, or **absent**, depending on the gene.
- On the basis of this domain combination the PAX family is grouped into **four structural classes**:

Class	Domain composition	Members	Principal organ system
I	Paired domain + octapeptide; no homeodomain	PAX1, PAX9	Axial skeleton (sclerotome), pharyngeal pouch derivatives, teeth
II	Paired domain + octapeptide + partial homeodomain	PAX2, PAX5, PAX8	Kidney, optic stalk, otic vesicle (PAX2); thyroid (PAX8); B-lymphocytes (PAX5)
III	Paired domain + octapeptide + complete homeodomain	PAX3, PAX7	Neural crest, dermomyotome, skeletal-muscle progenitors/satellite cells
IV	Paired domain + complete homeodomain; no octapeptide	PAX4, PAX6	Eye (lens, retina, iris), CNS, endocrine pancreas

- All nine genes act as **transcriptional activators or repressors** upstream of tissue-specific differentiation genes, and most are themselves switched on by diffusible morphogens (Sonic hedgehog/Shh, Wnts, Bmps, Fgfs) secreted from adjacent inducing tissues — i.e., PAX genes translate a positional signal into a stable programme of organ identity.

2. The individual PAX genes and their anatomical targets

Gene	Site of expression	Developmental role	Principal human disorder
PAX1	Sclerotome (ventro-medial somite)	Induced by notochordal Shh + noggin; required for chondrogenesis of the vertebral body and intervertebral disc	Vertebral body/disc malformations (undulated mouse model)

Gene	Site of expression	Developmental role	Principal human disorder
PAX2	Optic stalk, otic vesicle, metanephric/mesonephric duct, midbrain-hindbrain junction	Specifies optic stalk vs neural retina (antagonises PAX6); drives ureteric-bud/nephric-duct outgrowth; patterns the cochlea/sacculle	Renal-coloboma syndrome (optic-nerve coloboma + renal hypoplasia/dysplasia)
PAX3	Dorsal neural tube, neural crest, dermomyotome	Neural-crest cell specification (melanocytes, craniofacial skeleton); myotome formation; drives <i>MyoD</i> expression	Waardenburg syndrome type I/III ; fusion oncogene in alveolar rhabdomyosarcoma
PAX4	Pancreatic endocrine progenitors	Balances with <i>Arx</i> : high Pax4/ <i>Arx</i> ratio → beta/delta cell fate; low ratio → alpha cells	Rare monogenic diabetes/pancreatic agenesis (experimental)
PAX5	B-lymphocyte precursors	Commits lymphoid progenitors to the B-cell lineage (not organogenesis-related)	B-cell lymphomas (chromosomal rearrangement)
PAX6	Eye field, lens placode, neural retina, optic nerve, olfactory placode, CNS, pancreas	"Master control gene" for eye morphogenesis — orthologue of <i>Drosophila eyeless</i> ; required for lens induction, neural-retina multipotency, iris/ciliary body specification	Aniridia, Peters' anomaly , part of WAGR syndrome
PAX7	Central dermomyotome, satellite cells	Required for survival (not initiation) of muscle satellite cells; postnatal muscle regeneration	Fusion oncogene in alveolar rhabdomyosarcoma
PAX8	Thyroid follicular cell precursors, Wolffian/Müllerian duct, metanephros	With <i>Nkx2.1/Foxe1</i> drives thyroid-bud formation and thyroglobulin gene expression; cooperates with PAX2 in nephric-duct extension	Thyroid dysgenesis → congenital hypothyroidism

Gene	Site of expression	Developmental role	Principal human disorder
PAX9	Pharyngeal-pouch endoderm, odontogenic (dental) mesenchyme, limb, vertebral column	Marks the odontogenic field (induced by Fgf8, inhibited by Bmp4); required for tooth-bud invagination beyond the bud stage	Non-syndromic hypodontia (typically molars)

3. Regulatory logic — worked example: somite differentiation

The clearest illustration of how a PAX gene converts an inductive signal into organ identity is the **sclerotome–dermomyotome switch** in the somite:

- ▶ **Notochord/floor plate** secretes **Shh** (with **noggin**, a Bmp antagonist) → induces the ventro-medial somite to become **sclerotome** and switch on **Pax1** (and **Pax9**, **Bapx1**) → chondrogenic programme for the vertebral body and disc.
- ▶ **Dorsal neural tube/surface ectoderm** secretes **Wnts** (**Wnt1**, **Wnt3a**; **Wnt4**, **Wnt6**) → the dorso-lateral somite becomes **dermomyotome** and expresses **Pax3** (and later **Pax7**) → myogenic programme (dermomyotome → myotome → skeletal muscle) and, in the limb, long-range migration of Pax3-positive myoblasts.
- ▶ Pax3/Pax7-expressing central dermomyotome cells that persist postnatally become **satellite cells**; Pax7 is specifically required for their survival.

□ Fig 6.12 — Expression patterns of genes that regulate somite differentiation. *Son* (Langman's Medical Embryology — Sadler — 14e, p.103)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a **cross-section of a somite** at the four-compartment stage with labelled arrows for the inducing signals and the PAX genes they switch on:

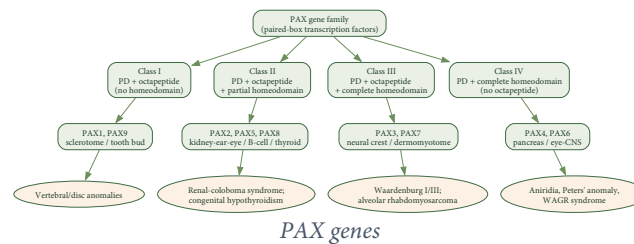
- ▶ **Notochord** (ventral midline) → arrow labelled "**Shh + Noggin**" → **Sclerotome** (ventro-medial) → label "**Pax1 (+ Pax9, Bapx1)**" → **vertebral body/disc**"
- ▶ **Surface ectoderm + dorsal neural tube** → arrow labelled "**Wnt1/3a/4/6**" → **Dermomyotome** (dorso-lateral) → label "**Pax3**" → **myotome**" and "**Pax3/Pax7**" → **satellite cells**"
- ▶ **Neural tube** (centre, for orientation) and **notochord** (ventral to neural tube) drawn as reference structures.

4. PAX genes in named organ systems

- ▶ **Eye:** Pax6 specifies the "eye field" in the anterior neural plate (with Six3, Rx, Lhx2); after optic-vesicle invagination it maintains the neural retina/lens while its mutual antagonism with Pax2 fixes the neural-retina–optic-stalk boundary. Loss of Pax6 arrests lens and iris development.
- ▶ **Kidney and ear:** Pax2 (with Pax8) is switched on in the intermediate mesoderm downstream of Osr1, driving Lhx1 and Ret expression essential for ureteric-bud outgrowth; the same gene, expressed in the ventral otocyst under Shh control, is required for cochlear/saccular development — explaining the combined eye–kidney–ear phenotype of PAX2 disease.

- **Axial skeleton and muscle:** Pax1/Pax9 (Shh-dependent) build the sclerotome-derived vertebral column; Pax3/Pax7 (Wnt-dependent) build the dermomyotome-derived musculature, including long-range limb myoblast migration.
- **Craniofacial and dental:** Pax9 marks the odontogenic field in the first pharyngeal arch (Fgf8-promoted, Bmp4-inhibited) and is essential for progression of the tooth bud into the cap stage; Pax3 governs neural-crest-derived craniofacial mesenchyme.
- **Endocrine organs:** Pax8 (with Nkx2.1, Foxe1) is required for thyroid follicular-cell differentiation; Pax4 (against Arx) sets the beta-vs-alpha balance in pancreatic islet progenitors.

5. Classification/relationship overview



Applied / Clinical anatomy

- **Aniridia (PAX6, chromosome 11p13):** congenital absence/hypoplasia of the iris; usually autosomal dominant with high new-mutation rate; associated glaucoma and cataract; can occur with **Peters' anomaly** (central corneal opacity with iridocorneal/lenticulo-corneal adhesion), reflecting Pax6's early role in the whole eye field.
- **WAGR syndrome:** a contiguous-gene deletion of 11p13 removing both **PAX6** and the adjacent **WT1** gene (only ~700 kb apart) — Wilms tumour, Aniridia, Genitourinary anomalies, mental Retardation.
- **Waardenburg syndrome type I/III (PAX3, neural-crest disorder):** dystopia canthorum (type I) ± limb/hand anomalies (type III, Klein–Waardenburg), heterochromia iridis, white forelock/premature greying, congenital sensorineural deafness — a classic **neurocristopathy**.
- **Renal-coloboma syndrome (PAX2):** optic-nerve/fundus coloboma with renal hypoplasia/dysplasia and vesico-ureteric reflux; illustrates the shared PAX2 programme of the optic stalk and the nephric duct.
- **Non-syndromic hypodontia (PAX9):** missing permanent molars (contrast with MSX1-related premolar/third-molar agenesis).
- **Alveolar rhabdomyosarcoma:** characteristic chromosomal translocations t(2;13)(q35;q14) and t(1;13)(p36;q14) create **PAX3-FOXO1** and **PAX7-FOXO1** chimeric transcription factors — the PAX DNA-binding domains are fused to the potent FOXO1 activation domain, converting a developmental master gene into an oncogene.
- **Thyroid dysgenesis/congenital hypothyroidism:** mutations of PAX8 (with NKX2.1, FOXE1) cause thyroid agenesis/hypoplasia — screened for on the neonatal TSH card test.
- **Vertebral segmentation anomalies:** Pax1 loss-of-function in the mouse (*undulated*) produces vertebral body/intervertebral disc dysplasia, the experimental correlate of human segmentation defects of the vertebral column.

High-yield exam pointers

- ▶ PAX = Paired boX gene; vertebrate homologue of *Drosophila paired*; defining motif is the **paired domain** ($\pm$ homeodomain $\pm$ octapeptide).
- ▶ 9 human PAX genes (PAX1–PAX9); classified into 4 **structural classes** by domain composition.
- ▶ PAX6 = **master control gene of the eye** (*Drosophila eyeless* orthologue); mutation $\rightarrow$ **aniridia** / Peters' anomaly / WAGR (with WT1, 11p13 deletion).
- ▶ PAX3 = **neural-crest gene**; mutation $\rightarrow$ **Waardenburg syndrome type I/III**; PAX3-FOXO1 translocation $\rightarrow$ **alveolar rhabdomyosarcoma** (shared with PAX7-FOXO1).
- ▶ PAX2/PAX8 pattern the intermediate mesoderm (kidney); PAX2 also patterns the optic stalk and otic vesicle $\rightarrow$ **renal-coloboma syndrome**.
- ▶ PAX1 (with Pax9, Bapx1) drives sclerotome $\rightarrow$ vertebral body/disc chondrogenesis, downstream of notochordal Shh + **noggin**.
- ▶ PAX9 marks the odontogenic field (tooth bud) and pharyngeal-pouch derivatives $\rightarrow$ non-syndromic **hypodontia** of molars.
- ▶ PAX7 maintains muscle **satellite-cell** survival (postnatal regeneration); shares the dermomyotome programme with PAX3.
- ▶ PAX8 builds the thyroid follicular epithelium (with Nkx2.1/Foxe1) $\rightarrow$ thyroid dysgenesis/congenital hypothyroidism.

SOURCE & COVERAGE

Langman's Medical Embryology (Sadler) 14e; Larsen's Human Embryology 6e; Moore's The Developing Human 9e; Gray's Anatomy (Standring) 41e; Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e.



Pedigree chart of sex-linked inheritance

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction — A **pedigree** is a graphic, symbol-based representation of a family's medical/genetic history across generations, constructed by an interviewer from a proband (index case) outward, and used to determine the mode of transmission of a trait or disorder. **Sex-linked inheritance** refers to genes carried on the sex chromosomes (X or Y); because the Y chromosome carries almost no genes besides the testis-determining (SRY/H-Y antigen) locus, "sex-linked" is, for practical exam purposes, synonymous with **X-linked** inheritance, which may be recessive or dominant. Pedigree analysis of sex-linked traits is a core tool in clinical genetics, genetic counselling and risk estimation.

1. Pedigree symbols and construction

Pedigrees are drawn with a standard, internationally-recognised symbol set (Thompson & Thompson Fig. 7-2; Gangane Table 6.1):

Symbol	Meaning
Square	Male
Circle	Female
Diamond	Sex unspecified
Filled (solid) square/circle	Affected individual
Open square/circle with a central dot	Carrier (heterozygote) female
Horizontal line joining a square and circle	Mating/union
Double horizontal line	Consanguineous union
Vertical line dropping from a union, with a horizontal sibship line	Offspring of that union, listed left to right in birth order
Diagonal line through a symbol	Deceased individual
Arrow	Proband (index case through whom the family was ascertained)
Roman numerals (I, II, III...) down the left margin	Successive generations
Arabic numerals below each symbol	Individual's serial number within that generation

Generations are numbered top to bottom, individuals left to right within each generation, so any member can be uniquely addressed (e.g., III-5).

2. X-linked recessive inheritance

Males are **hemizygous** for X-linked genes (only one X chromosome), so a single mutant allele is fully expressed — the trait is therefore expressed in **all males who inherit it**. In females the mutant allele must be present in **double dose (homozygous)** to produce disease; a heterozygous female is usually an unaffected **carrier**, though she may occasionally show mild features as a "manifesting heterozygote" (see Applied section).

Features on a pedigree (Gangane; Thompson & Thompson):

- ▶ The trait affects males much more frequently than females.
- ▶ It is transmitted from an affected male through **all his (carrier) daughters** to, on average, **half of their sons** — the classical "criss-cross" (skip-a-generation) pattern.
- ▶ **No male-to-male transmission** occurs, because a father passes only his Y chromosome to sons.
- ▶ In a kindred, affected males are related to one another **through carrier females**.
- ▶ A significant proportion of isolated (sporadic) cases arise from new mutation.

Two classical matings illustrate the outcome: (i) carrier mother × normal father → half the daughters are carriers, half the sons are affected; (ii) affected father × normal (non-carrier) mother → all daughters are obligate carriers, all sons are normal.

Examples: Haemophilia A (factor VIII/antihemophilic globulin deficiency; incidence ≈1 in 10,000 male births; historically traced through Queen Victoria's pedigree); Duchenne muscular dystrophy (DMD gene at Xp21, protein product dystrophin — the largest human gene, ~2300 kb with 79 exons;

incidence 1 in 3500 male births, with the allelic milder Becker muscular dystrophy at 1 in 20,000; serum creatine kinase markedly raised); X-linked colour blindness; and glucose-6-phosphate dehydrogenase (G6PD) deficiency.

□ Fig 39.4 — A pedigree chart showing X-linked recessive inheritance—A carrier mother has transmitted the gene to her son and daughter with the daughter becoming a carrier and the son getting affected; the affected male of the second generation transmits the gene to all daughters making them all carriers (IB Singh — Textbook of Anatomy Vol 3 — 6e, p.511)

3. X-linked dominant inheritance

The mutant allele is expressed in **both heterozygous and homozygous** states. Because females have two X chromosomes (twice the "target" for the mutation) but usually show a milder phenotype (a proportion of their cells inactivate the mutant X), the trait occurs **about twice as often in females as in males**, and affected females typically have a milder, more variable expression than affected males.

Features on a pedigree (Gangane; Thompson & Thompson):

- ▶ An **affected male transmits the trait to ALL his daughters and NONE of his sons** — this is the key feature that distinguishes X-linked dominant from autosomal dominant inheritance, and it can only be established by tracing the progeny of affected **males**.
- ▶ An affected **heterozygous female** transmits the trait to **half her children of either sex** — this closely mimics autosomal dominant transmission, so pedigrees through affected females alone cannot distinguish the two patterns.
- ▶ An affected homozygous female transmits the trait to all her children.
- ▶ Affected females are more common but more mildly affected than affected males.

Examples: X-linked hypophosphataemic (vitamin D-resistant) rickets — impaired renal tubular phosphate reabsorption, less severe in heterozygous females than in hemizygous affected males; and the Xg blood group.

A special variant is **X-linked dominant with male lethality**, where the disorder is lethal in hemizygous males in utero, so the pedigree shows transmission only through affected females, whose live-born offspring occur in the ratio of affected daughters : normal daughters : normal sons $\approx 1 : 1 : 1$, with no affected sons surviving. Rett syndrome (due to MECP2 mutation, presenting with normal early development followed by regression and neurological symptoms almost exclusively in girls) is a recognised example of this pattern.

□ Fig 39.3 — A pedigree chart showing X-linked dominant inheritance —From the pedigree chart, it can be seen that the affected mother of the first generation transmitted it to both son and daughter; the affected father of the second generation transmitted it only to the daughters (IB Singh — Textbook of Anatomy Vol 3 — 6e, p.511)

4. Y-linked (holandric) inheritance

Genes on the Y chromosome (essentially only the SRY/H-Y antigen locus and a handful of others) show **holandric inheritance**:

- ▶ Transmitted from an affected father to **all his sons**, and **never** to daughters (since daughters do not receive a Y chromosome).
- ▶ There is no carrier state and no skipping of generations — every male descendant in the direct male line is affected.

- The only clinically recognisable holandric trait cited classically is **hairy pinna** (hypertrichosis of the helix of the ear).

5. Distinguishing X-linked from autosomal patterns on a pedigree

Feature	Autosomal dominant	X-linked dominant	X-linked recessive
Sex ratio affected	Equal	Females $\approx 2 \times$ males (milder in females)	Males $\gg$ females
Affected male $\rightarrow$ daughters	50% affected	100% affected	All daughters carriers, none affected
Affected male $\rightarrow$ sons	50% affected	0% affected	0% affected (no male-to-male transmission)
Affected heterozygous female $\rightarrow$ children	50% of either sex	50% of either sex (mimics AD)	Sons: 50% affected; daughters: 50% carriers
Male-to-male transmission	Present	Absent	Absent

The single most useful discriminator on any pedigree suspected of being sex-linked is therefore the **absence of male-to-male transmission**; whether the pattern is recessive or dominant is then settled by whether affected males have unaffected daughters (recessive — daughters are only carriers) or all-affected daughters (dominant).

Applied / Clinical anatomy

- **Carrier detection:** biochemical/molecular assays supplement pedigree risk — e.g., serum creatine kinase is often elevated in DMD carrier females (though normal in many); factor VIII assay and molecular (linkage/mutation) testing refine haemophilia carrier risk.
- **Manifesting heterozygote:** an X-linked recessive carrier female may show mild disease features when random X-inactivation (Lyon hypothesis) is unfavourably skewed toward inactivation of the normal allele in the relevant tissue — important when counselling apparently "affected" females in an X-linked recessive family.
- **Genetic counselling and risk calculation:** pedigree analysis, combined with Bayesian conditional-probability methods, is used to refine a consultand's carrier or recurrence risk beyond the simple Mendelian expectation, particularly in X-linked recessive families with intervening unaffected male relatives.
- **Prenatal diagnosis:** in known X-linked recessive families, fetal sexing (ultrasound/free fetal DNA) combined with chorionic villus sampling/amniocentesis and molecular mutation analysis allows targeted testing of male fetuses at risk.
- **Medico-legal/forensic use:** pedigree charts are also used in disputed-paternity and hereditary-disease litigation to demonstrate a consistent mode of transmission.

High-yield exam pointers

- "Sex-linked" $\approx$ "X-linked" in practice; true Y-linked (holandric) traits are exceedingly few (SRY/H-Y locus; hairy pinna).

- ▶ **Absence of male-to-male transmission** is the single hallmark of any X-linked pedigree (recessive or dominant).
- ▶ X-linked recessive: males affected $\gg$ females; carrier daughters of an affected male; "criss-cross"/skip-a-generation pattern; hemophilia A, DMD, colour blindness, G6PD deficiency.
- ▶ X-linked dominant: affected male $\rightarrow$ all daughters affected, no sons affected; females affected $2\times$ more often but more mildly; vitamin D-resistant (hypophosphataemic) rickets, Xg blood group.
- ▶ X-linked dominant with male lethality (e.g., Rett syndrome/MECP2) shows transmission only through affected females, live-born ratio ≈ 1 affected daughter : 1 normal daughter : 1 normal son.
- ▶ Holandric (Y-linked): father $\rightarrow$ all sons affected, no carriers, no daughters ever affected.
- ▶ Standard pedigree symbols: square = male, circle = female, filled = affected, dot-in-open-symbol = carrier, arrow = proband, double line = consanguinity.
- ▶ Manifesting heterozygote in X-linked recessive disease = carrier showing mild disease due to skewed X-inactivation (Lyon hypothesis).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a 3-4 generation pedigree (label rows I, II, III, IV in Roman numerals down the left margin; number individuals 1, 2, 3... left to right within each generation) using the standard symbol key below, then draw two short illustrative pedigrees side by side:

- ▶ **Symbol key** (must be shown/labelled): square = male; circle = female; filled black = affected; open with small central dot = carrier female; horizontal line joining a couple = mating; double horizontal line = consanguineous mating; vertical + horizontal sibship line = offspring; arrow = proband; diagonal slash = deceased.
- ▶ **X-linked recessive pedigree:** generation I — unaffected carrier mother $\times$ normal father; generation II — one affected son, one carrier daughter; generation III — the affected male (from gen II, married to a normal/non-carrier female) has all daughters as carriers and all sons normal, showing the "criss-cross", skip-a-generation pattern and no male-to-male transmission.
- ▶ **X-linked dominant pedigree:** generation I — an affected male $\times$ normal female; generation II — ALL daughters affected, NO sons affected; then show an affected heterozygous female (from gen II) $\times$ normal male producing, in generation III, both sons and daughters affected in roughly equal (50%) proportion.
- ▶ **Holandric (Y-linked) pedigree:** a simple single-line diagram — affected male (gen I) $\rightarrow$ all sons affected (gen II) $\rightarrow$ all their sons affected (gen III); no female in the entire pedigree is ever affected or a carrier.

SOURCE & COVERAGE

Gangane — Human Genetics (5e), Chapter "Modes of Inheritance" (pedigree symbols, features of X-linked recessive/dominant inheritance, haemophilia, DMD, hairy pinna/holandric inheritance); Thompson & Thompson — Genetics in Medicine (8e), Chapter 7 "Patterns of Single-Gene Inheritance" (pedigree symbol conventions, characteristics of X-linked recessive/dominant inheritance, manifesting heterozygotes, X-linked dominant with male lethality/Rett syndrome); IB Singh — Textbook of Anatomy Vol 3 (6e), Chapter 39 "Inheritance of Genetic Disorders" (pedigree charts for X-linked dominant and recessive inheritance, Y-linked inheritance).



Population genetics

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction Population genetics is the quantitative study of the distribution of genetic variation within and between populations, and of how the frequencies of alleles and genotypes at a locus are maintained or altered over generations by evolutionary forces — mutation, non-random mating, selection, genetic drift and migration. Its central organising principle is the **Hardy-Weinberg law**, which states that in an ideal (large, randomly mating) population, allele and genotype frequencies remain constant from generation to generation. Population genetics is often nicknamed "**beanbag genetics**" — the total store of alleles at a locus in a population (the **gene pool**) is modelled as beans of two colours drawn at random from a bag. It has direct medical application in genetic counselling (carrier-risk estimation), mutation-rate calculation, and understanding ethnic differences in disease frequency.

1. Gene pool and allele/genotype frequency

- ▶ **Gene:** unit of heredity at a locus; **allele:** an alternative form of a gene at that locus.
- ▶ **Gene pool:** the total collection of all alleles at a locus carried by every member of a population. For an autosomal locus the pool size = $2 \times$ number of individuals (each person contributes two alleles).
- ▶ **Allele frequency:** proportion of one allelic form among all alleles at that locus in the gene pool (expressed as a fraction of unity, $p + q = 1$ for a two-allele system).
- ▶ **Genotype frequency:** proportion of a particular genotype (e.g., AA, Aa, aa) among all individuals in the population.
- ▶ **Worked example** (two-allele codominant system, e.g., blood-group alleles I^A/I^B): in a population of 1000 persons with genotypes AA = 200, AB = 500, BB = 300 — total genes in the pool = 2000. Allele A count = $(200 \times 2) + 500 = 900 \rightarrow$ frequency $p = 900/2000 = 0.45$. Allele B count = $(300 \times 2) + 500 = 1100 \rightarrow$ frequency $q = 1100/2000 = 0.55$; $p + q = 1$.

□ Fig 12.1 — Concept of "beanbag genetics". (Gangane — Human Genetics — 5e, p.210)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A gene-pool/Hardy-Weinberg schematic: a box labelled "Gene pool" with alleles A (freq. p) and a (freq. q), $p+q=1$; below it a 2×2 Punnett square — rows/columns labelled sperm p(A), q(a) and ova p(A), q(a) — with cells AA= p^2 , Aa=pq, aA=pq, aa= q^2 ; and the summed ratio $p^2 : 2pq : q^2 = 1$, looped back to itself labelled "unchanged next generation → equilibrium".

2. The Hardy-Weinberg law

- **Statement:** gene (allele) frequencies in a population remain constant from generation to generation provided no evolutionary force — mutation, migration, selection or drift — is operating; consequently genotype frequencies are also constant, in the fixed ratio $p^2 : 2pq : q^2$.
- **Historical note:** independently formulated in 1908 by G.H. Hardy (English mathematician) and Wilhelm Weinberg (German physician); the joint eponym was proposed by C. Stern in 1943.
- **Formula:** for two alleles A (frequency p) and a (frequency q), $(p + q)^2 = p^2 + 2pq + q^2 = 1$, where p^2 = frequency of AA, $2pq$ = frequency of Aa (heterozygote), q^2 = frequency of aa. For a locus with three alleles (p, q, r), the expansion $(p + q + r)^2$ is used.

Assumption required for Hardy-Weinberg equilibrium

Population is large, and mating is random with respect to the locus

No appreciable new-mutation rate at the locus

No selection — all genotypes equally capable of mating and reproducing

No significant migration/gene flow from a population with very different allele frequencies

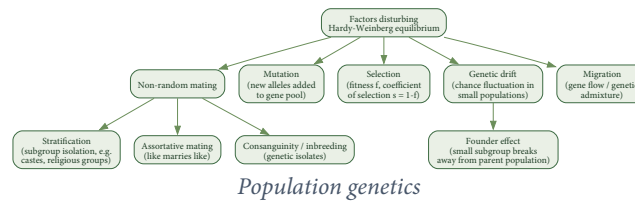
- **Two components:** (1) allele frequencies p, q fix genotype frequencies as $p^2 : 2pq : q^2$; (2) if p and q stay constant, this ratio is reproduced unaltered every generation — provable by summing all mating-type combinations (AA×AA, AA×Aa, Aa×Aa, etc.), which always reduce to $p^2(p+q)^2 : 2pq(p+q)^2 : q^2(p+q)^2$, i.e., $p^2 : 2pq : q^2$ again.
- Applies to all autosomal loci and the X chromosome in females, but **not** X-linked loci in males (hemizygous, single allele).

3. Applications of the Hardy-Weinberg law

- **Carrier-frequency estimation for autosomal recessive disease** (the single most important clinical use): q cannot be counted directly (carriers are phenotypically silent), but the affected-homozygote frequency q^2 is observable from disease incidence. Example — PKU in Ireland, incidence 1 in 4500: $q^2 = 1/4500$, $q \approx 0.015$, carrier frequency $2pq \approx 3\%$; the same calculation for Finland (PKU ≈ 1 in 200,000) gives only $\approx 0.6\%$ — carrier risk is population-specific.
- **X-linked application** (e.g., colour blindness): males have only two genotypes (mutant-allele frequency = q, read directly from male incidence); females show $p^2 : 2pq : q^2$. Since $q^2 \ll q$, an X-linked recessive trait is always far commoner in males (e.g., $\approx 8\%$ males colour blind vs affected females only $q^2 \approx 0.64\%$, though $\approx 15\%$ of females are silent carriers).
- **Mutation-rate calculation — direct method** (autosomal dominant, e.g. achondroplasia): of 96,000 births, 10 achondroplastics, 2 with an affected parent → 8 new mutations → rate ≈ 1 in

24,000 per gene ($\approx 42/\text{million genes/generation}$). *Indirect method*: $\mu = F(1-f)$ [autosomal], $\mu = \frac{1}{2}F(1-f)$ [X-linked recessive], where F = condition frequency, f = reproductive fitness.

4. Factors that disturb Hardy-Weinberg equilibrium

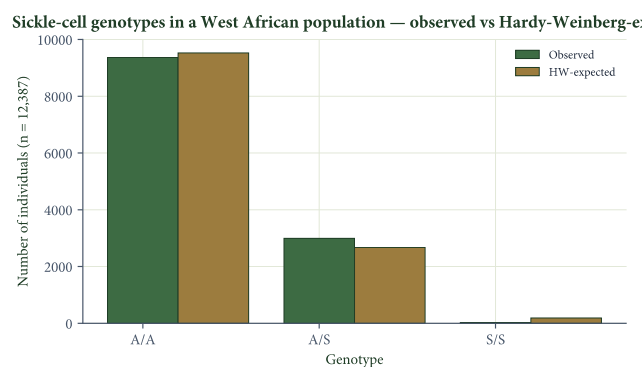


► Non-random mating

- *Stratification* — subgroups stay genetically separate for historical/cultural/religious reasons (e.g., different castes in India, Sunni/Shia communities, Orthodox Jews, French-Canadians); a higher-frequency subgroup allele produces more overall homozygotes than the pooled frequency predicts.
 - *Assortative mating* — preferential choice of a similar-trait mate (usually **positive**, e.g., stature, or shared disease like achondroplasia/deafness); increases homozygotes at the expense of heterozygotes.
 - *Consanguinity and inbreeding* — mating between relatives (or within a genetic isolate) lets a rare recessive allele from a common ancestor become homozygous, raising recessive-disease frequency. Example: Tay-Sachs disease is $\approx 100\times$ commoner among Ashkenazi Jews (1 in 3600) than elsewhere (1 in 360,000) — carrier frequency ≈ 1 in 30 vs ≈ 1 in 300.
- **Mutation** — spontaneous rate averages ≈ 1 in 100,000 per locus per generation (range 1 in 10,000 – 1,000,000); raised by radiation and mutagens (mustard gas, acridine orange). Slowly adds new alleles, causing gradual drift from equilibrium.
- **Selection and fitness** — fitness (f) = relative offspring-survival of affected persons; coefficient of selection $s = 1-f$; $s=1$ ($f=0$) is a **genetic lethal**. Selection against recessive alleles is far slower than against dominant alleles, since most recessive mutants hide in silent heterozygotes.
- **Genetic drift** — chance fluctuation of allele frequency, with far greater effect in small than large populations.
- **Founder effect** (a special form of drift): a small subgroup breaking off a larger population carries, by chance, different allele frequencies. Examples: **Huntington disease** clustered around Lake Maracaibo, Venezuela; **type I tyrosinemia** at 1 in 685 births in Saguenay–Lac-Saint-Jean, Quebec (vs 1 in 100,000 elsewhere), with 100% of patients sharing one founder mutation.
- **Migration and gene flow** — slow diffusion of genes across a reproductive barrier, merging a migrant group's alleles into the host gene pool (**genetic admixture**). Example: blood-group-B allele frequency exceeds 25% across Asia but falls below 10% in Scandinavia, reflecting Mongoloid migrations westward, 500–1500 AD.

5. Balanced selection / heterozygote advantage — deviation from equilibrium

- ▶ When a heterozygote genotype has a survival advantage over both homozygotes, the population shows a stable **excess of heterozygotes and deficit of homozygotes** compared with Hardy-Weinberg prediction — called balanced polymorphism.
- ▶ **Classic example — sickle-cell trait and malaria:** in a West African adult population of 12,387 individuals, genotypes A/A (normal), A/S (carrier) and S/S (sickle-cell disease) were observed as 9365 : 2993 : 29. Counting alleles gives $p(A) = 0.877$, $q(S) = 0.123$; the Hardy-Weinberg-predicted numbers would be 9527 : 2672 : 188. The observed heterozygote excess and homozygote deficit reflect **balanced selection** — the A/S heterozygote is relatively protected against falciparum malaria, while the S/S homozygote suffers sickle-cell disease and reduced fitness.



Heterozygote (A/S) excess and homozygote (S/S) deficit versus Hardy-Weinberg expectation demonstrate balanced selection (heterozygote advantage against malaria) (Thompson & Thompson 8e).

- ▶ Other examples: **thalassaemia trait** and **G6PD deficiency** persist at high frequency in malaria-endemic regions via heterozygote protection; a variant **APOL1** allele protects heterozygotes against trypanosomiasis while homozygotes carry markedly higher risk of focal segmental glomerulosclerosis (carrier frequency up to $\approx 45\%$ in some African populations).

6. Eugenics — a societal application of population-genetic principles

- ▶ **Eugenics** (Greek, "good birth"; concept introduced by **Francis Galton, 1883**) is the attempt to improve the hereditary quality of a population by selective breeding.
- ▶ **Positive eugenics** — encouraging reproduction by those considered genetically favourable; **negative eugenics** — discouraging/preventing reproduction by those considered genetically unfit (historically and infamously misused, e.g., in Nazi Germany, as a caution against its abuse).
- ▶ Modern medical practice touches on eugenic principles through **prenatal diagnosis with selective termination** and **carrier screening programmes**, raising ongoing ethical, social and legal questions rather than being practised as classical "eugenics."

Applied / Clinical anatomy

- ▶ **Genetic counselling:** Hardy-Weinberg-derived carrier risk (autosomal recessive) and recurrence risk (X-linked recessive) guide premarital and antenatal counselling.

- ▶ **Consanguinity counselling:** communities with consanguineous/intra-group marriage traditions (genetic isolates) carry disproportionately raised risk of rare autosomal recessive disorders — a standard pre-conception counselling point.
- ▶ **Founder-population screening:** recognised founder-effect diseases (Tay-Sachs in Ashkenazi Jews, tyrosinemia I in Quebec) underlie targeted community carrier-screening programmes.
- ▶ **Rh system:** Rh-negative is an autosomal recessive trait whose Hardy-Weinberg-derived population frequency determines maternal-sensitisation risk and the need for anti-D prophylaxis to prevent haemolytic disease of the newborn.
- ▶ **Malaria-linked haemoglobinopathies:** geographic correlation of sickle-cell/thalassaemia carrier frequency with historical malaria endemicity (balanced polymorphism) underlies antenatal haemoglobinopathy-screening programmes.
- ▶ **Ethnic/ancestry genetics:** allele-frequency differences between stratified subgroups (from founder effect, drift, limited gene flow) must be factored into counselling an individual from a different ancestral background.

High-yield exam pointers

- ▶ Population genetics = study of allele/genotype frequency change in populations; nicknamed "beanbag genetics".
- ▶ **Hardy-Weinberg law** (1908): $p^2 + 2pq + q^2 = 1$; frequencies constant if population large, mating random, and no mutation/selection/migration.
- ▶ Carrier frequency of autosomal recessive disease = $2pq$, from observed q^2 (PKU Ireland: $q^2=1/4500 \rightarrow \text{carrier} \approx 3\%$).
- ▶ X-linked recessive: affected males = q directly; affected females = q^2 (rare), carriers $\approx 2pq$.
- ▶ Four disturbing forces: **mutation, non-random mating (stratification/assortative mating/consanguinity), selection, migration/drift.**
- ▶ **Founder effect** (a form of drift): Huntington disease — Lake Maracaibo; tyrosinemia I — Quebec; Tay-Sachs — Ashkenazi Jews.
- ▶ **Balanced selection** (sickle-cell vs malaria) gives a stable heterozygote excess over Hardy-Weinberg prediction.
- ▶ Mutation rate: direct method (new cases $\div$ births) or $\mu = F(1-f)$ [autosomal] / $\frac{1}{2}F(1-f)$ [X-linked].
- ▶ Eugenics (Galton, 1883): improving population heredity by selective breeding; positive vs negative; historically misused.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e. Coverage note: Gray's Anatomy (Standring) 41e — the gross-anatomy spine text — has no equivalent content, since population genetics is a quantitative-genetics topic rather than a gross-anatomical one; grounding was therefore drawn entirely from the genetics specialty texts, per this topic's specialty designation.



Translocation

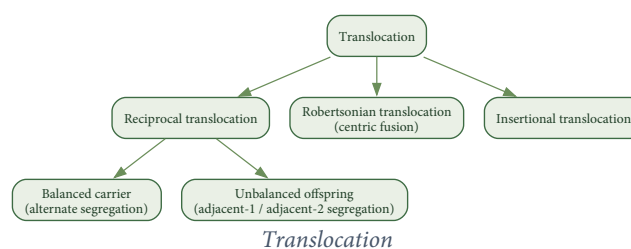
Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction

A **translocation** is a structural chromosomal aberration in which a segment of one chromosome is transferred to a different, non-homologous chromosome (or to a new site elsewhere in the genome) following one or more chromosome breaks. It rearranges genetic material without necessarily changing its total amount, and is detected on a G-banded karyotype (or by FISH/microarray) and reported using ISCN nomenclature. Translocations are classified functionally as **balanced** (no net gain or loss of chromosomal material — carrier is usually phenotypically normal) or **unbalanced** (net loss/gain — usually an abnormal phenotype), and structurally as **reciprocal**, **Robertsonian (centric fusion)**, or **insertional**. Their clinical importance lies chiefly in the risk of unbalanced gametes/offspring in balanced carriers, and in acquired somatic translocations that activate oncogenes in leukaemia and lymphoma.

Classification of translocations

Feature	Reciprocal	Robertsonian (centric fusion)	Insertional
Chromosomes involved	Any 2 non-homologous chromosomes	2 acrocentric chromosomes (D group 13,14,15; G group 21,22)	1 donor + 1 recipient non-homologous chromosome
Breakpoints	2 (one per chromosome)	2, at/near the centromere	3
Mechanism	Reciprocal exchange of the segments distal to each break	Fusion of long arms near centromere; short arms lost	Segment excised from one chromosome, inserted into another (same or inverted orientation)
Resultant chromosome number	Unchanged (46)	Reduced to 45 (functionally balanced)	Unchanged (46)
Reciprocity	Reciprocal — both derivative chromosomes retained	Non-reciprocal	Non-reciprocal



Reciprocal translocation

- ▶ **Mechanism:** breakage of two non-homologous chromosomes with reciprocal exchange of the broken-off segments; two new **derivative chromosomes** are formed, designated **der(A)** and **der(B)**; total chromosome number stays 46.
- ▶ Because material is only rearranged and not lost, carriers are usually phenotypically normal — such **balanced translocations** are found in as many as **1 in 500 individuals** in the general population.
- ▶ Balanced-translocation carriers are over-represented among couples with two or more spontaneous abortions and among infertile males.
- ▶ **Meiotic behaviour:** at meiosis I the two normal homologues and the two derivative chromosomes must pair as a cross-shaped **quadrivalent** (rather than the usual bivalents) so that homologous segments can align.
- ▶ **Segregation at anaphase** determines the gamete outcome:

Segregation pattern	What goes to each pole	Gamete outcome
Alternate (usual)	Both normal chromosomes to one pole; both derivatives to the other	Balanced — normal complement or balanced carrier
Adjacent-1	One normal + one derivative (homologous centromeres to opposite poles)	Always unbalanced
Adjacent-2	One normal + one derivative (homologous centromeres to the same pole)	Always unbalanced

- ▶ Net effect: the theoretical risk of an unbalanced conceptus in a reciprocal-translocation carrier can be as high as 50%, though the observed live-birth risk is usually lower because many unbalanced conceptuses abort spontaneously. A breakpoint can itself disrupt a gene and produce a phenotype even in an apparently "balanced" carrier.

Robertsonian translocation (centric fusion)

- ▶ Fusion of two **acrocentric** chromosomes (D group 13, 14, 15; G group 21, 22) near/at the centromere, with loss of both short arms.
- ▶ The short arms of acrocentric chromosomes carry only satellite DNA and repetitive ribosomal-RNA genes (nucleolar organiser regions), so their loss is **not deleterious** — the karyotype is functionally balanced despite having only **45 chromosomes**.
- ▶ Non-reciprocal; the fusion chromosome is often pseudodicentric.
- ▶ Commonest combinations: **rob(13;14)(q10;q10)** — found in about **1 in 1300** people, making it the single most common structural chromosome rearrangement in humans — and **rob(14;21)(q10;q10)**.
- ▶ The carrier is phenotypically normal (45 chromosomes) but at risk for unbalanced gametes and unbalanced offspring; the risk varies with which chromosomes are fused and with the sex of the carrier parent (carrier mothers transmit an unbalanced karyotype more often than carrier fathers).

□ Fig 1.9 — Karyotype of a Male With Down Syndrome Caused by Translocation (Larsen — Human Embryology — 6e, p.99)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Schematic mechanism of a Robertsonian translocation, **rob(14;21)** — label:

- ▶ Normal chromosome 14 (long arm 14q + short arm 14p with satellite/NOR)
- ▶ Normal chromosome 21 (long arm 21q + short arm 21p with satellite/NOR)
- ▶ Breakpoint at/near the centromere (q10) on each chromosome
- ▶ Both short arms (14p, 21p) lost — discarded, non-deleterious
- ▶ Fusion of the two long arms into a single translocation chromosome — **der(14;21)**
- ▶ Carrier karyotype: **45,XX (or XY),rob(14;21)(q10;q10)** — phenotypically normal
- ▶ Unbalanced offspring karyotype: **46,XX (or XY),rob(14;21)(q10;q10),+21** — translocation Down syndrome (trisomic for the whole of 21q despite only 46 chromosomes)

Insertional translocation

- ▶ A segment is excised from one chromosome (2 breaks) and inserted into a non-homologous chromosome (a further break) — 3 breaks in total, in the same or inverted orientation.
- ▶ Rare, because it requires three simultaneous chromosome breaks.
- ▶ The carrier is balanced/phenotypically normal, but abnormal segregation in offspring can produce duplication or deletion of the inserted segment; the average risk of an abnormal child can be up to 50%, so prenatal diagnosis is indicated in carriers.

Detection and nomenclature

- ▶ Conventional **G-banded karyotyping** remains the front-line test for structural rearrangements.
- ▶ **FISH** confirms and characterises specific translocations, including cryptic/subtelomeric rearrangements.
- ▶ **Chromosomal microarray (CGH/SNP array)** detects genome-wide copy-number (dosage) imbalance at high resolution but **cannot detect a truly balanced translocation** (there is no net dosage change) — karyotyping/FISH remains essential whenever a balanced rearrangement is suspected, e.g. in a recurrent-miscarriage work-up.
- ▶ **ISCN notation:** a reciprocal translocation between chromosomes A and B is written **t(A;B)** (breakpoint A;breakpoint B); the two resulting chromosomes are labelled **der(A)** and **der(B)**; a Robertsonian translocation is written **rob(13;14)(q10;q10)**.

Applied / Clinical anatomy

- ▶ **Translocation Down syndrome** — accounts for about **4% of Down syndrome cases**; usually a Robertsonian translocation between chromosome 21q and another acrocentric (commonly 14, sometimes 22), giving karyotype **46,XX/XY,rob(14;21)(q10;q10),+21** — 46 chromosomes yet trisomic for all of 21q. Unlike standard trisomy 21 (maternal-age related, population incidence ≈ 1 in 850 live births, with the meiotic error occurring in maternal meiosis in ~90% of cases), **translocation Down syndrome shows no relation to maternal age** but carries a much higher **familial recurrence risk** when a parent — especially the mother — is a carrier: unbalanced

offspring occur in roughly 10–15% of children of carrier mothers versus only a few percent for carrier fathers. Parental (and where relevant, sibling) karyotyping is therefore mandatory before genetic counselling.

- ▶ **21q21q translocation** (an isochromosome of 21q) is found in a few percent of Down syndrome cases; every gamete of such a carrier contains either a double dose of 21q (→ Down syndrome) or none (→ monosomy 21, usually non-viable) — i.e., a 100% risk of an abnormal pregnancy.
- ▶ **Philadelphia chromosome (chronic myeloid leukaemia)** — an acquired, reciprocal translocation between the long arms of chromosome 9 and chromosome 22, t(9;22), producing a shortened chromosome 22 ("22q-", the Philadelphia or Ph1 chromosome). It juxtaposes the *ABL* oncogene (chromosome 9) with the *BCR* gene (chromosome 22), generating a **BCR-ABL fusion gene/protein** with constitutive tyrosine-kinase activity — the first oncogenic translocation ever described (Nowell and Hungerford, 1960) and the paradigm for a diagnostic/prognostic cytogenetic marker that also guides targeted (tyrosine-kinase-inhibitor) therapy.
- ▶ **Acute promyelocytic leukaemia (APL)** — reciprocal translocation between chromosomes 15 and 17.
- ▶ **Burkitt lymphoma** — translocation between chromosomes 8 and 14 (occasionally variant t(2;8) or t(8;22)) that moves the *c-MYC* oncogene (8q24) next to the immunoglobulin heavy-chain locus (14q32), deregulating and markedly increasing MYC transcription.
- ▶ **Genetic counselling** — balanced-translocation carriers are phenotypically normal but are over-represented among couples with recurrent spontaneous abortion and among infertile men; karyotyping both partners is a standard step in the recurrent-pregnancy-loss and infertility work-up, followed by prenatal diagnosis (karyotype/FISH, not microarray alone) in any subsequent pregnancy.

□ Fig 10.3 — Formation of Philadelphia chromosome involves reciprocal translocation between *q* arms of chromosomes 9 and 22 (Gangane — Human Genetics — 5e, p.187)

High-yield exam pointers

- ▶ Translocation = exchange of chromosomal segment(s) between non-homologous chromosomes after breakage; **balanced** (no net material lost, usually normal phenotype) vs **unbalanced** (abnormal phenotype).
- ▶ Two main structural types: **reciprocal** (2 chromosomes, 2 breaks, chromosome number unchanged at 46) and **Robertsonian/centric fusion** (2 acrocentrics fuse near the centromere, short arms lost, chromosome number 45 but balanced); **insertional translocation** is a rarer 3-break variant.
- ▶ Reciprocal-translocation carriers form a **quadrivalent** at meiosis I; only **alternate segregation** gives balanced gametes — **adjacent-1** and **adjacent-2** segregation always give unbalanced gametes.
- ▶ Balanced translocations occur in ~1 in 500 people generally, and are enriched among couples with recurrent abortion or male infertility.
- ▶ **rob(13;14)** is the single commonest structural chromosome rearrangement in humans (~1 in 1300).

- ▶ **Translocation Down syndrome** (~4% of Down syndrome) is NOT maternal-age related but has a high familial recurrence risk — parental karyotyping is mandatory.
- ▶ Chromosomal microarray detects copy-number imbalance, not a truly balanced translocation — karyotyping/FISH is needed whenever one is suspected.
- ▶ Classic acquired/somatic oncogenic translocations: **t(9;22)** Philadelphia chromosome/*BCR-ABL* — chronic myeloid leukaemia; **t(15;17)** — acute promyelocytic leukaemia; **t(8;14)** *MYC-IGH* — Burkitt lymphoma.

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine (8e); Gangane Human Genetics (5e); Vishram Singh Textbook of Clinical Embryology; Larsen Human Embryology (6e) (karyotype figure). Gray's Anatomy (Standring) does not cover clinical cytogenetics; this topic is drawn entirely from the genetics specialty texts, as flagged in the topic spec.

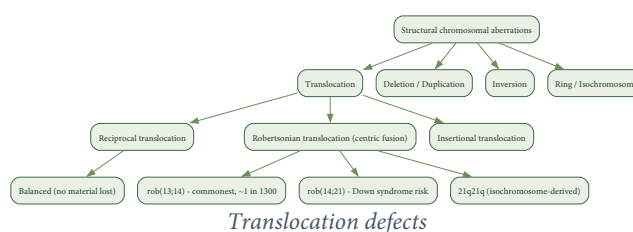


Translocation defects

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction A **translocation** is a structural chromosomal aberration in which a segment of one chromosome breaks off and is transferred to (or exchanged with) a non-homologous chromosome. Unlike numerical aberrations, the total genetic material may be completely conserved (a **balanced** translocation — carrier phenotypically normal) or altered (an **unbalanced** translocation — partial trisomy/monosomy, an abnormal phenotype). Translocations are of two principal types — **reciprocal** and **Robertsonian (centric fusion)** — and a third, rarer, **insertional** type. Their chief clinical importance in anatomy/genetics teaching is as the cause of "**familial**" or **translocation Down syndrome** and as recurrent, disease-defining rearrangements in certain malignancies (e.g. the Philadelphia chromosome).

Classification of translocations



Feature	Reciprocal translocation	Robertsonian translocation (centric fusion)
Chromosomes involved	Any two non-homologous chromosomes	Only the 5 acrocentric pairs (13,14,15,21,22)

Feature	Reciprocal translocation	Robertsonian translocation (centric fusion)
Mechanism	Breakage of both chromosomes with reciprocal exchange of the broken segments	Breakage near the centromere with fusion of the long arms ; the two short arms are lost
Reciprocity	Reciprocal (material exchanged, not lost)	Nonreciprocal — short arms discarded
Resulting chromosome number	46 (unchanged)	45 (two acrocentrics replaced by one fusion chromosome)
Net material lost	None	Short-arm satellite DNA/rRNA-gene repeats (functionally dispensable) — hence still balanced
Carrier phenotype	Normal	Normal
ISCN notation	t(2;8)(q22;p21)	rob(14;21)(q10;q10)
Commonest example	Various — e.g. t(9;22) in CML, t(8;14) in Burkitt lymphoma	rob(13;14) — commonest single chromosome rearrangement in humans (~1/1300); rob(14;21) — clinically important for Down syndrome

Reciprocal translocation

- ▶ Results from breakage of **two non-homologous chromosomes** with exchange of the broken-off segments; because the exchange is reciprocal, the total chromosome number stays **46** and, cytogenetically, no material is gained or lost — the rearrangement is **balanced**.
- ▶ At meiosis, the two normal chromosomes and their two translocated (derivative) partners must pair as a cross-shaped **quadrivalent** (rather than simple bivalents) to align homologous segments.
- ▶ Segregation from the quadrivalent at anaphase I can occur in three patterns:
 - **Alternate segregation** — the two normal chromosomes go to one pole and the two derivative (translocated) chromosomes to the other → gametes are **balanced** (either entirely normal or carrying the balanced translocation).
 - **Adjacent-1 segregation** — homologous centromeres separate to opposite poles but each gamete gets one normal + one derivative chromosome → always **unbalanced**.
 - **Adjacent-2 segregation** (rarer) — homologous centromeres go to the *same* pole → always **unbalanced**.
- ▶ Carriers are phenotypically normal but at increased risk of producing unbalanced gametes → recurrent spontaneous abortion, stillbirth, or a liveborn child with partial trisomy/partial monosomy and multiple congenital anomalies; the empirical risk of an abnormal liveborn child varies with the specific rearrangement, ranging from about **1% up to ~20%**.

- ▶ Balanced reciprocal translocations are found in as many as **1 in 500 individuals** in the general population and are disproportionately found in couples with recurrent miscarriage and in infertile men.
- ▶ A chromosome breakpoint can occasionally disrupt a gene directly, producing a phenotype in an apparently "balanced" carrier — an increasingly recognised cause of disease detectable by whole-genome sequencing.

Robertsonian translocation (centric fusion)

- ▶ Involves fusion of **two acrocentric chromosomes** (groups D: 13,14,15; and G: 21,22) near their centromeres, with loss of both short arms; because the short arms consist only of redundant satellite DNA and multiple copies of ribosomal RNA genes, their loss is **not deleterious** — the karyotype, though only **45 chromosomes**, is functionally balanced.
- ▶ The translocation is **nonreciprocal** and the resulting fusion chromosome is often **pseudodicentric** (two centromeres, one functionally inactivated).
- ▶ **rob(13;14)** is the single commonest structural chromosome rearrangement in humans (~1 in 1300 individuals); **rob(14;21)** is the type of chief clinical importance because it underlies translocation Down syndrome.
- ▶ A carrier is phenotypically normal (45 chromosomes total) but is at risk of producing **unbalanced gametes** — a gamete with both the translocation chromosome and a normal homologue of one of the involved chromosomes results in a trisomic zygote at fertilisation; a gamete lacking the fused chromosome and the homologue results in a monosomic (usually non-viable) zygote.
- ▶ Risk of an unbalanced (trisomic) child differs by parental sex: empirically, **carrier mothers** transmit an unbalanced complement in ~**10–15%** of pregnancies, whereas **carrier fathers** do so in only a **few percent** (~1–2%) — much lower than the theoretical 1-in-3 Mendelian expectation, because several of the theoretically possible unbalanced gametes are not compatible with viable conception.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Formation and inheritance of a Robertsonian translocation, rob(14;21) (label exactly):

- ▶ Two normal acrocentric chromosomes — **chromosome 14** and **chromosome 21** — each with a short arm (satellite stalk) and long arm
- ▶ **Centromeric breakpoint** on each, with loss of both short arms
- ▶ **Fusion of the two long arms** → single **translocation (derivative) chromosome, der(14;21)**, drawn alongside the remaining normal 14 and normal 21 → **carrier karyotype: 45 chromosomes total**
- ▶ Carrier's gametes at meiosis (three viable classes only): (i) **normal** (23, containing separate 14 and 21); (ii) **balanced** (24, containing der(14;21) alone); (iii) **unbalanced** (24, containing der(14;21) + normal chromosome 21)
- ▶ Fertilisation of the unbalanced gamete by a normal gamete → zygote **46,XX/XY,der(14;21)(q10;q10),+21** — **translocation Down syndrome**

□ Fig 1.9 — Karyotype of a Male With Down Syndrome Caused by Translocation (Larsen — Human Embryology — 6e, p.99)

Translocation ("familial") Down syndrome

Type	Karyotype	Approximate frequency among Down syndrome	Recurrence risk
Robertsonian rob(14;21) type	46,XX/XY,rob(14;21)(q10;q10),+21	~4% of Down syndrome cases	Carrier mother: ~10–15%; carrier father: a few percent (~1–2%); ~50% of translocation cases have a carrier parent, the rest are <i>de novo</i>
21q21q translocation (isochromosome-derived)	46 chromosomes, one being a 21q21q (centric-fusion/isochromosome) chromosome carrying a double dose of 21q, + one normal 21	A few percent of Down syndrome cases	If a parent carries the 21q21q chromosome, every viable pregnancy is either Down syndrome or non-viable monosomy 21 — effectively a 100% recurrence risk

- ▶ Although the *patient's* karyotype has 46 chromosomes with a Robertsonian translocation, all the genetic material of chromosome 21q is present in **triplicate**, so the phenotype is identical to standard trisomy 21.
- ▶ Unlike standard trisomy 21 (non-disjunctional, ~95% of cases), **translocation Down syndrome bears no relation to maternal age**; recurrence risk instead depends on whether a parent is a balanced carrier, making **parental karyotyping mandatory** whenever a child is found to have a translocation-type Down syndrome, for accurate genetic counselling of future pregnancies.
- ▶ Overall population incidence of Down syndrome (all types combined) is of the order of 1 in 700–850 live births.

Detection and nomenclature

- ▶ **Conventional karyotyping with G-banding** remains the reference method for identifying and characterising a translocation (reciprocal or Robertsonian) and for confirming a balanced-carrier state in parents.
- ▶ **FISH** (fluorescence in situ hybridisation) confirms specific translocation breakpoints and rapidly detects unbalanced segregants; **chromosomal microarray** and genome sequencing detect submicroscopic/cryptic rearrangements not visible by banding alone.
- ▶ **Prenatal diagnosis** (chorionic villus sampling or amniocentesis with karyotyping) is offered to known carrier parents; non-invasive prenatal screening of cell-free fetal DNA is increasingly used as a screening adjunct.
- ▶ **ISCN nomenclature** commonly tested: **t** = translocation (reciprocal), **rob** = Robertsonian translocation, **der** = derivative (translocation) chromosome, e.g. **46,XX,t(2;8)(q22;p21)** denotes a balanced reciprocal translocation carrier with breakpoints at 2q22 and 8p21.

Applied / Clinical anatomy

- ▶ **Translocation Down syndrome** — the single most important clinical translocation defect in genetic counselling; a young mother with a Down syndrome child and no advanced maternal age should always prompt parental karyotyping to exclude a Robertsonian carrier state.
- ▶ **Habitual/recurrent abortion and infertility** — balanced reciprocal or Robertsonian translocation carriers are over-represented among couples with recurrent pregnancy loss and among infertile males, owing to production of unbalanced gametes.
- ▶ **Cri-du-chat syndrome** (partial deletion of 5p) — about 10–15% of cases arise as an unbalanced segregant in the offspring of a parent carrying a balanced reciprocal translocation, illustrating how a translocation carrier can transmit a deletion/duplication syndrome quite different from the "classical" translocation Down syndrome picture.
- ▶ **Philadelphia chromosome (t(9;22)(q34;q11))** — a somatic reciprocal translocation acquired in haemopoietic stem cells in chronic myeloid leukaemia, fusing the **ABL** oncogene (chromosome 9) with the **BCR** gene (chromosome 22) to generate a constitutively active BCR-ABL tyrosine-kinase fusion protein; Ph-positive CML has a distinctly different clinical course from Ph-negative disease and is the basis of tyrosine-kinase-inhibitor therapy.
- ▶ **Burkitt lymphoma** — classic translocation t(8;14)(q24;q32) juxtaposes the **c-myc** oncogene (chromosome 8) next to the immunoglobulin heavy-chain gene locus (chromosome 14), driving markedly increased c-myc transcription; variant translocations t(2;8) and t(8;22) involve the kappa- and lambda-light-chain loci respectively.
- ▶ **Genetic counselling** — for a couple with a balanced translocation carrier, options discussed include prenatal diagnosis with selective termination, pre-implantation genetic testing, and, where risk is very high (e.g. a 21q21q carrier), use of donor gametes.

High-yield exam pointers

- ▶ Translocation = exchange/transfer of a chromosome segment to a non-homologous chromosome; **balanced** (no phenotype) vs **unbalanced** (abnormal phenotype).

- ▶ Two main types: **reciprocal** (any two chromosomes, 46 total, true exchange) and **Robertsonian/centric fusion** (only acrocentrics 13,14,15,21,22; 45 total; nonreciprocal, short arms lost).
- ▶ Reciprocal translocation carriers pair as a **quadrivalent** in meiosis; only **alternate segregation** gives balanced gametes — adjacent-1 and adjacent-2 always give unbalanced gametes.
- ▶ **rob(13;14)** is the commonest human chromosome rearrangement (~1/1300); **rob(14;21)** causes translocation Down syndrome.
- ▶ Translocation Down syndrome (~4% of all Down syndrome) shows **no maternal-age relationship**; recurrence risk depends on carrier parent's sex — **carrier mother** ~10–15%, **carrier father** ~1–2%.
- ▶ **21q21q translocation** carrier parent → essentially **100%** recurrence risk (Down syndrome or non-viable monosomy 21, no normal outcome).
- ▶ Parental karyotyping is mandatory after any translocation-type Down syndrome child, for genetic counselling of future pregnancies.
- ▶ Acquired somatic translocations underlie specific malignancies: **t(9;22) BCR-ABL** in CML (Philadelphia chromosome); **t(8;14) MYC-IGH** in Burkitt lymphoma.

SOURCE & COVERAGE

Thompson & Thompson — Genetics in Medicine (8e); Gangane — Human Genetics (5e); Larsen — Human Embryology (6e) (figure). **Coverage note:** Gray's Anatomy (Standring, 41e) has no dedicated cytogenetics/medical-genetics chapter, so this answer is grounded in the paper's designated genetics specialty texts rather than the spine text.



What is a 'GENE'? Discuss the different types of gene with examples

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction A **gene** is the fundamental unit of heredity — a segment of DNA at a fixed **locus** on a chromosome that specifies a functional product, a polypeptide or a functional RNA. The term was coined by the Danish botanist **Wilhelm Johannsen (1909)**, building on Mendel's "unit character." Classical anatomy texts cite **50,000–100,000** human genes; the sequence-based Human Genome Project figure is ~**20,000–25,000 protein-coding genes** plus a comparable number of **non-coding RNA genes** (~40,000–50,000 overall) — the difference reflects classical estimation versus whole-genome sequencing. Genes are classified by molecular product, internal structure, genomic organisation, expression pattern, chromosomal location, and phenotypic behaviour.

1. Structure of a typical human gene

- ▶ A gene = a **transcription unit** flanked by regulatory sequences.
- ▶ **Promoter** — a DNA sequence 5' (upstream) of the gene that allows RNA polymerase to bind and initiate transcription; contains conserved elements (the **TATA box**, **CAAT box**) in tissue-specific genes, or is **CG-rich (CpG island)** and TATA-less in housekeeping genes.

- ▶ **5' untranslated region (5'UTR)** — transcribed but not translated, lying between the promoter and the initiator codon.
- ▶ **Exons** — the coding (functional) segments; ultimately represented in mature mRNA.
- ▶ **Introns (intervening sequences)** — non-coding segments interrupting the exons; transcribed but then **spliced out** before translation.
- ▶ **3' untranslated region (3'UTR)** and a **polyadenylation signal (AATAAA)**, which directs addition of the poly-A tail to the mature transcript.
- ▶ Genes vary enormously in size: the β -globin gene has only 3 exons/2 introns (~1.6 kb); *BRCA1* has 24 exons; the dystrophin gene (Xp21) spans >2 Mb, with coding exons making up <1% of its length — the largest human gene.

□ Fig 17.14 — Structure of a gene. (Vishram Singh — General Anatomy — 4e, p.707)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ DNA double helix segment labelled, from one end to the other: **Intron — Exon — Intron** (alternating coding/non-coding blocks along the double helix).
- ▶ Show the helix **condensing upward** into a coiled **chromatin fibre**, and the chromatin fibre further condensing into a **metaphase chromosome** (paired sister chromatids joined at a centromere) — illustrating that the gene is one linear segment of the folded DNA that ultimately packages into a chromosome.
- ▶ Label the direction of transcription (5'→3'), the **promoter**, and mark "upstream"/"downstream" relative to the transcribed region.

2. Classification by molecular product

Type	Description	Example
Structural genes	Code for a specific amino acid sequence (a protein/enzyme)	β -globin gene; dystrophin gene
Operator genes	Do not code for a product themselves; act as the binding site that permits transcription of the adjacent structural gene(s) to proceed	Operator segment of the <i>lac</i> operon (classical Jacob–Monod model)
Regulator genes	Code for a repressor (or other regulatory) protein that acts on the operator to inhibit protein synthesis of the structural gene	Regulator gene of the <i>lac</i> operon; mutated <i>CFTR</i> alters regulated chloride-channel protein output
Protein-coding genes	Ultimate product is a polypeptide (~20,000–25,000 in the human genome)	Insulin gene, dystrophin gene

Type	Description	Example
RNA genes (non-coding)	Ultimate product is a functional RNA, not a protein; roughly equal in number to protein-coding genes	rRNA, tRNA, small nuclear/nucleolar RNA (snRNA/snoRNA), long non-coding RNA (lncRNA), microRNA (miRNA, ~22 bases, suppresses translation of target mRNAs)

3. Classification by internal (structural) organisation

- ▶ **Split (interrupted) genes** — the rule for human genes: coding exons are interrupted by one or more introns (e.g., β -globin: 3 exons/2 introns; *BRCA1*: 24 exons; *MYH7*: 40 exons).
- ▶ **Continuous (uninterrupted) genes** — a minority of human genes have no introns at all.
- ▶ **Overlapping genes** — two genes sharing the same stretch of DNA read in different frames or on opposite strands; well recognised in viral and mitochondrial genomes, less common in the nuclear genome.
- ▶ **Pseudogenes** — DNA sequences closely resembling a known gene but non-functional; tens of thousands are scattered through the genome, of two kinds:
 - **Non-processed pseudogenes** — "dead" duplicated genes inactivated in situ by mutation, still retaining introns and lying near their functional parent gene (found within both the β -globin and olfactory-receptor gene families).
 - **Processed pseudogenes** — formed by **retrotransposition**: the mRNA is reverse-transcribed to cDNA and reinserted elsewhere in the genome; consequently they lack introns and need not lie near the parent gene.

4. Classification by genomic organisation / copy number

- ▶ **Solitary genes** — present as a single copy in the haploid genome.
- ▶ **Multigene families** — groups of genes sharing closely related sequence and encoding related polypeptides, arising by duplication of an ancestral gene:
 - **Clustered families** — members lie together at one chromosomal locus, e.g. the **β -globin gene cluster** on chromosome 11 and the related **α -globin cluster** on chromosome 16 (arisen ~500 million years ago from a common precursor; each functional globin gene retains two introns at conserved positions, expressed sequentially from embryo to adult).
 - **Dispersed families** — members are scattered across many chromosomes, e.g. the **olfactory receptor (OR) gene family** (~1000 members, found on nearly every chromosome).
- ▶ **Repetitive gene-associated sequences** — tandem repeats (satellite DNA, α -satellite at centromeres) and dispersed repeats (**Alu** elements, ~300 bp, >1 million copies; **LINE/L1** elements, up to 6 kb, ~850,000 copies) are not themselves conventional genes but are closely linked to gene organisation and disease (see Jumping genes, below).

5. Classification by pattern of expression, and by chromosomal location

Basis	Type	Feature	Example
Expression pattern	Housekeeping genes	Constitutively expressed in virtually all cells; CG-rich, TATA-less promoters	<i>GAPDH</i> , <i>BRCA1</i> promoter
	Tissue-specific ("luxury") genes	Expressed selectively; promoters typically carry TATA/CAAT boxes	Globin genes (erythroid precursors only)
Chromosomal location	Autosomal genes	Carried on chromosomes 1–22; inheritance follows autosomal dominant/recessive patterns	Cystic fibrosis (<i>CFTR</i> , autosomal recessive)
	X-linked genes	Carried on the X chromosome; hemizygous expression in males	Haemophilia, red-green colour blindness (X-linked recessive)
	Y-linked (holandric) genes	Unpaired in males, hence always expressed, transmitted father→all sons only; the only confirmed human example is hairy pinna	Hypertrichosis of the pinna
	Mitochondrial genes	Located in mtDNA, transmitted exclusively through the mother (sperm mitochondria are excluded at fertilisation)	Leber's hereditary optic neuropathy; Pearson marrow–pancreas syndrome

6. Classification by phenotypic behaviour

- **Dominant genes** — express their effect whether present on one or both members of a chromosome pair.
- **Recessive genes** — express their effect only in the homozygous state (both alleles carrying the variant).
- **Jumping genes (transposons)** — mobile genetic elements that can relocate to and fro within the same chromosome or to an adjacent one; act as a recognised class of **mutagen**, causing insertional inactivation of a gene when they (or elements such as Alu/LINE) integrate within or near it.



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- ▶ **X-linked recessive disease** — haemophilia (defective clotting factor gene): hemizygous males (X^hY) are affected; heterozygous females (X^HX^h) are carriers; homozygous females (X^hX^h) are affected only if father is haemophilic and mother a carrier. Red–green colour blindness follows an identical X-linked recessive pattern.
- ▶ **Y-linked (holandric) inheritance** — the rare, purely paternal transmission of hypertrichosis of the pinna illustrates why, for practical purposes in medicine, "sex-linked" almost always means X-linked.
- ▶ **Mitochondrial (cytoplasmic) inheritance** — disorders such as Leber's hereditary optic neuropathy and Pearson marrow–pancreas syndrome are transmitted **only from affected mothers to all their children**, never from fathers, because mitochondria are maternally derived.
- ▶ **Gene-family/pseudogene relevance to disease** — the clustered β -globin gene family is the site of the mutations producing the thalassaemias; regulatory/promoter mutations in this cluster illustrate how a "regulatory gene" defect, not just a structural-gene defect, produces disease.
- ▶ **Large split genes and disease** — the exceptionally large, intron-rich dystrophin gene (X-linked) underlies Duchenne muscular dystrophy; *BRCA1* (24 exons, autosomal dominant) predisposes to hereditary breast/ovarian cancer; *MYH7* (40 exons) mutations cause inherited hypertrophic cardiomyopathy — all classic "structural gene" examples for viva.
- ▶ **Regulatory-gene disease** — cystic fibrosis (*CFTR*, autosomal recessive) is a defect of a chloride-channel-regulating gene, illustrating disease from a regulator/structural-gene axis rather than pure Mendelian dominance.
- ▶ **Jumping genes and mutagenesis** — Alu/LINE retrotransposon insertion into a medically important gene is a recognised, if uncommon, cause of inherited disease via insertional gene inactivation.

High-yield exam pointers

- ▶ Gene = unit of heredity (Johannsen, 1909); modern molecular definition = a DNA segment specifying a functional product (protein or RNA), including its regulatory flanking sequences.
- ▶ Structure: **promoter** → 5'UTR → **exon–intron–exon...** → 3'UTR → **poly-A signal**; introns are spliced out of the mature transcript.
- ▶ Two broad molecular categories: **protein-coding genes** (~20,000–25,000) and **non-coding RNA genes** (a comparable number) — rRNA, tRNA, snRNA/snoRNA, lncRNA, miRNA.
- ▶ **Split genes** (exons + introns) are the human rule; **pseudogenes** are non-functional gene copies, processed (no introns, retrotransposed) or non-processed (in situ, decayed).
- ▶ **Gene families**: clustered (β -globin cluster, chr 11) vs dispersed (olfactory receptor genes, ~1000 members, multiple chromosomes).
- ▶ **Housekeeping genes** (constitutive, CG-rich/TATA-less promoters) vs **tissue-specific/luxury genes** (TATA/CAAT-box promoters).
- ▶ **By location**: autosomal, X-linked (haemophilia, colour blindness), Y-linked/holandric (hairy pinna — the only human example), mitochondrial (strictly maternal — LHON, Pearson syndrome).

- ▶ **Operon concept:** operator gene + structural gene = operon (classical prokaryotic regulatory unit, Jacob–Monod).
- ▶ **Jumping genes (transposons)** — mobile elements (Alu, LINE) acting as a class of mutagen via insertional inactivation.
- ▶ Dominant genes express with one copy; recessive genes require both copies (homozygosity).

SOURCE & COVERAGE

Thompson & Thompson Genetics in Medicine 8e; Vishram Singh General Anatomy 4e; Gangane Human Genetics 5e. Coverage note: this is a molecular-genetics topic outside the scope of Gray's Anatomy (Standring) 41e, the spine text for this bank, which contains no dedicated treatment of gene structure or classification. Gangane's own dedicated "Molecular Genetics" chapter (its Chapter 3) is a known gap in this library's extracted corpus — only its end-of-chapter answer key survived extraction — so gene-structure and gene-type detail is drawn primarily from Thompson & Thompson's molecular genetics chapters and from Vishram Singh's General Anatomy "Medical genetics" chapter, which carries the classical anatomy-curriculum classification of gene types.



Dermatoglyphics

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Dermatoglyphics (Greek *derma* = skin, *glyphe* = carving) literally means "writing on the skin." It is the scientific study of the **epidermal (friction) ridge patterns** on the skin of the palms, digits and soles — patterns produced by the underlying dermal papillae. The scientific basis of the subject was laid down by **Galton**, and the term "dermatoglyphics" itself was introduced by **Cummins in 1961**. Because these ridge patterns are genetically determined, established before birth and remain permanent and unique to each individual throughout life, dermatoglyphics has important applications in clinical genetics, twin studies and forensic identification.

Development of epidermal ridges

- ▶ **Primary epidermal ridges** develop as regularly spaced downgrowths of epidermal cells, separated by corresponding dermal ridges, during the **2nd and 3rd months of gestation**.
- ▶ The ridge patterns on the hands and feet begin appearing around the **13th week** and are essentially completed by the **16th week** of intrauterine life.
- ▶ In the **5th month**, **secondary ridges** develop and the pattern becomes evident on the surface; this pattern is subsequently finalised by further remodelling in the postnatal period.
- ▶ The **pattern of the underlying dermal papillae determines the early development of the epidermal ridges** — the ridges follow the interlocking arrangement of dermal papillae with the epidermal rete pegs.
- ▶ Once formed, the arrangement is **stable for life**, making it a reliable and permanent marker of individual identity.

Histological basis of the ridge pattern

- Friction-ridge (palmo-plantar) skin is **thick, hairless skin** in which the dermal papillae are large, closely aggregated, and arranged in curved parallel rows that follow the surface ridge-and-groove pattern.
- Under each epidermal surface ridge lie **two longitudinal rows of dermal papillae**, one on either side of the epidermal rete peg, and the ducts of sweat glands pass between them to reach the surface.
- **Sweat pores open along the summit (crest) of each ridge** — this is why sweat/skin secretions deposited along the ridges form the basis of latent fingerprints used in forensic analysis.
- **Meissner's corpuscles**, situated in the dermal papillae, and dense free/tactile nerve endings beneath the ridges give friction-ridge skin its high discriminative tactile sensitivity.
- Functionally, the ridges **increase grip and prevent slippage** of the hands and feet during grasping and weight-bearing.

Classification of dermatoglyphic patterns

Fingerprint ridge patterns were classified by **Galton** on the basis of the number of **tri-radial** (junctional points where three sets of parallel ridges meet) into three basic types, with an additional composite/combination pattern:

Pattern	Tri-radial	Approx. frequency	Features
Arch	0	~5%	Ridges cross the pad transversely without recurving; simplest pattern; ridge count = 0
Loop	1	~70%	Ridges recurve upon themselves and exit on the same side they entered; subclassified as radial or ulnar loop depending on whether the loop opens towards the radial or ulnar side of the digit
Whorl	2 or more	~25%	Ridges form concentric circles or spirals; whorls are more common on the right hand , and males have more whorls and fewer arches than females
Composite	variable	uncommon	A single digit shows a combination of the above basic patterns

- **Ridge count** expresses the size of a finger pattern — the number of ridges counted between the tri-radius and the core of the pattern. The count of an arch is zero (no tri-radius). The sum of the

ridge counts of all ten fingers is the **total ridge count (TRC)**, which is inherited as a **multifactorial (polygenic) trait**.

□ Fig 5.5 — Schematic diagram of epidermal/friction ridges and the four types of fingerprints (Vishram Singh — *General Anatomy* — 4e, p.186)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw (A) a schematic cross-section of thick palmar skin showing the epidermis, dermal papillae, rete pegs and a sweat duct opening on the ridge crest; and (B) the four basic fingerprint patterns side by side, labelled:

- ▶ **Arch** — no tri-radius
- ▶ **Loop** — one tri-radius; mark radial/ulnar direction of opening
- ▶ **Whorl** — two tri-radial; concentric/spiral ridges
- ▶ **Composite** — combination pattern

Also sketch the palmar diagram with: four **digital tri-radial** (a, b, c, d), the **axial tri-radius** (t), the **atd angle**, thenar and hypothenar areas, and the **simian crease** and **Sydney line** as labelled flexion creases.

Palmar and plantar dermatoglyphics

Feature	Palmar pattern	Plantar pattern
Tri-radial mapped	Four digital tri-radial (a, b, c, d) at the base of digits II–V, plus one axial tri-radius (t) near the proximal palm	Less extensively studied than the palm
Key measurement	atd angle — angle subtended at the axial tri-radius by lines to the "a" and "d" digital tri-radial; used to record axial tri-radius displacement (as a fraction of palm length or as the atd angle)	Tibial arch pattern in the hallual (great toe) area
Other regional patterns	Interdigital recurving-ridge patterns between the digital tri-radial; thenar and hypothenar patterns may also be present	—
Flexion creases	"Heart," "head" and "life" lines (of palmistry) develop during the same period as the dermal ridges; simian crease = a single transverse palmar crease; Sydney line = a proximal palmar crease variant with the distal crease also present	—

Feature	Palmar pattern	Plantar pattern
Association with trisomy	Axial tri-radius is displaced distally (t') in Down syndrome and in trisomy 13; simian crease is seen in ~50% of Down syndrome patients (also in ~1% of normal individuals); Sydney line is seen in ~10% of normal individuals	Tibial arch pattern of the sole is seen in ~50% of Down syndrome patients

Applied / Clinical anatomy

- ▶ **Down syndrome (trisomy 21)** — the single most important dermatoglyphic association: a distally displaced axial tri-radius, a single transverse palmar (simian) crease in about half of cases, and a tibial arch pattern in the hallual area of the sole in about half of cases. A distally-placed axial tri-radius is also seen in **trisomy 13 (Patau syndrome)**.
- ▶ **Twin zygosity** — dermatoglyphic (fingerprint) patterns, together with other markers such as eye colour and the examination of fetal membranes/placenta, are used as supportive evidence in determining twin zygosity, since ridge patterns are strongly genetically influenced.
- ▶ **Forensic identification / criminology** — because the ridge pattern is unique to each individual and permanent for life, dermatoglyphic (fingerprint) analysis is used for personal identification in criminology and medico-legal practice. Sweat glands discharge along the crest of the friction ridges, and this sweat/secretion residue is the physical basis of a **latent fingerprint** lifted at a crime scene.
- ▶ **Adermatoglyphia** — an extremely rare **autosomal dominant** condition, caused by mutation of the **SMARCAD1** gene, in which fingerprint ridges are congenitally absent. Reduced or absent dermatoglyphics may also occur as a feature of other genetic disorders.
- ▶ **Genetic screening** — as a simple, non-invasive supportive investigation, dermatoglyphic analysis (axial tri-radius displacement, simian crease, ridge counts) contributes corroborative evidence in the work-up of suspected chromosomal disorders, alongside karyotyping.

High-yield exam pointers

- ▶ Dermatoglyphics = "writing on the skin"; scientific basis by **Galton**, term coined by **Cummins (1961)**.
- ▶ Three basic Galton patterns by tri-radii count: **Arch (0, ~5%)**, **Loop (1, ~70%)**, **Whorl (≥ 2 , ~25%)**; a **composite** pattern also occurs.
- ▶ Ridge pattern is laid down by **primary ridges (2nd–3rd month)** and **secondary ridges (5th month)**, complete by the **16th week**, and remains **permanent for life**.
- ▶ **Total ridge count (TRC)** = sum of ridge counts of all 10 digits; inherited as a **multifactorial trait**.
- ▶ Palmar landmarks: **4 digital tri-radii (a–d) + 1 axial tri-radius (t)**; **atd angle** quantifies axial tri-radius position.
- ▶ **Simian crease** and distally-displaced axial tri-radius (t') and **tibial arch of the sole** are classical findings in **Down syndrome (trisomy 21)**.
- ▶ **Sydney line** = proximal palmar crease variant, seen in ~10% of normal individuals.

- ▶ **Adermatoglyphia** — rare autosomal dominant, **SMARCAD1** gene mutation, absent fingerprints.
- ▶ Applications: supportive diagnosis of chromosomal disorders, **twin-zygosity** determination, and forensic/criminal identification.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Gangane — Human Genetics 5e; Vishram Singh — General Anatomy 4e; Vishram Singh — Textbook of Clinical Embryology; Vishram Singh — Textbook of Anatomy Vol 1 4e.



Karyotyping

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction **Karyotyping** is the laboratory technique of arresting dividing cells at metaphase, staining and photographing their chromosomes, and then arranging the chromosomes of that cell in a standard, ordered manner (by size, centromere position and banding pattern) to study the complete chromosomal complement of an individual. The resulting systematic array is called the **karyotype**; the term is also used for the photomicrograph itself. Karyotyping is the fundamental technique of clinical and applied cytogenetics — it identifies and numbers every chromosome of a cell and detects **numerical** (aneuploidy) and **structural** (deletion, duplication, translocation, inversion, ring formation) chromosomal abnormalities. It therefore forms the core practical exercise of "Principles of Microscopy/Cytogenetics" in the anatomy curriculum.

1. Chromosome morphology — the structural basis of karyotyping

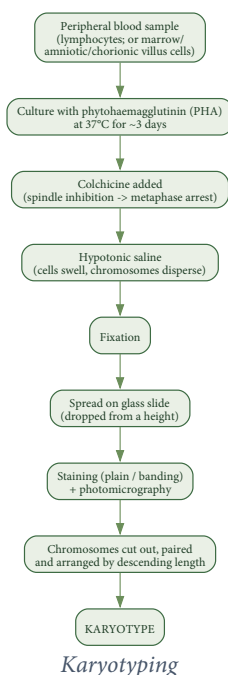
- ▶ A metaphase chromosome consists of two identical sister **chromatids** held together at a narrowed **primary constriction** or **centromere**; the mitotic spindle attaches here through a specialised region, the **kinetochore**.
- ▶ Each chromatid has two arms flanking the centromere — the short arm (**p**, French *petite*) and the long arm (**q**).
- ▶ Some chromosomes show an additional **secondary constriction**; the segment distal to it is called the **satellite**.
- ▶ Each arm ends in a **telomere** — a zone of repetitive nucleotide sequence (TTAGGG in vertebrates, repeated ~2500 times in humans) that caps and stabilises the chromosome end.
- ▶ Based on the **position of the centromere**, chromosomes are grouped morphologically into four types:

Type	Centromere position	Arms
Metacentric	At or near the centre	Both arms almost equal in length
Submetacentric	Between centre and end	One short arm, one long arm
Acrocentric	Near one end	One very short arm, one very long arm

Type	Centromere position	Arms
Telocentric	At the extreme end	Single arm only (not seen in normal human chromosomes)

2. Technique of karyotyping

- ▶ **Cell source:** the commonest source is **peripheral blood lymphocytes**; bone marrow cells, skin fibroblasts, amniotic-fluid cells and chorionic villi are used for specialised (marrow/prenatal) karyotyping.
- ▶ **Steps** (peripheral blood karyotyping):
- ▶ About 5 mL of venous blood is treated with **phytohaemagglutinin (PHA)**, a mitogen that stimulates the lymphocytes to divide, and cultured at 37 °C for **about 3 days**.
- ▶ **Colchicine** is added to arrest the dividing cells in **metaphase** by inhibiting spindle-fibre (microtubule) formation.
- ▶ The cells are treated with **hypotonic saline**, which makes them swell and disperses the chromosomes.
- ▶ The cells are **fixed**, then **spread** on a glass slide (classically by dropping the fixed-cell suspension from a height so the chromosomes splay apart).
- ▶ The metaphase spread is **stained** (plain or banding stains) and **photomicrographed**.
- ▶ From the photomicrograph, individual chromosomes are cut out, paired with their homologues, and **arranged in descending order of length** to construct the karyotype; computerised image-analysis systems now automate most of these manual steps.
- ▶ Chromosome analysis can also be performed by **banding techniques** and by **fluorescence in situ hybridisation (FISH)**, which bypasses the need for a dividing-cell culture.



3. Classification of the karyotype — Denver system and banding nomenclature

- The **Denver system of classification** (proposed at a Denver, Colorado, convention in 1960; accepted at London, 1963) arranges the 46 human chromosomes into **23 pairs**, ordered by **decreasing total length** and **centromere position**, into **seven groups labelled A to G**. The X chromosome (resembling chromosome 6) is placed in group C, and the Y chromosome (resembling chromosome 21) is placed in group G.

Group	Chromosome numbers	Morphology
A	1, 2, 3	Large, mainly metacentric
B	4, 5	Large, submetacentric
C	6–12 + X	Medium, mostly submetacentric
D	13, 14, 15	Medium, acrocentric, satellites on short arms
E	16, 17, 18	Small, submetacentric (16 metacentric)
F	19, 20	Short, metacentric
G	21, 22 + Y	Very short, acrocentric, satellites on 21 and 22

- The Denver system alone could not identify subtler alterations (deletions, translocations, malpositions), so the **Paris nomenclature (1971)** incorporated **banding** data: each chromosome arm is divided into numbered regions (1, 2, 3...) from the centromere outward, each subdivided into numbered bands — allowing precise localisation (e.g., 5p15, 7q21).
- In 1978 these were unified into the **International System for human Cytogenetic Nomenclature (ISCN)**, which is periodically updated and is the system used today.

□ Fig 17.7 — Karyotype of a normal male. (Vishram Singh — *General Anatomy* — 4e, p.695)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the karyotype of a normal male (46,XY) as a grid of 22 autosome pairs + XY, seven groups left to right:

- ▶ **Group A** (1–3, largest, metacentric) — **Group B** (4–5, large, submetacentric) — **Group C** (6–12 + X, medium, submetacentric) — **Group D** (13–15, medium, acrocentric, satellite on short arm) — **Group E** (16–18, small, submetacentric/16 metacentric) — **Group F** (19–20, short, metacentric) — **Group G** (21–22 + Y, shortest, acrocentric, satellites on 21/22).
- ▶ Label on one representative chromosome: **centromere**, **p arm** (short), **q arm** (long), **satellite**, **secondary constriction**, **telomere**.
- ▶ Caption the sex-chromosome pair distinctly (X in group C, Y in group G) to show 46,XY.

4. Chromosome banding techniques

Banding	Method	Bands seen
Q-banding	Chromosomes stained with quinacrine dyes; fluoresce under UV light	Q bands
G-banding	Chromosomes treated with trypsin, then Giemsa stain (the standard, most widely used clinical method)	G bands (dark/light alternating)
R-banding	Reverse of the Giemsa method (French technique)	R bands (reverse pattern of G bands)
C-banding	Demonstrates constitutive heterochromatin at the centromere	Prominent on chromosomes 1, 9, 16 and the long arm of Y

- ▶ G-banding has been the **gold standard** for detecting structural and numerical genomic abnormalities in both **constitutional (postnatal/prenatal)** and **acquired (cancer)** cytogenetic disorders.
- ▶ A routine metaphase G-banded karyotype resolves roughly **200–550 bands** (quoted variably across sources) and detects deletions/duplications larger than about **5–10 Mb**.
- ▶ **High-resolution (prometaphase) banding** — staining chromosomes at an earlier, less-condensed stage of mitosis — increases resolution to about **800–1400 bands**, revealing minor structural alterations missed on routine preparations.

5. Uses / indications of karyotyping

- ▶ **Confirming the clinical diagnosis** of a suspected chromosomal syndrome (dysmorphism, intersex states, unexplained intellectual disability).
- ▶ **Prenatal diagnosis** of genetic disease — chromosome analysis of cells obtained by **amniocentesis**, **chorionic villus biopsy**, or **fetal blood sampling**.
- ▶ **Gene mapping** — localising genes to specific chromosomal regions.

- **Prognosis of disease** — e.g., karyotyping in chronic myelogenous leukaemia, where an acquired chromosomal rearrangement carries prognostic significance.
- Detecting **numerical abnormalities** (trisomy, monosomy, polyploidy) and **structural abnormalities** (deletion, duplication, translocation, inversion, ring chromosome, isochromosome).
- Correlating with **sex chromatin (Barr body)** studies to confirm the number of X chromosomes.

6. Chromosomal disorders diagnosed by karyotyping

Syndrome	Karyotype	Key features
Down syndrome	Trisomy 21	Hypotonia, stocky build, slanting eyes, moderate intellectual disability
Edwards syndrome	Trisomy 18	Motor/renal/cardiac defects, characteristic clenched, overlapping fingers
Patau syndrome	Trisomy 13	Similar to Edwards but without the folded-finger sign
Cri-du-chat syndrome	5p deletion (5p–)	Cat-like cry, microcephaly, wide-set eyes
Jacobsen syndrome	Terminal 11q deletion	Variable physical/mental abnormalities
Wolf-Hirschhorn syndrome	4p deletion (4p–)	Growth retardation, facial changes, seizures
Klinefelter syndrome	47,XXY	Tall, infertile male, gynaecomastia, learning difficulty
Turner syndrome	45,X	Short stature, webbed neck, streak gonads, primary amenorrhoea
XYY syndrome	47,XYY	Tall, mild learning/behavioural issues
XXX syndrome	47,XXX	Tall, mild learning difficulty; usually fertile

Applied / Clinical anatomy

- **Sex chromatin / Barr body**: an inactivated, condensed X chromosome seen as a deeply staining body at the nuclear membrane in interphase cells of females (studied in a buccal smear; seen as a drumstick appendage in neutrophils). Per the **Lyon hypothesis (Mary Lyon, 1962)**, only one X chromosome is active per somatic cell in females, and the number of Barr bodies is always **one less than the total number of X chromosomes** — e.g., normal female (46,XX) shows 1 Barr body, normal male (46,XY) shows none, Klinefelter (47,XXY) shows 1, and 47,XXX shows 2. Barr-body screening is a quick adjunct to, but not a substitute for, full karyotyping.

- ▶ **Prenatal cytogenetic sampling:** amniocentesis (mid-trimester amniotic fluid, cultured for fetal karyotype), chorionic villus biopsy (earlier, first-trimester villus sampling), and fetal blood sampling all feed into karyotyping/molecular cytogenetic analysis for antenatal detection of aneuploidy and structural rearrangements.
- ▶ **Molecular cytogenetics: fluorescence in situ hybridisation (FISH)** uses fluorescent DNA probes to detect a chromosome/region directly in interphase or metaphase nuclei without needing a full dividing-cell culture, and is increasingly used alongside or in place of conventional G-banded karyotyping for rapid aneuploidy screening and detection of submicroscopic rearrangements.
- ▶ **Oncological use:** acquired chromosomal rearrangements detected by karyotyping/banding are used for diagnosis and prognostication in haematological malignancies (e.g., chronic myelogenous leukaemia).

High-yield exam pointers

- ▶ Karyotyping = culture → colchicine (metaphase arrest) → hypotonic saline → fixation → spread → stain → photomicrograph → arrange by length.
- ▶ Commonest cell source: **peripheral blood lymphocytes**, stimulated with PHA, cultured 37 °C × 3 days.
- ▶ **Denver system (1960):** 46 chromosomes → 23 pairs → 7 groups A–G by length and centromere position.
- ▶ Four centromere-based morphological types: **metacentric**, **submetacentric**, **acrocentric**, **telocentric** (telocentric absent in humans).
- ▶ Banding: **Q (quinacrine)**, **G (trypsin+Giemsa — gold standard)**, **R (reverse Giemsa)**, **C (centromeric heterochromatin)**; Paris nomenclature/ISCN localises bands by region and number.
- ▶ Resolution: routine ~300–550 bands (detects >5–10 Mb changes); high-resolution/prometaphase banding ~800–1400 bands.
- ▶ Uses: diagnosis of chromosomal syndromes, **prenatal diagnosis**, gene mapping, disease prognosis (e.g., CML).
- ▶ **Barr body count = number of X chromosomes – 1** (Lyon hypothesis, 1962).
- ▶ Karyotyping detects both **numerical** (trisomy/monosomy) and **structural** (deletion/translocation/inversion/ring) chromosomal abnormalities — e.g., Down (trisomy 21), Turner (45,X), Klinefelter (47,XXY).

SOURCE & COVERAGE

IB Singh Textbook of Anatomy Vol 3 (6e); Vishram Singh General Anatomy 4e; Vishram Singh Textbook of Clinical Embryology; Thompson & Thompson Genetics in Medicine 8e; Gangane Human Genetics 5e; Junqueira's Basic Histology 17e; Ross Histology 7e. Coverage note: Gray's Anatomy (Standring) 41e carries no cytogenetic-technique content; per this topic's note, grounding was drawn from the genetics/anatomy texts that carry the "Medical Genetics"/"Chromosomes" chapters, consistent with the Paper III "Principles of Microscopy" scope.



Write short notes on Philadelphia chromosome

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction

The Philadelphia (Ph1) chromosome is an abnormally short derivative chromosome 22 produced by a reciprocal translocation between the long arms of chromosomes 9 and 22 — $t(9;22)(q34;q11.2)$ — first described by Peter Nowell and David Hungerford in Philadelphia in 1960 in patients with chronic myeloid leukaemia (CML). The translocation juxtaposes the *ABL1* proto-oncogene (from chromosome 9) next to the *BCR* gene (on chromosome 22), creating the chimeric *BCR-ABL1* fusion gene whose constitutively active tyrosine-kinase product drives unregulated clonal proliferation of a haemopoietic precursor. It is the cytogenetic hallmark of CML and is also found in a subset of acute lymphoblastic leukaemia (ALL), making it both the first chromosomal abnormality ever linked consistently to a human malignancy and a landmark example bridging classical karyotyping/FISH cytogenetics with targeted molecular therapy.

Historical background

- ▶ **1960** — Peter C. Nowell (pathologist) and David A. Hungerford (graduate student), working in Philadelphia, identified an abnormally small chromosome in the leucocytes of patients with CML on routine chromosome analysis.
- ▶ It was named the **Philadelphia chromosome (Ph1)** after the city of discovery — the **first chromosomal abnormality shown to be consistently associated with a specific human cancer**, opening a new page in cancer cytogenetics.
- ▶ Later chromosome-banding studies established that the apparent shortening of chromosome 22 was not a simple deletion but the product of a **reciprocal translocation** — a small segment exchanged between chromosomes 9 and 22.
- ▶ The molecular basis (fusion of *ABL1* and *BCR*) was worked out subsequently, founding the modern discipline of cancer molecular genetics and, eventually, the first successful molecularly-targeted cancer drug (imatinib).

Cytogenetic basis — formation of the Philadelphia chromosome

- ▶ **Mechanism:** a reciprocal translocation between the long arm of chromosome 9 (band **q34**) and the long arm of chromosome 22 (band **q11.2**) — written **t(9;22)(q34;q11.2)**.
- ▶ Two derivative chromosomes result:
 - **der(9)** — chromosome 9 lengthened by the small segment received from chromosome 22 (usually functionally silent).
 - **der(22)** — chromosome 22 shortened because it has lost a segment to chromosome 9; this shortened chromosome is the Philadelphia chromosome (Ph1, also written **22q-**).
- ▶ The breakpoint on chromosome 9 lies within **intron 1 of ABL1**; the breakpoint on chromosome 22 falls within one of three breakpoint-cluster regions of BCR*. The two gene fragments are joined head-to-tail on der(22).
- ▶ On karyotyping (G-banded metaphase spread), Ph1 is recognised as an abnormally small chromosome 22.

□ Fig 10.3 — Formation of Philadelphia chromosome involves reciprocal translocation (Gangane — Human Genetics — 5e, p.187)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

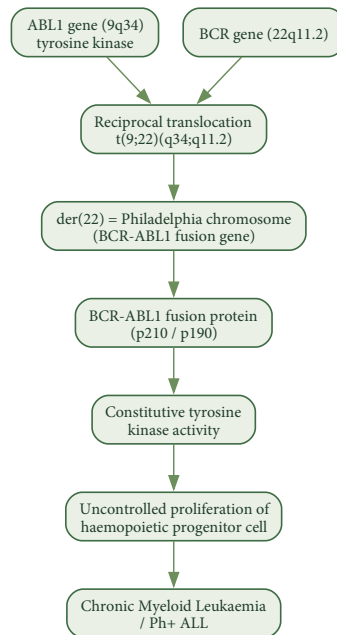
Draw the reciprocal translocation as a paired-chromosome scheme with these exact labels:

- ▶ Normal **chromosome 9**, band **9q34** marked, showing the **ABL1** locus.
- ▶ Normal **chromosome 22**, band **22q11.2** marked, showing the **BCR** locus.
- ▶ Breakpoint arrows on both chromosomes with a **reciprocal exchange (crossed arrows)** between the two long-arm segments.
- ▶ Resulting **der(9)** — chromosome 9 elongated by the segment received from 22.
- ▶ Resulting **der(22) = Philadelphia chromosome (Ph1, "22q-")** — chromosome 22 shortened, with the ***BCR-ABL1 fusion gene*** labelled at the junction point.
- ▶ A bracket/label reading **"t(9;22)(q34;q11.2)"** under the pair.

Molecular genetics — the BCR-ABL1 fusion gene and its protein product

- ▶ **ABL1 (Abelson murine-leukaemia-virus homologue)* — locus **9q34** — a *non-receptor tyrosine kinase**; the normal protein participates in the cell cycle, stress responses, integrin signalling and neural development.
- ▶ **BCR (breakpoint cluster region)* — locus **22q11*** — encodes a phosphoprotein with a coiled-coil dimerisation motif, a serine-threonine kinase domain, a GDP-GTP exchange domain, and a GTPase-activating domain.
- ▶ Fusion of the two gene fragments on der(22) generates a **giant chimeric BCR-ABL1 mRNA, spliced and translated into a BCR-ABL1 fusion protein*. The classic CML transcript retains roughly 900 amino acids of BCR fused to about 1100 amino acids of ABL1 (the larger "p210" fusion protein); a shorter BCR segment gives the smaller "p190*" variant typically seen in Ph-positive ALL.
- ▶ Unlike native ABL1, the fusion protein shows **constitutive (ligand-independent) tyrosine-kinase activity** and remains confined to the cytoplasm, where it binds actin microfilaments.

- It **phosphorylates multiple cytoplasmic substrates**, switching on signalling cascades that control growth, differentiation and adhesion of haemopoietic cells — the molecular basis of unregulated clonal proliferation.



Write short notes on Philadelphia chromosome

Laboratory identification (cytogenetic and molecular techniques)

Technique	Principle	Finding in Ph-positive leukaemia	Remarks
Karyotyping (conventional cytogenetics, G-banding)	Metaphase spread prepared from a cultured bone-marrow/peripheral-blood sample, banded and analysed under the microscope	Direct visualisation of the shortened chromosome 22 (Ph1)	Gold-standard, demonstrates the whole karyotype and any additional clonal changes; needs dividing cells and takes days
FISH — dual-colour, dual-fusion (D-FISH)	Differently fluorescently-labelled probes for <i>BCR</i> and <i>ABL1</i> co-localise on der(22), producing a yellow fusion signal	Fusion (yellow) signal confirms <i>BCR-ABL1</i> juxtaposition; can be done on interphase nuclei	Bypasses cell culture, usable on archival/paraffin material, detects cryptic or masked translocations missed by banding; D-FISH specifically reduces false-negative results and is the method of choice for demonstrating <i>BCR-ABL1</i> fusion

Technique	Principle	Finding in Ph-positive leukaemia	Remarks
RT-PCR / quantitative PCR	Amplifies and quantifies the <i>BCR-ABL1</i> fusion transcript	Confirms the specific transcript (p210/p190) and its quantity	Most sensitive method; used to monitor molecular response to therapy and detect minimal residual disease

Disease associations and frequency

Disease	Frequency of Ph chromosome / t(9;22)	Usual fusion transcript
Chronic myeloid leukaemia (CML)	~90–95% of cases	<i>BCR-ABL1</i> (p210)
Acute lymphoblastic leukaemia (ALL), adults	~10–15% of cases	<i>BCR-ABL1</i> (typically p190)
Acute myeloid leukaemia	Occasionally (rare)	<i>BCR-ABL1</i>

- ▶ CML evolves through a **biphasic/triphasic course**: an indolent **chronic phase** → an **accelerated phase** → **blast crisis** (an acute-leukaemia-like, rapidly fatal phase). Progression is accompanied, in 50–80% of cases, by **additional chromosomal changes** — trisomy 8, isochromosome 17q, trisomy 19, or a second Philadelphia chromosome.
- ▶ A minority (~5%) of clinically typical CML cases lack a visible Ph1 on karyotyping but still carry the *BCR-ABL1* fusion by FISH/PCR ("Ph-negative, BCR-ABL1-positive" — complex/variant/cryptic translocations).

Applied / Clinical anatomy

- ▶ **Clinical presentation**: middle-to-late adulthood; insidious fatigue, malaise, weight loss and splenomegaly (palpable spleen tip); peripheral smear shows leucocytosis with a full spectrum of maturing granulocytes and basophilia; bone marrow is hypercellular with an increased myeloid : erythroid ratio.
- ▶ **Epidemiology**: CML accounts for about 15% of adult leukaemias; incidence 1–2 per 100,000, with a higher age-adjusted incidence in men than women (≈1.3–1.7 : 1.0).
- ▶ **Prognostic point**: Ph-negative CML responds poorly to chemotherapy compared with Ph-positive disease — the Ph chromosome is thought to be a marker of, rather than the sole driver behind, disease behaviour.
- ▶ **Targeted therapy**: elucidation of the *BCR-ABL1* tyrosine-kinase mechanism led to **imatinib mesylate**, a tyrosine-kinase inhibitor and now first-line CML therapy — more than 85% of patients achieve a cytogenetic response (disappearance of t(9;22) on marrow karyotype). **Allogeneic bone-marrow transplantation** remains the only curative option, with the best results (~80% success) in patients under 40 years with an HLA-matched sibling donor; post-transplant surveillance for relapse uses RT-PCR for *BCR-ABL1* transcripts.

- ▶ **Inheritance:** the Ph chromosome/*BCR-ABL1* fusion is an **acquired (somatic) mutation** confined to the malignant clone — it is not present in the germline, so there is no risk of transmission to offspring.
- ▶ **Relevance to anatomical microscopy/cytogenetics:** Ph1 is a teaching example of how a structural chromosomal rearrangement, first suspected on light-microscopic karyotyping (G-banding of a metaphase spread), is confirmed and refined by FISH and molecular techniques — linking classical cytogenetic microscopy to modern molecular cytogenetics.

High-yield exam pointers

- ▶ Ph1 = **der(22)t(9;22)(q34;q11.2)** — a reciprocal translocation, not a simple deletion.
- ▶ First described by **Nowell and Hungerford in 1960** in Philadelphia — the first chromosomal abnormality linked consistently to a specific human cancer.
- ▶ Fuses ***ABL1** (*9q34, tyrosine kinase*) with **BCR** (*22q11*) → **chimeric BCR-ABL1*** gene → constitutively active tyrosine kinase.
- ▶ Present in **~90–95% of CML** and **~10–15% of adult ALL**; rarely in AML.
- ▶ Detected by **karyotyping** (G-banding, **direct visualisation of the small 22q– chromosome**), **FISH** (D-FISH, dual-fusion probes — works on interphase cells, **most rapid/sensitive for cryptic cases**), and **RT-PCR** (**quantifies the transcript, used for monitoring residual disease**).
- ▶ **Ph-negative CML** has a poorer response to chemotherapy than Ph-positive CML.
- ▶ Purely a **somatic (acquired)** abnormality — not heritable.
- ▶ Targeted therapy: **imatinib mesylate** (a **BCR-ABL1 tyrosine-kinase inhibitor**); **allogeneic bone-marrow transplant** is the only curative modality.
- ▶ CML runs a **triphasic course**: chronic phase → accelerated phase → blast crisis.

SOURCE & COVERAGE

Gangane — Human Genetics 5e; Thompson & Thompson — Genetics in Medicine 8e; Vishram Singh — General Anatomy 4e; Pranab Dey — Lab Techniques in Histopathology & Cytology 2e (FISH/D-FISH technique).



General histology (basic tissues)



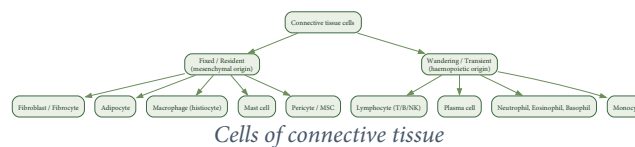
Cells of connective tissue

Asked in: Paper III May 2018 · Paper III Oct 2010 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — Connective tissue is composed of cells embedded in an extracellular matrix (fibres + ground substance) that it largely secretes. Its cells fall into two functional groups: **fixed (resident) cells**, of mesenchymal origin, which are permanent residents synthesising and maintaining the matrix; and **wandering (transient) cells**, of haemopoietic origin, which migrate in from the blood via venules to perform short-term defensive/immune functions and then die by apoptosis. This classification, together with the structure and function of each cell type, is high-yield for both gross/general anatomy and histology papers.

1. Classification of connective tissue cells

Fixed / resident cells (mesenchymal origin)	Wandering / transient cells (haemopoietic origin)
Fibroblast / fibrocyte, myofibroblast	Lymphocytes (T, B, NK)
Adipocyte (unilocular, multilocular)	Plasma cells
Macrophage (histiocyte)	Neutrophils
Mast cell	Eosinophils
Pericyte / mesenchymal (adult) stem cell	Basophils
Pigment cell (melanocyte)	Monocytes



□ Fig 7.10 — Cells of connective tissue. (Sontakke — Textbook of Human Histology — 2e, p.83)

2. Fibroblast and myofibroblast

- **Fibroblast** — the most numerous resident cell; arises locally from mesenchymal stem cells; spindle-shaped/fusiform with tapering eosinophilic cytoplasmic processes that blend with collagen bundles in H&E sections, making the cell body hard to distinguish from adjacent fibres.
- **Active fibroblast**: large euchromatic nucleus with prominent nucleolus, abundant basophilic cytoplasm rich in rough endoplasmic reticulum (rER) and a well-developed Golgi — reflects intense synthetic activity.
- **Quiescent fibroblast (fibrocyte)**: smaller, fewer organelles, flattened heterochromatic nucleus.
- **Function** — synthesises and secretes **collagen, elastin, reticular fibres**, and the GAGs/proteoglycans/multiadhesive glycoproteins of ground substance; a single fibroblast can produce all ECM components.
- In highly cellular organs (liver, spleen, lymphoid tissue, bone marrow) fibroblasts associated with type III collagen (reticular fibres) are called **reticular cells**.

- ▶ Rarely divide in adult tissue but re-enter the cell cycle under locally released growth factors (e.g., during repair).
- ▶ **Myofibroblast** — a specialised contractile fibroblast induced by TGF- β 1, expressing α -smooth muscle actin (α -SMA) and non-muscle myosin; resembles smooth muscle but, unlike it, lacks a surrounding basal lamina. Its actin bundles anchor to the plasma membrane at a site called the **fibronexus** (a cell-ECM anchoring junction). Responsible for **wound contraction** in granulation tissue; most undergo apoptosis once the wound closes.

3. Adipocyte (fat cell)

- ▶ Derived from mesenchymal stem cells; large cells specialised to store neutral fat or (less commonly) to generate heat.
- ▶ **Unilocular (white) adipocyte**: single large central lipid globule pushing a flattened, eccentric nucleus and thin cytoplasmic rim to the periphery — the "**signet-ring**" appearance seen after routine processing dissolves the lipid. Function: energy storage, cushioning, thermal insulation; forms the bulk of adult adipose tissue.
- ▶ **Multilocular (brown) adipocyte**: multiple smaller fat droplets, centrally placed round nucleus, numerous mitochondria containing the uncoupling protein **UCP1**, which uncouples oxidative phosphorylation for non-shivering **thermogenesis**. Classically interscapular in neonates; residual UCP1-positive ("beige") deposits are demonstrable in adults in the supraclavicular/neck/paravertebral regions on PET scanning.
- ▶ Also functions as an endocrine organ, secreting **adipokines**.

4. Macrophage (histiocyte) and the mononuclear phagocyte system

- ▶ Derived from circulating **monocytes** (themselves from bone-marrow haemopoietic stem cells) that cross venular walls and differentiate in the tissue; part of the **mononuclear phagocyte system (MPS)**.
- ▶ Structure: 10–30 μ m, irregular/kidney-shaped, heterochromatic nucleus with a prominent nucleolus; abundant lysosomes give a foamy cytoplasm; surface shows folds and finger-like projections (phagocytic pseudopodia) on EM.
- ▶ Functions: **phagocytosis** of bacteria, debris and apoptotic cells; **antigen presentation** via MHC-II to CD4 T-helper cells; **secretion** of cytokines, growth factors and ECM-degrading enzymes.
- ▶ Occur as **fixed** (attached to fibres) or **wandering/motile** forms; both are interconvertible.
- ▶ Can fuse on heavy antigenic load into multinucleated **Langhans/foreign-body giant cells** (up to ~100 nuclei).
- ▶ **Activation states**: **M1 (classically activated)** by IFN- γ /TNF- α /LPS — pro-inflammatory, microbicidal (NO), secretes IL-12; **M2 (alternatively activated)** by IL-4/5/10/13 — anti-inflammatory, promotes angiogenesis (VEGF) and ECM/tissue repair.

Site	Name of resident MPS cell
Connective tissue	Macrophage / histiocyte
Liver	Kupffer cell

Site	Name of resident MPS cell
Bone	Osteoclast
Skin (epidermis)	Langerhans cell
CNS	Microglia
Lung (alveoli)	Alveolar macrophage / dust cell
Placenta	Hofbauer cell
Lymph node / spleen	Dendritic cell

5. Mast cell

- ▶ Bone-marrow derived; circulates transiently as an agranular monocytoïd precursor, then matures after entering connective tissue, characteristically **perivascular** (also mucosal, e.g. gut/respiratory lamina propria).
- ▶ Structure: large, oval, ~20–30 µm, small central nucleus obscured by numerous cytoplasmic granules (0.3–2 µm); surface bears irregular microvilli (filopodia) and high-affinity **FcεRI** receptors for IgE.
- ▶ Granules are **metachromatic** (stain purple/red rather than blue with toluidine blue/alcan blue) because of highly sulfated heparin/GAGs; special fixatives (glutaraldehyde) are needed to preserve them for demonstration.
- ▶ **Preformed mediators**: heparin (anticoagulant), histamine (↑ vascular permeability, smooth-muscle contraction), neutral (serine) proteases (tryptase, chymase), eosinophil/neutrophil chemotactic factors.
- ▶ **Newly synthesised mediators** (from membrane phospholipids via phospholipase): leukotrienes and prostaglandin D₂.
- ▶ Two human subtypes by protease content: **MC_εT (tryptase + chymase)** — skin, mucosa, breast, axillary nodes; **MCT (tryptase only)** — lung, intestinal mucosa.
- ▶ IgE cross-linking on re-exposure to antigen triggers **degranulation** — the basis of immediate (Type I) hypersensitivity and anaphylaxis.
- ▶ Related to, but a separate lineage from, circulating basophils. Notably **absent from brain, spinal cord and spleen**.

6. Plasma cell and other wandering leukocytes

- ▶ **Plasma cell** — terminally differentiated from **B lymphocytes**; large, ovoid, basophilic cytoplasm rich in rER (site of immunoglobulin synthesis) with a pale-staining perinuclear Golgi "hof"; round, **eccentric nucleus** with peripheral heterochromatin clumps giving a characteristic "**clock-face**" / "**cart-wheel**" appearance.
- ▶ Function: synthesis and secretion of antibodies (immunoglobulins); short-lived, average lifespan **10–20 days**.
- ▶ Excess stored immunoglobulin may accumulate as **Russell bodies** (PAS-positive, dilated-rER inclusions); a plasma cell containing several such bodies is called a **Mott cell**.

- ▶ **Lymphocytes**: smallest wandering cells, thin cytoplasmic rim around a deeply heterochromatic nucleus; classified by CD-marker profile into **T cells** (cell-mediated immunity), **B cells** (humoral immunity, precursors of plasma cells) and **NK cells** (cytotoxic, non-antigen-specific).
- ▶ **Neutrophils, eosinophils, basophils, monocytes** — granulocytic/monocytic leukocytes that migrate into connective tissue chiefly during inflammation and typically survive only hours to days before undergoing apoptosis.
- ▶ **Pigment cells (melanocytes)** — neural-crest derived, star-shaped with long processes, synthesise melanin within membrane-bound melanosomes; found in skin, iris and choroid; protect deeper tissue from UV damage.
- ▶ **Pericytes / mesenchymal (adult) stem cells** — perivascular cells around capillaries/venules, embedded in a basal lamina continuous with that of the endothelium; retain multipotency (can give rise to fibroblasts, osteoblasts, chondrocytes, adipocytes) and are an important source of new myofibroblasts/fibroblasts in wound repair.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled composite of a loose connective-tissue field showing all the cell types together (as in the embedded plate above):

- ▶ **Fibroblast** (spindle-shaped, branching processes) → **Fibrocyte** (smaller, flattened)
- ▶ **Adipocyte** (signet-ring, single lipid vacuole)
- ▶ **Macrophage** (irregular outline, kidney-shaped nucleus, vacuolated cytoplasm)
- ▶ **Mast cell** (oval, granule-packed cytoplasm, central nucleus)
- ▶ **Plasma cell** (eccentric clock-face nucleus, perinuclear Golgi hof)
- ▶ **Neutrophil** (multilobed nucleus), **Eosinophil** (bilobed nucleus, granules), **Basophil** (granule-obscured nucleus)
- ▶ **Large and small lymphocyte** (round heterochromatic nucleus, thin cytoplasmic rim)
- ▶ Background: collagen fibre bundles + a small blood vessel (source of the wandering cells)

Applied / Clinical anatomy

- ▶ **Wound healing** — fibroblasts and myofibroblasts populate granulation tissue; myofibroblast-mediated wound contraction, if excessive/persistent, produces **hypertrophic scars** or **keloids**; pathological persistence and proliferation of myofibroblasts underlies **Dupuytren's contracture** (palmar fibromatosis) with flexion deformity of the ring/little fingers.
- ▶ **Vitamin C deficiency** impairs fibroblast collagen hydroxylation, producing **scurvy** (poor wound healing, gum bleeding).
- ▶ **Macrophage granulomas** — chronic antigenic stimulation (e.g. tuberculosis, sarcoidosis) causes macrophage fusion into **Langhans giant cells**, the histological hallmark of granulomatous inflammation.
- ▶ **Mast cell degranulation** underlies **Type I hypersensitivity** (urticaria, allergic rhinitis, asthma) and **anaphylaxis**; systemic mastocytosis is a clinically important mast-cell proliferative disorder.

- ▶ **Plasma cell dyscrasias** — neoplastic plasma cell proliferation (**multiple myeloma**, plasmacytoma) is often accompanied by prominent Russell bodies/Mott cells and monoclonal immunoglobulin (M-band) production.
- ▶ **Adipocyte tumours** — benign **lipoma** and malignant **liposarcoma** arise from adipose tissue; disorders of brown fat/UCP1 thermogenesis are relevant to neonatal cold stress and obesity research.
- ▶ MPS-cell pathology by site: **osteoclast** overactivity in osteoporosis/Paget's disease; **microglial** activation in neuroinflammation; **Langerhans cell histiocytosis** in the skin; **Kupffer cell** hyperplasia in chronic liver disease.

High-yield exam pointers

- ▶ Connective tissue cells = **fixed/resident** (mesenchymal origin: fibroblast, adipocyte, macrophage, mast cell, pericyte) + **wandering/transient** (haemopoietic origin: lymphocyte, plasma cell, neutrophil, eosinophil, basophil, monocyte).
- ▶ **Fibroblast** synthesises collagen/elastin/reticular fibres + ground substance; **fibrocyte** = its quiescent form; **myofibroblast** = α -SMA⁺, TGF- β 1-driven, mediates wound contraction (Dupuytren's if excessive).
- ▶ **Macrophage** = tissue histiocyte, part of MPS; named equivalents — Kupffer (liver), osteoclast (bone), Langerhans (skin), microglia (CNS), alveolar macrophage/dust cell (lung), Hofbauer cell (placenta); M1 (pro-inflammatory) vs M2 (reparative) activation; fuses into Langhans giant cells.
- ▶ **Mast cell** — metachromatic, toluidine-blue-staining granules; heparin + histamine + proteases (preformed) and leukotrienes/PGD₂ (newly synthesised); IgE-Fc ϵ RI mediated degranulation → Type I hypersensitivity; absent from brain, spinal cord and spleen.
- ▶ **Plasma cell** — B-lymphocyte derived, "clock-face" nucleus, antibody factory, Russell bodies/Mott cell, lifespan 10–20 days.
- ▶ **Adipocyte** — unilocular (white, signet-ring, energy store) vs multilocular (brown, UCP1, thermogenesis).
- ▶ Know the **mononuclear phagocyte system** table cold — it is a favourite short-note/viva question in isolation.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e.



Bone matrix

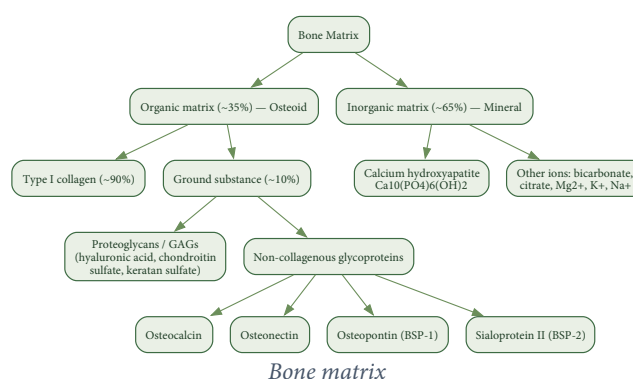
Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction Bone matrix is the mineralized extracellular material of osseous connective tissue, secreted initially by osteoblasts as an unmineralized organic framework (**osteoid**) that is then progressively calcified to form the definitive, rigid matrix in which osteocytes lie entombed within lacunae. It is a composite material — an **organic phase** (chiefly type I collagen and ground-substance proteins) contributing tensile strength and resilience, and an **inorganic/mineral phase** (calcium hydroxyapatite) contributing hardness and rigidity — and this dual composition is what gives bone its unique combination of strength and elasticity. Bone matrix also functions as the body's principal reservoir of calcium and phosphate.

1. Overall composition of bone matrix

Component	Approx. proportion (dry weight)	Chief constituents
Organic matrix (osteoid)	~35%	Type I collagen (~90% of organic matter) + ground substance (~10%)
Inorganic (mineral) matrix	~65%	Calcium hydroxyapatite crystals + other ions

- Dry, de-fatted bone consists of **65% inorganic salts** and **35% organic components**.
- Of the organic component, **collagen fibres form ~90%** and the amorphous **ground substance ~10%**.
- Of the inorganic salts, **crystalline calcium phosphate accounts for ~85%** of total mineral, chiefly as hydroxyapatite.
- On decalcification (acid removal of minerals), a bone becomes soft and pliable enough to be tied in a knot, showing that the residual collagenous framework alone determines shape but not rigidity; conversely, burning a bone destroys the organic component and leaves a brittle, chalky mineral skeleton.



2. Organic matrix (osteoid)

- **Collagen fibres (~90% of organic matrix):**
 - Almost entirely **type I collagen**, synthesised and secreted by osteoblasts; traces of type III, V, XI and XIII collagen are also found.

- Historically called **osteoid collagen**.
- Fibres are arranged in parallel bundles within a thin layer, or **lamella**; in **lamellar (mature) bone**, collagen fibres in one lamella run parallel to each other but are oriented roughly **90° to the fibres of the adjacent lamella** — a "plywood-like" arrangement that produces alternating bright and dark bands under polarised light (birefringence) and confers great mechanical strength.
- Functional correlation: collagen fibres provide **tensile strength and resilience**; deficient or defective collagen (as in osteogenesis imperfecta) causes bone fragility.
- ▶ **Ground substance (~10% of organic matrix):**
 - A small quantity of amorphous ground substance composed of **proteoglycans and glycosaminoglycans** — hyaluronic acid, chondroitin sulfate, keratan sulfate — that regulate collagen fibrillogenesis and matrix hydration.
 - **Non-collagenous (matricellular) proteins**, though a minor fraction by mass, are functionally critical and fall into four broad groups:
- ▶ **Small proteoglycans** (e.g., biglycan, decorin) — organise collagen fibril spacing.
- ▶ **Multiadhesive glycoproteins** — **osteonectin** (acts as a "glue" linking collagen to hydroxyapatite crystals), **osteopontin/bone sialoprotein-1 (BSP-1)** (mediates cell–matrix attachment), and **BSP-2**, which helps initiate calcium-phosphate deposition.
- ▶ **Gla (γ -carboxyglutamic acid) proteins** — chiefly **osteocalcin**, a vitamin K–dependent polypeptide that captures circulating Ca^{2+} and, together with matrix vesicles, promotes and localises mineralisation; also attracts and activates osteoclasts during remodelling.
- ▶ **Growth factors/cytokines** stored within the matrix (e.g., BMPs, TGF- β , IGF), released during resorption to couple bone formation to resorption.
 - **Alkaline phosphatase (ALP)**, produced by active osteoblasts, hydrolyses mineralisation-inhibiting pyrophosphate and raises local free phosphate — essential for calcification.
 - The newly secreted, still-unmineralised organic layer is termed **osteoid**; once mineralised it becomes definitive bone matrix, and the osteoblast trapped within it differentiates into an **osteocyte**.

3. Inorganic (mineral) matrix

- ▶ Inorganic material constitutes about **65% of the dry weight** of bone matrix.
- ▶ The principal mineral is **calcium hydroxyapatite**, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, deposited as tiny **needle-shaped crystals** lying **parallel to and embedded within the collagen fibrils** of each lamella.
- ▶ Other ions present in smaller amounts: **bicarbonate, citrate, magnesium, potassium, sodium**, and **calcium carbonate/calcium fluoride**; significant amounts of **non-crystalline (amorphous) calcium phosphate** also occur.
- ▶ A layer of **bound water** coats the hydroxyapatite crystal surface and facilitates ionic exchange between the mineral phase and the surrounding body fluids — the structural basis of bone's role as a **mineral ($\text{Ca}^{2+}/\text{PO}_4^{3-}$) reservoir**, mobilised under hormonal control (PTH, calcitonin, vitamin D).

- ▶ Association of the mineral with the collagen fibre framework gives bone its characteristic **hardness combined with resistance to fracture**; either component alone (pure mineral or pure collagen) is comparatively brittle or weak.

4. Mechanism of matrix mineralisation

- ▶ Osteoblasts first secrete osteoid — type I collagen plus the non-collagenous proteins described above.
- ▶ Osteoblasts release membrane-bound **matrix vesicles** into the newly formed osteoid; these vesicles concentrate Ca^{2+} and PO_4^{3-} ions and are rich in **alkaline phosphatase**.
- ▶ Local calcium-binding proteins (osteocalcin, and other Gla/glycoproteins) raise the Ca^{2+} concentration around the vesicles.
- ▶ Within/around the matrix vesicles, the **first hydroxyapatite crystals nucleate** — this is the first visible step of calcification.
- ▶ These crystals grow rapidly by accretion, coalesce with neighbouring nucleation sites, and eventually form a **confluent mass of calcified matrix** that engulfs the collagen fibrils and proteoglycans, converting osteoid into mineralised bone matrix.
- ▶ Osteoblasts that become completely surrounded by mineralising matrix transform into **osteocytes**; only 10–20% of osteoblasts follow this fate, the rest undergoing apoptosis or persisting as bone-lining cells.

□ Fig 8.4 — Mineralization in bone matrix. (Junqueira's Basic Histology — Text & Atlas — 17e, p.356)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A vertical labelled sequence showing progressive mineralisation of osteoid into bone, exactly as above:

- ▶ **Osteoblasts** (cuboidal cell layer) releasing **matrix vesicles**
- ▶ **Collagen fibres** with released **matrix vesicles** scattered among them
- ▶ **Early mineralisation** — small hydroxyapatite crystals appearing around individual vesicles
- ▶ **Matrix becoming confluent** — mineral deposits merging between vesicles
- ▶ Label the overall zones from top to bottom: **Osteoblasts** → **Osteoid layer (unmineralised)** → **Mineralised bone**

5. Woven vs lamellar organisation of the matrix

Feature	Woven (immature/primary) bone	Lamellar (mature/secondary) bone
Collagen fibre arrangement	Coarse, irregularly interwoven bundles	Fine fibres arranged in parallel layers (lamellae), orientation alternating $\sim 90^\circ$ between adjacent lamellae
Cellularity	More cells per unit area	Fewer cells per unit area
Ground substance	Relatively more	Relatively less

Feature	Woven (immature/primary) bone	Lamellar (mature/secondary) bone
Mineral content	Lower (more radiolucent)	Higher, more completely mineralised
Occurrence	First bone formed in the fetus and at a fracture site; temporary	Definitive adult bone (compact and cancellous); formed by remodelling of woven bone

Applied / Clinical anatomy

- ▶ **Osteogenesis imperfecta** — mutations in *COL1A1/COL1A2* causing deficient or structurally defective type I collagen; the matrix retains near-normal mineral content but lacks the collagenous scaffold that gives resilience, producing multiple fragility fractures, blue sclerae, and dentinogenesis imperfecta.
- ▶ **Rickets/osteomalacia** — vitamin D deficiency impairs mineralisation of newly formed osteoid; excess unmineralised organic matrix accumulates (widened osteoid seams), producing soft, deformable bone (bow-leg in children, Looser's zones radiographically in adults).
- ▶ **Osteoporosis** — imbalance favouring osteoclastic resorption over osteoblastic matrix formation (e.g., post-menopausal oestrogen deficiency, immobilisation, corticosteroid excess) reduces overall matrix quantity, especially trabecular bone, predisposing to fragility fractures (vertebrae, neck of femur, distal radius).
- ▶ **Osteopetrosis** — defective osteoclastic resorption of matrix leads to dense but structurally disorganised, brittle bone.
- ▶ **Fluorosis** — excess fluoride substitutes for the hydroxyl group of hydroxyapatite to form fluorapatite, increasing radiographic density but making bone brittle.
- ▶ **Tetracycline labelling** — tetracycline binds newly mineralising matrix and fluoresces under UV light, allowing measurement of the rate of new bone apposition — used experimentally and in some clinical bone-biopsy studies.
- ▶ **Biochemical markers**: serum **alkaline phosphatase** and **osteocalcin** reflect osteoblastic activity (formation markers); urinary hydroxyproline and pyridinoline cross-links reflect collagen breakdown (resorption markers) — used to monitor metabolic bone disease.
- ▶ **Decalcification for histology**: acid or EDTA decalcifying solutions are used to soften mineralised matrix for routine paraffin sectioning, exploiting the same organic–inorganic separation seen physiologically.

High-yield exam pointers

- ▶ Dry bone matrix = **65% inorganic + 35% organic** by weight.
- ▶ Organic matrix = **90% type I collagen + 10% ground substance**.
- ▶ Mineral phase = **calcium hydroxyapatite** $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, ~85% of total salts, plus bicarbonate/citrate/Mg/K/Na.
- ▶ Key non-collagenous proteins: **osteocalcin**, **osteonectin**, **osteopontin (BSP-1)**, **sialoprotein II (BSP-2)** — remember as calcium-binding/adhesive glycoproteins.
- ▶ **Osteoid** = unmineralised organic matrix secreted by osteoblasts before calcification.

- **Matrix vesicles** rich in alkaline phosphatase initiate hydroxyapatite nucleation — the first step of mineralisation.
- Collagen fibres run parallel within a lamella but $\sim 90^\circ$ to adjacent lamellae — basis of polarised-light birefringence and mechanical strength.
- Woven bone (coarse, more cells, less mineral) precedes lamellar bone in development and fracture repair.
- Clinical correlates: collagen defect $\rightarrow$ osteogenesis imperfecta; mineralisation defect $\rightarrow$ rickets/osteomalacia; matrix deficiency $\rightarrow$ osteoporosis.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas), 17e; Ross Histology — A Text and Atlas, 7e; IB Singh — Textbook of Human Histology, 9e; Sontakke — Textbook of Human Histology, 2e; Brijesh Kumar — Histology Text & Atlas, 3e; Gray's Anatomy (Standring) 41e.



Classify the connective tissue; describe its cells and fibers?

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction Connective tissue (CT) is a mesodermally-derived tissue (mostly from mesenchyme, with a neural-crest contribution in the head) that fills the interstices between more specialized tissues, binds and supports them, and mediates exchange of nutrients, metabolites and defensive cells. Unlike epithelium, it is characterized by a predominant **extracellular matrix** — fibers embedded in ground substance — secreted by a relatively sparse population of resident and migrant cells. CT = **Cells + Intercellular matrix (Fibers + Ground substance)**, and its classification is based chiefly on the type and proportion of these matrix components.

Classification of connective tissue

Connective tissue is classified, on the basis of structure and function, into three broad groups:

Group	Subtypes
Embryonic/fetal CT	Mesenchyme; Muroid CT (Wharton's jelly of umbilical cord)
Connective tissue proper	Loose (areolar) CT; Dense CT — regular and irregular; CT with special properties (elastic tissue, reticular tissue, adipose tissue)
Specialized CT	Cartilage; Bone; Blood and hemolymphatic tissue



Classify the connective tissue; describe its cells and fibers?

- **Loose (areolar) CT** — cells, fibers and ground substance in roughly equal proportion; delicate, flexible, poor stress resistance; e.g. superficial fascia, lamina propria, endomysium, mesentery.

- ▶ **Dense regular CT** — mostly parallel type I collagen bundles with few fibroblasts (fibrocytes) and little ground substance; e.g. tendon, ligament, aponeurosis.
- ▶ **Dense irregular CT** — collagen bundles interwoven in all directions, resisting stress from multiple directions; e.g. dermis, organ capsules, periosteum, dura mater.
- ▶ **CT with special properties** — **elastic tissue** (ligamentum nuchae, ligamenta flava, vocal ligament), **reticular tissue** (stroma of lymph node, spleen, bone marrow, liver), and **adipose tissue** (unilocular/white and multilocular/brown).

□ Fig 4.18 — Loose areolar connective tissue. (Vishram Singh — General Anatomy — 4e, p.164)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled drawing of a spread (stretch) preparation of loose areolar tissue (e.g. mesentery/omentum), showing:

- ▶ **Collagen fiber bundles** — broad, wavy, pink-staining
- ▶ **Elastic fibers** — thin, single, branching, refractile
- ▶ **Fibroblast/fibrocyte** — spindle-shaped with flattened nucleus, branching processes
- ▶ **Macrophage (histiocyte)** — irregular outline, small heterochromatic kidney-shaped nucleus
- ▶ **Mast cell** — round, granular cytoplasm
- ▶ **Plasma cell** — eccentric "cart-wheel" nucleus
- ▶ A small blood vessel with perivascular cells, embedded in ground substance

Cells of connective tissue

Cells of general CT fall into two functional groups (Gray's Anatomy):

Category	Cells
Resident (fixed/intrinsic) cells — maintain the matrix	Fibroblasts, mesenchymal (adult) stem cells, adipocytes, fixed macrophages, pigment cells (melanocytes)
Migrant/wandering (transient) cells — brought in via blood, mediate defence	Free macrophages, mast cells, lymphocytes, plasma cells, monocytes, eosinophils, neutrophils

Fibroblasts are the most numerous CT cells and the principal source of matrix fibers and ground substance. Active fibroblasts are spindle-shaped with a flattened euchromatic nucleus, prominent nucleolus and abundant rough endoplasmic reticulum (RER)/Golgi (reflecting active protein synthesis); when quiescent, with scanty cytoplasm and heterochromatic nucleus, they are termed **fibrocytes**. **Myofibroblasts**, sharing features of fibroblasts and smooth muscle (actin bundles), mediate wound contraction.

Macrophages (histiocytes/foam cells) are 15–20 µm cells with an indented, heterochromatic nucleus, a prominent nucleolus and lysosome-rich, faintly basophilic ("foamy") cytoplasm; derived from circulating monocytes of bone-marrow origin, they form part of the **mononuclear phagocyte system (MPS)**. They may be "fixed" (attached to fibers, resembling fibroblasts) or motile, and can fuse into multinucleate **foreign-body giant cells**. Tissue-specific names under the MPS: Kupffer cell (liver),

osteoclast (bone), Langerhans cell (skin), microglia (CNS), alveolar macrophage (lung), Hofbauer cell (placenta).

Mast cells are large ($\approx 20\ \mu\text{m}$) round/oval cells with a small central nucleus, filopodia on the surface, and cytoplasm packed with metachromatic granules (toluidine blue/Alcian blue — stain purple-red against a blue background) containing **heparin, histamine, tryptase and superoxide dismutase**; they are PAS-positive. Related in origin to blood basophils, they mediate immediate hypersensitivity/anaphylactic reactions and are notably absent from brain and spinal cord.

Plasma cells are round cells with abundant basophilic cytoplasm (extensive RER) and a juxtanuclear pale Golgi zone; the eccentric nucleus shows peripheral clumps of heterochromatin giving a classic "cart-wheel" (clock-face) appearance. They are terminally differentiated B-lymphocytes that synthesize and secrete antibodies, occasionally storing them as intracytoplasmic **Russell bodies**.

Other cells: adipocytes (fat storage; unilocular signet-ring cells or multilocular brown-fat cells), mesenchymal stem cells (pluripotent, source of repair/regeneration), pigment cells/melanocytes (dendritic, melanin-laden), and migrant leukocytes — lymphocytes (small, $6\text{--}8\ \mu\text{m}$, heterochromatic; B, T and NK subsets), monocytes, neutrophils and eosinophils (the latter increased in allergic states).

Fibers of connective tissue

Three types of fiber are recognized — **collagen, reticular and elastic** — all synthesized principally by fibroblasts (also by chondroblasts, osteoblasts and smooth muscle).

Feature	Collagen fibers	Elastic fibers
Colour (unstained)	White	Yellow
Arrangement	In bundles	Singly, branching
Ends	Straight	Curled
Function	Tensile strength	Elasticity/recoil
Turnover	Produced throughout life	Mainly fetal life

- **Collagen fibers** — the most abundant; bundles ($1\text{--}12\ \mu\text{m}$) of fibrils ($20\text{--}200\ \text{nm}$) made of microfibrils (tropocollagen, a triple helix of three polypeptide (α) chains with glycine at every third residue plus hydroxyproline/hydroxylysine); fibrils show 67-nm cross-banding. Stain pink with H&E, blue with Masson's trichrome, red with Van Gieson.
- **Reticular fibers** — a fine, branching, anastomosing variety of **type III collagen**; argyrophilic (black with silver impregnation); form the supporting meshwork of lymphoid organs (spleen, lymph node, bone marrow), liver, kidney, and basement membranes.
- **Elastic fibers** — thinner ($0.1\text{--}0.2\ \mu\text{m}$), branching, non-bundled fibers with a central amorphous core of **elastin** (rich in valine, alanine and the unique cross-linking amino acid **desmosine**) and a peripheral microfibrillar sheath of **fibrillin**; demonstrated by orcein, aldehyde-fuchsin or Verhoeff's stain; digested by elastase, otherwise chemically inert.

Major collagen types and location (high-yield):

Type	Principal location
I	Skin, tendon, bone, sclera, fascia (commonest)
II	Hyaline/elastic cartilage, vitreous humour, intervertebral disc
III	Reticular fibers — skin, blood vessels, lymphoid organs
IV	Basement membrane
V	Associated with type I, forms stroma

Ground substance

The amorphous, transparent, viscous **ground substance** fills the space between cells and fibers and is synthesized mainly by fibroblasts. It is composed of:

- ▶ **Proteoglycans** — protein core + glycosaminoglycans (GAGs, e.g. chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate); strongly negatively charged (sulfate/carboxyl groups), binding Na^+ and water to form a hydrated gel resisting compression.
- ▶ **Hyaluronic acid** — a huge, non-sulfated GAG not directly linked to protein; forms proteoglycan aggregates.
- ▶ **Multiadhesive glycoproteins** — fibronectin, laminin, tenascin and osteopontin — anchor cells to the matrix and regulate its function.

Applied / Clinical anatomy

- ▶ **Osteogenesis imperfecta** — mutation of type I collagen genes → brittle, fragile bones.
- ▶ **Ehlers–Danlos syndrome** — defective collagen/procollagen processing → hyperextensible skin and lax, hypermobile joints.
- ▶ **Marfan syndrome** — mutation in the **fibrillin-1** gene → defective elastic-fiber microfibrils, affecting aorta, lens and skeleton.
- ▶ **Scurvy** — vitamin C deficiency impairs hydroxylation of proline/lysine in collagen synthesis, causing poor wound healing and fragile vessels.
- ▶ **Wound healing** — fibroblasts proliferate and lay down type III (later remodelled to type I) collagen to form granulation tissue; myofibroblasts effect wound contraction; a keloid represents excessive, disorganized collagen deposition.
- ▶ **Mast-cell degranulation** — histamine release underlies urticaria and anaphylactic shock; brain/cord are protected as they lack mast cells.
- ▶ **Macrophage system (MPS)** clinically relevant across organs — e.g. Kupffer cells in hepatic clearance, microglial activation in neuroinflammation, osteoclastic bone resorption in metabolic bone disease.
- ▶ **Reticular fiber staining** (silver impregnation) is used histopathologically to outline the framework of liver, lymphoid organs and to assess basement-membrane integrity in renal biopsies.

High-yield exam pointers

- ▶ CT = Cells + Matrix (Fibers + Ground substance); mesodermal ($\pm$ neural crest) in origin.
- ▶ Three-tier classification: Embryonic (mesenchyme, mucoid) $\rightarrow$ CT proper (loose; dense regular/irregular) $\rightarrow$ Specialized (cartilage, bone, blood/adipose).
- ▶ Resident cells: fibroblast, mesenchymal stem cell, adipocyte, fixed macrophage, melanocyte. Migrant cells: macrophage, mast cell, lymphocyte, plasma cell, monocyte, eosinophil.
- ▶ Fibroblast $\rightarrow$ collagen/ground substance; macrophage $\rightarrow$ phagocytosis (MPS); mast cell $\rightarrow$ heparin/histamine granules (metachromatic); plasma cell $\rightarrow$ antibody synthesis, cart-wheel nucleus.
- ▶ Three fiber types: collagen (bundled, type I, tensile strength), reticular (type III, argyrophilic, supporting network), elastic (elastin core + fibrillin sheath, recoil).
- ▶ Collagen triple helix = 3 α -chains, glycine every 3rd residue; 67-nm banding; ≥ 25 collagen types, I–V most exam-relevant.
- ▶ Ground substance = proteoglycans (GAG + protein) + hyaluronic acid + multiadhesive glycoproteins (fibronectin, laminin).
- ▶ Clinical triad to remember: Osteogenesis imperfecta (type I collagen), Ehlers–Danlos (collagen processing), Marfan (fibrillin-1/elastic fibers).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology, 17e; IB Singh — Textbook of Human Histology, 9e; Sontakke — Textbook of Human Histology, 2e; Vishram Singh — General Anatomy, 4e.



Classify unilaminar and multilaminar epithelial tissues. Make a mention of cells and their functions. Add a brief note on the modifications of cells

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction

Epithelium is one of the four basic tissues — a sheet of closely apposed, polygonal cells with minimal intercellular space that covers external and internal body surfaces or lines tubes and cavities, resting on a **basal lamina** that separates it from the underlying (vascular) connective tissue. Epithelia are classified primarily by the **number of cell layers**: **unilaminar (single-layered, simple)** epithelium, in which a single layer of cells rests directly on the basal lamina, and **multilaminar** epithelium, in which the layer is more than one cell thick (stratified squamous, urothelium/transitional, and other slowly-renewing multilayered linings). Epithelial cells derive from all three germ layers, are avascular (nourished by diffusion), richly innervated, show marked apico-basal polarity, and have excellent regenerative capacity.

1. General Features and Functions of Epithelial Tissue

- ▶ **Minimal intercellular space** (~20 nm); cells bound tightly by junctional complexes.
- ▶ **Avascular** — nutrition by diffusion from capillaries in subjacent connective tissue; this limits epithelial thickness.
- ▶ Rests on a **basal lamina/basement membrane** (type IV collagen, laminin, entactin, proteoglycans), demonstrated by the PAS method.
- ▶ Shows **cell polarity** (distinct apical, lateral and basal domains) and **surface modifications**.
- ▶ **High regenerative capacity**; can undergo **metaplasia** (change from one epithelial type to another).
- ▶ Intermediate filament protein of all epithelia is **cytokeratin** — used immunohistochemically to identify carcinomas.

Functions: (i) **Protection** from abrasion, dehydration, chemical/mechanical injury; (ii) **Absorption** of luminal contents; (iii) **Secretion** of mucus, hormones, enzymes; (iv) **Exchange/transport** (transcellular movement of ions, gases, fluid); (v) **Sensation** (specialized sensory epithelia of olfactory, gustatory and vestibulocochlear systems contain sensory cells surrounded by supporting cells).

2. Classification of Unilaminar (Simple) Epithelia

Named according to the shape of their single layer of cells.

Type	Structure	Principal locations	Function
Simple squamous	Single layer of flat, polygonal ("tessellated") cells; nucleus bulges the surface	Alveoli; mesothelium (pleura, peritoneum, pericardium); endothelium of heart & vessels; parietal layer of Bowman's capsule; thin segment of renal tubule; parts of inner ear	Rapid diffusion of gases/fluids and filtration; tight (occluding) junctions force transcellular (not paracellular) transport
Simple cuboidal	Height ≈ width; round central nucleus; hexagonal in surface view	Thyroid follicles; ducts of exocrine glands; germinal (surface) epithelium of ovary; choroid plexus; pigment layer of retina; proximal convoluted tubule (with brush border)	Secretion, absorption, excretion
Simple columnar (non-ciliated)	Height > width; basal elongated nucleus; may bear goblet cells or a brush/striated border of microvilli	Gastric and large-intestinal mucosa; gallbladder	Secretion of mucus/enzymes; absorption
Simple columnar (ciliated)	As above, with apical cilia	Uterine tube, uterus, efferent ductules of testis, ependyma	Ciliary transport of ovum/CSF

Type	Structure	Principal locations	Function
Pseudostratified columnar	Single layer, all cells touch the basal lamina, but nuclei of tall and short (basal) cells lie at different levels giving a false impression of stratification	<i>Non-ciliated</i> : epididymis (with stereocilia), ductus deferens, membranous & penile urethra, part of auditory tube. <i>Ciliated (with goblet cells)</i> : trachea, large bronchi	Tall cells: secretory/absorptive; short basal cells: stem cells that replace tall cells; cilia clear mucus, stereocilia aid absorption

embed: ![Fig 4.7 — ■ Types of simple epithelium. (Vishram Singh — General Anatomy — 4e, p.145)]
(C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/4317be858801bf3d.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the four basic shapes in vertical section, resting on a basal lamina, and label: (A) simple squamous — flat cells, bulging nucleus; (B) simple cuboidal — square cells, round central nucleus; (C) simple columnar — tall cells, basal oval nucleus, basement membrane; (D) pseudostratified columnar — tall + short basal cells, nuclei at two levels, cilia at apex, goblet cell.

3. Classification of Multilaminar (Stratified/Compound) Epithelia

Named according to the shape of cells of the **most superficial** layer; the basal layer always rests on the basal lamina.

Type	Structure	Principal locations	Function
Stratified squamous, non-keratinized	Basal columnar cells → middle polyhedral cells → superficial flattened, living, nucleated cells (moist surface)	Oral cavity, oropharynx, oesophagus, true vocal folds, vagina	Protection of moist surfaces subject to friction
Stratified squamous, keratinized	As above, but surface cells are dead, anucleate, and replaced by keratin (cornification)	Epidermis of skin	Protection; prevents dehydration of underlying tissue
Stratified cuboidal	Two layers of cuboidal cells	Ducts of sweat glands	Protective
Stratified columnar	Deeper polyhedral/cuboidal layer + superficial columnar layer	Conjunctiva; large excretory ducts (parotid, pancreas); parts of male urethra	Protective; conducts glandular secretions

Type	Structure	Principal locations	Function
Transitional epithelium (urothelium)	4–6 cells thick; basal cuboidal/columnar cells → middle pear-shaped (polyhedral) cells → superficial large "umbrella" cells with specialized glycoprotein plaques in the plasma membrane; cells held by numerous desmosomes, allowing stretch without loss of integrity	Renal pelvis and calyces, ureter, urinary bladder, part of urethra	Distensibility; impermeable barrier resistant to the toxic effects of concentrated urine
Seminiferous epithelium (<i>special multilaminar tissue</i>)	Complex, unique multilayered germinal epithelium	Seminiferous tubules of testis	Site of spermatogenesis

embed: ![Fig 4.9 — ■ Types of compound/stratified epithelium. (Vishram Singh — General Anatomy — 4e, p.147)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/2fefaf3a8bd04652.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw stratified squamous **non-keratinized** (superficial flat nucleated cells) beside **keratinized** (superficial dead, keratin-filled, anucleate layer); and transitional epithelium in the **relaxed** (multilayered, umbrella cells rounded) and **stretched** (fewer apparent layers, umbrella cells flattened) states. Label: basal layer, intermediate/polyhedral layer, superficial layer, keratin layer, umbrella cells.

4. Cells of the Epithelium and Their Functions

An epithelium is not a homogeneous cell population; several distinct cell types recur across different epithelia:

Cell type	Function
Squamous cells	Least metabolically active (little cytoplasm, few organelles); permit rapid diffusion/filtration
Cuboidal/columnar cells	Rich in mitochondria and endoplasmic reticulum; highly active in secretion, absorption, ion transport
Goblet cells	Unicellular mucous glands interspersed in simple columnar/pseudostratified epithelia (gut, respiratory tract); secrete mucus for protection/lubrication
Ciliated cells	Bear motile cilia; propel mucus (respiratory tract) or ova (uterine tube)
Brush-border/absorptive cells	Apical microvilli increase surface area; specialized for absorption (intestine, renal PCT)

Cell type	Function
Basal (stem) cells	Undifferentiated cells at the base of pseudostratified and stratified epithelia; proliferate to replace superficial/tall cells
Myoepithelial cells	Contractile, basket-shaped cells around secretory acini and ducts (salivary, mammary, sweat glands); contraction expels secretion
Sensory (neuroepithelial) cells	Specialized receptor cells of olfactory mucosa, taste buds and the vestibulocochlear (hair cell) epithelium, surrounded by supporting cells
Pigment-containing cells	Seen in skin, retina and iris epithelium; contribute to photoprotection/light absorption

5. Modifications of Cells (Cell-Surface Specializations)

Epithelial cells modify their apical, lateral and basal domains to serve specific functions.

A. Apical modifications

Feature	Structure	Function/location
Microvilli	Finger-like projections (~1–2 µm long); core of actin microfilaments continuous with the terminal web; covered by glycocalyx; regular arrangement gives a striated border (small intestine), irregular gives a brush border (renal PCT)	Non-motile; greatly increase surface area for absorption
Stereocilia	Very long (5–10 µm), thick, non-motile microvilli	Absorption in epididymis; sensory transduction in cochlear/vestibular hair cells
Cilia	Motile hair-like organelles, ~10 µm long, 0.25 µm diameter; axoneme with 9 peripheral doublet + 2 central microtubules (9+2 pattern), dynein arms (motility) and nexin links, anchored by a basal body	Move mucus/secretions (trachea, bronchi) toward the pharynx; move ova along the uterine tube

B. Lateral modifications — the junctional complex (apical to basal order):

- **Zonula occludens (tight junction)** — a continuous, encircling band formed by claudin/occludin proteins; seals the paracellular space and forces transcellular transport; also fences apical and basolateral membrane domains apart.
- **Zonula adherens (adherent junction)** — encircling band just below the tight junction; cadherin-mediated, calcium-dependent adhesion linked to the actin terminal web; stabilizes the tight junction.

- ▶ **Macula adherens (desmosome)** — spot-like "rivet" of desmoglein/desmocollin cadherins linked via desmoplakin to intracytoplasmic cytokeratin (tonofilament) bundles; provides strong mechanical adhesion and tensile strength.
- ▶ **Gap junction (nexus)** — patches of connexon channels (six connexin subunits each) permitting direct intercellular exchange of ions and small molecules; mediates communication, not adhesion.

C. Basal modifications:

- ▶ **Hemidesmosomes** — anchor the basal cell surface to the basal lamina via transmembrane integrins.
- ▶ **Basal (basolateral) infoldings** — deep folds of the basal/lateral membrane that increase surface area, seen in renal tubular cells and striated ducts of glands, associated with abundant mitochondria for active ion transport.
- ▶ **Basal lamina** — thin extracellular layer of type IV collagen, laminin, entactin and proteoglycans; provides structural support, acts as a selective molecular filter/barrier, and influences epithelial polarity, proliferation and regeneration.

embed: ![Fig 5.7 — Types of cell junctions. Fig. 5.8: Microvilli, stereocilia, and cilia (Sontakke — Textbook of Human Histology — 2e, p.61)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/14f434b0e74d2778.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw an epithelial cell in vertical section with apical microvilli, and label from apex to base along the lateral membrane: **zonula occludens (tight junction)**, **zonula adherens**, **macula adherens (desmosome)**, **gap junction (connexon)**; then at the base label **hemidesmosome** and **basal lamina**.

Applied / Clinical anatomy

- ▶ **Metaplasia:** chronic irritation (e.g. cigarette smoke) converts the pseudostratified ciliated columnar epithelium of the bronchi into stratified squamous epithelium (**squamous metaplasia**), impairing mucociliary clearance.
- ▶ **Nomenclature of epithelial tumours:** a malignant tumour of squamous epithelium is a **squamous cell carcinoma**; one arising from glandular epithelium is an **adenocarcinoma**; the epithelial marker **cytokeratin** helps identify carcinomas immunohistochemically, including in poorly differentiated/metastatic tumours.
- ▶ **Immotile cilia syndrome (ICS):** autosomal recessive ciliary ultrastructural/functional defect causing impaired mucociliary clearance → recurrent chest infections; ciliary dysfunction in the uterine tube causes female infertility, and defective sperm-tail (flagellar) motility causes male infertility.
- ▶ **Kartagener's syndrome:** a variant of ICS with the triad of **situs inversus**, **chronic sinusitis** and **bronchiectasis**, due to defective ciliary/flagellar dynein motility.
- ▶ **Pemphigus vulgaris:** autoimmune antibodies against desmoglein disrupt desmosomes of the epidermis/oral mucosa, causing intraepidermal blistering.

- ▶ **Bacterial toxins on tight junctions:** *Clostridium perfringens* enterotoxin binds claudin and disrupts intestinal tight junctions (paracellular fluid loss/diarrhoea); *Helicobacter pylori* disrupts gastric tight-junction signalling, contributing to gastric ulceration.
- ▶ **Epithelial renewal rates** are clinically relevant to radiotherapy/chemotherapy toxicity: epidermis renews over ~28 days (basal layer to keratinized shedding); small-intestinal epithelium renews every 4–6 days.
- ▶ **Transitional epithelium** distension/relaxation is exploited in cystoscopic and radiological assessment of a full versus empty bladder.

High-yield exam pointers

- ▶ **Unilaminar** = single layer resting directly on the basal lamina; **multilaminar** = more than one cell layer (Gray's Anatomy terminology, synonymous with "simple" and "stratified").
- ▶ Simple epithelium is named by **cell shape**; stratified epithelium is named by the shape of its superficial layer.
- ▶ **Pseudostratified** ≠ **true stratified** — every cell touches the basal lamina; only the tall cells reach the free surface.
- ▶ **Transitional epithelium** = **urothelium**, confined to the urinary tract; umbrella cells form an osmotic/chemical barrier and permit stretch.
- ▶ Order of the epithelial **junctional complex** (apical → basal): zonula occludens → zonula adherens → macula adherens (desmosome); gap junctions are interspersed, not part of the linear sequence.
- ▶ **Cilia** (9+2 axoneme, motile) vs **microvilli** (actin core, non-motile, absorptive) vs **stereocilia** (long non-motile microvilli) — a favourite "differentiate" question.
- ▶ Basal specializations: **hemidesmosomes** anchor to basal lamina; **basal infoldings** increase surface area for active ion transport (renal tubules, striated ducts).
- ▶ Epithelial intermediate filament = **cytokeratin**; used to diagnose carcinomas.
- ▶ Classic clinicoanatomical correlates: **Kartagener's/immotile cilia syndrome**, **pemphigus vulgaris**, **squamous metaplasia** of bronchial epithelium in smokers.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh's Textbook of Human Histology 9e; Junqueira's Basic Histology 17e; Vishram Singh's Textbook of General Anatomy 4e; Sontakke's Textbook of Human Histology 2e.



Connective tissue cells

Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction Connective tissue (CT) proper is composed of cells embedded in an extracellular matrix (ground substance + fibres). Unlike epithelium, CT cells are widely separated by matrix that they themselves largely secrete and maintain. CT cells fall into two functional groups — **resident (fixed) cells**, which are permanent local inhabitants concerned with matrix synthesis and storage, and **wandering (transient/migrant) cells**, which arrive from the blood via venules to perform short-term defensive duties before dying by apoptosis. All CT cells trace their origin either to local embryonic **mesenchyme** (fibroblasts, adipocytes, mesenchymal stem cells) or to **haemopoietic stem cells** of bone marrow (macrophages, mast cells, plasma cells, leukocytes).

Classification of connective tissue cells

Group	Origin	Cells included
Resident (fixed) cells	Local mesenchyme	Fibroblast/fibrocyte, adipocyte, undifferentiated mesenchymal (adult stem) cell, pericyte, pigment cell (melanocyte)
Wandering (transient) cells	Bone-marrow haemopoietic stem cells, via blood monocytes/lymphocytes	Macrophage (histiocyte), mast cell, plasma cell, lymphocyte, monocyte, neutrophil, eosinophil, basophil

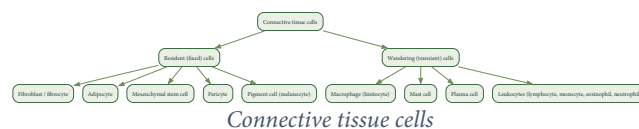


Fig 7.10 — Cells of connective tissue. (Sontakke — Textbook of Human Histology — 2e, p.83)

Fibroblast and fibrocyte

- **Fibroblasts** are the most numerous resident cells of CT proper; flattened, spindle-shaped in profile, with branching processes seen face-on.
- Nucleus large, euchromatic, with a prominent nucleolus; cytoplasm basophilic in the active cell owing to abundant rough endoplasmic reticulum (RER) and well-developed Golgi apparatus — reflecting their role as the principal synthesisers of collagen, elastin, glycosaminoglycans (GAGs), proteoglycans and multiadhesive glycoproteins of the ground substance.
- The quiescent, less active form with scanty cytoplasm, sparse RER and a flattened, heterochromatic nucleus is termed a **fibrocyte**.
- Where fibroblasts lay down type III collagen (reticular fibres) in liver, spleen, kidney and lymphoid tissue, they are called **reticular cells (reticulocytes)**, forming a fibrocellular reticular network.
- **Myofibroblasts** are contractile fibroblast-like cells rich in smooth-muscle actin, responsible for wound contraction during healing; current evidence suggests their precursor in wound repair

and fibrotic disease (e.g. hepatic cirrhosis) is the vascular **pericyte** rather than the ordinary fibroblast.

- ▶ Fibroblasts rarely divide in the healthy adult but re-enter the cell cycle under growth-factor stimulation, e.g. to form granulation tissue after injury. Collagen synthesis is impaired in vitamin C deficiency.

Macrophage (histiocyte) and the mononuclear phagocyte system

- ▶ **Macrophages** are large (10–30 μm), irregular cells with an eccentric, kidney-shaped, heterochromatic nucleus and a foamy, lysosome-rich cytoplasm — features of active phagocytosis.
- ▶ Derived from circulating **monocytes** that cross venule walls into CT and mature; hence part of the **mononuclear phagocyte system (MPS)**.
- ▶ Functions: phagocytosis of bacteria, debris and apoptotic cells (an innate-immune defence); antigen presentation to lymphocytes; secretion of cytokines, growth factors and matrix-degrading enzymes; fusion into multinucleate **giant cells** when strongly stimulated (e.g. foreign-body/Langhans giant cells in granulomas).
- ▶ Demonstrated experimentally by vital dyes such as trypan blue or India ink, which are taken up into the phagocytic cytoplasm.

Organ/tissue	Specialised macrophage (MPS) name
Connective tissue (general)	Macrophage / histiocyte
Liver	Kupffer cell
Bone	Osteoclast
Skin (epidermis)	Langerhans cell
Placenta	Hofbauer cell
Central nervous system	Microglia
Lung alveoli	Alveolar macrophage

Mast cell

- ▶ Oval/round cells, 7–20 μm (up to ~20 μm), with a small central nucleus obscured by cytoplasm packed with basophilic, membrane-bound secretory granules; numerous surface filopodia.
- ▶ Granules are strongly **PAS-positive** and show **metachromasia** with toluidine blue (blue dye stains granules purple/red) because of their sulphated GAG content.
- ▶ Granule contents: **heparin** (anticoagulant proteoglycan), **histamine** ($\uparrow$ vascular permeability, smooth-muscle contraction), **tryptase**, **chymase**, superoxide dismutase, and eosinophil/neutrophil chemotactic factors.
- ▶ Two human subtypes: **MC_{TC}** (tryptase + chymase, lattice-substructure granules — skin, intestinal submucosa, breast) and **MC_T** (tryptase only, scroll-substructure granules — lung, intestinal mucosa).

- ▶ Located characteristically **peri-vascularly** and beneath mucosal epithelia; arise from bone-marrow progenitors that circulate as agranular, basophil-like cells and mature only after entering tissue. Closely resemble but are lineage-distinct from blood basophils.
- ▶ **Mediator of immediate (type I) hypersensitivity**: first antigen exposure sensitises the cell via surface-bound IgE; re-exposure cross-links IgE receptors, triggering degranulation and release of histamine, leukotrienes and heparin — the basis of urticaria, angio-oedema and, in generalised release, anaphylactic shock. The brain and spinal cord are devoid of mast cells.

Plasma cell

- ▶ Large, ovoid, wandering cell derived from an antigen-stimulated **B lymphocyte** that has matured for antibody synthesis and lost the capacity for further division.
- ▶ Deeply basophilic cytoplasm (abundant RER) with a characteristic pale-staining juxtanuclear **Golgi hof** (site of antibody glycosylation).
- ▶ Nucleus round, eccentrically placed, with peripheral heterochromatin clumps giving a classic "**clock-face**"/"**cartwheel**" appearance.
- ▶ Secretes immunoglobulins (antibodies), which may act locally, enter the circulation, or accumulate as intracytoplasmic **Russell bodies**.
- ▶ Short-lived (average lifespan 10–20 days); scanty in normal CT, markedly increased in chronic inflammation and infected tissue.

Adipocyte (fat cell)

- ▶ Large, mesenchymally-derived cells specialised for lipid storage (white fat) or heat production (brown fat); embryologically arise, like fibroblasts, from mesenchymal stem cells.

Feature	White (unilocular) adipocyte	Brown (multilocular) adipocyte
Fat droplets	Single large central globule	Multiple small droplets
Nucleus	Flattened, peripheral	Rounded, more central
Mitochondria	Few	Numerous, with large cristae
Function	Energy storage; endocrine (adipokines)	Non-shivering thermogenesis via uncoupling protein UCP1
Location	Widespread subcutaneous/visceral fat	Interscapular in neonates; supraclavicular/paraspinal "beige fat" depots persist in some adults

- ▶ In routine H&E sections the lipid is dissolved by processing solvents, leaving only the thin peripheral cytoplasmic rim and compressed nucleus — the classic **signet-ring** appearance.
- ▶ Mature adipocytes do not divide; obesity results from lipid accumulation in and enlargement of existing cells, not new adipocyte formation.

Other resident and wandering cells

- ▶ **Undifferentiated mesenchymal (adult stem) cells:** inconspicuous, spindle/stellate cells retained from embryonic mesenchyme; can differentiate into fibroblasts, adipocytes or other CT cells during growth, turnover and wound repair — basis of mesenchymal stem-cell therapy research.
- ▶ **Pericytes:** perivascular cells surrounded by their own basal lamina around capillaries/venules; possess contractile and multipotent properties and are now regarded as the principal myofibroblast precursor in fibrosis.
- ▶ **Pigment cells (melanocytes):** stellate, melanin-containing cells, most numerous in skin, choroid and iris; melanin-engulfing but non-synthesising cells are chromatophores/melanophores.
- ▶ **Leukocytes** (lymphocytes, monocytes, neutrophils, eosinophils): true wandering blood cells that migrate through venule walls into CT, especially numerous during inflammation.

Applied / Clinical anatomy

- ▶ **Wound healing:** fibroblast proliferation and myofibroblast-driven wound contraction underlie granulation tissue formation and scar formation in tissues incapable of true regeneration (e.g. myocardium after infarction).
- ▶ **Chronic inflammation/granuloma:** activated macrophages fuse into Langhans/foreign-body giant cells (classically seen in tuberculosis and sarcoidosis).
- ▶ **Allergy and anaphylaxis:** IgE-mediated mast-cell degranulation is the basis of urticaria, allergic rhinitis and, systemically, anaphylactic shock; antihistamines and mast-cell stabilisers act at this level.
- ▶ **Plasma cell dyscrasias:** multiple myeloma is a neoplastic proliferation of plasma cells; Russell bodies are seen histologically.
- ▶ **Vitamin C deficiency (scurvy):** defective fibroblast collagen synthesis manifests as poor wound healing and capillary fragility.
- ▶ **Adipose tissue in imaging:** PET-detectable UCP1-positive "beige/brown" fat depots in the adult supraclavicular/paraspinal region are of interest in obesity and metabolic research.
- ▶ **MPS naming across organs** (Kupffer cells, osteoclasts, microglia, alveolar macrophages, Langerhans cells) is clinically relevant to site-specific chronic disease (e.g. viral hepatitis, osteopetrosis, neuroinflammation).

High-yield exam pointers

- ▶ Two functional groups: **resident** (fibroblast, adipocyte, mesenchymal stem cell, pericyte) vs **wandering** (macrophage, mast cell, plasma cell, leukocytes).
- ▶ Fibroblast = active, synthetic; fibrocyte = quiescent form; reticular cell = fibroblast associated with type III collagen.
- ▶ Macrophage = monocyte-derived member of the **mononuclear phagocyte system**; name changes by organ (Kupffer, osteoclast, microglia, Langerhans, Hofbauer, alveolar macrophage).
- ▶ Mast-cell granules: heparin + histamine + tryptase ± chymase; **metachromatic** with toluidine blue; IgE-mediated degranulation → type I hypersensitivity.
- ▶ Plasma cell: B-lymphocyte-derived antibody factory; **clock-face nucleus**, perinuclear Golgi hof, Russell bodies, short lifespan.

- ▶ Adipocyte: unilocular (white, energy storage) vs multilocular (brown, UCP1-mediated thermogenesis); signet-ring appearance on H&E.
- ▶ Myofibroblast (wound contraction) now thought to arise chiefly from the **pericyte**, not the ordinary fibroblast.
- ▶ All wandering cells reach CT from bone marrow via blood monocytes/lymphocytes crossing venule walls; all resident cells arise from local embryonic mesenchyme.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled field of loose areolar connective tissue showing, in one frame: **fibroblast** (spindle nucleus along collagen bundle), **fibrocyte**, **macrophage/histiocyte** (kidney-shaped nucleus, vacuolated cytoplasm), **mast cell** (granule-packed cytoplasm, central nucleus), **plasma cell** (eccentric clock-face nucleus, perinuclear Golgi hof), **adipocyte** (signet-ring, compressed peripheral nucleus), a small **lymphocyte**, and surrounding **collagen fibres/elastic fibres/a small blood vessel (venule)** through which the wandering cells are entering the tissue.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e.



Define and classify connective tissue

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

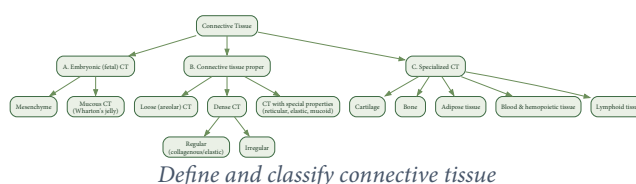
Definition / Introduction. Connective tissue is one of the four basic tissues of the body, defined as a tissue composed predominantly of **intercellular material (extracellular matrix)** — comprising **fibres** and a **ground substance** — secreted mainly by its own resident cells, with the cells themselves usually widely separated by this matrix. It is mesodermal in origin (derived from embryonic **mesenchyme**, or from **neural crest** in the head region), and it fills the interstices between more specialised tissues, binding, supporting, protecting and nourishing them. Unlike epithelium, connective tissue has a high matrix-to-cell ratio (except in adipose tissue, where the cellular component eventually dominates) and is well vascularised in most of its forms.

1. Classification of connective tissue

Connective tissue is classified, on the basis of its structure, cellularity and the nature of its matrix, into three broad groups.

Group	Subtypes	Typical sites
A. Embryonic (fetal) connective tissue	1. Mesenchyme 2. Mucous/mucoid connective tissue	Embryonic mesoderm; Wharton's jelly of umbilical cord, vitreous humour of eye

Group	Subtypes	Typical sites
B. Connective tissue proper (general CT)	1. Loose (areolar) CT 2. Dense CT — regular & irregular 3. CT with special properties — reticular, elastic, mucoid	Areolar: subcutaneous tissue, lamina propria, mesentery. Dense regular: tendon, ligament, aponeurosis. Dense irregular: dermis, periosteum, organ capsules, dura mater. Reticular: stroma of lymph node/spleen/bone marrow. Elastic: ligamentum nuchae/flava, elastic arteries
C. Specialized connective tissue	1. Cartilage (hyaline, elastic, fibro-) 2. Bone 3. Adipose tissue 4. Blood & hemopoietic tissue 5. Lymphoid (hemolymphoid) tissue	Skeletal support; subcutaneous/perinephric fat, marrow; blood vascular system; lymph nodes, spleen, bone marrow



- **Ordinary/general** connective tissues (loose, dense, adipose) are widely distributed; the **special skeletal types** (cartilage, bone) and **haemolymphoid tissue** (blood cells, lymphoid tissue and their precursors) are grouped with connective tissue because of a shared mesenchymal origin, even though structurally distinctive.

2. Components of connective tissue

Connective tissue = Cells + Intercellular matrix (fibres + ground substance).

(a) Cells

Cells are grouped as **resident (fixed)** cells, which maintain the matrix, and **wandering (transient/migrant)** cells, which are recruited from blood and mediate defence.

Type	Cell	Key feature / function
Resident	Fibroblast (fibrocyte when quiescent)	Most numerous cell; synthesises collagen, elastin and ground substance; spindle-shaped with euchromatic nucleus
	Myofibroblast	Fibroblast + smooth-muscle features (actin bundles); causes wound contraction
	Mesenchymal/adult stem cell	Undifferentiated, stellate; source of new fibroblasts/other CT cells
	Adipocyte	Stores neutral fat; signet-ring appearance on H&E

Type	Cell	Key feature / function
	Fixed macrophage (histiocyte/foam cell)	Part of the mononuclear phagocyte system (MPS) ; derived from blood monocytes; phagocytoses debris/microbes, presents antigen; kidney-shaped heterochromatic nucleus
	Mast cell	Round cell with metachromatic cytoplasmic granules (heparin, histamine, tryptase); mediates immediate hypersensitivity/allergic reactions; related to blood basophils
	Pigment cell (melanocyte)	Contains melanin; abundant in dermis, choroid, iris
Wandering	Lymphocyte	Immune surveillance/antibody-mediated defence; increases in inflammation
	Plasma cell	Matured B-lymphocyte; "cart-wheel" eccentric nucleus; basophilic cytoplasm rich in RER; synthesises and secretes antibodies (may store as Russell bodies)
	Monocyte, neutrophil, eosinophil, basophil	Recruited during inflammation/allergy; monocytes mature into macrophages

(Macrophage-lineage cells are renamed by site — *Kupffer cell in liver, osteoclast in bone, microglia in CNS, Langerhans cell in skin, alveolar macrophage in lung, Hofbauer cell in placenta* — but all belong to the MPS.)

(b) Fibres

Three types of fibre are recognised — collagen, reticular and elastic.

Fibre	Composition	Staining	Distinguishing feature	Typical sites
Collagen	Type I collagen (~90% of body collagen)	Eosin (pink, H&E); Masson's trichrome (blue); van Gieson (red)	Straight, unbranched fibres arranged in bundles; high tensile strength, inelastic; shows 67 nm cross-striations on EM	Tendon, ligament, bone, dermis, fascia, organ capsules

Fibre	Composition	Staining	Distinguishing feature	Typical sites
Reticular	Type III collagen	Silver impregnation (argyrophilic — Gordon & Sweet/Gomori); PAS-positive	Fine, branching fibres forming a supporting meshwork; do not form bundles	Liver, spleen, lymph node, bone marrow, endoneurium (absent in thymus)
Elastic	Elastin core + fibrillin-1 microfibrillar sheath	Orcein (brown), Verhoeff's method (black); poorly seen on H&E	Thin, branching, single fibres; recoil after stretch; no cross-striations	Ligamentum nuchae, ligamentum flava, elastic arteries, vocal ligaments, lung

- **Collagen types and disease correlation:** Type I — skin, tendon, bone (defect → **osteogenesis imperfecta**); Type II — hyaline/elastic cartilage, nucleus pulposus, vitreous; Type III (reticular) — deficiency associated with **Ehlers–Danlos syndrome**; Type IV — basement membrane (defect → **Alport syndrome** — haematuria, deafness, ocular lesions).
- **Fibrillin-1 (FBN1)** defects underlie **Marfan syndrome** (autosomal dominant) — tall stature, arachnodactyly, lens dislocation, aortic aneurysm.

(c) Ground substance (extracellular matrix)

- A transparent, hydrated gel occupying the space between cells and fibres; largely removed during routine tissue processing, giving loose CT its characteristically "empty" look on H&E.
- Composed of three groups of molecules:
- **Glycosaminoglycans (GAGs)** — hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate; long, negatively charged, unbranched chains that bind water to form a hydrated gel.
- **Proteoglycans** — a core protein with attached GAGs (e.g. aggrecan; syndecan is a transmembrane proteoglycan linking cell to matrix).
- **Glycoproteins** — fibronectin (binds cells to matrix), laminin (anchors epithelium to basal lamina), tenascin, osteonectin, chondronectin.
- **Function:** permits diffusion of gases, electrolytes and metabolites while acting as a selective barrier to large molecules and micro-organisms; stains metachromatically with toluidine blue and is PAS-positive.

3. Connective tissue proper — loose and dense CT

□ Fig 4.18 — ■ Loose areolar connective tissue. (Vishram Singh — General Anatomy — 4e, p.164)

Feature	Loose (areolar) CT	Dense regular CT	Dense irregular CT
Fibre arrangement	Loosely woven meshwork, in all directions	Collagen bundles parallel, orderly	Collagen bundles interlacing in all directions
Ground substance	Abundant	Scanty	Scanty

Feature	Loose (areolar) CT	Dense regular CT	Dense irregular CT
Cellularity	Rich — fibroblasts, macrophages, mast cells, etc.	Few — mainly fibrocytes between bundles	Few — mainly fibroblasts
Function	Binds structures, allows movement, supports vessels/nerves	Resists tension along one axis	Resists stress from all directions
Examples	Subcutaneous tissue (non-fatty areas), lamina propria of gut, mesentery, subperiosteal tissue, stroma of glands	Tendon, ligament, aponeurosis, central tendon of diaphragm, fibrous pericardium, dura mater	Dermis (reticular layer), periosteum, perichondrium, organ capsules (testis, sclera), adventitia of vessels

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a stretch/spread preparation of loose areolar tissue (mesentery/omentum), labelling:

- ▶ Wavy, pink-staining **collagen fibre bundles**
- ▶ Thin, branching **elastic fibres**
- ▶ Spindle-shaped **fibroblast** with an elongated euchromatic nucleus
- ▶ Kidney-shaped, heterochromatic **macrophage (histiocyte)**
- ▶ Granular-cytoplasm **mast cell**
- ▶ **Plasma cell** with cart-wheel eccentric nucleus
- ▶ A small **blood capillary**
- ▶ Clear "empty" spaces representing dissolved **ground substance**

4. Specialized connective tissue

- ▶ **Cartilage** — avascular, semi-rigid connective tissue with chondrocytes in lacunae within a matrix rich in type II collagen ($\pm$ elastic fibres/collagen bundles); three types — **hyaline**, **elastic**, **fibrocartilage**.
- ▶ **Bone** — mineralised (hydroxyapatite) connective tissue with osteocytes in lacunae, type I collagen matrix, organised as compact and cancellous bone; supports and protects, and is a store of calcium/phosphate.
- ▶ **Adipose tissue** — an aggregation of fat-storing **adipocytes** supported by reticular fibres and grouped into lobules; **yellow (unilocular)** adipose tissue is the adult type (subcutaneous panniculus adiposus, perinephric fat, marrow fat), while **brown (multilocular)** adipose tissue, rich in mitochondria, is abundant in the newborn and generates heat.
- ▶ **Blood** — a fluid (flowing) connective tissue; matrix is the liquid plasma, cellular elements are erythrocytes, leukocytes and platelets, produced by haemopoietic tissue (bone marrow).
- ▶ **Lymphoid (haemolymphoid) tissue** — reticular-fibre framework populated by lymphocytes and plasma cells; forms lymph nodes, spleen, tonsils, MALT; shares mesenchymal origin and defensive cell lineage with general connective tissue.

Applied / Clinical anatomy

- ▶ **Osteogenesis imperfecta** — mutation of type I collagen genes → brittle, easily fractured bones.
- ▶ **Ehlers–Danlos syndrome** — defective collagen/fibre synthesis → hyperextensible skin, joint laxity.
- ▶ **Marfan syndrome** — FBN1 (fibrillin-1) mutation → weak elastic fibres, causing tall stature with long limbs/digits, lens dislocation and aortic aneurysm/dissection.
- ▶ **Alport syndrome** — type IV collagen defect → haematuria, sensorineural deafness, ocular abnormalities (glomerular/lens/cochlear basement membranes).
- ▶ **Scurvy** — vitamin C deficiency impairs hydroxylation of proline/lysine during collagen synthesis, causing delayed wound healing and fragile capillaries.
- ▶ **Wound healing** — fibroblasts proliferate and lay down new collagen; myofibroblasts cause wound contraction, forming granulation tissue.
- ▶ **Oedema** — excess extracellular fluid accumulating within the ground substance/loose connective tissue.
- ▶ **Floating kidney** — loss of perinephric (adipose) connective tissue support allows abnormal renal mobility.
- ▶ **Special stains used diagnostically** — Masson's trichrome/van Gieson for collagen, silver impregnation for reticular fibres, orcein/Verhoeff for elastic fibres — routinely used in histopathology to characterise fibrotic, vascular and connective-tissue disease.

High-yield exam pointers

- ▶ Connective tissue = **cells + fibres + ground substance**; mesenchymal (or neural crest) in origin.
- ▶ Three-tier classification: **Embryonic (mesenchyme, mucoid) → Connective tissue proper (loose, dense-regular/irregular, special-property variants) → Specialized (cartilage, bone, adipose, blood, lymphoid tissue)**.
- ▶ Cells: **resident** (fibroblast, adipocyte, macrophage, mast cell, mesenchymal stem cell, pigment cell) vs **wandering** (lymphocyte, plasma cell, monocyte, granulocytes).
- ▶ Fibres: **collagen** (Type I, strongest, 90%), **reticular** (Type III, argyrophilic), **elastic** (elastin + fibrillin-1, recoil).
- ▶ Ground substance = **GAGs + proteoglycans + glycoproteins**; hyaluronic acid is the key hydrating GAG.
- ▶ Loose CT is cell/ground-substance-rich and mobile; dense CT is fibre-rich and resistant to stress (regular = one direction, irregular = all directions).
- ▶ Macrophage lineage = **mononuclear phagocyte system** — renamed by site (Kupffer, osteoclast, microglia, Langerhans, alveolar macrophage).
- ▶ Key eponymous/genetic disorders: **osteogenesis imperfecta** (Type I collagen), **Ehlers–Danlos** (collagen/fibre), **Marfan** (fibrillin-1), **Alport** (Type IV collagen).

SOURCE & COVERAGE

Gray's Anatomy (Standing) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh's Textbook of Human Histology 9e; Sontakke's Textbook of Human Histology 2e; Vishram Singh's General Anatomy 4e (figure).



Describe the microscopic anatomy of bone tissue and discuss the formation of osteoclasts

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction Bone (osseous tissue) is a specialised, mineralised connective tissue composed of a calcified extracellular matrix and three principal resident cell lineages — osteoprogenitor cells/osteoblasts, osteocytes, and osteoclasts. It provides mechanical support, protects viscera, forms a lever system for muscle action, houses the marrow, and acts as the body's principal reservoir of calcium and phosphate. Microscopically it exists as **woven** or **lamellar** bone, and macroscopically as **compact** (cortical) or **cancellous (trabecular/spongy)** bone; the histological unit of compact bone is the **osteon (Haversian system)**. Osteoclasts, uniquely among bone cells, are not of mesenchymal origin but arise by fusion of monocyte–macrophage lineage cells under cytokine control — the RANK–RANKL–OPG axis being central to their formation and regulation.

Bone matrix — composition and calcification

- ▶ **Inorganic component** ($\approx 50\%$ dry weight): mainly **calcium hydroxyapatite crystals** $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, plus bicarbonate, citrate, magnesium, potassium and sodium ions; a surface hydration shell allows ionic exchange with body fluid.
- ▶ **Organic component (osteoid)**: **type I collagen** forms $\sim 90\%$ of the organic matrix; the remainder is ground substance — small proteoglycans and multiadhesive glycoproteins (**osteonectin**), plus the calcium-binding, vitamin K–dependent protein **osteocalcin**.
- ▶ **Mineralisation**: osteoblasts release membrane-bound **matrix vesicles** rich in alkaline phosphatase, which locally raise PO_4^{3-} concentration; osteocalcin and glycoproteins concentrate Ca^{2+} ; hydroxyapatite nucleates within and around the vesicles and around collagen fibrils, and crystals grow by accretion to a confluent calcified mass.
- ▶ Decalcified bone is soft, pliable and strongly acidophilic (high collagen content) — the basis of histological sectioning after decalcification (or plastic embedding for undecalcified ground sections).

Cells of bone tissue

Cell	Origin	Location	Light-microscopic features	Function
Osteoprogenitor cell	Mesenchymal stem cell	Inner (cambium) layer of periosteum, endosteum, marrow stroma	Flattened/spindle-shaped, squamous nucleus; resembles fibroblast	Proliferates; differentiates into osteoblasts (driven by transcription factor CBFA1/RUNX2)
Osteoblast	Osteoprogenitor cell	Single layer on bone surface	Cuboidal to low-columnar, basophilic cytoplasm (abundant RER/Golgi), eccentric nucleus; joined by gap and adherens junctions	Synthesises and secretes type I collagen, osteocalcin, osteonectin (osteoid); mineralises matrix; expresses M-CSF and RANKL, driving osteoclast formation
Osteocyte	Osteoblast entrapped in matrix	Singly within a lacuna between lamellae	Ellipsoid cell body, scanty basophilic cytoplasm, condensed chromatin, reduced RER/Golgi vs osteoblast; long dendritic processes in canaliculi ($\approx 0.25\text{--}0.5\ \mu\text{m}$ wide)	Maintains matrix; mechanosensor ("mechanostat") for load and micro-damage via the lacuno-canalicular network; signals osteoblasts/osteoclasts; average lifespan measured in years
Bone-lining cell	Quiescent osteoblast/osteoprogenitor	Flattened layer on inactive surfaces (periosteal/endosteal)	Flat, attenuated	Regulates ionic traffic; a reserve of osteoprogenitor activity
Osteoclast	Fusion of monocyte lineage cells (haemopoietic, NOT mesenchymal)	Bone surface, in Howship's (resorption) lacunae	Large, multinucleate (many nuclei), acidophilic, foamy cytoplasm	Resorbs mineralised matrix during growth and remodelling

- Osteocytes are the most numerous bone cells; osteoclasts are the least numerous but largest (diameter $40\ \mu\text{m}$ or more).
- Osteocyte processes contact one another and neighbouring osteoblasts/lining cells via **gap junctions**, forming a continuous lacuno-canalicular network essential for nutrient diffusion (metabolites cannot cross calcified matrix directly) and mechanotransduction.

Osteoclasts — structure and resorptive function

- ▶ Osteoclasts sit within shallow enzymatically-etched depressions on the bone surface called **resorption lacunae** (Howship's lacunae).
- ▶ The cell membrane facing bone shows two specialised zones:
 - **Sealing (clear) zone:** a circumferential ring, poor in organelles, rich in actin and **integrins** ($\alpha\text{v}\beta 3$ vitronectin receptor, $\alpha 2\beta 1$ collagen receptor) that bind tightly to matrix proteins, sealing off a microenvironment beneath the cell.
 - **Ruffled border:** the central zone of deep membrane infoldings/microvilli facing the sealed subosteoclastic space; contains proton (V-type H^+ -ATPase) pumps and chloride channels.
- ▶ Within the sealed compartment the ruffled border pumps H^+ to acidify the space to $\sim\text{pH } 4.5$, dissolving hydroxyapatite, and secretes lysosomal/non-lysosomal hydrolytic enzymes (acid proteases, matrix metalloproteinases) that digest collagen and other organic matrix; degraded products are endocytosed and further digested intracellularly, while Ca^{2+} and phosphate are released into blood.
- ▶ **Tartrate-resistant acid phosphatase (TRAP)** is a histochemical/clinical marker of osteoclast activity and differentiation.
- ▶ Osteoclasts are **phagocytic, motile cells**; on a bone surface a single osteoclast can migrate, cutting a trench (seen well on scanning electron microscopy).

Formation of osteoclasts (osteoclastogenesis)

Unlike osteoblasts/osteocytes, osteoclasts are **not derived from mesenchyme** — they arise from **haemopoietic progenitor cells of the granulocyte–monocyte lineage** (GMP/CFU-GM), which in turn derive from the common myeloid progenitor (CFU-GEMM), by **fusion of several mononuclear cells** into one large multinucleate osteoclast.

- ▶ **Stromal/osteoblastic support and M-CSF:** differentiation occurs in close association with bone-marrow stromal cells/osteoblasts, which secrete **macrophage colony-stimulating factor (M-CSF)** — acting via receptor c-Fms to drive precursor proliferation (TNF, IL-1, IL-6 also contribute). This shared stromal dependency is why osteoclasts were once wrongly thought to share lineage with osteoblasts.
- ▶ **Early transcription factors:** committed precursors express **c-fos** and **NF- κB** , required for further differentiation, after which the receptor **RANK** appears on the precursor surface.
- ▶ **The RANK–RANKL–OPG axis** (central regulatory triad): **RANKL** (a TNF-family ligand) is expressed on osteoblast/stromal-cell surfaces — and, in inflammation, released in soluble form by activated T lymphocytes. Its binding to **RANK** on precursors is the obligatory signal for **differentiation, fusion and activation**. **Osteoprotegerin (OPG)**, also osteoblast-secreted, is a soluble **decoy receptor** that competitively blocks RANK–RANKL binding, restraining osteoclastogenesis; the local **RANKL:OPG ratio** sets the resorption/formation balance. PTH raises RANKL (and, with prolonged exposure, lowers OPG); PGE_2 from stressed osteocytes raises RANKL; oestrogen suppresses RANKL (its withdrawal underlies postmenopausal osteoporosis); calcitonin directly inhibits mature osteoclasts.
- ▶ **Fusion and maturation:** signalled precursors fuse into the large multinucleate osteoclast, which polarises against bone, forms its sealing zone and ruffled border, and begins resorption (markers:

- The outer boundary of each osteon is the **cement line**, rich in non-collagenous protein, poor in collagen.
- ▶ **Cancellous (trabecular/spongy) bone** ($\approx 20\%$ of skeletal mass; epiphyses, vertebral bodies, flat-bone diploë): also lamellar in the adult, but organised as anastomosing **trabeculae/spicules** without true osteons; osteocytes here rely on canalicular diffusion from adjacent marrow rather than a central Haversian vessel.
- ▶ **Periosteum**: outer fibrous (collagenous, vascular) layer + inner cellular (cambium) layer of osteoprogenitor cells/osteoblasts; anchored to bone by **Sharpey's (perforating) fibres**.
Endosteum: a thin, single-cell layer of osteoprogenitor/bone-lining cells covering trabeculae, the marrow cavity surface, and Haversian/Volkmann's canal walls.
- ▶ **Bone remodelling**: a continuous, coupled osteoclast–osteoblast "cutting cone" process — osteoclasts tunnel a cylindrical cavity, osteoprogenitor cells/capillaries follow, and osteoblasts line the tunnel depositing successive lamellae centripetally to build a new osteon, trapping osteocytes as they go; $\sim 5\text{--}10\%$ of adult bone turns over annually.

□ Fig 8.8 — Lamellar bone. (Junqueira's Basic Histology — Text & Atlas — 17e, p.366)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled transverse section through compact bone showing an osteon (Haversian system) — label:

- ▶ Haversian (central) canal with blood vessel
- ▶ Concentric lamellae (5–20, around the canal)
- ▶ Osteocyte lacunae (between lamellae) with radiating canaliculi
- ▶ Volkmann's (perforating) canal (oblique, connecting adjacent osteons)
- ▶ Cement line (outer boundary of the osteon)
- ▶ Interstitial lamellae (remnants of older eroded osteons, between intact osteons)
- ▶ Outer and inner circumferential lamellae
- ▶ Periosteum (outer surface) and endosteum (inner/canal surfaces)
- ▶ An osteoclast in a Howship's lacuna and an osteoblast row, shown at a remodelling front

Applied / Clinical anatomy

- ▶ **Osteoporosis**: resorption exceeds formation (postmenopausal oestrogen loss, immobilisation, age) via a raised RANKL:OPG ratio — reduced bone mineral density, increased fracture risk; assessed by **DEXA scan**.
- ▶ **Osteopetrosis ("marble bone disease")**: genetic defect of osteoclast function (commonly proton-pump/chloride-channel mutations) — ruffled border absent, resorption fails, bones dense but brittle, marrow cavities obliterated causing anaemia and leucopenia.
- ▶ **Anti-resorptive therapy**: **denosumab** (anti-RANKL monoclonal antibody, mimics OPG) and **bisphosphonates** (induce osteoclast apoptosis) both suppress osteoclast number/activity — used in osteoporosis and skeletal metastases.
- ▶ **Tetracycline double-labelling**: binds actively mineralising osteoid and fluoresces under UV — used on bone biopsy to measure the mineral apposition rate in metabolic bone disease.

- ▶ **PTH/calcitonin:** PTH acts on osteoblasts (osteoclasts lack PTH receptors — the resorptive effect is indirect, via RANKL) to raise serum Ca^{2+} ; calcitonin from thyroid C cells directly inhibits osteoclasts, lowering serum Ca^{2+} — used therapeutically in hypercalcaemia and Paget's disease.
- ▶ **Osteosarcoma** (malignant osteoprogenitor/osteoblastic lineage) and skeletal **metastasis** (breast, lung, prostate, kidney, thyroid) are the principal bone tumours encountered clinically; **fracture healing** recapitulates the woven-to-lamellar remodelling sequence described above.

High-yield exam pointers

- ▶ Three resident bone-forming cells (osteoprogenitor → osteoblast → osteocyte) are of **mesenchymal** origin; osteoclasts are of **haemopoietic (monocyte–macrophage/GMP)** origin — do not confuse the two lineages.
- ▶ Osteoclast formation is an obligatory **RANK–RANKL–OPG** signalling event: RANKL (osteoblast/stromal-derived) + RANK (on precursor) = differentiation and fusion; OPG = decoy receptor that blocks it.
- ▶ M-CSF is required upstream for osteoclast precursor proliferation; c-fos and NF- κ B are the early transcription factors in committed precursors.
- ▶ Osteoclast resorptive apparatus = **sealing (clear) zone** (integrin-anchored) + **ruffled border** (proton pumps, hydrolytic enzymes) around a Howship's lacuna.
- ▶ TRAP is the histochemical marker of osteoclast activity.
- ▶ Lamellar bone = mature, plywood-like, birefringent under polarised light; woven bone = immature, randomly arranged, seen in fetal bone, callus, and tumours.
- ▶ Osteon = Haversian canal + concentric lamellae + osteocyte lacunae/canaliculi; connected transversely by Volkmann's canals; older eroded osteon remnants = interstitial lamellae.
- ▶ Osteoporosis = $\uparrow$ RANKL:OPG (resorption > formation); osteopetrosis = defective osteoclast ruffled border (resorption failure).
- ▶ Denosumab (anti-RANKL) and bisphosphonates both act by suppressing osteoclast number/activity, though by different mechanisms.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e — Chapter 8, Bone (bone matrix, bone cells, periosteum/endosteum, lamellar/woven bone, osteon structure, osteoclast structure and RANKL/M-CSF signalling, remodelling, medical applications). Ross Histology: A Text and Atlas 7e — Chapter 8, Bone (osteoprogenitor cell/CBFA1, osteoclast origin from GMP/CFU-GM, detailed RANK–RANKL–OPG/c-fos/NF- κ B osteoclastogenesis pathway, TRAP, integrins of the sealing zone, denosumab). Gray's Anatomy (Standring) 41e — Chapter 5 (Cells, Tissues and Systems / Functional anatomy of the musculoskeletal system) for cross-confirmation of osteoblast/osteocyte/osteoclast histology, lamellar bone microarchitecture, Haversian/Volkmann canals, and the RANK–RANKL–osteoprotegerin pathway.



Histology of bone

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction Bone is a specialised, highly vascular form of connective tissue ("sclerous connective tissue") in which the collagenous extracellular matrix is impregnated with inorganic calcium salts, giving it rigidity combined with a degree of resilience. Despite its hardness, bone is a dynamic, living tissue that continuously undergoes remodelling (resorption + formation) throughout life to adapt to mechanical stress and to serve as the body's principal calcium/phosphate reservoir. Histologically, bone tissue is classified by maturity into **woven** and **lamellar** bone, and lamellar bone is further organised, at the gross-microscopic level, into **compact (cortical)** and **cancellous (trabecular/spongy)** bone.

General features and functions

- ▶ Rigid, hard connective tissue with power of regeneration and repair.
- ▶ Functions: bears weight/mechanical support; provides attachment for muscles and tendons (movement); protects vital organs (skull, thoracic cage); reservoir of calcium, phosphate and other ions; houses haematopoietic marrow.
- ▶ Bone surfaces are covered by **periosteum** (external) and **endosteum** (internal, lining marrow cavity, trabecular surfaces and Haversian canals).

Composition of bone tissue — cells

Cell	Origin	Location	Function
Osteoprogenitor cell	Mesenchymal stem cell	Inner (cambium) layer of periosteum, endosteum	Proliferates; differentiates into osteoblasts when bone formation is needed
Osteoblast	Osteoprogenitor cell	Rims surface of forming bone (single layer, cuboidal)	Secretes organic matrix (osteoid: type I collagen + ground substance); initiates mineralisation via alkaline-phosphatase-rich matrix vesicles
Osteocyte	Osteoblast trapped in its own matrix	Singly within lacunae between/within lamellae	Maintains matrix; mechanosensor; exchanges metabolites via canaliculi; can also participate in matrix removal/deposition
Osteoclast	Fusion of monocyte/macrophage precursors (haemopoietic lineage)	Large multinucleated cell in resorption bays (Howship's lacunae) on bone surface	Bone resorption — ruffled border + sealing zone create acidic microenvironment that dissolves hydroxyapatite and digests matrix

Composition of bone tissue — matrix

- **Organic matrix (osteoid)** — about 35% of dry weight: mainly **type I collagen** (~90% of organic matter, arranged in lamellae) plus ground substance — proteoglycans, and non-collagenous calcium-binding glycoproteins (**osteocalcin**, **osteonectin**) that promote and regulate mineralisation.
- **Inorganic matrix** — about 65% of dry weight: mainly **calcium hydroxyapatite crystals** $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, plus amorphous calcium phosphate, carbonate, citrate, magnesium and sodium; hydroxyapatite crystals lie parallel to and coat the collagen fibres, producing the lamellar birefringence seen under polarised light.
- Living bone also contains ~20% water by weight. Bone is the body's largest calcium store (~99% of total body calcium).

Classification of bone tissue — woven vs lamellar

Feature	Woven (primary/immature) bone	Lamellar (secondary/mature) bone
Collagen arrangement	Randomly oriented bundles	Organised in parallel sheets (lamellae), fibre direction shifting ~90° between adjacent lamellae
Cell density	More osteocytes, more irregularly shaped	Fewer, regularly arranged osteocytes
Mineralisation	Mineralises rapidly; less strength	Slower, highly ordered mineralisation; greater strength
Occurrence	First bone formed in the fetus, at fracture-repair sites, in Paget disease	Replaces woven bone in postnatal life; forms all adult compact and cancellous bone

Woven bone is always remodelled into lamellar bone by coordinated osteoclastic resorption followed by osteoblastic deposition.

Microscopic architecture of compact bone

Compact (cortical) bone forms the dense outer shell of all bones and the shaft of long bones — it constitutes about **80% of total adult bone mass**.

- **Haversian system (osteon)** — the structural unit of compact bone: a cylinder of **concentric lamellae** (typically 5–20 lamellae) surrounding a central **Haversian (osteonal) canal** that carries blood vessels, lymphatics and nerves. Osteons run mainly parallel to the long axis of the bone; there are an estimated **21 million osteons** in the adult skeleton, each **100–400 µm** in diameter; the Haversian canal itself has a mean diameter of about **50 µm**.
- **Types of lamellae:**
- **Circumferential lamellae** — outer (beneath periosteum) and inner (around marrow cavity), running parallel to the bone surface.
- **Concentric lamellae** — form the wall of each osteon around its Haversian canal.
- **Interstitial lamellae** — irregular, angular fragments between osteons, representing remnants of older, partly resorbed osteons.

- **Lacunae and canaliculi** — each lamella is separated from the next by flattened lacunae, each housing one osteocyte; fine canaliculi (~0.25–0.5 µm) radiate from every lacuna, interconnecting with those of neighbouring lacunae and ultimately with the Haversian canal, carrying the osteocytes' cytoplasmic processes and forming the diffusion pathway for nutrients/metabolites. The osteon's diameter ensures no osteocyte lies more than ~200 µm from a blood vessel.
- **Volkman's (perforating) canals** — transverse/oblique channels that connect adjacent Haversian canals to each other and to the periosteal and medullary surfaces; unlike Haversian canals, they are not surrounded by concentric lamellae.
- **Cement line** — a thin, collagen-poor, basophilic line marking the outer boundary of an osteon (where it abuts interstitial lamellae or an adjacent osteon); a few canaliculi cross it, but it otherwise arrests crack propagation, adding toughness.
- **Primary (atypical) vs secondary (typical) osteons** — the first osteons formed during ossification lack a fully lamellar pattern and no cement line surrounds them (primary/atypical Haversian systems); they are subsequently eroded by osteoclasts and refilled by concentrically deposited lamellae bounded by a cement line, becoming secondary (typical) Haversian systems — the basis of continuing bone remodelling (5–10% of adult compact bone turns over per year).

Microscopic architecture of cancellous bone

Cancellous (trabecular/spongy) bone forms the interior of bones — the epiphyseal ends of long bones and the interior of short/flat/irregular bones — and constitutes about **20% of total bone mass**.

- Composed of anastomosing **trabeculae** (bars/plates) enclosing marrow-filled intercommunicating spaces.
- Each trabeculum is made of several lamellae with lacunae/osteocytes and canaliculi, exactly as in compact bone, but is generally too thin to contain a true Haversian system; osteocytes are nourished by diffusion from the adjacent marrow via canaliculi rather than through a central vascular canal.
- Trabeculae are covered by vascular **endosteum** containing osteoblasts, osteoclasts and osteoprogenitor cells, and are oriented along the lines of mechanical stress (**Wolff's law**) — e.g., the biplanar trabecular pattern at the upper end of the femur resisting compressive and tensile forces.

Periosteum and endosteum

Layer	Composition	Function
Periosteum (outer fibrous layer)	Dense connective tissue, type I collagen, fibroblasts, blood vessels	Limiting membrane; nutritive; anchored to bone by Sharpey's (perforating) fibres embedded in the outer circumferential lamellae
Periosteum (inner osteogenic/cambium layer)	Osteoprogenitor cells, osteoblasts (active in growth/repair)	Appositional (width) growth of bone; fracture repair
Endosteum	Single layer of flattened osteoprogenitor cells/osteoblasts/bone-lining cells, sparse collagen	Lines marrow cavity, trabeculae and Haversian/Volkman canals; site of internal remodelling and resorption

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Compact bone in transverse section (a single osteon and its neighbours), labelling: **Haversian (central) canal** with blood vessels and nerve; **concentric lamellae**; **lacunae** with **osteocytes**; **canaliculi** radiating from lacunae; **cement line** demarcating the osteon; **interstitial lamellae** between osteons; **Volkman's (perforating) canal** connecting two Haversian canals; **outer and inner circumferential lamellae**; **periosteum** (with Sharpey's fibres) and **endosteum**. A second, simpler panel should show cancellous bone: **trabeculae**, intertrabecular **marrow space**, and **endosteal lining**.

 Fig 8.7 — Compact and cancellous bone. (Junqueira's Basic Histology — Text & Atlas — 17e, p.364)

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- ▶ **Osteoporosis** — resorption exceeds formation (postmenopausal women, immobilised patients), reducing bone mineral density; assessed clinically by **DEXA scan**; increases fracture risk (neck of femur, vertebrae, wrist).
- ▶ **Osteopetrosis ("marble bone disease")** — defective osteoclasts (ruffled-border/proton-pump or chloride-channel mutations) fail to resorb bone, producing dense, brittle bone with obliterated marrow cavities → anaemia and pancytopenia.
- ▶ **Paget disease (osteitis deformans)** — described by Sir James Paget (1877); disordered, accelerated remodelling with persistence of woven bone; monostotic or polyostotic; typically affects males over 50 years; produces weak, deformed, thickened bone.
- ▶ **Osteoma / osteosarcoma** — benign and malignant tumours of osteoblastic origin respectively; osteosarcoma classically arises around the knee (distal femur/proximal tibia) and metastasises haematogenously.
- ▶ **Fracture healing** — haematoma → procallus (granulation tissue) → soft callus (fibrocartilage/woven bone) bridging the fragments → hard (bony) callus by endochondral and intramembranous ossification → remodelling of woven callus into lamellar bone restores the original architecture; periosteal integrity accelerates healing.
- ▶ **Wolff's law** — trabecular architecture (and cortical thickness) is laid down along lines of maximum mechanical stress; clinically relevant to implant/prosthesis design and to the loss of trabecular pattern with disuse.
- ▶ **Osteomyelitis** — favours the metaphysis of growing long bones, where nutrient vessels form hairpin loops with sluggish flow, predisposing to bacterial seeding.
- ▶ **Decalcified bone** (histology sections) is rendered soft and acidophilic (high collagen content) once mineral is removed by acid/EDTA — the basis of routine bone histoprocessing.

High-yield exam pointers

- ▶ Three bone cells to name with function: osteoblast (forms), osteocyte (maintains), osteoclast (resorbs) — plus the osteoprogenitor stem cell.
- ▶ Matrix ratio: ~65% inorganic (hydroxyapatite) : ~35% organic (90% of which is type I collagen).

- ▶ Osteon = Haversian canal + concentric lamellae; three lamella types = circumferential, concentric, interstitial.
- ▶ Haversian canals run longitudinally and communicate via **transverse Volkmann's canals** (no concentric lamellae around the latter).
- ▶ Cement line = collagen-poor boundary of an osteon; canaliculi = diffusion channels linking lacunae to the vascular Haversian canal.
- ▶ Compact bone $\approx$ 80% of skeletal mass; cancellous bone $\approx$ 20%, organised as stress-oriented trabeculae (Wolff's law).
- ▶ Woven bone is remodelled into lamellar bone; osteoclasts resorb in Howship's lacunae, osteoblasts refill in a coordinated remodelling cycle.
- ▶ Periosteum anchored by Sharpey's fibres; osteogenic (inner) layer of periosteum and the endosteum are the reserve of osteoprogenitor cells for growth/repair.
- ▶ Clinical correlates: osteoporosis (DEXA), osteopetrosis, Paget disease, osteosarcoma, fracture callus, osteomyelitis of the metaphysis.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e.



Histology of Compact Bone

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction — Bone is a specialised, sclerous connective tissue in which the extracellular matrix is impregnated with inorganic salts (mainly calcium phosphate), giving it rigidity while retaining a capacity for continuous remodelling and repair. On naked-eye section it exists in two architectural forms — **compact (cortical) bone**, a dense wall with no visible spaces, forming the shaft (diaphysis) of long bones and the outer shell of all bones; and **spongy (cancellous) bone**, a meshwork of trabeculae found at bone ends and in the diploë. Compact bone is organised almost entirely as **lamellar bone**, its structural and functional unit being the **osteon or Haversian system** — a cylinder of concentric lamellae around a central vascular canal.

Cells of compact bone

Cell	Origin	Key features	Function
Osteoprogenitor cell	Mesenchymal stem cell	Fibroblast-like; lies on periosteal/endosteal surfaces	Divides and differentiates into osteoblasts when bone formation is needed

Cell	Origin	Key features	Function
Osteoblast	Osteoprogenitor cell	Cuboidal, basophilic cytoplasm (abundant RER + Golgi); rich in alkaline phosphatase; slender processes contact neighbours	Synthesises collagen I and ground substance (osteoid); initiates its mineralisation; regulates osteoclasts via RANKL/OPG
Osteocyte	"Imprisoned" osteoblast	Lies in a lacuna ; dendritic processes run in canaliculi and meet at gap junctions; sparse RER	Maintains the lacuno-canalicular network; mechanosensation; limited matrix turnover
Osteoclast	Monocyte–macrophage lineage (fusion of precursors)	Large, multinucleated (up to 20+ nuclei, 20–100 μm); ruffled border; acid-phosphatase- and cathepsin-K-rich lysosomes; occupies a Howship's lacuna (resorption bay)	Resorbs bone by acid demineralisation + enzymatic matrix digestion; stimulated by PTH, calcitriol, RANKL; inhibited by calcitonin and osteoprotegerin

Bone matrix

- **Organic matrix** ($\approx 35\%$ of dry weight; **osteoid before mineralisation**) — 90% type I collagen fibres, arranged in parallel bundles within each lamella; ground substance of glycosaminoglycans and proteoglycans; the glycoproteins **osteonectin** and **osteocalcin** bind calcium and promote mineralisation (vitamin D stimulates their synthesis).
- **Inorganic matrix** ($\approx 65\%$ of dry weight) — chiefly **hydroxyapatite crystals**, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, lying parallel to collagen fibres; about 85% of the mineral is calcium phosphate and 10% calcium carbonate; magnesium, citrate, fluoride and sodium are present in smaller amounts. Roughly 97% of total body calcium is stored in bone, exchanged with blood under parathormone and calcitonin control.
- **Decalcification** (weak acids/EDTA) removes minerals and leaves a soft, pliable organic framework; **calcination** (heat) destroys the organic component and leaves a brittle mineral framework — together these classical experiments demonstrate that mineral gives bone its hardness and collagen gives it tensile strength.

Bone membranes

- **Periosteum** covers all external bone surfaces except articular cartilage and tendon/ligament entheses; it has an **outer fibrous layer** and an **inner cellular (osteogenic/cambium) layer** rich in osteoprogenitor cells. Sharpey's perforating fibres anchor tendons/ligaments firmly into the outer lamellae. Periosteum carries the nutrient blood supply and is critical for fracture repair.
- **Endosteum** is a thin vascular layer lining the medullary cavity, trabecular surfaces and the Haversian canals; it too retains osteogenic potential and provides a very large surface ($\sim 7.5 \text{ m}^2$) for calcium exchange.

Lamellar organisation of compact bone

Compact bone is built from **lamellae** — thin (2–3 μm -thick collagen bundle), sheet-like layers of mineralised matrix, 3–7 μm thick, with the collagen orientation shifting by about 90° between successive lamellae (the basis of **birefringence** under polarised light, a key identification feature).

Type of lamella	Location	Description
Outer circumferential lamellae	Immediately beneath the periosteum	Parallel to the bone surface; encircle the whole shaft
Inner circumferential lamellae	Around the medullary cavity	Fewer in number than the outer set
Concentric (Haversian) lamellae	Around each central Haversian canal	5–20 (average ≈ 6) lamellae per osteon; the bulk of compact bone
Interstitial lamellae	Irregular gaps between osteons	Fragmentary remains of older osteons destroyed by remodelling

The osteon (Haversian system)

The osteon is the structural and functional unit of compact bone: a **central Haversian canal**, containing one or two capillaries, unmyelinated (occasionally myelinated) nerve fibres and endosteal lining, surrounded by concentric lamellae.

Parameter	Value
Osteon diameter	100–400 μm
Lamellae per osteon	5–20 (average ≈ 6)
Haversian canal mean diameter	$\approx 50 \mu\text{m}$
Canaliculus width	$\approx 0.25\text{--}0.5 \mu\text{m}$
Maximum osteocyte-to-vessel distance	$\approx 200 \mu\text{m}$ (diffusion limit)
Osteons in a typical adult skeleton	≈ 21 million

- ▶ Osteons run mainly **parallel to the long axis of the bone**, but may be spiral, may branch, and some end blindly; in cross-section they appear round, oval or ellipsoidal.
- ▶ **Canaliculi** radiating from each lacuna interconnect adjacent lacunae and open into the Haversian canal, forming the lacuno-canalicular network for diffusion of nutrients/metabolites between osteocytes and blood vessels; peripheral canaliculi of one osteon usually loop back within the same osteon and do not, as a rule, cross into the next.
- ▶ The outer boundary of each osteon is marked by a strongly basophilic **cement (reversal) line**, glycoprotein-rich and virtually devoid of collagen; a few canaliculi do cross it, linking osteon to interstitial lamellae.
- ▶ **Volkman's canals** run obliquely/transversely, connecting Haversian canals to each other and to the periosteal and marrow-cavity circulations; unlike Haversian canals they are **not** surrounded by concentric lamellae — the key feature distinguishing the two.

- **Primary osteons** (formed of woven bone, poorly lamellated) are progressively replaced during growth and remodelling by **secondary osteons** (true Haversian systems with well-formed concentric lamellae).

□ Fig 6.7 — Haversian system in compact bone (schematic representation). (IB Singh — Textbook of Human Histology — 9e, p.183)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

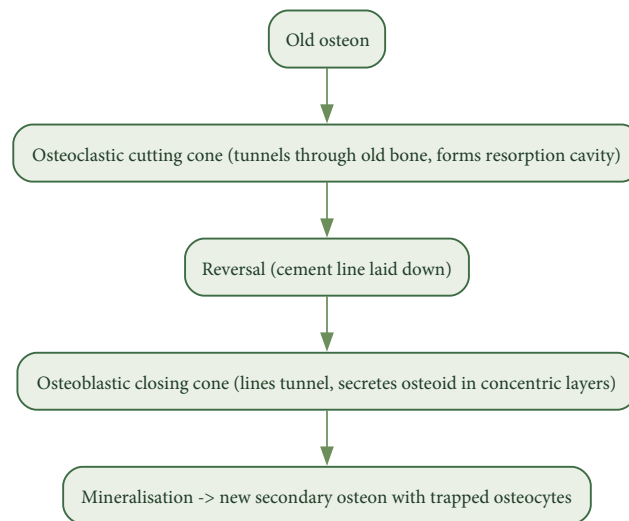
Draw a transverse ground section of compact bone and label:

- Haversian (central) canal — with blood vessel and nerve
- Concentric (Haversian) lamellae — 5–20 rings around the canal
- Lacunae with osteocytes (between lamellae)
- Canaliculi radiating from lacunae
- Cement (reversal) line — outer boundary of the osteon
- Interstitial lamellae — irregular fragments between osteons
- Outer and inner circumferential lamellae
- Volkmann's canal — transverse, connecting adjacent Haversian canals (no concentric lamellae)
- Periosteum (outer) and endosteum (lining canals/marrow cavity)

Remodelling: woven bone, lamellar bone, and osteon renewal

Feature	Woven (primary) bone	Lamellar (secondary) bone
Collagen arrangement	Random, interlacing bundles	Organised parallel sheets, 90° alternation between lamellae
When seen	Fetal bone, fracture callus, rapid remodelling	Almost all adult bone
Strength	Mechanically weaker	Stronger (crack-stopping lamellar interfaces)
Fate	Remodelled into lamellar bone	Stable structural form

Compact bone remodels continuously ($\approx 5\text{--}10\%$ of adult skeletal bone turns over annually):

*Histology of Compact Bone*

Applied / Clinical anatomy

- ▶ **Osteoporosis** — reduced bone mass (osteopaenia progressing to osteoporosis) with cortical thinning and enlargement of Haversian/marrow spaces; fragility fractures result; accelerated in postmenopausal women.
- ▶ **Osteomalacia / rickets** — defective mineralisation of osteoid (adult and growing skeleton respectively) leaving excess uncalcified osteoid seams; bone becomes soft and deformable.
- ▶ **Osteopetrosis** — a disorder of defective osteoclast function; failure of bone resorption produces abnormally dense but brittle bone.
- ▶ **Paget's disease (osteitis deformans)** — described by Sir James Paget (1877); disordered, excessive osteoclastic and osteoblastic remodelling produces abnormal persistence of woven bone, an irregular mosaic of cement lines, and weak, deformed bone; monostotic or polyostotic, chiefly in males over 50.
- ▶ **Osteoma / osteosarcoma** — benign and malignant tumours of osteoblastic origin respectively; osteosarcoma classically arises near the knee and metastasises haematogenously.
- ▶ **Fracture healing** — depends critically on an intact periosteum for osteoprogenitor supply; where periosteum is deficient (e.g., intracapsular femoral neck fracture) healing is slow/unreliable.
- ▶ **Tetracycline bone labelling** — tetracycline binds newly mineralising osteoid and fluoresces under UV light; double labelling at a known interval allows measurement of the rate of bone apposition — used diagnostically in osteomalacia and high-turnover states.
- ▶ **Age estimation from ground sections** — osteon number/density and the proportion of remodelled (secondary) versus primary bone increase with age, a principle used in forensic skeletal age estimation.
- ▶ **Radiology** — cortical (compact) bone is markedly radiopaque compared with the coarser trabecular pattern of cancellous bone; DEXA scanning quantifies cortical and trabecular bone mineral density for osteoporosis screening.

High-yield exam pointers

- ▶ Osteon (Haversian system) = structural + functional unit of compact bone; central Haversian canal + 5–20 (avg. 6) concentric lamellae.
- ▶ Three (four, counting outer/inner separately) types of lamellae: outer circumferential, inner circumferential, concentric (Haversian), interstitial.
- ▶ Osteon diameter 100–400 μm ; Haversian canal mean diameter $\approx 50 \mu\text{m}$; canaliculi $\approx 0.25\text{--}0.5 \mu\text{m}$ wide; no osteocyte lies $>200 \mu\text{m}$ from a vessel.
- ▶ Volkmann's canals are transverse/oblique and lack concentric lamellae — the distinguishing point from Haversian canals.
- ▶ Cement (reversal) line = outer boundary of an osteon, collagen-poor, strongly basophilic.
- ▶ Four cell types: osteoprogenitor cell, osteoblast, osteocyte, osteoclast — know origin, location, and one defining ultrastructural feature of each.
- ▶ Birefringence under polarised light (alternating bright/dark bands) reflects 90° alternation of collagen orientation between adjacent lamellae — a classical identification test.
- ▶ Woven bone (primary, random collagen) is always replaced by lamellar bone except at a few permanent sites (sutures, tendon insertions).
- ▶ Remodelling sequence: osteoclastic resorption (cutting cone) $\rightarrow$ reversal (cement line) $\rightarrow$ osteoblastic formation (closing cone) $\rightarrow$ mineralisation into a new secondary osteon.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Vishram Singh — General Anatomy 4e; Sontakke — Textbook of Human Histology 2e.



Histology of Skeletal muscle

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction

Skeletal (striated, voluntary) muscle is one of the three types of muscle tissue and constitutes 40–50% of total body mass. It is composed of long, cylindrical, multinucleated fibres (myocytes) that develop by fusion of mesenchymal myoblasts and contract rapidly and forcefully, usually under voluntary control, to move the skeleton. Its histological hallmark at light-microscopic and ultrastructural level is a regular, repeating banding pattern produced by the precise register of actin and myosin filaments within contractile units called sarcomeres. Study of its histology is organised at three levels — gross/connective-tissue architecture, light-microscopic fibre structure, and ultrastructure of the sarcomere — followed by its innervation, fibre-type diversity and associated proprioceptors.

1. Gross organization and connective-tissue coverings

- ▶ **Epimysium** — thick external sheath of dense irregular connective tissue enclosing the entire muscle; continuous with deep fascia and the tendon; carries the largest nerves and vessels.

- ▶ **Perimysium** — thin connective-tissue septa (from the epimysium) that divide the muscle into bundles called **fascicles**; a fascicle is the functional unit in which fibres contract together; nerves and vessels supplying a fascicle run in the perimysium.
- ▶ **Endomysium** — delicate layer of reticular fibres and scattered fibroblasts surrounding the external (basal) lamina of each individual muscle fibre within a fascicle; carries the rich capillary network and terminal nerve twigs that supply each fibre.
- ▶ All three layers contain collagen types I and III and are continuous with the dense regular connective tissue of the **tendon** at the myotendinous junction, where collagen bundles of the tendon insert among complex infoldings of the sarcolemma, transmitting the mechanical force of contraction to bone.

□ Fig 7.2 — Connective tissue present in relation to transverse section of skeletal muscle (IB Singh — *Textbook of Human Histology* — 9e, p.211)

2. Light-microscopic structure of the muscle fibre

- ▶ Each **skeletal muscle fibre** is a single, very long, multinucleated cell, 10–100 μm in diameter and up to several centimetres long, bounded by the **sarcolemma** (cell membrane) with **sarcoplasm** as its cytoplasm.
- ▶ **Nuclei** are numerous (up to several hundred per fibre), flattened, and characteristically located **peripherally**, just under the sarcolemma — a nuclear position unique to skeletal muscle (cardiac and smooth muscle nuclei are central).
- ▶ **Satellite cells** — a sparse population of reserve myoblasts lying between the sarcolemma and the external (basal) lamina — persist on mature fibres and are the source of post-injury regeneration and hypertrophy.
- ▶ In transverse section fibres are polygonal, closely packed, and their sarcoplasm has a stippled appearance from transversely cut myofibrils; packing density varies between muscles (loose in the extrinsic laryngeal muscles, dense in the muscles of mastication).
- ▶ In longitudinal section each fibre shows alternating dark and light transverse bands — the **cross-striations** — produced by thousands of parallel **myofibrils** ($\sim 1 \mu\text{m}$ diameter) filling the sarcoplasm, each itself made of a linear series of sarcomeres in exact lateral register with those of neighbouring myofibrils.
- ▶ The dark bands are the **A-bands** (anisotropic, birefringent under polarised light); the light bands are the **I-bands** (isotropic). Special stains or polarised light demonstrate this pattern most clearly.

3. Ultrastructure of the sarcomere

- ▶ The **sarcomere** is the structural and functional contractile unit of the myofibril, extending from one **Z-disc** to the next; it measures about **2.2 μm** in resting muscle.
- ▶ **Z-disc (Zwischenscheibe)** — a dense transverse line bisecting each I-band, formed of the actin-anchoring protein **α -actinin**, marking the boundary of each sarcomere.
- ▶ **A-band** — the dark, central zone occupied by the full length of the thick (myosin) filaments together with the overlapping ends of thin (actin) filaments.
- ▶ **I-band** — the pale zone containing only thin filaments (not overlapped by thick filaments); it is bisected by the Z-disc and narrows during contraction.

- ▶ **H-zone** — the paler central region of the A-band containing only the rod-like (non-globular) portion of the thick filaments, with no thin-filament overlap.
- ▶ **M-line** — bisects the H-zone; contains myomesin, which cross-links adjacent thick filaments, and creatine kinase, which regenerates ATP from phosphocreatine.

Component	Composition	Approx. dimensions
Thick filament	~200–500 myosin molecules (2 heavy chains + 4 light chains per molecule); myosin heads bind actin and ATP	15 nm diameter, ~1.5 μm long
Thin filament	F-actin (double helix) + tropomyosin (blocks myosin-binding site) + troponin complex (TnT–TnC–TnI)	8 nm diameter, ~1 μm long
Titin	Largest protein in the body (~3700 kDa); spans Z-disc to M-line; elastic scaffold anchoring thick filament	Spans half-sarcomere
Nebulin	Runs the length of the thin filament from the Z-disc; sets thin-filament length	Spans thin filament

- ▶ **Dystrophin**, an actin-binding protein on the inner face of the sarcolemma, links the cytoskeleton to the extracellular matrix and stabilises the sarcolemma during contraction (defective in Duchenne muscular dystrophy — see Applied anatomy).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a longitudinally sectioned sarcomere between two Z-discs (as in an EM/light-diagram), labelling: **Z-disc** (both ends) · **I-band** (either side, pale) · **A-band** (central, dark) · **H-zone** (pale centre of A-band) · **M-line** (centre of H-zone) · **thick (myosin) filament** · **thin (actin) filament** · **titin filament** (Z-disc to M-line) · myosin head/cross-bridge. Add a scale note: one sarcomere $\approx$ 2.2 μm resting.

4. Sarcoplasmic reticulum, T-tubule system and excitation–contraction coupling

- ▶ The **sarcoplasmic reticulum (SR)** is a specialised smooth ER surrounding each myofibril, storing Ca^{2+} (via calsequestrin) and possessing Ca^{2+} -ATPase pumps and voltage-gated Ca^{2+} -release channels in its terminal cisternae.
- ▶ **T-tubules (transverse tubules)** are deep, finger-like invaginations of the sarcolemma that encircle each myofibril at the A–I junction, conducting the action potential radially into the fibre interior.
- ▶ A T-tubule flanked by two terminal cisternae of SR constitutes a **triad**; this is the structural basis of excitation–contraction coupling.
- ▶ **Sequence:** NMJ depolarisation $\rightarrow$ sarcolemmal action potential propagates along T-tubules $\rightarrow$ triggers Ca^{2+} release from SR terminal cisternae $\rightarrow$ Ca^{2+} binds troponin-C $\rightarrow$ tropomyosin shifts,

exposing myosin-binding sites on actin → cross-bridge (myosin head–actin) cycling → **sliding filament mechanism**: thick and thin filaments slide past each other (filament lengths unchanged) drawing Z-discs together, narrowing I-band and H-zone while the A-band width stays constant; each myosin power stroke moves ~5–10 nm and is fuelled by ATP hydrolysis.

- On cessation of the nerve impulse, Ca^{2+} is pumped back into the SR, tropomyosin re-covers the binding sites, and the sarcomere relaxes. In the total absence of ATP the actin–myosin cross-bridges remain fixed, producing the post-mortem muscle stiffness of **rigor mortis**.

5. Innervation — neuromuscular junction and motor unit

- Each skeletal muscle fibre is supplied by a branch of a large myelinated **α -motor axon**; the terminal, ensheathed by Schwann cell processes, forms a **neuromuscular junction (motor end-plate)** in a trough on the fibre surface, opposite a sarcolemma thrown into deep junctional folds (sole plate) that increase acetylcholine-receptor surface area.
- On arrival of an action potential, **acetylcholine (ACh)** is released from synaptic vesicles, crosses the synaptic cleft, binds nicotinic ACh receptors, and depolarises the sarcolemma (end-plate potential); ACh action is terminated by **acetylcholinesterase**.
- A **motor unit** = one α -motor neurone + all the muscle fibres it innervates; fibres of a motor unit are scattered across the fascicle, not confined to it. Small motor units (~10 fibres) occur in muscles requiring fine control (extraocular, intrinsic laryngeal); large motor units (several hundred fibres) occur in bulky limb muscles used for gross power.
- Sensory (afferent) components of the "motor" nerve include group Ia/II afferents from muscle spindles and group Ib afferents from Golgi tendon organs.

6. Skeletal muscle fibre types

Fibres are classified histochemically (myosin-ATPase staining, pH-dependent) into three principal types, correlating with contractile speed and metabolic profile:

Feature	Type I (slow oxidative)	Type IIA (fast oxidative-glycolytic)	Type IIX (fast glycolytic)
Contraction speed	Slow	Fast	Fast
Myosin ATPase activity	Low	High	High
Oxidative metabolism / mitochondria	High	Intermediate	Low
Anaerobic glycolysis	Low	Intermediate	High
Capillary density, myoglobin	High (red fibres)	Intermediate	Low (pale/white fibres)
Fatigue resistance	Fatigue-resistant	Fatigue-resistant	Fatigue-susceptible (rapid fatigue)
Function	Sustained postural work	Fast, fatigue-resistant movement	Brief, powerful bursts

- ▶ All human muscles are physiologically **mixed** (fibres of different types intermingle), but the proportion determines the muscle's overall character; the classical "red" and "white" muscle description of some animals is largely superseded by this myosin-based typing.
- ▶ Note: in older/rodent literature the fast glycolytic fibre is termed **type IIB**; in humans the corresponding fibre is designated **type IIX**. Hybrid fibres (I/IIA, IIA/IIX) also occur.
- ▶ Clinical use: needle **muscle biopsy** with ATPase histochemistry (or fibre-type grouping patterns) helps distinguish myopathic from neurogenic causes of weakness.

7. Proprioceptors of skeletal muscle — spindle and Golgi tendon organ

- ▶ **Muscle spindle** — encapsulated, fusiform stretch receptor (~2 mm long) lying among extrafusal fibres, enclosing a few modified **intrafusal fibres** (nuclear bag and nuclear chain types) innervated by sensory Ia/II afferents (wrapping the intrafusal fibres) and motor **γ-efferents** (fusimotor fibres); it signals changes in muscle length, mediating stretch reflexes and postural regulation.
- ▶ **Golgi tendon organ** — smaller encapsulated receptor at the myotendinous junction, enclosing sensory (Ib afferent) axons among collagen bundles; it detects tension generated by active contraction and inhibits motor activity when tension becomes excessive, protecting the muscle-tendon unit.

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- ▶ **Duchenne muscular dystrophy** — X-linked mutation of the dystrophin gene disrupts the cytoskeleton–ECM linkage at the sarcolemma; repeated contraction tears the weakened membrane, producing progressive fibre necrosis, pseudohypertrophy (calf), and fibrofatty replacement.
- ▶ **Myasthenia gravis** — autoantibodies against nicotinic ACh receptors at the neuromuscular junction reduce effective receptor number, causing fatigable weakness; extraocular muscles are typically affected first (ptosis, diplopia).
- ▶ **Rigor mortis** — fixed actin–myosin cross-bridges in the total absence of ATP after death cause generalised stiffening, of forensic use in estimating time since death.
- ▶ **Muscle biopsy/fibre typing** — ATPase histochemistry distinguishes myopathic disease (random fibre atrophy, both types affected) from neurogenic disease (fibre-type grouping and angulated atrophic fibres after reinnervation).
- ▶ **Regeneration and denervation** — satellite-cell activation, proliferation and fusion regenerate fibres after injury/intense exercise (extensive trauma instead scars); denervated fibres atrophy and are reinnervated by collateral sprouting from adjacent motor units.
- ▶ **Motor point** — the zone in the muscle belly where terminal motor-nerve branches converge is the most electrically excitable site, used for EMG needle placement and botulinum toxin/nerve-block injections.

High-yield exam pointers

- ▶ Three connective-tissue coats: epimysium (whole muscle) → perimysium (fascicle) → endomysium (single fibre); continuous with tendon at the myotendinous junction.

- ▶ Fibre = multinucleate cell, peripheral nuclei (unique to skeletal muscle), satellite cells beneath the external lamina.
- ▶ Sarcomere = Z-disc to Z-disc, $\sim 2.2 \mu\text{m}$ resting; A-band (thick + overlap) is constant width during contraction; I-band and H-zone narrow — filament lengths never change (sliding filament theory).
- ▶ Thick filament = myosin (15 nm, $1.5 \mu\text{m}$); thin filament = actin + tropomyosin + troponin (8 nm, $1 \mu\text{m}$); titin (Z-to-M, elastic) and nebulin (thin-filament ruler) are key accessory proteins.
- ▶ Triad = 1 T-tubule + 2 terminal SR cisternae, located at the A-I junction; basis of excitation-contraction coupling.
- ▶ Ca^{2+} binds troponin-C $\rightarrow$ tropomyosin moves $\rightarrow$ myosin cross-bridge cycling (ATP-dependent) $\rightarrow$ contraction; ATP absence $\rightarrow$ rigor mortis.
- ▶ Fibre types: I (slow oxidative, fatigue-resistant, postural) / IIA (fast oxidative-glycolytic) / IIX (fast glycolytic, fatigues quickly) — myosin-ATPase histochemistry.
- ▶ Motor unit = one α -motor neurone + all fibres it supplies; size varies from ~ 10 (extraocular) to several hundred (limb muscles).
- ▶ Muscle spindle (stretch, intrafusal fibres, Ia/II + γ -efferent) vs Golgi tendon organ (tension, Ib afferent, at myotendinous junction).
- ▶ Applied triad to remember: dystrophin $\rightarrow$ Duchenne MD; ACh-receptor antibody $\rightarrow$ myasthenia gravis; satellite cell $\rightarrow$ regeneration/hypertrophy.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e.



Intercalated disc

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction. The intercalated disc is the specialised, irregular, step-like junctional complex that joins the ends of adjacent **cardiac muscle cells (myocytes)** to form a continuous cardiac muscle fibre. It is the histological hallmark that identifies cardiac muscle in a section and the structural basis of the two defining physiological properties of the myocardium — mechanical continuity strong enough to withstand a lifetime of rhythmic contraction, and electrical continuity that lets the heart behave as a **functional (electrical and mechanical) syncytium** while its constituent cells retain their individual identity. Unlike skeletal muscle, where a single fibre is a true multinucleated syncytium formed by myoblast fusion, cardiac muscle is composed of separate uninucleate (occasionally binucleate) cells joined end to end at intercalated discs — a developmental and structural distinction that is itself high-yield.

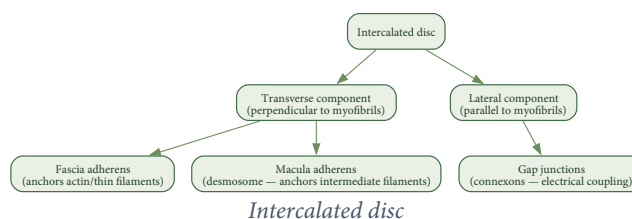
1. Light-microscopic appearance and location

- ▶ On H&E-stained **longitudinal sections**, intercalated discs appear as **dark-staining, transverse lines** crossing the muscle fibre at irregular intervals — the single most reliable light-microscopic feature distinguishing cardiac from skeletal muscle (viva/identification point).
- ▶ The line is often not perfectly straight but broken into short segments arranged in a **step-like ("stairway") pattern**, alternating between a **transverse** part (the "risers") running perpendicular to the myofibrils, and a short **lateral (longitudinal)** part (the "steps" or "treads") running parallel to the myofibrils, before turning again to form the next transverse segment.
- ▶ Discs are **not visible in every field** in routine H&E sections and their absence in a given field does not exclude cardiac muscle; they are best appreciated in well-oriented longitudinal sections.
- ▶ They characteristically lie **opposite the I-band** of the sarcomere (i.e., at the level of the Z-line), because the disc represents the junction between the terminal sarcomeres of two abutting cells.
- ▶ Individual myocytes are short, branching, cylindrical cells (roughly 15–30 µm in diameter and 85–120 µm long) with a single central nucleus; because cells join end-to-end and side-to-side through discs and their branches, the resulting fibre shows the characteristic **branching** pattern of cardiac muscle.

2. Ultrastructure — the three junctional components

Under the electron microscope the light-microscopic "line" resolves into a complex step-like junction with two structurally and functionally distinct parts, each carrying different types of intercellular junction.

Component of disc	Orientation	Junction(s) present	Molecular basis	Principal function
Transverse component ("riser")	Perpendicular to myofibril long axis; subject to the mechanical pull of contraction	Fascia adherens (major constituent) + Macula adherens (desmosomes)	Fascia adherens — cadherin-linked, analogous to the zonula adherens of epithelium, anchoring actin (thin) filaments of the terminal sarcomere via catenins; Desmosomes — desmoglein/desmo collin–plakoglobin–desmoplakin complex anchoring intermediate (desmin) filaments	Firm mechanical adhesion; transmits the contractile force of one cell to the next; prevents cells being pulled apart during rhythmic contraction
Lateral (longitudinal) component ("step/tread")	Parallel to myofibril long axis; not subject to shearing stress of contraction	Gap junctions (nexus)	Connexon channels (hexamers of connexin , principally connexin-43 in working ventricular myocardium) bridging a ~2–4 nm intercellular gap	Electrical/ionic coupling — low-resistance channel for rapid, direct spread of the action potential from cell to cell; this protected lateral position (away from the transverse pulling forces) preserves gap junction integrity during contraction



- **Fascia adherens** — the largest single component of the disc and the main reason the disc stains densely in H&E; it is a linear (non-encircling) counterpart of the epithelial zonula adherens, seen only at the intercalated discs of cardiac muscle among the three muscle types, and is the site at which actin filaments of the terminal sarcomere insert into the sarcolemma via a dense subsarcolemmal matrix, with **N-cadherin** as its principal transmembrane adhesion protein.
- **Macula adherens (desmosome)** — reinforces the fascia adherens; found in both the transverse and, to a lesser extent, the lateral parts of the disc; anchors the intermediate (desmin) filament

cytoskeleton rather than actin, distributing tensile stress and preventing separation of the constantly contracting cells.

- **Gap junctions** — confined to the lateral/longitudinal component of the disc; provide **ionic continuity** between adjoining myocytes, allowing the propagating action potential (and small signalling molecules) to pass directly from cell to cell.

3. Functional significance

- **Mechanical:** fascia adherens + desmosomes together anchor the contractile apparatus (thin filaments) and the intermediate-filament cytoskeleton across the cell boundary, so that the force generated by one cell's sarcomeres is transmitted efficiently, end-to-end, to the next — essential for the heart to act as a single pumping unit rather than a collection of independently contracting cells.
- **Electrical:** gap junctions convert the myocardium into a **functional syncytium** — depolarisation initiated at the SA node spreads cell-to-cell through gap junctions without needing a synapse at every cell, producing the coordinated, near-simultaneous contraction of large areas of myocardium (atria as one unit, ventricles as another, separated electrically by the fibrous cardiac skeleton except at the AV bundle).
- This dual mechanical–electrical role means the intercalated disc, though a single light-microscopic structure, actually performs the combined role that in skeletal muscle is divided between the myotendinous junction (force transmission) and the neuromuscular junction (excitation) — in cardiac muscle both roles are concentrated at one junctional complex between every pair of adjacent cells.
- The strategic placement of the mechanically-stressed junctions (fascia adherens, desmosomes) in the transverse plane and the electrically-important gap junctions in the protected lateral plane ensures that the shearing forces of contraction do not disrupt electrical coupling.

4. Distinguishing feature — comparison with skeletal and smooth muscle

Feature	Skeletal muscle	Cardiac muscle	Smooth muscle
Cell arrangement	True multinucleated syncytium (myoblast fusion)	Separate uninucleate cells joined by intercalated discs	Separate spindle cells, no discs
Cell-to-cell junction	None (single continuous cell)	Intercalated discs: fascia adherens + desmosomes + gap junctions	Gap junctions (nexus) only
Nucleus	Multiple, peripheral	Single (occasionally two), central	Single, central
T-tubule/SR junction	Triad (1 T-tubule + 2 terminal cisternae), at A–I junction	Diad (1 T-tubule + 1 terminal cisterna), at Z-line level	No T-tubules
Electrical coupling	Not required — single fibre, NMJ per fibre	Gap junctions — functional syncytium	Gap junctions — functional syncytium

- ▶ This table crystallises the single most important exam fact: **only cardiac muscle possesses true intercalated discs**; skeletal muscle needs none (it is already one continuous cell) and smooth muscle relies on gap junctions alone, without the elaborate adhesive apparatus of fascia adherens/desmosomes.
- ▶ The **conducting system of the heart** (SA node, AV node, bundle of His, Purkinje fibres) is composed of modified cardiac myocytes that still show intercalated discs (though the cells are larger, richer in glycogen, with peripherally displaced myofibrils and no T-tubules); the fundamental disc architecture is therefore retained throughout the specialised conduction pathway, not just the working myocardium.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 7.10 — Electron microscopic structure of part of an intercalated disc (schematic representation) (IB Singh — *Textbook of Human Histology* — 9e, p.225)

Reproduce a schematic longitudinal EM view of two abutting cardiac myocytes at their junction, labelling in sequence:

- ▶ Two adjoining **cardiac myocyte** ends meeting at an irregular **step-like line**
- ▶ **Transverse component** of the disc (perpendicular to myofibril axis) — label **Fascia adherens** (broad, electron-dense zone where **actin/thin filaments** of the terminal sarcomere insert) and an adjacent **Macula adherens (desmosome)** anchoring intermediate filaments
- ▶ **Lateral (longitudinal) component** of the disc (parallel to myofibril axis) — label **Gap junction (connexon)**
- ▶ Adjacent **mitochondria** and **myofibrils** flanking the junction
- ▶ Mark the overall "**staircase**"/stepwise profile of the disc as it crosses the fibre

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- ▶ **Histological identification:** the intercalated disc is the single most reliable light-microscopic feature (together with central nucleus and branching) used to identify cardiac muscle in a practical/viva section, since cross-striations alone are shared with skeletal muscle.
- ▶ **Ischaemia and myocardial infarction:** in acute ischaemia, connexins at the gap junction undergo dephosphorylation and lateralisation (redistribution away from the disc), impairing electrical coupling between myocytes; this disruption of intercalated-disc gap junctions slows and disorganises impulse conduction and is an important structural substrate for **re-entrant ventricular arrhythmias** after infarction.
- ▶ **Dilated cardiomyopathy:** familial dilated cardiomyopathy can result from mutations in cytoskeletal/junctional proteins that disrupt normal intercalated disc morphology, dissociating the junctions between myocytes and impairing myofibrillar organisation and contractile function, contributing to progressive chamber dilatation and pump failure.
- ▶ **Functional syncytium and arrhythmia spread:** because gap junctions allow essentially unimpeded cell-to-cell conduction, a focal electrical abnormality (ectopic focus, accessory pathway, scar-related re-entry circuit) can propagate rapidly through the myocardium — the

same property that guarantees coordinated contraction also underlies how arrhythmias spread once initiated.

- ▶ **Purkinje fibres:** although modified for rapid conduction rather than force generation, Purkinje fibres retain intercalated discs, underlining that the disc is a property of cardiac-lineage myocytes generally, not just the contractile working myocardium.
- ▶ **Lack of regenerative capacity:** adult cardiac myocytes essentially lack the capacity to regenerate after injury (no satellite-cell equivalent); myocardial infarction is therefore repaired by fibrous scar rather than by re-forming new intercalated-disc-linked fibre, which is why infarcted myocardium is mechanically and electrically discontinuous with the surrounding tissue.

High-yield exam pointers

- ▶ Intercalated disc = specialised junctional complex joining adjacent **cardiac myocytes**; seen on H&E as a **dark, irregular, step-like transverse line**, lying opposite the I-band.
- ▶ Two structural parts: **transverse component** (fascia adherens + macula adherens/desmosomes) and **lateral/longitudinal component** (gap junctions).
- ▶ **Fascia adherens** — largest component; anchors actin (thin) filaments via N-cadherin; analogous to zonula adherens.
- ▶ **Macula adherens (desmosome)** — reinforces fascia adherens; anchors intermediate (desmin) filaments via desmoglein/desmocollin–desmoplakin.
- ▶ **Gap junctions** — confined to the lateral component; connexon (chiefly connexin-43) channels give electrical/ionic continuity — basis of the **functional syncytium**.
- ▶ Only **cardiac muscle** has true intercalated discs; skeletal muscle is a true multinucleated syncytium (no discs needed); smooth muscle has gap junctions only.
- ▶ Cardiac muscle forms a **diad** (not a triad as in skeletal muscle) at the Z-line level.
- ▶ Purkinje fibres (conducting system) retain intercalated discs despite their modified structure.
- ▶ Clinical correlations: ischaemia-induced gap-junction dephosphorylation/lateralisation → **post-infarct re-entrant arrhythmia**; junctional-protein gene mutations → **familial dilated cardiomyopathy**.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh's Textbook of Human Histology 9e; Sontakke's Textbook of Human Histology 2e; Brijesh Kumar's Histology Text & Atlas 3e; Netter's Essential Histology (Ovalle) 3e (dilated cardiomyopathy/N-cadherin correlation).



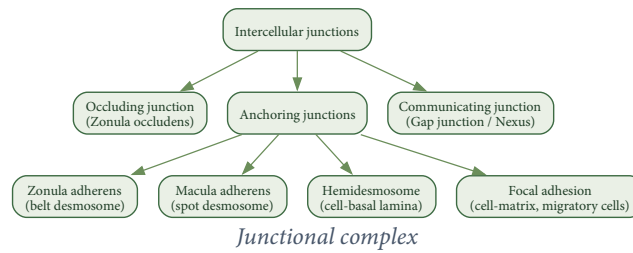
Junctional complex

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction A junctional complex is the characteristic apical arrangement of specialised intercellular junctions seen by electron microscopy just below the free (luminal) surface of adjoining epithelial cells, which binds the cells of an epithelial sheet into a mechanically and functionally coherent unit. The term was coined by Farquhar and Palade (1963) in their classic description of junctional complexes in various epithelia. In sequence from the lumen downward it comprises the **zonula occludens** (tight junction), the **zonula adherens**, and the **macula adherens** (desmosome); together these three correspond to the "**terminal bar**" seen at the luminal margin of columnar/cuboidal epithelial cells with the light microscope. Two further junctions are conventionally described alongside the complex to give the complete repertoire of epithelial cell junctions: the **gap junction** (a communicating junction) and the **hemidesmosome**/focal adhesion (basal, cell-to-matrix junctions).

1. Classification of intercellular junctions

Category	Junction	Also called	Shape	Principal adhesion molecule	Cytoskeletal link	Chief function
Ocluding	Zonula occludens	Tight junction	Encircling belt	Claudins, occludin, JAMs	Actin (via ZO-1/2/3)	Paracellular seal; apico-basolateral "fence"
Anchoring	Zonula adherens	Belt/intermediate junction	Encircling belt	E-cadherin (Ca ²⁺ -dependent) + catenins	Actin (terminal web)	Circumferential mechanical adhesion
Anchoring	Macula adherens	Desmosome, spot desmosome	Spot ("spot-weld")	Desmoglein, desmocollin + plakoglobin/desmoplakin	Intermediate filaments (keratin/desmin)	Strong focal adhesion, tensile strength
Anchoring (basal)	Hemidesmosome	—	Spot, basal	Integrin $\alpha 6\beta 4$ + BPAG1/BPAG2	Keratin intermediate filaments	Anchors cell to basal lamina
Communicating	Gap junction	Nexus	Patch of channels	Connexin hexamers (connexons)	None (forms a channel)	Ionic/metabolic intercellular coupling



2. Zonula occludens (tight junction)

- ▶ The **most apical** component of the complex; forms a **continuous encircling belt** ("zonula") near the apex of the lateral cell membrane of adjacent cells.
- ▶ Ultrastructure: adjacent plasma membranes appear fused along branching, anastomosing ridges; freeze-fracture preparations show these as strands of intramembranous particles on the protoplasmic (P) face.
- ▶ Molecular architecture: **claudins** (a family of ~20–27 kDa tetraspan proteins that form the backbone of the sealing strands and confer selective ionic permeability) and **occludin** (60 kDa, also tetraspan) provide the fusion strands; **junctional adhesion molecules** (JAM, ~40 kDa, immunoglobulin superfamily) and the **afadin–nectin complex** contribute extracellular adhesion; the cytoplasmic plaque proteins **ZO-1, ZO-2 and ZO-3** link all of these to the underlying actin cytoskeleton.
- ▶ Function: (i) a **sealing/barrier** function — forces transport across the epithelium to occur transcellularly rather than paracellularly; (ii) a **"fence"** function — prevents intermixing of apical and basolateral membrane lipids/proteins, the structural basis of epithelial cell polarity.
- ▶ The degree of "leakiness" correlates with the number of fused strands: epithelia with few strands (e.g. proximal renal tubule) are relatively permeable ("leaky" epithelium), whereas epithelia with many fused strands (e.g. urinary bladder) are relatively impermeable ("tight" epithelium).

3. Zonula adherens

- ▶ Lies immediately below the tight junction and, like it, forms a **continuous encircling belt**.
- ▶ Adhesion is mediated by transmembrane **E-cadherin**, a Ca^{2+} -dependent homophilic adhesion glycoprotein.
- ▶ The cytoplasmic tail of cadherin binds **catenins**, which link to the **actin filament bundle of the terminal web**; the complex also contains the **afadin–nectin** system.
- ▶ Function: firm circumferential adhesion that anchors the perijunctional actin ring, stabilising cell shape and contributing to epithelial morphogenesis and to the mechanical strength of the tight-junction belt above it.

4. Macula adherens (desmosome)

- ▶ A **spot-like, disc-shaped** plaque (not encircling) paired between two adjacent cells — likened to a "spot-weld."
- ▶ The intercellular gap here is wider (~25 nm) and is traversed by electron-dense transmembrane cadherin filaments.
- ▶ Transmembrane adhesion molecules are the larger cadherin-family members **desmoglein and desmocollin** (Ca^{2+} -dependent).

- ▶ The cytoplasmic attachment plaque contains **plakoglobin** (γ -catenin), **plakophilin** and **desmoplakin**, into which **intermediate filaments** insert — **keratin** (tonofilaments) in epithelia, **desmin** in cardiac muscle.
- ▶ Function: the **strongest** anchoring junction, providing tensile strength against mechanical stress; desmosomes are especially numerous and large in the **prickle-cell (stratum spinosum) layer of the epidermis**.

5. Gap junction (nexus / communicating junction)

- ▶ Not a sealing or adhesive junction but a **channel-forming** one, mediating direct intercellular communication.
- ▶ Ultrastructure: the two apposed membranes are separated by a narrow gap (~2–3 nm) bridged by clusters of transmembrane channels called **connexons**; each connexon is a hexamer of **connexin** subunits with a central hydrophilic pore (~1.5–2 nm). Connexons from the two adjoining cells dock end-to-end to form a complete channel linking the two cytoplasms; thousands of such channels may aggregate as a hexagonal-array "patch."
- ▶ Permits diffusion of ions and small molecules (< ~1.2 kDa — amino acids, sugars, nucleotides, second messengers, ions), producing **electrical and metabolic coupling** between cells.
- ▶ Widely distributed — hepatocytes, cardiac myocytes (rhythmic electrical coupling), smooth/visceral muscle, glial cells, and lens epithelium/fibres.

6. Hemidesmosome and focal adhesion (basal, cell–matrix junctions)

- ▶ **Hemidesmosome**: an asymmetric junction resembling "half" a desmosome, anchoring the basal surface of the epithelial cell to the underlying **basal lamina**. It uses **integrin $\alpha 6 \beta 4$** (not cadherin) linking through **plectin** and **BPAG1/BPAG2 (bullous pemphigoid antigens)** to keratin intermediate filaments; the integrins bind **laminin** in the basal lamina.
- ▶ **Focal adhesion (focal contact)**: smaller, more numerous, transient basal attachments seen chiefly in migrating/reorganising cells; integrins link — via talin, vinculin, paxillin and focal adhesion kinase (FAK) — to bundled **actin filaments** rather than intermediate filaments, and signal for cell motility, proliferation and gene expression.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 4.5 — *Epithelial cell junctional complex*. (Junqueira's Basic Histology — Text & Atlas — 17e, p.203)

Reproduce a labelled electron-microscopic-style sketch of the lateral membrane between two adjoining columnar epithelial cells (e.g. small intestine), from apex to base, with these exact labels:

- ▶ MV — microvilli (apical brush border)
- ▶ TJ — tight junction / zonula occludens (most apical, fused ridges)
- ▶ AJ — adherent junction / zonula adherens (immediately below TJ)
- ▶ D — desmosome / macula adherens (spot-like, below AJ)
- ▶ IF — intermediate (tonofilament) filaments converging on the desmosomal plaque
- ▶ (Extend the sketch basally to add) **Gap junction** — patch of connexons, and **Hemidesmosome** — at the basal lamina.

Applied / Clinical anatomy

- ▶ **Pemphigus vulgaris**: autoimmune IgG antibodies against **desmoglein-3** ($\pm$ desmoglein-1) disrupt desmosomes, causing intraepidermal acantholytic blistering of skin and oral mucosa.
- ▶ **Bullous pemphigoid**: autoantibodies against **BPAG1/BPAG2** of the hemidesmosome cause subepidermal blistering — distinguishing it clinically and histologically from pemphigus.
- ▶ ***Clostridium perfringens enterotoxin binds claudin***, preventing its incorporation into tight junctions and causing paracellular fluid loss into the gut lumen — a mechanism in food-poisoning diarrhoea.
- ▶ ***Helicobacter pylori injects CagA, which targets ZO-1*** and disrupts tight-junction integrity, contributing to gastric epithelial barrier breakdown in gastritis/peptic ulcer disease.
- ▶ Mutation of **claudin-16 or claudin-19** impairs paracellular Mg^{2+} (and Ca^{2+}) reabsorption in the renal tubule, causing familial hypomagnesaemia with hypercalciuria.
- ▶ Mutation of **connexin-26 (GJB2)** is the commonest cause of non-syndromic hereditary sensorineural **deafness**, from failure of K^+ recycling through cochlear gap junctions.
- ▶ Variation in tight-junction "tightness" (number of fused strands) explains why the **proximal renal tubule is leaky** while the **urinary bladder epithelium is impermeable** — clinically relevant to tubular transport physiology.
- ▶ Loss of **E-cadherin/catenin** function at the zonula adherens reduces cell-to-cell cohesion and is implicated in epithelial tumour invasion and metastasis.

High-yield exam pointers

- ▶ Sequence from apex to base: **Zonula occludens** → **Zonula adherens** → **Macula adherens** (+ gap junction and hemidesmosome basally).
- ▶ Coined by **Farquhar & Palade (1963)**; corresponds to the light-microscopic "**terminal bar**."
- ▶ Tight junction proteins: **claudin, occludin, JAM**, linked via **ZO-1/2/3** to actin.
- ▶ Zonula adherens & desmosome adhesion molecule is **cadherin** (E-cadherin; desmoglein/desmocollin respectively) — **Ca^{2+} -dependent**.

- ▶ Desmosomes anchor **intermediate filaments** (keratin/desmin); hemidesmosomes use **integrins**, not cadherins.
- ▶ Gap junction = **connexon** (hexamer of connexins); permits molecules < ~1.2 kDa.
- ▶ Clinical pairs to remember: **pemphigus** (desmoglein) vs **bullous pemphigoid** (hemidesmosome/BPAG); Cx26 → deafness; claudin-16/19 → renal Mg^{2+} wasting.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e.



Junctional complexes

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction

A junctional complex is the fixed, apex-to-base sequence of three specialized intercellular junctions — the **zonula occludens** (tight junction), **zonula adherens** (belt desmosome) and **macula adherens** (spot desmosome) — situated just deep to the apicolateral membrane of simple columnar/cuboidal epithelial cells (classically the intestinal absorptive cell). It was first resolved ultrastructurally by **Farquhar and Palade** (1963, J Cell Biol 17:375–412) and corresponds, under light microscopy, to the deeply-staining band earlier called the "**terminal bar**" (once attributed to a viscous "intercellular cement"). Functionally the complex seals the paracellular space and mechanically couples the cytoskeletons of neighbouring cells; **gap junctions** and **hemidesmosomes**, though not part of the complex proper, are conventionally described alongside it as the remaining members of the epithelial cell-junction family.

1. Classification of intercellular junctions

Category	Member junction(s)	Symmetry	Cytoskeletal link	Principal function
Occluding (tight) junction	Zonula occludens	Symmetric	F-actin (via ZO-1/2/3)	Paracellular seal; apicobasal "fence"
Anchoring junction	Zonula adherens (belt desmosome)	Symmetric	F-actin (terminal web)	Circumferential mechanical adhesion
Anchoring junction	Macula adherens (desmosome)	Symmetric	Intermediate filaments	Spot-weld mechanical adhesion
Anchoring junction	Fascia adherens	Symmetric	F-actin	Strip-like adhesion (non-epithelial cells)
Asymmetric anchoring junction	Hemidesmosome	Asymmetric	Intermediate filaments	Anchors basal cell to basal lamina

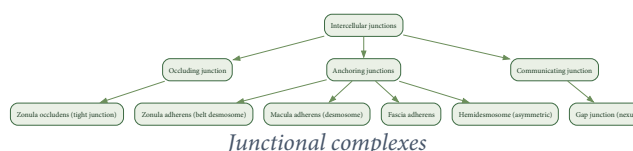
Category	Member junction(s)	Symmetry	Cytoskeletal link	Principal function
Communicating junction	Gap junction (nexus, macula communicans)	Symmetric	None (aqueous channel)	Ionic/metabolic cell-to-cell coupling

2. The apical junctional complex — sequence and real ultrastructure

- ▶ Location: apicolateral plasma membrane of epithelial cells, e.g. small-intestinal absorptive cells, renal tubular cells.
- ▶ Apex-to-base order: **zonula occludens** (most apical, encircles the cell as a continuous belt) → **zonula adherens** (immediately basal to it, also a continuous belt) → **macula adherens/desmosome** (spot-like, most basal of the three, scattered rather than continuous).
- ▶ On freeze-fracture and thin-section EM the complex appears as a compact, three-tiered structure at the lateral membrane just below the microvilli.

□ Fig 2.10 — Scheme to show a junctional complex. (IB Singh — Textbook of Human Histology — 9e, p.52)

□ Fig 4.5 — Epithelial cell junctional complex. (Junqueira's Basic Histology — Text & Atlas — 17e, p.203)



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw two adjacent epithelial cells' apicolateral membranes in vertical section (as in the embedded scheme) and label, apex to base:

- ▶ Microvilli at the apical surface.
- ▶ **Zonula occludens** — outer leaflets of the two membranes fused, obliterating the intercellular space; show branching sealing strands.
- ▶ **Zonula adherens** — thickened cytoplasmic plaques with **actin filaments of the terminal web** running into it; cadherins bridging a 15–20 nm gap.
- ▶ **Macula adherens (desmosome)** — cytoplasmic dense plaque with **keratin (tonofilament) intermediate filaments** inserting into it; cadherin filaments bridging a ~25 nm gap.
- ▶ Separately, in a side panel: a **hemidesmosome** at the basal surface anchoring keratin filaments to the **basal lamina**; and a **gap junction** showing paired **connexons** bridging a 3 nm gap between two lateral membranes.

3. Zonula occludens (tight/occluding junction)

- ▶ The most apical component; forms a continuous belt around the cell perimeter, connected to the actin cytoskeleton.
- ▶ At the site of contact the outer leaflets of the two plasma membranes fuse so that the intercellular space is obliterated; freeze-fracture shows branching, anastomosing sealing strands of protein particles on the P-face of the membrane.
- ▶ Molecular components:

- **Occludins and claudins** — tetraspanin-family proteins (4 transmembrane domains, 2 extracellular loops) forming the sealing strands.
 - **Junctional adhesion molecules (JAMs)** and the **afadin–nectin complex** — immunoglobulin-superfamily members forming trans-homodimers across the intercellular gap.
 - **Cytosolic zonula occludens proteins ZO-1, ZO-2, ZO-3** — link occludin, claudin and JAM to F-actin.
- Functions: (i) regulates **paracellular permeability** (gate function), preventing free diffusion of luminal contents into the intercellular space; (ii) maintains **epithelial cell polarity** by blocking lateral inter-mixing of apical and basolateral membrane lipids/proteins (fence function); (iii) also present between **vascular endothelial cells**.
- Mutation in **claudin-16** or **claudin-19** impairs paracellular Mg^{2+} reabsorption in the renal tubule, producing familial renal magnesium wasting.

4. Anchoring junctions — zonula adherens, macula adherens, fascia adherens, hemidesmosome

Feature	Zonula adherens (belt desmosome)	Macula adherens (desmosome)	Hemidesmosome
Position	Continuous belt, just basal to zonula occludens	Spot-like, scattered, basal to zonula adherens	Basal cell surface — basal lamina
Intercellular gap	15–20 nm	~25 nm, filled with electron-dense cadherin filaments	Asymmetric — no true intercellular gap (attaches to matrix)
Adhesion molecule	Cadherins (desmoglein/desmocollin family) + afadin–nectin complex	Cadherins — desmoglein, desmocollin	Integrin $\alpha 6\beta 4$; type XVII collagen (BPAG2)
Cytoplasmic plaque	Catenin complex, afadin	Desmoplakin, plakophilin, plakoglobin (γ-catenin)	BPAG1 (plakin family), plectin
Cytoskeletal filament	F-actin (forms the terminal web)	Intermediate filaments — keratin (epithelium) or desmin (cardiac muscle)	Keratin intermediate filaments
Matrix ligand	—	—	Laminin-332 of basal lamina
Disease correlate	—	Pemphigus vulgaris	Bullous pemphigoid; junctional epidermolysis bullosa

- **Zonula adherens**: the apical actin network it anchors forms the **terminal web**; contraction of the terminal web spreads out the bases of the microvilli, increasing the absorptive surface area.
- **Fascia adherens**: resembles a zonula adherens but forms only a strip or patch rather than a complete belt; found at the **intercalated discs of cardiac muscle**, between smooth muscle cells, and between glial cells and neurones.

- ▶ **Macula adherens (desmosome):** a plaque-like "spot-weld," extremely numerous in tissues under mechanical stress, e.g. the **prickle-cell layer of the epidermis**.
- ▶ **Hemidesmosome:** an asymmetric anchoring junction resembling half a desmosome; anchors the basal surface of epithelial cells to the basal lamina via integrin $\alpha 6 \beta 4$ and type XVII collagen (BPAG2), linked intracellularly to keratin filaments through the plaque protein BPAG1 and plectin. Uses integrins, not cadherins, distinguishing it molecularly from true desmosomes.

5. Gap junctions (communicating junctions, nexus, macula communicans)

- ▶ The two apposed lipid bilayers are separated by a gap of only ~ 3 nm, bridged by clusters of transmembrane channels called **connexons**.
- ▶ Each connexon is a ring of **six connexin** subunits; a connexon on one cell aligns with a connexon on the adjacent cell to form a continuous aqueous pore linking the two cytoplasms.
- ▶ Permits direct passage of ions and small molecules (Ca^{2+} , cAMP, metabolites) between cells without entering the extracellular fluid, creating a **physiological syncytium**.
- ▶ Channel opening/closing is regulated by intracellular Ca^{2+} concentration and pH.
- ▶ Found between **cardiac myocytes** and **smooth muscle cells** (permits synchronized excitation and contraction of the whole muscle mass), and also between **hepatocytes**, glial cells, and oocyte–granulosa cells.
- ▶ Not part of the apical junctional complex proper, but frequently co-exists with it in the lateral membrane, and forms part of the cardiac **intercalated disc** together with fascia adherens and desmosomes.

Applied / Clinical anatomy

- ▶ **Pemphigus vulgaris:** autoantibodies directed against desmosomal cadherins (desmogleins) cause loss of keratinocyte-to-keratinocyte adhesion, producing intraepidermal (suprabasal) blisters.
- ▶ **Bullous pemphigoid:** autoantibodies against the hemidesmosomal plaque proteins BPAG1/BPAG2 cause separation at the dermo-epidermal junction, producing large, tense, subepidermal blisters.
- ▶ **Junctional epidermolysis bullosa:** mutation of the **laminin-332** gene disrupts the hemidesmosome–basal lamina anchorage, producing a blistering skin disease.
- ▶ **Familial renal magnesium wasting:** mutation of claudin-16/claudin-19 impairs the tight-junction-dependent paracellular reabsorption of Mg^{2+} in the renal tubule.
- ▶ **Barrier function:** intact zonulae occludentes of gut, gallbladder and urinary bladder epithelium prevent luminal contents (digestive enzymes, urine solutes) from permeating into the intercellular space and underlying tissue.
- ▶ **Muscle correlate:** gap junctions at the cardiac intercalated disc and between smooth muscle cells permit coordinated, synchronous contraction of the whole muscle mass; their disruption uncouples cell-to-cell electrical continuity.
- ▶ **Neuroepithelium:** developing neuroepithelial cells of the neural tube remain attached to one another by terminal bars at the internal limiting membrane during early histogenesis.

High-yield exam pointers

- ▶ Apex-to-base sequence of the junctional complex: **zonula occludens** → **zonula adherens** → **macula adherens (desmosome)**.
- ▶ Classic description: **Farquhar and Palade, 1963**; light-microscopic correlate = **terminal bar**.
- ▶ Tight junction proteins: **occludin, claudins, JAM, ZO-1/2/3** — linked to F-actin.
- ▶ Zonula adherens anchors the apical actin **terminal web**, whose contraction spreads microvillar bases and increases absorptive area.
- ▶ Desmosome molecules: **desmoglein/desmocollin (cadherins)** + **desmoplakin/plakophilin/plakoglobin** + **keratin intermediate filaments**; ~25 nm intercellular gap.
- ▶ Hemidesmosome uses **integrin $\alpha 6\beta 4$** + **BPAG1/BPAG2** + **laminin-332** — molecularly distinct from a true desmosome (cadherin-based).
- ▶ Gap junction = **connexon (6 connexins)** bridging a **3 nm gap**; permits passage of molecules <1.2 kDa; creates a physiological syncytium.
- ▶ Disease triad to remember: **pemphigus vulgaris** (desmosome/cadherin), **bullous pemphigoid** (hemidesmosome/BPAG), **claudin-16/19 mutation** (renal Mg wasting).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



Ossification

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction Ossification (osteogenesis) is the process by which osteoprogenitor mesenchymal cells differentiate into osteoblasts that lay down osteoid, which is then mineralised by deposition of calcium hydroxyapatite to form bone. Almost every bone in the body is mesodermal (except most craniofacial bones, which are neural-crest derived) and is preceded by a mesenchymal model that is converted to bone by one of two mechanisms — **intramembranous** (direct, within a fibrous membrane) or **endochondral** (indirect, via a hyaline-cartilage model that is progressively eroded and replaced). Both processes initially produce woven bone, which is subsequently remodelled into stronger lamellar bone; the distinction lies only in the tissue the osteoblasts first invade, not in the final histological structure of mature bone.

1. Classification of bones by mode of ossification

Type	Precursor tissue	Bones formed	Examples
Membrane bones	Condensed fibrocellular mesenchyme	Flat bones of the skull vault, most facial bones	Frontal, parietal, part of occipital/temporal, maxilla, mandible

Type	Precursor tissue	Bones formed	Examples
Cartilage bones	Hyaline-cartilage model	Base of skull, vertebrae, ribs, all limb long bones	Femur, humerus, tibia, vertebral bodies
Membranocartilaginous (compound) bones	Part membrane + part cartilage	Bones ossifying by both routes	Clavicle (shaft — membranous; medial/sternal end — cartilaginous), mandible (body — membranous; condylar/coronoid processes — from secondary cartilages), scapula (body — membranous; coracoid, acromion, glenoid margin, borders — cartilaginous)

- The clavicle is the **first bone in the body to begin ossifying** (5th–6th week IUL) and one of the **last to complete** ossification (medial epiphysis fuses at 21–25 years) — a favourite fact for MCQs.

2. Intramembranous ossification

Occurs directly within a condensed sheet of embryonic mesenchyme, without an intervening cartilage stage.

- **Step 1 — Mesenchymal condensation:** mesenchymal cells aggregate into a richly vascular fibrous membrane; osteoprogenitor cells within it proliferate.
- **Step 2 — Ossification centre formation:** osteoprogenitor cells differentiate into **osteoblasts**, which arrange themselves around a capillary network and secrete **osteoid** (collagen type I + ground substance).
- **Step 3 — Mineralisation:** alkaline phosphatase secreted by osteoblasts drives calcium/phosphate deposition as **hydroxyapatite**, converting osteoid to mineralised **woven bone**; trapped osteoblasts become **osteocytes** within lacunae.
- **Step 4 — Fusion and remodelling:** adjoining spicules of woven bone fuse into **bony trabeculae**, then bony plates; peripheral condensed mesenchyme that does not ossify becomes the **periosteum** and **endosteum**; woven bone is remodelled into compact bone (outer/inner tables) enclosing cancellous diploë.
- Unossified islands of membrane persist postnatally as the **fontanelles** of the neonatal skull.

□ Fig 9.12 — Intramembranous ossification (Source: Textbook of Human Embryology, Yo (Sontakke — Textbook of Human Histology — 2e, p.113)

3. Endochondral (intracartilaginous) ossification

Occurs within a pre-formed hyaline-cartilage model and accounts for most bones of the body, best studied in a developing long bone.

- **Step 1:** A **hyaline-cartilage model**, invested by perichondrium, is laid down in the shape of the future bone.

- ▶ **Step 2 — Bone collar:** late in the first trimester, osteoblasts differentiating from the perichondrium around the mid-shaft lay down a thin **periosteal (subperiosteal) bone collar**, converting the perichondrium there into periosteum.
- ▶ **Step 3 — Chondrocyte hypertrophy and calcification:** the bone collar impedes diffusion; enclosed chondrocytes hypertrophy, compress the matrix, and calcify it (alkaline phosphatase, type X collagen); hypertrophied chondrocytes then die by apoptosis, leaving empty **primary areolae**.
- ▶ **Step 4 — Primary ossification centre:** a **periosteal bud** (blood vessels + osteoprogenitor cells) invades the calcified cartilage in the diaphysis; osteoclasts enlarge the spaces into **secondary areolae**, and osteoblasts lay osteoid on residual calcified-cartilage bars, forming woven bone — the **primary centre of ossification** (appears 7th–12th week IUL for most long bones).
- ▶ **Step 5 — Secondary ossification centres:** around/after birth, a similar sequence of vascular invasion and ossification occurs in each **epiphysis**, producing secondary centres separated from the primary (diaphysal) centre by the **epiphyseal (growth) plate**.
- ▶ **Step 6 — Growth and closure:** the epiphyseal plate keeps producing cartilage that is continuously converted to bone, allowing longitudinal growth, until it is finally replaced by bone (**epiphyseal fusion**), leaving only an **epiphyseal line**; **articular cartilage** at the joint surfaces persists lifelong.

□ Fig 9.13 — Endochondral ossification (Development of long bone) (Source: Textbook (Sontakke — Textbook of Human Histology — 2e, p.114)

Zones of the epiphyseal (growth) plate (epiphysis → diaphysis):

Zone	Histological activity
1. Zone of reserve/resting cartilage	Ordinary hyaline cartilage, cells quiescent
2. Zone of proliferation	Chondrocytes divide, align in longitudinal columns
3. Zone of hypertrophy	Chondrocytes swell, secrete type X collagen, compress matrix
4. Zone of calcification	Matrix around hypertrophied cells calcifies; chondrocytes apoptose
5. Zone of ossification (vascular invasion)	Capillaries + osteoprogenitor cells invade empty lacunae; osteoblasts deposit osteoid on calcified-cartilage bars → woven bone

4. Ossification centres and laws of ossification

- ▶ Ossification centres are classified as **primary** (form the **diaphysis**; appear before birth, usually 7th–12th week IUL — exceptions: carpal bones appear postnatally, tarsal centres for talus/calcaneum/cuboid appear in utero) and **secondary** (form the **epiphyses**; appear as a rule after birth, from birth to 5 years — exception: the lower femoral epiphysis appears in the 9th month IUL, occasionally also the upper tibial and upper humeral epiphyses).
- ▶ **Laws of ossification:**
- ▶ The **primary centre is single** and appears **before birth** (exception: carpal bones).
- ▶ **Secondary centres appear after birth** as a rule (exception: lower end of femur).

- ▶ **The centre that appears first fuses last** (exception: lower end of fibula, which appears first and also fuses first).
- ▶ **Growing end:** in a long bone, the two epiphyses do not fuse simultaneously; the end that fuses later is the **growing end**, and it is always the end whose secondary centre appeared first; the growing end lies **opposite** the direction of the nutrient foramen — mnemonic: *"to the elbow I go, from the knee I flee."* Growing ends: shoulder end of humerus, wrist ends of radius/ulna (upper limb); knee ends of femur/tibia/fibula (lower limb — fibula being the classic exception to the appears-first-fuses-first rule).
- ▶ **Medicolegal significance:** on radiography of a stillborn/newborn, the **shaft is radiopaque (bony)** while the ends are **radiolucent (cartilaginous)**; visibility of the **lower femoral epiphysis** on X-ray indicates a **full-term foetus** (appears ~36–40 weeks) — used in forensic determination of live birth/maturity.

5. Growth in length and width of a long bone

- ▶ **Growth in length** occurs entirely at the **epiphyseal plate** by the balanced, opposing processes of cartilage proliferation (epiphyseal side) and its replacement by bone (diaphyseal side); because the two rates are equal, plate thickness stays constant while the whole plate is progressively displaced away from mid-shaft, until epiphyseal fusion (complete by ~18–25 years) stops further lengthening.
- ▶ **Growth in width (circumference)** occurs by **appositional growth** — osteoprogenitor cells of the periosteum differentiate into osteoblasts that add new bone subperiosteally, while osteoclasts of the endosteum simultaneously resorb bone from the medullary surface, enlarging the marrow cavity without proportionately increasing bone weight.

6. Bone remodelling and regulation

- ▶ Throughout life, bone undergoes coupled **osteoclastic resorption** (osteoclasts cut a cylindrical "cutting cone" through existing bone) followed by **osteoblastic deposition** of concentric lamellae behind the advancing front, forming a new **osteon (Haversian system)** — the basis of continuous internal remodelling. Turnover is far more rapid in children than adults.
- ▶ Regulated by: **parathormone** (mobilises calcium via osteoclast activation), **calcitonin** (inhibits osteoclastic resorption), **vitamin D** (promotes osteoblast matrix protein synthesis and intestinal calcium absorption), growth hormone/thyroxine (stimulate epiphyseal cartilage proliferation), and local RANK–RANKL–osteoprotegerin signalling between osteoblasts and osteoclast precursors.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a longitudinal section of a developing long bone showing endochondral ossification, with these exact labels:

- ▶ Hyaline cartilage model with surrounding perichondrium.
- ▶ Periosteal (subperiosteal) bone collar around the mid-shaft.
- ▶ Primary ossification centre in the diaphysis, with invading periosteal bud (blood vessels).
- ▶ Zone of hypertrophied/calcified cartilage flanking the primary centre.
- ▶ Secondary ossification centre in each epiphysis.
- ▶ Epiphyseal (growth) plate separating epiphysis from diaphysis.
- ▶ Articular cartilage capping each end.
- ▶ Medullary (marrow) cavity.

Applied / Clinical anatomy

- ▶ **Cleidocranial dysostosis:** defective intramembranous ossification — absent/hypoplastic clavicles, wide-open fontanelles, delayed suture closure.
- ▶ **Achondroplasia:** defective endochondral ossification at the growth plate — disproportionate dwarfism with short limbs, normal trunk length, exaggerated lumbar lordosis.
- ▶ **Rickets (children) / Osteomalacia (adults):** vitamin D deficiency impairs mineralisation; growth plates become disorganised and widened, bones bend under load.
- ▶ **Osteogenesis imperfecta:** defective type I collagen synthesis by osteoblasts — abnormally fragile bone ("brittle bone disease").
- ▶ **Osteopetrosis:** defective osteoclast function — failure of remodelling, dense but brittle bone with obliterated marrow cavities.
- ▶ **Bone-age estimation:** sequential appearance/fusion of ossification centres on radiographs is used clinically (paediatric growth assessment) and forensically (age/maturity estimation, live-birth determination via the distal femoral epiphysis).
- ▶ **Fracture healing** recapitulates ossification — a periosteal/endosteal callus first forms fibrocartilage, which is converted to woven bone by the same endochondral/intramembranous mechanisms before final remodelling to lamellar bone.
- ▶ **Epiphyseal (Salter–Harris) injuries** in children can damage the growth plate and cause limb-length discrepancy or angular deformity because the plate — not the epiphysis or diaphysis — is the site of longitudinal growth.

High-yield exam pointers

- ▶ Two types: **intramembranous** (direct, in mesenchymal membrane — skull vault, mandible, clavicle shaft) and **endochondral** (via a cartilage model — most of the skeleton).
- ▶ **Clavicle:** first to start ossifying, last to finish; shaft membranous, medial end cartilaginous.
- ▶ **Primary centre → diaphysis** (before birth, exception carpals); **secondary centre → epiphysis** (after birth, exception lower femur).

- ▶ **Law:** centre appearing first fuses last (exception: lower fibula).
- ▶ **Growing end** lies opposite the nutrient foramen; mnemonic "to the elbow I go, from the knee I flee."
- ▶ **5 zones of the epiphyseal plate:** reserve → proliferation → hypertrophy → calcification → ossification.
- ▶ **Growth in length** = epiphyseal plate activity; **growth in width** = appositional (periosteal deposition + endosteal resorption).
- ▶ Lower femoral epiphysis on X-ray = medicolegal proof of full-term birth.
- ▶ Both intramembranous and endochondral routes first form **woven bone**, later remodelled to **lamellar bone**.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Vishram Singh — General Anatomy 4e; Vishram Singh — Textbook of Anatomy Vol 1 4e (scapula/clavicle ossification).



Osteon

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction An **osteon** (Haversian system) is the basic structural and functional unit of mature **compact (cortical) lamellar bone**. It is a cylindrical, sometimes spiral or branching, unit consisting of concentric rings (lamellae) of mineralized bone matrix arranged around a central longitudinal **Haversian canal** that carries the vessels and nerves supplying the osteocytes. An adult human skeleton contains an estimated **21 million osteons**, and their orderly stacking parallel to the long axis of a bone gives compact bone its characteristic strength and its ability to resist crack propagation.

Types of lamellae in compact bone

Compact bone is built entirely of bony lamellae, which are of three kinds:

Lamella type	Location	Description
Circumferential lamellae (outer & inner)	Outer: just deep to periosteum; Inner: bordering the medullary cavity	Continuous layers running around the whole circumference of the shaft; anchored by perforating (Sharpey's) fibres from periosteum
Concentric lamellae	Around each Haversian canal	5–20 (typically about 6) rings that constitute the osteon proper
Interstitial lamellae	Angular spaces between osteons	Irregular remnants of older osteons partly eroded during remodelling

Structure of the osteon (Haversian system)

Component	Description
Haversian (central) canal	Longitudinal canal at the axis of the osteon; mean diameter ~50 μm ; contains one or two fenestrated capillaries, unmyelinated/occasional myelinated axons, pericytes and an endosteal lining; communicates with adjacent osteons, the periosteum and the marrow cavity
Concentric lamellae	5–20 sheets of mineralized matrix (average ~6) wrapped around the canal; collagen fibres in successive lamellae alternate their helical pitch (mainly longitudinal vs mainly circumferential), which produces alternating bright and dark bands under polarized light — the phenomenon of birefringence
Lacunae with osteocytes	Small flattened spaces between adjoining lamellae, each housing one osteocyte
Canaliculi	Fine channels (~0.25–0.5 μm) radiating from each lacuna, interconnecting osteocytes with each other and with the Haversian canal; provide the sole diffusion route for nutrients, gases and waste since bone matrix itself is impermeable
Cement (reversal/growth-reversal) line	A hypermineralized, basophilic, collagen-poor line marking the outer boundary of the osteon; demarcates it from interstitial lamellae or neighbouring osteons; a few canaliculi cross it to allow limited exchange

The overall diameter of a mature osteon is **100–400 μm** . This dimension is not arbitrary: it keeps every osteocyte within about 0.2 mm of a Haversian vessel, the effective diffusion limit through canaliculi, since an osteon has no capillaries of its own beyond its central canal.

Volkmann's (perforating) canals

- Transverse or oblique canals that interconnect adjacent Haversian canals with each other and with vessels of the periosteum and the medullary cavity.
- **Do not** possess their own concentric lamellae — this is the key histological feature distinguishing them from Haversian canals in section.
- Together with Haversian canals they form a continuous three-dimensional vascular network permeating compact bone.

□ Fig 6.7 — Haversian system in compact bone (schematic representation). (IB Singh — *Textbook of Human Histology* — 9e, p.183)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a transverse section through one osteon and label:

- ▶ Haversian (central) canal — with blood vessel and nerve
- ▶ Concentric lamellae (4–6 rings)
- ▶ Lacunae with osteocytes (between lamellae)
- ▶ Canaliculi (radiating from lacunae, joining the canal)
- ▶ Cement (reversal) line — outer boundary of the osteon
- ▶ Volkmann's canal — connecting to a neighbouring Haversian canal
- ▶ Interstitial lamellae — in the angle between osteons

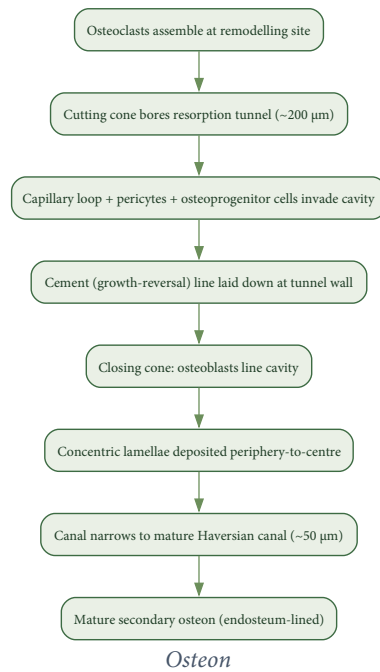
Formation and remodelling of the osteon (basic multicellular unit)

New (secondary) osteons are created by internal remodelling, carried out by a coupled **osteoclast–osteoblast team called the basic multicellular unit (BMU)**, which has two components:

- ▶ **Cutting cone** — a spearhead of osteoclasts that bores a cylindrical resorption tunnel (~200 µm diameter) through existing compact bone, followed closely by an advancing capillary loop, pericytes and osteoprogenitor cells.
- ▶ **Closing cone** — trailing osteoblasts that line the resorption cavity and deposit successive lamellae of osteoid from the periphery inward (the closing cone may contain up to ~4000 osteoblasts/mm²), progressively narrowing the canal until it attains the dimensions of a mature Haversian canal.

A hypermineralized cement (growth-reversal) line marks the boundary between the old bone removed by the cutting cone and the new osteon deposited by the closing cone. Osteons formed early in development around unresorbed woven bone are **primary osteons** (irregular, lack a true cement line); these are progressively replaced throughout life by **secondary osteons**, which alone show a well-defined cement line and fully organized concentric lamellae.

Osteoclast recruitment to the cutting cone is itself osteoblast-dependent: osteoblasts (driven by parathyroid hormone) secrete **M-CSF and RANKL**, which act on osteoclast precursors to trigger their differentiation and activation, while calcitonin opposes resorption. This osteoblast-to-osteoclast signalling is the molecular basis of the coupling that keeps cutting and closing cones moving together through the bone.



Applied / Clinical anatomy

- ▶ **Osteoporosis** — remodelling imbalance (resorption > formation), common in postmenopausal women and immobilized patients, reduces bone mineral density; assessed by DEXA scan.
- ▶ **Osteomalacia** — impaired matrix mineralization from calcium/vitamin D deficiency; demonstrated by tetracycline double-labelling of a bone biopsy, where the inter-label distance measures the rate of appositional bone growth.
- ▶ **Paget's disease (osteitis deformans)** — disorganized remodelling produces a chaotic "mosaic" of irregular cement lines and incompletely converted woven-to-lamellar bone rather than orderly concentric osteons.
- ▶ **Osteopetrosis** — genetic defect of osteoclast ruffled-border/proton-pump function; failure of resorption gives dense, brittle "marble bone" with obliterated marrow spaces.
- ▶ **Forensic age estimation** — the rate of Haversian remodelling slows with age, so the density/number of intact osteons versus fragmentary interstitial lamellae in a cross-section can be used to estimate age at death from skeletal remains.
- ▶ **Fracture healing** — internal remodelling by cutting/closing cones crosses the fracture line to knit new secondary osteons across the repair site, restoring continuity of the Haversian network.
- ▶ **Mechanical adaptation (Wolff's law)** — sustained loading increases cortical thickness and osteon density (e.g., up to 35% greater cortical thickness in the playing arm of elite tennis players), whereas disuse/bed rest accelerates resorption; sustained pressure (as in orthodontic tooth movement) locally favours resorption over deposition.

High-yield exam pointers

- ▶ Osteon = Haversian system = structural unit of compact bone; ~100–400 µm diameter; ~21 million per adult skeleton.
- ▶ Components: Haversian canal (vessels, nerve, endosteum) + 5–20 concentric lamellae + osteocytes in lacunae + canaliculi + cement (reversal) line.

- ▶ Volkmann's canals connect Haversian canals but have **no** concentric lamellae of their own — classic MCQ distinction.
- ▶ Alternating collagen orientation between lamellae → birefringence under polarized light.
- ▶ New osteons form via a coupled BMU: cutting cone (osteoclastic resorption) followed by closing cone (osteoblastic deposition, periphery→centre).
- ▶ Primary osteons (developmental, no true cement line) are replaced throughout life by secondary osteons (well-defined cement line).
- ▶ Diffusion via canaliculi limits osteocyte distance from a vessel to ~0.2 mm — the biological reason for the osteon's small diameter.
- ▶ Clinically anchors osteoporosis, osteomalacia, Paget's disease, osteopetrosis and forensic age estimation.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e.



Periosteum & its clinical importance

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction — The periosteum is the dense, vascular, fibrocellular connective-tissue membrane that invests the external surface of every bone except at sites covered by articular cartilage. It is united to the underlying cortex by extrinsic collagen bundles, the **Sharpey's fibres**, and functions simultaneously as a protective envelope, an attachment site for muscles/tendons/ligaments, a conduit for the periosteal blood supply, and — because it retains a population of osteoprogenitor cells throughout life — the principal source of new bone in growth, remodelling and fracture repair. Its clinical importance follows directly from this dual mechanical-and-osteogenic role.

1. Histological structure — two layers

The periosteum is organised into two poorly demarcated layers:

Layer	Composition	Cells present	Function
Outer fibrous layer	Dense, irregular collagenous (type I) connective tissue with fibroblasts and a rich network of blood vessels and nerves	Fibroblasts	Mechanical protection; anchors periosteum via Sharpey's fibres; carries the periosteal vessels and nerves

Layer	Composition	Cells present	Function
Inner cellular / osteogenic (cambium) layer	Loose, highly cellular connective tissue applied directly to bone surface	Osteoprogenitor cells, osteoblasts (active in growth/repair), occasional osteoclasts	Generates new osteoblasts for appositional bone growth and fracture repair; osteoprogenitor cells are morphologically indistinguishable from fibroblasts when quiescent

The osteogenic layer is thick and highly active in the fetus and growing child (2–3 cells deep, laying down woven bone during appositional growth) but becomes thin and relatively quiescent in the mature adult, re-activating promptly whenever injury or remodelling demands new bone.

2. Attachment to bone — Sharpey's fibres

- ▶ Coarse collagenous bundles from the outer fibrous layer, the **perforating (Sharpey's) fibres**, penetrate obliquely into the outer cortical (circumferential) lamellae.
- ▶ They act like "nails" bolting the periosteum to the bone, and are especially numerous and coarse at sites of tendon/ligament attachment (entheses), e.g. triceps brachii, pronator teres.
- ▶ Because muscle fibres never attach directly to bone, force is transmitted through this periosteal/tendinous connective-tissue continuity rather than to bone itself.

3. Where periosteum is absent

- ▶ Over **articular cartilage** — at the joint margin the periosteum becomes continuous with the fibrous joint capsule instead of continuing over the cartilage.
- ▶ Over **sesamoid bones** (e.g. patella-adjacent sesamoids, ossicles of the ear) — these are not covered by periosteum and ossify by multiple centres without it.
- ▶ Intracapsularly on the **femoral neck** — periosteum is absent within the hip joint capsule, which is why intracapsular (subcapital/transcervical) femoral neck fractures characteristically show slow, unreliable union and a high rate of avascular necrosis/non-union compared with extracapsular fractures.

4. Blood supply and nerve supply

- ▶ **Periosteal arteries** are numerous, ramify within the outer fibrous layer, and enter the cortex through **Volkman's canals** to nourish the outer one-third to one-half of compact bone; they are especially profuse beneath muscular and ligamentous attachments.
- ▶ These vessels anastomose with the medullary (nutrient) and epiphysal/metaphysal systems, so periosteal stripping compromises cortical nutrition.
- ▶ **Innervation** is dense — periosteum is one of the most richly innervated connective tissues in the body, carrying fine myelinated (pain, proprioceptive) and unmyelinated fibres. This explains its marked sensitivity to tearing, tension and pressure.

5. Functions of the periosteum

- ▶ Protects the bone and maintains its external shape/contour.
- ▶ Nourishes the outer cortex via the periosteal vascular plexus.
- ▶ Provides the attachment surface for muscles, tendons, ligaments and intermuscular septa.
- ▶ Supplies osteoprogenitor cells for appositional (circumferential) growth in width.
- ▶ Provides the principal regenerative/osteogenic capacity for fracture healing and remodelling.
- ▶ Transmits pain sensation, protecting bone from excessive mechanical stress.

6. Role in bone development and growth

- ▶ In **endochondral ossification**, the perichondrium around the mid-shaft of the cartilage model is converted into periosteum; its deep osteogenic layer sends **periosteal (osteogenic) buds** — capillary sprouts with osteoprogenitor cells and osteoclasts — into the calcified cartilage to establish the primary ossification centre, and lays down a **periosteal (bone) collar** around the diaphysis that impedes diffusion into the cartilage core and initiates cortical bone formation.
- ▶ Postnatally, **appositional growth** (increase in bone diameter) occurs exclusively by osteoblasts differentiating from periosteal osteoprogenitor cells depositing new circumferential lamellae, balanced by endosteal resorption that enlarges the medullary cavity — the classic internal remodelling seen as a long bone widens with age.

□ Fig 6.4 — Structure of compact bone: (A) Haversian systems and lamellae; (B) an osteon and (C) periosteum and outer circumferential lamellae of a compact bone. (Vishram Singh — General Anatomy — 4e, p.232)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

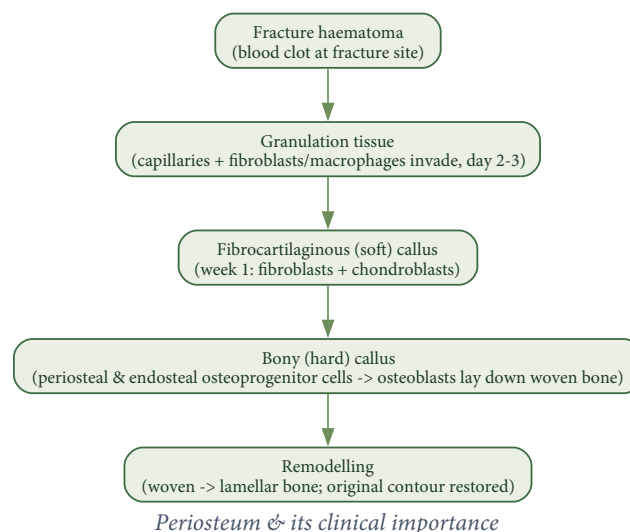
Draw a transverse section through compact bone and label:

- ▶ **Periosteum** — outer fibrous layer and inner osteogenic (cambium) layer
- ▶ **Sharpey's (perforating) fibres** penetrating outer circumferential lamellae
- ▶ **Periosteal blood vessel** entering via a **Volkmann's canal**
- ▶ **Outer circumferential lamellae**
- ▶ **Osteon (Haversian system)** with central Haversian canal and concentric lamellae
- ▶ **Endosteum** lining the medullary cavity, with osteoblasts/osteoprogenitor cells

Applied / Clinical anatomy

- ▶ **Fracture repair (external/periosteal callus)** — the osteoprogenitor cells of the periosteum (and endosteum) around a fracture proliferate intensely, forming a soft fibrocartilaginous callus that is progressively invaded and replaced by woven bone (bony/external callus) and finally remodelled into mature lamellar bone. Because this periosteal reaction is the dominant route of healing in children and in most diaphysial fractures, an intact, well-apposed periosteum is essential for sound union.
- ▶ **Slow union where periosteum is deficient** — intracapsular fractures of the femoral neck (periosteum absent within the hip capsule) and periosteum stripped by high-energy trauma or surgery both heal slowly/unreliably, since the main osteogenic surface is lost.

- ▶ **"Bone stripped of its periosteum will not survive"** — this underlies the surgical principle of minimising periosteal stripping during open reduction/internal fixation, and the rationale for **vascularised periosteal/bone grafts** in reconstructive and maxillofacial surgery, where the transferred periosteum carries its own osteogenic and blood supply.
- ▶ **Subperiosteal rib resection** — in thoracic surgery, a rib segment can be excised leaving the periosteal sheath intact; the rib subsequently regenerates (albeit imperfectly) from the retained periosteum, allowing the sheath itself to be used as a safe surgical entry plane.
- ▶ **Neonatal cephalohaematoma** — birth trauma can rupture minute periosteal (calvarial) vessels, producing a subperiosteal haemorrhage that is sharply limited by suture lines because the periosteum is firmly adherent there — a classic clinical sign distinguishing cephalohaematoma from caput succedaneum.
- ▶ **Extradural (epidural) haematoma** — the outer periosteal layer of the cranial dura is the periosteum of the inner table of the skull; a torn middle meningeal artery strips this layer from bone to create the extradural space and haematoma, again limited at firm sutural attachments.
- ▶ **Pain and clinical signs** — the dense sensory innervation of periosteum makes it exquisitely tender to percussion, tearing or tension; this is exploited clinically (point tenderness over a fracture or subperiosteal abscess) and explains why an intramuscular injection needle striking bone/periosteum causes sharp pain, and why drilling bone requires local anaesthesia infiltrated to the periosteum.
- ▶ **Sesamoid bones and articular ends** lack periosteum, which is why they are excluded from the classical description of "bones" in some classifications and why articular fractures/osteochondral injuries heal by a different (chondral/synovial) route rather than a periosteal callus.



High-yield exam pointers

- ▶ Two layers: **outer fibrous** (fibroblasts, vessels, nerves) and **inner osteogenic/cambium** (osteoprogenitor cells, osteoblasts).
- ▶ Attached to bone by **Sharpey's fibres**; absent over **articular cartilage**, **sesamoid bones**, and intracapsularly at the **femoral neck**.
- ▶ Periosteal arteries enter cortex via **Volkman's canals**; supply outer 1/3–1/2 of compact bone.

- ▶ Densely innervated → exquisitely pain-sensitive (basis of IM-injection pain, periosteal tenderness).
- ▶ Source of **appositional bone growth** (width) and the **periosteal (bone) collar** in endochondral ossification.
- ▶ Principal source of the **external/bony callus** in fracture repair — bone stripped of periosteum will not survive, hence its surgical preservation in grafting and rib resection.
- ▶ Clinical correlates: slow union of intracapsular femoral neck fractures, neonatal cephalohaematoma limited by sutures, extradural haematoma (periosteal layer of cranial dura), subperiosteal rib regeneration after thoracoplasty.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Vishram Singh — General Anatomy 4e; Junqueira's Basic Histology 17e; IB Singh — Textbook of Human Histology 9e; IB Singh — Textbook of Anatomy Vol 1 6e; Moore's Clinically Oriented Anatomy 9e.



Plasma cell

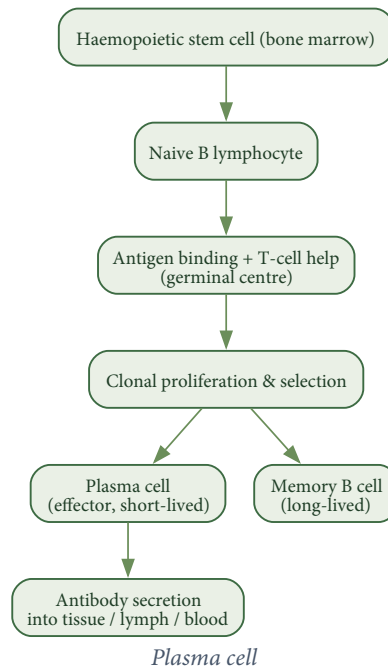
Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction A plasma cell (plasmacyte) is a terminally-differentiated, antibody-secreting effector cell derived from an antigen-activated B lymphocyte. It is a normal, though scanty, resident cell of loose (areolar) connective tissue and of the lymphoid organs, and is the structural and functional basis of humoral (antibody-mediated) immunity. Its light- and electron-microscopic features are classical "must-know" histology because they directly reflect its single-minded job of synthesising and exporting one specific immunoglobulin in bulk.

Origin and development

- ▶ Plasma cells arise from **B lymphocytes**, which themselves differentiate from haemopoietic stem cells in the bone marrow.
- ▶ After the naive B cell's surface immunoglobulin receptor binds its specific antigen, and with co-stimulation from helper T cells, the B cell proliferates within **germinal centres** of lymphoid tissue (lymph node, spleen, MALT/GALT).
- ▶ Progeny of this activated clone follow one of two fates:
- ▶ **Plasma cells** — large, basophilic, antibody-secreting cells, formed within or outside the lymphoid tissue.
- ▶ **Memory B cells** — long-lived cells that mount a faster, higher-affinity antibody response on re-exposure to the same antigen.
- ▶ Because the immunoglobulin gene's variable-region DNA has already undergone somatic recombination in the parent B cell, every plasma cell of a clone secretes antibody of **one specificity only**.

- Plasma cells have only limited migratory capacity once formed; they leave the lymphoid tissue and settle in connective tissue at the site where antibody is needed.



Distribution

- Small numbers occur in **most ordinary connective tissues**.
- Particularly numerous wherever antigens enter the body: **lamina propria of the gastrointestinal and respiratory tracts**, salivary gland stroma, and skin.
- Abundant in lymphoid organs — **lymph node medullary cords**, splenic red pulp, and diffuse lymphoid tissue of MALT/GALT.
- Plasma cells account for only **1–3% of cells in a resting lymphoid follicle/lymph node**, rising sharply during an active immune response.
- Absent from the thymus cortex proper but variable numbers are found in the thymic medulla.

Light microscopic structure

Feature	Description
Shape and size	Large, ovoid cell, about 20 μm in diameter
Cytoplasm	Strongly basophilic (deep blue/purple with H&E) due to abundant rough endoplasmic reticulum (RER) rich in ribosomal RNA
Perinuclear (negative) Golgi zone	A conspicuous pale-staining, unstained ("negative Golgi") area next to the nucleus, corresponding to a large Golgi apparatus
Nucleus	Spherical, eccentrically placed (pushed to one pole of the cell)

Feature	Description
Nuclear chromatin pattern	Coarse peripheral clumps of heterochromatin alternating with paler euchromatin, producing the classical " cart-wheel " / " clock-face " appearance
Cytoplasmic inclusions	May contain Russell bodies — rounded, PAS-positive, eosinophilic inclusions of excess stored immunoglobulin within dilated RER cisternae; a plasma cell packed with Russell bodies is called a Mott cell

□ Fig 6.25 — ▲ The plasma cell. a. This photomicrograph shows the typical features of a plasma cell as seen in a routine H&E preparation. Note clumps of peripheral heterochromatin alternating with clear areas of euchromatin in the nucleus. Also note the negative Golgi and basophilic cytoplasm. b. Electron micrograph shows that an extensive rER occupies most of the cytoplasm of the plasma cell (Ross Histology — A Text and Atlas — 7e, p.202)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw an ovoid cell and label:

- ▶ Cell membrane (limited motility, no further division)
- ▶ **Eccentric, spherical nucleus**
- ▶ **Cart-wheel / clock-face heterochromatin** — peripheral clumps of condensed chromatin resembling numerals of a clock, alternating with paler euchromatin
- ▶ **Perinuclear negative (pale) Golgi zone** — juxtannuclear clear halo
- ▶ Extensive **rough endoplasmic reticulum** filling most of the basophilic cytoplasm (parallel cisternae on EM)
- ▶ Occasional **Russell body** (rounded eosinophilic/PAS+ inclusion of stored immunoglobulin within a dilated RER cisterna)
- ▶ Secreted antibody molecules diffusing from cell surface into surrounding tissue

Ultrastructure (electron microscopic features)

- ▶ **Nucleus** — small, only slightly larger than a lymphocyte's nucleus, eccentric, with the heterochromatin arranged as peripheral clumps (the ultrastructural basis of the cart-wheel pattern).
- ▶ **Rough endoplasmic reticulum** — extensive, arranged as stacked parallel cisternae that occupy most of the cytoplasm; this is the site of immunoglobulin (heavy and light chain) synthesis.
- ▶ **Golgi apparatus** — large and well developed, positioned next to the nucleus; site of terminal glycosylation and packaging of the antibody (glycoprotein) before secretion — corresponds to the light-microscopic "negative Golgi" halo.
- ▶ **Mitochondria** — moderate numbers, providing energy for the high rate of protein synthesis and secretion.
- ▶ Few free ribosomes/polysomes are seen outside the RER because most translational machinery is membrane-bound, consistent with a cell dedicated to **export (secretory) protein synthesis**.

- ▶ Only a small segment of the genome remains transcriptionally active (that coding for one specific antibody), which explains why a cell engaged in such intense synthetic activity nonetheless shows a condensed, heterochromatin-rich nucleus.

Functional histology — antibody synthesis and secretion

- ▶ Plasma cells synthesise **immunoglobulins (antibodies)** — glycoproteins built of two identical heavy chains and two identical light chains linked by disulfide bonds, each with a constant (Fc) region and a variable antigen-binding (Fab) region.
- ▶ Antibody is synthesised on the RER, glycosylated and packaged in the Golgi, and released from the cell by **exocytosis**; secreted antibody may act locally in the tissue, enter blood/lymph, or in some cases (dilated RER, Russell bodies) accumulate transiently within the cell.
- ▶ **Five major immunoglobulin classes** are recognised, each with a distinct constant heavy-chain region: **IgG, IgA, IgM, IgD and IgE**.
 - **IgG** — the most abundant serum antibody (about 75–85% of circulating immunoglobulin); long half-life (>3 weeks); crosses the placenta, giving the fetus/neonate passive immunity.
 - **IgA** — secreted by plasma cells in the lamina propria of the digestive, respiratory and reproductive mucosae; assembled as a dimer joined by a **J chain** (made by the plasma cell) and combined with a **secretory component** (supplied by the covering epithelium) during transcytosis — the basis of **mucosal (secretory) immunity** in milk, saliva, tears and mucus.
 - **IgM** — the first antibody produced in a primary immune response.
 - IgD and IgE have specialised surface/effector roles (IgE mediates immediate hypersensitivity via mast cell/basophil Fc receptors).
- ▶ Each plasma cell (and its clonal progeny) secretes antibody of **one specificity only**, directed against the antigen that originally activated its parent B lymphocyte.
- ▶ Plasma cells are **short-lived**, with an average lifespan of roughly 10–30 days; they die once the antigenic stimulus is cleared.

Applied / Clinical anatomy

- ▶ **Multiple myeloma** — a malignant, monoclonal proliferation of plasma cells (typically in bone marrow), producing large amounts of a single, homogeneous immunoglobulin or its light chain fragment; the basis of monoclonal antibody technology conceptually derives from this single-clone, single-specificity behaviour of plasma cells.
- ▶ **Plasmacytoma** — a localised (solitary) neoplastic mass of plasma cells.
- ▶ **Mott cells** — plasma cells distended with multiple Russell bodies (stored immunoglobulin in dilated RER); seen in chronic inflammatory and some neoplastic states.
- ▶ **Reactive plasmacytosis** — a marked rise in tissue/lymph node plasma cell numbers is a reliable histological marker of chronic inflammation or ongoing immune stimulation (e.g., chronically inflamed lamina propria, chronic gastritis, syphilis).
- ▶ **Mucosal immunity/IgA deficiency** — because IgA is the principal secretory antibody made by mucosal plasma cells, IgA deficiency (a relatively common primary immunodeficiency) predisposes to recurrent mucosal (respiratory/GI) infections.

- ▶ **Diagnostic/teaching relevance** — the cart-wheel nucleus and negative Golgi hof are classic identification points in histology practicals and in interpreting inflamed connective tissue or chronic granulation tissue sections.

High-yield exam pointers

- ▶ Plasma cell = terminally differentiated **B lymphocyte**; effector cell of humoral immunity.
- ▶ Ovoid, ~15–20 µm; **basophilic cytoplasm** (RER-rich) + **pale perinuclear Golgi halo**.
- ▶ **Eccentric nucleus** with **cart-wheel/clock-face heterochromatin**.
- ▶ **Russell bodies** = stored Ig in dilated RER; a plasma cell full of them = **Mott cell**.
- ▶ Short-lived cell (~10–30 days); accounts for only **1–3%** of cells in a resting lymph node, rising during immune response.
- ▶ Secretes **one antibody specificity** per clone; five Ig classes — **IgG, IgA, IgM, IgD, IgE**.
- ▶ IgA is unique among mucosal secretions — dimeric, joined by **J chain**, exported with epithelial **secretory component**.
- ▶ Sites: lamina propria of GI/respiratory tract, lymph node medullary cords, splenic red pulp, salivary gland stroma.
- ▶ Clinical correlate: **multiple myeloma** (malignant monoclonal plasma cell proliferation) and **plasmacytoma**.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



Stratified Epithelium

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Epithelium is a sheet of closely apposed cells covering external body surfaces or lining internal tubes and cavities, resting on a basement membrane. When two or more layers of cells are present, with only the basal layer resting on the basement membrane, the tissue is called **stratified (multilayered) epithelium** — as opposed to simple (unilayered) or pseudostratified epithelium. Stratified epithelium is named according to the **shape of the cells of its most superficial (surface) layer**, giving four types: stratified squamous, stratified cuboidal, stratified columnar, and transitional epithelium (urothelium). Its multilayered architecture sacrifices some absorptive/secretory efficiency for greater mechanical, chemical and microbial protection.

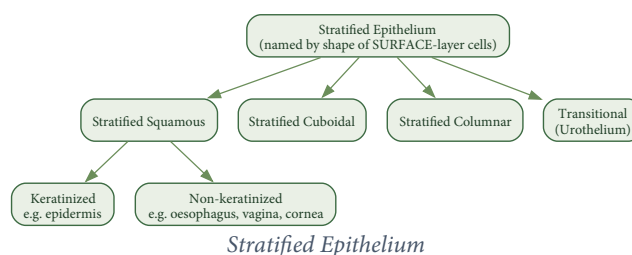
General Features and Functions of Epithelium

Feature	Description
Cellularity	Cells tightly packed, minimal intercellular space (~20 nm)

Feature	Description
Vascularity	Avascular; nourished by diffusion from underlying connective tissue
Attachment	Basal layer rests on a basement membrane
Polarity	Distinct apical, lateral and basal cell domains
Surface specialisation	Site-specific microvilli, cilia or stereocilia on the apical surface
Regenerative capacity	High; basal layer is mitotically active
Plasticity	Capable of metaplasia — reversible change to another epithelial type
Embryological origin	Derived from all three germ layers (ectoderm, mesoderm, endoderm)

- **Functions:** protection from abrasion/injury; absorption; secretion (mucus, hormones, enzymes); exchange/transcellular transport.

Classification of Stratified Epithelium



Type	Layers (base → surface)	Principal locations	Function
Stratified squamous (keratinized)	Basal columnar → polyhedral (prickle) → progressively flattened → anucleate keratin squames	Epidermis; mucocutaneous junctions (lips, nostrils, distal anal canal); outer tympanic membrane; gingivae, hard palate, filiform papillae of tongue	Protection against friction, water loss, microbial invasion
Stratified squamous (non-keratinized)	Basal columnar → polyhedral → flattened but nucleated superficial cells	Buccal cavity (except sites above), oropharynx, laryngopharynx, oesophagus, true vocal folds, part of anal canal, vagina, distal uterine cervix, distal urethra, cornea, inner eyelids, nasal vestibule	Protection with distensibility in a moist environment

Type	Layers (base → surface)	Principal locations	Function
Stratified cuboidal	2 layers of cuboidal cells	Ducts of sweat glands; anorectal transition zone of the anal canal	Greater strength/protection than simple epithelium
Stratified columnar	Basal cuboidal/polyhedral layer + superficial columnar layer	Conjunctiva (palpebral/fornix); large excretory ducts of salivary glands and pancreas; parts of the male urethra (membranous, penile)	Protection; conducts glandular secretions
Transitional (urothelium)	4–6 layers: basal cuboidal → intermediate polyhedral/pear-shaped → superficial dome-shaped "umbrella" cells	Renal pelvis and calyces, ureter, urinary bladder, proximal part of the urethra	Distensible, impermeable barrier protecting tissue from toxic urine

Stratified Squamous Epithelium (Keratinized and Non-keratinized)

- Commonest and most extensive type of stratified epithelium; found on surfaces subject to friction.
- **Basal layer** — columnar cells resting on the basement membrane; site of frequent mitosis, replacing cells constantly shed at the surface.
- **Middle layer(s)** — polyhedral/polygonal cells (the "prickle cell" layer), held together by numerous desmosomes.
- **Superficial layer** — progressively flattens toward the surface.

Feature	Keratinized	Non-keratinized
Surface	Dry	Moist
Superficial cells	Anucleate, dead, flattened squames packed with keratin	Flattened but living, retain nuclei until desquamation
Distinguishing layer	Stratum corneum (keratin + keratohyalin granules)	No stratum corneum
Typical sites	Entire epidermis; mucocutaneous junctions (lips, nostrils, distal anal canal); outer tympanic membrane; gingivae, hard palate, filiform papillae of tongue	Buccal cavity, oropharynx, laryngopharynx, oesophagus, true vocal folds, vagina, distal cervix, distal urethra, cornea, inner eyelids, nasal vestibule
Variant	Orthokeratinized — fully keratinized (epidermis)	Parakeratinized — partial keratinization at high-abrasion oral sites
Vitamin A effect	—	Deficiency induces keratinization of normally non-keratinized epithelium; excess induces mucus-secreting metaplasia

□ Fig 4.15 — Stratified epithelium. (Junqueira's Basic Histology — Text & Atlas — 17e, p.223)

Stratified Cuboidal and Stratified Columnar Epithelium

- ▶ **Stratified cuboidal** — only two layers of cuboidal cells; found in the **ducts of sweat glands**, and at the anorectal transition zone where colonic simple columnar epithelium abruptly changes at the anal canal.
- ▶ **Stratified columnar** — a deeper polyhedral-to-cuboidal layer plus a superficial columnar layer; found in the **conjunctiva** (palpebral and fornical), certain **large excretory ducts** (salivary glands, pancreas), and parts of the **male urethra**.
- ▶ Both are relatively rare, are not continually replaced by basal mitoses (unlike stratified squamous), but can repair themselves if damaged.
- ▶ Function: mainly **protective**, with a secondary role in conducting glandular secretions.

Transitional Epithelium (Urothelium)

- ▶ A specialised stratified epithelium, 4–6 cells thick, lining the **urinary tract** from the ends of the renal collecting ducts through the renal pelvis, ureter and bladder to the proximal urethra — hence also called **urothelium**.
- ▶ Layers: **basal** cuboidal/columnar cells; **intermediate** polyhedral or pear-shaped cells; **superficial "umbrella" (dome) cells**, which are large, may be binucleate or polyploid, and are the only fully differentiated layer.
- ▶ Umbrella-cell plasma membranes contain thickened **intramembranous plaques** (glycoprotein-rich), rendering the surface impermeable and resistant to the toxic, hypertonic contents of urine.
- ▶ Cells are firmly bound by numerous **desmosomes**, so they retain their relative position whether the epithelium is relaxed or stretched.
- ▶ In the **relaxed** state the epithelium appears 4–6 layers thick with rounded umbrella cells; when **stretched** (e.g. a full bladder) the epithelium thins, cells flatten, and apparent layering decreases — all without loss of barrier integrity.
- ▶ Basal mitoses are infrequent compared with stratified squamous epithelium, since normal surface erosion is minimal.

□ Fig 4.9 — Transitional epithelium in (a) relaxed state and (b) stretched state. (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.58)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A. Stratified squamous epithelium (layered section, keratinized vs non-keratinized) — label: Basement membrane → Stratum basale (columnar cells, mitotic figures) → Stratum spinosum/prickle-cell layer (polyhedral cells, desmosomes) → Stratum granulosum (keratohyalin granules — keratinized type only) → Stratum corneum (anucleate keratin squames, keratinized) or superficial nucleated flattened squames (non-keratinized).

B. Transitional epithelium — relaxed vs distended — label: Basement membrane; Basal cuboidal layer; Intermediate polyhedral/pear-shaped layer; Superficial umbrella (dome) cells with surface plaques; annotate "relaxed — rounded, 4–6 layers apparent" vs "stretched — flattened, fewer apparent layers".

Basement Membrane and Intercellular Junctions

- ▶ **Basement membrane** — thin membrane of protein fibres and mucopolysaccharides separating epithelium from underlying connective tissue; not distinct on H&E but well demonstrated with PAS stain.
- ▶ Ultrastructurally comprises: **basal lamina** (lamina lucida, adjacent to cell membranes + lamina densa, containing fibrils) and an underlying **reticular (fibroreticular) lamina** merging with connective tissue.
- ▶ **Intercellular junctions** binding stratified epithelial cells laterally: **desmosomes**, **zonulae adherens**, and **zonulae occludens (tight junctions)** — these ensure materials crossing the epithelium pass through cells rather than between them, and mechanically bind the multiple layers into a coherent sheet.
- ▶ **Renewal**: basal-layer mitosis continuously replaces cells lost by surface desquamation, maintaining a steady state (e.g. epidermal renewal from basal mitosis to keratinization takes about 3–4 weeks).

Applied / Clinical anatomy

- ▶ **Squamous cell carcinoma** arises from stratified squamous epithelium — classically skin (sun-exposed epidermis), oral cavity, oesophagus, uterine cervix and anal canal.
- ▶ **Cervical squamous metaplasia** at the transformation zone (squamocolumnar junction) is a *physiological* change of endocervical columnar epithelium into stratified squamous epithelium under the hormonally-influenced vaginal environment; this zone is the site most vulnerable to HPV-related dysplasia (CIN) and is the basis of **Pap-smear** screening.
- ▶ **Barrett's oesophagus** — chronic gastro-oesophageal reflux replaces the normal non-keratinized stratified squamous lining of the lower oesophagus with mucus-secreting **columnar (gastric-type) epithelium**; this is a *pathological*, premalignant gastric metaplasia.
- ▶ **Bronchial squamous metaplasia** — the ciliated respiratory (pseudostratified columnar) epithelium of the upper airway undergoes squamous metaplasia in response to irritants in cigarette smoke; a recognised precursor change in chronic smokers.

- ▶ **Vitamin A deficiency** worsens/induces keratinization of normally non-keratinized epithelia (e.g. conjunctival xerosis/Bitot spots), whereas excess vitamin A can convert keratinizing epithelium into a mucus-secreting one.
- ▶ **Transitional cell (urothelial) carcinoma** is the commonest bladder neoplasm, arising from the urothelium; tumours are often multifocal because the entire urothelial lining is exposed to the same carcinogenic urinary field.
- ▶ **Exfoliative cytology** exploits the continuous desquamation of stratified squamous epithelium — the basis of the cervical Pap smear and the buccal smear (sex-chromatin/Barr-body study).
- ▶ **Burns** destroying only the epidermis regenerate from stratum basale mitoses; full-thickness burns destroying the basal layer heal by scarring rather than epithelial regeneration.
- ▶ **Psoriasis** shows hyperproliferation of the basal/prickle layers with abnormal, accelerated (para)keratinization.

High-yield exam pointers

- ▶ Stratified epithelium is named by the shape of its **surface (not basal)** layer — squamous, cuboidal, columnar, or transitional.
- ▶ Only **four** types exist: stratified squamous (keratinized/non-keratinized), stratified cuboidal, stratified columnar, and transitional (urothelium).
- ▶ Mitosis for renewal occurs only in the **basal layer**; superficial cells are progressively pushed up and desquamate.
- ▶ Keratinized = dry, anucleate surface (epidermis); non-keratinized = moist, nucleated surface (oesophagus, vagina, cornea, true vocal folds).
- ▶ **Parakeratinized** = partial keratinization at high-friction oral sites (gingiva, hard palate); **orthokeratinized** = full keratinization (epidermis).
- ▶ Transitional epithelium = **urothelium**; surface umbrella cells bear membrane plaques that make it impermeable to urine and allow stretch without loss of integrity.
- ▶ Stratified epithelium trades absorptive/secretory efficiency for greater mechanical, microbial and chemical **protection** compared with simple epithelium.
- ▶ Desmosomes, zonulae adherens and zonulae occludens bind adjoining epithelial cells and make the barrier tight.
- ▶ **Metaplasia** typically converts columnar to squamous epithelium under chronic irritation (physiological in the cervix; pathological in bronchus/oesophagus).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; Brijesh Kumar — Histology Text & Atlas 3e; Vishram Singh — General Anatomy 4e.



Transitional Epithelium

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction Transitional epithelium, now more precisely termed **urothelium**, is a specialised multilayered (stratified) epithelium found exclusively in the urinary passages, extending from the ends of the renal collecting ducts, through the calyces, pelvis, ureter and bladder, to the proximal part of the urethra. It is called "transitional" because, on stretching to accommodate variable volumes of urine, it shows an apparent transition in form between a relaxed stratified-cuboidal appearance and a distended stratified-squamous appearance. Its defining features — a variable number of cell layers, large surface "umbrella" cells with a plaque-stiffened apical membrane, and tight intercellular junctions — together create a highly distensible yet virtually impermeable barrier between urine and the underlying tissues/bloodstream.

1. Distribution

Region	Lining	Key point
Collecting ducts (renal papilla) → minor/major calyces, renal pelvis	Urothelium, thinnest here (as few as 2 cell layers in the minor calyces)	Continuous with simple/stratified columnar epithelium of the papilla proximally
Ureter	Urothelium, 4–5 layers	Continuous proximally with the pelvis, distally with the bladder
Urinary bladder	Urothelium, 4–7 layers relaxed, attenuated to 2–3 layers when fully distended	Thickest part of the urothelium; lines the trigone as well as the rest of the bladder
Prostatic urethra (male)	Urothelium, continuous with bladder neck	Becomes intermittent and is replaced by stratified/pseudostratified columnar epithelium in the membranous urethra, then stratified squamous distally in the penile urethra
Female urethra	Urothelium near the internal urethral orifice only	Remainder of the urethra is lined by stratified squamous epithelium; urothelium extends only as far as the urogenital membrane

2. Histological structure — the three functional layers

- **Basal layer** — a single row of small, cuboidal, basophilic cells (rich in ribosomes) resting on the basal lamina; uninucleate and diploid; mitotically active and forms the germinal/stem-cell layer from which the upper layers arise; normal cell turnover is slow, but regeneration is rapid after injury.
- **Intermediate layer(s)** — several rows of polygonal or pear-shaped cells with the capacity to stretch and flatten; nuclei may be binucleate or polyploid; these cells can differentiate further to replace lost surface cells.

- ▶ **Superficial (umbrella/dome) layer** — a single layer of the largest cells in the epithelium (some binucleate, occasionally polyploid up to octoploid), bulging into the lumen as dome-shaped cells in the relaxed state; it is the only fully differentiated layer, has a thickened eosinophilic glycocalyx, shows degenerative cytoplasmic changes with age, and its cells are ultimately exfoliated into the urine.

□ Fig 4.9 — Transitional epithelium in (a) relaxed state and (b) stretched state. (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.58)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the ureter/bladder mucosa in **two panels** — **relaxed and stretched** — and label:

- ▶ Lumen
- ▶ Superficial (umbrella/dome-shaped) cell layer — large, rounded, some binucleate
- ▶ Intermediate (polygonal/pear-shaped) cell layer — several rows
- ▶ Basal (cuboidal) cell layer — resting on basement membrane
- ▶ Basement membrane
- ▶ Lamina propria (underlying connective tissue)
- ▶ Annotate: relaxed panel shows 4–7 rows and dome-shaped surface cells; stretched panel shows the same sheet flattened to 2–3 rows with squamous-looking surface cells.

3. Ultrastructure of the umbrella cell — the permeability (blood–urine) barrier

- ▶ The apical (luminal) plasma membrane of the umbrella cell is an **asymmetric unit membrane (AUM)** — rigid plaques of hexagonally packed, ~16 nm **uroplakin** protein particles, separated by narrow, flexible **hinge** regions of thinner interplaque membrane.
- ▶ On relaxation, the plaques are partially internalised by folding of the hinge regions and stored in cytoplasmic **fusiform vesicles**; on stretching, these vesicles fuse back with the surface and the plaques re-emerge — allowing a large change in apparent surface area without loss of membrane integrity.
- ▶ These plaques, together with the thickened glycocalyx, stiffen the umbrella-cell surface and form the **permeability barrier** that prevents reabsorption of hypertonic, acidic and potentially toxic urinary solutes into the bloodstream, and prevents loss of plasma constituents into the urine.
- ▶ Adjacent umbrella cells are sealed by extensive apical **tight junctions (zonulae occludentes)**; numerous **desmosomes** bind all three layers into a strong, contiguous, mechanically coherent sheet.

4. Developmental origin

- ▶ The whole urothelium is of **endodermal** lineage, though from two different embryonic sources.
- ▶ The urothelium of the **ureter, renal pelvis, major and minor calyces, and the collecting tubules** is derived from the epithelium of the **ureteric bud**, an outgrowth of the caudal mesonephric duct.
- ▶ The **entire epithelium of the urinary bladder** is derived directly from the **endoderm of the vesical part of the urogenital sinus**; the other layers of the bladder wall (lamina propria,

muscularis, part of the trigone connective tissue) develop from adjacent splanchnic mesenchyme and the absorbed caudal mesonephric ducts.

- ▶ The urothelium of the **prostatic urethra** is continuous with that of the bladder neck (urogenital-sinus endoderm); more distally, the membranous and penile urethral epithelium have different origins and are no longer urothelium.

5. Functional correlations

- ▶ **Distensibility** — the apparent number of cell layers and epithelial thickness change reversibly with luminal volume (without disruption of the sheet or its junctions), which is essential for urine storage in the bladder and for peristaltic transport in the ureter.
- ▶ **Barrier function** — the AUM plaques, glycocalyx and tight junctions together form a near-impermeable barrier, protecting deeper tissues from the mechanical, chemical and microbial hazards of urine.
- ▶ **Slow but adaptable renewal** — basal cells are normally quiescent (very low mitotic index) but can proliferate rapidly to regenerate the epithelium after damage (e.g. following catheterisation, infection or calculus trauma).

Applied / Clinical anatomy

- ▶ **Von Brunn's nests / cystitis cystica**: islands or nests of urothelium may become pinched off from the surface during development and lie embedded in the underlying lamina propria; these can undergo central cystic degeneration to produce **cystitis cystica**, a common benign finding on cystoscopy/histology of the bladder mucosa.
- ▶ **Urinary tract infection**: uropathogenic *Escherichia coli* (responsible for ~85% of UTIs) attach via **FimH adhesins** at the tip of their filamentous attachment organelles, which interact directly with the uroplakins of the umbrella-cell plaques and trigger bacterial invasion of the urothelium — the structural basis for ascending and recurrent UTI.
- ▶ **Urothelial (transitional cell) carcinoma**: the commonest primary neoplasm of the renal pelvis, ureter and bladder arises from this epithelium; because a single continuous sheet of urothelium lines the whole tract, tumours are frequently multifocal/papillary ("field change") and require examination of the entire urinary tract on follow-up. Urine/effusion cytology and immunocytochemical markers are used to identify urothelial carcinoma cells and their nodal metastases.
- ▶ **Exfoliative cytology**: superficial umbrella cells are physiologically shed into urine, providing the cytological basis for non-invasive screening and monitoring of urothelial malignancy.
- ▶ **Instrumentation tolerance**: the epithelium's distensibility and rapid regenerative capacity allow it to withstand catheterisation and endoscopic instrumentation, though repeated trauma or chronic irritation can still provoke reactive or metaplastic change.

High-yield exam pointers

- ▶ Urothelium (transitional epithelium) is unique to the urinary passages — renal calyces/pelvis, ureter, bladder, and proximal urethra.
- ▶ "Transitional" refers to the apparent change in form between relaxed (stratified cuboidal-like) and stretched (stratified squamous-like) states.

- ▶ Three functional layers: basal (stem cells, cuboidal) → intermediate (polygonal, can be binucleate) → superficial umbrella cells (largest, only fully differentiated layer).
- ▶ Layer count varies inversely with distension: as few as 2 layers in minor calyces, 4–5 in ureter, 4–7 relaxed / 2–3 fully distended in bladder.
- ▶ Umbrella-cell apical membrane = asymmetric unit membrane with hexagonal ~16 nm uroplakin plaques, hinge regions, and fusiform vesicles for surface-area reserve.
- ▶ Tight junctions + desmosomes complete the permeability (blood–urine) barrier and give mechanical strength.
- ▶ Whole urothelium is endodermal: ureteric bud → pelvis/calyces/ureter; vesical part of urogenital sinus → bladder.
- ▶ Von Brunn's nests → cystitis cystica is the classic benign developmental correlate.
- ▶ Urothelial (transitional cell) carcinoma is the commonest urinary-tract tumour and is often multifocal because of the continuous shared epithelial lining.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; Moore — The Developing Human 9e; Langman's Medical Embryology (Sadler) 14e; Pranab Dey — Lab Techniques in Histopathology & Cytology 2e (urothelial carcinoma cytology markers).



Systemic histology - cardiovascular, blood & lymphoid



Compare the microscopic structure of the lymphoid organs

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction Lymphoid organs are aggregations of lymphoid tissue supported by a reticular (or, in the thymus, an epithelial) framework, and are classified as **primary** (thymus, bone marrow — site of lymphocyte generation and antigen-independent maturation) or **secondary/peripheral** (lymph node, spleen, tonsil and other MALT — sites of antigen-dependent proliferation). Lymph node, spleen and thymus share a basic plan of an outer **capsule** giving off **trabeculae/septa** that divide the organ into compartments, but each is built around a different physiological task — filtering lymph, filtering blood, or "schooling" T-lymphocytes — and this functional difference is what produces their distinct histology. The palatine tonsil, in contrast, has no capsule or cortex-medulla organisation. The four organs are compared systematically below.

1. Lymph node

□ Fig 11.5 — Structure of a lymph node. (Vishram Singh — General Anatomy — 4e, p.490)

- ▶ Small, bean-shaped bodies (0.1–2.5 cm) along the course of lymphatics; up to **450 lymph nodes** are present in the adult body (60–70 head/neck, ~100 thorax, ~250 abdomen and pelvis).

- ▶ **Capsule** of collagen and elastin sends **trabeculae** inward; just deep to the capsule is the **subcapsular sinus**, which receives several **afferent lymphatics** piercing the convex surface.
- ▶ **Cortex** (outer, darkly staining):
 - **Outer cortex** — spherical **lymphoid follicles/nodules** of B-lymphocytes and follicular dendritic cells (FDCs). A **primary follicle** is a uniform mass of small resting B-cells; a **secondary follicle** has a pale **germinal centre** (proliferating centroblasts in a "dark zone", centrocytes undergoing affinity selection in a "light zone") surrounded by a rim of small lymphocytes, the **mantle zone/corona**.
 - **Paracortex (inner/deep cortex)** — no follicles; populated mainly by T-lymphocytes and interdigitating dendritic cells, hence called the **thymus-dependent zone**. Contains **high endothelial venules (HEV)**, lined by cuboidal endothelium, through which circulating lymphocytes extravasate into the node.
- ▶ **Medulla** (lighter staining, continuous with hilum): branching, anastomosing **medullary cords** (mainly B-lymphocytes, plasma cells, macrophages) separated by **medullary sinuses**.
- ▶ **Sinus system**: subcapsular sinus → radial cortical (intermediate/peritrabecular) sinuses → medullary sinuses → single **efferent lymphatic** at the hilum. Sinuses are criss-crossed by reticular cells/fibres and macrophages that filter particulate matter and antigen from lymph.
- ▶ **Vasculature**: artery and vein traverse the hilum; arterioles/capillaries loop through cortex and medulla; the reticular network is of type III collagen (reticulin), produced by fibroblastic reticular cells.
- ▶ **Function**: lymph filtration, antigen presentation, and lymphopoiesis (both B- and T-cell proliferation).

2. Spleen

□ Fig 11.8 — Spleen. (Vishram Singh — General Anatomy — 4e, p.496)

- ▶ Largest lymphoid organ; unlike the other three, it filters **blood**, not lymph, and receives **no afferent lymphatics**.
- ▶ **Capsule** (dense connective tissue, elastin-rich) gives off **trabeculae** carrying trabecular vessels into the parenchyma.
- ▶ **White pulp** — grey nodules seen in the fresh unstained organ:
 - **Periarterial lymphatic sheath (PALS)** — a cylindrical cuff of predominantly T-lymphocytes around a **central artery** (centrally placed in PALS).
 - **Splenic nodules (Malpighian corpuscles)** — local expansions of PALS containing B-lymphocytes, often with a germinal centre; here the arteriole lies **eccentrically**.
- ▶ **Marginal zone** — at the PALS/red-pulp interface; rich in sinusoids, macrophages and antigen-presenting cells; traps blood-borne antigen and is the only site in the body where an immune response can be mounted against antigen confined to circulating blood.
- ▶ **Red pulp** — **splenic (Billroth) cords** of reticular cells, macrophages, plasma cells and blood cells, interspersed with wide **splenic sinusoids** lined by elongated "**stave cells**" with discontinuous basal lamina, permitting free passage of cells.

- ▶ **Splenic circulation:** splenic artery → trabecular arteries → central artery (through PALS) → **penicillar arterioles** → sheathed capillaries (ellipsoids of macrophages) → either directly into sinusoids (**closed circulation**) or first into red-pulp cords (**open circulation**, the predominant pattern in humans) → sinusoids → trabecular veins → splenic vein.
- ▶ **Function:** destruction of aged/defective erythrocytes ("graveyard of RBCs"), immune response to blood-borne antigen, lymphopoiesis, and (in fetal life) haemopoiesis.

3. Thymus

□ Fig 8.15 — Histology of thymus. (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.180)

- ▶ A bilobed, **primary lymphoid organ** with a **dual embryonic origin** — parenchymal epithelial reticulum from the endoderm of the **third pharyngeal pouch**, lymphocytes from mesenchyme/bone marrow stem cells (unlike all other lymphoid organs, which are purely mesenchymal).
- ▶ Weighs 10–15 g at birth, 30–40 g at puberty, thereafter undergoes progressive fatty involution, though T-cell output continues lifelong.
- ▶ **Capsule** with septa dividing each lobe into **incomplete lobules** (~2 mm) — the septa do not reach the centre, so the **medulla of adjoining lobules is continuous**, while the cortex is confined to one lobule (a key distinguishing feature from lymph node).
- ▶ **Cortex** — densely packed, darkly staining immature T-cells (**thymocytes**); no lymphoid nodules are ever formed here. Supported by **epithelioreticular cells (epitheliocytes)**, not by reticular fibres — a reticulum of cellular processes joined by desmosomes, unique to the thymus.
- ▶ **Epitheliocyte types** (six described): Type 1 lines the capsule/septa/vessels and forms the **blood-thymus barrier**; Types 2 and 3 compartmentalise the outer and inner cortex respectively ("thymic nurse cells", which eliminate self-reactive thymocytes); Type 4 forms the cortico-medullary barrier; Type 5 provides the medullary framework; Type 6 forms **Hassall's corpuscles**.
- ▶ **Blood-thymus barrier** (cortex only) — from within out: endothelium + its basal lamina, perivascular connective tissue with macrophages, basal lamina of epitheliocytes, Type 1 epitheliocytes. It sequesters developing thymocytes from circulating antigen, so lymphoid nodules and plasma cells never form in the thymus.
- ▶ **Medulla** — fewer, more mature lymphocytes; prominent **epithelioreticular cells**; characteristic **Hassall's (thymic) corpuscles** — concentric whorls of keratinising epitheliocytes with a core of degenerating cells/macrophages, of unknown but probably immunoregulatory function.
- ▶ **Hormones** (from epitheliocytes): thymulin, thymopoietin, thymosin- α 1, thymosin- β 4, thymic humoral factor — drive proliferation/maturation of T-cell subsets. Only 1–3% of thymocytes survive intrathymic selection to become circulating immunocompetent T-cells; the rest die by apoptosis and are removed by macrophages.

4. Palatine tonsil

□ Fig 14.17 — Histology of palatine tonsil. (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.280)

- ▶ Paired **secondary lymphoid/MALT organ** in the lateral wall of the oropharynx, between the palatoglossal and palatopharyngeal folds; part of **Waldeyer's ring** along with the pharyngeal (adenoid), lingual and tubal tonsils.

- ▶ Medial (luminal) surface covered by **stratified squamous non-keratinised epithelium**, continuous with the oral mucosa; this epithelium invaginates to form **15–20 tonsillar crypts**.
- ▶ Deep to the epithelium/crypts is **diffuse lymphoid tissue** containing numerous **lymphoid follicles**, often with germinal centres; lymphocytes and desquamated cells frequently fill the crypt lumen.
- ▶ Lateral (deep) surface is separated from underlying muscle by a **hemicapsule** — a partial, one-sided capsule (unlike the complete capsule of lymph node, spleen and thymus).
- ▶ **No cortex–medulla organisation** and **no afferent lymphatics or lymph sinuses** — it is a discrete but incompletely encapsulated MALT aggregate, not a filtering organ.

Comparative table

Feature	Lymph node	Spleen	Thymus	Palatine tonsil
Type	Secondary	Secondary	Primary	Secondary (MALT)
Capsule	Complete	Complete	Complete (bilobed)	Hemicapsule (one surface only)
Cortex–medulla	Present; medulla confined to node	Absent (white pulp/red pulp instead)	Present; medulla continuous across lobules	Absent
Compartmentalising stroma	Reticular fibres (type III collagen)	Reticular fibres	Epithelioreticular cells (endodermal)	Reticular fibres (no true framework)
Nodules/follicles	Outer cortex (B-cells)	Splenic nodules in white pulp (B-cells)	None (blood–thymus barrier excludes antigen)	Numerous, in diffuse tissue
T-cell zone	Paracortex	PALS	Cortex (thymocytes)	Interfollicular diffuse tissue
Characteristic structure	Germinal centre, HEV	Central artery, stave-cell sinusoids, Billroth cords	Hassall's corpuscles	Crypts lined by stratified squamous epithelium
Afferent supply	Afferent lymphatics + artery	Artery only (no lymphatics)	Artery only (no afferent lymph)	None (no capsule/sinus system)
Filters	Lymph	Blood	Nothing (isolates, doesn't filter)	Nothing
Embryonic origin	Mesenchyme	Mesenchyme	Endoderm (3rd pouch) + mesenchyme	Mesenchyme + pharyngeal endoderm

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ **Lymph node** (low power, longitudinal): capsule → subcapsular sinus → trabecula → outer cortex with lymphoid follicle (germinal centre + mantle zone) → paracortex → medullary cords and medullary sinuses → hilum with afferent/efferent lymphatics, artery and vein.
- ▶ **Spleen**: capsule → trabecula with trabecular artery/vein → white pulp (PALS around a central artery; splenic/Malpighian nodule with eccentric arteriole) → marginal zone → red pulp (splenic cords of Billroth + sinusoids).
- ▶ **Thymus**: capsule → septum → lobule showing outer dark cortex (thymocytes) and inner light medulla with Hassall's corpuscles; note medulla continuous between adjoining lobules.
- ▶ **Palatine tonsil**: stratified squamous non-keratinised epithelium → tonsillar crypt → diffuse lymphoid tissue with lymphoid follicles (germinal centres) → hemicapsule.

Applied / Clinical anatomy

- ▶ **Lymphadenitis** — painful nodal enlargement from leucocytic infiltration/oedema stretching the capsule; acute (bacterial) or chronic (e.g. tuberculous). Sentinel-node and regional-node examination guides cancer staging, since malignant cells spread via afferent lymphatics to the subcapsular sinus first.
- ▶ **Lymphangitis/lymphoedema** — inflamed or obstructed lymphatics (malignant infiltration, post-irradiation fibrosis, filariasis) produce lymphoedema/elephantiasis; ruptured lymphatics cause chylothorax, chylopericardium or chyloperitoneum.
- ▶ **Splenomegaly** — infections (malaria, kala-azar), portal hypertension/cirrhosis, haemolytic anaemias (thalassaemia, spherocytosis), lympho-haematological malignancies (Hodgkin/non-Hodgkin lymphoma), and storage disorders (Gaucher's, Niemann–Pick). Splenectomy predisposes to overwhelming post-splenectomy infection because marginal-zone-dependent responses to blood-borne encapsulated bacteria are lost.
- ▶ **DiGeorge syndrome** — failure of development of the 3rd (and 4th) pharyngeal pouch derivatives → thymic (and parathyroid) aplasia, profound T-cell deficiency with preserved B-cell/humoral immunity.
- ▶ **Thymoma** — tumour of thymic epithelial cells, frequently associated with **myasthenia gravis** (autoantibodies against acetylcholine receptors at the motor end-plate); thymectomy often improves the myasthenic weakness.
- ▶ **Tonsillitis/tonsillectomy** — recurrent streptococcal tonsillitis is the classical indication for surgical removal; because the tonsil is only hemicapsulated, dissection plane and haemorrhage risk during tonsillectomy relate directly to this histology.

High-yield exam pointers

- ▶ Primary lymphoid organs = thymus + bone marrow; secondary = lymph node, spleen, tonsil/MALT.

- ▶ Only the **lymph node** filters lymph via a true sinus system with both afferent and efferent lymphatics; the **spleen** filters blood and has no afferent lymphatics; **thymus** and **tonsil** are not filtering organs at all.
- ▶ **Cortex–medulla** distinction is present in lymph node and thymus but **absent in spleen (white/red pulp) and tonsil**.
- ▶ Framework: reticular fibres in lymph node/spleen/tonsil vs. unique **epithelioreticular cells** (endodermal) in thymus.
- ▶ **Paracortex** = thymus-dependent zone of lymph node; **PALS** = thymus-dependent zone of spleen.
- ▶ Central artery of white pulp: **central** in PALS, **eccentric** in a splenic (Malpighian) nodule.
- ▶ **Hassall's corpuscles** are pathognomonic of thymic medulla; germinal centres are common to lymph node follicles, splenic nodules and tonsillar follicles but never occur in the thymus (blood–thymus barrier).
- ▶ Only the thymus has a **dual germ-layer origin** (3rd pharyngeal pouch endoderm + mesenchyme); the other three lymphoid organs arise purely from mesenchyme (tonsil epithelium from pharyngeal endoderm, but its lymphoid tissue is mesenchymal).
- ▶ Palatine tonsil is distinguished by its **hemicapsule** and **crypts lined by stratified squamous non-keratinised epithelium** — no other lymphoid organ has either feature.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh Human Histology 9e; Brijesh Kumar Histology Text & Atlas 3e; Vishram Singh General Anatomy 4e; BD Chaurasia Human Anatomy Vol 3–4, 8e.



Describe in detail about the microstructure of mucosa associated lymphoid tissue

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction

Mucosa-associated lymphoid tissue (MALT) is the collective term for the **unencapsulated aggregations of lymphocytes and accessory immune cells** that lie in the lamina propria and submucosa of the mucous membranes lining tracts open to the exterior — the respiratory, alimentary and urogenital tracts (and, more diffusely, the skin). Together with the lymph nodes and spleen it constitutes a **secondary (peripheral) lymphoid tissue**, where lymphocytes that have already matured in the bone marrow/GALT (B cells) or thymus (T cells) undergo antigen-dependent activation and proliferation. MALT is not a single organ but a widely dispersed system that, taken together, is one of the largest lymphoid masses in the body — containing up to 70% of all the body's immune cells — reflecting the huge surface area of mucosa it protects.

1. Definition, distribution and classification

- ▶ **Sites:** diffuse lymphocytes and organized nodules throughout the lamina propria/submucosa of the GI tract, respiratory tract, and urogenital tract; organized examples include the **tonsils**

(Waldeyer's ring), Peyer's patches of the ileum, lymphoid nodules of the vermiform appendix, and solitary lymphatic follicles scattered along the entire gut and airway.

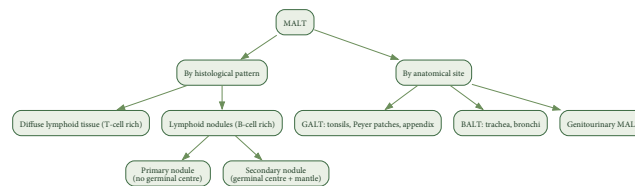
► **Classification by anatomical region/name:**

- **GALT** (gut-associated lymphoid tissue) — tonsils, Peyer's patches, appendix, solitary follicles of the gut.
- **BALT** (bronchus-associated lymphoid tissue) — small aggregations in the walls of the trachea and large bronchi.
- **Genitourinary MALT** — nodules in the mucosa of the urinary and reproductive tracts.

► **Classification by histological pattern (Flowchart, Sontakke):**

- **Diffuse lymphoid tissue** — lymphocytes scattered singly through the lamina propria without a discrete boundary.
- **Dense (nodular) lymphoid tissue** — lymphocytes aggregated into rounded **lymphatic follicles/nodules**, further subdivided into **primary nodules** (no germinal centre) and **secondary nodules** (with a germinal centre).

► **MALT vs discrete lymphoid organs:** lymphoid tissue as a whole is divided into **discrete lymphoid organs** (thymus, lymph nodes, spleen — each individually encapsulated) and **MALT**, which is unencapsulated and structurally continuous with the mucosa it guards (IB Singh).



Describe in detail about the microstructure of mucosa associated lymphoid tissue

2. General microarchitecture common to all MALT

- **No capsule, no septa:** unlike lymph node/spleen/thymus, MALT aggregations are **not** surrounded by a **connective-tissue capsule** and possess no trabeculae; only a **supporting meshwork of reticular fibres** (produced by fibroblastic reticular cells) holds the lymphoid cells.
- **Diffuse component:** lymphocytes (predominantly **T cells**) are scattered singly through the lamina propria between the nodules — this inter-nodular diffuse zone is sometimes termed the **parafollicular zone**; plasma cells, macrophages, dendritic (antigen-presenting) cells, eosinophils and mast cells are admixed.
- **Nodular component:** rounded **lymphatic follicles**, structurally identical to the nodules of a lymph node, are predominantly **B-cell** in composition.
 - **Primary nodule:** uniform population of small, resting lymphocytes; no germinal centre.
 - **Secondary nodule:** shows a pale-staining **germinal centre** — the zone of B-cell proliferation, containing larger, lightly stained **lymphoblasts and plasmoblasts** — surrounded by a darker rim of small, densely packed lymphocytes, the **mantle (corona) zone**.
- **Relationship to the covering epithelium:** the nodules lie immediately deep to the surface epithelium of the mucosa; individual lymphocytes constantly **infiltrate the epithelium and migrate through it into the lumen**. Over well-developed nodules (Peyer's patches, tonsillar

crypts) the epithelium is specialised as a **follicle-associated epithelium** studded with M (microfold) cells:

- M cells are flattened epithelial cells with short irregular **apical microfolds** instead of the brush border/glycocalyx of neighbouring enterocytes.
 - Their **basolateral membrane is deeply invaginated**, forming a large intraepithelial "pocket" that houses transient **lymphocytes and dendritic cells**.
 - The pocket opens to the underlying lymphoid tissue through a porous, **sieve-like basement membrane**.
 - Function: M cells **endocytose/transcytose luminal antigens and microorganisms** and deliver them to the immune cells in the pocket, which then present antigen to helper T cells and trigger the local B-cell response — the structural basis of mucosal immune surveillance.
- **High endothelial venules (HEVs):** present in the deeper, more organised parts of large MALT aggregates (tonsils, Peyer's patches), exactly as in the lymph node paracortex; their plump endothelium mediates **homing of recirculating lymphocytes** from blood into the tissue.
- **No afferent lymphatics, no lymph sinuses:** MALT does **not receive afferent lymphatic vessels** and has no subcapsular/medullary sinuses, so — unlike a lymph node — it **does not filter lymph**. It is purely a site of local lymphocyte production; lymphocytes generated here drain to regional lymph nodes via **efferent lymphatics**, while others pass directly through the epithelium into the lumen.
- **Local antibody (IgA) production:** plasma cells derived from locally activated B cells are abundant in the lamina propria beneath the epithelium. They secrete **dimeric IgA**, which is taken up and transported across the overlying epithelial cells and released into the lumen as **secretory IgA** — the principal effector of mucosal humoral immunity.

3. Waldeyer's ring — tonsillar MALT

The tonsils form an incomplete ring of lymphoid tissue, **Waldeyer's ring**, guarding the naso-oropharyngeal isthmus.

Tonsil	Location	Covering epithelium	Crypts	Capsule
Palatine tonsil	Either side of the oropharyngeal isthmus (between palatoglossal & palatopharyngeal folds)	Stratified squamous non-keratinised, continuous with oral mucosa	12–15 crypts; largest is the intratonsillar cleft	Hemicapsule — a partial fibrous capsule (extension of pharyngobasilar fascia) on the lateral/deep aspect only
Pharyngeal tonsil (adenoid)	Posterior wall of nasopharynx (midline)	Pseudostratified ciliated columnar (respiratory) with mucosal folds	Folds, not true crypts	No capsule

Tonsil	Location	Covering epithelium	Crypts	Capsule
Lingual tonsil	Posterior third of tongue dorsum	Stratified squamous	Single shallow crypt per nodule, often drained by a mucous/serous gland	No capsule
Tubal tonsil	Around the pharyngeal opening of the auditory (Eustachian) tube	Respiratory epithelium	Minimal	No capsule

Palatine tonsil — microstructure in detail:

- Consists of **diffuse lymphoid tissue** in which numerous **lymphatic nodules** (typically secondary, with germinal centres) are embedded; there is **no differentiation into cortex and medulla** (unlike a lymph node).
- The oral surface is covered by stratified squamous non-keratinised epithelium that dips deeply into the substance of the tonsil to form the tonsillar crypts; the crypt walls are lined by nodules pressing against the epithelium from beneath.
- The crypt epithelium is heavily infiltrated by migrating lymphocytes — so heavily that it may appear discontinuous/"reticulated" — and the crypt lumen typically contains desquamated epithelial cells, lymphocytes and bacteria.
- The lateral (deep) surface is covered only by a loose **hemicapsule**, firmly adherent anteroinferiorly (forming the suspensory ligament of the tonsil), but only loosely applied to the superior constrictor elsewhere — hence pus/infection can track laterally (peritonsillar abscess/quinsy).
- Only **efferent lymphatics** leave the tonsil (to the jugulodigastric and upper deep cervical nodes); there are **no afferent lymphatics**.

□ Fig 14.17 — Histology of palatine tonsil (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.280)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a section through the palatine tonsil: (1) stratified squamous non-keratinised surface epithelium, (2) tonsillar crypt lumen (with desquamated cells/lymphocytes), (3) lymphocyte-infiltrated ("reticulated") crypt epithelium, (4) secondary lymphoid nodule with pale germinal centre and dark mantle zone, (5) diffuse lymphoid tissue between nodules, (6) hemicapsule (lateral/deep aspect only), (7) efferent lymphatic vessel (no afferent vessel present).

4. Peyer's patches — ileal GALT

- **Location:** the antimesenteric border of the ileum; roughly 200 patches can be identified along the human gut, becoming progressively larger and more numerous towards the terminal ileum; each measures ~2–10 cm in length.

- ▶ **Architecture:** multiple (up to several dozen) aggregated **lymphatic nodules** — mostly secondary, with prominent germinal centres — occupy the lamina propria and extend into the submucosa; there is **no capsule** separating them from surrounding gut wall tissue.
- ▶ **The dome:** the mucosa directly overlying a Peyer's patch is flattened, with villi few, rudimentary or absent, forming a **dome**. The dome epithelium is a specialised **follicle-associated epithelium** containing scattered **M cells** interspersed among enterocytes — structurally and functionally identical to the M cells described above — that continuously sample luminal antigens/microorganisms for the underlying immune cells.
- ▶ **Germinal centres** here are the site of intense B-cell proliferation and immunoglobulin class-switching to IgA; locally generated plasma cells populate the adjacent lamina propria and secrete IgA that is transported into the gut lumen.
- ▶ **Muscularis mucosae** is frequently deficient or interrupted beneath large nodules, allowing the nodule to bulge from mucosa into submucosa.

□ Fig 14.16 — Photomicrograph of aggregated nodules in the wall of the ileum (Ross Histology — A Text and Atlas — 7e, p.477)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a longitudinal section of ileum at a Peyer's patch: (1) villi with cores (away from the patch), (2) dome region with flattened/absent villi, (3) follicle-associated epithelium with M (microfold) cells, (4) M-cell basolateral pocket containing lymphocytes/dendritic cells, (5) secondary lymphoid nodule with germinal centre extending from lamina propria into submucosa, (6) diffuse (parafollicular) T-cell zone between nodules, (7) high endothelial venule, (8) interrupted muscularis mucosae, (9) antimesenteric border.

5. Vermiform appendix — "abdominal tonsil"

- ▶ The lamina propria and submucosa are packed with numerous confluent **lymphatic nodules**, often effacing the intestinal glands otherwise seen in large-bowel mucosa.
- ▶ Lymphoid tissue is **best developed around 10–12 years of age**, after which it progressively involutes with advancing age — hence its designation as the "abdominal tonsil."
- ▶ The muscularis externa is deficient at places where large nodules bulge outward.
- ▶ The narrow lumen typically contains intestinal flora and debris.

Applied / Clinical anatomy

- ▶ **Tonsillitis:** recurrent infection of the palatine tonsil (via the crypts, which trap bacteria/debris) causes sore throat and, if frequent, hypertrophy warranting **tonsillectomy**.
- ▶ **Adenoid hypertrophy:** enlargement of the pharyngeal tonsil in children can obstruct the pharyngeal opening of the auditory tube, predisposing to **secretory (serous) otitis media** and mouth breathing.
- ▶ **Peritonsillar abscess (quinsy):** infection tracking laterally through the loosely attached hemicapsule of the palatine tonsil.
- ▶ **Typhoid ulcer:** *Salmonella typhi* selectively invades and ulcerates the lymphoid nodules of the Peyer's patches along the antimesenteric border of the ileum, causing swelling followed by

sloughing/ulceration — the anatomical basis of intestinal haemorrhage and perforation in typhoid fever.

- ▶ **Appendicitis:** luminal obstruction (often by lymphoid hyperplasia in children, or a faecolith in adults) precipitates inflammation of the lymphoid-tissue-rich appendiceal wall.
- ▶ **M cells as a portal of entry:** the same transcytotic pathway that samples antigen for immunity is exploited by pathogens (e.g. *Salmonella*, poliovirus) to breach the mucosal barrier.
- ▶ MALT is the tissue of origin of **extranodal marginal-zone (MALT) lymphomas**, classically arising in gastric mucosa after chronic antigenic stimulation.

High-yield exam pointers

- ▶ MALT = unencapsulated secondary lymphoid tissue of mucosa (GI, respiratory, urogenital); GALT/BALT are its regional names.
- ▶ No capsule, no septa, no afferent lymphatics, no sinuses → does NOT filter lymph (key contrast with lymph node).
- ▶ Diffuse zone = mainly T cells; nodules = mainly B cells; germinal centre = zone of B-cell proliferation.
- ▶ M (microfold) cells overlie nodules, lack brush border/glycocalyx, transcytose antigen to an intraepithelial pocket of lymphocytes/dendritic cells.
- ▶ Locally produced IgA is transported across the epithelium into the lumen — hallmark of mucosal immunity.
- ▶ Waldeyer's ring = palatine + pharyngeal + lingual + tubal tonsils; only the palatine tonsil has a (partial) hemicapsule.
- ▶ Peyer's patches lie on the antimesenteric border of the ileum, mucosa over them is a villus-free "dome."
- ▶ Appendix = "abdominal tonsil"; lymphoid tissue peaks around age 10–12 then involutes.
- ▶ Typhoid ulcer classically involves and ulcerates ileal Peyer's patches.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh's Textbook of Human Histology 9e; Sontakke's Textbook of Human Histology 2e; Brijesh Kumar's Histology Text & Atlas 3e; BD Chaurasia's Human Anatomy Vol 3–4 8e (Waldeyer's ring/palatine tonsil gross-histological detail and figure).



Describe in detail about the microstructure of Spleen, compare with lymph node

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction The spleen is the largest lymphoid organ of the body, containing the largest single mass of lymphoid tissue in direct continuity with the blood circulation; it is a secondary lymphoid organ that filters **blood**, removing effete/abnormal blood cells and mounting immune responses to blood-borne antigens. The lymph node is a small, bean-shaped secondary lymphoid organ interposed along lymphatic vessels, filtering **lymph** draining from a defined tissue field. Both share capsule, trabeculae, reticular framework, and lymphocyte aggregations — but the spleen's parenchyma is organised into **white pulp** and **red pulp**, unlike the cortex-paracortex-medulla plan of a lymph node.

1. Fibrous framework and general architecture of the spleen

- ▶ Except at the hilum, the spleen is covered by peritoneum (serosa); deep to this is a true connective-tissue **capsule**, about 1.5 mm thick, with outer and inner laminae of differing collagen-fibre direction (adding strength), abundant type I collagen and some elastin.
- ▶ Numerous **trabeculae** extend from the capsule and branch progressively, forming a scaffold that carries the splenic vessels and nerves; the largest enter at the hilum.
- ▶ Within the pulp, this framework becomes continuous with a delicate network of **reticular (type III collagen) fibres** pervading both white and red pulp, maintained by resident fibroblasts (reticular cells).
- ▶ Parenchyma is grossly divisible into **white pulp** (5–20% of tissue) and **red pulp** (up to 90% of volume), named from the cut surface of a fresh spleen — basophilic grey-white nodules (white pulp) in a deep reddish-purple background (red pulp).

2. White pulp

- ▶ Branches of the splenic artery ramify within the trabeculae; in their terminal few millimetres the adventitia is replaced by a sheath of **T lymphocytes**, the **peri-arteriolar lymphatic sheath (PALS)**.
- ▶ The PALS is locally expanded into **lymphoid follicles** (0.25–1 mm diameter) of **B lymphocytes**, seen as white semi-opaque dots on the cut surface.
- ▶ Follicles typically abut the arteriole's terminal branch eccentrically, so the **central (follicular) artery** occupies an off-centre position within the nodule. This **eccentric arteriole** is the single most useful feature distinguishing a splenic follicle from a lymph-node follicle, which contains no vessel at all.
- ▶ After antigenic stimulation, follicles develop pale **germinal centres** (secondary follicles, also called **Malpighian bodies**) where B cells proliferate under follicular dendritic cells presenting antigen — histologically similar to lymph-node germinal centres; they regress once the stimulus abates and atrophy with age.
- ▶ The **perifollicular (marginal) zone**, at the white-red pulp interface, contains loosely arranged lymphocytes, antigen-presenting cells, and a **peri-arteriolar macrophage sheath**; it is where blood enters the red pulp and lymphocytes migrate into the white pulp. This zone is unique to

the spleen — the only site for an immune response against antigens confined to **circulating blood** (lymph nodes receive antigen only via afferent lymph from tissues).

3. Red pulp and splenic microcirculation

- ▶ Red pulp consists of a three-dimensional continuum of **venous sinusoids** separated by intersinusoidal tissue — the **splenic cords (of Billroth)** — containing reticular fibroblasts, macrophages, and all formed elements of blood.
- ▶ Sinusoids are elongated, ovoid, about 50 µm in diameter, and lined by a unique, discontinuous endothelium of long, spindle-shaped "**stave**" **cells** (named for their resemblance to the planks of a barrel), attached to neighbours by intermittent junctions alternating with intercellular slits; the abluminal basal lamina is also discontinuous.
- ▶ **Splenic microcirculation**: segmental (trabecular) arteries → arterioles traversing the white pulp → **penicillar arterioles** → cross the marginal zone → red pulp. Evidence favours a dominantly "**open**" **circulation** in humans (blood empties into the cord meshwork and squeezes back into a sinusoid through the stave-cell slits); a minor "**closed**" **circulation** (capillaries opening directly into sinusoids) also occurs — some texts call this a **compromise theory** (open in a distended spleen, closed in a contracted one).
- ▶ The narrow slits admit pliable normal erythrocytes, platelets, and motile leukocytes, but trap stiff, aged (~120-day) or abnormal RBCs, which cord macrophages then phagocytose — the basis of splenic **culling**; macrophages also perform **pitting** (removing inclusions like Howell–Jolly bodies without destroying the cell) and recycle iron (ferritin/transferrin) for erythropoiesis, converting iron-free haem to bilirubin for biliary excretion.
- ▶ Sinusoidal blood drains via red-pulp veins → **trabecular veins** → **splenic vein**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 14.29 — Schematic diagram and photomicrograph of splenic structure. (Ross Histology — A Text and Atlas — 7e, p.486)

Reproduce and label: **capsule** → **trabecula** (with trabecular artery/vein) → **white pulp** (PALS around a **central/follicular arteriole**, eccentrically placed, with a **germinal centre**) → **marginal (perifollicular) zone** → **red pulp** (**splenic cords of Billroth** and **venous sinusoids** lined by **stave cells**) → **penicillar arterioles** → **trabecular/splenic vein**. Show the **open** and **closed** circulation pathways as two alternative routes from arteriole to sinusoid.

4. Microstructure of the lymph node

- ▶ Bean-shaped; its convex surface receives several **afferent lymphatics**, while its concave **hilum** carries blood vessels in/out and a (usually single) **efferent lymphatic vessel**.
- ▶ **Capsule**: thin, mainly collagenous with some elastic fibres/smooth muscle; sends **trabeculae (septa)** inward that incompletely divide the node into lobules; the hilum is occupied by dense fibrous tissue.
- ▶ **Sinus system**: beneath the capsule lies the **subcapsular sinus** (receiving afferent lymph); radial **cortical (trabecular) sinuses** run to the medulla, uniting into **medullary sinuses**, which converge on the efferent vessel. Sinuses have attenuated/discontinuous endothelium with reticular cells

and macrophages, so lymphocytes/macrophages move freely into/out of them while particulate matter and bacteria are phagocytosed.

- ▶ **Cortex** (outer, dark): the superficial/**nodular cortex** contains **lymphatic follicles** — **primary** (dense small lymphocytes) and **secondary** (pale **germinal centre** of proliferating B-lymphoblasts ringed by a **mantle zone/corona**) — the B-cell zone. Deep to it, the **paracortex** (thymus-dependent) holds mainly **T lymphocytes** and **high endothelial venules (HEVs)** — cuboidal postcapillary venules bearing receptors that mediate lymphocyte homing from blood into the node.
- ▶ **Medulla** (inner, pale): lymphocytes form branching **medullary cords** (B cells, plasma cells, macrophages) separated by **medullary sinuses**.
- ▶ **Blood supply**: arteries enter at the hilum, traverse the medulla, and end in cortical arteriolar/capillary loops draining into the paracortical HEVs.
- ▶ **Function**: site of B- and T-lymphocyte proliferation; macrophages phagocytose antigen/bacteria arriving via afferent lymph; antigen presentation here initiates humoral and cell-mediated responses against antigens draining from the tissue field the node serves.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 14.17 — Structure of a lymph node. (Ross Histology — A Text and Atlas — 7e, p.478)

Reproduce and label: **capsule** → **afferent lymphatics** → **subcapsular sinus** → **trabecula** with **trabecular sinus** → **cortex** (**primary** and **secondary follicle** with **germinal centre** and **mantle zone**) → **paracortex** (with **high endothelial venule**) → **medulla** (**medullary cords** and **medullary sinuses**) → **hilum** with **efferent lymphatic vessel** and **blood vessels**.

5. Comparative histology: spleen vs lymph node

Feature	Spleen	Lymph node
Fluid filtered	Blood	Lymph
Position in circulatory pathway	Interposed directly in the bloodstream (hilar artery/vein only)	Interposed along lymphatic vessels; afferents enter the convex surface, efferent leaves at hilum
Afferent lymphatics	Absent — spleen has no afferent lymph vessels	Present , entering the convex surface
Capsule	Thick (~1.5 mm), two laminae of collagen + some elastin; largely vestigial smooth muscle in humans (does not contract)	Thin, mainly collagen with some elastic fibres/smooth muscle
Gross parenchymal organisation	White pulp and red pulp — no cortex/medulla	Distinct cortex (outer) and medulla (inner)
Lymphocyte zones	PALS (T cells) + follicles (B cells) around an eccentric central arteriole ; paracortex-equivalent not separately named	Nodular/superficial cortex (B-cell follicles) + paracortex (diffuse T cells) + medullary cords (B cells, plasma cells)

Feature	Spleen	Lymph node
Key identification feature	Follicle contains an eccentric arteriole (central artery)	Follicle contains no vessel
Sinuses	Venous sinusoids (blood-filled) in red pulp, lined by stave cells	Lymph sinuses — subcapsular, cortical/trabecular, medullary — lined by discontinuous endothelium/reticular cells
Marginal/perifollicular zone	Present — unique interface zone for blood-borne antigen capture	Not described as a separate zone
High endothelial venules (HEV)	Absent	Present in the paracortex, mediate lymphocyte recirculation
Principal functions	Destruction of aged/damaged RBCs and platelets ("graveyard of RBCs"), blood reservoir, immune response to blood-borne antigens, fetal haemopoiesis (can resume postnatally in disease)	Filtration of lymph, immune response to antigens draining from a tissue field , site of lymphocyte proliferation
Consequence of surgical removal	Splenectomy → lifelong risk of overwhelming post-splenectomy sepsis; other functions largely taken over by liver and bone marrow	Removal of a regional node does not carry a comparable systemic risk; lymph reroutes via collateral channels

Applied / Clinical anatomy

- **Splenomegaly**: from portal/splenic venous obstruction (reversed flow, congestion, varices), haemolytic anaemias, infections, or infiltrative/malignant disease (lymphoma, myelofibrosis); red pulp expands as reticular cells proliferate.
- **Splenic rupture**: the splenic capsule is relatively thin for the organ's vascularity, so the spleen is one of the most frequently injured organs in blunt abdominal trauma (lower-left rib fractures may co-exist); management ranges from conservative/partial splenectomy (preserving a pole with its own vascular pedicle) to splenorrhaphy, to preserve splenic tissue where possible.
- **Splenectomy** removes a major site of RBC/bacterial clearance; its recognised long-term risk is severe/overwhelming post-splenectomy sepsis, historically quoted at about 2% mortality in otherwise healthy adults and higher in children, the elderly, and those with chronic disease — hence pre-operative vaccination against encapsulated organisms (pneumococcus, meningococcus, *H. influenzae*) and splenic-conserving surgery are preferred whenever feasible.
- **Accessory spleen (splenunculus)**: a persistent splenic lobule in roughly 10% of individuals, usually near the hilum in the gastrosplenic ligament/greater omentum; if missed at splenectomy for haematological disease it can cause recurrence.
- **Hyposplenism**: peripheral smear shows Howell–Jolly bodies and target cells, reflecting loss of splenic pitting function. Splenic imaging (ultrasound, CT, MRI, ^{99m}Tc -sulphur-colloid scintigraphy) assesses size, vasculature, and phagocytic function.
- **Lymph node pathology**: reactive lymphadenopathy shows follicular (B-zone) hyperplasia in bacterial/reactive processes or paracortical (T-zone) expansion in viral infections; metastases

characteristically appear first in the subcapsular sinus — the rationale for **sentinel lymph node biopsy** in cancer staging (e.g. axillary node in breast carcinoma).

High-yield exam pointers

- ▶ Spleen = largest lymphoid organ, largest mass of lymphoid tissue in continuity with the circulation; filters **blood**, not lymph.
- ▶ White pulp = PALS (T cells) + follicles (B cells, germinal centres = Malpighian bodies) around an **eccentric** central arteriole — the key point distinguishing it from a lymph-node follicle (which has no vessel).
- ▶ Red pulp = splenic cords of Billroth + venous sinusoids lined by **stave cells** with a discontinuous basal lamina — the structural basis of RBC culling and pitting.
- ▶ The **marginal (perifollicular) zone** is unique to the spleen — the only site for immune responses to blood-borne antigen.
- ▶ Splenic circulation in humans is predominantly "**open**"; a minor "**closed**" component also exists.
- ▶ Lymph node has a true **cortex** (B-follicles) — **paracortex** (T cells, HEVs) — **medulla** (cords + sinuses); spleen has no cortex/medulla, only white/red pulp.
- ▶ Only the lymph node has **afferent lymphatics** and **HEVs**; the spleen has neither — blood enters/leaves via hilar vessels, and only efferent lymph leaves the spleen (from the white pulp).
- ▶ Clinically: thin splenic capsule is vulnerable to rupture; splenectomy mandates anti-pneumococcal/meningococcal/Hib vaccination because of overwhelming post-splenectomy sepsis risk; accessory spleen occurs in ~10% of people.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; BD Chaurasia Human Anatomy Vol 2 8e.



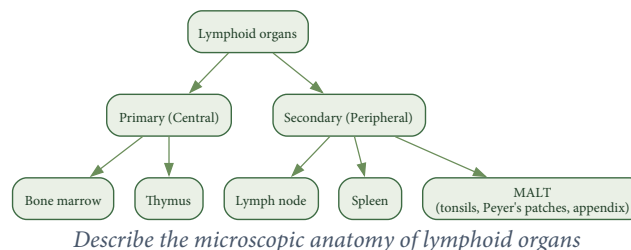
Describe the microscopic anatomy of lymphoid organs

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Lymphoid organs are the tissues in which lymphocytes are produced, undergo differentiation, and are activated to mount an immune response. They are classified as **primary (central)** lymphoid organs — bone marrow and thymus, where lymphocytes arise de novo from stem cells and acquire antigen-receptor diversity — and **secondary (peripheral)** lymphoid organs — lymph node, spleen and mucosa-associated lymphoid tissue (MALT, including the tonsils) — where mature, antigen-naïve lymphocytes are trapped, presented with antigen by accessory cells, and activated. Each organ has a distinctive microanatomy that reflects this functional role.

Classification of lymphoid organs

Feature	Primary (central)	Secondary (peripheral)
Examples	Bone marrow, thymus	Lymph node, spleen, tonsils, Peyer's patches, appendix
Process	De novo lymphopoiesis; antigen-independent	Antigen-dependent activation/proliferation
Antigen exposure	Lymphocytes shielded from antigen	Lymphocytes actively exposed to antigen
Output	Immunocompetent, naïve B/T cells released into circulation	Effector/memory lymphocytes and plasma cells



Microscopic anatomy of the lymph node

- ▶ **Capsule and framework:** a thin collagenous capsule (with some elastic fibres and smooth muscle) surrounds the node and sends **trabeculae** radially inward, dividing it into incomplete lobules. A fine type III collagen (**reticulin**) network, produced by fibroblastic reticular cells, pervades the node and supports lymphocytes, macrophages and dendritic cells.
- ▶ **Cortex (outer, densely stained zone):**
 - **Lymphoid follicles/nodules** — aggregates of B cells. A **primary follicle** is a uniform mass of small quiescent B cells; a **secondary follicle** has a pale **germinal centre** (proliferating centroblasts in the dark zone → centrocytes in the light zone interacting with follicular dendritic cells for affinity maturation; apoptotic B cells cleared by tingible-body macrophages), surrounded by a rim of small B cells — the **mantle zone/corona**.
 - **Paracortex (deep/thymus-dependent cortex)** — lies between the follicles and the medulla; populated by T cells (CD4 and CD8), interdigitating dendritic cells (including migrated

Langerhans cells), and rich in **high endothelial venules (HEVs)**, the site of blood-borne lymphocyte entry via cuboidal endothelium.

- ▶ **Medulla:** lymphocytes (mainly B cells, plasma cells) are arranged as branching, anastomosing **medullary cords**, separated by **medullary sinuses**; the reticulin network is easily seen here. The cortex is absent at the hilum, where the medulla reaches the surface.
- ▶ **Sinus system and lymph flow:** afferent lymphatics pierce the capsule peripherally → **subcapsular sinus** → radial **cortical sinuses** → **medullary sinuses** → single **efferent lymphatic vessel** at the hilum. All sinuses are lined by a discontinuous/loose endothelium that permits free passage of lymphocytes and phagocytosis of particulate matter by sinus macrophages.
- ▶ **Blood supply:** artery and vein enter/leave at the hilum, traverse the medulla, and form dense arteriolar/capillary arcades in the cortex, draining via HEV-rich postcapillary venules in the paracortex.

Diagram to draw — see the box below (Lymph node) for exact labels.

□ Fig 4.8 — A lymph node (human) showing cortex (C) and medulla (M), and lymphoid follicles (F), some with germinal centres (G) (Gray's Anatomy — Standring — 41e, p.127)

Microscopic anatomy of the spleen

The largest lymphoid organ; a fetal haemopoietic organ and, postnatally, the "graveyard" of aged RBCs.

- ▶ **Capsule and trabeculae:** covered by peritoneum (serosa) except at the hilum; deep to this, a fibroelastic capsule sends trabeculae (rich in elastic fibres) into the parenchyma, carrying the trabecular vessels.
- ▶ **White pulp:** dense lymphocytic sheaths around a small artery.
 - **Periarterial lymphatic sheath (PALS)** — T cells cuffing the **central artery** (eccentrically placed).
 - **Splenic (Malpighian) nodules** — localized B-cell expansions of the PALS, with germinal centres when antigen-stimulated; distinguished from lymph-node follicles by the eccentric arteriole running through them.
 - **Marginal zone** — the red-pulp rim immediately surrounding white pulp, rich in sinusoids and antigen-presenting cells; the unique site where blood-borne antigen is filtered and presented (lymph nodes only sample antigen delivered via lymph).
- ▶ **Red pulp:** a sponge-work of **splenic sinusoids**, lined by elongated "**stave cells**" (banana-shaped endothelium with contractile fibrils that open/close intercellular slits), separated by cellular **splenic cords of Billroth** containing macrophages, lymphocytes and all formed elements of blood.
- ▶ **Splenic circulation:** central artery → straight **penicillar arterioles** → some sheathed by macrophages ("sheathed capillaries") → open into the splenic cords (**open circulation**, the human pattern, forcing blood through macrophage-rich cords before it re-enters sinusoids) or directly into sinusoids (**closed circulation**) → sinusoids drain into trabecular veins.
- ▶ **Function correlate:** white pulp = immune surveillance/response (PALS = T zone, nodules = B zone, as in lymph-node cortex); red pulp = filtration and destruction of senescent/abnormal RBCs and blood-borne organisms by the largest macrophage population in the body (mononuclear phagocyte system).

Microscopic anatomy of the thymus

A bilobed, lymphoepithelial primary lymphoid organ with a dual embryonic origin (unlike all other lymphoid tissue, which is purely mesenchymal).

- ▶ **Capsule and lobulation:** each lobe has a connective-tissue capsule; septa extend inward, incompletely subdividing it into lobules (~2 mm each). The **cortex is confined to one lobule**, but the **medulla is continuous** between adjoining lobules.
- ▶ **Cortex:** darkly stained owing to densely packed developing T cells (**thymocytes**). The supporting stroma is a cellular epithelial reticulum (**epitheliocytes**, endodermal, non-phagocytic, joined by desmosomes), not a fibrous reticulin network as in node/spleen.
- ▶ **Epithelial reticular cell types** (6 types described):
 - *Type 1* — lines the capsule, septa and vessels; forms the **blood–thymus barrier** together with endothelial tight junctions, endothelial and epithelial basal laminae, and perivascular connective tissue — it shields cortical thymocytes from circulating antigen.
 - *Types 2 & 3* — outer and inner cortex respectively; "thymic nurse cells" that compartmentalize the cortex and eliminate self-reactive thymocytes.
 - *Type 4* — deep cortex/corticomedullary junction; forms the cortex–medulla barrier.
 - *Type 5* — structural framework of the medulla.
 - *Type 6* — forms **Hassall's corpuscles**, concentric whorls of degenerating keratinized epitheliocytes with lipid/keratohyalin, characteristic of the medulla.
- ▶ **Medulla:** lighter-staining (lymphocytes less densely packed than cortex), containing more mature thymocytes, dendritic cells at the corticomedullary junction, and Hassall's corpuscles. There is **no blood–thymus barrier** here, permitting the negative selection of self-reactive cells.
- ▶ **Selection and output:** thymocytes proliferate in the outer cortex, migrate inward through positive selection (survival of cells recognising self-MHC) in the cortex and negative selection (deletion of strongly self-reactive clones) at the corticomedullary junction/medulla; only a small minority (~1–3%) survive both checkpoints to emigrate as immunocompetent, self-tolerant T cells via post-capillary venules.

Microscopic anatomy of tonsils (MALT)

Tonsils are the largest, encapsulated-to-unencapsulated members of MALT, forming **Waldeyer's ring** (palatine, pharyngeal, tubal and lingual tonsils) at the oropharyngeal isthmus.

- ▶ **Palatine tonsil:** covered by **stratified squamous non-keratinized epithelium**, continuous with that of the mouth/pharynx, which invaginates deeply as **tonsillar crypts** (lumen often containing desquamated cells, lymphocytes and bacteria). Deep to the epithelium is diffuse lymphoid tissue with numerous **secondary lymphoid nodules** (germinal centres) clustered around the crypts; a **partial (hemi)capsule** of dense connective tissue lies on its deep aspect only.
- ▶ **Lingual tonsil:** similar epithelium and crypts, but **lacks a distinct capsule**.
- ▶ **Pharyngeal tonsil (adenoid):** covered by **pseudostratified ciliated columnar (respiratory) epithelium**; mucosa thrown into shallow folds rather than true crypts; has a **thin capsule**.
- ▶ **General MALT features:** unencapsulated (except the partial/thin capsules above), consist of diffuse lymphoid tissue plus lymphoid nodules (predominantly B cells; diffuse T-cell

parafollicular zones), supported by reticular fibres, with no afferent lymphatics or lymph sinuses — they are centres of lymphocyte production rather than lymph filters, draining via efferents to regional nodes. Includes **GALT** (Peyer's patches of ileum, appendix) and **BALT** (bronchial wall aggregates).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ **Lymph node** (low power): capsule → trabeculae → subcapsular sinus → cortex with primary/secondary follicles (germinal centre, mantle zone) → paracortex (HEV) → medulla (medullary cords, medullary sinuses) → hilum with afferent/efferent lymphatics and blood vessels.
- ▶ **Spleen**: capsule/trabeculae → central artery (eccentric) → PALS (T zone) → splenic/Malpighian nodule with germinal centre (B zone) → marginal zone → red pulp (splenic cords of Billroth, splenic sinusoids).
- ▶ **Thymus**: capsule → septa → lobule with outer dark cortex (thymocytes, blood–thymus barrier) → inner pale medulla (Hassall's corpuscle) → corticomedullary junction.
- ▶ **Palatine tonsil**: stratified squamous epithelium → tonsillar crypt → diffuse lymphoid tissue with secondary nodules → partial capsule (deep aspect).

Applied / Clinical anatomy

- ▶ **Lymph node biopsy / sentinel node**: reactive follicular hyperplasia (enlarged germinal centres) indicates antigenic stimulation; architecture effacement is a histological clue to lymphoma. Sentinel-node biopsy exploits the orderly afferent → subcapsular sinus drainage pattern to detect the first-echelon node in tumour spread (e.g. breast carcinoma, melanoma).
- ▶ **Splenectomy**: loss of the marginal zone/red-pulp macrophage filter predisposes to **overwhelming post-splenectomy infection (OPSI)** with encapsulated organisms, since the spleen is the only site where blood-borne (as opposed to tissue-derived) antigen triggers an immune response.
- ▶ **Thymic pathology**: thymic hypoplasia/aplasia (failure of third/fourth pharyngeal pouch derivatives, as in DiGeorge syndrome) causes profound T-cell deficiency; age-related thymic involution (replacement by fat after puberty) underlies declining T-cell output and immunosenescence; thymoma may arise from the epithelial component.
- ▶ **Tonsils**: recurrent tonsillitis/adenotonsillar hypertrophy (crypts trap debris/bacteria) is the indication for tonsillectomy/adenoidectomy; hypertrophied pharyngeal tonsil (adenoids) in children can obstruct the nasopharynx.
- ▶ **HEV-mediated homing**: paracortical high endothelial venules are the physiological gateway for lymphocyte recirculation and the target of chronic inflammatory lymphocyte trafficking.

High-yield exam pointers

- ▶ Primary lymphoid organs (bone marrow, thymus) = lymphopoiesis, antigen-independent; secondary (lymph node, spleen, MALT/tonsils) = antigen-dependent activation.
- ▶ Lymph node cortex = B-cell follicles (germinal centre, mantle zone); paracortex = T-cell zone with HEVs; medulla = medullary cords (B, plasma cells) and sinuses.

- ▶ Spleen: white pulp (PALS = T zone around central artery; Malpighian nodules = B zone) vs red pulp (cords of Billroth + sinusoids lined by stave cells); marginal zone filters blood-borne antigen.
- ▶ Thymus: cortex (dense thymocytes, blood–thymus barrier, positive selection) vs medulla (Hassall's corpuscles, negative selection, no barrier); six epitheliocyte types.
- ▶ Tonsils form Waldeyer's ring; only the palatine tonsil has crypts + a partial (hemi)capsule; pharyngeal tonsil has respiratory epithelium and no crypts; lingual tonsil has no capsule.
- ▶ MALT (tonsils, Peyer's patches, appendix, BALT) is unencapsulated and lacks afferent lymphatics/lymph sinuses.
- ▶ Spleen = only lymphoid organ responding to blood-borne antigen; splenectomy risks OPSI.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh Textbook of Human Histology 9e; Junqueira's Basic Histology 17e; Sontakke Textbook of Human Histology 2e.

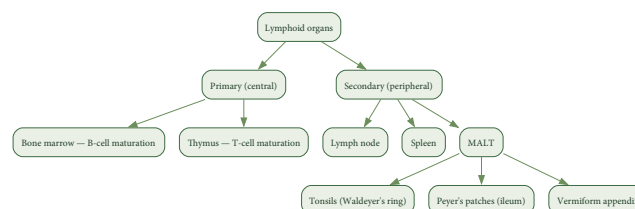


Describe the microscopic features of Lymphoid organs and their role

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction. Lymphoid organs are the sites where lymphocytes are produced, mature, and are activated to mount the immune response. They are divided into **primary (central)** organs — bone marrow and thymus, where lymphocytes acquire antigen-receptor diversity independent of antigen — and **secondary (peripheral)** organs — lymph node, spleen, and mucosa-associated lymphoid tissue (MALT: tonsils, Peyer's patches, appendix) — where mature lymphocytes encounter antigen, proliferate, and differentiate into effector/memory cells. Except for the thymus (which uses an **epithelial cytotreticulum**), all lymphoid organs share a common stromal scaffold of **reticular fibres (type III collagen)** secreted by fibroblastic reticular cells, on which lymphocytes, plasma cells, macrophages and dendritic cells are suspended.

Classification and general histological plan



Describe the microscopic features of Lymphoid organs and their role

- ▶ **Diffuse lymphoid tissue** — loosely scattered lymphocytes in mucosal lamina propria, no defined boundary.
- ▶ **Lymphoid nodule (follicle)** — a compact spherical aggregate of proliferating B cells around follicular dendritic cells (FDCs).
 - **Primary nodule** — small, uniformly dark-staining lymphocytes, no germinal centre.

- **Secondary nodule** — a pale-staining **germinal centre** of proliferating centroblasts (undergoing immunoglobulin hypermutation) surrounded by a darker peripheral **mantle zone** of small naive B cells; forms only after antigenic stimulation and lasts 2–3 weeks.
- Common cellular residents: lymphocytes (B, T), plasma cells, macrophages, dendritic cells (interdigitating — MHC-II-restricted antigen presentation to T cells; follicular — trap antigen–antibody complexes for B cells), and reticular cells.

Thymus — microscopic structure and role

Primary lymphoid organ; bilobed, in the superior mediastinum; develops from the endoderm of the **3rd pharyngeal pouch**, colonised by lymphoblasts from bone marrow. Fully active before puberty, then undergoes progressive **involution** with replacement by adipose tissue (islands of cortex/medulla persist).

Feature	Description
Capsule/septa	Dense CT capsule sends septa in, creating incompletely separated lobules (cortex/medulla continuous between lobules)
Cortex	Outer, densely basophilic — packed T-lymphoblasts (thymocytes) on a cytotreticulum of thymic epithelial cells (TECs) , not reticular fibres
Medulla	Inner, lighter-staining — fewer, larger, mature lymphocytes; contains Hassall's corpuscles
Lymphoid nodules	Absent (key distinguishing negative feature vs lymph node/spleen)
Cortical TECs	Squamous (subcapsular/perivascular, form blood–thymus barrier), stellate (cytotreticulum, MHC-II+ APCs, secrete cytokines), corticomedullary-junction TECs
Hassall's corpuscles	Concentric whorls of TECs, up to 100 µm, unique to medulla; secrete cytokines that drive dendritic-cell activity and regulatory T-cell development
Blood–thymus barrier	Squamous TECs + endothelium + pericytes isolate the cortex from circulating antigen; absent in medulla (mature T cells exit here via venules/efferent lymphatics)

Role:

- Site of **T-lymphocyte maturation and selection**, over ~2 weeks: **positive selection** in the cortex — only thymocytes whose T-cell receptors (TCR) recognise self-MHC-I/II survive (~80% fail and are removed by macrophages); **negative selection** in the medulla — TECs expressing the **AIRE** gene present organ-specific "self" peptides, and thymocytes binding these too strongly undergo apoptosis. Only ~2% of thymocytes survive both checkpoints as immunocompetent CD4+ or CD8+ T cells.
- This two-stage selection produces **central immune tolerance**; regulatory T cells generated in the medulla (under Hassall's-corporuscle cytokines) supplement it as peripheral tolerance.

- **Clinical:** Failure of 3rd (and 4th) pharyngeal pouch development → **DiGeorge syndrome** (thymic hypoplasia/aplasia, depressed cell-mediated immunity). Thymic hyperplasia/thymoma is classically associated with **myasthenia gravis**.

Lymph node — microscopic structure and role

□ Fig 4.8 — A lymph node (human) showing cortex (C) and medulla (M), and lymphoid follicles (F), some with germinal centres (G). (Gray's Anatomy — Standring — 41e, p.127)

Bean-shaped, encapsulated, $\sim 1 \times 2.5$ cm; 400–450 nodes lie along lymphatics of the axilla, groin, neck, thorax, abdomen and mesenteries. A **convex surface** receives several **afferent lymphatics**; a **concave hilum** carries the single **efferent lymphatic** plus the nodal artery, vein and nerve. Dense CT **capsule** sends **trabeculae** inward.

Zone	Contents
Cortex (outer)	Subcapsular sinus (receives afferent lymph) → radial cortical/trabecular sinuses ; lymphoid nodules (mostly B cells, primary or secondary with germinal centres) organised around FDCs
Paracortex (deep cortex, no nodules)	T-cell zone; high endothelial venules (HEVs) — cuboidal postcapillary venules through which $\approx 90\%$ of circulating lymphocytes enter the node
Medulla	Medullary cords (T & B cells, numerous plasma cells) alternating with medullary sinuses (rich in macrophages — final lymph filter), converging at the hilum as the efferent lymphatic

Role:

- **Sequential filtration of lymph** — every lymph channel drains through at least one node before reaching the blood; sinus macrophages remove particulate matter, microorganisms and tumour cells.
- **Antigen presentation and lymphocyte activation** — B cells contact antigen on FDCs in follicles (germinal-centre reaction) and differentiate into plasma cells (mostly in medullary cords, secreting non-IgA antibody into efferent lymph); T cells are activated in the paracortex.
- **Clinical:** the first node draining a tumour bed (**sentinel node**) is biopsied to stage malignancy; reactive/neoplastic proliferation → **lymphadenopathy/lymphoma**.

Spleen — microscopic structure and role

□ Fig 8.15 — Histology of thymus (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.180)

Largest lymphoid organ and the **only one that filters blood** ($\sim 12 \times 7 \times 3$ cm); dense CT capsule with trabeculae carrying splenic vessels from the hilum. Parenchyma = **pulp**, of two types:

Component	Structure
White pulp (~20% of organ)	Periarteriolar lymphoid sheath (PALS) — T cells ($\pm$ macrophages, DCs) cuffing a central arteriole ; B cells within the PALS may form a lymphoid nodule with germinal centre (arteriole then eccentric); a peripheral marginal zone separates white from red pulp
Red pulp	Splenic cords (of Billroth) — reticular tissue rich in macrophages, lymphocytes and RBCs — separated by splenic sinusoids lined by elongated stave cells with a discontinuous basal lamina

Blood flow: splenic artery $\rightarrow$ trabecular artery $\rightarrow$ **central arteriole** (white pulp) $\rightarrow$ **penicillar arterioles** $\rightarrow$ sheathed capillaries $\rightarrow$ either **closed circulation** (capillaries open directly into sinusoids) or **open circulation** (blood dumped into cords, must squeeze between stave cells to re-enter sinusoids) $\rightarrow$ red-pulp veins $\rightarrow$ trabecular veins $\rightarrow$ splenic vein.

Role:

- **Blood filtration and old-RBC destruction** — stiff, effete erythrocytes (120-day lifespan) fail to pass the stave-cell slits and are phagocytosed by cord macrophages; iron is recycled via ferritin/transferrin, haem via hemopexin/bilirubin.
- **Immune surveillance of blood-borne antigen** — PALS/marginal-zone lymphocytes respond to blood-borne antigens; antibody and activated lymphocytes are delivered directly into the bloodstream.
- **Clinical:** splenomegaly (lymphoma, infections, haemolytic anaemias) risks traumatic rupture; **splenectomy** $\rightarrow$ Howell–Jolly bodies in circulating RBCs and increased susceptibility to encapsulated-organism sepsis (loss of the organ's filtering/opsonising function).

Tonsils and other MALT — microscopic structure and role

MALT (mucosa-associated lymphoid tissue) collectively houses up to 70% of the body's immune cells, as diffuse lymphocytes/IgA-plasma cells plus discrete nodular aggregates — tonsils, Peyer's patches, appendix.

Tonsil	Epithelium	Crypts	Capsule
Palatine (posterior oral cavity; paired with lingual & pharyngeal, forms Waldeyer's ring)	Stratified squamous non-keratinised	10–20 deep crypts, densely lymphocyte-infiltrated	Partial (deep to nodules)
Lingual (base of tongue)	Stratified squamous	Shallower crypts	No distinct capsule
Pharyngeal (nasopharyngeal roof/posterior wall — single, midline)	Pseudostratified ciliated columnar	Shallow infoldings only, no true crypts	Thin capsule

Deep to the epithelium in all three: diffuse lymphoid tissue studded with numerous secondary **lymphoid nodules** bearing germinal centres.

- ▶ **Peyer's patches** (ileal submucosa) — large, non-encapsulated clusters of nodules; overlying epithelium contains specialised **M (microfold) cells** that transcytose luminal antigen to underlying dendritic cells/lymphocytes, driving local IgA-plasma-cell formation.
- ▶ **Appendix** — mucosa/submucosa almost completely replaced by lymphoid nodules.

Role: first line of adaptive immune surveillance at mucosal portals of entry (oropharynx, gut); local IgA production neutralises luminal pathogens. **Clinical:** chronic pharyngeal-tonsil hypertrophy → **adenoids**, may obstruct the Eustachian tube → recurrent middle-ear infection; palatine tonsillitis is common in children.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ **Lymph node** (as in the embedded plate) — label: capsule, afferent lymphatics, subcapsular sinus, trabecula, cortex with primary/secondary lymphoid follicle (germinal centre + mantle), paracortex with HEV, medullary cords, medullary sinus, hilum, efferent lymphatic, artery/vein.
- ▶ **Thymic lobule** — label: capsule, interlobular septum, cortex (densely packed thymocytes), corticomedullary junction, medulla, Hassall's corpuscle, thymic epithelial cell (cytoreticulum), blood vessel with blood–thymus barrier.
- ▶ **Spleen** (low-power schematic) — label: capsule, trabecula with trabecular artery/vein, white pulp (PALS around central arteriole, lymphoid nodule with germinal centre), marginal zone, red pulp (splenic cords of Billroth, splenic sinusoids), penicillar arteriole.
- ▶ **Palatine tonsil** — label: stratified squamous epithelium, tonsillar crypt, lymphoid nodule with germinal centre, diffuse lymphoid tissue, partial capsule.

Applied / Clinical anatomy

- ▶ Sentinel-lymph-node biopsy for cancer staging; lymphadenopathy vs reactive hyperplasia vs lymphoma on node histology.
- ▶ DiGeorge syndrome (thymic aplasia, 3rd/4th pouch defect) — absent T-cell immunity; thymoma associated with myasthenia gravis.
- ▶ Splenectomy — Howell–Jolly bodies, target cells, and lifelong risk of overwhelming infection by encapsulated organisms (pneumococcus, meningococcus, H. influenzae) — indication for prophylactic vaccination.
- ▶ Traumatic splenic rupture (thin capsule, highly vascular) — surgical emergency.
- ▶ Adenoid hypertrophy in children — Eustachian tube obstruction, secretory otitis media; recurrent tonsillitis — tonsillectomy indications.
- ▶ Peyer's patch M cells are the portal for enteric pathogens (e.g., Salmonella typhi invasion in typhoid) as well as for oral vaccine uptake.

High-yield exam pointers

- ▶ Primary lymphoid organs = bone marrow + thymus (lymphopoiesis); secondary = lymph node, spleen, MALT (antigen-driven activation).

- ▶ Thymus is the **only** lymphoid organ with an epithelial (not reticular-fibre) framework and **no lymphoid nodules**.
- ▶ Hassall's corpuscles are diagnostic of thymic medulla; blood–thymus barrier is confined to the cortex.
- ▶ Lymph node is the only organ with **both afferent and efferent lymphatics**; HEVs in the paracortex are the main lymphocyte entry portal.
- ▶ Spleen is the only lymphoid organ that filters **blood**; stave cells and cords of Billroth are unique to red pulp.
- ▶ White pulp = PALS (T cell) + nodule (B cell); red pulp = cords + sinusoids.
- ▶ Waldeyer's ring = pharyngeal + 2 palatine + 2 tubal + lingual tonsils.
- ▶ Germinal centre = antigen-activated secondary nodule; primary nodule has no germinal centre.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Gray's Anatomy (Standing) 41e; BD Chaurasia's Human Anatomy Vol 3–4 8e; Sontakke's Textbook of Human Histology 2e; Ross Histology (A Text and Atlas) 7e.



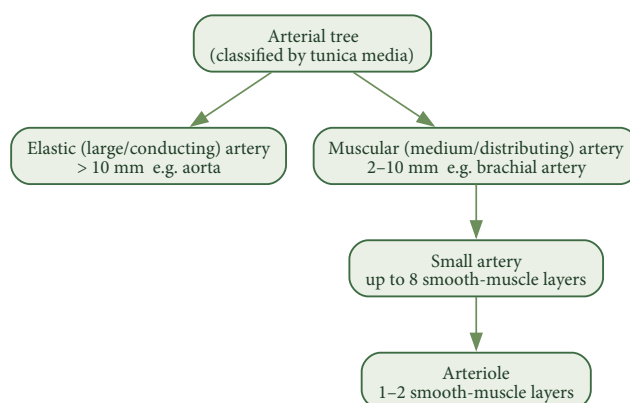
Differences between Histology of Muscular and Elastic artery

Asked in: Paper III 02-Aug-2021 — RGUHS MD/MS Anatomy

Definition / Introduction Arteries are classified histologically, on the basis of the composition of their **tunica media**, into elastic (large/conducting) arteries, muscular (medium/distributing) arteries, small arteries and arterioles. **Elastic arteries** (aorta and its major branches) lie closest to the heart and convey blood by means of elastic recoil, damping the pulsatile ventricular output into a near-continuous flow. **Muscular arteries** (most named arteries of the body) lie distal to elastic arteries and regulate the volume of blood delivered to individual organs and regions by active vasoconstriction/vasodilatation. Both share the same basic three-tunic wall plan (intima, media, adventitia), but the relative proportion of elastic tissue to smooth muscle in each tunic — and consequently the appearance and thickness of each layer — differs sharply between the two, and this distinction is the basis of the question.

Basis of classification — the arterial continuum

- ▶ Arteries are graded by size and by the make-up of the tunica media, not by a sharp cut-off; the change from elastic to muscular artery is **gradual**, with progressive reduction of elastic lamellae and progressive increase of smooth muscle as the vessel diameter falls.
- ▶ Small arteries are distinguished from arterioles by the number of smooth muscle layers in the media — small arteries may have up to **8 layers**, arterioles only **1–2 layers**.



Differences between Histology of Muscular and Elastic artery

Tunica intima — comparison

Feature	Elastic artery	Muscular artery
Endothelium	Simple squamous, resting on basal lamina, long axis parallel to blood flow	Same as elastic artery
Subendothelial connective tissue	Well developed/significant — contains collagen, elastic fibres, myointimal cells	Scanty/sparse — basal lamina may almost abut the internal elastic lamina
Internal elastic lamina (IEL)	Not clearly/distinctly identifiable — merges with the multiple elastic lamellae of the media	Prominent, distinct, wavy (undulating because of medial smooth-muscle contraction); often the single best identification feature

Tunica media — the key discriminator

- **Elastic artery:** the media is the **thickest layer** and consists predominantly of concentric, **fenestrated elastic lamellae** (sheets) alternating with thin layers of smooth muscle cells, collagen and fine elastic fibres. Each elastic lamella + adjoining smooth-muscle zone forms a "lamellar unit". The **human aorta has approximately 40–70 (~52) such elastic lamellae/lamellar units**, each about 11 μm thick; the number and thickness increase after birth (from ~40 lamellae at birth) and further increase with age and in hypertension. Tunica media does **not** contain fibroblasts.
- **Muscular artery:** the media is composed **predominantly of circularly/spirally arranged smooth muscle cells** (roughly **75% of medial mass**, up to ~40 layers), with only a small amount of interspersed elastic fibres and collagen. Smooth muscle cells are joined by gap junctions for coordinated, rapid contraction. A well-defined **external elastic lamina (EEL)** — a compact elastic sheet — separates media from adventitia in the larger muscular arteries; in elastic arteries the EEL is not clearly distinguishable from the other elastic lamellae of the media.

Tunica adventitia — comparison

Feature	Elastic artery	Muscular artery
Relative thickness	Relatively thin (usually less than half the thickness of the media)	Relatively thick — can approach or exceed the thickness of the media in smaller muscular arteries
Composition	Longitudinally running collagen fibres, fine elastic fibres, fibroblasts, occasional macrophages	Fibroelastic connective tissue — collagen predominant, with fibroblasts and some elastic fibres
Vasa vasorum / nervi vasorum	Present, supplying/innervating the outer wall	Present and generally more prominent, penetrating well into the media

Master comparison table

Parameter	Elastic (large/conducting) artery	Muscular (medium/distributing) artery
Also called	Conducting artery	Distributing artery
Diameter	> 10 mm	2–10 mm
Tunica intima	Subendothelial CT well developed; IEL indistinct	Subendothelial CT scanty; IEL prominent and wavy
Tunica media (dominant tissue)	Elastic lamellae (up to ~40–70 fenestrated sheets)	Smooth muscle (~75% of medial mass)
External elastic lamina	Not distinctly separable from medial lamellae	Usually well defined
Tunica adventitia	Thin	Thick
Main function	Windkessel/pressure-reservoir effect — elastic recoil in diastole converts pulsatile ventricular ejection into continuous flow, maintains diastolic pressure	Active vasoconstriction/vasodilatation — regulates blood volume delivered to the region supplied
Examples	Aorta, pulmonary trunk/arteries, brachiocephalic trunk, common carotid, subclavian, common iliac arteries	Brachial, radial, ulnar, femoral, popliteal and coronary arteries; most named arteries of the limbs

□ Fig 11.9 — Elastic artery. (Junqueira's Basic Histology — Text & Atlas — 17e, p.554)

□ Fig 10.3 — Transverse section of a muscular (medium) artery. Notice that T. media has relatively more smooth muscle. (Brijesh Kumar — Histology Text & Atlas — 3e, p.145)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw two labelled transverse-section wall diagrams side by side for comparison:

Panel A — Elastic artery wall (label, inside → outside):

- ▶ Lumen
- ▶ Endothelium (tunica intima)
- ▶ Subendothelial connective tissue (well developed)
- ▶ Internal elastic lamina (merging with medial lamellae — label "indistinct")
- ▶ Tunica media — draw 5–6 concentric wavy elastic lamellae with smooth muscle in between (label "up to ~50 lamellae in vivo")
- ▶ External elastic lamina (label "not distinct from other lamellae")
- ▶ Tunica adventitia (thin) with vasa vasorum

Panel B — Muscular artery wall (label, inside → outside):

- ▶ Lumen
- ▶ Endothelium (tunica intima)
- ▶ Subendothelial connective tissue (scanty)
- ▶ Internal elastic lamina — draw as a single thick wavy/undulating line (label "prominent")
- ▶ Tunica media — shade densely to show smooth muscle predominance, with only a few fine elastic fibres
- ▶ External elastic lamina (label "well defined")
- ▶ Tunica adventitia (thick) with vasa vasorum and nervi vasorum

Applied / Clinical anatomy

- ▶ **Atherosclerosis** preferentially affects elastic arteries and large/medium muscular arteries (aorta, coronaries, carotids) — intimal accumulation of an atheromatous plaque containing smooth muscle cells (migrated from the media), macrophage-derived and smooth-muscle-derived foam cells, collagen and cholesterol crystals, causing luminal narrowing.
- ▶ **Aneurysm** — a sac-like dilatation from weakening of the tunica media, with replacement of elastic fibres by collagen; classically affects the elastic-artery wall (aortic aneurysm) in atherosclerosis, syphilis, Marfan syndrome and Ehlers–Danlos syndrome.
- ▶ **Hypertension** increases the number and thickness of elastic lamellae in elastic arteries and increases smooth muscle bulk/collagen in muscular arteries and arterioles, raising peripheral vascular resistance.
- ▶ **Thromboangiitis obliterans (Buerger disease)** — inflammatory thrombosis of small and medium (muscular) arteries and veins of the limbs, strongly linked to tobacco use.
- ▶ **Surgical/radiological correlate:** the thick, smooth-muscle-rich wall of muscular arteries (radial, internal thoracic) makes them suitable autografts in coronary artery bypass grafting; elastic-artery compliance is exploited clinically as pulse-wave velocity, a surrogate marker of arterial stiffness.

- ▶ **Loss of elastic recoil** with age/atherosclerosis in elastic arteries widens pulse pressure and contributes to isolated systolic hypertension in the elderly.

High-yield exam pointers

- ▶ The **single best distinguishing feature** on a slide: elastic artery = multiple fenestrated elastic lamellae dominate the media, IEL not distinct; muscular artery = smooth muscle dominates the media, IEL is a single prominent wavy line.
- ▶ Elastic artery diameter > 10 mm; muscular artery 2–10 mm.
- ▶ Aorta has ~40–70 (~52) elastic lamellae in the media; number increases with age and hypertension.
- ▶ ~75% of the muscular-artery medial mass is smooth muscle.
- ▶ Tunica adventitia is thin in elastic arteries, thick in muscular arteries.
- ▶ Neither tunica media contains fibroblasts (fibroblasts are confined to adventitia/subendothelium).
- ▶ Transition elastic → muscular artery is gradual, not abrupt.
- ▶ Function: elastic artery = conduit + diastolic (Windkessel) pump; muscular artery = distributing/regulating vessel via vasomotion.
- ▶ Examples: elastic = aorta, pulmonary trunk, brachiocephalic, common carotid, subclavian, common iliac; muscular = brachial, radial, femoral, coronary.

SOURCE & COVERAGE

Gray's Anatomy (Standing) 41e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology 17e (figure); Brijesh Kumar — Histology Text & Atlas 3e (figure).



Histology of blood vessels

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

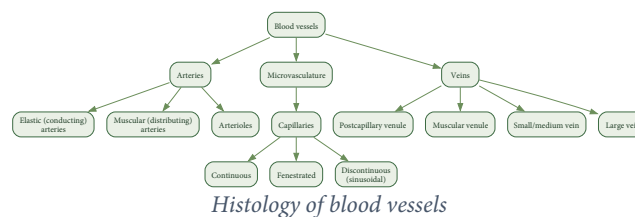
Definition / Introduction

Blood vessels are tubular conduits — arteries, arterioles, capillaries, venules and veins — that together form the closed cardiovascular circuit carrying blood from and back to the heart. Except for capillaries and venules, every vessel wall is built on a common plan of three concentric coats or **tunics**: the tunica intima, tunica media, and tunica adventitia, corresponding roughly to the endocardium, myocardium and epicardium of the heart. The relative thickness and composition of these tunics — chiefly the ratio of elastic tissue to smooth muscle — varies systematically along the vascular tree and defines the histological subtype (elastic artery, muscular artery, arteriole, capillary, venule, vein), which in turn dictates its physiological role in conduction, distribution, exchange or capacitance.

General plan of the vascular wall

Tunic	Composition	Salient features
Tunica intima (innermost)	Simple squamous endothelium on a basal lamina + thin subendothelial connective tissue (loose CT, occasional myointimal smooth-muscle-like cells); bounded externally by a fenestrated sheet of elastin, the internal elastic lamina (IEL) , in arteries	Endothelial long axis parallel to flow; antithrombogenic surface; secretes vWF (Weibel–Palade bodies), heparin, tPA, endothelin-1/ACE (vasoconstrictors), NO/prostacyclin (vasodilators)
Tunica media (middle)	Concentric, helically arranged smooth muscle cells interspersed with elastic lamellae/fibres, reticular fibres and proteoglycans, all secreted by the SMCs themselves; an external elastic lamina (EEL) separates it from the adventitia in larger muscular arteries	Thickest layer in arteries; by far thinnest (or absent) in capillaries and venules; determines vessel tone, elasticity and, in arterioles, peripheral resistance
Tunica adventitia (externa) (outer)	Longitudinally oriented type I collagen and elastic fibres, fibroblasts, mast cells; contains the vasa vasorum (arterioles/capillaries/venules nourishing the outer wall) and nervi vasorum (unmyelinated sympathetic vasomotor fibres)	Merges imperceptibly with surrounding stroma, anchoring the vessel; thickest tunic in large veins; vasa vasorum are needed once wall thickness exceeds ~1 mm (diffusion from the lumen alone is then inadequate)

Capillaries possess only an endothelium and basal lamina ($\pm$ pericytes) — media and adventitia are absent; venules acquire a media only once their outer diameter exceeds $\sim 50 \mu\text{m}$ ("muscular venules").



□ Fig 11.9 — Elastic artery. (Junqueira's Basic Histology — Text & Atlas — 17e, p.554)

Elastic (conducting) arteries

- Include the **aorta, pulmonary trunk and their major branches** (brachiocephalic, common carotid, subclavian, common iliac); function to conduct blood and smooth out the pulsatile output of the heart ("Windkessel effect").
- **Tunica intima**: endothelium + well-developed subendothelial CT (elastic fibres, type I collagen, myointimal cells that accumulate lipid with age); IEL present but less distinct because it merges with the elastic lamellae of the media.
- **Tunica media** — the thickest tunic: numerous **fenestrated elastic lamellae** alternate with layers of smooth muscle, collagen and fine elastic fibres, arranged as regular "lamellar units." The **adult**

aorta has ~50 elastic lamellae (about 40 at birth, increasing with age and further increasing in hypertension); lamellar units measure ~11 μm and number ~52 in the human aorta.

- ▶ During **systole** the elastic lamellae stretch and store energy; during **diastole** they recoil, propelling blood onward and converting intermittent ventricular ejection into continuous peripheral flow.
- ▶ **Tunica adventitia**: relatively thin, rich in elastic fibres, fibroblasts, macrophages, mast cells, vasa vasorum and lymphatics.

Muscular (distributing) arteries

- ▶ The majority of named arteries (e.g. coronary, radial, femoral, splenic) belong to this group; they regulate regional blood flow by contracting/relaxing their smooth muscle, hence also called **distributing arteries**.
- ▶ **Tunica intima**: thin subendothelial layer; a distinct, often wavy (post-mortem contraction) **internal elastic lamina** — more prominent than in elastic arteries.
- ▶ **Tunica media**: up to ~40 layers of **smooth muscle**, constituting roughly 75% of the **medial mass**, arranged circularly/spirally with fine elastic fibres interposed; a distinct **external elastic lamina** is present in the larger members of this group.
- ▶ **Tunica adventitia**: fibroelastic connective tissue that can be as thick as the media in smaller muscular arteries; contains vasa vasorum and nervi vasorum.

Arterioles

- ▶ The terminal, smallest branches of the arterial tree (**muscular arterioles** $\approx 100\text{--}50\text{ }\mu\text{m}$, **terminal arterioles** $< 50\text{ }\mu\text{m}$, down to as little as $\sim 12\text{ }\mu\text{m}$), marking entry into the organ's microvasculature.
- ▶ **Intima**: endothelium on a basal lamina; IEL absent or reduced to fenestrated remnants.
- ▶ **Media**: only 1–2 (up to 3) layers of **circularly arranged smooth muscle**; no elastic lamellae.
- ▶ **Adventitia**: thin, inconspicuous, merging with surrounding connective tissue.
- ▶ Densely innervated by sympathetic (adrenergic) vasomotor fibres; sustained smooth-muscle tone makes arterioles the principal site of **peripheral vascular resistance** and hence the chief regulator of systemic blood pressure. Terminal arterioles give off **metarterioles**, whose muscle cells form **precapillary sphincters** that open and close rhythmically (about 5–10 cycles/min), producing pulsatile perfusion of the capillary bed.

Capillaries

The smallest vessels (average diameter **4–10 μm** , length rarely exceeding 50 μm) consist of only an endothelial tube on a basal lamina, with scattered contractile **pericytes**. Over 90% of total vascular length is capillary. Three structural/functional types are recognised:

Type	Endothelial wall	Basement membrane	Typical sites
Continuous	Uninterrupted, sealed by tight (occluding) junctions	Continuous	Muscle, lung, connective tissue, nervous tissue (with very tight junctions — blood–brain barrier), skin
Fenestrated	Circular pores (~50–100 nm) through the cell, usually closed by a thin diaphragm	Continuous	Intestinal villi, endocrine/exocrine glands, choroid plexus, renal glomerulus (fenestrae here lack a diaphragm)
Discontinuous (sinusoidal)	Wide gaps between and through cells; larger caliber (30–40 μm)	Discontinuous/incomplete	Liver, spleen, bone marrow, adenohypophysis, suprarenal medulla

Capillary beds are perfused via **metarterioles/thoroughfare channels**; alternative pathways include **arteriovenous anastomoses** (direct arteriole-to-venule shunts, important in skin thermoregulation) and **venous portal systems** (e.g. hepatic portal and hypothalamo-hypophyseal systems, in which one capillary bed drains into a second via an intervening vein).

Venules and veins

- **Postcapillary venules** (10–30 μm) resemble capillaries but are larger, with occasional pericytes; they are the principal site of **leukocyte emigration** during inflammation because their endothelial junctions are the loosest and most permeable in the vasculature. Specialised **high endothelial venules (HEVs)** with tall cuboidal endothelium occur in lymph nodes, thymus and MALT, mediating lymphocyte homing.
- Venules acquire a true media (2–3 smooth-muscle layers) once diameter exceeds ~50 μm , becoming **muscular venules**, then converge into **small and medium veins** (usually running alongside the corresponding muscular artery) and finally **large veins** near the heart.
- Compared with an artery of similar lumen, a **vein has a thinner wall, a larger, often collapsed/irregular lumen, and tunics that are not sharply demarcated**; the **adventitia — not the media — is its thickest layer**, and in large veins (e.g. inferior vena cava) it may contain longitudinal smooth-muscle bundles.
- Most small and medium veins (especially of the lower limb) possess **bicuspid valves** — folds of intima rich in elastic fibres, covered on both faces by endothelium — that enforce unidirectional flow toward the heart; valves are absent in venae cavae, and in veins of the cranial cavity, vertebral canal, and very small veins.
- Venous pressure is normally under 5 mmHg (falling from ~25 mmHg at the capillary to ~5 mmHg at the venule), so onward flow depends on the muscular pump of surrounding skeletal muscle and residual medial tone rather than on wall elasticity.

Feature	Artery	Vein
Lumen	Round, comparatively narrow	Large, often irregular/collapsed
Wall thickness	Thick	Thin
Thickest tunic	Tunica media	Tunica adventitia
Tunic boundaries	Sharply demarcated	Poorly demarcated
Internal elastic lamina	Prominent (muscular type)	Absent/indistinct
Media composition	Predominantly smooth muscle + elastic lamellae	Thin, mainly collagen; little muscle/elastin
Valves	Absent	Present (except venae cavae, cranial/vertebral veins)

□ Fig 13.7 — Photomicrograph. Medium-sized artery (muscular artery) and medium-sized vein (H&E stain). (Sontakke — Textbook of Human Histology — 2e, p.171)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a transverse section of a generalized medium (muscular) blood vessel wall and label:

- ▶ **Lumen**
- ▶ **Tunica intima** — endothelium, basal lamina, subendothelial connective tissue
- ▶ **Internal elastic lamina**
- ▶ **Tunica media** — circularly arranged smooth muscle cells, interspersed elastic lamellae/fibres
- ▶ **External elastic lamina**
- ▶ **Tunica adventitia** — collagen and elastic fibres, fibroblasts
- ▶ **Vasa vasorum** (in adventitia/outer media)
- ▶ **Nervi vasorum**

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- ▶ **Atherosclerosis** begins with endothelial dysfunction and oxidised-LDL deposition in the tunica intima, driving monocyte recruitment, foam-cell (lipid-laden macrophage) formation and fatty streaks that progress to fibro-fatty **atheromas**; affects elastic arteries and large/medium muscular arteries. Predisposing factors: dyslipidaemia, hyperglycaemia, hypertension, smoking.
- ▶ In elastic arteries, atheromatous weakening of the media predisposes to **aneurysm** (e.g. abdominal aortic aneurysm) and dissection; in muscular arteries (coronary, cerebral), luminal occlusion causes **ischaemic heart disease/stroke**.
- ▶ **Hypertension** increases the number and thickness of aortic elastic lamellae and drives arteriolar smooth-muscle hypertrophy, raising peripheral resistance further.
- ▶ Incompetent venous valves (long saphenous system) with weakened media/adventitia produce **varicose veins**; venous stasis, endothelial injury and hypercoagulability (Virchow's triad) predispose to **deep vein thrombosis**, particularly in the thin-walled, low-pressure veins of the leg.

- ▶ **Diabetic microangiopathy**: diffuse thickening of capillary basal laminae reduces exchange, underlying diabetic retinopathy, nephropathy and impaired wound healing.
- ▶ **Carotid sinus** (dilated internal carotid origin, thinned media, glossopharyngeal [CN IX] baroreceptors) and **aortic arch** (vagal [CN X] baroreceptors) monitor blood pressure — relevant in carotid sinus massage/hypersensitivity and carotid endarterectomy. **Carotid and aortic bodies** contain neural-crest-derived glomus cells that chemosense hypoxia/hypercapnia/acidosis; carotid body tumours (paragangliomas/chemodectomas) arise here.
- ▶ **Vasa vasorum** insufficiency is implicated in vasculitis and in the pathogenesis of aortic dissection; per IB Singh the coronary arteries are the largest vasa vasorum in the body, and vasa vasorum are required once wall thickness exceeds ~1 mm.
- ▶ **Arteriovenous anastomoses** (glomus bodies) in digital skin regulate heat loss — clinically relevant in Raynaud phenomenon and frostbite.
- ▶ Endothelial **Weibel–Palade bodies** release von Willebrand factor — assayed in von Willebrand disease; anti-VEGF agents exploit the angiogenesis pathway in tumour and retinal disease.
- ▶ Understanding of tunica composition guides **vascular access** (arterial lines, CVP catheterisation) and use of the great saphenous vein as a **CABG conduit**.

High-yield exam pointers

- ▶ Three tunics — intima (endothelium + subendothelial CT + IEL), media (smooth muscle + elastic lamellae + EEL), adventitia (collagen + vasa/nervi vasorum); capillaries have neither media nor adventitia.
- ▶ Elastic (conducting) arteries — aorta/pulmonary trunk; ~50 elastic lamellae in adult aorta; thick media; Windkessel/recoil function.
- ▶ Muscular (distributing) arteries — most named arteries; media up to 40 SMC layers (~75% of medial mass); prominent IEL and EEL.
- ▶ Arterioles — 1–2/3 SMC layers, no elastic lamina; chief determinant of peripheral resistance; metarterioles have precapillary sphincters.
- ▶ Capillaries — continuous, fenestrated, discontinuous (sinusoidal); diameter 4–10 µm; wall = endothelium + basal lamina ± pericyte.
- ▶ Vein vs artery — thinner wall, larger/collapsed lumen, adventitia (not media) thickest, valves present, poorly demarcated tunics.
- ▶ Vasa vasorum nourish adventitia/outer media once wall thickness exceeds ~1 mm; nervi vasorum are sympathetic vasomotor fibres.
- ▶ Carotid sinus/aortic arch = baroreceptors (CN IX/CN X); carotid/aortic bodies = chemoreceptors (glomus cells).
- ▶ Postcapillary venules — most permeable segment; principal site of leukocyte emigration; HEVs mediate lymphocyte homing in lymphoid organs.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e (figure).



Histology of lymphnode

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Lymph nodes are small, encapsulated, bean- or oval-shaped **secondary (peripheral) lymphoid organs** interposed along the course of lymphatic vessels, so that lymph draining any region of the body (with few exceptions) passes through one or more nodes before reaching the bloodstream. A normal young adult has up to about **450 lymph nodes** — roughly 60–70 in the head and neck, 100 in the thorax and as many as 250 in the abdomen and pelvis, with particular concentration in the neck, mediastinum, posterior abdominal wall, mesenteries, pelvis and proximal limbs. Functionally they act simultaneously as **mechanical/biological filters** of lymph (via phagocytic macrophages) and as **organized sites of antigen presentation, lymphocyte activation, proliferation and differentiation**, generating mature, antigen-primed B and T cells and, ultimately, antibody-secreting plasma cells.

1. General organisation

- ▶ Size: **0.1–2.5 cm** long, oval or kidney-shaped, lying along the course of lymphatic channels.
- ▶ Each node shows a slight indentation on one aspect, the **hilum**, through which blood vessels enter and leave and a single (or few) **efferent lymphatic vessel** emerges; several **afferent lymphatic vessels** pierce the convex capsule around the periphery.
- ▶ On low-power section the node shows a densely cellular, darkly staining outer **cortex** and a paler, less densely populated inner **medulla**; the cortex is absent at the hilum, where the medulla reaches the surface.

2. Capsule, trabeculae and reticular stroma

- ▶ **Capsule**: composed mainly of collagen fibres with elastin fibres (more prominent in its deeper layers) and a few fibroblasts; occasional smooth muscle fibres may be present.
- ▶ **Trabeculae** of dense connective tissue, carrying small blood vessels, extend radially from the capsule into the interior, incompletely dividing the node into lobules.
- ▶ **Reticular (reticulin) framework**: fine type III collagen fibrils continuous with the trabeculae branch and interconnect to form a dense meshwork throughout the cortex, paracortex and medulla (sparser within germinal centres), providing attachment for lymphocytes, macrophages and dendritic cells; the network is produced and maintained by **fibroblastic reticular cells (FRCs)**.
- ▶ **Conduit system**: a specialized arrangement of collagen fibres sheathed by FRC processes forms a sponge-like network of micro-channels that not only supports lymphocytes but also rapidly conducts antigen and chemokines deep into the node; dendritic cells sample antigen from within these conduits.

3. Cortex — superficial (nodular) cortex and deep cortex (paracortex)

Zone	Predominant cells	Key features
Superficial/nodular cortex	B lymphocytes, follicular dendritic cells (FDCs)	Contains rounded lymphoid follicles (nodules)
Paracortex (deep cortex, thymus-dependent zone)	T lymphocytes (CD4+ and CD8+), interdigitating dendritic cells	No follicles; site of high endothelial venules (HEVs)

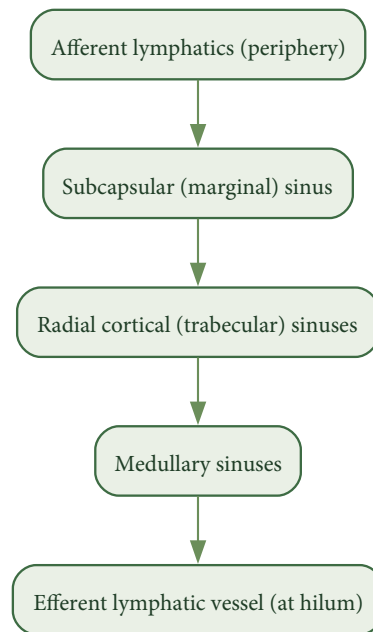
- ▶ **Primary follicle:** uniformly populated by small, quiescent (unstimulated) lymphocytes.
- ▶ **Secondary follicle:** develops a **germinal centre** once antigen-stimulated — the germinal centre allows *affinity maturation* of the B-cell response and has two histological zones:
 - **Dark zone** — rapidly proliferating **centroblasts** undergoing somatic hypermutation of their antibody genes.
 - **Light zone** — **centrocytes** that migrate here and compete for binding to intact antigen displayed by FDCs; helper T cells assist survival and induce class switching, while cells failing to bind with sufficient affinity die by apoptosis and are engulfed by macrophages, whose cytoplasm then fills with debris to form **tingible-body macrophages**.
 - Surrounding the whole follicle is the **mantle zone (corona)** — small, deeply basophilic quiescent B cells (marginalized as the germinal centre expands), plus a few helper T cells, FDCs and macrophages.
- ▶ **Paracortex:** populated mainly by T cells (not organized into follicles) together with **interdigitating dendritic cells**, including Langerhans cells that migrate here as "veiled cells" via the afferent lymphatics from skin/mucosal epithelia to present processed antigen to T cells. This zone expands markedly in T-cell-dominated immune responses.

4. Medulla — cords and sinuses

- ▶ Lymphocytes are less densely packed than in the cortex and are arranged in irregular, branching **medullary cords**, in which the reticulin network is readily visible.
- ▶ Medullary cords contain a mixture of **B lymphocytes**, **plasma cells** (many antibody-secreting plasma cells mature here), **macrophages** and dendritic cells.
- ▶ The cords are separated by dilated **medullary sinuses**, which converge toward the hilum.

5. Sinus system and pathway of lymph flow

- ▶ Afferent lymphatics entering at many points on the periphery form a dense intracapsular plexus and open into the **subcapsular (marginal) sinus**, a cavity peripheral to the whole cortex except at the hilum.
- ▶ From here, numerous **radial cortical (trabecular) sinuses** run through the cortex to the medulla, where they coalesce into larger **medullary sinuses**; these in turn become confluent at the hilum with the single **efferent lymphatic vessel**.
- ▶ All sinus spaces are traversed by fine reticular fibres and stellate reticular cells whose processes project into the lumen, forming a final filtering meshwork; sinus macrophages phagocytose bacteria and particulate matter as lymph percolates through, and lymphocytes freely enter or leave via these channels.

*Histology of lymphnode*

6. Vascular supply and high endothelial venules (HEV)

- ▶ Arteries and veins enter/leave the node through the hilum; the artery gives straight branches that traverse the medulla (with minor branches along the way) to reach the cortex, where they form dense arcades of arterioles and capillaries in anastomosing loops before draining into venules — capillaries are especially profuse around follicles, which themselves contain relatively few vessels.
- ▶ **Postcapillary high endothelial venules (HEVs)** are abundant in the paracortex and are lined by unusually plump/cuboidal endothelial cells (rather than the flattened endothelium of ordinary venules).
- ▶ HEVs are the principal site of **lymphocyte recirculation**, accounting for the entry of most (about 90%) of circulating lymphocytes from blood into the node: **L-selectin** on circulating lymphocytes recognizes sugar-rich addressin ligands on the HEV endothelial surface, causing lymphocytes to tether and stop; integrin-mediated adhesion then allows the lymphocytes to cross the vessel wall (diapedesis through labile endothelial junctions) into the paracortical parenchyma.
- ▶ Capillary/HEV density increases markedly during antigenic stimulation. Veins leave the node through the principal trabeculae and capsule.

7. Cell populations and functional correlation

- ▶ **B lymphocytes** — concentrated in follicles (cortex); on antigenic stimulation proliferate in the germinal centre, giving rise to memory B cells (which rejoin the recirculating pool) and to plasma cells (which mature in the medullary cords or migrate to peripheral tissues) that secrete antibody into efferent lymph.
- ▶ **T lymphocytes (CD4/CD8)** — concentrated in the paracortex ("thymus-dependent" zone); expand and disperse during cell-mediated immune responses.
- ▶ **Macrophages** — line the sinuses and populate the germinal centre and medulla; phagocytose bacteria/particulate matter from lymph and apoptotic cells (tingible-body macrophages).

- ▶ **Follicular dendritic cells (FDCs)** — trap and display intact antigen–antibody complexes to B cells in the germinal centre light zone (do not process/present antigen via MHC).
- ▶ **Interdigitating/Langerhans (veiled) dendritic cells** — process and present antigen via MHC class II to T cells in the paracortex.
- ▶ Net effect: the lymph node filters lymph mechanically and immunologically, and functions as an organized meeting point where naïve lymphocytes (entering via HEVs) and antigen-bearing dendritic cells (arriving via afferent lymph) interact to mount an adaptive immune response.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 4.8 — A lymph node (human) showing cortex (C) and medulla (M), and lymphoid follicles (F), some with germinal centres (G); also shown are the subcapsular sinus (arrow) and medullary blood vessels (V) (Gray's Anatomy — Standring — 41e, p.127)

Reproduce a labelled longitudinal-section schematic of a lymph node with the following exact labels, in sequence from outside inward:

- ▶ **Capsule**, with **afferent lymphatics** piercing it at the periphery
- ▶ **Subcapsular sinus**
- ▶ **Trabeculae** extending radially inward (carrying an **artery** and **vein**)
- ▶ **Cortex**, showing a **primary lymphoid follicle** and a **secondary lymphoid follicle with germinal centre**
- ▶ **Paracortex**, with a **high endothelial venule (HEV)** marked within it
- ▶ **Medulla**, showing **medullary cords** and **medullary sinus**
- ▶ **Hilum**, with the **efferent lymphatic vessel** leaving

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- ▶ **Lymphadenitis** — infection draining into a node produces painful, tender enlargement (acute lymphadenitis); persistent infections such as tuberculosis cause chronic lymphadenitis, classically with caseation necrosis and matting of cervical nodes.
- ▶ **Reactive (follicular) hyperplasia** — antigenic stimulation enlarges and multiplies germinal centres, a benign histological pattern that must be distinguished from lymphoma.
- ▶ **Metastatic spread of carcinoma** — malignant cells detach from a primary tumour, enter afferent lymphatics and lodge first in the **sentinel lymph node** (the first node draining the tumour bed); the presence and number of involved nodes is a key determinant of cancer staging (e.g. the "N" component of TNM), and sentinel node biopsy now guides the extent of nodal dissection in breast cancer and melanoma.
- ▶ **Lymphoma** — Hodgkin and non-Hodgkin lymphomas efface the normal cortex–paracortex–medulla architecture; excisional lymph node biopsy (commonly cervical, axillary or inguinal) remains the gold-standard diagnostic procedure.
- ▶ **Loss of HEV/paracortical integrity** underlies impaired T-cell recirculation seen in some immunodeficiency states, while paracortical (T-zone) expansion is a marker of vigorous cell-mediated response, e.g. in viral lymphadenitis.

- ▶ Superficial node groups (cervical, axillary, inguinal, epitrochlear, popliteal) are palpable clinically and are the standard sites for diagnostic lymph node biopsy.

High-yield exam pointers

- ▶ Cortex = **B-cell zone** (follicles ± germinal centres); paracortex = **T-cell (thymus-dependent) zone**, site of HEVs; medulla = **cords (B cells, plasma cells) + sinuses**.
- ▶ Cortex is **absent at the hilum**, where medulla reaches the surface — the classic low-power identification point.
- ▶ Lymph flow sequence: afferent lymphatics → subcapsular sinus → cortical (trabecular) sinuses → medullary sinuses → efferent lymphatic vessel at the hilum.
- ▶ Germinal centre has a dark zone (centroblasts, hypermutation) and a light zone (centrocytes selected on FDC-displayed antigen) — the site of **affinity maturation**.
- ▶ **HEVs** (cuboidal, "high" endothelium, paracortex) are the principal portal for lymphocyte entry from blood — L-selectin/addressin mediated, ~90% of recirculating lymphocytes.
- ▶ Reticular framework = type III collagen (reticulin), made by fibroblastic reticular cells; also forms the antigen/chemokine-conducting "conduit system."
- ▶ Several afferents but usually a single efferent vessel — this slows lymph flow through the node, maximizing time for filtration and antigen contact.
- ▶ Sentinel lymph node = first node draining a tumour bed — anatomical basis of sentinel node biopsy in cancer staging.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e.



Histology of palatine tonsil

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction The palatine tonsils are the largest paired masses of mucosa-associated lymphoid tissue (MALT) in the body, situated in the tonsillar fossa on the lateral wall of the oropharynx, between the palatoglossal and palatopharyngeal arches. Together with the pharyngeal, tubal and lingual tonsils they form **Waldeyer's lymphatic ring**, guarding the entrance to the digestive and respiratory tracts. Unlike a lymph node, the tonsil is fixed in the mucosa, has **no afferent lymphatics**, and is not organised into cortex and medulla; instead it is built of four histological components — covering epithelium, tonsillar crypts, lymphoid parenchyma, and a fibrous hemicapsule with septa — arranged so that a very large surface area of specialised epithelium is exposed to antigen.

Waldeyer's lymphatic ring — components

Tonsil	Location	Lining epithelium
Palatine tonsil	Tonsillar fossa/sinus, between palatoglossal (anterior) and palatopharyngeal (posterior) arches	Nonkeratinized stratified squamous, with patches of specialised reticulated epithelium in the crypts
Pharyngeal tonsil (adenoid)	Roof and posterior wall of the nasopharynx	Ciliated pseudostratified columnar (respiratory) anteriorly, changing to nonkeratinized stratified squamous posteriorly
Tubal tonsil	Mucosa around the pharyngeal (nasopharyngeal) opening of the auditory tube	Respiratory-type ciliated epithelium, transitional near the tubal elevation
Lingual tonsil	Posterior one-third (root) of the tongue, as dome-shaped nodules	Nonkeratinized stratified squamous; each nodule has a single central crypt

Covering epithelium and tonsillar crypts

- ▶ The free (medial) surface of the tonsil is covered by **nonkeratinized stratified squamous epithelium**, continuous with that of the oropharynx and mouth.
- ▶ This surface epithelium invaginates deeply to form numerous **tonsillar crypts** — narrow, blind-ending, often-branching tubular diverticula that extend through almost the whole thickness of the tonsil, nearly reaching the hemicapsule. The human tonsil is described as **polycryptic**.
- ▶ Crypt number is given as **10–20** (Gray's Anatomy); BD Chaurasia states 12–15, and Brijesh Kumar 15–20 — all texts agree the tonsil is multicryptic, with the largest crypt being the **intratonsillar cleft** (recessus palatinus), a remnant of the second pharyngeal pouch, opening in the upper part of the medial surface.
- ▶ Crypt lumina are frequently packed with **desquamated epithelial cells, lymphocytes and bacteria**, which may calcify to form a **tonsillolith**.

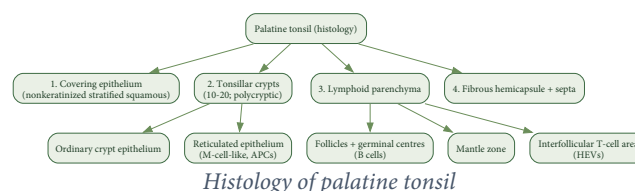
- ▶ Crypt epithelium is of two kinds: most of it resembles the ordinary oropharyngeal stratified squamous epithelium, but there are patches of thinner **reticulated epithelium**, described below, which subserves the immunological function of the tonsil.

Reticulated (crypt) epithelium — functional histology

- ▶ Reticulated epithelium lacks the orderly laminar arrangement of stratified squamous epithelium; its base is deeply and irregularly invaginated so that epithelial cells, with slender branched processes, form a coarse mesh infiltrated by lymphocytes and macrophages.
- ▶ The **basal lamina is discontinuous**, and in places only a tenuous cytoplasmic layer separates the crypt lumen from the underlying lymphoid tissue. Epithelial cells are linked by small desmosomes anchored to keratin filaments; **interdigitating dendritic cells (antigen-presenting cells)** are interspersed within it.
- ▶ Functionally these cells resemble the **M (microfold) cells of the gut**, allowing direct transport of luminal antigen to the underlying lymphoid tissue.
- ▶ Because of the branched crypt architecture, the total surface area of reticulated epithelium is very large — estimated at $\sim 295 \text{ cm}^2$ for an average palatine tonsil.

Lymphoid parenchyma

- ▶ Deep to the epithelium the tonsil is a mass of **dense lymphoid tissue**, supported throughout by a delicate meshwork of fine type III collagen (reticular) fibres.
- ▶ Gray's Anatomy describes **four lymphoid compartments**:
- ▶ **Lymphoid follicles/nodules**, many with **germinal centres**, arranged in rows roughly parallel to the connective-tissue septa; size and cellularity vary with immunological activity (B-cell compartment).
- ▶ **Mantle zone** — a dense cap of closely packed small lymphocytes on the side of each follicle nearest the mucosal surface, formed of B lymphocytes produced within the germinal centre.
- ▶ **Extrafollicular (interfollicular) T-lymphocyte area**, containing specialised microvasculature — **high endothelial venules (HEVs)** — through which circulating lymphocytes enter the tonsillar parenchyma.
- ▶ Lymphoid tissue of the **reticulated crypt epithelium**, containing predominantly IgG- and IgA-producing B lymphocytes (including mature plasma cells), T lymphocytes and antigen-presenting cells.
- ▶ Unlike a lymph node, the tonsil shows **no differentiation into cortex and medulla**.



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power coronal/sagittal section of the palatine tonsil and label:

- ▶ **Oropharyngeal (free/medial) surface** — nonkeratinized stratified squamous epithelium
- ▶ **Tonsillar crypt** (blind, branching) with desquamated debris in the lumen
- ▶ **Intratonsillar cleft** (largest crypt, opening in upper part)
- ▶ **Reticulated crypt epithelium** with infiltrating lymphocytes
- ▶ **Lymphoid follicle with germinal centre and mantle zone**
- ▶ **Interfollicular (diffuse) lymphoid tissue** — T-cell area
- ▶ **Connective-tissue septum** (reticular fibres + elastin)
- ▶ **Fibrous hemicapsule** on the deep/lateral surface

Real reference plate (paste directly into notes for comparison):

 Fig 14.17 — *Histology of palatine tonsil* (BD Chaurasia — *Human Anatomy* Vol 3-4 — 8e, p.280)

Capsule, septa and tonsillar bed

- ▶ The lateral (deep) surface of the tonsil is covered by a **fibrous hemicapsule**, a condensation of the pharyngobasilar fascia. Reticular septa from the parenchyma merge at their outer ends with this hemicapsule and, on the pharyngeal surface, with the lamina propria.
- ▶ The hemicapsule is only **loosely attached** to the muscular pharyngeal wall (superior constrictor and styloglossus) but is **firmly adherent anteroinferiorly to the tongue** (the suspensory ligament of the tonsil), which anchors the tonsil during swallowing.
- ▶ The **tonsillar bed**, from within outward, is formed by the pharyngobasilar fascia, the superior constrictor and palatopharyngeus muscles, the buccopharyngeal fascia, styloglossus (lower part), and the glossopharyngeal nerve — knowledge essential to safe tonsillectomy.

Development (histogenesis)

- ▶ The palatine tonsil develops from the **endoderm of the ventral part of the second pharyngeal pouch**; the pouch endoderm proliferates and its buds are secondarily invaded by surrounding **mesenchyme**, forming the tonsillar primordium.
- ▶ Between the **3rd and 5th fetal months**, the primordium is infiltrated by **lymphatic tissue** (lymphocytes are of mesodermal origin) to form the lymphoid follicles.
- ▶ Part of the original pouch persists in the adult as the **tonsillar fossa/intratonsillar cleft**. Thus the covering and crypt **epithelium is endodermal**, while the **reticular stroma and lymphocytes are mesodermal**.

Applied / Clinical anatomy

- ▶ **Tonsillitis** — infection of the palatine tonsil, usually viral or bacterial (classically *Streptococcus pyogenes*); a common cause of sore throat, more so in children in whom the tonsils are physiologically larger.
- ▶ **Peritonsillar abscess (quinsy)** — collection of pus in the peritonsillar space between the superior constrictor and the tonsillar hemicapsule; often begins in the intratonsillar cleft.

- ▶ **Tonsillectomy** — indicated for recurrent tonsillitis or airway obstruction from hypertrophied tonsils; performed in the plane of the fibrous hemicapsule. Post-tonsillectomy haemorrhage is checked by removal of clot from the raw tonsillar bed — tonsil and the postpartum uterus are the only two sites in the body where bleeding is controlled by clot removal rather than encouragement of clotting.
- ▶ **Referred otalgia** — the glossopharyngeal nerve supplies both the tonsil and (via its tympanic branch) the middle ear mucosa, so tonsillitis/tonsillectomy commonly causes referred ear pain.
- ▶ **Salivary corpuscles** — desquamated epithelial cells and lymphocytes shed from the tonsil mix with saliva.
- ▶ **Age changes** — tonsils enlarge rapidly in the first 5–6 years of life, reach maximum size at puberty (average **20–25 mm vertical**, **10–15 mm transverse**), and thereafter undergo progressive involution.
- ▶ **Surgical hazard** — the internal carotid artery lies only about 25 mm posterolateral to the tonsil; an elongated styloid process may also deviate toward the tonsillar bed.
- ▶ **Septic focus** — chronic tonsillar infection has been implicated as a focus for distant disease (e.g. rheumatic fever, glomerulonephritis, pulmonary tuberculosis).

High-yield exam pointers

- ▶ Palatine tonsil = largest component of **Waldeyer's ring**; secondary/peripheral lymphoid MALT organ with **no afferent lymphatics** and **no cortex-medulla differentiation**.
- ▶ Three structural pillars of the histology: **covering epithelium** → **crypts** → **lymphoid follicles**, supported by a **reticular fibre meshwork** and bounded by a **fibrous hemicapsule**.
- ▶ Covering and crypt-surface epithelium = **nonkeratinized stratified squamous**; specialised **reticulated epithelium** lines part of the crypts and is functionally M-cell-like.
- ▶ **10–20 crypts**; largest = **intratonsillar cleft**, a remnant of the 2nd pharyngeal pouch.
- ▶ Four lymphoid compartments (Gray's): follicle with germinal centre, mantle zone, interfollicular T-cell zone with HEVs, and crypt-associated IgA/IgG-producing lymphoid tissue.
- ▶ Developmentally: **epithelium/crypts** = **endoderm (2nd pharyngeal pouch)**; **lymphoid stroma** = **mesoderm**, infiltrated in the 3rd–5th fetal months.
- ▶ Applied triad to remember: **tonsillitis** → **peritonsillar abscess (quinsy)** → **tonsillectomy**, with referred otalgia via the glossopharyngeal nerve and internal carotid artery ~25 mm posterolateral as the key surgical hazard.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; BD Chaurasia's Human Anatomy Vol 3-4 8e; Brijesh Kumar Histology Text & Atlas 3e; Sontakke Textbook of Human Histology 2e; IB Singh Textbook of Human Histology 9e; IB Singh Textbook of Anatomy Vol 3 6e; Langman's Medical Embryology (Sadler) 14e.



Lymphoid tissue

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction

Lymphoid tissue is a specialised reticular connective tissue packed with lymphocytes, plasma cells and antigen-presenting cells (APCs), supported by a stroma of reticular cells and type III collagen (reticulin) fibres — except in the thymus, where the stroma is epithelial. It forms the structural basis of the immune system and exists either diffusely in mucosae or as discrete, capsulated organs. Functionally it is divided into **primary (central) lymphoid organs**, where lymphocytes are produced and mature, and **secondary (peripheral) lymphoid organs**, where mature lymphocytes contact antigen and proliferate.

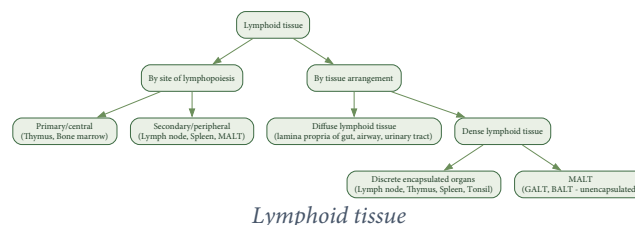
1. Classification of lymphoid tissue

Basis	Types	Examples
Site of lymphopoiesis	Primary/central lymphoid organs	Thymus (T cells), bone marrow (B cells)
	Secondary/peripheral lymphoid organs	Lymph node, spleen, MALT (Peyer's patches, appendix, tonsils)
Tissue arrangement	Diffuse lymphoid tissue	Loosely arranged lymphocytes/plasma cells in lamina propria of gut, trachea, bronchi, urinary tract
	Dense lymphoid tissue	Aggregated lymphocytes forming nodules (follicles)

Dense lymphoid tissue is further subdivided into:

- **Discrete encapsulated organs** — thymus, lymph node, spleen, tonsil.
- **Mucosa-associated lymphoid tissue (MALT)** — unencapsulated aggregates in mucosae; regional names include **GALT** (gut-associated: Peyer's patches of ileum, appendix, tonsils) and **BALT** (bronchus-associated, in trachea/large bronchi).

Lymphoid follicles (nodules) themselves are **primary** (uniform small dark lymphocytes, no germinal centre — unstimulated) or **secondary** (a pale-staining germinal centre of proliferating B-lymphoblasts, ringed by a darker follicular mantle of naive cells — antigen-stimulated).



2. Cells of lymphoid tissue

- **Lymphocytes** — majority cell; small, round, dense basophilic nucleus, scanty cytoplasm (hence dark blue on H&E).

- ▶ **Plasma cells** — eccentric "clock-face" nucleus, basophilic cytoplasm; secrete antibody, concentrated in medullary cords/red pulp.
- ▶ **Macrophages/APCs** — phagocytose antigen and apoptotic debris; present antigen via MHC class II.
- ▶ **Follicular dendritic cells (FDCs)** — mesenchymal (not haemopoietic) in origin; trap antigen-antibody complexes on their processes within follicles, driving B-cell activation; do **not** use MHC II, unlike interdigitating dendritic cells of the paracortex.
- ▶ **Reticular cells** — fibroblast-like cells that synthesise and maintain the type III collagen (reticulin) framework on which all the above cells sit (absent in thymus, replaced by epithelial reticular cells).

3. Lymph node — structure

Bean-shaped, encapsulated nodes along the course of lymphatics; a convex surface receives several **afferent lymphatics**, and a concave **hilum** gives exit to a single **efferent lymphatic** and carries the nodal artery/vein. A dense connective-tissue **capsule** sends **trabeculae** inward carrying blood vessels. Valves in afferent/efferent lymphatics ensure one-way lymph flow.

Region	Composition	Key feature
Cortex (outer)	Primary/secondary lymphoid follicles, mostly B cells ; subcapsular sinus beneath capsule	Follicles absent from paracortex — this is the distinguishing point
Paracortex (deep/inner cortex)	Diffuse T cells , interdigitating dendritic cells	Site of high endothelial venules (HEVs) — cuboidal post-capillary venules through which ~90% of circulating lymphocytes enter the node (L-selectin/addressin mediated)
Medulla	Medullary cords (T & B cells, numerous plasma cells) separated by medullary sinuses	Sinuses converge at the hilum as the efferent lymphatic; lymph is progressively filtered cortex → paracortex → medulla

□ Fig 11.2 — Structure of the lymph node (sectional view). (Sontakke — Textbook of Human Histology — 2e, p.136)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Bean-shaped lymph node in longitudinal section, labelling: **Capsule** → **Trabecula** → **Afferent lymphatic vessel** → **Subcapsular (marginal) sinus** → **Cortex with lymphoid follicles (germinal centre + mantle)** → **Paracortex (with HEV)** → **Medullary cords** → **Medullary sinuses** → **Hilum** → **Efferent lymphatic vessel** → **Artery and Vein at hilum**.

4. Thymus — structure

Bilobed lympho-epithelial organ in the superior mediastinum; unique among lymphoid organs in having a **dual embryonic origin** — epithelial reticulum from **endoderm of the third pharyngeal pouch**, populated by lymphoid precursors of mesenchymal (bone-marrow) origin. Weighs 10–15 g at birth,

increases to 30–40 g at puberty, then progressively involutes and is replaced by fat, though some T-cell output continues lifelong. It has **no afferent lymphatics** — only efferent.

- ▶ Capsule → septa → incompletely-divided **lobules** (~2 mm each), each with an outer dark **cortex** (densely packed thymocytes) and a paler **medulla** (continuous between adjacent lobules, unlike the cortex).
- ▶ **Epithelioreticular cells (epitheliocytes)** form the structural cytotreticulum (not reticulin fibres); six types are described:

Type	Location	Function
Type 1	Lines capsule, septa, vessels	Forms blood–thymus barrier
Type 2 & 3	Outer and inner cortex	Compartmentalise cortex; "thymic nurse cells"; eliminate self-reactive thymocytes
Type 4	Cortico-medullary junction	Cortex–medulla barrier
Type 5	Medulla	Structural framework of medulla
Type 6	Medulla	Form Hassall's corpuscles

- ▶ **Blood–thymus barrier** (isolates cortical thymocytes from circulating antigen) is made up of five layers: endothelial tight junctions → endothelial basal lamina → perivascular connective tissue → epitheliocyte basal lamina → Type 1 epitheliocytes.
- ▶ **Hassall's corpuscles** — concentric whorls of degenerating Type 6 epitheliocytes with keratohyalin/lipid, pathognomonic of thymic medulla; secrete cytokines that promote medullary dendritic-cell activity and regulatory T-cell development.
- ▶ **T-cell selection**: positive selection (cortex) retains thymocytes whose TCR recognises self-MHC; negative selection (medulla, aided by **AIRE**-expressing epitheliocytes presenting peripheral-tissue antigens) deletes strongly self-reactive clones — the basis of central immune tolerance. Only ~2% of thymocytes survive to emigrate as mature T cells.

5. Spleen — structure

Largest single mass of lymphoid tissue (~12 × 7 × 3 cm); the only lymphoid organ that filters **blood** rather than lymph. Capsule of dense connective tissue gives trabeculae carrying the splenic artery/vein branches from the hilum.

- ▶ **White pulp** (~20% of organ) — lymphoid tissue: a **periarteriolar lymphoid sheath (PALS)**, mainly T cells, surrounds the **central arteriole**; B cells within the PALS form lymphoid follicles (with germinal centres when stimulated), pushing the arteriole eccentrically; a marginal zone of B cells lies at the white–red pulp interface.
- ▶ **Red pulp** — **splenic cords (of Billroth)**, reticular tissue rich in macrophages, plasma cells and all blood cell types, alternating with venous **sinusoids** lined by elongated **stave cells** with a discontinuous basal lamina.
- ▶ **Circulation**: central arteriole → penicillar arterioles → either **closed circulation** (capillary opens directly into a sinusoid) or **open circulation** (capillary empties into the cordal stroma; cells must

squeeze between stave cells to re-enter a sinusoid) — the open route is where senescent/rigid RBCs are trapped and phagocytosed.

- Functions: blood filtration/immune surveillance, destruction of aged erythrocytes with iron recycling (ferritin/haemosiderin, bilirubin excreted via liver), antibody production, and a site of fetal haemopoiesis (extramedullary haemopoiesis in disease).

6. Tonsils and mucosa-associated lymphoid tissue (MALT)

Palatine, lingual, pharyngeal ($\pm$ tubal) tonsils together form **Waldeyer's ring** guarding the pharyngeal isthmus.

Tonsil	Epithelium	Crypts	Capsule
Palatine	Stratified squamous	10–20 deep branching crypts	Partial (dense CT)
Lingual	Stratified squamous	Shallow crypts	Absent
Pharyngeal (adenoid)	Pseudostratified ciliated columnar (patches of squamous)	Shallow infoldings, no true crypts	Thin

Each shows diffuse lymphoid tissue with numerous secondary follicles beneath the epithelium. **GALT** (Peyer's patches of ileum — nodule clusters with no capsule, overlain by antigen-sampling **M cells**; vermiform appendix — mucosa almost replaced by lymphoid follicles) and **BALT** (trachea/bronchi) complete the MALT system, together housing up to 70% of the body's immune cells.

Applied / Clinical anatomy

- **Lymph node**: draining nodes enlarge in infection (**lymphadenitis**) or tumour spread (**lymphadenopathy**); the **sentinel node** concept (first node draining a tumour bed) underlies surgical staging of cancer.
- **Lymphatics**: acute lymphangitis (streptococcal/staphylococcal) shows tender red streaking; chronic obstruction (filarial, post-irradiation, malignant) causes **lymphoedema**; ruptured lymphatics give chylothorax/chyloperitoneum/chyluria.
- **Thymus**: hyperplasia/thymoma associates with **myasthenia gravis** (autoantibodies to acetylcholine receptor); failure of 3rd/4th pharyngeal pouch development causes thymic aplasia/hypoplasia in **DiGeorge syndrome**, with defective cell-mediated immunity.
- **Spleen**: **splenomegaly** in malaria, portal hypertension, haemolytic anaemias, lymphomas; thin capsule predisposes an enlarged spleen to traumatic rupture requiring splenectomy; post-splenectomy blood shows Howell–Jolly bodies (nuclear remnants normally culled by splenic macrophages) and increased infection risk from encapsulated organisms.
- **Tonsil**: recurrent **tonsillitis**; adenoidal hypertrophy in children obstructs the eustachian tube, predisposing to otitis media — basis for tonsillectomy/adenoidectomy.

High-yield exam pointers

- Primary organs = thymus + bone marrow; secondary = lymph node, spleen, MALT — know this distinction cold.

- ▶ Only the lymph node filters **lymph**; only the spleen filters **blood**.
- ▶ Paracortex = T-cell zone with HEVs (no follicles); cortex = B-cell follicles — this differentiates cortex from paracortex on a slide.
- ▶ Thymus stroma is epithelial (epitheliocytes), not reticular fibre — unlike every other lymphoid organ.
- ▶ Hassall's corpuscles = thymic medulla identification point; blood–thymus barrier has 5 components, formed by Type 1 epitheliocytes.
- ▶ White pulp (PALS + follicles) around a central arteriole = spleen identification point; red pulp = cords of Billroth + sinusoids with stave cells.
- ▶ Waldeyer's ring = palatine + lingual + pharyngeal (+ tubal) tonsils.
- ▶ FDCs (mesenchymal, trap antigen, no MHC II) ≠ interdigitating dendritic cells (haemopoietic, present via MHC II) — a frequently confused pair.
- ▶ Germinal centre = secondary follicle = antigen-stimulated B-cell proliferation site.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Gray's Anatomy (Standing) 41e.



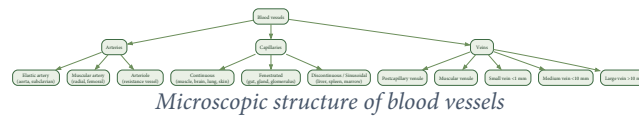
Microscopic structure of blood vessels

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction — Blood vessels form a closed system of tubes — arteries, arterioles, capillaries, venules and veins — that convey blood from the heart to the tissues and back. Except capillaries and venules, every vessel wall is built of three concentric coats: the **tunica intima**, **tunica media** and **tunica adventitia**, whose relative thickness and composition change progressively along the vascular tree and determine each segment's function — conduction, resistance, exchange or capacitance. Arteries are further subdivided structurally into **elastic** and **muscular** types, and capillaries into **continuous**, **fenestrated** and **discontinuous (sinusoidal)** varieties, each correlating with a distinct physiological role.

Classification of blood vessels

- ▶ **Functional classes** (Gray's): conducting/distributing vessels (large & medium arteries); resistance vessels (small arteries, mainly **arterioles**); exchange vessels (capillaries, sinusoids, postcapillary venules); capacitance vessels (veins) — veins hold the largest proportion of the total blood volume.
- ▶ **Structural classes** of arteries: **elastic (large)**, **muscular (medium)**, **arterioles**.
- ▶ From the aorta, the systemic tree gives rise to about 4×10^6 **arterioles** and roughly four times as many capillaries; total cross-sectional area rises from $\sim 7 \text{ cm}^2$ at the aortic root (outer diameter $\sim 30 \text{ mm}$) to $\sim 150 \text{ cm}^2$ at arteriolar level — over 200 times greater — so blood flow is fastest near the heart and slowest in the microcirculation.



General plan of the vessel wall

Tunica	Composition	Key features
Intima (innermost)	Endothelium (simple squamous) on a basal lamina + subendothelial connective tissue, limited externally by the internal elastic lamina (IEL)	Endothelium lines the entire vascular tree, including the heart and lymphatics; thickens with age
Media (middle)	Concentrically/spirally arranged smooth muscle cells, elastic fibres and collagen, limited by the external elastic lamina (EEL)	Thickest layer in arteries; comparatively thin in veins; absent in capillaries
Adventitia (outer)	Longitudinally arranged collagen and elastic fibres, fibroblasts, vasa vasorum and nervi vasorum	Anchors the vessel to surrounding tissue; vasa vasorum appear once wall thickness exceeds ~1 mm (diffusion from the lumen alone becomes inadequate)

□ Fig 7.1 — Layers in the wall of a typical artery (IB Singh — Textbook of Anatomy Vol 1 — 6e, p.77)

Elastic (large) arteries vs muscular (medium) arteries

Feature	Elastic artery	Muscular artery
Examples	Aorta, brachiocephalic, common carotid, subclavian, common iliac	Radial, femoral, coronary, axillary, palmar arteries
Role	Conducting vessel — recoils in diastole to smooth the pulsatile output (Windkessel effect)	Distributing vessel — alters lumen size to regulate regional blood flow
Intima	Well-developed subendothelial layer; IEL present but not sharply distinct from medial lamellae	IEL thin, distinct, and prominent; thrown into wavy folds when the media contracts
Media	Thickest layer; fenestrated concentric elastic lamellae alternating with smooth muscle — about 40 lamellar units at birth , ~52 in the adult (each ~11 µm thick); number/thickness increase with age and in hypertension	~75% of medial mass is smooth muscle, arranged circularly/spirally; only fine elastic fibres interspersed
Adventitia	Relatively thin compared with the media; contains vasa vasorum, fibroblasts, mast cells, nerves and lymphatics	Fibroelastic; can be as thick as the media in smaller muscular arteries

Arterioles

- ▶ Continuation of muscular arteries once diameter falls below $\sim 100\ \mu\text{m}$; **muscular arterioles** measure $100\text{--}50\ \mu\text{m}$, **terminal arterioles** are $<50\ \mu\text{m}$ (smallest $\sim 10\text{--}12\ \mu\text{m}$).
- ▶ All three tunics are present but markedly thinned; in the smallest arterioles the wall thickness equals about half the outer radius.
- ▶ IEL is absent or highly fenestrated; media is only **one or two layers of circumferentially wound smooth muscle**.
- ▶ Adventitia is very thin.
- ▶ Arterioles are the principal **resistance vessels**; their contraction/relaxation, regulated mainly by local metabolic factors (and central sympathetic drive), governs peripheral vascular resistance and blood pressure — they act functionally as **precapillary sphincters**.
- ▶ Densely innervated by unmyelinated sympathetic (mostly adrenergic) fibres, with neuroeffector gaps as small as $50\text{--}100\ \text{nm}$.

Capillaries

Type	Endothelium	Basal lamina	Typical sites
Continuous	Uninterrupted; cells joined by tight junctions	Continuous	Skin, muscle, lung, connective tissue, brain (basis of blood–brain barrier via very tight junctions)
Fenestrated	Circular pores $50\text{--}100\ \text{nm}$ in diameter, often closed by a thin diaphragm (glycoprotein PV-1)	Continuous	Renal glomeruli, intestinal mucosa, endocrine and exocrine glands
Discontinuous (sinusoidal)	True gaps between adjacent, fenestrated endothelial cells; larger and irregular calibre	Discontinuous or absent	Liver, spleen, bone marrow, suprarenal medulla, adenohypophysis

- ▶ Capillary diameter is **$4\text{--}8\ \mu\text{m}$** (mean $\sim 8\ \mu\text{m}$) — just wide enough for a single erythrocyte to pass, often with deformation.
- ▶ Wall = a single endothelial cell + basal lamina + occasional **pericytes** (contractile, mesenchymal, support the wall and modulate lumen calibre; absent from fenestrated capillaries, more continuous around postcapillary venules).
- ▶ **Precapillary sphincters** (smooth muscle cuffs at the origin of capillaries from metarterioles/terminal arterioles) and **thoroughfare channels** together form the microcirculatory unit that regulates regional perfusion.

Venous system — venules to large veins

Segment	Diameter	Wall structure
Postcapillary venule	10–30 μm	Endothelium + basal lamina + pericytes; no true tunica media — as permeable as capillaries; site of leucocyte diapedesis and (in MALT/lymph nodes) high endothelial venules
Muscular venule	up to ~0.1 mm	Acquires 1–2 layers of smooth muscle (a true media appears)
Small vein	0.1–1 mm	All three tunics recognisable but thin
Medium vein	up to 10 mm	Media thinner than in an artery of equal calibre; valves are a characteristic feature (e.g. radial, tibial, popliteal veins)
Large vein	>10 mm	Media relatively thin; adventitia is the thickest layer , containing longitudinal smooth muscle (e.g. superior/inferior vena cava, portal vein)

- ▶ General differences from arteries: thinner wall, larger lumen for the same blood flow, media contains more collagen and less elastin/muscle, and adventitia (not media) is usually the thickest coat.
- ▶ Pressure falls from ~25 mmHg in capillaries to ~5 mmHg at the postcapillary venule, and does not normally exceed 5 mmHg in the venous system — hence veins act as low-pressure capacitance vessels.
- ▶ Valves are semilunar folds of intima (endothelium over a collagen/elastic core); absent in venae cavae, very small veins, and veins of the cranial and vertebral canals.
- ▶ Muscle is characteristically absent from veins of erectile tissue, cancellous bone, dural venous sinuses, retinal and placental veins.

Endothelium, vasa vasorum, nervi vasorum & arteriovenous anastomoses

- ▶ **Endothelium**: simple squamous monolayer on a basal lamina, joined by tight and gap junctions; functions include a selective permeability barrier, secretion of anticoagulant/antithrombogenic factors (heparin, tissue plasminogen activator), storage/release of **von Willebrand factor** in **Weibel–Palade bodies**, and secretion of vasoactive substances — vasodilators (nitric oxide, prostacyclin) and vasoconstrictors (endothelin, angiotensin-converting enzyme).
- ▶ **Vasa vasorum** ("vessels of vessels"): small arteries in the adventitia (and outer media) of vessels whose wall thickness exceeds ~1 mm, since diffusion from the lumen alone is then insufficient; the **coronary arteries are the largest vasa vasorum** in the body.
- ▶ **Nervi vasorum**: unmyelinated sympathetic vasomotor fibres (mostly adrenergic) plus some myelinated sensory afferents in the adventitia.

- ▶ **Arteriovenous (AV) anastomoses:** direct thick-walled, richly sympathetically-innervated channels connecting arteries to veins that bypass the capillary bed; found in the skin of the nose, lips, fingertips and ear, in gut mucosa, and in erectile tissue. A specialised coiled form — an afferent artery, one or more S-shaped connecting vessels and an efferent vein enclosed in connective tissue — constitutes a **glomus body**, important in thermoregulation.

Applied / Clinical anatomy

- ▶ **Atherosclerosis:** begins with endothelial injury and LDL oxidation in the tunica intima of large elastic and muscular arteries; monocytes migrate into the intima and become foam cells, while medial smooth muscle cells migrate inward — the basis of the atheromatous plaque, and the major risk factor for myocardial infarction and stroke.
- ▶ **Hypertension** (BP > 140/90 mmHg): causes smooth-muscle contraction and hyperplasia in the media of small arteries/arterioles (arteriolosclerosis), raising peripheral resistance and, over time, left ventricular hypertrophy.
- ▶ **Varicose veins:** permanent dilatation and tortuosity of superficial leg veins (chiefly the long saphenous vein and tributaries) due to valvular incompetence; affects 10–12% of the population, more often women, especially in pregnancy (uterine compression of iliac veins causing venous stasis).
- ▶ **Deep vein thrombosis (DVT):** thrombus formation in the deep veins of the lower limb, associated with immobilisation (post-surgical bed rest, casts, long-haul travel); a dislodged clot risks pulmonary embolism.
- ▶ **Myocardial sleeves:** cardiac muscle extensions persist in the adventitia of the superior/inferior venae cavae and pulmonary veins; these ectopic foci are implicated in atrial fibrillation.
- ▶ **Vascular tumours:** haemangioma (capillary or cavernous type) arises from vessel endothelium; **glomus tumour** is a rare, painful neoplasm from the glomus body, typically subungual or on the fingertip/foot.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw transverse sections of an **elastic artery**, a **muscular artery**, a **vein** of comparable calibre, and the three capillary types, with these labels:

- ▶ Lumen → Endothelium → Subendothelial connective tissue → **Internal elastic lamina** → **Tunica media** (smooth muscle + elastic lamellae, shown as multiple concentric fenestrated sheets in the elastic artery, circular muscle bulk in the muscular artery) → **External elastic lamina** → **Tunica adventitia** (collagen, elastic fibres) → **Vasa vasorum** and **nervi vasorum** in the adventitia.
- ▶ For the vein: thinner media, thicker adventitia (with longitudinal smooth muscle), larger collapsed lumen, and a **valve** (two semilunar endothelial cusps).
- ▶ For capillaries: (a) continuous — uninterrupted endothelium + continuous basal lamina + occasional pericyte; (b) fenestrated — endothelial pores with a thin diaphragm, continuous basal lamina; (c) discontinuous/sinusoidal — wide intercellular gaps, discontinuous basal lamina.

High-yield exam pointers

- ▶ Three tunics — intima, media, adventitia — present in all vessels except **capillaries and venules** (no true media).
- ▶ Elastic artery media = fenestrated elastic lamellae (~52 in adult aorta, from ~40 at birth); muscular artery media = ~75% smooth muscle.
- ▶ Arterioles: muscular 100–50 µm, terminal <50 µm (down to ~10–12 µm); act as precapillary sphincters and are the chief resistance vessels.
- ▶ Three capillary types — continuous, fenestrated, discontinuous (sinusoidal) — correlate exactly with organ function (BBB, glomerulus/gut, liver/spleen/marrow).
- ▶ Capillary diameter 4–8 µm; pressure falls from ~25 mmHg (capillary) to ~5 mmHg (venule).
- ▶ Veins: adventitia is usually the **thickest** coat (opposite of arteries); valves are characteristic of medium veins, absent in venae cavae.
- ▶ Vasa vasorum supply the adventitia/outer media once wall thickness >1 mm; coronary arteries are the largest vasa vasorum in the body.
- ▶ Endothelium is not a passive lining — it is secretory (NO, prostacyclin, endothelin, ACE, vWF/Weibel–Palade bodies, heparin, tPA).
- ▶ Applied correlates: atherosclerosis (intima), arteriolosclerosis (hypertension), varicose veins and DVT (venous system), glomus tumour/haemangioma (AV anastomoses/endothelium).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology 17e.



Microscopic structure of spleen

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction The spleen is the largest single mass of lymphoid tissue in the body and the only secondary lymphoid organ interposed directly in the systemic circulation, giving it a unique dual role in blood filtration and immune surveillance. Grossly it measures about $12 \times 7 \times 3$ cm and lies in the left hypochondrium. On cut section the parenchyma (splenic pulp) shows two grossly and microscopically distinct components — **white pulp** (basophilic, lymphoid) and **red pulp** (blood-filled) — named from their colour on a fresh cut surface, both supported by a connective-tissue capsule and trabecular framework.

Capsule and trabecular framework

- ▶ The spleen is covered by peritoneal serosa except at the hilum and the sites of peritoneal ligament attachment.
- ▶ Deep to the serosa is a dense connective-tissue **capsule**, about 1.5 mm thick, containing type I collagen with some elastin, arranged as an outer and an inner lamina whose fibre directions differ (adds strength). Human splenic capsule/trabeculae contain very little smooth muscle, so the spleen does not contract actively.
- ▶ **Trabeculae** extend from the capsule into the substance of the organ and branch repeatedly to form a connective-tissue scaffold; the largest trabeculae enter at the hilum and carry the segmental branches of the splenic vessels, lymphatics and nerves into the pulp.
- ▶ Within the pulp, the trabecular collagen continues as a delicate meshwork of **type III (reticular) fibres** that pervades both red and white pulp, maintained by resident fibroblasts/reticular cells — this reticulum is the structural stroma on which all pulp cells sit.

White pulp

- ▶ Accounts for roughly 5–20% of splenic tissue (average ~20%).
- ▶ Branches of the splenic artery run in the trabeculae and, in their terminal few millimetres, lose their connective-tissue adventitia, which is replaced by a sheath of **T lymphocytes** — the **periarteriolar lymphoid sheath (PALS)** — around what is now called the **central arteriole**.
- ▶ The PALS is expanded at intervals by aggregates of **B lymphocytes** forming **lymphoid follicles (nodules)**, 0.25–1 mm in diameter, visible to the naked eye on a fresh cut surface as pale semi-opaque dots against the deep red pulp.
- ▶ Follicles form near the terminal branches of the arteriole and typically bulge to one side, so the arteriole appears **eccentrically placed** within an enlarged follicle (in contrast to the PALS, where the arteriole is central).
- ▶ After antigenic stimulation, follicles develop pale-staining **germinal centres** (B-cell proliferation aided by follicular dendritic cells), which regress once the stimulus subsides; follicles atrophy with age and may be sparse or absent in the very elderly.
- ▶ Arterioles branch laterally within the follicles as parallel terminal **penicillar arterioles**, which then leave the white pulp to supply the red pulp.

Marginal zone

- ▶ The **marginal (perifollicular) zone** lies at the interface between white and red pulp — the site where blood is actually delivered into the red pulp and where circulating lymphocytes leave the blood to migrate into the T- and B-cell zones of the white pulp.
- ▶ Lymphocytes here are more loosely arranged than in the PALS/follicle proper, held within a dense network of reticular fibres and cells.
- ▶ Arterioles leaving the white pulp are surrounded here by a small cuff of macrophages — the **peri-arteriolar macrophage sheath** — positioned to screen blood-borne antigens as they enter the red pulp.

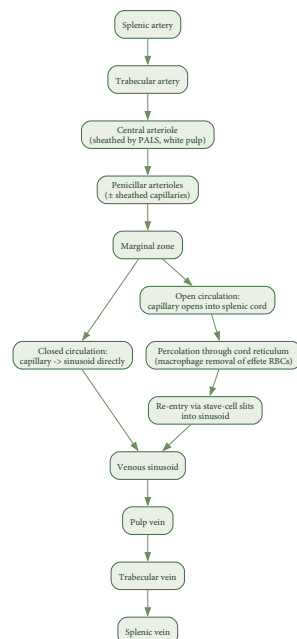
Red pulp — splenic cords and sinusoids

- ▶ Constitutes up to 90% of splenic volume; it is the unique filtration device that clears particulate material and aged cells from perfusing blood.
- ▶ Composed of **venous sinusoids** and the intervening **splenic cords (cords of Billroth)**.
 - **Splenic cords:** a reticular meshwork of collagen, reticular fibroblasts and resident **macrophages**, packed with red cells, lymphocytes and other leukocytes; in two-dimensional section they look like separate strips of tissue but are actually a continuous three-dimensional space around the sinusoids.
 - **Venous sinusoids:** elongated, ovoid vessels about 50 µm in diameter, supported externally by circumferential and longitudinal reticular fibres. They are lined by a **discontinuous endothelium unique to the spleen** — long, narrow "stave" cells aligned parallel to the long axis of the sinusoid (like the staves of a barrel), joined to neighbouring cells only at intervals by short junctional stretches alternating with **intercellular slits**. A discontinuous basal lamina lies on the abluminal aspect.
 - Large stellate **reticular cells (fibroblasts)** surround the sinusoids, synthesising the collagen/proteoglycan matrix and compartmentalising the reticular space with their cytoplasmic processes.
- ▶ Blood emptied from open-ended penicillar-arteriole capillaries percolates through the reticular spaces of the cords; cord macrophages phagocytose particulate material and ageing/damaged erythrocytes there.

Feature	White pulp	Red pulp
Proportion of spleen	~5–20%	up to ~90%
Main cell type	T cells (PALS), B cells (follicles)	Macrophages, all blood-cell types
Vessel	Central arteriole	Venous sinusoids (stave-cell lining)
Stroma	Reticular fibres + PALS/follicle lymphocytes	Reticular fibres of splenic cords (of Billroth)
Principal function	Immune surveillance, antibody production	Blood filtration, erythrocyte destruction, iron recycling

Splenic microcirculation — open vs closed theories

- ▶ Segmental splenic arteries ramify as **trabecular arteries**, tapering into arterioles that traverse the white pulp (enclosed by PALS) as **central arterioles**, then emerge as short, straight **penicillar arterioles** that traverse the marginal zone and enter the red pulp; some of these are surrounded by macrophage-rich "sheathed capillaries" for additional immune screening of blood.
- ▶ Two theories of terminal blood flow are described:
 - **Closed circulation** — capillaries connect directly to the lumen of a sinusoid, so blood remains endothelium-enclosed throughout.
 - **Open circulation** — capillaries end openly in the splenic cords; blood percolates through the reticular meshwork of the cords and must squeeze through the intercellular slits between stave cells to re-enter the sinusoidal lumen.
- ▶ Current evidence favours a **dominantly open circulation in humans**. This slow, cell-by-cell passage through narrow slits is what allows the spleen to test the deformability of each erythrocyte: pliable, healthy cells slip through easily, while stiff or effete cells (normal RBC lifespan ~120 days) are trapped and phagocytosed by cord macrophages.
- ▶ From the sinusoids, blood drains into small red-pulp (pulp) veins, which converge as **trabecular veins** (thin-walled, sparse smooth muscle, essentially channels hollowed in the trabecular connective tissue) and finally form the **splenic vein**.



Microscopic structure of spleen

□ Fig 9.9 — Spleen: Red and White Pulp. Stain: Hematoxylin-eosin. Medium magnification. (diFiore's Atlas of Histology — Eroschenko, p.144)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power section of spleen and label:

- ▶ Capsule (dense connective tissue, outer + inner lamina)
- ▶ Trabecula, with trabecular artery and trabecular vein
- ▶ Central arteriole
- ▶ Periarteriolar lymphoid sheath (PALS) — T cells
- ▶ Lymphoid follicle with germinal centre — B cells (arteriole shown eccentric)
- ▶ Marginal zone (interface of white and red pulp) with peri-arteriolar macrophage sheath
- ▶ Penicillar arteriole / sheathed capillary
- ▶ Splenic (venous) sinusoids — stave-cell (discontinuous) endothelium, intercellular slits
- ▶ Splenic cords (of Billroth) — reticular fibres, macrophages, mixed blood cells
- ▶ Reticular fibre meshwork (stroma) throughout red and white pulp

Applied / Clinical anatomy

- ▶ **Accessory spleen (splenunculus):** a splenic lobule that fails to fuse with the main organ persists as a fully functional supernumerary spleen in roughly 10% of individuals — relevant when spleen must be removed for haematological disease, since a missed accessory spleen can cause relapse.
- ▶ **Splenomegaly:** enlargement occurs in portal hypertension, Gaucher's disease, myelofibrosis (extramedullary haemopoiesis — reactivation of the fetal haemopoietic role of red pulp), thalassaemia, lymphoma and other malignant infiltration. The capsule is relatively thin, so an enlarged spleen is vulnerable to rupture with life-threatening haemoperitoneum; absent restraining peritoneal ligaments can also allow a "wandering"/mobile spleen.
- ▶ **Splenic trauma:** because the segmental arterial supply is end-arterial, **partial/subtotal splenectomy** can be performed along avascular planes between segments, preserving splenic tissue supplied by the short gastric or left gastroepiploic vessels; conservative management and laparoscopic approaches now often avoid total splenectomy.
- ▶ **Total splenectomy:** intraoperative risk of injury to pancreas, stomach or colon (close hilar relations); early complications include left basal atelectasis, pleural effusion, subphrenic abscess, and transient leukocytosis/thrombocytosis with thrombotic risk. Longer term there is an increased, occasionally fatal, risk of infection, especially with encapsulated organisms — the rationale for pre-splenectomy pneumococcal/meningococcal/Hib vaccination and for autotransplantation of splenic fragments ($\geq 20\%$ of splenic mass, with portal venous drainage) to preserve function when the whole organ cannot be salvaged.
- ▶ **Loss of erythrocyte "quality control":** after splenectomy, the number of morphologically abnormal erythrocytes (nuclear/inclusion remnants) in the circulation rises because the red-pulp filtering mechanism is lost; most such cells are then removed instead by macrophages in the sinusoids of bone marrow and liver.
- ▶ **Fetal haemopoiesis:** the spleen is a major haemopoietic organ in fetal life and can resume this role postnatally in certain pathological (myeloproliferative) states.

High-yield exam pointers

- ▶ Spleen = largest lymphoid organ; only secondary lymphoid organ filtering **blood** (not lymph); two pulps — white (lymphoid) and red (vascular).
- ▶ Capsule ~1.5 mm, collagen + some elastin, minimal smooth muscle; trabeculae carry vessels/nerves; reticular (type III collagen) stroma throughout.
- ▶ White pulp (5–20%): PALS = T cells around central arteriole (arteriole central); follicles = B cells with germinal centres (arteriole eccentric).
- ▶ Marginal zone = interface zone, site of blood entry and lymphocyte trafficking; peri-arteriolar macrophage sheath here.
- ▶ Red pulp (up to 90%): splenic cords (of Billroth, macrophage-rich) + venous sinusoids lined by discontinuous "stave" cells with intercellular slits.
- ▶ Human splenic circulation is dominantly the **open** type — deformability testing of RBCs occurs as they squeeze through stave-cell slits; effete RBCs are trapped and destroyed by cord macrophages, iron recycled.
- ▶ Functions: blood filtration/removal of aged RBCs and particulate matter, iron/haem recycling, immune surveillance and antibody production, fetal (and reactivated pathological) haemopoiesis.
- ▶ Applied: accessory spleen ~10%; thin capsule → rupture risk; segmental vascular supply permits partial splenectomy; post-splenectomy sepsis risk from encapsulated organisms.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Brijesh Kumar Histology Text & Atlas 3e; diFiore's Atlas of Histology (Eroschenko).



Structure of lymph node

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction — Lymph nodes are small, encapsulated, bean- or oval-shaped secondary (peripheral) lymphoid organs sited along the course of lymphatic vessels, through which afferent lymph is filtered before returning to the venous blood. Each node has a connective-tissue capsule and a reticular framework that is compartmentalised, but not epithelially separated, into an outer **cortex**, a **paracortex**, and an inner **medulla**; the framework is populated by lymphocytes, macrophages and dendritic cells and permeated by a system of lymph-conducting sinuses. Functionally, the node is the principal site of antigen presentation, lymphocyte activation/proliferation and antibody production.

General features

- ▶ **Shape and size:** small, oval or kidney (bean)-shaped bodies, usually **0.1–2.5 cm** long, lying along lymphatic vessels; an average node measures roughly 10 mm × 2.5 cm.
- ▶ **Number and distribution:** the adult body contains up to **~450 lymph nodes** — about 60–70 in the head and neck, 100 in the thorax, and as many as 250 in the abdomen and pelvis. They are

especially numerous in the neck, mediastinum, posterior abdominal wall, abdominal mesenteries, pelvis, and the proximal limbs (axillary and inguinal groups).

- ▶ **Hilum:** a slight indentation on one (concave) side through which the **artery and vein enter/leave** and the single **efferent lymphatic vessel** leaves.
- ▶ **Afferent lymphatics:** several enter the node around the periphery of the convex surface.
- ▶ Each group of nodes has a defined territory of drainage — the basis of regional lymph-node examination and of sentinel-node biopsy in oncology.

Capsule, trabeculae and reticular framework

- ▶ **Capsule:** a thin coat of collagen fibres, with some elastic fibres and occasional smooth muscle, that surrounds the whole node.
- ▶ **Trabeculae:** septa that extend inward from the capsule (and from the hilar fibrous mass), carrying blood vessels and dividing the node incompletely into lobules.
- ▶ **Subcapsular (marginal) sinus:** a clear space immediately beneath the capsule that receives afferent lymph.
- ▶ **Reticular framework (stroma):** a delicate meshwork of reticular fibres and associated **reticular cells** (fibroblastic reticular cells) fills the remaining space; associated with it are **macrophages**, **interdigitating dendritic cells**, and, within the follicles, **follicular dendritic cells (FDCs)**. Collagen fibres of the conduit system, ensheathed by these fibroblastic reticular cells, carry small lymph-borne molecules rapidly deep into the node.

Cortex and paracortex

The node shows three functional (not physically separate) compartments — outer cortex, paracortex, and medulla.

Zone	Location	Principal cells	Key feature
Superficial (nodular) cortex	Outer cortex, deep to subcapsular sinus	B lymphocytes; follicular dendritic cells	Contains lymphoid follicles/nodules ; absent over the hilum
Paracortex (deep cortex)	Between nodular cortex and medulla	T lymphocytes (CD4 and CD8), interdigitating dendritic cells	No follicles; site of high endothelial venules (HEVs) ; also called the <i>thymus-dependent zone</i>
Medulla	Central, adjacent to hilum	B cells, plasma cells, macrophages	Medullary cords separated by medullary sinuses

- ▶ **Lymphoid follicles (nodules):** rounded aggregates within the cortex.
 - **Primary follicles** — aggregates of small, resting (naïve) B cells, uniformly and densely stained, with no germinal centre.
 - **Secondary follicles** — develop after antigenic stimulation; each has a pale-staining **germinal centre** of large, rapidly dividing, antigen-activated B lymphoblasts, surrounded by a **mantle zone (corona)** of small quiescent B cells with condensed, heterochromatic nuclei.

- The germinal centre provides the microenvironment for **affinity maturation** of the antibody response; its B cells give rise to memory B cells (which rejoin the recirculating pool) and to antibody-secreting plasma cells (which mature in the medulla or in peripheral tissues).
- ▶ **Paracortex:** lacks nodules; populated mainly by T cells and interdigitating dendritic cells (including Langerhans cells that have migrated in via afferent lymphatics); it enlarges markedly during T-cell-mediated (cellular) immune responses.

Medulla

- ▶ **Medullary cords:** branching, anastomosing cords of lymphoid tissue extending from the paracortex, containing B lymphocytes, plasma cells, macrophages and dendritic cells within the reticular framework.
- ▶ **Medullary sinuses:** dilated, endothelium-lined spaces that separate the cords; their lumen is traversed by reticular-cell processes that act as a final lymph filter and is rich in macrophages (increased further in an infected/draining node).
- ▶ Cortex is absent over the hilum, which is occupied by dense fibrous tissue and the entering/leaving vessels.

Sinus system and circulation of lymph

- ▶ Afferent lymphatics → **subcapsular sinus** → **radial cortical (trabecular) sinuses** running through the cortex → convergence to form **medullary sinuses** → one (occasionally more) **efferent lymphatic vessel** leaving at the hilum.
- ▶ Sinuses are lined by an endothelium whose walls permit free passage of lymphocytes and macrophages, and their lumen is bridged by reticular-cell processes.
- ▶ As lymph percolates through the sinus meshwork it comes into intimate contact with macrophages, which remove bacteria and particulate matter — the essential **filtering function** of the node.
- ▶ Valves within the afferent/efferent lymphatics ensure unidirectional lymph flow.

Blood supply and high endothelial venules

- ▶ Arteries enter at the hilum, traverse the medulla giving off branches, and form dense arteriolar/capillary arcades in the cortex that drain into venules.
- ▶ **Post-capillary venules of the paracortex are lined by unusually tall, cuboidal endothelium — high endothelial venules (HEVs).** Their endothelial cells bear surface glycoproteins/receptors recognised by circulating lymphocytes.
- ▶ HEVs are the principal portal (about 90%) by which circulating **B and T lymphocytes leave the blood** and enter the node parenchyma, recirculating lymphocytes that subsequently leave again via the efferent lymphatic.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 11.5 — Structure of a lymph node. (Vishram Singh — General Anatomy — 4e, p.490)

Label a longitudinal schematic section with:

- ▶ Fibrous capsule
- ▶ Trabecula
- ▶ Afferent lymph vessel
- ▶ Subcapsular sinus
- ▶ Lymph nodule (with germinal centre)
- ▶ Outer cortex (B-cell area)
- ▶ Inner cortex / paracortex (thymus-dependent, T-cell area)
- ▶ Anastomosing medullary cords
- ▶ Medullary sinuses (draining toward hilum)
- ▶ Efferent lymph vessel (at hilum)

Applied / Clinical anatomy

- ▶ **Lymphadenitis:** inflammation/enlargement of a node draining an infected area (e.g. reactive follicular hyperplasia with enlarged, prominent germinal centres); tender, palpable node on clinical examination.
- ▶ **Lymphangitis:** inflammation of the afferent lymphatics (commonly β -haemolytic streptococci/staphylococci), seen as painful red streaks in the skin; may become chronic (e.g. tuberculosis).
- ▶ **Lymphoedema:** swelling from lymph accumulation — primary (idiopathic) or secondary (malignant infiltration of nodes, post-irradiation fibrosis, or filarial obstruction producing elephantiasis).
- ▶ **Chylous leaks:** rupture of dilated lymphatics can release chyle into the peritoneum, pleura, pericardium or urinary tract (chyloperitoneum/chylothorax/chylopericardium/chyluria).
- ▶ **Nodal metastasis and staging:** carcinoma cells reach nodes via afferent lymphatics; the node enlarges and its architecture may be effaced — histological/FNAC examination of regional and **sentinel lymph nodes** is central to cancer staging and guides surgical clearance.
- ▶ **Fine needle aspiration cytology (FNAC):** the accessibility of superficial nodes (cervical, axillary, inguinal) makes FNAC a quick, safe first investigation for lymphadenopathy.
- ▶ **Lymphoma:** malignant proliferation within follicles/paracortex can obliterate the normal cortex–medulla architecture, converting the node into a diffusely enlarged mass (lymphadenopathy).

High-yield exam pointers

- ▶ Node has 3 **functional zones**, not epithelially separated: cortex (B cells, follicles), paracortex (T cells, HEVs), medulla (cords + sinuses).
- ▶ **Primary follicle** = no germinal centre (naïve B cells); **secondary follicle** = germinal centre + mantle zone (antigen-stimulated).

- ▶ **Paracortex = thymus-dependent zone**; site of HEVs — 90% of lymphocyte entry from blood.
- ▶ Lymph pathway: afferent vessel → subcapsular sinus → cortical sinus → medullary sinus → efferent vessel (single, at hilum).
- ▶ Reticular framework = reticular fibres + fibroblastic reticular cells + macrophages + dendritic/follicular dendritic cells.
- ▶ ~450 nodes in the body; largest numbers in abdomen/pelvis (~250), then thorax (~100), then head & neck (60–70).
- ▶ Cortex is **absent at the hilum**.
- ▶ Node is a **secondary lymphoid organ** — site of antigen presentation and lymphocyte proliferation, not lymphocyte origin.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Vishram Singh — General Anatomy 4e (figure).



Sub endothelium of a blood vessel

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction — The wall of every blood vessel (except a capillary) is built of three concentric tunics — intima, media and adventitia. The **tunica intima**, the innermost tunic, is itself made of an endothelium resting on a basal lamina, an internal elastic lamina (in arteries), and, sandwiched between endothelium and internal elastic lamina, a layer of loose connective tissue called the **subendothelial connective tissue** (also termed the **lamina propria** of the vessel). It is a thin but functionally important layer — it cushions the endothelium, contributes to vascular elasticity and repair, and is the tissue plane in which several major vascular diseases (atherosclerosis, venous valve incompetence) actually arise. Functionally, the endothelium and its basal lamina form the selective, antithrombogenic barrier between blood and vessel wall, whereas the subendothelial connective tissue immediately beneath it is the structural, reparative and haemostatic-reserve component of the intima — the two together constitute the tunica intima proper.

1. Location and basic composition

- ▶ Lies immediately deep to the endothelium and its basal lamina, and superficial to the internal elastic lamina (IEL) — i.e., it is the middle component of the tunica intima.
- ▶ Composed of a typical **fibrocollagenous extracellular matrix** — type I collagen fibrils and elastic fibres — sparsely populated by cells.
- ▶ Cellular constituents:
 - A few **fibroblasts**.

- Occasional **smooth-muscle-like cells**, termed **myointimal cells**, which are contractile, mesenchymal in character, and synthesise collagen, elastin and other ECM components of this layer.
- ▶ Thickness is **variable and vessel-dependent** — well developed in large elastic arteries, thin in muscular arteries, and **absent in the smallest vessels** (arterioles, capillaries, venules), where the endothelium is instead supported directly by **pericytes**.
- ▶ **Pericytes**, which substitute for the subendothelial layer at this level, are perivascular mesenchymal cells that partly encircle the endothelium, share and fuse with its basal lamina, and possess a contractile actin–myosin–tropomyosin cytoskeleton; they help regulate microvascular blood flow, contribute to the blood–brain barrier in the CNS, and can proliferate and differentiate into smooth muscle during vascular repair/remodelling.

2. Relationship to the internal elastic lamina

- ▶ In arteries, the subendothelial layer is bounded externally by the **internal elastic lamina** — a fenestrated sheet of elastin marking the intima–media junction.
- ▶ In the largest elastic arteries (e.g. aorta), the IEL is **not sharply distinct** from the elastic lamellae of the adjacent tunica media, because the subendothelial layer itself already contains abundant elastic fibres.
- ▶ In muscular (medium-sized) arteries the IEL is a **thin, well-defined, sometimes duplicated** layer, often thrown into wavy folds by medial smooth-muscle tone.
- ▶ In arterioles the IEL is thin and **gradually disappears**, and in capillaries and venules it is absent altogether.

3. Variation of the subendothelial layer across vessel types

Vessel type	Subendothelial connective tissue	Internal elastic lamina
Elastic (large) artery — e.g. aorta	Well developed; elastic fibres + type I collagen + fibroblasts + myointimal cells	Present, but merges with elastic lamellae of media
Muscular (medium) artery — e.g. brachial, radial	Thin layer	Prominent, distinct, sometimes duplicated
Arteriole	Very thin, then absent	Thin, gradually disappears
Capillary	Absent — only endothelium + basal lamina (pericytes substitute)	Absent
Vein (small–medium)	Thin but variable; occasional smooth-muscle cells	Usually absent
Large vein (e.g. vena cava)	Present; forms the core of venous valves	Present only in the largest veins
High endothelial venule (postcapillary venule of lymphoid tissue)	Replaced by pericytes + a small amount of connective tissue; endothelium is cuboidal	Absent

4. Functions of the subendothelial connective tissue

- ▶ **Structural/mechanical support** to the overlying endothelium and contribution to the overall distensibility and elastic recoil of the vessel wall through its collagen and elastin content.
- ▶ **Haemostatic reservoir** — endothelial **von Willebrand factor (vWF)**, a large multimeric glycoprotein stored in rod-shaped endothelial **Weibel–Palade bodies** ($\sim 0.2 \times 2\text{--}3\ \mu\text{m}$) along with P-selectin, is released and concentrates by binding collagen within the subendothelial layer. When the overlying endothelium is breached, the exposed subendothelial collagen–vWF complex binds platelet glycoprotein Ib receptors, mediating **platelet adhesion**, especially efficiently under high-flow/high-shear-stress conditions — the first step of haemostasis at a site of vascular injury.
- ▶ **Site of vascular smooth-muscle/myointimal cell activity** — these cells synthesise the ECM of the layer and can migrate/proliferate to help repair damaged endothelium; with age they progressively accumulate lipid, which in its extreme form is the substrate for **atheroma formation**.
- ▶ **Forms the core of venous valves** — in large veins, a fold of intima projecting into the lumen (the valve cusp) is essentially subendothelial connective tissue covered by endothelium on both surfaces.
- ▶ Contributes to the **age-related, physiological thickening of the intima**, which is more marked in the distal than the proximal aorta.

5. Cellular identity of myointimal cells

- ▶ Myointimal cells are **mesenchymal-derived, smooth-muscle-like cells** resident in (or capable of migrating into) the subendothelial layer, distinct in location from the concentrically arranged contractile smooth muscle cells of the tunica media proper, though similar in staining and ultrastructural character.
- ▶ They are multifunctional — synthesising collagen, elastin and ground substance of the subendothelial ECM, and retaining the capacity to **migrate and proliferate to re-endothelialise/repair a site of endothelial denudation**.
- ▶ With advancing age (and accelerated by endothelial injury), these cells undergo **fatty change**, accumulating lipid within their cytoplasm; aggregates of lipid-laden myointimal cells are the earliest morphological step towards an atheromatous plaque.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled transverse-section diagram of a muscular/elastic artery wall, with these exact labels (lumen to periphery):

- ▶ **Lumen**
- ▶ **Endothelium** (simple squamous, resting on basal lamina)
- ▶ **Basal lamina**
- ▶ **Subendothelial connective tissue** — show elastic fibres, collagen fibrils, a fibroblast, and a myointimal (smooth-muscle-like) cell
- ▶ **Internal elastic lamina** (fenestrated)
- ▶ **Tunica media** — concentric smooth muscle + elastic lamellae
- ▶ **External elastic lamina**
- ▶ **Tunica adventitia** — collagen fibres, vasa vasorum, nervi vasorum

□ Fig 11.9 — Elastic artery. (*Junqueira's Basic Histology — Text & Atlas — 17e, p.554*)

Applied / Clinical anatomy

- ▶ **Atherosclerosis:** the atheromatous plaque develops primarily **within the tunica intima of large elastic and muscular arteries**, following endothelial injury/dysfunction; lipid-laden myointimal cells of the subendothelial layer are central to plaque formation, and this is the principal risk factor for myocardial infarction and stroke.
- ▶ **Age-related intimal thickening:** myointimal cell proliferation and lipid accumulation in the subendothelial layer cause progressive intimal thickening with age, more pronounced in the distal than the proximal aorta.
- ▶ **Von Willebrand disease:** hereditary deficiency or dysfunction of vWF impairs platelet adhesion to exposed subendothelial collagen after vascular injury, producing a bleeding tendency — the physiological basis for this is the normal vWF–subendothelial collagen interaction described above.
- ▶ **Varicose veins:** incompetence of venous valves — whose cusps are folds of subendothelial connective tissue covered by endothelium — with venous stasis in the lower limb (e.g. long saphenous vein and tributaries), commoner in women and aggravated by pregnancy (uterine compression of the iliac veins).
- ▶ **Vessel identification in histology practicals:** presence/absence and thickness of the subendothelial layer, together with the prominence of the IEL, is a key criterion used to distinguish elastic artery vs muscular artery vs vein vs capillary in a slide.

High-yield exam pointers

- ▶ Subendothelial connective tissue = middle component of the tunica intima, between endothelium/basal lamina and the internal elastic lamina.
- ▶ Also called the **lamina propria** of the vessel wall.
- ▶ Composed of fibrocollagenous ECM (collagen I, elastic fibres) + a few fibroblasts + smooth-muscle-like **myointimal cells**.

- ▶ **Absent in the smallest vessels** (arterioles, capillaries) — pericytes substitute for structural support there.
- ▶ **Best developed in large elastic arteries**, thin in muscular arteries and veins.
- ▶ Endothelial **vWF concentrates here**; exposure after injury triggers platelet adhesion (haemostasis).
- ▶ Myointimal cells accumulate lipid with age → substrate for **atherosclerosis**.
- ▶ Forms the **core of venous valve cusps**.
- ▶ Vascular smooth-muscle/myointimal proliferation here underlies **intimal hyperplasia** and age-related intimal thickening.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e.



Systemic histology - gastrointestinal & associated glands



Cells of Gastric Mucosa

Asked in: Paper III 16-Nov-2019 · Paper III Nov 2018 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — The gastric mucosa is lined throughout by a simple columnar, mucus-secreting surface epithelium that invaginates to form millions of **gastric pits** (60–100/mm², ~70 µm wide, ~0.2 mm deep). Several tubular **gastric glands** open into the base of each pit; their type and cellular composition vary regionally into **cardiac**, **fundic (principal/gastric)** and **pyloric** glands. The fundic gland — the most highly specialised and the commonest exam focus — is built of **five distinct cell types**: chief, parietal, mucous neck, undifferentiated/stem, and neuroendocrine cells, together with the surface mucous cells lining the pit itself. Each cell type has a characteristic light-microscopic appearance, ultrastructure and secretory product that together produce ~2 L/day of gastric juice.

Regional distribution of glands and cells

Region	Gastric pits	Gland type	Dominant cells
Cardia (narrow zone at oesophagogastric junction)	Shallow	Cardiac glands — simple tubular or compound tubulo-alveolar	Mucus-secreting cells predominate; parietal/chief cells sparse

Region	Gastric pits	Gland type	Dominant cells
Fundus and body (bulk of stomach)	Shallow, occupy $\leq 1/4$ of mucosal thickness	Fundic (principal/gastric) glands — simple, branched tubular, 3–7 opening per pit	Chief, parietal, mucous neck, stem and neuroendocrine cells
Pyloric antrum/pylorus	Deep, occupy $\sim 2/3$ of mucosal thickness	Pyloric glands — coiled, simple/branched tubular, 2–3 opening per pit	Mucus-secreting cells predominate; numerous G cells ; occasional parietal cells

Cell types of the fundic (principal) gland

The gland is divided, from the pit downward, into an **isthmus** (junction with the pit base), a **neck**, and a **base/fundus**.

Cell type	Location in gland	Light-microscopic features	Ultrastructure	Secretory product	Function
Surface mucous cells	Line the entire luminal surface and the gastric pits	Simple columnar; apical mucin granules (often washed out in processing $\rightarrow$ vacuolated/"empty" look); basal, flattened/oval nucleus	Basal rER giving faint basophilia; supranuclear Golgi	Thick, viscous, alkaline "visible" (cloudy) mucus, rich in HCO_3^- and K^+	Forms the mucus–bicarbonate barrier protecting the epithelium from acid and pepsin; renewed every 3–5 days
Mucous neck cells	Neck of gland, interspersed among parietal cells	Shorter, less columnar than surface cells; spherical nucleus pushed basally; less mucinogen	Apical secretory (mucinogen) granules	Soluble, less alkaline (near-neutral/acidic, sialomucin) mucus, chemically distinct from surface mucus	Considered immature precursors of surface mucous cells; release is vagally induced (not secreted at rest); lifespan ~ 1 week

Cell type	Location in gland	Light-microscopic features	Ultrastructure	Secretory product	Function
Parietal (oxyntic) cells	Most numerous in the upper half/neck, extend as far as the isthmus and into the base; bulge against the basement membrane giving a "beaded" appearance	Large, round/pyramidal/triangular, often binucleate; central nucleus; strongly eosinophilic cytoplasm	Deep apical invaginations forming intracellular canaliculi lined by microvilli; abundant mitochondria; a tubulovesicular membrane system carrying reserve H^+/K^+ -ATPase pumps that fuses with the canalicular membrane on stimulation	Hydrochloric acid and intrinsic factor (Castle's factor, a glycoprotein)	HCl (pH 1–2) activates pepsinogen $\rightarrow$ pepsin and is bactericidal; intrinsic factor binds vitamin B_{12} for ileal absorption; stimulated via gastrin, histamine (H_2) and acetylcholine (M_3) receptors ; longest cell lifespan, ~150–200 days
Chief (peptic/zymogenic) cells	Most numerous in the base/deeper part of the gland	Cuboidal to low columnar; basal, round nucleus; basophilic cytoplasm (apical eosinophilia when granules preserved)	Abundant basal rough endoplasmic reticulum, prominent Golgi, apical zymogen granules	Pepsinogen and a weak gastric lipase	Pepsinogen is converted to active pepsin by HCl at pH < 5; pepsin hydrolyses proteins into peptides; lifespan ~60–90 days
Neuroendocrine (enteroendocrine) cells	Base of gland, between chief cells and the basal lamina; mostly "closed" type not reaching the lumen	Pale/flattened, pyramidal cells with basally placed granular cytoplasm; formerly called argentaffin/argyrophil cells for their silver-staining granules	Basal, membrane-bound dense-core secretory granules	Site- and type-specific hormones — G cells (gastrin, chiefly in the pyloric antrum), D cells (somatostatin), ECL cells (histamine), enterochromaffin (EC) cells (serotonin)	Part of the diffuse neuroendocrine system (DNES); regulate acid secretion, motility and gut blood flow; lifespan ~60–90 days

Cell type	Location in gland	Light-microscopic features	Ultrastructure	Secretory product	Function
Undifferentiated (stem) cells	Isthmus of the gland and the base of the gastric pit	Columnar cells bearing a few short apical microvilli	Relatively undifferentiated cytoplasm	None (progenitor pool)	Mitotically active; progeny migrating upward become surface mucous cells, progeny migrating downward differentiate into mucous neck, parietal, chief and neuroendocrine cells; proliferate briskly during mucosal repair (e.g. after a gastric ulcer)

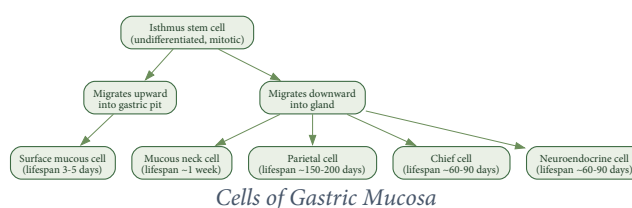
□ Fig 15-17 — Gastric glands: throughout the fundus and body the gastric pits lead into gastric glands showing surface mucous, mucous neck, parietal and chief cells (Junqueira's Basic Histology 17e, p.762)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a longitudinal section of one gastric pit + fundic gland, labelling from lumen to muscularis mucosae:

- ▶ **Gastric pit** (surface mucous cells lining pit lumen)
- ▶ **Isthmus** — undifferentiated stem cells (dividing cells)
- ▶ **Neck** — mucous neck cells (with interspersed parietal cells)
- ▶ **Base/fundus** — chief cells (majority), parietal cells, neuroendocrine cells
- ▶ **Basal lamina and surrounding lamina propria**
- ▶ Arrows showing bidirectional migration of stem-cell progeny (upward → surface mucous cell; downward → neck/parietal/chief/neuroendocrine cells)

Stem-cell lineage of the fundic gland epithelium



Cardiac and pyloric glands — cellular composition

- ▶ **Cardiac glands:** confined to a small zone at the cardiac orifice; simple tubular or compound tubulo-alveolar; **mucus-secreting cells predominate**, with sparse parietal and chief cells; occasional neuroendocrine cells.
- ▶ **Pyloric glands:** coiled, simple/branched tubular glands emptying by 2–3 short ducts into deep gastric pits; lined mainly by **mucus-secreting cells resembling surface mucous cells**; parietal and chief cells scarce (parietal cells are, however, always present in fetal and postnatal pyloric mucosa); rich in **neuroendocrine G cells** secreting gastrin, stimulated by mechanical distension/vagal activity.

HCl synthesis in the parietal cell — key steps

- ▶ CO₂ diffuses into the cell and combines with H₂O (catalysed by **carbonic anhydrase**) to form H₂CO₃ → dissociates to H⁺ and HCO₃⁻.
- ▶ H⁺ is pumped into the canalicular lumen by the apical **H⁺/K⁺-ATPase** (the target of proton-pump inhibitors) in exchange for K⁺.
- ▶ Cl⁻ enters the canaliculus through Cl⁻ channels; H⁺ + Cl⁻ → HCl.
- ▶ HCO₃⁻ leaves across the basolateral membrane in exchange for Cl⁻ (the "alkaline tide").

Applied / Clinical anatomy

- ▶ **Pernicious anaemia:** autoimmune destruction of parietal cells (or antibodies against intrinsic factor/parietal-cell H⁺/K⁺-ATPase) → achlorhydria and loss of intrinsic factor → megaloblastic anaemia from vitamin B₁₂ malabsorption.
- ▶ **Zollinger–Ellison syndrome:** a gastrin-secreting tumour (gastrinoma) of G cells, usually in the duodenum or pancreatic islets, drives relentless parietal-cell HCl secretion → severe, often multiple, peptic ulcers.
- ▶ **Peptic ulcer disease:** chiefly caused by *Helicobacter pylori*, whose urease neutralises local acid, allowing mucosal colonisation and chronic damage to surface mucous cells; healed/ulcerated mucosa often has reduced intrinsic-factor output.
- ▶ **Post-gastrectomy vitamin B₁₂ deficiency:** partial/total gastrectomy removes parietal-cell mass, necessitating lifelong B₁₂ replacement.
- ▶ **Pharmacology correlate:** H₂-receptor antagonists (ranitidine, cimetidine) and proton-pump inhibitors (omeprazole) both suppress parietal-cell acid output; PPIs act directly on the H⁺/K⁺-ATPase and spare intrinsic-factor secretion.
- ▶ **Mucosal repair:** the isthmus stem-cell niche is the basis for regeneration of ulcerated or gastrectomy-margin mucosa.
- ▶ **Carcinoid (neuroendocrine) tumours:** arise from enterochromaffin/ECL cells; can cause carcinoid syndrome (flushing, diarrhoea) via serotonin/histamine release.

High-yield exam pointers

- ▶ Five cell types of the fundic gland: **chief, parietal, mucous neck, stem, neuroendocrine** — plus surface mucous cells lining the pit.

- ▶ Parietal cells secrete **HCl + intrinsic factor**; identified by intracellular canaliculi and tubulovesicular system on EM.
- ▶ Chief cells secrete **pepsinogen** (+ weak lipase); rich basal rER and apical zymogen granules.
- ▶ **G cells** (gastrin) are concentrated in the **pyloric antrum**; **D cells** secrete somatostatin (inhibitory); **ECL cells** secrete histamine.
- ▶ Isthmus = **stem-cell niche**; bidirectional migration gives surface mucous cells upward and all other gland cells downward.
- ▶ Turnover: surface mucous cells 3–5 days < mucous neck cells ~1 week < chief/neuroendocrine cells 60–90 days < parietal cells 150–200 days (longest-lived).
- ▶ Cardiac and pyloric glands are mucus-dominant with sparse parietal/chief cells; principal (fundic) glands alone have the full complement of five cell types.
- ▶ Loss of parietal cells → achlorhydria + pernicious anaemia; gastrinoma of G cells → Zollinger–Ellison syndrome.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh's Textbook of Human Histology 9e; Sontakke's Textbook of Human Histology 2e; Gray's Anatomy (Standring) 41e.



Histology of Liver

Asked in: Paper III 11-May-2022 · Paper III Nov 2017 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The liver is the largest gland in the body, an epithelial–mesenchymal outgrowth of the caudal foregut that remains connected to the gut via the biliary tree. It is functionally a mixed exocrine (bile-secreting) and endocrine (plasma-protein-, glucose- and lipid-regulating) gland. Microscopically it is built of anastomosing one-cell-thick plates of hepatocytes, perfused by a unique dual (portal venous + arterial) blood supply flowing through fenestrated sinusoids, and drained by a centrally placed venule. Three complementary schemes — the classic lobule, the portal lobule, and the liver acinus — are used to describe its microarchitecture.

Capsule and stroma

- ▶ **Glisson's capsule**: thin (50–100 µm) connective-tissue capsule covering the liver, itself covered by visceral peritoneum (serosa) except at the bare area.
- ▶ Capsule sends **septa/trabeculae** into the parenchyma; these carry branches of the **hepatic artery, hepatic portal vein and bile ductules/ducts** — together called **portal tracts (portal canals)**.
- ▶ The combination of an arteriole, a portal venule and a bile duct within one portal tract is the **portal triad**.
- ▶ Stroma is scanty in the human liver (unlike species such as the pig, where thick interlobular septa clearly delineate lobules); hence lobular boundaries are not sharply visible in human histological sections.

Classic hepatic lobule

- ▶ **Structural unit** of the liver — a roughly **hexagonal** mass of parenchyma, about **1–2 mm** across.
- ▶ **Central vein** (terminal hepatic venule) occupies the long axis/centre of the hexagon; **portal triads** lie at the six vertices.
- ▶ **Hepatocytes** are arranged as **plates/laminae**, one cell thick in the adult (two cells thick up to about 7 years of age, and again several cells thick with multinucleated cells during regeneration), radiating outward from the central vein.
- ▶ Plates are separated by **hepatic sinusoids**, which anastomose freely between plates and converge on the central vein — blood flow within the lobule is therefore **centripetal**.
- ▶ At the lobule periphery, hepatocyte plates fuse to form a continuous **limiting plate** abutting each portal tract; a narrow **periportal space (space of Mall)**, containing the smallest lymphatic radicles, separates the limiting plate from the portal connective tissue.
- ▶ Because there is little interlobular septum in humans, the classic lobule is best regarded as a venous drainage territory around one central vein rather than a true anatomically-bounded structural unit.

Portal lobule and liver acinus — the three microarchitectural concepts

Concept	Shape / axis	Periphery	Functional emphasis
Classic (hepatic) lobule	Hexagonal; central vein at centre, portal triads at corners	Portal triads at 6 corners	Endocrine function — plasma constituents pass from hepatocyte to blood flowing toward the central vein
Portal lobule	Triangular; portal triad (bile duct) at centre, three central veins at corners (drawn joining the 3 nearest central veins around one portal triad)	Central veins at corners	Exocrine function — bile drainage toward the interlobular duct at the axis
Liver (portal) acinus	Lozenge/diamond-shaped; short axis = terminal portal-triad branches, long axis joins the 2 nearest central veins	Central veins at the 2 poles	Best correlates blood perfusion with metabolic activity and pathology — divided into 3 concentric zones of oxygenation

▶ Zonation of the acinus:

- **Zone 1 (periportal)** — nearest the afferent vessels; best oxygenated/nourished; first to receive (and show damage from) toxins and bile stasis; last to undergo ischaemic necrosis; first to regenerate.
- **Zone 2** — intermediate, transitional metabolic profile.

- **Zone 3 (pericentral/perivenular)** — farthest from afferent supply, least oxygenated; first to show **ischaemic (centrilobular) necrosis**, fatty change, and the earliest site of **hepatic fibrosis**; last to respond to toxins/bile stasis.

Blood supply and the hepatic sinusoid

- ▶ **Dual blood supply:** the **hepatic portal vein** (venous, nutrient-rich, providing roughly 70% of hepatic blood/nutrition) and the **hepatic artery** (oxygen-rich, providing the remaining ~30%); both enter at the **porta hepatis** and both perfuse the sinusoids, so sinusoidal blood is a mixture of venous and arterial blood.
- ▶ Preterminal arterioles empty mostly via a fine peri-portal capillary plexus into the portal-venous inflow to sinusoids, with a small direct arteriolar contribution to sinusoids.
- ▶ **Sinusoidal endothelium** is thin, discontinuous and **fenestrated** (fenestrae grouped in clusters, mean diameter ~100 nm) and **lacks a continuous basal lamina** — it behaves as a dynamic blood filter, allowing free passage of plasma (but not cells) to the hepatocyte surface.
- ▶ **Space of Disse (perisinusoidal space):** a narrow (0.2–0.5 μm , widening in hypoxia) gap between sinusoidal lining and hepatocyte surface; contains hepatocyte microvilli, interstitial fluid, hepatic stellate cells and fine (mostly type III) collagen fibres — the site of exchange between plasma and hepatocyte, and the origin of hepatic lymph.
- ▶ Blood drains centripetally, sinusoid → **central (terminal hepatic) vein** → sublobular/interlobular veins → **hepatic veins** → inferior vena cava.

Hepatocyte — structure and surface specializations

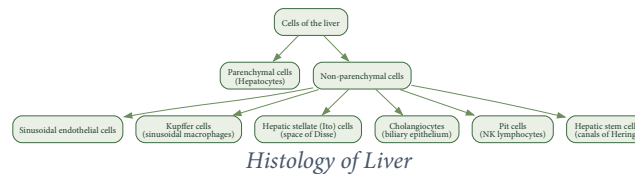
- ▶ Polyhedral parenchymal cell, 5–12 sides, 20–30 μm across; makes up ~80% of liver volume and ~60% of its cell population.
- ▶ **Nucleus:** round, euchromatic, often binucleate or polyploid/tetraploid.
- ▶ **Cytoplasm:** abundant rough and smooth endoplasmic reticulum, numerous mitochondria, well-developed Golgi, lysosomes, peroxisomes, glycogen granules and lipid droplets, and storage vacuoles for ferritin/haemosiderin — reflecting high synthetic, detoxifying and storage activity.
- ▶ Three cell-surface domains (each sealed from the others by junctional complexes):

Surface	Approx. % of hepatocyte surface	Features	Function
Sinusoidal surface	~70%	Numerous microvilli (~0.5 μm) projecting into space of Disse; coated pits	Exchange/transfer of substances with plasma
Canalicular surface	~15%	Paired grooves of adjacent hepatocytes forming the bile canaliculus , sealed by tight junctions	Secretion of bile; tight junctions form the blood–bile barrier

Surface	Approx. % of hepatocyte surface	Features	Function
Intercellular surface	~15%	Gap junctions and desmosomes with neighbouring hepatocytes	Cell-to-cell communication/adhesion

- **Functions of the hepatocyte** (as an exocrine + metabolic cell): carbohydrate/protein/lipid metabolism; synthesis of plasma proteins, fibrinogen, prothrombin; glucose/lipid regulation; storage of glycogen, lipid, vitamin A, iron; vitamin D processing and vitamin K utilisation; detoxification of drugs/alcohol; bilirubin conjugation and excretion in bile; hormone (thyroxine, GH, insulin, glucagon) modification/degradation; site of fetal haemopoiesis.

Non-parenchymal cells of the liver



Cell	Location	Origin	Key features / function
Sinusoidal endothelial cell	Lines sinusoid	—	Flat, fenestrated, no basal lamina; forms a dynamic filter
Kupffer cell	Attached to endothelial (luminal) surface of sinusoid	Bone-marrow monocyte	Resident macrophage of mononuclear phagocyte system; removes microbial/cellular debris and senile/damaged RBCs (fully takes over this role after splenectomy); secretes cytokines
Hepatic stellate cell (Ito cell)	Perisinusoidal space of Disse	Mesenchymal	Stores vitamin A (retinyl esters) in lipid droplets; secretes matrix components (type III collagen); on liver injury, activates into myofibroblasts producing collagen scar — the basis of hepatic fibrosis/cirrhosis (first seen in zone 3, around central veins)
Cholangiocyte	Lines bile ductules/ducts, canals of Hering	Biliary epithelium of foregut	Modifies bile; cuboidal to columnar

Cell	Location	Origin	Key features / function
Pit cell	Sinusoidal lumen	Bone marrow	Natural-killer lymphocyte; immune surveillance
Hepatic stem cell	Around canals of Hering	Fetal ductal plate	Undifferentiated, bipotential; can differentiate to hepatocyte or cholangiocyte lineage

Biliary canalicular system

- ▶ **Bile canaliculi** (~1 µm diameter): minute channels formed by apposed, grooved plasma membranes of adjacent hepatocytes, sealed by tight junctions; bile flow is therefore **centrifugal** — opposite to the direction of sinusoidal blood flow.
- ▶ Canaliculi drain into the **canals of Hering** (trough-shaped, lined by both hepatocytes and cholangiocytes, at the lobular periphery) → **bile ductules** (cholangiocyte-lined) → **interlobular bile ducts** within the portal triads → right/left hepatic ducts → common hepatic duct.

□ Fig 19.6 — Photomicrograph. Histology of liver (low magnification on left, portal triad at high magnification on right) (Sontakke — Textbook of Human Histology — 2e, p.262)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a hexagonal classic hepatic lobule with these labels:

- ▶ **Central vein** at the centre
- ▶ **Cords/plates of hepatocytes**, one cell thick, radiating from the central vein
- ▶ **Hepatic sinusoids** between plates, lined by endothelial cells and Kupffer cells
- ▶ **Portal triads** at the six corners, each showing: branch of **hepatic artery**, branch of **hepatic portal vein**, **interlobular bile duct**
- ▶ **Limiting plate** of hepatocytes bordering the portal tract, with the **space of Mall** between it and the portal connective tissue
- ▶ **Space of Disse** between hepatocyte surface and sinusoidal endothelium (inset, high magnification)
- ▶ **Bile canaliculi** between adjacent hepatocytes, draining toward the portal tract (arrow opposite to blood flow)
- ▶ Direction arrows: blood flow **centripetal** (periphery → central vein); bile flow **centrifugal** (centre → portal tract)

Applied / Clinical anatomy

- ▶ **Fatty liver disease (steatosis)**: reversible triglyceride accumulation in hepatocytes (SER-related), seen in alcoholism/obesity; may progress to steatohepatitis.

- ▶ **Congestive hepatopathy:** zone 3 (pericentral) hepatocytes are most hypoxia-sensitive — congestive cardiac failure/shock produces **centrilobular (zone 3) ischaemic necrosis**, best explained using the acinar model.
- ▶ **Hepatic fibrosis/cirrhosis:** activation of hepatic stellate (Ito) cells into collagen-producing myofibroblasts, beginning around zone 3/central veins, progressively destroys lobular architecture and normal blood flow.
- ▶ **Jaundice:** failure of hepatocyte SER glucuronosyl-transferase to conjugate bilirubin (e.g. neonatal hyperbilirubinaemia due to immature SER) causes unconjugated hyperbilirubinaemia; phototherapy converts bilirubin to a water-soluble photoisomer.
- ▶ **Blood–bile barrier:** tight junctions sealing the canalicular surface prevent bile reflux into plasma; breakdown of these junctions (e.g. in cholestasis) causes conjugated hyperbilirubinaemia.
- ▶ **Regeneration:** hepatocytes retain strong regenerative capacity via compensatory hyperplasia/hypertrophy (plates transiently several cells thick, with multinucleated cells) — the basis of living-donor liver transplantation and recovery after partial hepatectomy; hepatic stem cells near canals of Hering also contribute.
- ▶ **Liver biopsy interpretation** (toxic injury, viral hepatitis, cirrhosis grading) relies on recognising portal tracts, lobular architecture and zonal (acinar) distribution of damage.
- ▶ **Splenectomy:** Kupffer cells assume the entire burden of aged/damaged RBC removal previously shared with the spleen.

High-yield exam pointers

- ▶ Three ways to view liver microarchitecture: **classic lobule** (endocrine emphasis), **portal lobule** (exocrine/bile emphasis), **liver acinus** (metabolic zonation, best correlates with pathology).
- ▶ Portal triad = branch of hepatic artery + branch of portal vein + interlobular bile duct, all within a portal tract (an extension of Glisson's capsule).
- ▶ Blood flow is **centripetal** (periphery → central vein); bile flow is **centrifugal** (opposite direction).
- ▶ Hepatocyte surface: ~70% sinusoidal, ~15% canalicular, ~15% intercellular.
- ▶ Space of Disse (0.2–0.5 µm) — site of plasma–hepatocyte exchange and origin of hepatic lymph; contains stellate (Ito) cells.
- ▶ Sinusoidal endothelium is fenestrated (~100 nm fenestrae) with no basal lamina — a dynamic filter.
- ▶ Kupffer cells (sinusoidal macrophages, MPS) vs Ito/stellate cells (perisinusoidal, store vitamin A, fibrogenic when activated) — a frequently confused pair.
- ▶ Zone 3 (pericentral) hepatocytes are most vulnerable to hypoxia/ischaemia and earliest site of fibrosis; zone 1 (periportal) hepatocytes are most vulnerable to toxins/bile stasis and regenerate first.
- ▶ Dual blood supply: hepatic portal vein (~70% of flow, nutrient-rich) + hepatic artery (~30%, oxygen-rich) mix within the sinusoid.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e.



Histology of serous and mucous Salivary glands

Asked in: Paper III 02-Aug-2021 · Paper III 18-Jul-2020 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction Salivary glands are compound tubuloacinar (racemose) exocrine glands of the oral cavity that discharge saliva by merocrine (exocytotic) secretion. Three paired major glands — parotid, submandibular and sublingual — together with numerous minor glands scattered through the oral mucosa build their secretory units from two histologically distinct cell types: **serous cells**, which produce a thin, protein-rich watery secretion, and **mucous cells**, which produce a viscous, mucin-rich secretion. A gland (or acinus) made of only one cell type is **homocrine**; one containing both is **heterocrine/mixed**, with mucous tubules typically capped by a crescent of serous cells (**serous demilune**). The parotid is purely serous, the sublingual is predominantly mucous, and the submandibular is mixed (serous-predominant) — this triad is the classic slide-identification exercise in this topic.

1. General organisation of a salivary gland

- ▶ **Stroma:** a connective-tissue capsule sends septa into the gland, dividing it into lobes and lobules; blood vessels, lymphatics, nerves and the larger excretory ducts run in these septa.
- ▶ **Parenchyma** has two components: (i) the **secretory (end) part** — acini/tubules (the *portio terminalis*), and (ii) the **conducting (duct) part**.
- ▶ **Salivon** — the basic secretory-excretory unit of a salivary gland — comprises the **acinus + intercalated duct + striated duct** (draining finally into an excretory duct).
- ▶ A secretory unit with a single cell type is **homocrine** (e.g., parotid — all serous); one with both serous and mucous cells is **heterocrine** (submandibular, sublingual).

2. The serous acinus — structure and secretion

- ▶ Serous cells are arranged around a small central lumen as a rounded, "berry-shaped" **acinus**.
- ▶ Each cell is **pyramidal**, with a broad base and narrow apex, and a single **round nucleus placed basally** (pushed basal by apical secretory material).
- ▶ Cytoplasm shows classic **bipolar staining**: the base is **basophilic** (abundant rough endoplasmic reticulum for protein synthesis) and the apex is **eosinophilic**, packed with apical **zymogen/secretory granules** (protein-secreting cell morphology).
- ▶ Adjacent cells are joined apically by tight and adherent junctions, closing off the small acinar lumen.

- ▶ Secretory products: **α -amylase** (starch digestion), proline-rich antimicrobial proteins, and — from submandibular/sublingual serous cells — **lysozyme** (bacteriolytic); serous acinar cells also make the **secretory component** that carries IgA into saliva.
- ▶ Distribution: exclusively in the **parotid**; predominant (with demilunes) in the **submandibular**; a minority (as demilunes) in the **sublingual**.

3. The mucous acinus/tubule and serous demilunes

- ▶ Mucous cells are more **columnar** than serous cells and tend to form **tubules** rather than rounded acini; the tubular lumen is wider than a serous acinar lumen.
- ▶ The nucleus is **flattened, condensed and pressed against the base** of the cell.
- ▶ Cytoplasm is pale, foamy and poorly stained in routine H&E because it is filled with hydrophilic **mucinogen (mucus)** granules, which push the nucleus basally.
- ▶ **Serous demilune (crescent of Giannuzzi)**: a cap of serous cells at the blind end of a mixed (mucous) tubule; the serous component drains into the shared lumen through fine intercellular **canaliculi** that run between the mucous cells rather than directly into the main lumen.
- ▶ Exam-important nuance: the crescentic, sharply demarcated demilune seen in routine aldehyde-fixed sections is now regarded as **at least partly a fixation artifact** — the mucous cells swell disproportionately during conventional fixation and displace the serous cells to the periphery; tissue fixed rapidly (e.g., by immersion or in plastic-embedded sections) shows serous cells distributed more evenly around the acinus rather than as a discrete crescent.

4. Myoepithelial cells

- ▶ Small, flattened, stellate ("basket") cells that lie **within the basal lamina**, i.e., between the basal lamina and the acinar/ductal epithelial cells.
- ▶ They contain contractile **actin and myosin filaments** and extend branching processes around the secretory unit.
- ▶ Present around **acini and the proximal (intercalated) duct** segments.
- ▶ Function: contraction squeezes secretion out of the acinus/ intercalated duct and propels it along the conducting system.

5. Duct system

Duct segment	Epithelium	Key feature	Function
Intercalated duct	Low cuboidal / squamous	Smallest, myoepithelial-covered; long & conspicuous in parotid, short/inconspicuous in mucous-predominant glands	Initial conduit; connects acinus to striated duct

Duct segment	Epithelium	Key feature	Function
Striated duct (intralobular)	Simple columnar	Basal striations = infoldings of the basolateral membrane packed with vertically-oriented mitochondria; central (not basal) nucleus	Reabsorbs Na^+ (and Cl^-), secretes K^+ and HCO_3^- into the lumen — makes resting saliva hypotonic ; with rapid flow, reabsorption cannot keep pace and saliva becomes isotonic-to-hypertonic
Interlobular / excretory duct	Simple to stratified cuboidal/columnar, pseudostratified in places	Runs in connective-tissue septa; lumen widens and epithelium becomes taller/stratified toward the mouth	Collects saliva from lobules; becomes nonkeratinized stratified squamous just before opening into the oral cavity

- **Mucosal immunity:** plasma cells in the periductal connective tissue secrete IgA, which is complexed with the **secretory component** synthesised by serous acinar and intralobular duct cells and transcytosed into saliva — an important first-line antimicrobial mechanism.

6. Comparative histology of the three major salivary glands

Feature	Parotid	Submandibular	Sublingual
Acinar type	Purely serous	Mixed, serous-predominant, with numerous demilunes	Mixed, mucous-predominant, with occasional demilunes
Intercalated ducts	Long, prominent	Present, shorter than parotid	Short/inconspicuous, often not identifiable
Striated ducts	Long, very conspicuous	Prominent (more so than parotid)	Sparse
Stromal fat	Numerous adipocytes (aid slide identification)	Adipocytes present, fewer	Least adipose tissue
Excretory duct	Stensen's (parotid) duct → vestibule opposite upper 2nd molar	Wharton's (submandibular) duct → sublingual caruncle beside the frenulum	8–20 small ducts opening along the sublingual fold + an anterior Bartholin's duct joining near the submandibular orifice
Share of resting saliva	~20% (rises to ~50% on stimulation)	~65% (produces roughly two-thirds of total saliva)	~15% (with minor glands)

□ Fig 16.16 — Histology of parotid (serous) salivary gland (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.224)

□ Fig 16.18 — Histology of submandibular (mixed) salivary gland (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.225)

□ Fig 16.20 — Histology of sublingual (predominantly mucous) salivary gland (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.226)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power lobule for each gland and label: **serous acini** (parotid/submandibular), **mucous acini** (submandibular/sublingual), **serous demilune**, **intercalated duct**, **striated duct**, **interlobular (excretory) duct**, **interlobular connective tissue septum**, **blood vessel**, **adipocyte**. Add small inset circles showing cross-sections of a serous acinus, a mucous acinus with a serous demilune, an intercalated duct, and a striated duct, with **myoepithelial cells** ringed around each.

7. Saliva — composition, quantity and functions

- ▶ Total output: $\approx 0.5\text{--}1.5$ L/day; resting flow ≈ 0.3 mL/min, rising to 1.5–2 mL/min on stimulation.
- ▶ Functions tied directly to the histology above: **lubrication/moistening** (mucus — mucous cells), **digestion** (salivary α -amylase from serous cells; lingual lipase from serous **von Ebner glands**), **antimicrobial defence** (lysozyme, lactoferrin, secretory IgA), **buffering** (HCO_3^- added by striated ducts), and a **solvent role for taste**.
- ▶ Minor salivary glands: labial and buccal glands are mixed; palatal and (anterior) lingual glands are mucous; the small serous **glands of von Ebner** lie deep to the circumvallate papillae and wash taste stimuli over the taste buds.

Applied / Clinical anatomy

- ▶ **Xerostomia** (dry mouth) follows destruction/atrophy of acinar tissue — mumps (viral parotitis), therapeutic irradiation of the glands, or anticholinergic drugs — and predisposes to dental caries and periodontal disease.
- ▶ The parotid (Stensen's) and submandibular (Wharton's) ducts can be outlined radiologically by **sialography**.
- ▶ Obstruction of a minor salivary gland duct produces a **mucocele**; obstruction of the sublingual gland's duct(s) produces a **ranula**, a tense bluish cystic swelling in the floor of the mouth (a "plunging ranula" herniates through the mylohyoid dehiscence into the submental region); treatment is excision of the sublingual gland.
- ▶ $\sim 80\%$ of salivary gland tumours are benign and most arise in the **parotid**; the commonest is the **pleomorphic adenoma (mixed tumour)**, containing epithelial (ductal) and myoepithelial-derived components admixed with a chondromyxoid stroma — reflecting the dual epithelial–myoepithelial histology of the normal gland.
- ▶ Histological identification on a biopsy: purely serous acini + abundant adipocytes = parotid; mixed acini with numerous demilunes = submandibular; predominantly pale mucous tubules with sparse ducts = sublingual/minor gland.

High-yield exam pointers

- ▶ Parotid = **purely serous**; submandibular = **mixed, serous-predominant**; sublingual = **mixed, mucous-predominant** — the classic three-gland identification triad.
- ▶ **Salivon** = acinus + intercalated duct + striated duct — the basic secretory-ductal unit.
- ▶ Serous cell = pyramidal, basal round nucleus, bipolar staining (basophilic base, eosinophilic apex); mucous cell = columnar, flattened basal nucleus, pale foamy cytoplasm.

- ▶ **Serous demilune** caps a mucous tubule and drains via intercellular canaliculi; largely a fixation artifact of conventional processing.
- ▶ **Striated duct** = basal striations from basolateral infoldings + mitochondria; reabsorbs Na^+ , secretes $\text{K}^+/\text{HCO}_3^- \rightarrow$ hypotonic saliva at rest.
- ▶ **Myoepithelial cells** are contractile (actin/myosin), lie inside the basal lamina, surround acini and intercalated ducts.
- ▶ Named ducts: **Stensen's** (parotid), **Wharton's** (submandibular), **Bartholin's** (major sublingual duct).
- ▶ Secretory-component-IgA complex from serous acinar/duct cells provides mucosal immunity in saliva.
- ▶ ~80% of salivary tumours are benign, mostly parotid (pleomorphic adenoma).

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e (figures); Gray's Anatomy (Standring) 41e.



Describe in detail the histological structure of alimentary canal. Correlate the structure with the functions

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction The alimentary canal (gastrointestinal tract) is the musculomembranous tube extending from the oesophagus to the anal canal, specialised for the transport, digestion and absorption of food and the elimination of waste. Despite marked regional variation, its wall throughout is built on a constant four-layered plan — mucosa, submucosa, muscularis externa and serosa/adventitia. Each layer is regionally modified — epithelial type, glandular content, muscle arrangement, lymphoid tissue — to serve the locally dominant function: protection and transport in the oesophagus, secretion and churning in the stomach, digestion and absorption in the small intestine, and mucus-aided water reabsorption in the large intestine. This structure-function correlation is the essence of gut histology.

General Plan of the Gut Wall (Four Layers)

Layer	Sublayers/ components	Key features	Functional correlation
Mucosa	Epithelium, lamina propria, muscularis mucosae	Epithelium varies regionally (squamous vs columnar); lamina propria = loose connective tissue with glands, vessels, GALT; muscularis mucosae = thin smooth muscle (2 layers)	Epithelial type dictates protective vs absorptive/secretory role; muscularis mucosae throws mucosa into folds and moves villi

Layer	Sublayers/ components	Key features	Functional correlation
Submucosa	Dense irregular connective tissue, larger vessels, lymphatics, Meissner's (submucosal) plexus ; glands in oesophagus & duodenum	Highly vascular; supports mucosa	Regulates mucosal blood flow, secretion and local mucosal movement
Muscularis externa	Inner circular + outer longitudinal smooth muscle (oblique layer added in stomach); Auerbach's (myenteric) plexus between the two coats	Circular layer thickens into sphincters (lower oesophageal, pyloric, ileocaecal, internal anal)	Coordinated contraction produces peristalsis and segmentation — mechanical propulsion/mixing
Serosa / adventitia	Serosa = visceral peritoneum (mesothelium + thin connective tissue); adventitia = plain connective tissue merging with surroundings	Serosa where intraperitoneal (most of stomach, small intestine); adventitia where retroperitoneal/mediastinal (most of oesophagus, duodenum, ascending/descending colon)	Serosa allows frictionless mobility; adventitia anchors fixed segments

Diagram to draw (real plate below): a labelled transverse section through the generalised gut wall, showing — mucosa (epithelium, lamina propria, muscularis mucosae) → submucosa (with Meissner's plexus and vessels) → muscularis externa (inner circular, outer longitudinal, Auerbach's plexus between) → serosa (mesothelium).

□ Fig 16.1 — Layers of the gut (schematic representation). (IB Singh — Textbook of Human Histology — 9e, p.414)

Oesophagus

- Mucosa: **nonkeratinized stratified squamous epithelium** — resists abrasion by the masticated bolus; lamina propria has occasional lymphoid nodules and, near the stomach, mucous cardiac glands; muscularis mucosae is unusually thick and purely longitudinal.
- Submucosa: contains **oesophageal glands proper** — mucous, tubuloacinar, secrete lubricating mucus discharged through ducts opening on the surface.
- Muscularis externa (inner circular, outer longitudinal): **upper third skeletal muscle** (voluntary initiation of swallowing), **middle third mixed** skeletal + smooth, **lower third pure smooth muscle** (involuntary, autonomically controlled peristalsis); thickening of the circular layer at the lower end forms the physiological **lower oesophageal sphincter**.
- Outer coat: mostly **adventitia** (oesophagus is largely extraperitoneal/mediastinal); only the terminal 1–2 cm, which projects into the peritoneal cavity, has a true serosa.
- Functional correlation: stratified squamous epithelium → mechanical protection; glandular mucus → lubrication of the bolus; proximal skeletal muscle → voluntary initiation of swallowing;

distal smooth muscle and sphincter → involuntary peristaltic transport and prevention of gastro-oesophageal reflux.

Stomach

The gastric mucosa is thrown into **rugae** (allowing distension) and its surface is studded with **gastric pits**, into which branched tubular glands open; gland type differs by region — **cardiac glands** (mucous), **fundic/gastric glands** (body and fundus — the principal digestive glands) and **pyloric glands** (mucous, with concentrated gastrin-secreting cells).

Cell type (fundic gland)	Location in gland	Secretion	Functional correlation
Mucous neck cells	Neck	Soluble mucus	Lubricates and protects surface epithelium
Parietal (oxyntic) cells	Upper part/body, more numerous proximally	HCl and intrinsic factor	HCl activates pepsinogen and kills ingested microbes; intrinsic factor is essential for vitamin B12 absorption in the ileum — its loss causes pernicious anaemia
Chief (zymogenic/peptic) cells	Base, more numerous distally	Pepsinogen (and gastric lipase)	Pepsinogen is converted to active pepsin by HCl at pH < 5, initiating protein digestion
Enteroendocrine cells (G, D, ECL etc.)	Scattered throughout	Gastrin, somatostatin, histamine	Paracrine/endocrine regulation of acid secretion and motility
Stem cells	Isthmus/neck	—	Rapid renewal of all lineages; surface mucous cells are replaced every few days

- ▶ Muscularis externa is **three-layered** — an added innermost **oblique** layer beneath the usual circular and longitudinal coats — permitting the powerful churning needed to convert food into **chyme**; the circular layer thickens markedly at the pylorus to form the **pyloric sphincter**, which regulates gastric emptying.
- ▶ Serosa covers the whole stomach except the bare areas at the curvatures where the omenta attach.
- ▶ Functional correlation: rugae permit storage/distension; pits and glands supply gastric juice for chemical digestion of protein and antimicrobial defence; the extra oblique muscle layer enables mechanical churning; the pyloric sphincter controls the rate of gastric emptying into the duodenum.

Small Intestine

Absorption is maximised by three progressively finer surface amplifiers: **plicae circulares** (permanent mucosal-submucosal folds, tallest and closest in the jejunum) > **villi** (finger-like mucosal projections,

tallest in the duodenum, shortening distally) > **microvilli** (the enterocyte brush border, further coated by a glycocalyx bearing digestive enzymes).

- ▶ Mucosal epithelium (villi): **enterocytes** (tall absorptive columnar cells with brush border) and **goblet cells** (mucus, increasing in number toward the ileum); the villus core (lamina propria) contains a capillary plexus, a central **lacteal** (lymphatic, absorbs chylomicrons) and smooth muscle strands from the muscularis mucosae that pump the villus.
- ▶ **Crypts of Lieberkühn** (between villus bases): lined by enterocytes, goblet cells, **Paneth cells** (base of crypt — eosinophilic granules of lysozyme/defensins, innate antibacterial defence) and stem cells that continuously renew the epithelium (turnover $\approx$ 4–6 days).
- ▶ Submucosa: in the **duodenum**, large branched tubuloacinar **Brunner's glands** occupy the submucosa (and encroach on the mucosa), secreting a distinctly **alkaline mucus** (pH 8–9) that neutralises the acidic chyme entering from the stomach — an identification feature unique to the duodenum. In the **ileum**, aggregated lymphoid follicles — **Peyer's patches** — lie in the lamina propria/submucosa of the antimesenteric border, overlain by specialised **M cells** that sample luminal antigen and present it to underlying immune cells (part of GALT).
- ▶ Muscularis externa (inner circular, outer longitudinal) with **Auerbach's plexus** between the two coats controls peristalsis and segmentation.
- ▶ Serosa is complete except over the retroperitoneal part of the duodenum, which has an adventitia.

Diagram to draw (real plate below): a labelled villus-and-crypt unit — villus tip with enterocytes and goblet cells, brush border, capillary plexus, central lacteal; crypt of Lieberkühn with Paneth cells at its base and mitotic stem cells; muscularis mucosae sending fibres into the villus core.

□ Fig 18.2 — Villi of small intestine. (Sontakke — *Textbook of Human Histology* — 2e, p.243)

Functional correlation: villi + microvilli give the vast surface area needed for absorption of digested nutrients; rapid crypt-based renewal replaces enterocytes damaged by digestive/microbial insult; goblet mucus lubricates and protects; Paneth cells and lysozyme maintain a controlled bacterial flora; lacteals allow lymphatic absorption of dietary fat; Brunner's alkaline secretion protects the duodenal mucosa from acid injury; Peyer's patches/M cells provide continuous immune surveillance of the largest antigen-exposed surface in the body.

Large Intestine, Rectum and Anal Canal

- ▶ Mucosa is **smooth** — **no villi and no plicae circulares** (the colon's job is reabsorption, not digestion); it shows only **crypts of Lieberkühn**, which are longer, straighter and more closely packed than in the small intestine and contain far **more goblet cells** (mucus lubricates the increasingly solid faecal stream); **Paneth cells and M cells are normally absent** in the adult colon (present only in the small intestine).
- ▶ Muscularis externa: inner circular layer is complete, but the outer longitudinal layer condenses into **three taenia coli** (except in the rectum and appendix, where it is again complete); because the taeniae are shorter than the gut tube, the wall puckers into **haustra**, separated internally by semilunar folds.
- ▶ Serosa bears small peritoneal fat tags, the **appendices epiploicae**.

- ▶ **Vermiform appendix:** narrow, often obliterated lumen; mucosa and submucosa packed with lymphoid follicles (GALT) that may efface the muscularis mucosae — functions as a lymphoid organ ("tonsil of the abdomen").
- ▶ **Anal canal:** epithelium transitions in three steps — upper third **simple columnar** (rectal type), middle third **nonkeratinized stratified squamous**, and the lower third, below the **pectinate (dentate) line**, **keratinized stratified squamous** continuous with perianal skin (bearing hair follicles and sebaceous glands). The **internal anal sphincter** is a thickening of the inner circular smooth muscle (involuntary); the **external anal sphincter** is skeletal muscle (voluntary).
- ▶ **Functional correlation:** loss of villi with lengthened, mucus-rich crypts shifts the organ's role from nutrient absorption to **reabsorption of water and electrolytes** and lubrication of faeces; taeniae/hausta permit slow mixing and mass movements needed for that reabsorption; the appendix's dense lymphoid tissue provides mucosal immunity at the ileocaecal junction; the epithelial transition and dual (smooth + skeletal) sphincters at the anal canal underpin both continence and the clinically important shift in blood supply, lymphatic drainage and nerve supply across the pectinate line.

Applied / Clinical anatomy

- ▶ **GERD and Barrett's oesophagus:** an incompetent lower oesophageal sphincter allows acidic reflux; chronic injury can replace squamous epithelium with metaplastic columnar (intestinal-type) epithelium — a premalignant change.
- ▶ **Peptic ulcer disease:** breach of the gastric/duodenal mucosal barrier from acid-pepsin/*H. pylori* imbalance; parietal and chief cell activity is central to pathogenesis.
- ▶ **Pernicious anaemia:** autoimmune destruction of parietal cells (atrophic gastritis) → loss of intrinsic factor → vitamin B12 malabsorption → megaloblastic anaemia with subacute combined degeneration of the cord.
- ▶ **Coeliac disease:** gluten-triggered villous atrophy with crypt hyperplasia in the duodenum/jejunum, producing malabsorption.
- ▶ **Hirschsprung's disease:** congenital absence/reduction of ganglion cells in Auerbach's (and Meissner's) plexus of the distal colon, due to failure of neural crest cell migration, causing a non-peristaltic aganglionic segment and proximal megacolon.
- ▶ **Acute appendicitis:** luminal obstruction of the narrow, lymphoid-rich appendix predisposes to inflammation and perforation.
- ▶ **Hemorrhoids:** dilated venous plexuses above (internal — columnar mucosa, visceral innervation, painless) and below (external — squamous skin, somatic innervation, painful) the pectinate line, reflecting the epithelial/neural transition at that line.
- ▶ **Rectal/anal carcinoma spread:** tumours above the pectinate line drain to internal iliac nodes, those below to superficial inguinal nodes — a direct consequence of the embryological hindgut-vs-proctodeum boundary at that line.

High-yield exam pointers

- ▶ Four constant layers of the gut wall: mucosa (epithelium + lamina propria + muscularis mucosae), submucosa, muscularis externa (circular + longitudinal), serosa/adventitia.

- ▶ Oesophagus: nonkeratinized stratified squamous epithelium; upper third skeletal, middle mixed, lower third smooth muscle in the muscularis externa.
- ▶ Fundic gland cells and secretions: mucous neck (mucus), parietal (HCl + intrinsic factor), chief (pepsinogen), enteroendocrine (hormones); stomach uniquely has a third, innermost **oblique** muscle layer.
- ▶ Small intestinal surface amplifiers, largest to smallest: plicae circulares > villi > microvilli.
- ▶ Duodenum is identified by **Brunner's glands** (submucosa, alkaline mucus); ileum by **Peyer's patches** (antimesenteric border, GALT, overlain by M cells).
- ▶ Large intestine: no villi; longer, straighter, more goblet-cell-rich crypts; taenia coli/haustra; Paneth cells and M cells normally absent.
- ▶ Anal canal epithelial transition at the pectinate line: simple columnar → nonkeratinized stratified squamous → keratinized stratified squamous — the single most clinically loaded histological landmark in the GIT (vascular, lymphatic and nerve supply all change here).
- ▶ Auerbach's (myenteric) plexus → motility/peristalsis; Meissner's (submucosal) plexus → secretion and mucosal blood flow.
- ▶ Master structure-function axiom: epithelial type = protection vs absorption vs secretion; muscle coats = motility; glands and GALT = secretion and mucosal immunity.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; Moore's Clinically Oriented Anatomy 9e (anal canal applied anatomy).



Describe the histology of the derivatives of foregut and correlate the histology with function

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction The foregut is the cranial-most, endoderm-lined division of the primitive gut tube, extending from the pharynx up to the duodenum **proximal to the opening of the common bile duct** (at the major duodenal papilla). Endoderm forms only the epithelial lining and glandular parenchyma of its derivatives; the muscularis and connective tissue come from splanchnopleuric mesoderm. Apart from the pharynx and lower respiratory tract (described under those systems), the alimentary/glandular foregut derivatives with distinctive, examinable histology are the **oesophagus, stomach, proximal duodenum, liver, extrahepatic biliary apparatus and pancreas** — described below with structure-function correlation.

Foregut: extent and derivatives

- ▶ Foregut derivatives in the abdomen are supplied by the **coeliac trunk** ("artery of the foregut"), dividing into **left gastric, common hepatic and splenic arteries** — supplying the lower oesophagus, stomach, proximal duodenum, liver, spleen and most of the pancreas.

- The **superior pancreaticoduodenal artery** (from the gastroduodenal, a common-hepatic branch) supplies the foregut part of the duodenum; distal to the bile-duct opening the **inferior pancreaticoduodenal artery** (from the SMA, artery of the midgut) takes over — this arcade is the foregut–midgut arterial watershed.



Describe the histology of the derivatives of foregut and correlate the histology with function

General architectural plan of the tubular derivatives

- The oesophagus, stomach and duodenum share the universal 4-layered gut plan — **mucosa** (epithelium + lamina propria + muscularis mucosae) → **submucosa** → **muscularis externa** (inner circular, outer longitudinal) → **serosa/adventitia** — with only the mucosa (and, regionally, the submucosal glands) varying enough to explain regional function.
- Epithelium switches from **protective stratified squamous** where the tube merely conducts (oesophagus) to **secretory/absorptive simple columnar** where digestion/absorption occurs (stomach, duodenum) — a direct structure-function correlation.

Oesophagus

- A 25 cm muscular tube from the cricoid cartilage to the cardia; purely a **conduit**, so it carries no digestive glands in its epithelium.
- **Mucosa**: stratified squamous non-keratinised epithelium (resists abrasion by the bolus) on connective-tissue papillae; longitudinal folds efface on distension. **Oesophageal cardiac glands** (mucous) lie in the lamina propria at the upper and lower ends. Muscularis mucosae is unusually thick and longitudinal.
- **Submucosa**: compound tubuloalveolar **oesophageal glands proper** (mucous), concentrated in the upper half — lubricate the lumen and buffer refluxed acid.
- **Muscularis externa** shows a functional gradient — **upper third skeletal (voluntary)**, **middle third mixed**, **lower third smooth (involuntary)** — matching the switch from voluntary initiation of swallowing to involuntary peristalsis via the myenteric (Auerbach's) plexus.
- **Adventitia** covers most of the tube; only the terminal intra-abdominal segment has a serosa. No anatomical sphincter exists at the cardia, though a physiological lower oesophageal sphincter is present.

Stomach

Converts the bolus into chyme; histology is dominated by acid/enzyme-secreting glands whose density and cell composition vary regionally.

Region	Gastric pits	Glands present	Principal secretion
Cardia	Shallow	Cardiac glands (mucous, simple/compound tubuloalveolar)	Mucus

Region	Gastric pits	Glands present	Principal secretion
Fundus/body	Shallow ($\leq \frac{1}{4}$ mucosal depth)	Gastric (fundic) glands	HCl, pepsinogen, intrinsic factor, mucus
Pylorus	Deep ($\frac{2}{3}$ mucosal depth)	Pyloric glands (coiled, tubular)	Mucus, gastrin

- **Fundic gland cell types:** **mucous neck cells** (weakly-alkaline mucus, protective); **chief/peptic cells** (basal, basophilic, rER-rich; apical granules contain **pepsinogen**, activated to pepsin by HCl); **parietal/oxynitic cells** (large, eosinophilic, mitochondria-packed; intracellular canaliculi secrete HCl and **intrinsic factor of Castle**, needed for vitamin B12 absorption); **enteroendocrine cells** (basal, secrete **gastrin/serotonin**); **stem cells** (isthmus/neck, replenish all other types).
- **Muscularis externa** has an extra **inner oblique layer** (unique to stomach) for churning; the circular layer thickens into the **pyloric sphincter**, regulating gastric emptying. Surface mucous cells secrete an alkaline mucus-bicarbonate layer protecting the epithelium from the acid/enzymes produced beneath it.

□ Fig 15.17 — Gastric glands. (Junqueira's Basic Histology — Text & Atlas — 17e, p.762)

Duodenum (the foregut segment, proximal to the major duodenal papilla)

- Follows the general 4-layer plan, but the **submucosa contains Brunner's glands** — the defining feature of duodenal histology. These branched, mucous, **bicarbonate-rich alkaline glands** neutralise acidic chyme from the stomach, protecting the mucosa and creating the near-neutral pH needed for pancreatic enzyme activity.
- **Mucosa:** broad villi (absorptive enterocytes with a microvillous striated border, plus goblet cells) and crypts of Lieberkühn with **Paneth cells** (lysozyme — antibacterial) and stem cells. Plicae circulares and villi together maximise absorptive surface exactly where bile and pancreatic secretions first meet the food.
- The **major duodenal papilla**, where bile duct and main pancreatic duct join to form the hepatopancreatic ampulla, marks the foregut–midgut junction.

Liver

The largest gland in the body; a modified exocrine gland with added metabolic functions.

- Covered by **Glisson's capsule**, whose septa carry portal triads (hepatic artery + portal vein + interlobular bile duct branches) into the gland.
- Parenchyma is organised into three overlapping conceptual units:

Unit	Centre	Periphery	Functional significance
Classic (hepatic) lobule	Central vein	Portal triads	Venous drainage unit
Portal lobule	Portal triad	3 central veins	"Nutritional"/bile-secretion unit
Liver acinus (of Rappaport)	Line joining 2 portal triads	2 central veins	Metabolic zonation unit

- ▶ The acinus is divided into **zone 1** (periportal, best oxygenated — first to be damaged by toxins/bile stasis but first to regenerate), **zone 2**, and **zone 3** (pericentral, least oxygenated — first to undergo ischaemic necrosis and fatty change).
- ▶ **Hepatocytes** form one-cell-thick anastomosing plates; abundant rER/SER, mitochondria, Golgi and glycogen/lipid stores correlate with the liver's synthetic (plasma proteins, clotting factors), metabolic and detoxification roles. Each cell has a sinusoidal surface (microvilli into the space of Disse), a canalicular surface (forms bile canaliculi, sealed by junctional complexes — the blood–bile barrier), and an intercellular surface.
- ▶ **Sinusoids** are fenestrated capillaries lined by endothelium and **Kupffer cells** (macrophages of the mononuclear phagocyte system — clear damaged erythrocytes and gut-derived antigens). The **space of Disse** contains **Ito (stellate) cells**, which store vitamin A and, when activated by injury, transform into collagen-producing myofibroblasts (basis of fibrosis).
- ▶ Blood flows **centripetally** (portal + arterial blood at the periphery → sinusoids → central vein) while bile flows **centrifugally** towards the interlobular bile ducts — opposite directions in the same lobule. Dual blood supply ($\approx 70\%$ nutrient-rich portal vein, $\approx 30\%$ oxygen-rich hepatic artery) matches its dual metabolic/detoxification workload.

□ Fig 19.6 — Histology of liver, low magnification with portal triad at high magnification. (Sontakke — Textbook of Human Histology — 2e, p.262)

Extrahepatic biliary apparatus and pancreas

Gallbladder — stores and concentrates bile.

- ▶ Mucosa thrown into honeycomb-like folds; lined by **simple tall columnar epithelium with a striated (microvillous) border**, correlating with its role in actively pumping Na^+ and water out of stored bile to concentrate it.
- ▶ **No muscularis mucosae and no submucosa** (unlike the rest of the gut) — a key identification point. **Fibromuscular coat**: randomly-oriented smooth muscle contracting under cholecystokinin stimulation to expel bile. Deep mucosal diverticula into the muscularis (**Rokitansky–Aschoff sinuses**) predispose to bile stasis and stone formation.
- ▶ Extrahepatic ducts (hepatic, cystic, bile ducts) share a tall columnar epithelium with some smooth muscle in the wall; at the **hepatopancreatic ampulla** the folded mucosa forms a valve, and the **sphincter of Oddi** (choledochus + pancreaticus + ampullae components) controls flow into the duodenum and diverts bile into the gallbladder between meals.

Exocrine pancreas — a compound tubuloacinar **serous** gland, structurally similar to the parotid.

- ▶ **Acinar cells**: pyramidal, basal spherical nucleus, strongly basophilic base (packed rER) — matched to synthesis of digestive enzymes (trypsinogen, chymotrypsinogen, amylase, lipase); apical eosinophilic zymogen granules store them until exocytosis.
- ▶ **Centroacinar cells**: pale, flattened cells that appear to lie within the acinar lumen (actually the innermost, invaginated cells of the intercalated duct) — secrete a watery, bicarbonate-rich fluid (stimulated by secretin) that flushes enzymes down the duct and further neutralises duodenal acid.
- ▶ Duct system: intercalated → interlobular ducts → main pancreatic duct, joining the bile duct at the major papilla.

Endocrine pancreas — islets of Langerhans, about one million, most numerous in the tail, richly vascularised.

Cell	Location in islet	% of cells	Secretion	Function
Alpha (A)	Peripheral	~20%	Glucagon	Raises blood glucose
Beta (B)	Central	~70%	Insulin	Lowers blood glucose
Delta (D)	Peripheral	Minority	Somatostatin/ gastrin	Paracrine inhibition of A and B cells

- ▶ Shared endodermal origin and common portal venous drainage to the liver link the exocrine and endocrine metabolic functions of the pancreas.

□ Fig 23.19 — Histology of pancreas. (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.383)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ **Gastric fundic gland:** gastric pit, isthmus (stem cells), neck (mucous neck cells), body/fundus (chief, parietal, enteroendocrine cells), muscularis mucosae.
- ▶ **Classic liver lobule:** central vein, radiating hepatocyte plates, sinusoids with Kupffer cells, space of Disse, peripheral portal triad (arteriole, venule, bile ductule), limiting plate.
- ▶ **Pancreatic acinus with islet:** pyramidal acinar cells (basal basophilia, apical zymogen granules), centroacinar cell, intercalated duct, adjoining islet (alpha peripheral, beta central), capillary plexus.
- ▶ **Duodenal wall:** villi (enterocytes, goblet cells), crypts with Paneth cells, muscularis mucosae, submucosal Brunner's glands, muscularis externa, serosa.

Applied / Clinical anatomy

- ▶ **Barrett's oesophagus:** chronic reflux causes columnar (intestinal-type) metaplasia of the lower oesophageal squamous epithelium — a precursor of adenocarcinoma. **Achalasia:** failure of LES relaxation → progressive dysphagia and megaesophagus.
- ▶ **Peptic ulcer/gastritis:** breakdown of the mucus-bicarbonate barrier or acid excess; **Zollinger–Ellison syndrome** (gastrin-secreting tumour) causes refractory ulceration. **Pernicious anaemia:** autoimmune parietal-cell loss → no intrinsic factor → B12 malabsorption and megaloblastic anaemia.
- ▶ **Cirrhosis:** hepatocyte loss with Ito-cell-derived fibrous replacement distorts the sinusoidal sponge → portal hypertension → oesophageal varices at the portosystemic anastomosis near the cardia → risk of fatal haematemesis.
- ▶ **Cholelithiasis/cholecystitis:** stones (favoured by Rokitsansky–Aschoff sinuses) cause biliary colic; duct obstruction produces obstructive jaundice.

- ▶ **Acute pancreatitis:** premature enzyme activation causes autodigestion (raised amylase early, lipase later). **Islet-cell tumours** (insulinoma, glucagonoma, gastrinoma) and beta-cell failure (diabetes mellitus) reflect endocrine pancreatic disease.

High-yield exam pointers

- ▶ Foregut = pharynx to duodenum **proximal** to the bile-duct opening; artery of foregut = **coeliac trunk** (left gastric, splenic, common hepatic).
- ▶ Oesophagus muscularis externa gradient: upper 1/3 skeletal → middle 1/3 mixed → lower 1/3 smooth; stomach adds a unique **inner oblique** layer for churning plus three glandular zones (cardiac, fundic, pyloric).
- ▶ Parietal cells → HCl + intrinsic factor; chief cells → pepsinogen; mucous neck/surface cells → protective mucus.
- ▶ Brunner's glands (duodenal submucosa) secrete alkaline, bicarbonate-rich mucus — the histological hallmark separating duodenum from jejunum.
- ▶ Liver's three units: classic lobule (venous drainage), portal lobule (bile secretion), liver acinus (metabolic zonation, zones 1–3).
- ▶ Kupffer cells = hepatic macrophages; Ito cells = vitamin A storage/fibrosis; space of Disse = hepatocyte–plasma exchange site.
- ▶ Gallbladder mucosa lacks a muscularis mucosae and submucosa — a key differentiating point from intestine.
- ▶ Pancreatic islets: beta (70%, central, insulin) > alpha (20%, peripheral, glucagon) > delta (somatostatin); centroacinar cells are unique to pancreas among serous glands.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Junqueira's Basic Histology — Text & Atlas 17e; Brijesh Kumar — Histology Text & Atlas 3e; BD Chaurasia — Human Anatomy Vol 1 & 2, 8e; Moore — The Developing Human 9e; Moore — Clinically Oriented Anatomy 9e; Larsen — Human Embryology 6e. Coverage note: Gray's Anatomy is gross-anatomy oriented and contributes mainly the arterial/foregut-extent grounding; the detailed cellular histology is drawn from the dedicated histology texts (IB Singh, Ross, Sontakke, Junqueira, Brijesh Kumar) as indicated in the topic spec.



Describe the microanatomy of tongue

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction The tongue is a highly muscular organ of taste, mastication, deglutition and speech, covered by a mucous membrane that is directly bound to the underlying skeletal muscle with no intervening submucosa. Its dorsal mucosa is divided by the V-shaped **sulcus terminalis** into an anterior (presulcal, oral) part bearing four types of lingual papillae, and a posterior (postsulcal, pharyngeal) part that is papilla-free but carries the lymphoid lingual tonsil. Microanatomically the tongue must be described under: mucosa and papillae, taste buds, lingual glands, lingual tonsil, and the underlying striated muscle.

1. General mucosal architecture

- **Dorsal mucosa:** thicker than the ventral mucosa; epithelium is **stratified squamous**, ranging from fully **keratinized** over the filiform papillae anteriorly to **non-keratinized** posteriorly; it is firmly adherent to the muscle with **no discernible submucosa**.
- **Lamina propria:** dense fibrous connective tissue rich in elastic fibres, continuous with the perimysium between muscle fascicles; carries the vessels, nerves, lymph plexuses and lingual glands that supply the papillae.
- **Ventral (inferior) mucosa:** smooth, thin, non-keratinized, reflected onto the floor of the mouth; connected anteriorly by the lingual frenulum.
- **Sulcus terminalis and foramen caecum:** the sulcus separates the anterior two-thirds from the posterior one-third; its apex shows the **foramen caecum**, the vestigial site of origin of the embryonic thyroglossal duct.

2. Lingual papillae — types and histology

Papillae are mucosal projections limited to the presulcal (anterior) part of the dorsum; they produce its roughness and increase surface area for contact with food. Four types are described, and all except the filiform bear taste buds.

Papilla	Location	Shape / epithelium	Taste buds	Key histological feature
Filiform	Most numerous; cover the entire presulcal dorsum in diagonal rows parallel to the sulcus terminalis (transverse at the apex)	Minute conical/cylindrical projections; epithelium fully keratinized , tips may split into fine secondary processes	Absent	Purely mechanical — increases friction for food handling; no vascular core visible through the thick keratin
Fungiform	Scattered among filiform papillae, more numerous at the tip and lateral margins	Mushroom-shaped, rounded, deep red; epithelium thin, non-keratinized	Present , on the apical surface (sparse, ~50 per papilla)	Highly vascular connective-tissue core gives the red colour seen as pink dots on gross inspection

Papilla	Location	Shape / epithelium	Taste buds	Key histological feature
Foliate	4–5 vertical mucosal folds bilaterally, near the sulcus terminalis on the lateral margin	Parallel leaf-like ridges; non-keratinized epithelium	Present, numerous, on facing walls of adjacent ridges	Rudimentary/ regressed in adult humans (well developed in children and in many other mammals)
Circumvallate (vallate)	8–12 papillae in a V-shaped row immediately anterior to the sulcus terminalis	Large, cylindrical, dome-shaped, 1–2 mm diameter; narrower at the base than the apex; non-keratinized	Present, in great numbers on the lateral walls (average ~250 per papilla)	Each papilla is surrounded by a circular sulcus/trench; ducts of serous glands of von Ebner open into the floor of the trench and continuously flush it

3. Taste buds — microanatomy

Taste buds are intraepithelial, barrel/onion-shaped neuroepithelial (chemosensory) structures present on all lingual papillae except the filiform, and scattered elsewhere on the dorsal/lateral tongue, soft palate and epiglottis.

- ▶ **Dimensions:** a cluster of roughly 50–150 **spindle-shaped cells**, about 70 μm tall and 40 μm across, resting on a basal lamina; opens onto the mucosal surface through a narrow **gustatory (taste) pore** (~2 μm wide).
- ▶ **Cell types** (classical three-cell model taught for PG anatomy):
- ▶ **Gustatory (neuroepithelial/taste) cells** — elongated cells extending from the basal lamina to the taste pore; apical microvilli project into the pore; their base synapses with afferent gustatory nerve fibres; continuously renewed with a ~7–10 day life span.
- ▶ **Supporting (sustentacular) cells** — similarly elongated, interspersed among taste cells, providing structural support and secreting material at the pore.
- ▶ **Basal (stem) cells** — small, slowly dividing cells at the base of the bud that give rise to both gustatory and supporting cells.
- ▶ **Innervation:** a fine fasciculus of afferent fibres penetrates the basal lamina and spirals around the sensory cells (perigemmal plexus around the bud, intragemmal plexus within it).
 - **Chorda tympani** (branch of facial nerve, VII) — taste from the anterior two-thirds of the tongue, *excluding* the circumvallate papillae.
 - **Glossopharyngeal nerve** (IX) — taste and general sensation from the posterior one-third, and taste from the circumvallate and foliate papillae.
 - **Vagus** (X, via internal laryngeal nerve) — taste from the extreme posterior tongue and epiglottis.

- Central gustatory afferents ascend in the tractus solitarius to the rostral nucleus solitarius.
- ▶ Chemical tastants (salty, sour, sweet, bitter, umami) dissolved in saliva diffuse through the pore to depolarise the gustatory cells via ion channels (salt, sour) or G-protein-coupled receptors (sweet, bitter, umami).

□ Fig 15.4 — Tongue, lingual papillae, and taste buds. (Junqueira's Basic Histology — Text & Atlas — 17e, p.738)

□ Fig 16.9 — Taste bud. (Sontakke — Textbook of Human Histology — 2e, p.220)

4. Lingual glands

- ▶ **Serous glands of von Ebner:** purely serous; lie in the connective tissue deep to the circumvallate (and foliate) papillae; their ducts open into the floor of the circumvallate trench, continuously flushing tastants away so the taste buds can respond to fresh stimuli.
- ▶ **Anterior lingual glands (glands of Blandin and Nuhn):** the largest lingual glands, situated on the ventral aspect near the apex; **mixed mucous and serous** acini.
- ▶ **Posterior/mucous lingual glands:** numerous small **mucous** glands scattered among the muscle fibres of the posterior third; their ducts open onto the surface, including into the crypts of the lingual tonsil.

5. Lingual tonsil (posterior one-third mucosa)

- ▶ The postsulcal (pharyngeal) mucosa is devoid of papillae and shows low, dome-shaped elevations formed by aggregates of lymphoid follicles — the **lingual tonsil**.
- ▶ Each dome-shaped group of follicles is arranged around a **central deep crypt/invagination** opening onto the surface epithelium; ducts of the mucous lingual glands open into the base of these crypts, keeping them flushed.
- ▶ Isolated lymphoid follicles also occur beneath the lingual mucosa elsewhere.

6. Musculature — histological features

- ▶ Core of the tongue is formed by interlacing **intrinsic** (longitudinal, transverse, vertical) and **extrinsic** skeletal muscle bundles arranged in three mutually perpendicular planes — an arrangement unique to the tongue that permits its exceptional mobility for speech and swallowing.
- ▶ Muscle fascicles are separated by variable amounts of **adipose tissue**, increasing posteriorly.
- ▶ No submucosa: lamina propria merges directly with the perimysium of the muscle.
- ▶ Many transverse intrinsic fibres show multiple "en grappe" motor end-plates (a slow-tonic-fibre specialisation) that aid fine control of tongue shape.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Longitudinal section through the anterior tongue (mucosa + papillae) — label:

- ▶ Stratified squamous epithelium — keratinized over a filiform papilla, non-keratinized over a fungiform papilla
- ▶ Lamina propria (connective-tissue core) of each papilla, with capillary loop
- ▶ A taste bud on the apical/lateral epithelium of the fungiform papilla
- ▶ Underlying skeletal muscle bundles (longitudinal, transverse, vertical)
- ▶ Adipose tissue and a mixed lingual gland acinus deep to the muscle

Circumvallate papilla (low power) — label: papilla core, surrounding circular sulcus/trench, taste buds studding the lateral walls, duct of von Ebner's gland opening into the trench floor, serous acini of von Ebner's gland beneath the papilla.

Taste bud (high power) — label: gustatory pore, apical microvilli, gustatory (neuroepithelial) cell, supporting (sustentacular) cell, basal (stem) cell, afferent nerve fibril, basal lamina.

Applied / Clinical anatomy

- ▶ **Geographic tongue (benign migratory glossitis):** irregular, migrating patches of filiform-papilla atrophy on the dorsum, exposing the deeper red fungiform papillae.
- ▶ **Hairy tongue:** hypertrophy and elongation of filiform papillae, discoloured black/brown/yellow by chromogenic bacteria/food pigments — a purely keratin/papillary abnormality.
- ▶ **Atrophic (bald) glossitis:** loss of papillae producing a smooth, red tongue, classically seen in vitamin B12, iron or riboflavin deficiency and pernicious anaemia.
- ▶ **Fissured tongue:** genetically determined furrowing of the dorsal mucosa.
- ▶ **Lingual thyroid:** failure of thyroid descent leaves functioning thyroid tissue at the **foramen caecum**, the embryological landmark at the apex of the sulcus terminalis; may present as a midline swelling at the tongue base.
- ▶ **Median rhomboid glossitis:** a smooth, papilla-free patch just anterior to the foramen caecum, associated with *Candida* infection.
- ▶ Squamous cell carcinoma most often arises on the **lateral border/ventral surface**, where the epithelium is non-keratinized and thinner — clinically the most important site for biopsy.
- ▶ Ageusia/dysgeusia can follow chorda tympani injury (anterior two-thirds taste loss) or glossopharyngeal injury (posterior one-third).

High-yield exam pointers

- ▶ Dorsal lingual mucosa has **no submucosa** — lamina propria is directly continuous with muscle perimysium.
- ▶ **Four papillae** — filiform (no taste buds, keratinized), fungiform, foliate (rudimentary in adults), circumvallate (8–12, V-shaped row, non-keratinized).
- ▶ Only the **circumvallate and foliate** papillae have serous **glands of von Ebner** opening into their trenches/clefts.

- ▶ Taste bud = intraepithelial barrel-shaped structure of ~50–150 cells (gustatory, supporting, basal), opening via a 2 µm gustatory pore; gustatory cells turn over every 7–10 days.
- ▶ Taste nerve supply: **chorda tympani (VII)** — anterior two-thirds (except circumvallate); **glossopharyngeal (IX)** — posterior one-third + circumvallate/foliate; **vagus (X)** — epiglottis/extreme posterior tongue. Motor supply of tongue muscle is by the **hypoglossal nerve (XII)**.
- ▶ Lingual glands: **von Ebner (serous)**, **Blandin–Nuhn (anterior, mixed)**, posterior **mucous** glands.
- ▶ **Lingual tonsil** = dome-shaped lymphoid follicle groups around a central crypt, in the papilla-free posterior one-third.
- ▶ **Foramen caecum** = embryological site of the thyroglossal duct — key link between microanatomy and lingual thyroid/thyroglossal cyst.
- ▶ Tongue muscle is uniquely arranged in three interlacing planes (longitudinal, transverse, vertical), unlike any other skeletal muscle.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — Text and Atlas 7e; Sontakke's Textbook of Human Histology 2e; IB Singh's Textbook of Human Histology 9e; Brijesh Kumar's Histology Text & Atlas 3e.



Describe the microscopic features of stomach and their significance

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction The stomach is a dilated, muscular part of the gut tube interposed between the oesophagus and duodenum, anatomically divided into **cardia, fundus, body and pylorus**. Histologically, however, its mucosa falls into only **three functional zones** — **cardiac, fundic (principal/body) and pyloric** — each with a distinct gland population. Like the rest of the gut, the wall shows four concentric layers (mucosa, submucosa, muscularis externa, serosa), but the mucosal glandular architecture is unique to the stomach and underlies its two central functions: mechanical/chemical digestion (HCl, pepsin) and mucosal self-protection.

General plan and gross-to-microscopic correlation

- ▶ **Rugae**: longitudinal folds of mucosa + submucosa, most marked at the pyloric end and greater curvature; they flatten out completely when the stomach distends — of practical significance in barium studies and endoscopy (folds disappear with air insufflation).
- ▶ **Gastric pits (foveolae)**: numerous surface depressions, approximately **60–100 pits/mm²**, each about 70 µm in diameter and 0.2 mm deep (Standring), into whose base 3–7 principal (fundic) glands open in the body/fundus, and 2–3 short pyloric glands open in the antrum.
- ▶ **Gastric canals**: longitudinal mucosal folds along the lesser curvature — the "food/magenstrasse" pathway allowing fluids to bypass the fundus and pass rapidly toward the pylorus.

- ▶ **Visible mucus:** a thick, alkaline, gel-like coat secreted by surface mucous cells that overlies the entire lining epithelium — the first line of mucosal defence.
- ▶ The columnar surface epithelium begins abruptly at the **Z-line** (gastro-oesophageal junction), replacing the stratified squamous epithelium of the oesophagus — the anatomical landmark for reflux/Barrett's change.

Histology of the cardiac region

- ▶ Small zone (1–3 cm) around the cardiac orifice.
- ▶ **Epithelium:** simple columnar surface mucous cells (no goblet cells, no microvilli — these distinguish stomach from intestine).
- ▶ **Lamina propria:** simple to compound branched tubular **cardiac glands**, lined predominantly by mucus-secreting cells; parietal and chief cells are present but sparse.
- ▶ Ducts of cardiac glands open into the gastric pits.
- ▶ Muscularis mucosae, submucosa, muscularis externa and serosa follow the general gastric pattern (see below).

Histology of the fundic (body) region — the principal glands

Histologically identical in fundus and body; these are the most highly specialised gastric glands.

Zone of gland	Cell types present	Key feature
Isthmus (junction with pit)	Undifferentiated stem cells	Mitotically active; migrate up to renew surface mucous cells and down to renew gland cells
Neck (narrow, long)	Mucous neck cells, parietal cells	Mucous neck cells produce soluble mucus
Base/fundus (short, wide, branches into 2–3 limbs)	Chief cells, some parietal and enteroendocrine cells	Chief cells predominate basally

Cells of the gastric (fundic) glands and their functional significance

Cell type	Location	Morphology / staining	Secretion	Functional significance
Surface mucous cells	Line pits + luminal surface	Simple columnar, basal oval nucleus, apical mucinogen (vacuolated on H&E, magenta on PAS); turnover ~3 days	Thick, alkaline, insoluble mucus	Physical + chemical barrier against acid/pepsin; traps HCO_3^- to buffer surface pH
Mucous neck cells	Neck of gland, below the pit	Columnar/irregular, foamy apical mucinogen; turnover ~1 week	Soluble , less-alkaline mucus	Lubrication; a source of replicating stem cells

Cell type	Location	Morphology / staining	Secretion	Functional significance
Parietal (oxyntic) cells	Upper part/neck of gland	Large, rounded/pyramidal, central nucleus, strongly eosinophilic; EM shows intracellular canaliculi, abundant mitochondria, tubulovesicular system	HCl and intrinsic factor; lifespan 150–200 days	HCl activates pepsinogen and sterilises ingested food; intrinsic factor is obligatory for ileal vitamin B12 absorption — its loss causes pernicious anaemia
Chief (zymogenic/peptic) cells	Base of gland	Basal position, basophilic base (abundant rER) with eosinophilic apical granules	Pepsinogen and gastric lipase	Pepsinogen is acid-activated to pepsin (optimal pH 1.8–3.5) for protein digestion; lipase aids fat digestion
Enteroendocrine (APUD/DNES) cells	Scattered basally, deeper gland	Clear on H&E; argentaffin/argyrophilic on silver stains; "closed" (basal only) or "open" (microvillous, luminal) type	G cells—gastrin; D cells—somatostatin; ECL cells—histamine; EC cells—serotonin; P/D1 cells—ghrelin	Paracrine/endocrine control of acid secretion and motility
Stem cells	Isthmus	Undifferentiated	—	Continuous renewal of all epithelial lineages

Mechanism of HCl secretion (significance for pharmacology and pathology): carbonic anhydrase converts $\text{CO}_2 + \text{H}_2\text{O}$ to H^+ and HCO_3^- inside the parietal cell; H^+ is actively pumped into the intracellular canaliculi via the H^+/K^+ -ATPase (the target of proton-pump inhibitors) in exchange for K^+ , while HCO_3^- leaves basally in exchange for Cl^- ; Cl^- then diffuses into the canaliculus to combine with H^+ , forming HCl in the gland lumen. Parietal cell activity is stimulated via **ACh (vagal)**, **gastrin** and **histamine (H₂) receptors** — the pharmacological basis of anti-secretory drugs (PPIs, H₂-blockers, anticholinergics).

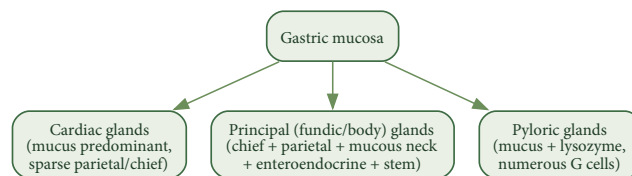
Histology of the pyloric region

Feature	Fundus/body (principal glands)	Pyloric region
Gastric pits	Shallow — occupy upper 1/3 of mucosal thickness	Deep — occupy about 2/3 of mucosal thickness
Glands	Long, occupy lower 2/3 of mucosa, less coiled, narrow lumen	Short, coiled, branched, occupy lower 1/3, wider lumen

Feature	Fundus/body (principal glands)	Pyloric region
Cell types	Parietal, chief, mucous neck, enteroendocrine, stem	Almost entirely mucus-secreting cells (similar to neck mucous cells) + lysozyme; parietal cells scarce but always present; chief cells essentially absent
Endocrine cells	Enteroendocrine cells scattered, mostly EC/ECL/D	Numerous G cells (gastrin)
Muscularis externa	Three ill-defined layers	Thick circular layer forms the pyloric sphincter

Other layers common to all regions

- **Muscularis mucosae**: thin inner circular + outer longitudinal smooth muscle.
- **Submucosa**: dense irregular connective tissue with blood vessels, lymphatics, and **Meissner's** (submucosal) plexus.
- **Muscularis externa**: classically three smooth-muscle layers — outer longitudinal, middle circular, inner oblique (the oblique layer is unique to stomach and helps churning); circular layer thickens distally into the pyloric sphincter; **myenteric (Auerbach's) plexus** lies between the layers; **interstitial cells of Cajal** (pacemaker cells) are interspersed and generate the gastric slow wave.
- **Serosa**: mesothelium-covered visceral peritoneum, continuing along the curvatures as the lesser/greater omentum and gastrosplenic/gastrophrenic ligaments.



Describe the microscopic features of stomach and their significance

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power longitudinal section of the fundic mucosa showing, top to bottom: **surface epithelium** → **gastric pit (foveola)** → **isthmus** → **neck of gland** → **base of gland** → **muscularis mucosae**. Label the cell types at their correct level:

- ▶ Surface mucous cells (lining pit and surface)
- ▶ Mucous neck cells (neck)
- ▶ Parietal/oxyntic cells (neck/upper gland, large pink cells)
- ▶ Chief/zymogenic cells (base, blue basal cytoplasm)
- ▶ Enteroendocrine cell (base, clear/unstained)
- ▶ Stem cell (isthmus)

Alongside, sketch a full-thickness diagram of the stomach wall labelling the four coats — **mucosa** (epithelium, lamina propria, muscularis mucosae) → **submucosa** (with Meissner's plexus) → **muscularis externa** (outer longitudinal, middle circular, inner oblique, with myenteric/Auerbach's plexus) → **serosa**.

□ Fig 19.15 — Histology of mucous membrane of body/fundus of stomach (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.328)

□ Fig 15.20 — Ultrastructure of parietal, chief, and enteroendocrine cells (Junqueira's Basic Histology — Text & Atlas — 17e, p.768)

Applied / Clinical anatomy

- ▶ **Peptic ulcer disease:** loss of the mucus–bicarbonate barrier (H. pylori infection, NSAID-induced prostaglandin suppression) allows acid-pepsin autodigestion of the mucosa; NSAIDs suppress PGE₂, which normally stimulates mucus and bicarbonate secretion.
- ▶ **Atrophic (autoimmune) gastritis:** antibodies against parietal cells/intrinsic factor destroy oxyntic mucosa → achlorhydria and loss of intrinsic factor → vitamin B₁₂ malabsorption → **pernicious anaemia** (megaloblastic).
- ▶ **Zollinger–Ellison syndrome:** a gastrin-secreting tumour (gastrinoma) causes parietal cell hyperplasia and refractory peptic ulceration.
- ▶ **Carcinoid tumours** arise from enterochromaffin (EC) cells; excess serotonin causes flushing, diarrhoea and mucosal vasoconstriction.
- ▶ **Gastric carcinoma:** usually an adenocarcinoma of the surface/glandular epithelium, classically preceded by the chronic gastritis → atrophy → intestinal metaplasia → dysplasia sequence.
- ▶ Pharmacological correlation: **proton-pump inhibitors** block the parietal cell H⁺/K⁺-ATPase; **H₂-receptor antagonists** block histamine action on parietal cells — both exploit the microscopic secretory mechanism above.
- ▶ Rugae flattening with distension is exploited in double-contrast barium studies and endoscopic insufflation to visualise the mucosa.

High-yield exam pointers

- ▶ Stomach wall = 4 layers; mucosa has 3 sub-layers (epithelium, lamina propria, muscularis mucosae).
- ▶ Three histological zones: cardiac, fundic/body (principal), pyloric — NOT the same as the four gross anatomical regions.
- ▶ Gastric pits: 60–100/mm²; 3–7 principal glands per pit in body/fundus; 2–3 pyloric glands per pit.
- ▶ No goblet cells, no microvilli in gastric surface epithelium (unlike intestine) — classic identification point.
- ▶ Principal gland cells: surface mucous, mucous neck, parietal (oxyntic — HCl + intrinsic factor), chief (peptic — pepsinogen + lipase), enteroendocrine, stem cells.
- ▶ Parietal cell lifespan 150–200 days; H⁺/K⁺-ATPase pumps H⁺ into intracellular canaliculi.
- ▶ Cardiac and pyloric glands lack (or nearly lack) parietal and chief cells — mucus-dominant; pyloric glands are rich in G cells (gastrin).
- ▶ Pyloric sphincter = thickened circular smooth muscle of muscularis externa.
- ▶ Clinical hooks: intrinsic factor loss → pernicious anaemia; parietal cell H⁺/K⁺-ATPase → PPI target; NSAID-PGE₂-mucus axis → gastric ulcer.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Sontakke — Textbook of Human Histology 2e; Junqueira's Basic Histology — Text & Atlas 17e; BD Chaurasia — Human Anatomy Vol 2 8e (figure).



Discuss the microscopic structure of gastric mucosa with special reference to ultra structure of oxyntic cells

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction The gastric mucosa is the innermost of the four layers of the stomach wall (mucosa, submucosa, muscularis externa, serosa) and is thrown into gastric pits that lead into long tubular gastric glands occupying the lamina propria. It is a secretory mucosa whose principal (fundic) glands contain five distinct cell types — surface mucous, mucous neck, chief, parietal (oxyntic) and neuroendocrine cells — each contributing a specific component of gastric juice. The parietal or oxyntic cell is the most structurally distinctive of these, possessing a unique intracellular canalicular/tubulovesicular ultrastructure that underlies its function of secreting hydrochloric acid and intrinsic factor.

Gross features of the gastric mucosa

- ▶ Mucosa is thick, soft and velvety, reddish-brown (pink in the pyloric region).
- ▶ Thrown into longitudinal folds or **rugae** in the contracted stomach, most marked along the greater curvature and towards the pylorus; rugae reflect folding of the submucosa and are obliterated on distension.

- ▶ The smooth mucosa along the lesser curvature forms the **gastric canal (magenstrasse)**, which fast-tracks fluids to the pylorus.
- ▶ **Areae gastricae** — subtle cobblestone mucosal undulations seen especially in the antrum on double-contrast barium studies.
- ▶ Epithelium changes abruptly from oesophageal stratified squamous to gastric simple columnar at the **Z line**, at the cardiac orifice.

General microscopic architecture of the stomach wall

Layer	Composition
Mucosa	Surface epithelium + lamina propria + muscularis mucosae
Submucosa	Loose connective tissue, collagen and elastin bundles, vessels, Meissner's (submucosal) plexus
Muscularis externa	Oblique (innermost, incomplete), circular (thickened distally as the pyloric sphincter) and longitudinal layers; Auerbach's (myenteric) plexus between the muscle coats; interstitial cells of Cajal generate slow-wave rhythmicity
Serosa	Visceral peritoneum with mesothelial lining

Surface epithelium and gastric pits

- ▶ The luminal surface and pits are lined by **simple columnar surface mucous cells** with apical mucinogen granules (vacuolated on routine H&E after mucus loss) and basal oval nuclei; they secrete a thick, adherent, bicarbonate-rich mucus that protects the mucosa from self-digestion.
- ▶ The surface is honeycombed by **gastric pits (foveolae)** — approximately 60–100 pits per mm² of mucosa, each about 70 µm in diameter and 0.2 mm deep.
- ▶ Each gastric pit receives the openings of **3–7 long, coiled tubular gastric glands** that extend through the full thickness of the lamina propria to the muscularis mucosae.
- ▶ Each gland is divided into an **isthmus** (junction with the pit, contains stem cells), a **neck**, and a **base**.

Cell types of the gastric (fundic/principal) glands

Cell type	Location in gland	Light-microscopic features	Secretion / function
Surface mucous cells	Surface and pits	Tall columnar, apical mucin, basal nucleus	Visible (insoluble) mucus + bicarbonate — protective barrier; renewed every ~3 days
Mucous neck cells	Neck region	Clear/foamy apical cytoplasm, flattened basal nucleus	Soluble mucus, biochemically distinct from surface mucus; renewed weekly

Cell type	Location in gland	Light-microscopic features	Secretion / function
Chief (peptic/zymogenic) cells	Predominantly base	Basophilic (basal, rER-rich), apical eosinophilic zymogen granules	Pepsinogen (converted to pepsin by acid) and gastric lipase
Parietal (oxyntic) cells	Numerous in upper half/neck, fewer at base	Large, rounded/pyramidal, one central nucleus, intensely eosinophilic (mitochondria-rich) cytoplasm; often bulge into lamina propria ("beaded" appearance)	HCl and intrinsic factor of Castle
Neuroendocrine cells	Deep parts of gland, basally situated	Pale/inconspicuous on H&E; argyrophil/argentaffin with silver stains	G cells – gastrin; D cells – somatostatin; ECL cells – histamine; enterochromaffin cells – serotonin; P/D1 cells – ghrelin
Stem cells	Isthmus/base of pit	Columnar, few short microvilli, mitotically active	Give rise to all other epithelial lineages; proliferate briskly after mucosal injury (e.g. ulcer healing)

□ Fig 17.10 — Photomicrograph. Fundus/body region of stomach (entire stomach wall on left, X40 and low magnification of gastric mucosa on right, X100; H&E stain) (Sontakke — Textbook of Human Histology — 2e, p.236)

Regional variation of gastric glands

Feature	Cardia	Fundus / body	Pylorus
Gastric pits	Shallow	Shallow (occupy superficial ¼ of mucosa)	Deep (occupy ~⅔ of mucosal depth)
Glands	Cardiac glands — simple tubular or compound tubulo-alveolar	Principal (fundic) glands — simple/branched tubular, 3–7 open per pit	Pyloric glands — coiled, simple/branched tubular, open via 2–3 short ducts
Dominant cells	Mucus-secreting; parietal/chief cells sparse	Full complement — chief, parietal, mucous neck, neuroendocrine, stem	Mucus-secreting cells + G cells (gastrin); parietal/chief cells scarce
Function	Mucus protection at gastro-oesophageal junction	HCl, pepsinogen, intrinsic factor secretion	Mucus protection + gastrin-mediated regulation of acid secretion

Ultrastructure of oxyntic (parietal) cells — the specific ask

Light microscopy: Large, ovoid-to-pyramidal cells with a single (occasionally binucleate) centrally placed round nucleus and strikingly eosinophilic, granular-looking cytoplasm — the eosinophilia and the "oxyntic" (Gr. *oxys*, sharp/acid) name both stem from the extremely high mitochondrial content. They lie singly, wedged between chief cells and the basement membrane, more numerous towards the isthmus/neck than the base of the gland, and often bulge outward into the lamina propria producing a beaded contour on section.

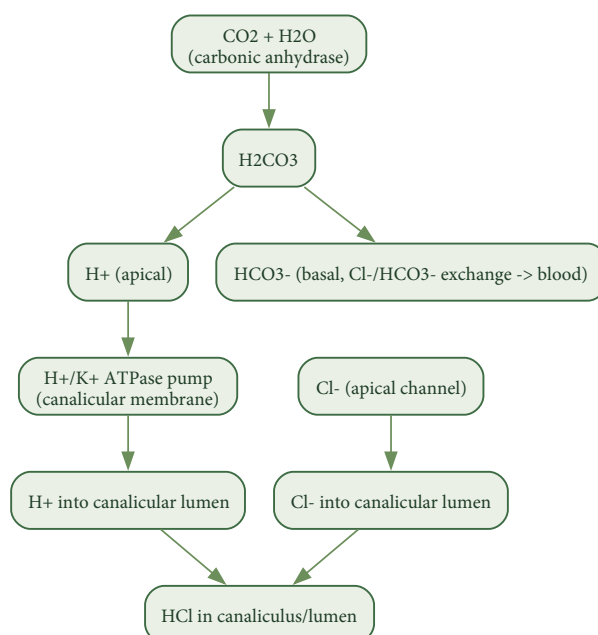
Electron microscopy — resting cell: The apical membrane shows only short, shallow intracellular canaliculi with few microvilli. The apical cytoplasm instead contains an abundant system of smooth-membraned **tubulovesicles (TV)**, in continuity with, but distinct from, the canalicular membrane.

Electron microscopy — stimulated (active) cell: On stimulation, the tubulovesicular membrane rapidly fuses with the canalicular membrane, so that:

- ▶ the apical plasma membrane invaginates deeply to form long, tortuous **intracellular canaliculi** that communicate with the glandular lumen;
- ▶ the walls of these canaliculi bear **thousands of long microvilli**, increasing the luminal membrane surface area up to five-fold;
- ▶ the perinuclear/peri-canalicular cytoplasm is packed with **numerous large mitochondria** (occupying up to 30–40% of cell volume), providing the ATP required to drive ion transport;
- ▶ a well-developed Golgi complex is present, but the cell characteristically lacks visible secretory granules, since HCl is generated by membrane ion pumps rather than stored as a preformed secretory product.

On cessation of stimulation the process reverses — microvilli are resorbed and canalicular membrane retreats back into cytoplasmic tubulovesicles.

Molecular basis of acid secretion: The canalicular (microvillous) plasma membrane is uniquely rich in the **H⁺/K⁺-ATPase (gastric proton pump)**. Carbonic anhydrase in the cytoplasm catalyses $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-$. HCO_3^- leaves the cell basolaterally in exchange for Cl^- (the "alkaline tide" into the mucosal venous blood), while H^+ is actively pumped into the canalicular lumen by the **H⁺/K⁺-ATPase** in exchange for K^+ ; Cl^- follows through apical channels. H^+ and Cl^- combine in the canalicular lumen to form HCl.



Discuss the microscopic structure of gastric mucosa with special reference to ultra structure of oxyntic cells

Parietal cells also secrete **intrinsic factor of Castle**, a glycoprotein that binds dietary (extrinsic) vitamin B12 to form a complex absorbed in the terminal ileum. Secretory activity is controlled by three convergent pathways: direct **parasympathetic** (vagal, **muscarinic-M3**) stimulation, **paracrine histamine** (via H₂ receptors, released from neighbouring ECL cells) and **endocrine gastrin** (via CCK-B/gastrin receptors, from antral G cells). Parietal cells have an estimated lifespan of 150–200 days.

□ Fig 16.4 — Electron microscope structure of an oxyntic cell (schematic representation) (IB Singh — Textbook of Human Histology — 9e, p.427)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a schematic parietal (oxyntic) cell (resting vs stimulated) with:

- ▶ Central round nucleus
- ▶ Numerous mitochondria (perinuclear/peri-canalicular)
- ▶ Intracellular (secretory) canaliculus opening into the glandular lumen
- ▶ Microvilli lining the canaliculus (short/few — resting; long/numerous — stimulated)
- ▶ Tubulovesicular system (apical cytoplasm, resting cell)
- ▶ Golgi complex
- ▶ Basolateral membrane with Cl⁻/HCO₃⁻ exchanger
- ▶ Canalicular membrane labelled "H⁺/K⁺-ATPase (proton pump)"
- ▶ Arrows showing H⁺ and Cl⁻ entering the canaliculus to form HCl

Applied / Clinical anatomy

- ▶ **Peptic ulcer disease / H. pylori gastritis** — the organism's urease locally neutralises acid and disrupts the mucous barrier, permitting acid-peptic injury through the surface epithelium; ulcers characteristically penetrate through the muscularis mucosae, unlike erosions.

- ▶ **Autoimmune (Type A) atrophic gastritis** — antibodies against parietal cells and intrinsic factor cause loss of oxyntic cells in the fundus/body, achlorhydria, and failure of intrinsic-factor-mediated B12 absorption, producing **pernicious anaemia** (megaloblastic anaemia).
- ▶ **Zollinger–Ellison syndrome** — a gastrin-secreting tumour (gastrinoma) causes parietal cell hyperplasia, hypergastrinaemia and refractory/recurrent peptic ulceration.
- ▶ **Proton-pump inhibitors** (e.g. omeprazole class) irreversibly inhibit the parietal cell's canalicular H^+/K^+ -ATPase — the definitive pharmacological target derived directly from this ultrastructure; **H2-receptor blockers** act one step upstream by blocking histamine-mediated stimulation.
- ▶ **Ménétrier's disease** — hypertrophic gastropathy with mucous cell hyperplasia and glandular/parietal cell atrophy, presenting with protein-losing enteropathy.
- ▶ **Endoscopic correlation** — antral biopsies are preferred for *H. pylori* detection because pyloric mucosa lacks the acid barrier of oxyntic mucosa; gastroscopic "magenstrasse" and rugal pattern along the lesser curvature guide biopsy and foreign-body/scope passage.
- ▶ **Gastrectomy for peptic ulcer/carcinoma** — loss of oxyntic mucosa (partial/total gastrectomy) removes the intrinsic-factor source, mandating lifelong parenteral B12 supplementation.

High-yield exam pointers

- ▶ Four layers of stomach wall: mucosa, submucosa, muscularis externa, serosa; mucosa itself has epithelium + lamina propria + muscularis mucosae.
- ▶ Gastric pits: 60–100/mm², ~70 µm diameter, ~0.2 mm deep; each pit receives 3–7 gastric glands.
- ▶ Five cell types of fundic glands: surface mucous, mucous neck, chief, parietal (oxyntic), neuroendocrine — plus stem cells at the isthmus.
- ▶ Parietal cells are eosinophilic (mitochondria-rich), secrete HCl + intrinsic factor of Castle.
- ▶ Unique ultrastructure: intracellular canaliculi with microvilli + tubulovesicular system that interconvert on stimulation (up to 5-fold surface increase).
- ▶ Molecular pump: H^+/K^+ -ATPase on canalicular membrane — target of PPIs.
- ▶ Regulation: vagal (ACh), paracrine (histamine via H2), endocrine (gastrin via CCK-B) — all converge on the parietal cell.
- ▶ Pyloric mucosa has deep pits, short mucus-secreting glands, and G cells; fundic/body mucosa has shallow pits and the full complement of secretory cells.
- ▶ Clinical anchors: pernicious anaemia (autoimmune parietal cell/intrinsic factor loss), Zollinger–Ellison syndrome, PPI mechanism.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Junqueira's Basic Histology — Text & Atlas 17e.



Enterochromaffin system

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction The enterochromaffin (EC) system is the collection of individually-scattered, hormone-secreting cells embedded within the epithelium of the gastrointestinal mucosa (stomach to colon) and the pancreatic islets/ducts. Classically identified by a positive silver (argentaffin) and chromate (chromaffin) reaction in their basal granules, these cells are now known to comprise several distinct functional populations that together make up the diffuse neuroendocrine system (DNES), also called the APUD (amine precursor uptake and decarboxylation) cell system. Although never aggregated into a discrete gland, the DNES is, by cell number, the largest endocrine organ in the body and coordinates digestion, motility, appetite and satiety through endocrine, paracrine and neurocrine mechanisms.

Nomenclature and general characteristics

- ▶ **Argentaffin cells:** the basal granules directly reduce ammoniacal silver nitrate (the true argentaffin reaction, classically demonstrated by the Masson-Fontana stain) without need for an added reducing agent — first described by Kulchitsky (1897) in intestinal crypts.
- ▶ **Chromaffin reaction:** the same granules also blacken with chromium/potassium-dichromate salts — hence the composite name **enterochromaffin (EC) cells**.
- ▶ **Argyrophil cells:** other enteroendocrine cells stain with silver only after an external reducing agent is added (e.g. the Grimelius silver method) — a wider population than the true argentaffin (EC) cells.
- ▶ **APUD cells:** because most of these cells take up amine precursors and decarboxylate them into biogenic amines/polypeptide hormones, Pearse grouped them functionally as APUD cells.
- ▶ **Diffuse neuroendocrine system (DNES):** the modern collective term for the enteroendocrine cells of the gut together with similar dispersed cells of the respiratory mucosa and pancreas — sharing dense-core secretory granules, chromogranin expression and paracrine/endocrine signalling with neurons.
- ▶ On routine H&E staining the cells are pale/unstained and inconspicuous; special silver/chromate stains or immunohistochemistry are needed for identification.

Distribution and cytological types

Region	Principal enteroendocrine cells present
Stomach — body & fundus (deep in glands, among chief cells)	D (somatostatin), ECL (histamine), EC (serotonin), P/D1 (ghrelin)
Stomach — antrum & pylorus	G (gastrin), D (somatostatin)
Duodenum & jejunum	I (CCK), S (secretin), K (GIP), Mo (motilin)
Ileum & colon	L (PYY, GLP-1), EC (serotonin)
Pancreatic islets/ducts	Cells forming part of the same gastroenteropancreatic (GEP) endocrine system

Two morphological/functional types occur throughout:

- **Closed type:** the cell apex does not reach the glandular lumen and is overlain by neighbouring epithelial cells; the base rests on the basal lamina, discharging granules towards the lamina propria/capillaries (paracrine/endocrine release). Most gastric D, ECL and EC cells are of this type.
- **Open type:** a constricted apical process bearing microvilli with chemoreceptors (of the taste-receptor T1R/T2R family) reaches the lumen to sample luminal nutrients, pH and osmolarity, triggering basal hormone release. Most gastrin (G) cells and the intestinal I, S and K cells are of this type.

Classification of enteroendocrine (DNES) cell types

Category	Hormone	Cell	Principal site	Major action
Endocrine	Gastrin	G cells	Stomach (antrum/pylorus)	Stimulates gastric acid secretion
Endocrine	Ghrelin	Gr (P/D1) cells	Stomach	Stimulates GH secretion & appetite
Endocrine	Cholecystokinin (CCK)	I cells	Duodenum, jejunum	Gallbladder contraction, pancreatic enzyme secretion; inhibits gastric emptying
Endocrine	Secretin	S cells	Duodenum	Pancreatic bicarbonate & enzyme secretion; inhibits gastric acid secretion
Endocrine	Gastric inhibitory peptide (GIP)	K cells	Duodenum, jejunum	Insulin release; inhibits gastric acid secretion
Endocrine	Motilin	Mo cells	Duodenum, jejunum	Gastric & intestinal motility
Endocrine	Pancreatic polypeptide	PP cells	Pancreas	Inhibits pancreatic secretion & gut motility
Endocrine	PYY / GLP-1	L cells	Ileum, colon	Inhibit gastric acid secretion/emptying; GLP-1 promotes insulin release
Paracrine	Somatostatin	D cells	Throughout GI mucosa	Inhibits gastrin release & gastric acid secretion

Category	Hormone	Cell	Principal site	Major action
Paracrine	Histamine	ECL (enterochromaffin-like) cells	Gastric mucosa	Stimulates gastric acid secretion via parietal-cell H ₂ receptors
Paracrine	Serotonin (5-HT)	EC cells	Throughout GI mucosa (most numerous cell type)	Increases gut motility; local paracrine action
Neurocrine	Bombesin/GRP, enkephalins, VIP	Mucosal/smooth-muscle neuroendocrine cells	Throughout gut	Modulate motility and secretion; VIP relaxes smooth muscle & sphincters

(Note the important distinction: true EC cells secrete serotonin; ECL — "enterochromaffin-like" — cells secrete histamine. This is a frequently tested confusion.)

Ultrastructure and histochemical identification

- ▶ On electron microscopy, enteroendocrine cells are small, basally-situated, pleomorphic cells with membrane-bound, dense-core secretory granules (about 0.3 µm in the gastric mucosa), concentrated towards the base near the basal lamina.
- ▶ Granules show an electron-dense core with an electron-lucent halo, a hallmark shared with neurosecretory vesicles of the CNS.
- ▶ Chromogranin (chromogranin A) is expressed within the granules and regulates their biosynthesis — the standard immunohistochemical marker used to confirm neuroendocrine differentiation in tissue sections.
- ▶ **True argentaffin reaction** (e.g. Masson-Fontana stain): granules reduce silver salts directly — positive in EC cells.
- ▶ **Argyrophil reaction** (e.g. Grimelius silver stain): requires an added external reducing agent — positive in many other enteroendocrine cells (e.g. gastric G cells, pancreatic A cells).
- ▶ These cells are not seen with routine H&E and require the above special stains, or immunoperoxidase against their specific peptide product, for reliable identification.

Embryological origin

- ▶ In the developing gut, enteroendocrine cells appear in the small-intestinal epithelium between the 9th and 11th weeks of intrauterine life — after goblet cells (from 8 weeks) but before Paneth's cells (weeks 11–12) and M cells (from 14 weeks).
- ▶ They differentiate from the **same multipotent stem-cell progenitors** in the intestinal crypt as enterocytes, goblet cells and Paneth cells — that is, they are of **endodermal**, not neural-crest, origin.
- ▶ This is an important refinement of Pearse's original APUD hypothesis, which proposed a shared neuroectodermal/neural-crest origin for all amine-handling cells (true of the adrenal medullary chromaffin cells, thyroid parafollicular C cells, and carotid-body glomus cells). The

functional/histochemical term "APUD" is retained, but it does **not** imply a common embryological origin for gut enteroendocrine cells — two cell populations may share secretory products without sharing lineage.

- The wider APUD/DNES cell family (beyond the gut) additionally includes cells of the adenohypophysis, hypothalamic neurosecretory neurons, parathyroid chief cells, thyroid C-cells, adrenal medullary and SIF cells, carotid-body glomus cells, melanocytes, pineal cells, cardiac myoendocrine cells, and renin-producing renal cells.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

embed: ![Fig 15.20 — Ultrastructure of parietal, chief, and enteroendocrine cells. (Junqueira's Basic Histology — Text & Atlas — 17e, p.768)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/db0c5cc86ffc91ac.jpg)

Hand-drawn schematic (longitudinal section of a gastric gland with an inset intestinal crypt "open" cell) — label:

- Gland lumen
- Surface mucous cell
- Mucous neck cell
- Parietal (oxyntic) cell
- Chief (zymogenic) cell
- Enteroendocrine cell, **closed type** — basal secretory granules, apex not reaching lumen
- Basal lamina
- Capillary in lamina propria (site of paracrine/endocrine discharge)
- Inset — Enteroendocrine cell, **open type** (intestinal crypt) — apical microvilli/chemoreceptors reaching the lumen



Applied / Clinical anatomy

- **Carcinoid tumour:** a neoplasm of serotonin-secreting EC cells; the vermiform appendix is the commonest gastrointestinal site of origin. When hepatic metastases allow serotonin to bypass first-pass hepatic inactivation, systemic **carcinoid syndrome** results — diarrhoea (from serotonin), episodic flushing, bronchoconstriction, and right-sided cardiac valve disease.
- **Gastroenteropancreatic neuroendocrine tumours (GEP-NETs):** the modern umbrella term for neoplastic transformation of any DNES/enteroendocrine cell of the gut or pancreas; rare but often hormonally active.
- **Zollinger-Ellison syndrome:** a gastrinoma — a tumour of gastrin-producing (G-type) enteroendocrine cells, usually in the duodenum or pancreatic islets — causes continuous parietal-cell stimulation, leading to recurrent, often multiple, peptic ulcers and diarrhoea.

- ▶ **Diagnosis:** chromogranin-A and silver (argentaffin/argyrophil) positivity are used histologically to confirm neuroendocrine differentiation in a gut biopsy or resected tumour, both in routine practice and historically before immunohistochemistry became available.

High-yield exam pointers

- ▶ EC = enterochromaffin cells: positive with **both** silver (argentaffin) **and** chromate (chromaffin) salts; secrete **serotonin**.
- ▶ ECL ("enterochromaffin-like") ≠ EC: ECL cells secrete **histamine**, not serotonin — a classic point of confusion.
- ▶ **Closed type** = base only, paracrine/endocrine release; **Open type** = apical microvilli reach the lumen, chemoreceptor sampling function.
- ▶ All gut enteroendocrine cells, together with similarly dispersed cells elsewhere in the body, constitute the **DNES/APUD cell system** (Pearse).
- ▶ Gut enteroendocrine cells are of **endodermal** origin (same crypt stem cells as enterocytes/goblet cells) — unlike the truly **neural-crest-derived** APUD cells (adrenal medulla, thyroid C-cells, carotid-body glomus cells).
- ▶ Key cell-hormone pairs: **G cell** → **gastrin** (antrum); **D cell** → **somatostatin** (throughout gut); **I cell** → **CCK** (duodenum/jejunum); **S cell** → **secretin** (duodenum); **EC cell** → **serotonin**; **ECL cell** → **histamine**.
- ▶ **Carcinoid tumour** = EC-cell neoplasm; **appendix** is the commonest site; systemic carcinoid syndrome occurs only after hepatic metastasis.
- ▶ Diagnostic immunohistochemical marker: **chromogranin A**.

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas, 17e; Ross Histology — A Text and Atlas, 7e; IB Singh, Textbook of Human Histology, 9e; Sontakke, Textbook of Human Histology, 2e; Gray's Anatomy (Standring), 41e.



Histological features of small intestine

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction The small intestine is a ~5 m long mucomuscular tube extending from the pylorus to the ileocaecal junction, divided into **duodenum** (25 cm), **jejunum** (proximal two-fifths, ~2 m) and **ileum** (distal three-fifths, ~3 m). It is the principal site of terminal digestion and nutrient absorption, and its histology is built around three surface-amplifying devices — plicae circulares, villi and microvilli — superimposed on the standard four-layered gut-tube plan. The three segments share a common architecture but differ in villus shape, glandular content and lymphoid tissue, which is the commonest basis for a spot/slide identification.

1. General plan of the gut wall

Layer	Composition
Mucosa	Epithelium (simple columnar) + lamina propria (loose connective tissue with capillaries, lacteals, lymphocytes) + muscularis mucosae (inner circular, outer longitudinal smooth muscle)
Submucosa	Dense connective tissue, larger vessels/lymphatics, submucosal (Meissner) plexus ; site of Brunner's glands in the duodenum
Muscularis externa	Inner circular + outer longitudinal smooth muscle, with the myenteric (Auerbach) plexus between the two coats — drives peristalsis
Serosa / adventitia	Mesothelium-covered connective tissue where the segment is intraperitoneal (most of jejunum/ileum); adventitia where retroperitoneal (most of duodenum)

2. Devices that increase the absorptive surface area

- **Plicae circulares (valves of Kerckring)** — permanent transverse/spiral folds of mucosa **and** submucosa that do not flatten on distension; best developed in the distal duodenum and jejunum, become sparse and disappear in the terminal ileum. They slow transit and increase surface area ~3-fold.
- **Villi** — finger-/leaf-like projections of the mucosa (epithelium + lamina propria core) covering the entire small-intestinal surface; increase area a further ~10-fold. Villus height and density fall progressively from duodenum to ileum.
- **Microvilli** — regularly packed apical projections of the enterocyte (the striated/brush border), each with a core of actin filaments anchored to the terminal web; increase area another ~20-fold. Combined, the three devices raise the absorptive surface to over 200 m².

□ Fig 18.6 — Histology of jejunum (typical features of small intestine). Low magnif (Sontakke — Textbook of Human Histology — 2e, p.248)

3. Structure of the villus

- ▶ Central **core of lamina propria** carrying fenestrated blood capillaries, a blind-ending central lymphatic (the **lacteal**), fibroblasts, smooth-muscle fibres (derived from muscularis mucosae), lymphocytes and plasma cells.
- ▶ Covered by simple columnar epithelium — enterocytes with numerous interspersed goblet cells.
- ▶ Contraction of the intrinsic smooth-muscle fibres produces rhythmic villus movement that promotes lymph flow from the lacteal into submucosal lymphatics.
- ▶ Villus : crypt ratio is about 4 : 1 in the proximal small intestine and 2–3 : 1 more distally.

4. Cell types of the epithelium

Cell	Identification	Function
Enterocyte (absorptive columnar cell)	Tall columnar, basal oval nucleus, apical striated/brush border of microvilli covered by glycocalyx	Terminal digestion (disaccharidases/peptidases in glycocalyx) and absorption; lifespan ~4–6 days, shed by apoptosis at the villus tip
Goblet cell	Glass-shaped, apical mucinogen vacuoles (empty on H&E), basal flattened nucleus; increase in number toward the ileum	Secretes protective/lubricating mucus
Paneth cell	Clusters at crypt base; large eosinophilic apical secretory granules; lifespan ~20–30 days, migrates basally (not villus-ward)	Releases lysozyme, α -defensins/cryptidins and phospholipase A2 — innate immunity of the crypt niche
Enteroendocrine cell ("open" or "closed" type, argentaffin)	Scattered basal-granule cells, argyrophilic/argentaffin	Secretes CCK, secretin, GIP, motilin and other gut peptides regulating motility and secretion
M (microfold) cell	Overlies lymphoid follicles; apical microfolds (no true brush border); deep basolateral pocket housing lymphocytes/macrophages	Trans-epithelial transport of luminal antigens to mucosal immune cells (GALT)
Stem/undifferentiated cell	Columnar cell with short irregular microvilli, in the lower crypt	Pluripotent progenitor for all other epithelial lineages

□ Fig 18.3 — Cells of small intestine. (Sontakke — Textbook of Human Histology — 2e, p.244)

5. Crypts of Lieberkühn and epithelial renewal

- ▶ Simple tubular **intestinal glands** formed by downgrowth of the surface epithelium into the lamina propria, opening at the base of the intervillous space; their lining is continuous with that of the villus.
- ▶ Contain stem cells (lower crypt), differentiating enterocytes/goblet/enteroendocrine cells (migrating up toward the villus) and Paneth cells (fixed at the base).

- Overall epithelial renewal time for absorptive and goblet cells is **4–6 days**; daughter cells move up the crypt–villus axis and are shed by apoptosis at the tip within about a week — one of the fastest-renewing epithelia in the body.

6. Regional histological variation — duodenum vs jejunum vs ileum

Feature	Duodenum	Jejunum	Ileum
Plicae circulares	Present (absent in first 5–6 cm)	Most pronounced, tall and closely set	Sparse, absent in terminal ileum
Villi	Numerous, broad/leaf-shaped	Long, numerous, tongue-shaped	Shorter, fewer, finger-shaped; absent over Peyer's patches
Goblet cells	Few	Moderate	Increase progressively, most numerous
Muscularis mucosae	Well formed	Well formed	Thin, may be absent over large lymphoid masses
Submucosal glands	Brunner's (duodenal) glands — branched tubulo-acinar, alkaline mucus (pH 8.1–9.3), ducts open at crypt bases	Absent	Absent
Lymphoid tissue	Solitary follicles only	Small, few solitary follicles	Peyer's patches — large aggregated lymphoid follicles, most numerous and largest
Serosa/adventitia	Mostly adventitia (retroperitoneal); 1st inch peritonealised	Serosa (mesenteric)	Serosa (mesenteric)

7. Gut-associated lymphoid tissue (GALT)

- **Peyer's patches** — elongated aggregates of lymphoid follicles in the lamina propria (may extend across the muscularis mucosae into the submucosa), located along the **antimesenteric border of the terminal ileum**; about 100 are present, most prominent in childhood/adolescence and regressing with age.
- Overlying epithelium contains **M cells**, which sample and transport luminal antigens to underlying lymphocytes, macrophages and dendritic cells — the afferent limb of mucosal immunity.
- Solitary lymphoid follicles occur throughout the small intestine but are sparse in the jejunum.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Longitudinal section through one villus and its adjoining crypt of Lieberkühn (label exactly):

- ▶ Villus tip with simple columnar epithelium
- ▶ Enterocyte with striated (brush) border of microvilli
- ▶ Goblet cell (mucin vacuole)
- ▶ Lamina propria core of villus — fenestrated capillary
- ▶ Central lacteal (blind lymphatic)
- ▶ Smooth-muscle fibres of villus core (from muscularis mucosae)
- ▶ Crypt of Lieberkühn opening at base of villus
- ▶ Stem/undifferentiated cells (lower crypt)
- ▶ Paneth cells with eosinophilic granules (crypt base)
- ▶ Enteroendocrine cell (basal granules)
- ▶ Muscularis mucosae (inner circular, outer longitudinal)
- ▶ Submucosa — Brunner's gland (duodenum only) / Peyer's-patch follicle (ileum only)
- ▶ Muscularis externa — inner circular + outer longitudinal, with myenteric (Auerbach) plexus between
- ▶ Serosa/adventitia

Applied / Clinical anatomy

- ▶ **Coeliac (gluten) disease** — immune reaction to gluten causes villous atrophy with crypt hyperplasia, flattening the mucosa and reducing enterocyte absorptive capacity → malabsorption; duodenal/jejunal biopsy is the diagnostic gold standard.
- ▶ **Meckel's diverticulum** — a congenital ileal diverticulum found in 2–3% of individuals, the remnant of the proximal vitello-intestinal duct; projects from the **antimesenteric border of the terminal ileum**, typically 50–100 cm proximal to the ileocaecal junction, 2–5 cm long. Its mucosa is usually ileal but may contain heterotopic gastric (or pancreatic) epithelium whose acid secretion can ulcerate the adjacent ileal mucosa, causing painless bleeding; inflammation can mimic appendicitis, with pain referred to the periumbilical region since both structures are midgut derivatives.
- ▶ **Typhoid fever (*Salmonella typhi*)** preferentially colonises and ulcerates the Peyer's patches of the terminal ileum, with risk of haemorrhage or perforation at the antimesenteric border — a direct consequence of the regional concentration of GALT there.
- ▶ **Carcinoid (neuroendocrine) tumours** arise from the enteroendocrine cells of the crypts, most commonly in the ileum.
- ▶ Villus/microvillus loss (e.g. in infective enteritis, tropical sprue) reduces the effective absorptive area disproportionately because of the multiplicative (plicae × villi × microvilli) amplification of surface area.

- ▶ **Lacteal obstruction/lymphatic disease** (e.g. intestinal lymphangiectasia) causes protein-losing enteropathy and fat malabsorption because chylomicrons are transported almost exclusively via the lacteals, not blood capillaries.

High-yield exam pointers

- ▶ Three amplifiers of surface area, in order of increasing multiplication: plicae circulares ($\times 3$), villi ($\times 10$), microvilli ($\times 20$) — combined effect ~ 600 -fold, total area $> 200 \text{ m}^2$.
- ▶ Six epithelial cell types to name with one identifying feature each: enterocyte (brush border), goblet cell (mucin vacuole), Paneth cell (basal eosinophilic granules), enteroendocrine cell (basal granules, argentaffin), M cell (overlies Peyer's patches), stem cell (crypt base).
- ▶ Villus: crypt ratio 4:1 proximally, 2–3:1 distally; epithelial turnover 4–6 days.
- ▶ Brunner's glands = submucosal, duodenum only, alkaline mucus; Peyer's patches = lamina-propria/submucosal lymphoid aggregates, ileum only, antimesenteric border, ~ 100 in number.
- ▶ Regional villus shape: duodenum leaf-shaped, jejunum tongue-shaped and tallest, ileum short/finger-shaped and fewest.
- ▶ Lacteal = central lymphatic of villus, carries absorbed chylomicrons to the thoracic duct.
- ▶ Myenteric (Auerbach) plexus between the two muscularis externa coats; submucosal (Meissner) plexus in the submucosa.
- ▶ Meckel's diverticulum and Peyer's-patch pathology (typhoid) are the classic applied correlates.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Anatomy Vol 2, 6e; Brijesh Kumar — Histology Text & Atlas 3e; Vishram Singh — Textbook of Anatomy Vol 2, 4e.



Histological structure of the gall bladder

Asked in: Paper III Nov 2016 — RGUHS MD/MS Anatomy

Definition / Introduction — The gall bladder is a pear-shaped, mucomuscular, blind-ending sac lodged in the fossa on the visceral (inferior) surface of the right lobe of the liver, with a capacity of about 30-50 mL. It is a secondary derivative of the embryonic foregut, developing as an evagination (cystic bud) of the hepatic diverticulum that connects the primitive liver to the developing duodenum, and it functions to concentrate and temporarily store bile delivered by the cystic duct until cholecystokinin (CCK)-induced contraction expels it into the duodenum. Histologically its wall is deceptively simple — only **three coats** (mucosa, fibromuscular coat, serosa/adventitia) — but several distinctive features (absent muscularis mucosae/submucosa, Rokitsky-Aschoff sinuses, absorptive columnar epithelium) make it a favourite practical/viva and theory topic.

1. General plan of the wall

Layer	Composition	Distinctive point
Mucosa	Simple columnar epithelium + lamina propria, thrown into numerous temporary folds (rugae)	No muscularis mucosae
Fibromuscular coat (muscularis externa)	Smooth muscle bundles interlaced with collagen and elastic fibres	No submucosa; muscle fibres randomly oriented (not layered as in gut)
Serosa / Adventitia	Mesothelium + connective tissue (serosa where free; adventitia where attached to liver)	Peritoneal covering only on the free (inferior) surface

Unlike the rest of the alimentary canal, the gall bladder wall therefore has **only three layers** — the muscularis mucosae and submucosa, present everywhere else in the gut tube, are absent (Ross; Junqueira; IB Singh; Sontakke).

 Fig 16.20 — Gallbladder. (Junqueira's Basic Histology — Text & Atlas — 17e, p.845)

2. Mucosa

- ▶ **Surface configuration:** interior shows numerous branching, temporary mucosal folds that flatten out and disappear when the organ distends with bile; this gives a characteristic **honeycomb appearance** on cut section.
- ▶ **Lining epithelium:** **simple tall columnar epithelium** with a basally placed, ovoid nucleus and pale eosinophilic cytoplasm.
 - Apical surface bears numerous short **microvilli**, described classically as a **striated (brush) border** — these increase surface area for water/electrolyte absorption.
 - Apical junctional complexes seal the lumen from the lateral intercellular space; complex basolateral membrane infoldings and abundant apical/basal mitochondria support active $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ transport (Na^+-K^+ ATPase on lateral membranes) followed by passive water movement through **aquaporin-1 and aquaporin-8** water channels — the structural basis of bile concentration.

- **Goblet cells are absent** (key differentiating feature from intestine).
- ▶ **Lamina propria:** loose, cellular connective tissue rich in **fenestrated capillaries** and small venules; contains lymphocytes and plasma cells but characteristically **has no lymphatic vessels**.
 - Occasional **mucosal crypts** appear owing to tangential sectioning of the deep folds.
 - **Mucous (mucin-secreting) glands** may be present in the lamina propria, particularly near the **neck** of the gall bladder, and are more prominent in chronic cholecystitis; scattered enteroendocrine-like cells occur in these glands.
 - At the neck, the mucosal folds become spiral, continuing into the cystic duct as the **spiral valve (of Heister)**.
- ▶ **No muscularis mucosae and no submucosa** anywhere in the gall bladder wall.

3. Fibromuscular coat (muscularis externa)

- ▶ Composed of bundles of **smooth muscle cells** with interspersed **collagen and elastic fibres**.
- ▶ Smooth muscle is **not organised into distinct layers** (unlike the circular/longitudinal layers of the gut) — fibres run in haphazard, criss-crossing directions, an arrangement that allows uniform, omnidirectional contraction to expel bile efficiently.
- ▶ This combined muscle + connective-tissue layer is often termed the **fibromuscular coat**; a similar fibromuscular arrangement is seen in the extrahepatic biliary tree and the ureter.
- ▶ Contraction is stimulated hormonally by **cholecystokinin (CCK)** released from duodenal enteroendocrine cells in response to luminal fat, reinforced by parasympathetic (vagal) stimulation, which also relaxes the sphincter of Oddi.

4. Rokitansky-Aschoff sinuses

- ▶ **Deep diverticula/invaginations of the mucosal epithelium** that herniate through the fibromuscular coat into, or through, the muscularis externa.
- ▶ Named for **Carl Freiherr von Rokitansky** (1804-1878) and **Ludwig Aschoff** (1866-1942), pathologists.
- ▶ Thought to arise from epithelial hyperplasia with herniation through the muscle coat; they are lined by the same simple columnar epithelium as the surface mucosa and may resemble glands, but are true mucosal extensions, not true glands.
- ▶ **Clinical significance:** bacteria and biliary sludge can lodge within these sinuses, predisposing to chronic inflammation and acting as a nidus/risk factor for gallstone formation and chronic cholecystitis.

□ Fig 18.17 — ▲ Photomicrograph of the Rokitansky-Aschoff sinuses in the wall of the (Ross Histology — A Text and Atlas — 7e, p.660)

5. Serosa / adventitia

- ▶ Consists of a thick layer of dense connective tissue carrying large blood vessels, an extensive lymphatic network, autonomic nerve fibres, elastic fibres, and adipose tissue.
- ▶ The surface **not** attached to the liver (fundus and part of the body, projecting into the peritoneal cavity) is covered by true **serosa** — a mesothelium (simple squamous epithelium/visceral peritoneum) resting on this connective tissue.

- The surface applied to the hepatic fossa, where the gall bladder is attached to the liver by loose areolar tissue with venous communications, is covered only by **adventitia** (no mesothelial lining).

6. Histological differentiation of the gall bladder from the small intestine

A recurring practical/viva point — mucosal folds of the collapsed gall bladder can superficially mimic intestinal villi.

Feature	Gall bladder	Small intestine
Surface folds	Irregular, variable size/shape mucosal folds (rugae); disappear on distension	Regular, uniform-sized villi in a given segment
Goblet cells	Absent	Present
Muscularis mucosae	Absent	Present
Submucosa	Absent	Present
Muscularis externa	Poorly organised, random smooth-muscle bundles (fibromuscular coat)	Well-organised inner circular/outer longitudinal layers
Special mucosal feature	Rokitansky-Aschoff sinuses	Crypts of Lieberkühn, Peyer's patches (ileum)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a cross-section through the gall bladder wall (low power, with an inset of the mucosal epithelium at high power):

- Lumen (containing bile)
- Mucosal fold (rugae) lined by simple columnar epithelium
- Surface epithelial cell — tall columnar, basal nucleus, apical striated (microvillous) border
- Lamina propria — loose connective tissue with fenestrated capillaries (no lymphatics); occasional mucous glands near the neck
- Rokitansky-Aschoff sinus — mucosal diverticulum penetrating the fibromuscular coat
- Fibromuscular coat (muscularis externa) — randomly oriented smooth-muscle bundles with interspersed collagen/elastic fibres
- Perimuscular connective tissue — large vessels, lymphatics, autonomic nerve plexus, adipose tissue
- Serosa (mesothelium, free/peritoneal surface) vs Adventitia (hepatic/attached surface)

Applied / Clinical anatomy

- **Cholecystitis:** acute or chronic inflammation of the gall bladder, commonly precipitated by gallstones obstructing the cystic duct/neck; bacteria trapped in Rokitansky-Aschoff sinuses perpetuate chronic inflammation.

- ▶ **Cholelithiasis:** gallstone formation follows supersaturation of bile with cholesterol (cholesterol stones) or excess unconjugated bilirubin, e.g. in chronic haemolytic states such as sickle cell disease (pigment stones); the concentrating (water-absorptive) function of the mucosal epithelium is central to this pathogenesis.
- ▶ **Adenocarcinoma of the gall bladder** typically arises against a background of chronic cholecystitis and long-standing gallstones; the columnar mucosal epithelium is the tissue of origin.
- ▶ **Porcelain gall bladder:** dystrophic calcification of a chronically inflamed wall — a recognised (though debated) risk factor for carcinoma.
- ▶ **Cholecystography/USG:** the epithelium's ability to concentrate contrast/bile is exploited diagnostically (oral cholecystography historically; ultrasonography is now the imaging modality of choice for wall thickness, stones, and sludge).
- ▶ **Empyema/mucocele of the gall bladder** can follow persistent cystic duct obstruction, distending the organ with pus or mucus respectively.
- ▶ **Surgical correlate:** laparoscopic cholecystectomy is guided by knowledge of the mucosal/fibromuscular anatomy at Calot's triangle and the neck-cystic duct junction (spiral valve of Heister).

High-yield exam pointers

- ▶ Gall bladder wall = **only 3 layers** — mucosa, fibromuscular coat, serosa/adventitia; **no muscularis mucosae, no submucosa**.
- ▶ Mucosa = simple tall columnar epithelium with **striated (microvillous) border**, basal nucleus, **no goblet cells**; folds create a **honeycomb** appearance.
- ▶ Lamina propria has **fenestrated capillaries but no lymphatics**; mucous glands may occur near the neck.
- ▶ **Rokitansky-Aschoff sinuses** = mucosal diverticula penetrating the muscularis externa; risk factor for stones/chronic cholecystitis.
- ▶ Muscularis externa = smooth muscle **randomly/haphazardly arranged**, not layered as elsewhere in the gut.
- ▶ Capacity ≈ **30-50 mL**; epithelium actively concentrates bile via $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ transport and aquaporin (AQP1, AQP8) water channels.
- ▶ Classic differentiators from small intestine: absent villi (only folds), absent goblet cells, absent submucosa, disorganised muscularis externa.
- ▶ Serosa covers the free (peritoneal) surface; adventitia covers the hepatic (attached) surface.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology 17e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; Vishram Singh Textbook of Anatomy Vol 2 4e (gross/developmental correlation).



Histology of Gastro intestinal tract

Asked in: Paper III 02-Aug-2021 — RGUHS MD/MS Anatomy

Definition / Introduction The gastrointestinal tract (GIT) — oesophagus, stomach, small intestine and large intestine — is a mucomuscular tube built throughout on a common four-layered plan: **mucosa, submucosa, muscularis externa and serosa/adventitia**. Superimposed on this constant framework, each region shows characteristic regional modifications of the mucosa (and occasionally the other layers) that reflect its specific function — protection in the oesophagus, secretion/digestion in the stomach, absorption in the small intestine, and water reabsorption/storage in the large intestine. A complete answer must give the general plan, the region-specific histology of each segment with its cell types, the enteric nerve plexuses and gut-associated lymphoid tissue (GALT), and the applied correlates.

1. General plan of the gastrointestinal wall

Layer	Composition	Notes
Mucosa	Lining epithelium + lamina propria (loose connective tissue, glands, lymphoid tissue) + muscularis mucosae (inner circular, outer longitudinal smooth muscle)	The only layer that shows major regional variation along the GIT
Submucosa	Dense connective tissue with larger blood vessels/lymphatics; ganglionated submucosal (Meissner's) plexus	May contain glands (oesophageal glands, Brunner's glands of duodenum)
Muscularis externa	Inner circular + outer longitudinal smooth muscle (three layers in stomach — inner oblique, middle circular, outer longitudinal); myenteric (Auerbach's) plexus lies between the two principal coats	Drives peristalsis; the two intrinsic plexuses form the enteric nervous system , derived from neural-crest cells that migrate craniocaudally into the gut wall
Serosa / adventitia	Mesothelium + thin connective tissue (serosa) where intraperitoneal; connective tissue merging with adjacent structures (adventitia) where retroperitoneal	Duodenum and ascending/descending colon have both serosa (anterior) and adventitia (posterior)

2. Oesophagus

- **Epithelium:** non-keratinised stratified squamous — purely protective; changes abruptly to simple columnar at the **cardio-oesophageal junction**.
- **Lamina propria:** a few lymphoid follicles; muscularis mucosae is a thin, longitudinally-arranged smooth-muscle layer.
- **Submucosa:** contains **oesophageal glands** (mucous, tubulo-acinar) that lubricate the lumen; near the stomach end, **cardiac oesophageal glands** are also present.

- ▶ **Muscularis externa** is unique in composition along its length: **upper one-third** — skeletal muscle; **middle one-third** — mixed skeletal and smooth muscle; **lower one-third** — smooth muscle only.
- ▶ **Outer coat**: adventitia in the thoracic part; serosa in the short abdominal part.
- ▶ **Cardio-oesophageal junction**: abrupt epithelial transition (stratified squamous → simple columnar); lamina propria of the cardiac stomach shows gastric pits and mucus-secreting cardiac glands; oesophageal glands persist in the submucosa just proximal to the junction.

3. Stomach

The stomach has four gross regions — **cardia, fundus, body and pylorus**; the mucosa of fundus and body is histologically identical and differs from that of the pylorus.

- ▶ **Mucosa**: simple columnar surface-mucous epithelium invaginates to form **gastric pits**, into whose base open the **gastric glands** (branched, simple tubular; parts = isthmus, neck, base).
- ▶ The **ratio of pit depth : gland length** is the key discriminator between regions — **1 : 3 in fundus/body** (short pits, long glands) versus **3 : 1 in pylorus** (long pits, short, more-coiled glands).

Cell type	Location in gland	Product / feature
Surface mucous cell	Surface epithelium, gastric pit	Protective mucous film over the mucosa
Mucous neck cell	Neck	Basal round nucleus; secretes mucus
Parietal (oxyntic) cell	Isthmus and neck, few at base	Eosinophilic (numerous mitochondria); intracellular canaliculus with microvilli; secretes HCl and intrinsic factor (B12 absorption); bulges laterally giving the gland a beaded look
Chief / zymogen cell	Base (majority)	Basophilic (abundant RER); secretes pepsinogen, rennin and gastric lipase
Enteroendocrine cell	Base (fundus/body: mainly serotonin-secreting EC cells); in contact with the lumen in the pylorus (G cells → gastrin)	Part of the diffuse neuroendocrine system (DNES/APUD cells)

□ Fig 19.15 — Histology of mucous membrane of body/fundus of stomach (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.328)

Feature	Fundus/Body	Pylorus
Gastric pit	Short	Long
Gastric gland	Long, less coiled	Short, more coiled
Parietal cells	Numerous	Very few
Chief (zymogen) cells	Numerous	Absent

Feature	Fundus/Body	Pylorus
Predominant secretory cell	Parietal + chief	Mucus-secreting cells; G cells (gastrin)

- **Muscularis externa** of stomach has **three** layers — inner oblique, middle circular, outer longitudinal; the middle circular thickens at the pylorus to form the **pyloric sphincter**.
- Submucosa of stomach has **no glands** (unlike oesophagus).

4. Small intestine (duodenum, jejunum, ileum)

The small intestine amplifies absorptive surface area through three superimposed devices — permanent mucosal-and-submucosal folds (**plicae circulares/valves of Kerckring**, $\sim 3\times$), finger-like mucosal projections (**villi**, a further $\sim 10\times$) and apical **microvilli** (striated/brush border, $\sim 20\times$), together raising the effective area to over 200 m^2 .

Cell (epithelium + crypts)	Identification	Function
Enterocyte	Tall columnar, striated (brush) border	Terminal digestion and absorption
Goblet cell	Mucin vacuoles, basal flattened nucleus; increase distally	Protective/lubricating mucus
M (microfold) cell	Overlies lymphoid follicles	Transports luminal antigens to GALT
Paneth cell	Crypt base, eosinophilic apical granules	Secretes lysozyme and defensins — innate immunity
Enteroendocrine cell	Base of crypt, basal granules	Gut hormones
Stem cell	Lower half of crypt	Renews all epithelial lineages

□ Fig 13.16 — Villi and crypt of Lieberkuhn. (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.203)

- Each villus carries a lamina-propria core with fenestrated capillaries and a central blind-ending lymphatic, the **lacteal**, plus smooth-muscle fibres from the muscularis mucosae that produce rhythmic villus movement.
- **Crypts of Lieberkühn** are simple tubular intestinal glands between villus bases; new cells arise from crypt stem cells, migrate up the crypt-villus axis and are shed at the tip — overall epithelial renewal is about **4–6 days**, among the fastest in the body.

Feature	Duodenum	Jejunum	Ileum
Villi	Leaf-shaped	Tongue-shaped, tallest	Finger-shaped, short, fewest
Submucosal glands	Brunner's (duodenal) glands — alkaline mucus, ducts open into crypt bases	Absent	Absent

Feature	Duodenum	Jejunum	Ileum
Lymphoid tissue	Solitary follicles only	Sparse solitary follicles	Peyer's patches — large lymphoid aggregates on the antimesenteric border
Fourth layer	Serosa + adventitia (retroperitoneal)	Serosa	Serosa

5. Large intestine, appendix and anal canal

The large intestine (caecum, colon, rectum, anal canal) has a larger diameter than the small intestine and its structure is uniform throughout except the anal canal.

- **Mucosa:** no villi and no plicae circulares; simple columnar epithelium with **more goblet cells** than the small intestine; crypts of Lieberkühn are present but contain **no Paneth cells**; lamina propria has numerous lymphoid follicles.
- **Muscularis externa:** inner circular + outer longitudinal, but the outer longitudinal coat condenses into three bands, the **taenia coli**.
- The peritoneum shows fat-filled outpouchings, the **appendices epiploicae**.
- **Fourth layer:** serosa in intraperitoneal parts (transverse, sigmoid colon); serosa + adventitia in retroperitoneal parts (ascending, descending colon).

□ Fig 18.12 — Histology of large intestine (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.252)

Feature	Small intestine	Large intestine
Villi / plicae	Present	Absent
Goblet cells	Present	More numerous
M cells	Present	Absent
Paneth cells (crypts)	Present	Absent

- **Appendix:** resembles colon, but lamina propria has abundant lymphoid follicles that extend into the submucosa, **no taenia coli**, and a smaller lumen.
- **Anal canal:** upper mucosa shows 6–10 permanent longitudinal folds, the **anal columns of Morgagni**, joined below by anal valves at the level of the **pectinate line**. Epithelium changes from simple columnar (colonic) → **stratified squamous non-keratinised** at the pectinate line → **stratified squamous keratinised** (epidermis) below, with apocrine circumanal glands in the lowest zone. Submucosa contains the **internal and external haemorrhoidal venous plexuses**. The inner circular muscularis-externa layer thickens into the **internal anal sphincter** (involuntary smooth muscle); the **external anal sphincter** is skeletal muscle under voluntary control; the fourth layer is adventitia.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Cross-section through a typical segment of gut wall, labelling the four layers and their contents (base this on the region asked, e.g. jejunum):

- ▶ Mucosa — lining epithelium (simple columnar with goblet cells)
- ▶ Lamina propria (loose connective tissue, capillaries, lymphocytes)
- ▶ Muscularis mucosae (inner circular, outer longitudinal)
- ▶ Submucosa with blood vessel and **submucosal (Meissner's) plexus**
- ▶ Muscularis externa — inner circular layer
- ▶ **Myenteric (Auerbach's) plexus** between the two muscle coats
- ▶ Muscularis externa — outer longitudinal layer
- ▶ Serosa (mesothelium + connective tissue) or adventitia

Applied / Clinical anatomy

- ▶ **Barrett's oesophagus** — chronic gastro-oesophageal reflux causes metaplastic replacement of the lower oesophageal squamous epithelium by columnar epithelium; a recognised risk factor for oesophageal adenocarcinoma.
- ▶ **Gastric ulcer** — breach of the gastric mucosa, most commonly due to *Helicobacter pylori* infection (also cigarettes, alcohol, aspirin/NSAIDs); may be benign or malignant.
- ▶ **Duodenal ulcer** — unlike gastric ulcers, duodenal ulcers essentially never turn malignant.
- ▶ **Coeliac disease** (gluten-sensitive enteropathy) — gluten sensitivity damages small-intestinal villi, producing villous atrophy with crypt hyperplasia and malabsorption.
- ▶ **Typhoid ulcer** — *Salmonella typhi* invades the ileal epithelium and ulcerates the Peyer's patches, with risk of haemorrhage or perforation at the antimesenteric border.
- ▶ **Ulcerative colitis** — an inflammatory bowel disease with mucosal/submucosal ulceration of colon and rectum, producing bloody diarrhoea.
- ▶ **Hirschsprung disease** — arrested craniocaudal migration of neural-crest cells leaves an aganglionic segment lacking Meissner's and myenteric plexuses; the uncoordinated, contracted segment causes functional obstruction with proximal constipation and distension.
- ▶ **Appendicitis** — inflammation of the appendix with mucosal ulceration and neutrophilic infiltration.
- ▶ **Carcinoid tumours** arise from enteroendocrine (EC) cells and can cause carcinoid syndrome through serotonin overproduction.

High-yield exam pointers

- ▶ Four constant layers everywhere: mucosa, submucosa, muscularis externa, serosa/adventitia — only the mucosa (and occasionally submucosal glands) shows major regional variation.
- ▶ Oesophagus: non-keratinised stratified squamous epithelium; muscularis externa skeletal (upper 1/3) → mixed (middle 1/3) → smooth (lower 1/3).

- ▶ Stomach: gastric pit : gland ratio 1:3 in fundus/body vs 3:1 in pylorus; parietal cells (HCl + intrinsic factor) and chief cells (pepsinogen) confined to fundus/body; chief cells absent in pylorus; three muscle layers with pyloric sphincter.
- ▶ Small intestine surface amplifiers: plicae circulares (~3×), villi (~10×), microvilli (~20×); Brunner's glands = duodenum only; Peyer's patches = ileum, antimesenteric border only; epithelial renewal ~4–6 days.
- ▶ Large intestine: no villi/plicae, more goblet cells, no Paneth cells, taenia coli and appendices epiploicae are unique gross-histological markers.
- ▶ Anal canal: pectinate line marks the columnar-to-squamous transition and the portal-to-systemic venous drainage change (internal vs external hemorrhoidal plexus).
- ▶ Enteric nervous system: submucosal (Meissner's) plexus + myenteric (Auerbach's) plexus, both neural-crest derived — their absence underlies Hirschsprung disease.
- ▶ GALT: M cells, Peyer's patches (ileum), appendix and diffuse lymphoid follicles form the mucosal immune surveillance system of the gut.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Brijesh Kumar — Histology Text & Atlas 3e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; BD Chaurasia — Human Anatomy Vol 2, 8e.



Histology of liver – structural and functional correlations

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction — The liver is the largest gland in the body (~1500 g) and a modified compound tubuloacinar exocrine gland (bile secretion) that also performs vast metabolic, synthetic, storage and detoxification functions. Its microarchitecture — the arrangement of hepatocyte plates around a dual afferent (portal triad) and single efferent (central vein) vascular system, the sinusoidal lining, and the space of Disse — is built to place every hepatocyte in direct contact with plasma, so that structure directly explains function: exchange, synthesis, storage, detoxification and bile secretion all occur across the same sinusoidal surface.

Gross-to-microscopic organization

- ▶ **Capsule:** liver is enclosed by a connective tissue capsule, the **capsule of Glisson**; this extends into the parenchyma along the portal triads (portal vein, hepatic artery, bile duct) as periportal connective tissue.
- ▶ **Stroma:** a fine meshwork of **reticular (type III collagen) fibres** supports hepatocyte plates; these are argyrophilic (blackened on silver impregnation).
- ▶ **Parenchyma:** **hepatocytes**, large (20–30 µm) polygonal cells with a round, euchromatic (open-faced) nucleus, 1–2 nucleoli; many are binucleate or polyploid (4n), reflecting high metabolic/synthetic activity; lifespan ~5 months with strong regenerative capacity.

- **Dual blood supply:** **portal vein** (~75–80%), nutrient-rich but less oxygenated blood from the gut/spleen/pancreas, and **hepatic artery** (~20–25%), oxygen-rich blood — both enter the liver at the porta hepatis and, via the portal triads, empty into the hepatic sinusoids, which drain centripetally to the **central (terminal hepatic) vein** → **sublobular vein** → **hepatic vein** → IVC (hepatic veins have no valves).
- **Bile flow** runs in the opposite (centrifugal) direction — from bile canaliculi towards the portal triad — the reverse of blood flow, a key structure-function point.

Three models of liver lobulation

Model	Shape	Centre	Periphery/ vertices	Functional emphasis
Classic hepatic lobule	Hexagonal	Central (terminal hepatic) vein	Portal triads at the six corners	Best depicts direction of blood flow (structural unit)
Portal lobule	Triangular	Portal triad (bile duct as its axis)	Central veins at the corners	Emphasises exocrine bile secretion (functional unit for bile)
Liver acinus (of Rappaport)	Diamond/lozenge-shaped	Short axis = line between 2 nearest portal triads; long axis = line between 2 nearest central veins	Central veins at the two poles	True structural AND functional unit — explains metabolic zonation and the pattern of hepatic injury

- In humans the classic lobules are not sharply demarcated by connective tissue septa (unlike in the pig, where dense septa outline them clearly).
- **Portal triad** (portal canal) = a branch each of the **hepatic artery**, **hepatic portal vein** and **bile ductule**, running together in periportal connective tissue at the corners of the classic lobule; the central/hepatic vein is **not** part of the portal triad (a common MCQ trap).

Metabolic zonation of the liver acinus (the core structure–function link)

Zone	Position	Blood supply	Functional/ pathological correlation
Zone 1 (periportal)	Nearest short axis/portal triad	Richest in oxygen & nutrients, but first exposed to absorbed toxins	Oxidative energy metabolism — gluconeogenesis, β -oxidation of fatty acids, ureagenesis, cholesterol/bile-acid synthesis; first cells to show changes with bile-duct occlusion/cholestasis ; last to die in ischaemia; first to regenerate
Zone 2	Intermediate	Intermediate; no sharp boundary	Transitional metabolic activity
Zone 3 (centrilobular/perivenous)	Nearest central vein	Least oxygenated (last to receive sinusoidal blood)	Rich in smooth ER/cytochrome P450 — glycolysis, lipogenesis, drug/alcohol detoxification; most vulnerable to hypoxia — first to undergo ischaemic (centrilobular) necrosis , e.g. in shock or congestive cardiac failure (" cardiac cirrhosis "/nutmeg liver, with minimal nodular regeneration unlike true cirrhosis); also most susceptible to many hepatotoxins bio-activated by cytochrome P450

This zonal concept is the standard framework used to interpret patterns of hepatocyte degeneration, regeneration and toxic/ischaemic injury seen on liver biopsy.

Hepatocyte ultrastructure–function correlation

Organelle/feature	Functional correlation
Round, euchromatic, often binucleate/polyploid nucleus, 1–2 nucleoli	High synthetic activity; regenerative reserve
Abundant rough ER	Synthesis of plasma proteins — albumin, fibrinogen, clotting factors

Organelle/feature	Functional correlation
Abundant smooth ER	Detoxification (cytochrome P450 system), cholesterol and bile-acid synthesis; glycogen granules cluster around SER
Well-developed Golgi complex	Packaging/secretion of plasma proteins and lipoproteins (VLDL)
Numerous mitochondria	High ATP yield for synthetic, detoxifying and secretory work
200–300 peroxisomes/cell	Catalase-mediated H ₂ O ₂ detoxification (e.g. alcohol), fatty-acid β -oxidation, gluconeogenesis, purine metabolism
Lysosomes concentrated near the bile canaliculus	Autophagy; accumulate lipofuscin pigment with age
Glycogen (PAS+) and lipid (Sudan+) inclusions	Storage of glucose and lipid

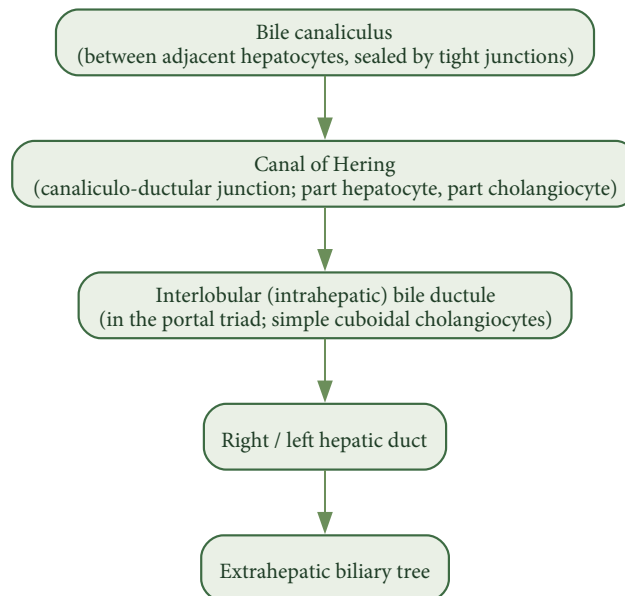
Three specialised hepatocyte surfaces underlie this traffic:

- ▶ **Sinusoidal surface** — bears microvilli projecting into the space of Disse, plus coated pits for endo-/exocytosis; the main site of plasma exchange.
- ▶ **Canalicular surface** — forms the wall of the bile canaliculus (~0.5 μ m) with the adjoining hepatocyte, sealed off from the intercellular space by tight junctions.
- ▶ **Intercellular (lateral) surface** — joined to neighbouring hepatocytes by gap junctions (metabolic/electrical coupling) and desmosomes.

Sinusoids and the perisinusoidal (Disse) space

- ▶ **Hepatic sinusoids** are wide, irregular vascular channels between hepatocyte plates (which are **one cell thick** in adults; two cells thick up to ~7 years of age, a pattern that recurs during regeneration).
- ▶ Lined by a **thin, discontinuous, fenestrated endothelium lacking a continuous basal lamina** — this "leaky" sieve-plate arrangement permits free plasma exchange with hepatocytes and explains the liver's rapid metabolic turnover.
- ▶ **Kupffer cells** — stellate sinusoidal macrophages derived from circulating monocytes, resident within the sinusoidal lumen attached to the endothelium; they phagocytose aged erythrocytes, bacteria and gut-derived endotoxin delivered by portal blood — the liver's first line of defence against enteric antigens.
- ▶ **Pit cells** — natural-killer lymphocytes within the sinusoidal lumen, providing immune surveillance.
- ▶ **Perisinusoidal space of Disse** (0.2–0.5 μ m, widens in hypoxia) — lies between the sinusoidal endothelium and the hepatocyte microvilli; site of hepatocyte–plasma exchange, of **hepatic lymph formation**, and (in fetal life) of **haematopoiesis**.
- ▶ **Hepatic stellate cells (Ito cells)** — mesenchymal cells within the space of Disse that store **vitamin A and lipid droplets**; in chronic liver injury they transdifferentiate into **myofibroblasts**, depositing collagen and driving **hepatic fibrosis/cirrhosis** — a key clinical correlation.

- **Space of Mall** (periportal space) — a smaller lymph-forming space between the portal connective tissue and the outermost hepatocytes.



Histology of liver – structural and functional correlations

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a **classic hepatic lobule** in cross-section and label:

- Central (terminal hepatic) vein at the centre
- Radiating, anastomosing **hepatocyte plates** (one cell thick), separated by **sinusoids**
- A **portal triad** at one corner — branch of hepatic artery, branch of portal vein, and a bile ductule
- Within a sinusoid: the fenestrated **endothelial lining**, a **Kupffer cell**, and the **space of Disse** with a **hepatic stellate (Ito) cell**
- **Bile canaliculi** between hepatocytes, with arrows showing **bile flow towards the portal triad** and, alongside, arrows showing **blood flow from the portal triad towards the central vein** (opposite directions)
- Superimpose the **acinus zones 1–2–3** radiating from the portal triad (short axis) to the central vein

□ Fig 16.12 — Hepatic lobule. (Junqueira's Basic Histology — Text & Atlas — 17e, p.829)

Applied / Clinical anatomy

- **Centrilobular (zone 3) necrosis** — the pattern seen in shock and congestive cardiac failure ("nutmeg liver"/cardiac cirrhosis); zone 3 is farthest from oxygenated inflow and dies first, while zones 1–2 remain largely unaffected; unlike true cirrhosis, nodular regeneration is minimal.
- **Periportal (zone 1) injury** — the pattern seen with cholestasis/bile-duct occlusion and with toxins absorbed directly from portal blood, since zone 1 hepatocytes are exposed first.
- **Chronic hepatitis/alcoholic liver disease** — sustained Kupffer-cell activation and cytokine release drive Ito cells to transform into myofibroblasts, laying down collagen in the space of

Disse; progressive fibrosis converts the normal lobular architecture into fibrous septa enclosing regenerative nodules — the histological basis of **cirrhosis**.

- ▶ **Regenerative capacity** — hepatocyte hyperplasia/hypertrophy (recapitulating the two-cell-thick plate pattern of early childhood) underlies recovery after partial hepatectomy and living-donor liver transplantation.
- ▶ **Kupffer cell dysfunction** in advanced cirrhosis contributes to portal bacteraemia/endotoxaemia.
- ▶ Liver biopsy interpretation in clinical hepatology is built on this **zonal (acinar) concept** to localise and pattern-recognise ischaemic, toxic, viral and cholestatic injury.

High-yield exam pointers

- ▶ Three descriptive models of liver architecture: **classic hepatic lobule** (structural, blood-flow based), **portal lobule** (functional, bile-based), **liver acinus of Rappaport** (true structural + functional unit).
- ▶ **Portal triad** = branch of hepatic artery + branch of portal vein + bile ductule — the **central vein** is **never part of the portal triad**.
- ▶ **Zone 1 (periportal)**: oxygen-rich, first to regenerate, last to undergo ischaemic necrosis, first affected by cholestasis/portal toxins.
- ▶ **Zone 3 (centrilobular)**: oxygen-poor, first to undergo ischaemic (centrilobular) necrosis, richest in cytochrome P450 — most vulnerable to many hepatotoxins.
- ▶ Sinusoids have a **discontinuous, fenestrated endothelium without a continuous basal lamina**, plus resident **Kupffer cells**.
- ▶ **Space of Disse** — site of hepatic lymph formation, fetal haematopoiesis, and home to **Ito (hepatic stellate) cells** whose activation causes fibrosis/cirrhosis.
- ▶ Hepatocyte plates are **one cell thick** in adults (two cells thick till ~7 years; the childhood pattern reappears during regeneration).
- ▶ **Bile flows centrifugally** (canaliculus → canal of Hering → interlobular duct → portal triad), **opposite** to the centripetal direction of blood flow (portal triad → sinusoid → central vein).
- ▶ Hepatocyte organelle profile (abundant RER/SER, mitochondria, peroxisomes) directly explains its roles in protein synthesis, detoxification and metabolism — the classic structure–function correlation asked in this question.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology — Text & Atlas 17e.



Histology of pancreas

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction The pancreas is a retroperitoneal, mixed exocrine-endocrine gland extending from the concavity of the duodenum to the hilum of the spleen, with head, neck, body and tail regions. Structurally it is a **compound tubuloacinar (tubuloalveolar) serous gland**, closely resembling the parotid gland, but distinguished from it by three cardinal histological features — presence of **islets of Langerhans**, presence of **centroacinar cells**, and **absence of striated ducts**. About 98% of the gland's mass is exocrine tissue producing digestive enzymes; the remaining 1–2% is endocrine tissue (the islets) that produces glucagon and insulin. Both components share a common embryonic origin from endodermal outgrowths (dorsal and ventral pancreatic buds) of the foregut, which explains the intermixing of exocrine and endocrine tissue in the mature organ.

Gross framework relevant to sectioning

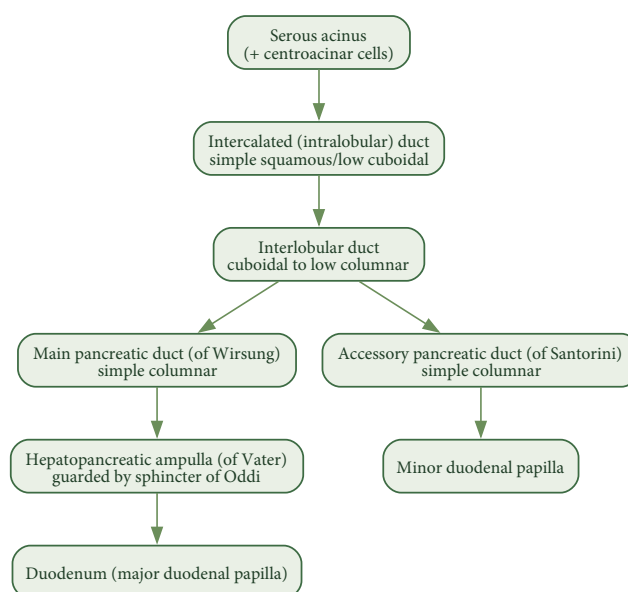
- ▶ A thin **capsule** of loose connective tissue surrounds the gland; septa extend from it into the substance of the organ, incompletely dividing the parenchyma into **ill-defined lobules** (unlike the well-demarcated lobules of salivary glands).
- ▶ Within lobules, a sparse stroma of loose/reticular connective tissue surrounds the acini, giving a rich capillary network but very little periacinar collagen.
- ▶ Larger interlobular septa carry the bigger ducts, blood vessels, autonomic nerves, ganglia and adipose tissue; small mucous glands may be found in the connective tissue around the main duct.
- ▶ **Pancreatic stellate cells** — myofibroblast-like cells in the peri-acinar, perivascular and periductal connective tissue — are normally quiescent but become activated in chronic pancreatitis and pancreatic cancer.

Exocrine pancreas — acinar structure

 Fig 23.19 — *Histology of pancreas (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.383)*

- ▶ The secretory unit is the **serous acinus** — a round-to-oval cluster of pyramidal cells around a very small lumen, without myoepithelial cells (myoepithelial cells are seen in salivary glands but not the pancreas).
- ▶ Each acinar cell is polarised:
 - **Basal region** — spherical, basally placed nucleus surrounded by abundant basophilic rough endoplasmic reticulum (basal basophilia on H&E).
 - **Apical region** — packed with eosinophilic **zymogen granules** (apical eosinophilia) and a prominent supranuclear Golgi complex; granules diminish after a secretory episode and increase during fasting.
- ▶ Zymogen granules store digestive enzyme precursors: proteases (trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase, kallikreinogen), α -amylase, lipases, and nucleases (DNAase, RNAase). All are released by exocytosis in an inactive form.
- ▶ **Centroacinar cells** are pale-staining, flattened (squamous-to-low-cuboidal) cells found in the centre of the acinar lumen — a feature unique to the pancreas among glandular acini. They represent the invaginated beginning of the intercalated duct ("a balloon with a straw poked into it").

Duct system of the exocrine pancreas



Histology of pancreas

- **Intercalated ducts** begin within the acinus as centroacinar cells and are lined by simple squamous to very low cuboidal epithelium; their cells secrete a large, HCO_3^- -rich fluid that alkalinises and hydrates the enzymatic secretion.
- Intercalated ducts unite to form **interlobular ducts** (cuboidal to low columnar epithelium, increasingly tall), which in turn drain into the **main pancreatic duct (of Wirsung)** running the length of the gland; an **accessory pancreatic duct (of Santorini)**, a vestige of the two embryonic ductal primordia, commonly drains a separate segment at the minor duodenal papilla.
- The main pancreatic duct joins the common bile duct to form the **hepatopancreatic ampulla (of Vater)**, opening at the major duodenal papilla; the opening is guarded by the **sphincter of Oddi** (with its component sphincter choledochus, sphincter pancreaticus and sphincter ampullae), which regulates flow and prevents duodenal reflux.
- **Important negative point (frequently tested):** the pancreas has **no striated ducts** — striated ducts are a feature of the parotid and submandibular salivary glands, not of the pancreas.
- Net exocrine output is ~1.5 L/day of alkaline pancreatic juice, whose HCO_3^- neutralises acidic gastric chyme in the duodenum and establishes optimal pH for enzyme activity.

Endocrine pancreas — islets of Langerhans

- **Islets of Langerhans** are compact, spherical-to-ovoid clusters of pale-staining polygonal endocrine cells embedded within the exocrine acinar tissue; on H&E they appear as pale islands surrounded by darker-staining acini, and individual cell types cannot be distinguished without special stains.
- The human pancreas contains **more than one million islets**, mostly 100–200 μm in diameter, constituting only about 1–2% of **total pancreatic volume**; they are **most numerous in the tail**.
- Each islet is separated from surrounding acinar tissue by a thin **reticular capsule** and is very richly vascularised — arterioles enter the islet and break up into a dense network of **fenestrated capillaries**, allowing rapid, bidirectional exchange of hormones between islet cells and blood,

before draining as efferent capillaries into the surrounding acinar capillary bed (the **insulo-acinar portal system**), by which islet hormones modulate neighbouring acinar secretion.

- Islets receive both sympathetic and parasympathetic autonomic innervation; about 10% of islet cells have direct nerve endings, and gap junctions couple neighbouring islet cells electrically.

Islet cell types — secretions, proportions and identification

Cell type	Hormone	Approx. proportion	Location in islet	Identification/special stain
A (alpha)	Glucagon	~15–20% (classical teaching; newer data cite 30–40%)	Peripheral	Stains red with Mallory–Azan; brightly with acid fuchsin; argyrophilic with Grimelius silver stain
B (beta)	Insulin	~60–70% (classical teaching; newer data cite ~50%) — the most abundant cell	Central	Stains brownish-orange with Mallory–Azan; aldehyde fuchsin positive
D (delta)	Somatostatin	~5–10%	Peripheral	Stains blue with Mallory–Azan
PP (F) cells	Pancreatic polypeptide	Minor population, more numerous in islets of the head	Scattered	Identified by immunohistochemistry
D1 cells	Vasoactive intestinal peptide (VIP)	Minor	Scattered; also in exocrine ducts	Immunohistochemistry
EC (enterochromaffin) cells	Serotonin, motilin, substance P	Minor	Scattered; also in acini/ducts	Immunohistochemistry
Epsilon cells	Ghrelin	Minor	Scattered	Immunohistochemistry

Note on figures: older/classical Indian-curriculum texts (Ross, IB Singh, Sontakke) give B ≈ 60–70% and A ≈ 15–20%; Gray's Anatomy 41e cites more recent human-islet data with B ≈ 50% and A ≈ 30–40% — both proportions are used in the literature and either may be quoted, provided B is stated as the majority cell type.

- Unlike rodent islets (where B cells form a discrete central core surrounded by A cells), **human islets show no strict cell-type-specific zonation** — cell types are more uniformly interspersed with extensive intercellular contact, permitting paracrine cross-talk.
- All islet cell types other than B cells are functionally analogous to the diffuse neuroendocrine (enteroendocrine) cells of the gut mucosa, reflecting the shared endodermal lineage of pancreas and gut.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a low-power (H&E) field of pancreas showing:

- ▶ Thin fibrous **capsule** and an interlobular **septum**
- ▶ A lobule of **serous acini** (pyramidal cells, basal nucleus, basal basophilia, apical eosinophilic zymogen granules)
- ▶ A **centroacinar cell** within an acinar lumen
- ▶ **Intercalated (intralobular) duct** — low cuboidal epithelium
- ▶ **Interlobular duct** — cuboidal-to-columnar epithelium, embedded in connective tissue with vessels
- ▶ A pale-staining **islet of Langerhans** with its capillary network, clearly demarcated from surrounding acini
- ▶ Adjacent **adipose tissue** (commonly present in adult pancreatic septa)

Regulation of exocrine and endocrine secretion

- ▶ **Cholecystokinin (CCK)**, released by duodenal enteroendocrine cells on entry of fat/protein, stimulates **acinar** cells to release digestive enzymes.
- ▶ **Secretin**, released by duodenal S cells on acid entry, stimulates **duct (centroacinar/intercalated)** cells to secrete a copious HCO_3^- -rich fluid.
- ▶ Parasympathetic (vagal) stimulation increases both acinar and duct secretion; sympathetic fibres inhibit exocrine flow.
- ▶ Blood glucose >70 mg/dL stimulates B cells to release insulin and inhibits A-cell glucagon release; glucose <70 mg/dL stimulates glucagon release. Somatostatin from D cells acts in a paracrine fashion to inhibit both insulin and glucagon release and also suppresses exocrine secretion. Parasympathetic stimulation increases secretion of both insulin and glucagon; sympathetic stimulation increases glucagon and inhibits insulin release.
- ▶ The pancreas protects itself from autodigestion by (i) secreting proteases as inactive zymogens, activated only in the duodenum by enteropeptidase (which converts trypsinogen to trypsin, triggering a proteolytic cascade), (ii) packaging a **trypsin inhibitor** alongside trypsinogen in the secretory granules, and (iii) maintaining a high intraductal/intra-acinar pH (via HCO_3^-) that keeps enzymes inactive until secretion.

Applied / Clinical anatomy

- ▶ **Acute pancreatitis**: premature intra-glandular activation of proenzymes causes autodigestion, inflammation, oedema and fat necrosis; causes include gallstones, alcohol, drugs and trauma; serum amylase and lipase are markedly elevated.
- ▶ **Chronic pancreatitis**: repeated acute insults lead to progressive fibrosis (activated pancreatic stellate cells) and loss of both exocrine and endocrine function.
- ▶ **Pancreatic carcinoma**: usually a ductal-cell adenocarcinoma, most often in the head of the gland near the duodenum; the sparse periductal connective tissue facilitates early local spread, and the tumour typically remains silent until advanced, giving a poor prognosis.

- ▶ **Diabetes mellitus:** Type 1 (insulin-dependent) results from autoimmune destruction of B cells; Type 2 (non-insulin-dependent) reflects inadequate insulin secretion together with peripheral insulin resistance, often linked with obesity.
- ▶ **Islet-cell tumours:** **insulinoma** (B-cell tumour — hypoglycaemia), **glucagonoma** (A-cell tumour — hyperglycaemia, necrolytic migratory erythema), and gastrin-secreting islet-cell tumours causing **Zollinger–Ellison syndrome** (severe peptic ulceration from unopposed acid secretion).
- ▶ The **sphincter of Oddi** and hepatopancreatic ampulla are surgically important in obstructive jaundice and ERCP; the accessory duct of Santorini, when it is the dominant drainage channel (pancreas divisum), is clinically relevant to recurrent pancreatitis.

High-yield exam pointers

- ▶ Pancreas = compound tubuloacinar **serous** gland; resembles parotid but differs by islets, centroacinar cells, and **no striated ducts**.
- ▶ Centroacinar cells are unique to the pancreas — beginning of the intercalated duct, invaginated into the acinar lumen.
- ▶ Duct sequence: acinus (+ centroacinar cell) → intercalated duct → interlobular duct → main duct of **Wirsung** (± accessory duct of **Santorini**) → **ampulla of Vater** → duodenum, guarded by **sphincter of Oddi**.
- ▶ Islets = 1–2% of pancreatic volume, >1 million in number, most numerous in the **tail**; richly vascularised by fenestrated capillaries.
- ▶ B (beta, insulin) cells are the most numerous islet cell and lie centrally; A (alpha, glucagon) cells are next most numerous and lie peripherally; D (delta) cells secrete somatostatin.
- ▶ CCK drives acinar enzyme secretion; secretin drives ductal HCO_3^- /water secretion.
- ▶ Zymogens are secreted inactive and activated only in the duodenum (trypsinogen → trypsin via enteropeptidase), protecting the gland from autodigestion.
- ▶ Special stains to identify islet cell types: Mallory–Azan (A = red, B = brownish-orange, D = blue), aldehyde fuchsin, Grimelius silver stain, and immunohistochemistry.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Gray's Anatomy (Standring) 41e; BD Chaurasia Human Anatomy Vol 2 8e (figure).



Microscopic anatomy of small intestine

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction The small intestine is the ~5 m long segment of the gastrointestinal tract extending from the pylorus to the ileocaecal junction, divided into **duodenum** (~25 cm), **jejunum** (~2 m) and **ileum** (~3 m). It is the principal site of terminal digestion and absorption of nutrients. Histologically its wall follows the general four-layered plan of the alimentary canal — mucosa, submucosa, muscularis externa and serosa — but is distinguished by three orders of mucosal folding (plicae circulares, villi, microvilli) that amplify the absorptive surface area by factors of approximately 2, 7 and 13 respectively, yielding an estimated total absorptive surface area of almost 30 m².

1. General plan of the wall

Layer	Composition
Mucosa	Epithelium + lamina propria + muscularis mucosae
Submucosa	Loose connective tissue, vessels, nerves, Meissner's (submucosal) plexus; Brunner's glands in duodenum
Muscularis externa	Inner circular + outer longitudinal smooth muscle; myenteric (Auerbach's) plexus between the two layers
Serosa / adventitia	Visceral peritoneum (mesothelium + subserous connective tissue); adventitia only over the retroperitoneal duodenum and the mesenteric border

2. Mucosal surface-amplifying folds

- ▶ **Plicae circulares (valves of Kerckring)** — permanent transverse/oblique folds of mucosa and submucosa (do not flatten on distension, unlike gastric rugae); absent in the first 2–5 cm of the duodenum, largest and most closely set in the distal duodenum and proximal jejunum, progressively diminish through the ileum and are almost absent in the terminal ileum. Individual folds may be up to 8 mm deep.
- ▶ **Villi** — finger- or leaf-shaped projections of mucosa covering the entire surface, 0.5–1.0 mm tall, 10–40/mm² in density. They are broad ridges in the first part of the duodenum, become tall and foliate in the distal duodenum/proximal jejunum, and shorten to a finger-like form through the distal jejunum and ileum — i.e. **large and numerous in duodenum/jejunum, smaller and fewer in ileum**.
- ▶ **Intestinal glands/crypts (of Lieberkühn)** — simple tubular invaginations of the epithelium between the bases of villi, extending a short distance into the lamina propria; most prominent in the proximal small intestine. They house the stem-cell compartment.
- ▶ **Microvilli** — apical brush border on enterocytes, up to ~3000 per cell, ~1 µm thick collectively (resolvable only by electron microscopy), covered by a glycocalyx.

Real histology plate:

□ Fig 18.6 — Histology of jejunum (typical features of small intestine). Low magnif (Sontakke — Textbook of Human Histology — 2e, p.248)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a transverse section through the wall of the small intestine (jejunum) at low power, then a magnified inset of one villus tip. Label:

- ▶ Mucosal folds (plicae circulares) and villi
- ▶ Epithelium (simple columnar) lining the villi
- ▶ Lamina propria (core of the villus) with a central lacteal
- ▶ Intestinal glands/crypts of Lieberkühn opening between villi bases
- ▶ Muscularis mucosae
- ▶ Submucosa (with a lymphatic nodule/Peyer's patch)
- ▶ Muscularis externa — inner circular and outer longitudinal layers, with myenteric (nerve) plexus between them
- ▶ Serosa
- ▶ Inset of the epithelium: absorptive enterocyte with microvilli/basal lamina, goblet cell, Paneth cell, enteroendocrine cell, intervillous space

3. Villus core and lamina propria

- ▶ **Villus core** — delicate connective tissue containing a single (occasionally double) blind-ending central lymphatic, the **lacteal**, which drains chylomicrons absorbed from dietary fat; fenestrated capillaries for rapid nutrient uptake; smooth muscle fascicles extending from the muscularis mucosae, whose contraction propels lacteal contents into the deeper lymphatic plexus; nerve fibres.
- ▶ **Lamina propria** — connective tissue rich in fibroblasts, plasma cells, lymphocytes, eosinophils, macrophages, mast cells and a dense capillary/lymphatic plexus; it also carries solitary and aggregated lymphoid follicles that may extend through the muscularis mucosae into the submucosa.

4. Epithelial cell types of the mucosa

Cell type	Location	Salient feature
Enterocytes (absorptive columnar cells)	Cover villi; also line crypts	~20 µm tall; brush border of up to ~3000 microvilli; lifespan ~5 days — arise by mitosis at crypt base, migrate up the villus, undergo apoptosis at the tip
Goblet cells	Villi and crypts	Mucin-secreting; increase in number from duodenum to a maximum in the terminal ileum

Cell type	Location	Salient feature
Paneth cells	Base of crypts only	Eosinophilic secretory granules; secrete lysozyme, defensins and TNF-α — innate antibacterial defence; also sustain the stem-cell niche; may persist a month or more
Enteroendocrine (neuroendocrine) cells	Scattered in crypt walls, occasionally over villi	Secrete gastrin, cholecystokinin, secretin basally into the lamina propria
Stem cells	Crypt base, above the Paneth cells	Give rise to all epithelial lineages (enterocytes, goblet, Paneth, enteroendocrine, tuft cells); progeny reach the villus tip in 4–5 days
M (microfold) cells	Dome epithelium overlying lymphoid follicles/Peyer's patches	Broad microfolds rather than microvilli; sample luminal antigen by endocytosis and deliver it to intraepithelial lymphocytes
Intraepithelial lymphocytes	Basolateral to enterocytes/goblet cells, near M cells	Migratory cells from underlying lymphoid tissue; mucosal immune defence

5. Submucosa, Brunner's glands, muscularis externa and serosa

- **Submucosa** — loose connective tissue carrying larger vessels, lymphatics and the submucosal (Meissner's) plexus; its ridges form the cores of the plicae circulares.
- **Brunner's (submucosal) glands** — confined to the **duodenal submucosa only**; branched tubuloacinar glands lined by low columnar cells secreting an **alkaline (pH 9) mucoïd fluid** that neutralises acidic gastric chyme; ducts pierce the muscularis mucosae to open at the base of the crypts; largest and most numerous near the pylorus, forming a near-complete layer in the proximal descending duodenum, then diminishing and disappearing at the duodenojejunal junction.
- **Muscularis externa** — inner circular (thicker) and outer longitudinal (thinner) smooth muscle, thicker proximally; contains the myenteric plexus and networks of **interstitial cells of Cajal (ICC)**, the pacemaker cells generating slow-wave electrical activity (the migrating motor complex).
- **Serosa** — visceral peritoneum over most of the small bowel; the retroperitoneal duodenum and the mesenteric border are covered by adventitia instead.

6. Regional histological variation — duodenum, jejunum, ileum

Feature	Duodenum	Jejunum	Ileum
Length	~25 cm	~2 m	~3 m

Feature	Duodenum	Jejunum	Ileum
Lumen / wall	Wide lumen, thick wall	Wide lumen, thick wall	Narrow lumen, thin wall
Plicae circulares	Large, thick folds (absent in first 2–5 cm)	Large, thick, closely set folds	Few, small folds; absent in terminal ileum
Villi	Numerous, broad	Numerous, tall	Few, short, slender
Submucosal glands	Brunner's glands present	Absent	Absent
Lymphoid follicles	Absent/scanty	Occasional solitary follicles	Numerous Peyer's patches , extending into the submucosa, on the antimesenteric border

7. Gut-associated lymphoid tissue (GALT) and Peyer's patches

- ▶ **Peyer's patches** are aggregated lymphoid follicles, each composed of dozens of nodules without a connective-tissue capsule, situated in the lamina propria (extending into submucosa) along the **antimesenteric border of the ileum**.
- ▶ The overlying **dome epithelium** contains scattered **M cells** that transcytose luminal antigen to underlying lymphocytes and macrophages, initiating mucosal immune responses.
- ▶ Solitary follicles become more numerous, and aggregated follicles (Peyer's patches) larger, caudally along the small intestine; like other MALT they are most prominent around puberty and diminish (though many persist) with age.
- ▶ Functionally, GALT maintains the homeostatic balance with commensal flora and provides the first line of adaptive immune defence against enteric pathogens.

Applied / Clinical anatomy

- ▶ **Coeliac disease (coeliac sprue)** — gluten-triggered immune injury of the proximal small intestinal mucosa causing **villous atrophy**, crypt hyperplasia and malabsorption; diagnosed on **duodenal/jejunal biopsy**.
- ▶ **Crohn disease** — chronic granulomatous inflammatory bowel disease affecting most commonly the ileum (and colon); transmural lymphocytic infiltration through any/all wall layers causing pain, bleeding, malabsorption and diarrhoea.
- ▶ **Meckel's diverticulum** — persistence of the intestinal end of the vitello-intestinal (omphalomesenteric) duct; "rule of 2s" — occurs in ~2% of people, ~2 inches (5 cm) long, ~2 feet (60 cm) proximal to the ileocaecal junction on the antimesenteric border of the ileum; may contain ectopic gastric or pancreatic mucosa, giving rise to peptic ulceration, bleeding, perforation or an inflammation mimicking appendicitis.
- ▶ **Typhoid ileitis** — the ulcers of enteric fever classically overlie the Peyer's patches of the terminal ileum, along their long axis, and may perforate.
- ▶ **Brunner's gland dysfunction/hyperplasia** and failure of duodenal alkaline neutralisation contribute to duodenal peptic ulceration.

- ▶ **Radiology/endoscopy** — plicae circulares produce the "feathery" mucosal pattern of the jejunum on barium studies and CT/MR enterography, in contrast to the relatively featureless terminal ileum — a useful way to distinguish jejunal from ileal loops on imaging.

High-yield exam pointers

- ▶ Small intestine = duodenum (25 cm) + jejunum (~2 m) + ileum (~3 m) $\approx$ 5 m; surface area amplified $\sim 2\times$ (plicae) $\times \sim 7\times$ (villi) $\times \sim 13\times$ (microvilli), giving an estimated total absorptive surface area of almost 30 m².
- ▶ Four wall layers: mucosa, submucosa, muscularis externa, serosa — as in the rest of the gut.
- ▶ Villi and plicae circulares are large/numerous in duodenum–jejunum and small/few in ileum; crypts are most prominent proximally.
- ▶ Six epithelial cell types: enterocyte, goblet cell, Paneth cell, enteroendocrine cell, stem cell, M cell.
- ▶ **Brunner's glands = duodenum only** (alkaline mucus, submucosal); **Peyer's patches = ileum, antimesenteric border** (aggregated lymphoid follicles with M-cell dome epithelium).
- ▶ Each villus core carries a central lacteal draining chylomicrons.
- ▶ Enterocytes turn over in ~5 days (crypt $\rightarrow$ villus tip $\rightarrow$ apoptosis); Paneth cells escape this flow and remain at the crypt base for a month or more.
- ▶ Applied triad to remember: coeliac disease $\rightarrow$ villous atrophy; Meckel's diverticulum $\rightarrow$ rule of 2s; typhoid $\rightarrow$ ulceration over Peyer's patches.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Junqueira's Basic Histology 17e; BD Chaurasia Human Anatomy Vol 2 8e; Vishram Singh Textbook of Clinical Embryology (for Meckel's diverticulum detail).



Microscopic structure of Liver

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction — The liver is the largest gland/internal organ in the body (about 1.5 kg, ~2% of adult body weight), developing as an epithelial–mesenchymal outgrowth of the caudal foregut with which it stays connected via the biliary tree. Its parenchyma is a repeating epithelial unit — the **hepatocyte plate** — perfused by a dual afferent blood supply and drained by a unique bile-collecting system that runs in the direction opposite to blood flow. Three complementary models (classic lobule, portal lobule, liver acinus) are used to describe its functional microarchitecture, and it retains a strong capacity for regeneration despite a normally slow rate of cell turnover.

1. Capsule and connective-tissue framework

- ▶ The liver surface is covered by a serosa (visceral peritoneum); beneath this lies a thin (50–100 μ m) fibrous **capsule** (Glisson's capsule).

- ▶ Septa/trabeculae extend from the capsule into the parenchyma; branches of the **hepatic artery**, **hepatic portal vein** and **bile ductules/ducts** run within these trabeculae, called **portal tracts** (portal canals).
- ▶ Human liver has only sparse interlobular connective tissue, so classic lobule boundaries are not sharply demarcated histologically (unlike, e.g., the pig liver) — lobular territory has to be inferred from the position of portal tracts and central veins.

2. Structural and functional units — three models

The parenchyma can be viewed three ways, each emphasising a different function:

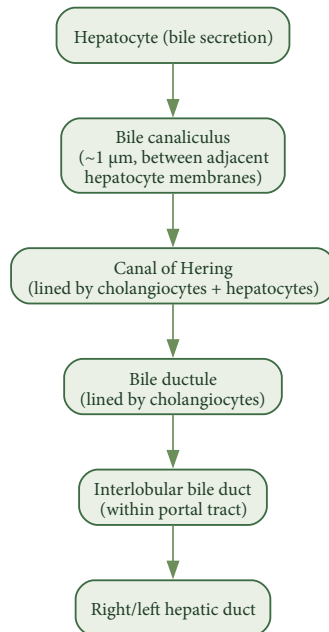
Model	Shape	Axis (centre)	Periphery	Functional emphasis
Classic hepatic lobule	Hexagonal	Central (terminal hepatic) vein	Portal triads at the six vertices	Traditional structural unit; blood flow-oriented
Portal lobule	Triangular	Bile duct of a portal triad	Three nearest central veins	Exocrine (bile-secretory) function
Liver (portal) acinus	Diamond/ovoid	Terminal branches of hepatic arteriole + portal venule (equator)	Two central veins at the poles	Best correlates with metabolic zonation, hypoxic injury and toxic/hepatocellular pathology

- ▶ Within the **liver acinus**, hepatocytes are arranged in three concentric, ill-defined **zones**:
 - **Zone 1** (periportal) — closest to the incoming blood; richest in oxygen/nutrients; first to regenerate; first to show morphological change after bile-duct occlusion (bile stasis).
 - **Zone 2** — intermediate zone; morphology/function intermediate between zones 1 and 3.
 - **Zone 3** (centrilobular/perivenular) — farthest from the afferent vessels, nearest the central vein; least oxygenated; first to undergo ischaemic (centrilobular) necrosis and the site where hepatic fibrosis first appears around the central vein.

3. Portal triad and vascular architecture

- ▶ A **portal triad** = a branch of the **hepatic portal vein** (largest profile) + a branch of the **hepatic artery** + an **interlobular bile duct**, usually accompanied by lymphatics and nerve fibres, all within a portal tract.
- ▶ **Dual blood supply**: the hepatic portal vein contributes the major share (~75% of hepatic blood flow — nutrient-rich but relatively deoxygenated blood from the gut, pancreas and spleen); the hepatic artery (a coeliac trunk branch) contributes the remaining ~25% as oxygenated blood. Both mix within the sinusoids before reaching the hepatocytes.
- ▶ Pre-terminal hepatic arterioles chiefly drain into a fine capillary plexus that joins portal-vein tributaries, with a small direct arterial contribution to sinusoids.

- ▶ Blood flow through the sinusoids is **centripetal** — from the portal tract at the periphery towards the central vein. Central veins from adjacent lobules unite as interlobular/sublobular veins and then **hepatic veins**, draining into the inferior vena cava.
- ▶ Bile flow is **centrifugal** — from the hepatocyte, via canaliculi, towards the bile duct in the portal tract — i.e., opposite in direction to blood flow (see diagram below).



Microscopic structure of Liver

4. Hepatocytes — structure

- ▶ Hepatocytes make up about **80% of liver volume** and **60% of its cell population**; the remainder is non-parenchymal (sinusoidal, Kupffer, stellate, biliary epithelial, stem cells).
- ▶ **Polyhedral** cells with 5–12 sides, **20–30 µm** across, arranged mainly in **plates (cords)** one cell thick (normally two cells thick until ~7 years of age), separated by sinusoids; plates thicken again transiently during regeneration.
- ▶ Nuclei are round, euchromatic, and frequently **binucleate/polyploid/tetraploid** — reflecting high synthetic activity.
- ▶ Cytoplasm is rich in rough and smooth endoplasmic reticulum, numerous mitochondria, lysosomes, a well-developed Golgi apparatus, glycogen granules, lipid vacuoles and peroxisomes (some containing urease crystals) — correlating with the hepatocyte's protein synthetic, detoxification and metabolic roles. Storage vacuoles containing ferritin/haemosiderin crystals reflect its role in iron metabolism.
- ▶ Each hepatocyte has two functionally distinct surfaces:
 - **Sinusoidal surface** — bears numerous microvilli (~0.5 µm) facing the perisinusoidal space of Disse, forming ~70% of the cell surface exposed to plasma — the site of secretory/absorptive exchange with blood.
 - **Canalicular (biliary) surface** — lateral membrane grooves of adjacent hepatocytes appose to form the **bile canaliculus**, sealed by tight junctions (occludins, claudins, junctional adhesion

molecules) that create a blood–bile barrier preventing bile reflux into plasma; hepatocytes are further linked by gap junctions and desmosomes.

- ▶ Hepatocytes are therefore functionally exocrine (bile secretion into the canaliculus → gut) as well as the site of the liver's major metabolic/endocrine activities.

5. Hepatic sinusoids and their lining cells

- ▶ **Sinusoids** are wide, irregular vascular channels between hepatocyte plates, lined by a thin, highly **fenestrated endothelium** (fenestrae clustered, ~100 nm mean diameter) that characteristically **lacks a continuous basal lamina**, allowing plasma free access to hepatocytes — this constitutes a dynamic blood filter.
- ▶ **Kupffer cells** — stellate, monocyte/bone-marrow-derived resident macrophages attached to the luminal sinusoidal surface; part of the mononuclear phagocyte system; remove aged/damaged erythrocytes, cellular/microbial debris, and secrete cytokines (a function taken over entirely from the spleen after splenectomy).
- ▶ **Hepatic stem cells** — pluripotential cells residing around the canals of Hering (derived from the fetal ductal plate); small (8–18 µm), oval-nucleated progeny express both biliary and hepatocyte markers and contribute to regeneration (also termed "oval cells").
- ▶ **Pit cells** — natural-killer lymphocytes within the sinusoidal lumen (immune surveillance).

6. Perisinusoidal space of Disse and hepatic stellate cells

- ▶ The **space of Disse** is a narrow perisinusoidal gap (normally 0.2–0.5 µm, widening in hypoxia) between the fenestrated endothelium and the hepatocyte plate, containing interstitial fluid, hepatocyte microvilli, fine type III (and some type I/IV) collagen fibres, and occasional non-myelinated nerve terminals — it is a major site of origin of hepatic lymph (drained via the periportal space of Mall to portal-tract lymphatics).
- ▶ **Hepatic stellate (Ito, fat-storing) cells** lie within the space of Disse; mesenchymal in origin, they store **vitamin A** in cytoplasmic lipid droplets and secrete intralobular matrix components (including type III collagen) under normal conditions. On liver injury they become activated, transform into **myofibroblast-like cells**, and lay down collagenous scar tissue — the basis of **hepatic fibrosis**, which characteristically begins in zone 3 around the central vein and, if it progresses, destroys the normal parenchymal/vascular architecture as **cirrhosis**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a hexagonal classic hepatic lobule in cross-section with the following exact labels:

- ▶ **Central vein** at the centre, receiving radiating **sinusoids**.
- ▶ **Hepatocyte plates (cords)**, one cell thick, radiating between sinusoids.
- ▶ **Portal triads** at the six peripheral vertices, each showing a **branch of the hepatic portal vein** (largest lumen), a **branch of the hepatic artery** (smaller, thicker-walled), and a **bile duct** (cuboidal epithelium).
- ▶ **Interlobular septum/connective tissue** around the portal tract.
- ▶ Along one sinusoid: label the fenestrated **sinusoidal endothelial cell**, a **Kupffer cell** projecting into the lumen, the **space of Disse** with a **hepatic stellate (Ito) cell**, and **bile canaliculi** between adjacent hepatocytes.
- ▶ Arrows showing **centripetal blood flow** (portal tract → sinusoid → central vein) and **centrifugal bile flow** (hepatocyte → canaliculus → canal of Hering → bile ductule → interlobular bile duct), in opposite directions.

 Fig 16.12 — *Hepatic lobule.* (Junqueira's Basic Histology — Text & Atlas — 17e, p.829)

Applied / Clinical anatomy

- ▶ **Centrilobular (zone 3) necrosis** — the hepatocytes farthest from the afferent vessels and closest to the central vein are the first to undergo ischaemic necrosis in shock, congestive cardiac failure ("nutmeg liver") and halothane hepatitis, since they receive the least-oxygenated blood after it has already perfused zones 1 and 2.
- ▶ **Periportal (zone 1) injury** — the first zone affected by bile stasis (extrahepatic bile-duct obstruction) and typically the site of piecemeal necrosis in chronic viral hepatitis; also the first zone to show regenerative activity after injury.
- ▶ **Alcoholic/toxic fatty liver** — fatty change and toxic (drug/alcohol-metabolite) injury characteristically begin in zone 3, the site of maximal cytochrome-mediated biotransformation.
- ▶ **Hepatic fibrosis and cirrhosis** — activation of hepatic stellate cells (space of Disse) with excess collagen deposition begins peri-centrally (zone 3) and, on progression, destroys normal lobular architecture and sinusoidal fenestration ("capillarisation" of sinusoids), producing regenerative nodules, portal hypertension and impaired hepatocyte–plasma exchange.
- ▶ **Kupffer-cell (reticuloendothelial) function** — clearance of senescent erythrocytes and bilirubin production; overload or dysfunction contributes to jaundice; Kupffer cells assume the red-cell-clearance role of the spleen after splenectomy.
- ▶ **Liver biopsy interpretation** — recognising the classic lobule/portal triad vs central vein orientation, and the acinar zonal concept, underlies histopathological grading of viral hepatitis, drug toxicity and cirrhosis.
- ▶ **Regenerative capacity** — despite a normally slow parenchymal turnover, the liver has a strong regenerative capacity (hepatocyte mitosis restoring lost mass, with a contribution from peri-ductular stem/oval cells), which underlies the feasibility of living-donor partial hepatectomy for transplantation.

High-yield exam pointers

- ▶ Hepatocytes: **80% of liver volume, 60% of cell number**; polyhedral, 5–12 sides, 20–30 µm; frequently binucleate/polyploid.
- ▶ **Portal triad** = branch of hepatic artery + branch of hepatic portal vein + bile duct, at each of the six corners of the classic hexagonal lobule.
- ▶ Three descriptive models: **classic lobule** (hexagonal, central-vein-centred), **portal lobule** (triangular, bile-duct-centred — exocrine emphasis), **liver acinus** (diamond-shaped, zones 1–3 — best for metabolic/pathological correlation).
- ▶ Blood flow is **centripetal** (portal tract → sinusoid → central vein → hepatic vein → IVC); bile flow is **centrifugal** (hepatocyte → canaliculus → canal of Hering → bile ductule → interlobular duct) — opposite directions.
- ▶ Dual blood supply: ~75% **portal vein** (nutrient-rich) + ~25% **hepatic artery** (oxygen-rich), mixing in the sinusoids.
- ▶ Sinusoids: **fenestrated endothelium without a basal lamina** + luminal **Kupffer cells** (macrophages).
- ▶ **Space of Disse** (perisinusoidal space) houses **hepatic stellate (Ito) cells** — vitamin A storage normally; myofibroblastic transformation drives fibrosis/cirrhosis.
- ▶ **Bile canaliculi** (~1 µm) are intercellular grooves sealed by tight junctions, forming a blood–bile barrier; they drain via **canals of Hering** to bile ductules and interlobular ducts.
- ▶ Zone 3 (centrilobular) is most vulnerable to hypoxia and toxins; Zone 1 (periportal) is first to regenerate and first affected by bile stasis.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e (text and Fig. 16.12).



Systemic histology - respiratory & urinary



Histology of kidney

Asked in: Paper III 11-May-2022 · Paper III May 2019 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — The kidney is a compound tubular retroperitoneal organ whose parenchyma is built of about 1–1.3 million **nephrons** per kidney (renal corpuscle + a convoluted-and-straight renal tubule) plus a branching **collecting-duct system**. The nephron is the structural and functional unit of the kidney: it filters plasma at the renal corpuscle and then selectively modifies the filtrate along the tubule to form urine. On section the kidney shows an outer **cortex** and inner **medulla** organised into pyramids, with the same tubular components recurring in a predictable cortex/medulla distribution that is the key to reading a kidney slide.

1. General organisation — cortex, medulla, lobes and lobules

- ▶ **Capsule** — thin connective-tissue capsule with an outer fibrocollagenous layer and an inner layer containing contractile myofibroblasts.
- ▶ **Cortex** — outer, granular, reddish-brown zone containing renal corpuscles, proximal and distal convoluted tubules, connecting tubules and cortical blood vessels.
 - **Cortical labyrinth** — the area between medullary rays, containing the renal corpuscles and convoluted tubules.
 - **Medullary rays (of Ferrein)** — vertical striations projecting from the medulla into the cortex, made of straight tubules and cortical collecting ducts.
- ▶ **Medulla** — inner, paler zone made of **8–18 renal pyramids** (striated by the loops of Henle, collecting ducts, vasa recta). Each pyramid has a base facing the cortex and an apex, the **papilla**, perforated by the **area cribrosa** and projecting into a minor calyx.
- ▶ **Renal columns (of Bertin)** — cortical tissue extending between adjacent pyramids, histologically identical to the rest of the cortex.
- ▶ **Lobe** — one pyramid + its overlying/flanking cortex (visible as surface convexities in the fetal kidney).
- ▶ **Lobule** — a medullary ray with the nephrons draining into its collecting duct; the functional equivalent of a secretory unit of an exocrine gland.

2. The nephron

Feature	Detail
Number/kidney	≈1–1.3 million (quoted up to ~1.25 million, Junqueira)
Components	Renal corpuscle → PCT → loop of Henle → DCT → connecting tubule → (joins) collecting duct
"Uriniferous tubule"	Nephron + its collecting duct (nephron and collecting duct have separate embryological origins — metanephric blastema vs ureteric bud — and unite secondarily)

Types of nephron (by position of renal corpuscle):

- ▶ **Cortical (subcapsular) nephrons** — corpuscle in outer cortex; short loop of Henle confined to the outer medulla; the majority (~85%) of nephrons.
- ▶ **Juxtamedullary nephrons** — corpuscle near the corticomedullary junction; long loop of Henle extending deep into the inner medulla; about one-eighth of nephrons; essential for the countercurrent urine-concentrating mechanism.
- ▶ **Midcortical (intermediate) nephrons** — corpuscle in mid-cortex; loop of intermediate length.

□ Fig 20.3 — Photomicrograph. Histology of kidney (low magnification on left, cortex and medulla at high magnification on right, H&E stain) (Sontakke — Textbook of Human Histology — 2e, p.274)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Low-power kidney section with labels: **Capsule** → **Cortex** → **Medulla**; in the cortex — **renal corpuscle (glomerulus + Bowman's capsule)**, numerous **PCT profiles** (more numerous, darker, brush border), fewer **DCT profiles** (lighter, no brush border), **medullary ray**; in the medulla — parallel **thin segments**, **thick ascending limbs**, **collecting ducts**, converging toward the **papilla**.

3. Renal corpuscle and the glomerular filtration barrier

The **renal corpuscle** (Malpighian corpuscle), ~200 µm in diameter, consists of the **glomerulus** (a tuft of 10–20 anastomosing fenestrated capillary loops fed by an afferent arteriole and drained by an efferent arteriole) invaginated into the double-layered **Bowman's (glomerular) capsule**:

- ▶ **Parietal layer** — simple squamous epithelium resting on a basal lamina; continuous with the PCT epithelium at the **urinary pole**.
- ▶ **Visceral layer** — made of **podocytes**, whose primary processes give secondary processes (**pedicels/foot processes**) that interdigitate on the glomerular basement membrane, leaving **filtration slits** (~40 nm) bridged by a **slit diaphragm**.
- ▶ The **vascular pole** is where the afferent/efferent arterioles enter/leave; it lies opposite the urinary pole.
- ▶ **Urinary (Bowman's) space** lies between the two capsular layers and is continuous with the PCT lumen.

Glomerular filtration barrier (three layers, from lumen outward):

- ▶ **Fenestrated endothelium** of glomerular capillaries — pores 70–90 nm, without a diaphragm (unlike fenestrated capillaries elsewhere).
- ▶ **Glomerular basement membrane (GBM)** — thick (~300–370 nm), a fused product of endothelium and podocytes, with three zones — lamina rara externa, lamina densa (type IV collagen — the physical filter), lamina rara interna — rich in heparan-sulfate proteoglycans (the charge barrier).
- ▶ **Podocyte foot processes with filtration-slit diaphragm** — a zipper-like sheet built of the transmembrane protein **nephrin**, reinforced by Neph1/Neph2, P-cadherin, FAT1/FAT2, anchored to the podocyte actin cytoskeleton — together excluding plasma proteins ≥~70 kDa (near albumin's size) and charge-selecting against anions.

Mesangium — intraglomerular mesangial cells and their matrix, enclosed within the GBM at the vascular stalk and between capillary loops. Functions: phagocytosis/clearance of trapped

macromolecules from the GBM, structural support to capillary loops, contractility (modulating glomerular blood flow), and secretion of cytokines (IL-1, PDGF, PGE₂). Mesangial cells and extraglomerular ("lakis") mesangial cells arise from smooth-muscle-cell precursors of the metanephric mesenchyme.

4. Renal tubule

Feature	Proximal convoluted tubule (PCT)	Distal convoluted tubule (DCT)
Origin	Urinary pole of Bowman's capsule	Ascending limb of loop of Henle (after macula densa)
Epithelium	Cuboidal/low columnar, strongly acidophilic	Cuboidal, less acidophilic/basophilic
Apical surface	Prominent brush border (long microvilli)	No brush border
Diameter	40–60 µm; longer, more coiled	20–50 µm; ~1/3 the length of PCT, less coiled
Basal cytoplasm	Basal striations — vertical infoldings packed with elongated mitochondria (Na ⁺ /K ⁺ -ATPase-driven transport)	Basal infoldings with mitochondria, less prominent
Number in section	More profiles seen (longer, more convoluted)	Fewer profiles
Main function	Bulk (isosmotic) reabsorption — ~65–80% of filtrate: Na ⁺ , water, glucose, amino acids, HCO ₃ ⁻ , phosphate	Na ⁺ reabsorption/K ⁺ secretion (aldosterone-responsive), HCO ₃ ⁻ reabsorption with H ⁺ secretion

Loop of Henle — the U-shaped segment linking the PCT to the DCT:

- **Proximal straight tubule (thick descending limb)** — cuboidal, brush border, continuous with PCT.
- **Thin segment** (descending + ascending limbs) — simple squamous epithelium; four EM cell subtypes (I–IV) differ by height/microvilli/interdigitation per their role in the countercurrent multiplier; thin descending limb is water-permeable (aquaporin-1), thin ascending limb is water-impermeable but Na⁺/Cl⁻-permeable.
- **Distal straight tubule (thick ascending limb)** — cuboidal, no brush border, actively pumps out Na⁺/K⁺/Cl⁻ (Na-K-2Cl cotransporter); the "diluting segment"; secretes **uromodulin** (Tamm–Horsfall protein).
- Near its end the thick ascending limb becomes taller/columnar and forms the **macula densa** at the vascular pole, before continuing as the DCT.

Connecting tubule and collecting duct — the DCT drains via a short connecting tubule (some forming an "arched connecting tubule") into the **cortical collecting duct**, which continues as the outer- then inner-medullary collecting duct, and finally the large **papillary ducts (of Bellini)** opening on the area cribrosa into a minor calyx.

5. Juxtaglomerular apparatus (JGA)

Located at the vascular pole of the renal corpuscle, the JGA has three components:

□ Fig 20.7 — Structure of renal corpuscle and JG apparatus (Sontakke — Textbook of Human Histology — 2e, p.276)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Vascular pole of the renal corpuscle showing: **afferent arteriole** (with **juxtaglomerular/JG cells** in its tunica media — epithelioid cells with rounded nuclei and secretory granules), **efferent arteriole**, **macula densa** (tall, densely packed columnar cells of the distal tubule apposed to the afferent arteriole), **extraglomerular mesangial (lakis) cells** filling the triangular space between afferent and efferent arterioles and the macula densa, glomerular capillaries, podocytes and Bowman's capsule.

- ▶ **Macula densa** — modified, tall, closely packed columnar cells at the point where the thick ascending limb of the loop of Henle contacts its own afferent arteriole; senses luminal Na^+/Cl^- concentration and tubular flow rate.
- ▶ **Juxtaglomerular (JG) cells** — modified smooth-muscle cells (epithelioid, with rounded nuclei) in the tunica media of the afferent arteriole (occasionally the efferent arteriole); store and release **renin**; also act as intrarenal baroreceptors and receive sympathetic innervation.
- ▶ **Extraglomerular mesangial cells (lakis cells / cells of Polkissen)** — polyhedral cells occupying the triangular space between the macula densa and the two arterioles; connect the macula densa signal to the JG cells.

Function — the JGA integrates a chemoreceptor signal (macula densa Na^+ sensing) and a baroreceptor signal (JG-cell stretch) to regulate **renin** release, driving the RAAS: renin converts angiotensinogen → angiotensin I → (via pulmonary ACE) angiotensin II → vasoconstriction + adrenal aldosterone release → aldosterone acts on collecting-duct principal cells to increase Na^+ /water reabsorption and K^+ secretion, raising blood volume and pressure.

6. Collecting duct system

Simple epithelium throughout, becoming taller (cuboidal → columnar) from cortex to papilla; contains two cell types:

- ▶ **Principal (light/clear) cells** — majority; few organelles, some microvilli, basal infoldings; carry aquaporin-2 (ADH-responsive, apical) and aquaporin-3/4 (basolateral) water channels and cytoplasmic mineralocorticoid receptors — the true target of ADH and **aldosterone**, mediating final water reabsorption and Na^+/K^+ exchange.
- ▶ **Intercalated (dark) cells** — fewer, mitochondria-rich, apical microvilli; concerned with acid-base balance — **α -intercalated cells** secrete H^+ (acidify urine), **β -intercalated cells** secrete HCO_3^- ; α -cells outnumber β -cells, consistent with the net acid load of a normal diet.

Collecting ducts converge as **papillary ducts (of Bellini)**, opening through the **area cribrosa** into a minor calyx.

Applied / Clinical anatomy

- ▶ **Goodpasture syndrome** — autoantibodies to the NC1 domain of the $\alpha 3$ -chain of type IV collagen in the GBM (cross-reacting with pulmonary alveolar basement membrane) cause crescentic glomerulonephritis with pulmonary haemorrhage; crescents form from fibrin deposition and proliferation of parietal Bowman's-capsule cells with monocyte influx.
- ▶ **Alport syndrome** — mutation of the $\alpha 5$ -chain of type IV collagen; irregularly thickened, laminated GBM — hereditary nephritis with haematuria, proteinuria, progressive renal failure (often with deafness).
- ▶ **Congenital nephrotic syndrome** — nephrin gene (NPHS1) mutation disrupting the slit diaphragm, causing massive proteinuria and oedema.
- ▶ **IgA nephropathy (Berger disease), diabetic nephropathy, membranoproliferative and lupus nephritis** — all show mesangial cell proliferation/matrix expansion.
- ▶ **Glomerulonephritis** — proliferation of parietal epithelial cells, immune-complex deposition, loss of GBM anionic charge (minimal-change disease) → selective albuminuria.
- ▶ **RAAS and hypertension** — basis for ACE inhibitors, angiotensin-receptor blockers and renin inhibitors; JGA hyperplasia occurs in renal artery stenosis.
- ▶ **Urinalysis** — normal urinary protein <150 mg/day; urinary casts form from precipitated uromodulin (Tamm–Horsfall protein).
- ▶ **Polycystic kidney disease** — cystic dilatation from any nephron-tubule epithelium, disrupting cortical/medullary architecture.
- ▶ **Renal biopsy** — H&E/PAS/silver/Masson trichrome light microscopy + immunofluorescence + electron microscopy of a corticomedullary core is the standard diagnostic triad; PAS highlights the thick GBM and brush border.

High-yield exam pointers

- ▶ Nephron = functional unit; ~1–1.3 million per kidney; renal corpuscle + PCT + loop of Henle + DCT (+ connecting tubule → collecting duct).
- ▶ Two main nephron types: cortical/subcapsular (short loop, ~85%) and juxtamedullary (long loop, ~1/8, concentrating mechanism).
- ▶ Renal corpuscle $\approx 200 \mu\text{m}$; vascular pole (afferent/efferent arterioles) vs urinary pole (PCT origin).
- ▶ Filtration barrier = fenestrated endothelium + GBM (lamina rara externa/densa/rara interna) + podocyte foot processes with slit diaphragm (nephrin); excludes proteins $\geq 70 \text{ kDa}$ and negatively charged molecules.
- ▶ PCT: brush border + basal striations, ~65–80% bulk reabsorption; DCT: no brush border, less convoluted, ends at macula densa proximally.
- ▶ Thin limb of loop of Henle = simple squamous (four EM subtypes); thick ascending limb secretes uromodulin and is the diluting segment.
- ▶ JGA = macula densa (DCT) + JG cells (afferent arteriole, renin) + extraglomerular mesangial/lacis cells → RAAS.

- ▶ Collecting duct = principal cells (ADH/aquaporin-2 + aldosterone target) and intercalated cells (α : H^+ secretion; β : HCO_3^- secretion).
- ▶ Mesangial cells: phagocytic, structural, contractile — proliferate in IgA nephropathy, diabetic and lupus nephropathy.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas, 7e; Junqueira's Basic Histology — Text & Atlas, 17e; IB Singh Textbook of Human Histology, 9e; Sontakke Textbook of Human Histology, 2e.



Histology of lung

Asked in: Paper III 30-May-2025 · Paper III May 2018 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The lung is the site of pulmonary gas exchange and is built around a progressively branching airway system embedded in a highly vascular connective-tissue stroma. Functionally the intrapulmonary airways are divided into a **conducting portion** (intrapulmonary bronchi → bronchioles → terminal bronchioles) that only transmits air, and a **respiratory portion** (respiratory bronchioles → alveolar ducts → alveolar sacs → alveoli) where gas exchange actually occurs. Along this pathway, cartilage, glands and pseudostratified epithelium progressively disappear while smooth muscle and elastic tissue become relatively more prominent, until the wall is reduced to the simple squamous epithelium of the alveolus applied directly to a capillary. The whole lung is invested by pleura, a serous membrane continuous with the parietal pleura at the hilum.

1. Intrapulmonary bronchi

- ▶ Each segmental (tertiary) bronchus divides repeatedly; the wall resembles the trachea but cartilage forms **irregular plates** (not complete C-shaped rings) that become smaller and more scattered as the bronchus narrows.
- ▶ **Mucosa**: pseudostratified ciliated columnar epithelium (respiratory epithelium) resting on an unusually thick basement membrane; contains ciliated columnar cells, goblet cells, basal (stem) cells, brush (chemosensory) cells and small granule/Kulchitsky (neuroendocrine) cells.
- ▶ **Lamina propria**: crisscrossing spiral bands of smooth muscle and elastic fibres, producing the folded mucosal appearance seen in cross-section on contraction.
- ▶ **Submucosa**: seromucous glands draining onto the luminal surface, lying between the muscle and cartilage layers.
- ▶ **Adventitia**: contains blood vessels, lymphatics and nerves; bronchus-associated lymphoid tissue (BALT) is present, especially at branching points, and becomes relatively more abundant as the bronchi become smaller.

2. Bronchioles

- ▶ Intralobular airways ≤ 1 mm in diameter, formed after about the tenth generation of branching; they **lack both cartilage and submucosal glands** — this is the histological criterion separating a bronchiole from a bronchus.

- ▶ Epithelium simplifies progressively: ciliated pseudostratified columnar in larger bronchioles → simple ciliated columnar → simple cuboidal in the smallest bronchioles.
- ▶ The relative proportion of smooth muscle in the wall increases (it is proportionately greater than in bronchi), producing the folded mucosa typical of contracted bronchioles; this muscle is the target of bronchospasm in asthma.
- ▶ **Terminal bronchioles** are the last purely conducting airways (no alveoli in their walls). Their cuboidal epithelium consists largely of non-ciliated **Clara (club) cells** with dome-shaped apical secretory granules that (i) secrete surfactant lipoproteins/mucins, (ii) detoxify inhaled xenobiotics via smooth-ER cytochrome P450 enzymes, and (iii) secrete antimicrobial peptides/cytokines. A **secondary pulmonary lobule**, the smallest macroscopic (pyramid-shaped) subdivision of the lung with apex toward the hilum, contains about 5–7 terminal bronchioles.

3. Respiratory bronchioles and the pulmonary acinus

- ▶ Each terminal bronchiole divides into two or more **respiratory bronchioles** — the transitional segment that is partly conducting, partly respiratory, because a few alveoli open directly into its wall.
- ▶ Epithelium is cuboidal (Clara + occasional ciliated cells) proximally, becoming simple squamous at the alveolar openings; a thin layer of smooth muscle and elastic tissue persists in the lamina propria.
- ▶ The **acinus** (the functional respiratory unit of the lung) lies distal to a terminal bronchiole and comprises 3–4 orders of respiratory bronchioles, each giving rise to 3–8 orders of alveolar ducts. The **primary lobule**, a smaller subdivision, is the lung tissue distal to a single respiratory bronchiole.

4. Alveolar ducts, alveolar sacs and alveoli

- ▶ **Alveolar ducts** are elongated air passages whose entire wall is made up of the mouths of alveoli; a thin lamina propria with knob-like bands of smooth muscle encircles each alveolar opening, supported by a web of elastic and collagen fibres.
- ▶ Alveolar ducts terminate in clusters of alveoli called **alveolar sacs**.
- ▶ **Alveoli** are saccular evaginations (~200 µm diameter) responsible for the spongy texture of the lung; both lungs together contain ~300–500 million alveoli, giving a combined alveolar–capillary surface area of about 70–100 m² for gas exchange.

5. The interalveolar septum and blood–air barrier

- ▶ Adjacent alveoli are separated by a thin **interalveolar septum** containing a dense, single-layered network of continuous (non-fenestrated) capillaries, supported by elastic and reticular (collagen III) fibres, fibroblasts and alveolar macrophages.
- ▶ The **blood–air (respiratory) barrier** across which O₂ and CO₂ diffuse consists of three layers: (i) the attenuated **type I pneumocyte**, (ii) the fused basal laminae of the epithelial and endothelial cells, and (iii) the thin **capillary endothelial cell**. Overall thickness ranges from as little as ~0.2 µm in the thin diffusion-optimised portions up to ~1.5 µm in the thicker connective-tissue-bearing portions of the septum.

- **Pores of Kohn** — small interalveolar pores (up to 7 per alveolus, 2–13 μm), usually lined by type II cells — connect neighbouring alveoli, equalise air pressure if a bronchiole is obstructed, and also serve as a route for macrophage/bacterial migration.

Cell/structure	Key features
Type I pneumocyte	Extremely attenuated squamous cell; forms >90% of alveolar surface area; organelles clustered perinuclearly to keep the diffusion barrier thin; tight junctions prevent fluid leak; post-mitotic — replaced by differentiating type II cells after injury
Type II pneumocyte (septal cell)	Cuboidal, contributes <10% of surface area; bulges at septal angles, often near pores of Kohn; cytoplasm contains lamellar bodies (concentric phospholipid whorls) that store/secrete pulmonary surfactant ; acts as the stem cell of the alveolar epithelium (normal turnover $\approx 1\%$ /day)
Alveolar macrophage ("dust cell")	Derived from bone-marrow monocytes; phagocytoses inhaled particulates, excess surfactant and extravasated erythrocytes; becomes a haemosiderin-laden " heart-failure cell " in pulmonary venous congestion; exits via the mucociliary escalator or lymphatics

- **Surfactant** is a film of phospholipid (chiefly dipalmitoylphosphatidylcholine) plus surfactant proteins SP-A/SP-B/SP-C/SP-D and cholesterol, secreted continuously by type II cells (and, in a different composition, by Clara cells); it lowers alveolar surface tension, preventing alveolar collapse on expiration and easing the work of inspiration. Surfactant only appears in adequate quantity in the last weeks of gestation.

6. Vasculature, lymphatics and nerves of the lung

- The lung has a dual blood supply: the **pulmonary circulation** (functional — thin-walled arteries accompanying the bronchial tree; capillary networks begin at the level of the respiratory bronchioles and densely invest every alveolar septum) and the much smaller **bronchial circulation** (nutritive, from the systemic circulation), which supplies the conducting airways down to the respiratory bronchioles, where it anastomoses with the pulmonary vessels.
- **Lymphatics** run in two networks — a deep peribronchial set following the bronchial tree to hilar nodes, and a superficial set beneath the visceral pleura — but **alveoli themselves have no lymphatic vessels**; lymphatics stop at the level of the alveolar ducts.
- **Nerves**: both parasympathetic and sympathetic autonomic fibres, plus visceral afferents, run mainly in the connective tissue around the larger bronchi and control bronchiolar smooth-muscle tone and glandular secretion.

7. Pleura

- The lung surface is covered by **visceral pleura**, continuous at the hilum with the **parietal pleura** lining the thoracic wall; both consist of a simple squamous mesothelium on a thin connective-tissue layer rich in collagen and elastic fibres (the visceral pleural elastic fibres are continuous

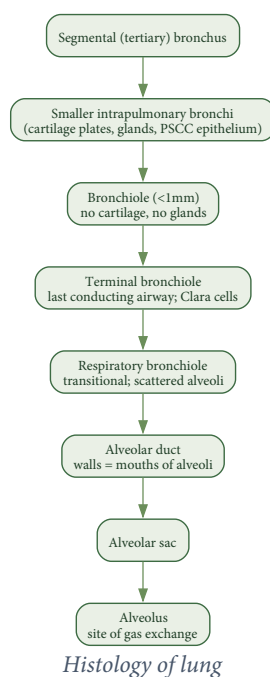
with those of the underlying parenchyma). The intervening pleural cavity is lined by mesothelium producing a thin serous lubricating film.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- Fig 15.13 — *Histology of lung (practice figure)*. (Sontakke — *Textbook of Human Histology* — 2e, p.208)
- Fig 15.15 — *Photomicrograph. Histology of lung (low magnification, H&E stain)*. (Sontakke — *Textbook of Human Histology* — 2e, p.209)

For the hand-drawn exam diagram, label a low-power field showing:

- ▶ **Intrapulmonary bronchus** — mucosa (pseudostratified ciliated columnar epithelium, lamina propria), smooth muscle layer, irregular cartilage plate, seromucous glands, connective tissue
- ▶ **Terminal bronchiole** — simple cuboidal epithelium, smooth muscle layer, connective tissue layer (no cartilage/glands)
- ▶ **Respiratory bronchiole** — leading into an **alveolar duct** and clusters of **alveoli**
- ▶ Surrounding lung parenchyma — honeycomb pattern of thin-walled alveoli with interalveolar septa



Applied / Clinical anatomy

- ▶ **Bronchogenic carcinoma** correlates with cell of origin: squamous cell carcinoma arises from metaplastic squamous epithelium of segmental bronchi (strongly smoking-linked); adenocarcinoma, the commonest lung cancer in non-smokers, arises more peripherally from bronchiolar/alveolar epithelium; small-cell carcinoma arises from neuroendocrine (Kulchitsky) cells of the bronchial epithelium.
- ▶ **Squamous metaplasia** of the normally pseudostratified respiratory epithelium occurs with chronic smoking/pollution and is a precursor lesion for dysplasia/carcinoma.

- ▶ **Asthma:** bronchiolar smooth-muscle bronchospasm triggered by mast-cell degranulation; the bronchiolar muscle layer is proportionately thicker than in bronchi, explaining the severity of obstruction.
- ▶ **Emphysema:** destruction of interalveolar septa and loss of elastic recoil (protease–antiprotease imbalance, e.g. α 1-antitrypsin deficiency), causing permanent airspace enlargement distal to terminal bronchioles.
- ▶ **Infant (neonatal) respiratory distress syndrome / hyaline membrane disease:** failure of type II pneumocytes to differentiate and produce adequate surfactant in preterm infants, causing alveolar collapse; managed with antenatal corticosteroids, exogenous surfactant and CPAP.
- ▶ **ARDS / diffuse alveolar damage:** injury to type I pneumocytes and capillary endothelium from infection, toxic gas inhalation or hyperoxia, causing exudation and hyaline membrane formation.
- ▶ **Pneumoconiosis/asbestosis:** macrophage-mediated chronic inflammation and fibrosis from indigestible inhaled particulates (coal dust, asbestos fibres); asbestos additionally predisposes to pleural mesothelioma.
- ▶ **Heart-failure cells:** haemosiderin-laden alveolar macrophages seen in sputum/lung in pulmonary venous congestion (mitral stenosis, left ventricular failure).
- ▶ **Primary ciliary dyskinesia (Kartagener syndrome):** defective ciliary ultrastructure impairs mucociliary clearance, producing bronchiectasis, chronic sinusitis and situs inversus.
- ▶ **Atelectasis:** airway obstruction (mucus plug/aspiration) leads to gas absorption and collapse of the dependent lobule; reversible if the obstruction is relieved.
- ▶ **Pneumothorax/pleural effusion/pleurisy:** reflect disruption of the mesothelial-lined pleural cavity and its serous fluid film.
- ▶ Type II pneumocytes bear the ACE2 receptor and are the principal target cell for SARS-CoV-2, explaining surfactant loss and alveolar collapse in severe COVID-19 pneumonia.

High-yield exam pointers

- ▶ Conducting portion ends and respiratory portion begins at the **respiratory bronchiole**.
- ▶ Loss of cartilage + glands is the defining histological criterion for bronchiole vs bronchus.
- ▶ **Terminal bronchiole** = last purely conducting airway; epithelium dominated by **Clara (club) cells**.
- ▶ **Acinus** = functional respiratory unit distal to a terminal bronchiole (3–4 orders of respiratory bronchioles → 3–8 orders of alveolar ducts).
- ▶ **Type I pneumocyte** >90% of alveolar surface (gas exchange, non-dividing); **Type II pneumocyte** <10% (surfactant secretion via lamellar bodies, stem cell of alveolar epithelium).
- ▶ Blood–air barrier = type I pneumocyte + fused basal lamina + capillary endothelium (~0.2–1.5 μ m thick).
- ▶ Both lungs: ~300–500 million alveoli; combined surface area ~70–100 m².
- ▶ **Pores of Kohn** equalise inter-alveolar pressure and allow collateral air flow/macrophage transit.
- ▶ **Alveoli have no lymphatic vessels** — lymphatics end at the alveolar ducts.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e (figures).



Histology of urinary bladder

Asked in: Paper III Nov 2017 · Paper III Oct 2010 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The urinary bladder is an extraperitoneal, musculo-membranous reservoir organ that stores urine between successive acts of micturition. Its wall follows the general hollow-viscus plan of mucosa, muscularis and serosa/adventitia, but it is histologically distinctive because the mucosa is lined by a specialized stratified epithelium — the **transitional epithelium** or **urothelium** — that forms a near-impermeable barrier to hypertonic, cytotoxic urine while still being able to stretch and flatten as the organ fills. Both the epithelial cell-layer number and the overall wall thickness vary reciprocally with the state of distension, and the mucosa over the **trigone** is structurally and developmentally distinct from the rest of the bladder. A firm grasp of this histology explains cystitis, urothelial carcinoma, vesico-ureteric reflux and the cystoscopic landmarks used clinically.

1. General plan of the bladder wall

Layer (inside → outside)	Composition	Key features
Mucosa	Urothelium (transitional epithelium) + lamina propria	Thrown into folds/rugae when empty, flattened when full; smooth and non-rugose over the trigone
"Submucosa"	Loose-to-dense fibroelastic connective tissue	Many authors regard this as simply the deeper, looser part of the lamina propria — there is no true muscularis mucosae and no distinct submucosal layer as seen in the gut
Muscularis (detrusor)	Three loosely demarcated, interlacing smooth-muscle layers	Inner longitudinal, middle circular/oblique, outer longitudinal; thickens at the bladder neck to form the sphincter vesicae
Serosa / Adventitia	Peritoneal mesothelium (only the superior surface) / fibroelastic connective tissue (rest)	Only the superior and part of the posterior surface is truly peritonealised — the remainder is covered by adventitia carrying vessels, nerves and lymphatics

2. Mucosa — the urothelium: a three-layered cytology

- ▶ **Basal layer** — a single row of small, low-cuboidal cells resting on a thin basement membrane; this layer holds the stem-cell population that regenerates the urothelium.
- ▶ **Intermediate layer** — one to several layers of cuboidal-to-low-columnar cells of variable thickness; these cells can differentiate to replace lost superficial cells.
- ▶ **Superficial ("umbrella") layer** — large, dome-shaped (bulbous) cells up to 100 µm in diameter, often binucleate, occasionally with polyploid nuclei. Each umbrella cell's cytoplasm spreads like an umbrella over several underlying intermediate cells, allowing the epithelium to distend without disrupting cell-cell junctions — hence the name.
- ▶ **Distension-dependent thickness** — the epithelium is ~5–7 cell layers thick when the bladder is empty/contracted (some texts describe up to 6), thinning to 2–3 cell layers when fully distended. This change is achieved not by cell loss but by cells sliding and changing shape (cuboidal ↔ squamous) and by insertion of pre-formed apical membrane from sub-surface vesicles.

3. Ultrastructure of the umbrella cell — the urothelial permeability barrier

- ▶ The apical plasma membrane of umbrella cells is thrown into rigid, plate-like **urothelial plaques** separated by narrow, flexible **hinge (inter-plaque) regions**.
- ▶ On electron microscopy the plaque membrane is asymmetric — its outer lipid leaflet is twice as thick as the inner leaflet — hence **asymmetric unit membrane (AUM)**.
- ▶ The thickened outer leaflet carries a crystalline, hexagonally packed array of 16-nm particles built from a family of five transmembrane proteins, the **urolakins** — **UPIa, UPIb, UPII, UPIIIa and UPIIIb** — which render the membrane virtually impermeable to water, urea and protons. Together with apical tight junctions, these plaques constitute the **urothelial permeability barrier**.
- ▶ Beneath the apical surface, umbrella cells contain numerous discoid **fusiform vesicles** built of the same AUM. On filling of the bladder these fuse with the apical membrane by exocytosis (adding surface area and flattening the cell); on emptying, the added membrane is retrieved by endocytosis — the structural basis of the cyclical stretch–recoil of the epithelium.
- ▶ **Clinical link:** urolakins in the plaque are the receptor exploited by the **FimH adhesin** of uropathogenic *Escherichia coli* (the cause of roughly 85% of urinary tract infections), which binds urothelial urolakins to gain a foothold on the mucosa before invading the epithelium.

4. Lamina propria, detrusor muscle and serosa/adventitia

- ▶ **Lamina propria** — a thick, richly vascularised fibroelastic connective tissue immediately beneath the urothelium; contains occasional mucous glands near the internal urethral orifice; thrown into rugae in the empty bladder that flatten on filling.
- ▶ **Detrusor (muscularis)** — three poorly delineated, interlacing smooth-muscle layers (inner longitudinal, middle circular/oblique, outer longitudinal) forming a true meshwork rather than discrete sheets; its coordinated contraction empties the bladder during micturition. At the bladder neck the middle circular fibres thicken to form the **sphincter vesicae** (internal urethral

sphincter) — anatomically well defined in the male, functionally present but less discretely organised in the female.

- **Serosa/Adventitia** — only the superior surface (and part of the posterior surface) bears true peritoneal serosa; the remaining external surface is covered by a fibroelastic adventitia.

5. The trigone — a histological exception

- A smooth, triangular area on the postero-inferior bladder wall, bounded antero-inferiorly by the internal urethral orifice and postero-laterally by the two slit-like ureteric orifices; its superior boundary is the slightly curved **inter-ureteric bar (crest)**, formed by continuation of the ureters' longitudinal muscle — seen at cystoscopy as a pale band that guides identification of the ureteric orifices.
- Unlike elsewhere in the bladder, the trigonal mucosa is **thin, always smooth (never rugose even when the bladder is empty)**, and **firmly adherent** to the underlying muscle, because it lacks the loose areolar attachment present over the rest of the bladder.
- Trigonal smooth muscle is described in two components: a **deep trigonal (detrusor) layer**, indistinguishable from ordinary detrusor muscle, and a finer-bundled **superficial trigonal muscle** continuous proximally with the intramural ureteric musculature and distally with the smooth muscle of the proximal urethra.
- **Embryological note:** trigone mucosa was classically taught to be mesodermal (incorporated from the absorbed caudal mesonephric ducts), in contrast to the endodermal urothelium elsewhere. Gray's Anatomy (41e) records that later lineage-tracing work (Viana et al. 2007; Tanaka et al. 2010) has shown the trigone epithelium is in fact **endodermal**, like the rest of the bladder, and that trigonal muscle is derived predominantly from the detrusor rather than from the ureter — worth quoting if the examiner asks why the trigone is histologically distinct.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 20.15 — Photomicrograph. Histology of transverse section of urinary bladder (low magnification, H&E stain).
(Sontakke — Textbook of Human Histology — 2e, p.282)

Reproduce a labelled low-power transverse section of the bladder wall with:

- Transitional epithelium (urothelium), thrown into mucosal folds
- Umbrella (superficial) cells at the luminal surface
- Lamina propria with blood vessels
- Detrusor muscle — inner longitudinal, middle circular/oblique, outer longitudinal layers
- Serosa / adventitia (outermost)

Add a small high-power inset of a single umbrella cell showing: basal cell → intermediate cell → umbrella cell sequence, urothelial plaques (asymmetric unit membrane), hinge regions, and sub-surface fusiform vesicles.

Applied / Clinical anatomy

- **Cystitis** — inflammation of the urothelium, most often from ascending urinary tract infection (uropathogenic *E. coli* via FimH–uropilin adhesion); chronic cystitis can produce an "unstable urothelium" with hyperplastic or metaplastic change.

- ▶ **Urothelial (transitional cell) carcinoma** — the commonest primary bladder malignancy, usually papillary, arising from the urothelium; recognised predisposing factors include smoking, occupational exposure to dyes/textiles, arsenic, and chronic irritation from bladder stones. Cystoscopic biopsy grading and staging are read directly against this layered histology (depth of detrusor invasion determines stage).
- ▶ **Cystoscopic landmark** — the pale inter-ureteric bar of the trigone is the surgeon's guide to locating the ureteric orifices, e.g. for ureteric catheterisation or reimplantation surgery.
- ▶ **Vesico-ureteric reflux** — the oblique intramural course of the terminal ureter through the detrusor acts as a flap-valve, compressed by detrusor contraction; a short or maldeveloped intramural segment predisposes to reflux and ascending pyelonephritis.
- ▶ **Bladder exstrophy (ectopia vesicae)** — failure of fusion of the infra-umbilical mesenchyme and anterior bladder wall leaves the trigone and posterior bladder mucosa exposed on the anterior abdominal wall, with chronic squamous metaplasia of the exposed urothelium.
- ▶ **Catheter-related change** — chronic indwelling catheters mechanically irritate the urothelium and predispose to squamous metaplasia.

High-yield exam pointers

- ▶ Bladder mucosa is lined by **transitional epithelium/urothelium** — shared only with the ureter and (in part) the prostatic urethra.
- ▶ Three cytological layers: **basal (stem cells) → intermediate → umbrella (superficial, dome-shaped, up to 100 µm, sometimes binucleate)**.
- ▶ Epithelial thickness varies inversely with distension: **~5–7 layers empty vs 2–3 layers distended**.
- ▶ Umbrella cell apex = **asymmetric unit membrane (AUM)** studded with **uoplakin plaques (UPIa, UPIb, UPII, UPIIIa, UPIIIb)** — the urothelial permeability barrier; fusiform vesicles recycle membrane on filling/emptying.
- ▶ **No true muscularis mucosae or distinct submucosa** — only a thick lamina propria beneath the urothelium.
- ▶ **Detrusor** = three interlacing smooth-muscle layers (inner longitudinal, middle circular, outer longitudinal); thickens at the neck as the sphincter vesicae.
- ▶ Serosa present only over the superior surface; the rest of the bladder is covered by adventitia.
- ▶ **Trigone**: smooth, non-rugose, adherent mucosa bounded by the two ureteric orifices and the internal urethral orifice, marked superiorly by the inter-ureteric bar; current evidence favours an **endodermal** origin of its epithelium, with its muscle derived mainly from the detrusor.
- ▶ Clinically: FimH–uoplakin binding underlies most UTIs; urothelial carcinoma arises from this epithelium; the trigone is the cystoscopic key landmark, and failure of the intramural ureteric valve causes vesico-ureteric reflux.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Gray's Anatomy (Standing) 41e.

**Podocytes**

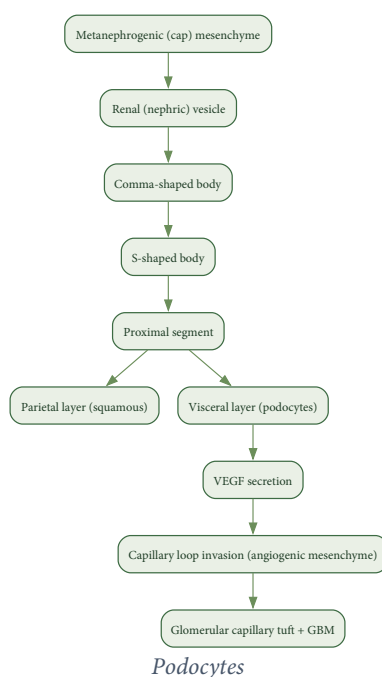
Asked in: Paper III Nov 2017 · Paper III Sep 2007 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction

Podocytes (visceral epithelial cells) are the highly specialized, terminally differentiated cells that form the inner (visceral) layer of Bowman's capsule and clothe the outer aspect of every glomerular capillary loop. Each podocyte is a stellate ("octopus-shaped") cell whose primary processes branch into numerous interdigitating secondary processes, or foot processes (pedicels), which rest on the glomerular basement membrane (GBM) and leave narrow filtration slits between them, bridged by a slit diaphragm. Together with the fenestrated glomerular endothelium and the GBM, podocytes constitute the three-layered glomerular filtration barrier, and disorders of podocyte structure are the commonest histological basis of proteinuric renal disease — which is why this is a recurring EM-spotter/short-essay topic.

Development and origin

- ▶ The metanephros (definitive kidney) develops from two components: the **ureteric bud** (from the mesonephric duct) and the **metanephrogenic (cap) mesenchyme** derived from intermediate mesoderm.
- ▶ Reciprocal induction between the ureteric bud tip and the cap mesenchyme converts clusters of mesenchymal cells into a **renal (nephric) vesicle**, which successively remodels into a **comma-shaped body** and then an **S-shaped body**.
- ▶ The **proximal segment of the S-shaped body** differentiates into the outer parietal layer (simple squamous epithelium) and the inner visceral layer — the **podocytes** — of the future Bowman's capsule.
- ▶ Podocyte progenitors at this proximal pole secrete **VEGF**, which attracts ingrowing endothelial precursors (angiogenic mesenchyme) into the cleft of the S-shaped body; the resulting capillary loops are invested by the differentiating podocytes to form the glomerular tuft.
- ▶ Podocytes and the invading endothelium jointly lay down the components of the GBM (type IV collagen, laminin, fibronectin) as the capillary loops mature.
- ▶ **Mesangial cells** are contributed by a separate (stromal/angiogenic) mesenchymal population and come to occupy the spaces between capillary loops not covered by podocyte processes.
- ▶ Once differentiated, **podocytes are permanently post-mitotic** — they cannot re-enter the cell cycle or replicate; podocyte loss is therefore not compensated by proliferation, which underlies the progressive, irreversible nature of podocyte-depletion glomerulopathies.



Location and light-microscopic features

- ▶ Podocytes constitute the **visceral (juxtacapillary) layer of Bowman's capsule**, in contrast to the outer **parietal layer**, which is lined by simple squamous epithelium resting on a basement membrane.
- ▶ The visceral and parietal layers are separated by the **urinary (Bowman's) space**, continuous at the urinary pole with the lumen of the proximal convoluted tubule.
- ▶ On routine light microscopy (H&E/PAS), podocyte cell bodies appear as large, pale, ovoid nuclei bulging into the urinary space around the periphery of the capillary tuft; their delicate cytoplasmic processes cannot be resolved by LM.
- ▶ **Mesangial cell nuclei**, lying between capillary loops, stain more darkly than podocyte nuclei and are the principal LM clue used to distinguish the two cell types within the glomerular tuft.

Ultrastructure of the podocyte

Component	Description
Cell body (perikaryon)	Stellate/octopus-shaped; bulges into the urinary space; contains nucleus, well-developed Golgi, mitochondria and abundant rough endoplasmic reticulum (active protein secretion)
Primary (major) processes	A few long, thick processes radiating from the cell body, running roughly parallel to the long axis of the capillary
Secondary processes (pedicels/foot processes)	Numerous fine processes arising from primary processes; rest directly on the GBM; interdigitate tightly with pedicels of the <i>neighbouring</i> podocyte
Filtration slits	Elongated spaces, 25–30 nm wide , between interdigitating pedicels

Component	Description
Slit diaphragm	A thin, zipper-like membrane bridging each filtration slit, through which the ultrafiltrate must pass to reach the urinary space

□ Fig 20.7 — Structure of renal corpuscle and JG apparatus. (Sontakke — Textbook of Human Histology — 2e, p.276)

Molecular architecture of the slit diaphragm

Protein	Gene	Role
Nephrin	<i>NPHS1</i>	Key transmembrane protein; extracellular domains from opposing foot processes interdigitate homophilically to form the zipper-like central density of the slit diaphragm
Podocin	<i>NPHS2</i>	Anchors nephrin-based complex to the podocyte actin cytoskeleton at the base of the foot process
Podocalyxin	—	Most abundant polyanionic sialoglycoprotein of the luminal/slit-diaphragm surface coat; confers the high negative surface charge central to charge-selectivity
Neph1, Neph2, P-cadherin, FAT1, FAT2	—	Additional adhesion molecules reinforcing the slit-diaphragm sheet
α -Actinin, synaptopodin, GLEPP-1	—	Cytoskeletal/regulatory proteins that anchor the slit diaphragm to actin filaments within the foot process and maintain slit patency

Glomerular filtration barrier and functions of podocytes

The filtration barrier separating capillary blood from the urinary space has three components, and podocytes contribute to two of them:

- **Fenestrated capillary endothelium** — blocks blood cells and platelets but is freely permeable to plasma and small solutes.
- **Glomerular basement membrane (GBM)** — a thick (~300–370 nm), fused basal lamina of endothelial and podocyte origin, composed chiefly of type IV collagen, laminin and heparan-sulphate proteoglycans; restricts passage of proteins larger than ~70 kDa and repels anionic molecules.
- **Podocyte foot processes and slit diaphragm** — the final, size-selective checkpoint before filtrate enters the urinary space.

Functions of podocytes:

- ▶ **Size-selective barrier:** the slit diaphragm's zipper architecture restricts filtration of macromolecules by molecular size.
- ▶ **Charge-selective barrier:** the polyanionic glycocalyx (podocalyxin, sialoglycoproteins, heparan sulphate) repels negatively charged plasma proteins such as albumin.
- ▶ **Synthesis and maintenance of the GBM:** podocytes (jointly with endothelium) produce laminin, type IV collagen and fibronectin.
- ▶ **Structural/mechanical support:** foot processes counteract high intracapillary hydrostatic pressure and maintain the contour of the capillary loops, preventing capillary ballooning.
- ▶ **Dynamic regulation of filtration slit calibre:** via the actin cytoskeleton anchored to the slit diaphragm, allowing modulation of filtration surface area.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a cross-section through a glomerular capillary loop showing:

- ▶ Capillary lumen with red blood cell
- ▶ Fenestrated endothelial cell (with fenestrae)
- ▶ Glomerular basement membrane — lamina rara interna, lamina densa, lamina rara externa
- ▶ Podocyte cell body with nucleus, bulging into urinary space
- ▶ Primary (major) process
- ▶ Secondary process/pedicle (foot process), interdigitating with the neighbouring podocyte's pedicels
- ▶ Filtration slit (25–30 nm) between adjacent pedicels
- ▶ Slit diaphragm spanning the filtration slit
- ▶ Urinary (Bowman's) space
- ▶ Mesangial cell/matrix filling the space between capillary loops not covered by podocytes

Applied / Clinical anatomy

- ▶ **Podocytes are terminally differentiated and post-mitotic:** once lost (by detachment or apoptosis), they cannot be replaced by proliferation of surviving podocytes — a key reason why podocyte-depletion diseases progress to irreversible **glomerulosclerosis**.
- ▶ **Nephrotic syndrome** (massive proteinuria, hypoalbuminaemia, generalized oedema, hyperlipidaemia, lipiduria) results from injury to the slit diaphragm and/or loss of the anionic charge barrier, allowing plasma proteins to leak into the filtrate.
- ▶ **Minimal change disease** — the commonest cause of childhood nephrotic syndrome: light microscopy and immunofluorescence are essentially normal, but electron microscopy shows **diffuse foot process effacement** with no electron-dense immune deposits; this ultrastructural finding is the diagnostic hallmark and is why EM is indispensable in renal biopsy work-up.
- ▶ **Focal segmental glomerulosclerosis (FSGS):** segmental capillary tuft sclerosis with diffuse foot process effacement on EM — an essential criterion for diagnosis.

- ▶ **Membranous glomerulonephritis:** sub-epithelial immune-complex deposits produce GBM "spikes"; overlying podocyte foot processes are effaced over the deposits.
- ▶ **Diabetic glomerulosclerosis** (Kimmelstiel–Wilson nodules): GBM thickening and mesangial matrix expansion with variable foot process effacement.
- ▶ **Congenital nephrotic syndrome of the Finnish type:** an autosomal recessive mutation of *NPHS1* (nephrin) causing a structurally deficient slit diaphragm and massive proteinuria from birth — the classic eponymous example of a slit-diaphragm-protein disease; *NPHS2* (podocin) mutations similarly disrupt the nephrin complex.
- ▶ **Alport syndrome:** primarily a defect of GBM type IV collagen (*COL4A3/4/5*), but foot processes are secondarily "smudged"/effaced as the GBM becomes irregular and lamellated.
- ▶ **Electron microscopy is the essential technique** for assessing podocyte foot-process integrity on renal biopsy — a portion of every diagnostic renal biopsy is fixed in glutaraldehyde for TEM processing (in addition to formalin for light microscopy and a frozen portion for immunofluorescence), since foot process effacement, GBM texture and immune deposit location can only be resolved at the ultrastructural level.
- ▶ **Surface marking/functional correlate:** because podocyte injury (not tubular injury) is the initiating lesion in most primary glomerulopathies, proteinuria without haematuria or overt inflammation classically points to a podocytopathy.

High-yield exam pointers

- ▶ Podocytes = visceral epithelial layer of Bowman's capsule; opposite the parietal (squamous) layer across the urinary space.
- ▶ Cell body → primary processes → secondary processes (pedicels/foot processes) → interdigitate with neighbouring podocyte → filtration slits (25–30 nm) → bridged by slit diaphragm.
- ▶ Slit diaphragm proteins: **nephrin** (*NPHS1*), **podocin** (*NPHS2*), podocalyxin, Nep1/2, P-cadherin, FAT1/FAT2 — anchored to actin via α -actinin/synaptopodin/GLEPP-1.
- ▶ Filtration barrier = fenestrated endothelium + GBM + podocyte slit diaphragm — size- and charge-selective.
- ▶ Podocytes are **terminally differentiated/post-mitotic** — cannot replicate once lost.
- ▶ Develop from the proximal segment of the S-shaped body of the metanephric nephron; VEGF from podocyte progenitors recruits ingrowing capillaries.
- ▶ Diffuse **foot process effacement** on EM is the hallmark of minimal change disease and a required feature of FSGS.
- ▶ Congenital nephrotic syndrome of the Finnish type = *NPHS1* (nephrin) mutation; classic genetic slit-diaphragm disease.
- ▶ Mesangial cells (not podocytes) occupy the intercapillary spaces and are phagocytic/contractile — do not confuse the two cell types.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Bancroft's Theory & Practice of Histological Techniques (transmission electron microscopy chapter); Larsen — Human Embryology 6e.



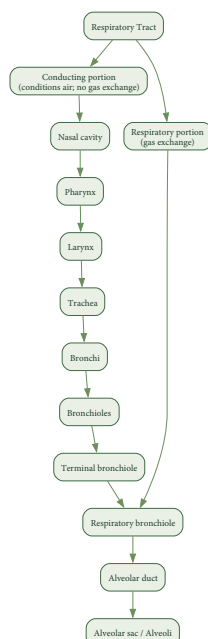
Describe the histology of the different parts of the respiratory tract. Discuss how the structure is related to its functions

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction The respiratory tract is a continuous, mucosa-lined tube extending from the external nares to the pulmonary alveoli. Anatomically it has **upper** (nasal cavities, pharynx) and **lower** (larynx, trachea, bronchi, lungs) parts. Histologically and functionally it is better divided into a **conducting portion** — nasal cavities to terminal bronchioles, which conditions (warms, humidifies, filters) inspired air — and a **respiratory portion** — respiratory bronchioles to alveoli, where gas exchange occurs. Along this length the epithelium, glands, cartilage, smooth muscle and connective-tissue framework of the wall change progressively; each regional modification is a direct structural adaptation for a specific function — mechanical support, mucociliary clearance, phonation, or diffusion — which is the structure–function theme this answer develops region by region.

1. Functional and Anatomical Organisation

- ▶ **Conducting portion:** nasal cavities → pharynx → larynx → trachea → bronchi → bronchioles → terminal bronchioles. No gas exchange; conditions air and provides low-resistance conduits.
- ▶ **Respiratory portion:** respiratory bronchioles → alveolar ducts → alveolar sacs → alveoli. Site of the system's principal function, O₂–CO₂ exchange.
- ▶ A combination of cartilage, smooth muscle, and collagen/elastic fibres gives the conducting portion both rigid support (patency) and flexibility (extensibility) — a recurring design principle in every region below.



Describe the histology of the different parts of the respiratory tract. Discuss how the structure is related to its functions

2. Nasal Cavity, Nasopharynx and Paranasal Sinuses

- ▶ **Vestibule:** skin-lined, with sebaceous/sweat glands and coarse **vibrissae** that filter particulate matter; epithelium loses keratinisation and transitions to respiratory epithelium deeper in.
- ▶ **Respiratory epithelium** covers the middle and inferior conchae and most of the conducting portion: **ciliated pseudostratified columnar epithelium** on an unusually thick basement membrane, with five cell types —
 - **Ciliated columnar cells** (most numerous; 250–300 cilia/cell) — drive the mucociliary escalator.
 - **Goblet cells** — secrete mucin glycoproteins that trap particles/microbes.
 - **Brush cells** — chemosensory, with sparse blunt microvilli.
 - **Small granule (Kulchitsky) cells** (~3%) — part of the diffuse neuroendocrine system (DNES).
 - **Basal cells** — mitotically active stem/progenitor cells (~30% of the epithelium) for the other four types.
- ▶ Vascular conchae (turbinates) create turbulence; subepithelial capillary loops and seromucous glands warm and humidify air; surface IgA (from lamina-propria plasma cells) neutralises trapped organisms.
- ▶ **Olfactory epithelium** covers the superior conchae/roof (~10 cm², up to 100 μm thick); pseudostratified, with **bipolar olfactory neurons** (dendritic knob bearing ~20 long non-motile cilia bearing odorant receptors; axons cross the cribriform plate as CN I to the olfactory bulb), **sustentacular (supporting) cells**, and **basal cells** (replace neurons every 1–2 months). **Bowman's (olfactory) glands** in the lamina propria secrete a serous fluid containing lysozyme and odorant-binding proteins.
- ▶ **Paranasal sinuses:** thin respiratory epithelium with goblet cells over a sparsely glandular lamina propria continuous with periosteum; drain into the nasal cavity by ciliary action.

- ▶ **Nasopharynx:** respiratory mucosa, contains the pharyngeal tonsil and the auditory-tube openings. **Oropharynx/laryngopharynx:** nonkeratinised stratified squamous epithelium (withstands abrasion by swallowed food), with palatine/lingual tonsils.

□ Fig 17.2 — *Respiratory epithelium. Ciliated pseudostratified columnar epithelium on a thick basement membrane, with goblet cells and a well-vascularised lamina propria. (Junqueira's Basic Histology — Text & Atlas — 17e, p.857)*

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a cross-section of respiratory epithelium: (1) thick basement membrane, (2) basal (stem) cell, (3) ciliated columnar cell with cilia, (4) goblet cell with apical mucin granules, (5) brush cell (blunt microvilli), (6) small granule/Kulchitsky cell, (7) well-vascularised lamina propria with a capillary and a seromucous gland duct.

3. Larynx

- ▶ Rigid framework: **hyaline cartilage** (thyroid, cricoid, inferior arytenoids) + **elastic cartilage** (epiglottis, cuneiform, corniculate, superior arytenoids), moved by skeletal muscles for airway patency and phonation.
- ▶ **Epiglottis:** lingual surface — stratified squamous epithelium; laryngeal surface — transitions to ciliated pseudostratified (respiratory) epithelium; mixed seromucous glands in the lamina propria.
- ▶ **Vestibular (false) folds:** covered by respiratory epithelium overlying seromucous glands and occasional lymphoid nodules — add resonance, not sound.
- ▶ **Vocal (true) folds:** **nonkeratinised stratified squamous epithelium** (resists abrasion from rapidly moving air), a dense elastic **vocal ligament**, and the striated **vocalis muscle**. Adduction of the folds narrows the **rima glottidis**; expelled air vibrates the ligament-supported mucosal edge to produce sound (phonation); tension/length/rima-width changes alter pitch.

4. Trachea

- ▶ **10–12 cm long**, lined by typical respiratory epithelium; lamina propria has abundant **seromucous glands**.
- ▶ Wall reinforced by **16–20 incomplete, C-shaped rings of hyaline cartilage** (Gray's Anatomy 41e), open posteriorly against the oesophagus and bridged there by the smooth **trachealis muscle** and a fibroelastic membrane.
- ▶ Trachealis relaxes during swallowing (allows oesophageal bulging) and contracts strongly in the cough reflex (narrows the lumen, increases expiratory air velocity, expels debris).

5. Bronchial Tree — Bronchi, Bronchioles and Terminal Bronchioles

- ▶ Trachea bifurcates into primary bronchi → 3 (right)/2 (left) secondary (lobar) bronchi → tertiary (segmental) bronchi, each supplying an independent **bronchopulmonary segment** with its own artery and connective-tissue capsule (basis of segmental lung resection).
- ▶ As airway calibre falls, the histological organisation progressively simplifies:

Feature	Bronchi	Bronchioles (≤ 1 mm)	Terminal bronchioles
Epithelium	Pseudostratified ciliated columnar, goblet cells	Ciliated simple columnar $\rightarrow$ simple cuboidal	Simple cuboidal; mostly non-ciliated club (Clara) cells
Cartilage	Rings $\rightarrow$ irregular isolated plates (decreasing)	Absent	Absent
Submucosal glands	Present (seromucous)	Absent	Absent
Smooth muscle	Spiral bands in lamina propria	Proportionately more prominent	Present, 1–2 layers, folds the mucosa
Goblet cells	Present	Progressively fewer	Absent
MALT/lymphoid tissue	Present, more at branch points	More prominent	Present

- **Club (Clara) cells** of terminal bronchioles: secrete surfactant lipoproteins/mucins, detoxify inhaled xenobiotics (SER/P450 enzymes), secrete antimicrobial peptides/cytokines, and act as the bronchiolar stem-cell pool. Chemosensory brush cells and DNES small-granule cells persist alongside them.

6. Respiratory Portion — Respiratory Bronchioles, Alveolar Ducts and Alveoli

- **Respiratory bronchioles**: mucosa like terminal bronchioles (club + squamous cells) but interrupted by scattered alveolar openings — the first gas-exchanging component.
- **Alveolar ducts**: a continuous linear series of alveolar openings, each ringed by a knob of smooth muscle; end in clusters — **alveolar sacs**.
- **Alveoli**: ~ 200 μm diameter saccules; ~ 200 million/adult lung; total surface area $\approx 75 \text{ m}^2$. **Interalveolar septa** contain fibroblasts, a dense capillary meshwork (richest in the body), and elastic + reticular fibres — elastic fibres permit passive expansion/recoil, reticular fibres resist over-distension and collapse. **Pores of Kohn** ($10\text{--}15 \mu\text{m}$) connect adjacent alveoli, equalising pressure and permitting collateral air flow.
- **Alveolar wall cell types**:

Cell	Structure	Function
Type I pneumocyte	Extremely attenuated squamous cell; $\sim 95\%$ of alveolar surface; organelles perinuclear	Forms the alveolar side of the blood–air barrier; minimal diffusion distance
Type II pneumocyte (septal cell)	Cuboidal, apical microvilli, cytoplasmic lamellar bodies	Synthesises/secretates pulmonary surfactant (DPPC + cholesterol + SP-A, SP-B, SP-C, SP-D); acts as the progenitor/stem cell replacing damaged Type I cells

Cell	Structure	Function
Alveolar macrophage ("dust cell")	Free in alveolus/septum	Phagocytoses particulates, surfactant turnover, migrated erythrocytes (hemosiderin)

- ▶ **Blood–air barrier** (0.1–1.5 μm thick): Type I pneumocyte + fused basal laminae + continuous (non-fenestrated) capillary endothelium — the minimal-diffusion-distance design that enables rapid passive O_2/CO_2 exchange.
- ▶ **Surfactant** (SP-A most abundant, innate-immune; SP-B/SP-C orient the phospholipid DPPC film; SP-D innate-immune) lowers alveolar surface tension, preventing end-expiratory collapse and reducing the work of breathing.
- ▶ **Pleura**: simple squamous mesothelium on a thin elastic/collagenous connective-tissue layer, continuous between visceral (lung surface) and parietal (thoracic wall) layers at the hilum; secretes serous fluid that lubricates respiratory movements.

□ Fig 15.18 — Blood–air barrier: type I alveolar cell, fused basal laminae, and continuous capillary endothelium. (Sontakke — *Textbook of Human Histology* — 2e, p.213)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label the alveolar wall/blood-air barrier in cross-section: (1) alveolar lumen, (2) Type I pneumocyte (attenuated squamous), (3) fused epithelial–endothelial basal lamina, (4) capillary endothelial cell (continuous, non-fenestrated), (5) erythrocyte in the capillary lumen, (6) Type II pneumocyte with lamellar bodies bulging into an adjacent alveolus, (7) alveolar macrophage, (8) pore of Kohn connecting two alveoli, (9) interalveolar septum with elastic/reticular fibres.

7. Structure–Function Correlation

Structural feature	Region	Functional significance
Ciliated pseudostratified columnar epithelium + goblet cells	Nasal cavity $\rightarrow$ bronchioles	Mucociliary escalator — traps and clears particles/microbes
Vascular conchae + seromucous glands	Nasal cavity	Warms and humidifies inspired air
Olfactory bipolar neurons with direct CN I connection	Superior concha/roof	Special sense of smell — unique neuroepithelial transduction
Nonkeratinised stratified squamous epithelium + vocal ligament + vocalis muscle	Vocal folds	Withstands abrasion of vibrating airflow; enables phonation
C-shaped hyaline cartilage rings (open posteriorly)	Trachea, large bronchi	Keeps lumen patent against negative intrathoracic pressure yet allows oesophageal bulging
Progressive loss of cartilage/glands, relative rise in smooth muscle	Bronchi $\rightarrow$ bronchioles	Permits autonomic control of airway calibre where rigid support is no longer needed

Structural feature	Region	Functional significance
Club (Clara) cells	Terminal bronchioles	Surfactant/mucin secretion, xenobiotic detoxification, innate defence, stem-cell reserve
Progressive epithelial thinning (columnar → cuboidal → squamous)	Bronchioles → alveoli	Shortens diffusion distance in preparation for gas exchange
Extreme attenuation of Type I pneumocyte + fused basal lamina	Alveolar wall	Blood–air barrier as thin as 0.1 μm — rapid passive O_2/CO_2 diffusion
Type II pneumocyte lamellar bodies/surfactant	Alveolar wall	Lowers surface tension (Laplace's law), prevents alveolar collapse at end-expiration
Elastic + reticular fibres in septa/pleura	Alveoli, pleura	Elastic recoil on expiration; reticular meshwork resists over-distension
Independent bronchus + artery per bronchopulmonary segment	Lung	Functional/surgical unit — segmental resection without injuring adjacent lung

Applied / Clinical anatomy

- ▶ **Squamous metaplasia:** chronic smoking/pollution converts respiratory epithelium (pseudostratified columnar) to stratified squamous, particularly in bronchi — a precancerous change.
- ▶ **Primary ciliary dyskinesia (Kartagener syndrome):** defective ciliary ultrastructure → chronic sinusitis, bronchitis, bronchiectasis (with situs inversus).
- ▶ **Laryngitis/croup:** viral oedema of laryngeal/vocal-fold lamina propria → hoarseness; **singer's nodules** are reactive polyps of the vocal-fold squamous epithelium.
- ▶ **Asthma:** bronchiolar smooth-muscle bronchospasm from mast-cell degranulation; the bronchiolar muscle layer is proportionately thicker than in bronchi.
- ▶ **Atelectasis:** mucus/aspirate obstruction of bronchi → lobular collapse as blood absorbs alveolar gas.
- ▶ **Emphysema:** chronic smoking-related dilation/destruction of distal airspaces beyond terminal bronchioles, irreversible loss of gas-exchange surface.
- ▶ **Infant respiratory distress syndrome:** incomplete Type II pneumocyte differentiation/surfactant deficiency in prematurity; adult ARDS from diffuse alveolar/endothelial injury; COVID-19 targets Type II pneumocytes via the ACE2 receptor, depleting surfactant.
- ▶ **"Heart failure cells":** hemosiderin-laden alveolar macrophages seen in pulmonary venous congestion (e.g. mitral stenosis).
- ▶ **Lung cancer histogenesis:** squamous cell carcinoma from segmental bronchial epithelium (smoking-linked); adenocarcinoma from peripheral bronchiolar/alveolar epithelium (commonest in non-smokers); small-cell carcinoma from neuroendocrine (Kulchitsky) cells of bronchial epithelium.

- ▶ **Pneumothorax/pleurisy/pleural effusion:** disruption or inflammation of the pleural mesothelial lining and its serous-fluid-filled potential space.

High-yield exam pointers

- ▶ Conducting portion (nasal cavity → terminal bronchiole) vs respiratory portion (respiratory bronchiole → alveoli) is the key functional classification.
- ▶ Respiratory epithelium = ciliated pseudostratified columnar with 5 cell types: ciliated, goblet, brush, small granule (Kulchitsky), basal.
- ▶ Olfactory epithelium (superior concha/roof): bipolar neurons + sustentacular + basal cells; Bowman's glands.
- ▶ Vocal folds = nonkeratinised stratified squamous epithelium + vocal ligament + vocalis muscle (only region with this epithelium apart from vestibule/epiglottis).
- ▶ Trachea: 16–20 C-shaped hyaline cartilage rings, open posteriorly, bridged by trachealis muscle.
- ▶ Cartilage (rings → plates) and glands disappear by the bronchiole level; smooth muscle becomes relatively more prominent.
- ▶ Terminal bronchioles: simple cuboidal epithelium dominated by club (Clara) cells — no cartilage, no glands.
- ▶ Alveolus: Type I pneumocyte (~95% surface area, blood–air barrier) + Type II pneumocyte (surfactant, lamellar bodies, stem cell) + alveolar macrophage.
- ▶ Blood–air barrier = Type I cell + fused basal lamina + continuous capillary endothelium (0.1–1.5 μm).
- ▶ Surfactant (DPPC + SP-A/B/C/D) lowers surface tension, prevents alveolar collapse, eases work of breathing.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Gray's Anatomy (Standring) 41e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



Describe the histology of urinary tract and its role in excretion and storage

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction The urinary tract comprises the **kidneys** — the secretory organs that manufacture urine — and the **excretory passages** (minor and major calyces, renal pelvis, ureters, urinary bladder and urethra) that conduct, temporarily store, and periodically evacuate urine without altering its composition. The **nephron**, the structural and functional unit of the kidney, together with the **collecting-duct system**, carries out filtration, selective reabsorption, secretion and hormone-regulated concentration of urine (excretion). The lower urinary tract, lined almost throughout by a unique stretchable epithelium — the **urothelium (transitional epithelium)** — is histologically adapted for distension and impermeable storage of urine, with a coordinated detrusor–sphincter mechanism for continence and micturition.

1. Kidney parenchyma — organisation

- Kidney substance is divided into an outer **cortex** (renal corpuscles, convoluted tubules, medullary rays) and inner **medulla**, organised into 8–18 conical **renal pyramids**.
- The apex of each pyramid, the **renal papilla**, projects into a **minor calyx**; 2–3 minor calyces unite to form a **major calyx**, and 2–3 major calyces unite to form the **renal pelvis**, which is continuous below with the ureter.
- Each kidney contains roughly one million **nephrons**, each opening into a **collecting duct** (derived from the ureteric bud) that finally drains into a minor calyx.

□ Fig 20.2 — Histology of kidney (low magnification). (Sontakke — Textbook of Human Histology — 2e, p.273)

2. Nephron — renal corpuscle and tubular segments

Segment	Epithelium / lining	Key histological features	Principal function
Renal corpuscle (Bowman's capsule + glomerulus)	Parietal layer — simple squamous; Visceral layer — podocytes	Glomerulus = tuft of fenestrated capillaries fed by afferent, drained by efferent arteriole; Bowman's space continuous with PCT lumen at urinary pole	Ultrafiltration of plasma
Proximal convoluted tubule (PCT)	Simple cuboidal with prominent brush border (microvilli)	Acidophilic cytoplasm (numerous basal mitochondria giving basal striations); indistinct lumen; longest, most convoluted segment, confined to cortex	Bulk (~65–80%) reabsorption of Na ⁺ , water, glucose, amino acids, HCO ₃ ⁻

Segment	Epithelium / lining	Key histological features	Principal function
Loop of Henle — thin segments	Simple squamous	Descending thin limb freely permeable to water; thin ascending limb impermeable to water but permeable to NaCl	Countercurrent multiplication
Thick ascending limb / Distal convoluted tubule (DCT)	Simple cuboidal, no brush border , distinct lumen	Basal striations present; macula densa at the point DCT abuts its own afferent arteriole	Active NaCl reabsorption, dilutes tubular fluid
Collecting duct	Cuboidal (cortex) → columnar (medulla); principal (light) cells + intercalated (dark) cells	Principal cells bear ADH-regulated aquaporin-2 channels; intercalated cells regulate acid–base balance	Final, hormonally-tuned regulation of water and electrolyte reabsorption

Glomerular filtration barrier (three layers, size- and charge-selective):

- ▶ **Fenestrated endothelium** of glomerular capillaries (fenestrae without diaphragms).
- ▶ **Glomerular basement membrane** (~300 nm thick) — a central electron-dense **lamina densa** (type IV collagen meshwork, physical barrier) flanked by electron-lucent **lamina rara interna** and **externa** (rich in heparan sulfate, the charge barrier).
- ▶ **Podocyte foot processes (pedicels)** interdigitating on the basement membrane; adjacent pedicels are bridged by a **filtration slit diaphragm** (nephrin-based).

Together these restrict passage of plasma proteins above ~70 kDa while allowing free passage of water and small solutes.

3. Juxtaglomerular apparatus (JGA)

Located at the vascular pole, where the DCT contacts its own afferent (and part of the efferent) arteriole; three components:

- ▶ **Macula densa** — tall, closely packed, columnar modification of DCT epithelium; chemoreceptor sensing luminal Na^+/Cl^- concentration.
- ▶ **Juxtaglomerular (granular) cells** — modified smooth-muscle (myoepithelioid) cells in the tunica media of the afferent arteriole, containing renin granules; act as baroreceptors, releasing **renin** on a fall in perfusion pressure.
- ▶ **Extraglomerular mesangial cells (lacis cells / cells of Polkissen)** — polyhedral cells between macula densa and the arterioles, forming a lace-like network; probable signal-relay role between the other two components.

4. Ureter

- ▶ Muscular tube, **25–30 cm long**, **3–4 mm diameter**, carrying urine from renal pelvis to bladder by peristalsis.

- ▶ **Mucosa** — transitional epithelium (urothelium), 4–5 cell layers thick, resting on lamina propria; thrown into longitudinal folds giving a **stellate (star-shaped)** lumen when empty, which flattens on distension.
- ▶ **Muscularis** — upper two-thirds: inner longitudinal + outer circular layer (the reverse of the gut pattern); lower one-third: an additional outer longitudinal layer appears, continuous with the bladder detrusor.
- ▶ **Adventitia** — fibroelastic connective tissue carrying vessels, nerves and fat.
- ▶ Peristaltic waves propel urine in boluses; the oblique intramural (submucosal) course of the ureter through the bladder wall, compressed further by detrusor contraction, constitutes the physiological anti-reflux valve.

5. Urinary bladder

Wall, from within outward: **mucosa** → **submucosa** → **muscularis (detrusor)** → **serosa/adventitia**.

- ▶ **Mucosa** — urothelium (no muscularis mucosae) on lamina propria; folded when empty, smoothed on filling — except over the **trigone**, where mucosa is firmly adherent and never folds.
- ▶ **Urothelium** is 5–7 cell layers thick relaxed, thinning to only 2–3 layers when fully distended (cells change shape and slide over one another, not by cell loss).
- ▶ Superficial **umbrella cells** — large, dome-shaped, often binucleate/polyploid; their apical membrane is thickened by paracrystalline **urolakin plaques** ("asymmetric unit membrane") hinged by narrow membrane regions — plaques are internalised as discoidal vesicles when the bladder empties and reinserted into the apical membrane as it fills, allowing huge surface-area change without loss of the permeability barrier. Extensive tight junctions between umbrella cells complete this blood–urine barrier.
- ▶ **Muscularis (detrusor)** — three interlacing smooth-muscle layers (inner longitudinal, middle circular/oblique, outer longitudinal); the circular layer thickens at the internal urethral orifice to form the **sphincter vesicae (internal sphincter)** in the male.
- ▶ **Serosa** covers the superior surface (peritoneum); the rest of the bladder has fibroelastic **adventitia**.

□ Fig 20.15 — Photomicrograph. Histology of transverse section of urinary bladder (low magnification). (Sontakke — *Textbook of Human Histology* — 2e, p.282)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a nephron + JGA schema with: **Bowman's capsule** (parietal layer, Bowman's space), **glomerulus** (afferent arteriole, efferent arteriole, capillary tuft), **podocyte with foot processes/filtration slits**, **proximal convoluted tubule** (brush border), **descending thin limb**, **ascending thin limb**, **thick ascending limb/distal convoluted tubule**, **macula densa**, **juxtaglomerular cells**, **extraglomerular mesangial (laci) cells**, **collecting duct**, **vasa recta**.

6. Urethra

- ▶ **Male urethra** (prostatic → membranous → spongy/penile): prostatic part lined by **transitional epithelium** (continuous with bladder); membranous part by **stratified/pseudostratified columnar epithelium**; spongy (penile) part by **stratified/pseudostratified columnar**, changing to

stratified squamous non-keratinised at the fossa navicularis near the external meatus. Mucous glands of Littre open throughout, most numerous in the spongy part.

- ▶ **Female urethra** — lined predominantly by **stratified squamous non-keratinised** epithelium, with **transitional epithelium** only near the bladder neck; para-urethral mucous glands present.
- ▶ **Muscularis** — inner longitudinal + outer circular smooth muscle (well defined in the female urethra and in the male membranous/prostatic parts). The **membranous urethra** (both sexes) is additionally encircled by **striated muscle** forming the voluntary **external urethral sphincter**.

7. Functional correlation — excretion and storage

Excretion (urine formation)

- ▶ **Filtration** at the glomerulus is size- and charge-selective, governed directly by the three-layered filtration barrier described above.
- ▶ **PCT** performs bulk isotonic reabsorption of Na^+ , glucose, amino acids and HCO_3^- — its brush border (huge luminal surface area) and basal infoldings packed with mitochondria (basal striations) provide the structural basis for active, energy-dependent transport.
- ▶ **Loop of Henle**: differential water/solute permeability of the thin and thick limbs generates the **countercurrent multiplier**, building a hyperosmotic medullary interstitial gradient.
- ▶ **DCT/collecting duct** fine-tune the filtrate under hormonal control — aldosterone (Na^+/K^+ exchange), PTH (Ca^{2+}), and **ADH (vasopressin)**, which inserts **aquaporin-2** channels into principal-cell apical membranes, determining final urine concentration or dilution.
- ▶ The **JGA** links tubular flow/composition to systemic haemodynamics through the renin–angiotensin–aldosterone axis and tubuloglomerular feedback, regulating GFR and blood pressure.

Storage

- ▶ The urothelium's umbrella-cell **uroplakin plaques** plus inter-cellular tight junctions render the bladder mucosa impermeable to urinary solutes despite the marked change in surface area between the empty (~50 mL) and full (~400–500 mL) states.
- ▶ **Detrusor** smooth muscle undergoes receptive relaxation as the bladder fills; submucosal stretch receptors signal via pelvic afferents, triggering the **micturition reflex** once a threshold volume/pressure is reached.
- ▶ Continence between voidings is maintained by the involuntary **internal (vesical) sphincter** (thickened detrusor circular fibres) and the voluntary **external urethral sphincter** (striated muscle at the membranous urethra).

Applied / Clinical anatomy

- ▶ **Nephrotic syndrome / minimal change disease** — loss of the basement membrane's negative charge and podocyte foot-process effacement → heavy proteinuria.
- ▶ **Diabetes insipidus** (central or nephrogenic/AQP-2 defect) — failure of ADH-mediated water reabsorption at the collecting duct → large volumes of dilute urine.
- ▶ **Renal calculi** — commonly form in calyces/pelvis; Randall plaques originate in the basement membranes of the thin loop of Henle in the medulla.

- ▶ **Vesico-ureteric reflux** — results when the oblique intramural ureteric course/submucosal tunnel is inadequate (common in children), permitting retrograde urine flow and ascending infection.
- ▶ **Urinary tract infection** — more frequent in females owing to a short urethra lined largely by non-keratinised stratified squamous epithelium and its proximity to the anus.
- ▶ **Urothelial (transitional cell) carcinoma** — the commonest bladder malignancy; because urothelium lines the entire tract from renal pelvis to proximal urethra, tumours are frequently multifocal ("field change").
- ▶ **Renal biopsy** interpretation (light/electron/immunofluorescence microscopy) of glomerular basement membrane thickening and foot-process effacement underlies diagnosis of glomerulonephritides and diabetic nephropathy.

High-yield exam pointers

- ▶ Nephron = renal corpuscle + PCT + loop of Henle + DCT; ~1 million nephrons/kidney; collecting duct is of ureteric-bud origin, not part of the nephron proper.
- ▶ PCT vs DCT: PCT has brush border, acidophilic cytoplasm, indistinct lumen; DCT has no brush border, distinct lumen, fewer cells lining a wider lumen.
- ▶ Glomerular filtration barrier = fenestrated endothelium + basement membrane (lamina rara interna–densa–externa) + podocyte foot processes with slit diaphragm.
- ▶ JGA = macula densa (DCT) + juxtaglomerular/granular cells (afferent arteriole, secrete renin) + extraglomerular mesangial/lacis cells.
- ▶ Ureter and bladder are both lined by transitional epithelium (urothelium); ureteric muscularis is inner-longitudinal/outer-circular — reversed compared to the gut.
- ▶ Bladder urothelium: 5–7 layers relaxed → 2–3 layers distended; umbrella cells bear uroplakin plaques (asymmetric unit membrane) forming the blood–urine barrier.
- ▶ Detrusor = three interlacing smooth-muscle layers; internal sphincter is smooth muscle (involuntary), external urethral sphincter is striated muscle (voluntary) at the membranous urethra.
- ▶ Male urethra epithelium sequence: transitional (prostatic) → stratified/pseudostratified columnar (membranous, spongy) → stratified squamous (fossa navicularis); female urethra is mainly stratified squamous.
- ▶ ADH acts on collecting-duct principal cells via aquaporin-2 to concentrate urine; aldosterone acts on DCT/collecting duct for Na⁺ reabsorption.

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas (17e); Ross Histology — A Text and Atlas (7e); IB Singh — Textbook of Human Histology (9e); Sontakke — Textbook of Human Histology (2e).



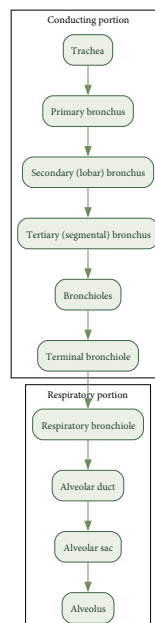
Describe the light and ultra microscopic features of the respiratory system and discuss their functional importance

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction The respiratory system is histologically divisible into a **conducting portion** (nasal cavity to terminal bronchiole), which conditions inspired air and provides no gas exchange, and a **respiratory portion** (respiratory bronchiole to alveolus), the site of O_2 - CO_2 exchange. The mucosal epithelium throughout is of **endodermal origin**, arising from the laryngotracheal (respiratory) diverticulum of the foregut, while the surrounding cartilage, smooth muscle and connective-tissue stroma differentiate from the enveloping splanchnic mesenchyme. Progressing distally, the epithelium becomes thinner, glands and cartilage disappear, and the wall is ultimately reduced — at the alveolus — to a single layer of attenuated epithelium apposed to a capillary, the basis of efficient gas exchange. Light microscopy (LM) reveals this region-by-region change in epithelium, glands, muscle and cartilage; electron microscopy (EM) reveals the fine structure of the pneumocytes and the blood–air barrier that underlies respiratory function.

1. Functional organisation of the respiratory tract

Portion	Components	Function
Conducting portion	Nasal cavity, nasopharynx, larynx, trachea, bronchi, bronchioles, terminal bronchioles	Warms, humidifies and filters air; no gas exchange
Respiratory portion	Respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli	Site of gas exchange (respiration)



Describe the light and ultra microscopic features of the respiratory system and discuss their functional importance

2. Light microscopic features — conducting portion

Region	Epithelium	Wall specialisation
Vestibule (nasal)	Stratified squamous (skin continuation)	Vibrissae + sebaceous glands trap large particles
Respiratory region (nasal cavity, sinuses, most of tract)	Ciliated pseudostratified columnar — " respiratory epithelium "	Vascular lamina propria with capillary loops (warming) + seromucous glands (humidifying)
Olfactory region (superior concha/roof)	Pseudostratified, ~60–100 µm thick, no goblet cells	Bipolar olfactory neurons + sustentacular cells + basal cells; Bowman's (olfactory) glands in lamina propria
Larynx	Vocal folds — nonkeratinised stratified squamous (abrasion resistance); rest — respiratory epithelium	Hyaline cartilages (thyroid, cricoid, arytenoid) + elastic cartilages (epiglottis, cuneiform, corniculate); vocal ligament + vocalis muscle
Trachea	Respiratory epithelium on an unusually thick basement membrane	~16–20 C-shaped hyaline cartilage rings; trachealis muscle bridges the posterior gap; submucosal seromucous glands
Bronchi (primary→secondary→tertiary)	Respiratory epithelium	Cartilage rings → irregular plates as calibre falls; spiral smooth-muscle bands appear and thicken
Bronchioles (<1 mm)	Simple columnar → cuboidal ciliated	No cartilage, no submucosal glands ; smooth muscle proportionately most prominent here
Terminal bronchiole	Cuboidal, dominated by non-ciliated club (Clara) cells	Club cells secrete surfactant lipoprotein, detoxify xenobiotics (SER/P450), secrete antimicrobial peptides; last purely conducting segment

Respiratory epithelium (the pseudostratified ciliated columnar type lining most of the tract) is composed of five cell types confirmed across sources: **ciliated columnar cells** (up to ~250–300 cilia each, beat frequency ~12–16/s, driving the mucociliary escalator), **goblet cells** (mucin secretion; ~6000–7000/mm² in the trachea, increasing with irritation), **brush cells** (chemosensory, blunt microvilli, afferent synapse), **small granule/Kulchitsky (neuroendocrine) cells** (dense-core granules ~100–300 nm, part of the diffuse neuroendocrine system) and **basal cells** (mitotic stem cells for the other four types).

□ Fig 17.2 — Respiratory epithelium. (Junqueira's Basic Histology — Text & Atlas — 17e, p.857)

3. Light microscopic features — respiratory portion

- **Respiratory bronchiole:** epithelium continues from the terminal bronchiole (cuboidal, club cells) but its wall is now interrupted by a few alveolar openings — the first site of gas exchange.

- ▶ **Alveolar duct:** an elongated airway whose wall consists almost entirely of alveolar openings, each rimmed by a knob of smooth muscle.
- ▶ **Alveolar sac:** a cluster of alveoli opening into a common space at the termination of a duct.
- ▶ **Alveolus:** thin-walled polyhedral sac ~200 μm in diameter; each adult lung has been classically described as containing ~200 million alveoli with ~75 m^2 of surface (Junqueira, IB Singh), though Gray's Anatomy gives a wider, more precise range of **300–500 million alveoli and 70–100 m^2 total surface area**, rising from ~10 million at birth mainly over the first 8 years. The **interalveolar septum** is a thin connective-tissue partition (elastic + reticular fibres, fibroblasts) carrying the densest capillary network in the body; elastic fibres allow passive recoil on expiration, reticular fibres resist over-distension.

4. Ultrastructural (EM) features — pneumocytes and the blood–air barrier

Cell/structure	Ultrastructure	Function
Type I pneumocyte	Extremely attenuated squamous cell (cytoplasm as thin as 0.05–0.2 μm); ~40% of alveolar cell population but lines >90–95% of the alveolar surface ; joined by tight junctions; cannot divide	Forms the epithelial component of the blood–air barrier; minimises diffusion distance
Type II pneumocyte (septal cell)	Cuboidal, apical microvilli, rounded nucleus; characteristic lamellar bodies (100–400 nm, concentric/stacked phospholipid membrane whorls) formed via RER→Golgi→multivesicular body; ~60% of cell population, <5–10% of surface	Synthesises, stores and secretes pulmonary surfactant ; also the progenitor/stem cell that repopulates type I cells after injury
Blood–air (respiratory) barrier	(i) Type I pneumocyte, (ii) fused basal laminae of epithelium and endothelium, (iii) continuous (non-fenestrated) capillary endothelial cell	Total thickness 0.2–1.5 μm , as little as ~0.1–0.2 μm at the thinnest points — allows rapid passive diffusion of O_2/CO_2 ; capillary transit time ~0.75 s at rest, halving on exercise
Alveolar macrophage ("dust cell")	Derived from blood monocytes; phagocytic, often laden with carbon/iron pigment	Clears particulate matter, surfactant turnover, and effete erythrocytes (→ "heart-failure cells" in pulmonary congestion)
Pores of Kohn	Small openings (~2–15 μm) through the interalveolar septum (up to ~7 per alveolus)	Equalise air pressure and permit collateral ventilation if a bronchiole is obstructed; also a route for macrophage/microbial migration

Surfactant is a lipoprotein film (mainly the phospholipid **dipalmitoylphosphatidylcholine, DPPC**, plus cholesterol) together with four surfactant proteins: **SP-A** and **SP-D** (hydrophilic, innate-immune defence and feedback regulation of secretion) and **SP-B** and **SP-C** (hydrophobic, orient DPPC within the film). It appears in the fetal lung from about the last weeks of gestation (type II cell differentiation)

and lowers alveolar surface tension, preventing end-expiratory collapse (Laplace's law) while reducing the work of breathing.

□ Fig 15.18 — Blood–air barrier. (Sontakke — *Textbook of Human Histology* — 2e, p.213)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

(a) **Cross-section of trachea/bronchus wall** — label: pseudostratified ciliated columnar (respiratory) epithelium → ciliated columnar cell, goblet cell, basal cell, brush cell, small granule (Kulchitsky) cell → thick basement membrane → lamina propria with seromucous glands → submucosa → C-shaped/irregular hyaline cartilage → adventitia with vessels/nerves.

(b) **Alveolar wall / blood–air barrier (EM diagram)** — label: alveolar lumen → type I pneumocyte (thin cytoplasm) → type II pneumocyte with lamellar bodies → fused epithelial and endothelial basal lamina → capillary endothelial cell → erythrocyte in capillary lumen → interalveolar septum (elastic/reticular fibres) → alveolar macrophage → pore of Kohn.

6. Functional importance — structure–function correlation

- ▶ **Ciliated cells + mucus blanket** → mucociliary escalator clears trapped particles/organisms toward the pharynx (impaired in primary ciliary dyskinesia/Kartagener syndrome).
- ▶ **Vascular nasal mucosa + seromucous glands** → warms and humidifies inspired air before it reaches the alveoli.
- ▶ **Progressive loss of cartilage and glands, gain of smooth muscle** (trachea → bronchiole) → shifts control of airway calibre from rigid support to autonomically-regulated muscular tone (relevant to asthma/bronchospasm).
- ▶ **Club (Clara) cells** → surfactant-like secretion, xenobiotic detoxification and antimicrobial defence in the smallest conducting airways, which lack glands.
- ▶ **Extreme thinness of type I pneumocytes + fused basal laminae + continuous capillary endothelium** → the anatomical basis of the blood–air barrier, permitting gas exchange across ~70–100 m² by simple diffusion.
- ▶ **Type II pneumocyte surfactant** → prevents alveolar collapse at low lung volumes and reduces the work of breathing; its absence causes neonatal respiratory distress syndrome.
- ▶ **Pores of Kohn** → collateral ventilation, limiting the extent of atelectasis distal to an obstructed bronchiole.

Applied / Clinical anatomy

- ▶ **Squamous metaplasia** of respiratory epithelium (chronic smoking/pollution) is reversible but predisposes to dysplasia and **squamous cell carcinoma**, typically arising from segmental bronchial epithelium.
- ▶ **Adenocarcinoma** (commonest lung cancer in non-smokers) arises more peripherally from bronchiolar/alveolar epithelium; **small cell carcinoma** arises from neuroendocrine (Kulchitsky) cells.
- ▶ **Primary ciliary dyskinesia / Kartagener syndrome** — defective ciliary ultrastructure → impaired mucociliary clearance, chronic sinusitis and bronchiectasis (with situs inversus).

- ▶ **Bronchial asthma** — goblet-cell hyperplasia, thickened basement membrane, bronchiolar smooth-muscle hypertrophy and eosinophilic infiltration.
- ▶ **Neonatal (infant) respiratory distress syndrome** — surfactant deficiency from incomplete type II pneumocyte differentiation in prematurity; treated with exogenous surfactant + antenatal corticosteroids.
- ▶ **COVID-19 / diffuse alveolar damage** — type II pneumocytes are the principal target (ACE2 receptor); loss of surfactant production contributes to alveolar collapse and hypoxia.
- ▶ **Emphysema** — destruction of alveolar walls and capillary bed, most often smoking-related, causing irreversible loss of gas-exchange surface.
- ▶ **Atelectasis, pneumothorax, pleural effusion** relate to the thin serosal pleura (simple squamous mesothelium on loose connective tissue) lining lung and thoracic wall.

High-yield exam pointers

- ▶ Conducting portion = nasal cavity to terminal bronchiole (no gas exchange); respiratory portion begins at the respiratory bronchiole.
- ▶ Five cell types of respiratory epithelium: ciliated columnar, goblet, brush, small granule (Kulchitsky), basal.
- ▶ Cartilage: complete rings (trachea) → irregular plates (bronchi) → absent (bronchioles).
- ▶ Club (Clara) cells characterise the terminal bronchiole — surfactant-like secretion + detoxification.
- ▶ Type I pneumocyte: ~40% of cells, >90% of surface, forms blood–air barrier, cannot divide.
- ▶ Type II pneumocyte: cuboidal, lamellar bodies, surfactant (DPPC + SP-A/B/C/D), progenitor for type I cells.
- ▶ Blood–air barrier = type I pneumocyte + fused basal lamina + capillary endothelium; thickness ~0.2–1.5 μm .
- ▶ Pores of Kohn permit collateral air flow between alveoli.
- ▶ Surfactant deficiency (prematurity) → neonatal RDS; type II cells are the COVID-19 target cell.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Gray's Anatomy (Standring) 41e.

Coverage note: total alveolar count/surface area is cited slightly differently across texts (Junqueira/IB Singh: ~200 million, 75 m^2 ; Gray's 41e: 300–500 million, 70–100 m^2) — both figures are given above with attribution rather than resolved to a single number, since this reflects genuine inter-textbook variation rather than a gap in the corpus.



Describe the micro-anatomy of kidney

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — The kidney is a compound tubular gland whose functional unit is the **nephron** (renal corpuscle + renal tubule), of which each kidney contains about **1 million** (both kidneys $\approx$ 2 million); after birth no new nephrons form. Each nephron drains, via a connecting tubule, into a separate **collecting-duct system** (ureteric-bud derivative) that concentrates and conducts urine to a minor calyx. Microscopically the kidney is organised into an outer **cortex** and inner **medulla**, and its parenchyma is built of three histological compartments — the nephron, the collecting-duct system, and the interstitium/vasculature.

1. General architecture — cortex, medulla, lobes and lobules

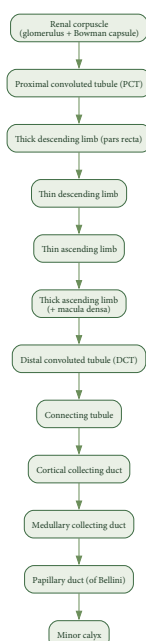
- ▶ **Capsule** — thin fibrous connective-tissue capsule covering the cortex and hilum.
- ▶ **Cortex** — outer, granular-looking (renal corpuscles + convoluted tubule cross-sections); arches over the pyramid bases and dips between them as **renal columns (of Bertin)**.
- ▶ **Medulla** — inner, pale, striated, made of **8–15 conical renal pyramids**, base towards the cortex, apex (**renal papilla**) projecting into a **minor calyx**; papillary surface studded with pores (**area cribrosa**) where papillary ducts open.
- ▶ **Renal lobe** = one pyramid + the cortical tissue over its base and along its sides (drained by one minor calyx).
- ▶ **Renal lobule** = one **medullary ray** (a column of parallel straight tubules/collecting ducts radiating from the medulla into the cortex) + the surrounding cortical tissue it drains.
- ▶ **Cortical labyrinth** = cortical tissue between medullary rays, containing renal corpuscles and convoluted tubules.

□ Fig 20.2 — Histology of kidney (low magnification). (Sontakke — Textbook of Human Histology — 2e, p.273)

2. The nephron — types and overview

Feature	Cortical nephron (~85%)	Juxtamedullary nephron (~15%)
Corpuscle position	Outer/mid cortex	Near corticomedullary junction
Loop of Henle	Short, barely enters medulla	Long, deep into inner medulla
Associated vessels	Peritubular capillary plexus	Vasa recta (countercurrent exchange)
Main role	Bulk reabsorption	Urine concentration

- ▶ A nephron = **renal corpuscle** (filtration) + **renal tubule**: proximal convoluted tubule (PCT) → loop of Henle (thick descending, thin descending, thin ascending, thick ascending limbs) → distal convoluted tubule (DCT) → connecting tubule → joins the collecting-duct system.



Describe the micro-anatomy of kidney

3. Renal corpuscle and the glomerular filtration barrier

- ▶ **Renal (Malpighian) corpuscle** — a rounded structure (~200 µm) with a **vascular pole** (afferent arteriole enters, efferent arteriole leaves) and an opposite **urinary pole** (continues as PCT).
- ▶ **Bowman capsule** — double-walled: outer **parietal layer** (simple squamous epithelium) and inner **visceral layer** made of **podocytes**; between the two is the **Bowman (urinary) space**, continuous with the tubule lumen.
- ▶ **Podocytes** — large cells with primary (major) processes giving rise to interdigitating secondary processes (**pedicels/foot processes**) that embrace the glomerular capillaries; gaps between interdigitating pedicels are **filtration slits**, bridged by a zipper-like **slit diaphragm** (nephrin-based).
- ▶ **Mesangium** — **mesangial cells** + mesangial matrix occupy the interstices between capillary loops not covered by podocytes; functions: structural/contractile support, phagocytosis of trapped macromolecules/immune complexes, matrix turnover. **Extraglomerular (laci) mesangial cells** lie outside the corpuscle at the vascular pole as part of the JGA.
- ▶ **Filtration barrier** (three layers, blood → filtrate):

Layer	Structure	Restriction
Fenestrated endothelium	Capillary endothelial cells with 70–100 nm pores, usually without diaphragms	Excludes blood cells; freely permeable to plasma
Glomerular basement membrane (GBM)	Thick (300–360 nm), fused endothelial + podocyte basal laminae; type IV collagen, laminin, heparan-sulfate proteoglycans	Size barrier + negative-charge barrier
Filtration slit diaphragm	Between interdigitating podocyte pedicels	Fine molecular sieve (final size selectivity)

□ Fig 20.8 — Photomicrograph. Structure of renal corpuscle. (Sontakke — Textbook of Human Histology — 2e, p.276)

4. Proximal convoluted tubule (PCT)

- ▶ Longest, most coiled part of the nephron; begins at the urinary pole and occupies most of the cortical labyrinth.
- ▶ Lined by **simple cuboidal/low columnar epithelium** with a tall, **brush border** (long microvilli) on the apical surface — greatly increases luminal surface area.
- ▶ Cytoplasm strongly **eosinophilic** (numerous mitochondria); **basal striations** from basal plasma-membrane infoldings interdigitating with mitochondria (Na^+/K^+ -ATPase-driven transport).
- ▶ Cell boundaries indistinct; lumen appears narrow/irregular (brush border occupies it) compared with DCT.
- ▶ Functionally reabsorbs ~2/3 of filtered water, Na^+ , and virtually all glucose and amino acids by Na^+ -coupled cotransport; apical endocytic vacuoles/canaliculi reclaim filtered small proteins.

5. Loop of Henle and distal convoluted tubule (DCT)

- ▶ **Thick descending limb** (pars recta of PCT, in medullary ray) → **thin descending limb** (simple squamous epithelium, permeable to water) → **thin ascending limb** (squamous, impermeable to water; may be short/absent in cortical nephrons) → **thick ascending limb** (cuboidal, active NaCl reabsorption, water-impermeable) — establishes the medullary osmotic gradient.
- ▶ Thick ascending limb returns to the cortex and contacts its own corpuscle's vascular pole as the **macula densa**, then continues as the **DCT**.
- ▶ **DCT** — shorter and less coiled than PCT; cuboidal epithelium, **no brush border**, cell boundaries distinct, lumen wide and clear; diameter ~20–50 μm .

Feature	PCT	DCT
Brush border	Prominent	Absent
Lumen	Narrow, indistinct	Wide, clear
Staining	Strongly eosinophilic	Paler
Length/coiling	Longer, more coiled	Shorter, less coiled

6. Juxtaglomerular apparatus (JGA)

- ▶ A specialised sensor/effector complex at the vascular pole of the renal corpuscle, made of three components:
- ▶ **Macula densa** — tall, closely packed columnar cells of the thick ascending limb/early DCT where it touches the afferent arteriole; apical nuclei; senses tubular fluid NaCl .
- ▶ **Juxtaglomerular (granular) cells** — modified smooth-muscle cells in the afferent (occasionally efferent) arteriolar wall, containing **renin**-filled secretory granules; also act as mechanoreceptors (baroreceptors) for arteriolar pressure.
- ▶ **Extraglomerular mesangial (laci) cells** — polyhedral cells filling the triangular space between macula densa, afferent and efferent arterioles; relay/integrate signals.
- ▶ Function: **tubuloglomerular feedback** (macula densa NaCl sensing adjusts afferent arteriolar tone and GFR) and **renin–angiotensin–aldosterone** control of systemic blood pressure.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a schematic of the **renal corpuscle with the juxtaglomerular apparatus**: Bowman capsule (parietal layer) • Bowman/urinary space • Visceral layer (podocytes) • Glomerular capillary tuft • Mesangial cells (intraglomerular) • Vascular pole • Afferent arteriole • Efferent arteriole • Juxtaglomerular (granular) cells • Macula densa (distal tubule) • Extraglomerular mesangial (lakis) cells • Urinary pole • Proximal convoluted tubule.

7. Collecting-duct system, interstitium and blood supply

- ▶ **Connecting tubule** → **cortical collecting duct** (in medullary rays) → **outer medullary collecting duct** → **inner medullary collecting duct** → **papillary duct of Bellini** (opens at area cribrosa into minor calyx).
- ▶ Lined by two cell types:
 - **Principal cells** — pale-staining, columnar, few organelles, sparse microvilli, distinct cell boundaries; ADH (via aquaporin-2)-regulated water and Na^+ reabsorption.
 - **Intercalated cells** — fewer, darker-staining, interspersed among principal cells; **type A** (α) secrete H^+ (acid), **type B** (β) secrete HCO_3^- — acid–base regulation.
- ▶ **Interstitium** — sparse in cortex, more prominent in medulla, especially near the papillae; medullary interstitial cells elaborate lipid droplets/prostaglandins.
- ▶ **Blood supply** (single functional end-artery pattern): renal artery → segmental → **interlobar arteries** (between pyramids) → **arcuate arteries** (at corticomedullary junction) → **interlobular (cortical radiate) arteries** → **afferent arteriole** → glomerular capillary tuft → **efferent arteriole** → (cortical nephrons) **peritubular capillary plexus** around PCT/DCT, or (juxtamedullary nephrons) **vasa recta** — hairpin capillary loops descending/ascending through the medulla in a countercurrent-exchange arrangement that preserves medullary hypertonicity. Venous drainage mirrors the arterial pattern.

Applied / Clinical anatomy

- ▶ **Podocyte foot-process effacement** (fusion) on electron microscopy is the hallmark of **minimal-change disease**, a common cause of nephrotic-range proteinuria in children.
- ▶ **Goodpasture syndrome** — autoantibodies against the NC1 domain of the $\alpha 3$ chain of type IV collagen in the GBM (shared with alveolar basement membrane) cause crescentic glomerulonephritis with pulmonary haemorrhage.
- ▶ Loss of the GBM's negatively-charged heparan-sulfate proteoglycans (e.g. in diabetic nephropathy, where the GBM also thickens progressively) impairs the charge barrier and produces albuminuria.
- ▶ Immune-complex deposition in the mesangium/GBM underlies IgA nephropathy and post-streptococcal glomerulonephritis; mesangial cells phagocytose trapped complexes.
- ▶ **Juxtaglomerular cell hyperplasia/reninoma** and **renal artery stenosis** raise renin and cause secondary hypertension via the renin–angiotensin–aldosterone axis.
- ▶ **Loop diuretics** (e.g. furosemide) act on the $\text{Na}^+ \text{--} \text{K}^+ \text{--} 2\text{Cl}^-$ cotransporter of the thick ascending limb — direct clinical correlate of loop histology/function.

- ▶ **Central and nephrogenic diabetes insipidus** reflect failure of ADH secretion or of the principal cell's aquaporin-2/V2-receptor response, producing polyuria.
- ▶ **Distal (type 1) renal tubular acidosis** results from defective H^+ secretion by type-A intercalated cells.
- ▶ Nephrons are fixed in number at birth (no post-natal neogenesis); this underlies compensatory hypertrophy after unilateral nephrectomy and progressive nephron loss with ageing/chronic kidney disease. Cystic dilatation of any nephron segment or the collecting ducts is the basis of polycystic kidney disease.

High-yield exam pointers

- ▶ Nephron = renal corpuscle + renal tubule; ~1 million nephrons/kidney; two types — cortical (short loop) and juxtamedullary (long loop, drives concentration).
- ▶ Renal lobe = pyramid + overlying cortex; renal lobule = medullary ray + surrounding cortical labyrinth.
- ▶ Filtration barrier = fenestrated endothelium + GBM + podocyte slit diaphragm (size + charge selective).
- ▶ JGA = macula densa + juxtaglomerular (granular, renin-secreting) cells + extraglomerular mesangial (lakis) cells — mediates tubuloglomerular feedback and renin release.
- ▶ PCT: brush border, eosinophilic, basal striations, narrow lumen — vs DCT: no brush border, clear wide lumen.
- ▶ Collecting duct: principal cells (ADH/water) and intercalated cells (type A H^+ / type B HCO_3^-).
- ▶ Vasa recta (from juxtamedullary efferent arterioles) — countercurrent exchange maintaining medullary hypertonicity.
- ▶ Vascular route: interlobar → arcuate → interlobular → afferent → glomerulus → efferent → peritubular capillaries/vasa recta.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e (figures).



Describe the microscopic structure of Lung and its functions

Asked in: Paper III 02-Aug-2021 — RGUHS MD/MS Anatomy

Definition / Introduction The lung is a paired, air-filled respiratory organ whose parenchyma is built around a progressively branching intrapulmonary airway system set in a richly vascular connective-tissue stroma. Functionally this airway system is divided into a **conducting portion** (intrapulmonary bronchi → bronchioles → terminal bronchioles), which only transmits air, and a **respiratory portion** (respiratory bronchioles → alveolar ducts → alveolar sacs → alveoli), where gas exchange occurs. Along this pathway cartilage, submucosal glands and pseudostratified epithelium progressively disappear while smooth muscle and elastic tissue become relatively more prominent, until the wall is reduced to a single squamous cell applied directly to a capillary. The microscopic organisation of every level directly explains a specific respiratory or non-respiratory function of the lung.

1. Conducting vs respiratory portion — overview

Feature	Conducting portion	Respiratory portion
Components	Intrapulmonary (lobar/segmental) bronchi → bronchioles → terminal bronchioles	Respiratory bronchioles → alveolar ducts → alveolar sacs → alveoli
Epithelium	Pseudostratified ciliated columnar → simple ciliated/cuboidal	Cuboidal (Clara) proximally → simple squamous at alveoli
Cartilage/glands	Present in bronchi, progressively reduced; absent in bronchioles	Absent
Function	Air conduction, filtration, humidification, mucociliary defence	Gas exchange

2. Intrapulmonary bronchi and bronchioles

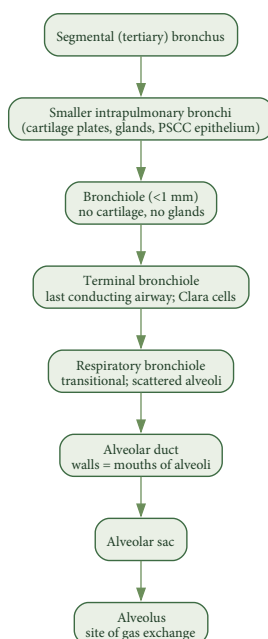
- ▶ Each **segmental (tertiary) bronchus** divides repeatedly; the wall resembles the trachea but cartilage forms **irregular, progressively smaller plates** rather than complete C-shaped rings.
- ▶ **Mucosa**: pseudostratified ciliated columnar (respiratory) epithelium containing ciliated cells, goblet cells, basal (stem) cells, brush (chemosensory) cells and neuroendocrine (Kulchitsky) cells.
- ▶ **Lamina propria**: crisscrossing spiral bands of smooth muscle and elastic fibres producing the folded mucosa seen on contraction.
- ▶ **Submucosa**: seromucous glands draining onto the lumen, lying between muscle and cartilage.
- ▶ **Adventitia**: vessels, nerves and increasing bronchus-associated lymphoid tissue (BALT), especially at branching points.
- ▶ **Bronchioles** are intralobular airways ≤ 1 mm in diameter, arising after about the tenth generation of branching; they **lack cartilage and submucosal glands** — the defining histological criterion separating bronchiole from bronchus. Epithelium simplifies from ciliated pseudostratified columnar to simple cuboidal in the smallest bronchioles, while the proportion of smooth muscle in the wall increases.

- **Terminal bronchioles** are the last purely conducting airways. Their epithelium is dominated by non-ciliated **Clara (club) cells** with dome-shaped apical secretory granules that (i) secrete surfactant lipoproteins/mucins, (ii) detoxify inhaled xenobiotics via smooth-ER cytochrome-P450 enzymes, and (iii) secrete antimicrobial peptides/cytokines and the secretory component for IgA transport. A **secondary pulmonary lobule** (the smallest, pyramid-shaped macroscopic subdivision, apex toward the hilum) contains about five to seven terminal bronchioles.

Characteristic	Bronchus	Bronchiole
Diameter	>1 mm	<1 mm
Epithelium	Pseudostratified ciliated columnar with goblet cells	Simple columnar (few cilia/goblet cells) → simple cuboidal, no cilia/goblet cells
Smooth muscle	Discrete layer between mucosa and cartilage	Well-defined muscle + elastic layer beneath mucosa
Cartilage	Present, irregular plates	Absent
Glands	Serous and mucous acini present	Absent

3. Respiratory bronchioles, alveolar ducts, alveolar sacs and the pulmonary acinus

- Each terminal bronchiole divides into two or more **respiratory bronchioles**, the transitional segment that is partly conducting and partly respiratory because a few alveoli open directly from its wall; epithelium is cuboidal (Clara cells) proximally, becoming simple squamous at the alveolar openings, with a thin persisting layer of smooth muscle and elastic tissue.
- The **acinus**, the functional respiratory unit of the lung, lies distal to a terminal bronchiole and comprises three to four orders of respiratory bronchioles, each giving rise to three to eight orders of **alveolar ducts**; the **primary lobule** is the smaller subdivision of lung distal to a single respiratory bronchiole.
- **Alveolar ducts** are elongated passages whose entire wall consists of the mouths of alveoli; a thin lamina propria with knob-like bands of smooth muscle encircles each alveolar opening, supported by a web of elastic and collagen fibres. Alveolar ducts terminate in clusters of alveoli called **alveolar sacs**.
- **Alveoli** are saccular evaginations about 200 μm in diameter responsible for the spongy texture of the lung; each lung contains approximately **200 million alveoli**, giving an internal alveolar surface area of about **75 m²** (with a still larger capillary surface area, about 125 m², available for gas exchange).



Describe the microscopic structure of Lung and its functions

4. The alveolus: wall structure and the blood–air barrier

- ▶ Adjacent alveoli are separated by a thin **interalveolar septum** containing a dense, single-layered network of continuous (non-fenestrated) capillaries — among the richest capillary networks in the body — supported by elastic and reticular fibres, fibroblasts and alveolar macrophages.
- ▶ The **blood–air (respiratory) barrier**, across which O_2 and CO_2 diffuse, consists of three layers: (i) an attenuated **type I pneumocyte**, (ii) the fused basal laminae of the epithelial and endothelial cells, and (iii) the thin **capillary endothelial cell**; overall thickness ranges from as little as ~ 25 nm up to about $1.5\ \mu\text{m}$ across different parts of the septum.
- ▶ **Pores of Kohn** ($10\text{--}15\ \mu\text{m}$ diameter) perforate the interalveolar septa, connecting neighbouring alveoli that open onto different bronchioles; they equalise air pressure and permit collateral circulation of air if a bronchiole becomes obstructed.

Cell/structure	Key features
Type I pneumocyte (alveolar cell)	Extremely attenuated squamous cell forming the great majority ($\sim 90\text{--}95\%$) of the alveolar lining; organelles clustered perinuclearly to keep the diffusion barrier minimal; tight junctions prevent leakage of tissue fluid into the airspace; post-mitotic, replaced by differentiating type II cells
Type II pneumocyte (septal cell)	Cuboidal, bulging cell making up the remainder of the lining, often at septal angles; apical microvilli; cytoplasm contains lamellar bodies ($100\text{--}400$ nm, membrane-bound granules with stacked lipid lamellae) that store and secrete pulmonary surfactant ; normal turnover $\approx 1\%$ /day; acts as progenitor for both pneumocyte types after injury

Cell/structure	Key features
Alveolar macrophage ("dust cell")	Migrates in from blood monocytes; phagocytoses inhaled particulates, excess surfactant and extravasated erythrocytes; becomes a haemosiderin-laden " heart-failure cell " in pulmonary venous congestion; exits via the mucociliary escalator or lymphatics

- ▶ **Surfactant** is a film of phospholipid (chiefly dipalmitoylphosphatidylcholine) with cholesterol and surfactant proteins SP-A, SP-B, SP-C and SP-D, secreted continuously by type II cells; SP-A/SP-D contribute to innate immune defence while SP-B/SP-C orient the phospholipid film. It appears in adequate quantity only in the last weeks of gestation.

5. Vasculature, lymphatics, nerves and pleura

- ▶ The lung has a **dual blood supply**: the low-pressure (about 25/5 mmHg) **pulmonary circulation**, whose arterial branches accompany the bronchial tree and whose capillary networks begin at the level of the respiratory bronchioles to densely invest every alveolar septum; and the much smaller, nutritive **bronchial circulation**, derived from the systemic circulation, which supplies the conducting airways down to the respiratory bronchioles, where it anastomoses with the pulmonary vessels.
- ▶ **Lymphatics** run as a deep peribronchial network following the bronchial tree to hilar nodes and a superficial network beneath the visceral pleura; lymphatic vessels are **not present in the terminal portions of the bronchial tree beyond the alveolar ducts** — the alveoli themselves have no lymphatics.
- ▶ **Nerves**: both parasympathetic and sympathetic autonomic fibres, together with general visceral afferents carrying poorly localised pain, run mainly in the connective tissue around the larger elements of the bronchial tree, controlling smooth-muscle tone and glandular secretion, and exit at the hilum.
- ▶ **Pleura**: the lung surface is covered by **visceral pleura**, continuous at the hilum with the **parietal pleura**; both are simple squamous mesothelium on a thin connective-tissue layer rich in elastic fibres, blood vessels, nerves and lymphatics, with considerable adipose tissue beneath the parietal layer.

6. Functions of the lung — structure–function correlation

- ▶ **Gas exchange (primary function)**: enormous alveolar–capillary surface area (~200 million alveoli, ~75 m² per lung) plus a minimal blood–air barrier (as little as ~25 nm) permit rapid O₂/CO₂ diffusion.
- ▶ **Prevention of alveolar collapse**: type II pneumocyte surfactant lowers surface tension, preventing collapse on expiration and easing inspiratory work; deficiency in preterm infants causes respiratory distress syndrome.
- ▶ **Mucociliary defence and clearance**: goblet/seromucous mucus traps particles/organisms, cleared proximally by ciliated epithelium (mucociliary escalator); Clara cells detoxify xenobiotics and secrete antimicrobial peptides/IgA component; alveolar macrophages phagocytose particulates reaching alveoli; BALT provides local immune surveillance.

- ▶ **Collateral ventilation:** pores of Kohn redistribute air between alveoli of adjacent lobules if a bronchiole is obstructed, limiting atelectasis.
- ▶ **Elastic recoil and lung mechanics:** elastic/reticular fibres of septa and bronchiolar walls allow inspiratory distension and passive expiratory recoil while preventing over-distension, underpinning compliance.
- ▶ **Blood reservoir and filtration:** the low-pressure pulmonary vascular bed reservoirs blood for the left ventricle and filters small venous emboli/microthrombi before the systemic circulation.
- ▶ **Metabolic function:** pulmonary capillary endothelium is a major site of angiotensin-converting enzyme (ACE) activity, converting angiotensin I to angiotensin II and inactivating bradykinin.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 15.15 — Photomicrograph. Histology of lung (low magnification, H&E stain). (Sontakke — Textbook of Human Histology — 2e, p.209)

□ Fig 15.18 — Blood–air barrier. (Sontakke — Textbook of Human Histology — 2e, p.213)

For the hand-drawn exam diagram, label a low-power field showing:

- ▶ **Intrapulmonary bronchus** — pseudostratified ciliated columnar epithelium, lamina propria with smooth muscle, irregular cartilage plate, seromucous glands
- ▶ **Terminal bronchiole** — simple cuboidal epithelium (Clara cells), smooth muscle layer, no cartilage/glands
- ▶ **Respiratory bronchiole** → **alveolar duct** → **alveolar sac** → **alveoli**
- ▶ **Interalveolar septum** — capillary network, type I and type II pneumocytes, alveolar macrophage
- ▶ **Pore of Kohn** connecting adjacent alveoli
- ▶ Surrounding honeycomb-pattern lung parenchyma

Applied / Clinical anatomy

- ▶ **Bronchogenic carcinoma** correlates with cell of origin: squamous cell carcinoma from metaplastic squamous epithelium of segmental bronchi (smoking-linked); adenocarcinoma, commonest in non-smokers, from peripheral bronchiolar/alveolar epithelium; small-cell carcinoma from neuroendocrine (Kulchitsky) cells.
- ▶ **Asthma:** mast-cell-triggered bronchospasm; the bronchiolar muscle layer is proportionately thicker than in bronchi, explaining severity of obstruction.
- ▶ **Emphysema:** protease–antiprotease imbalance (e.g. α 1-antitrypsin deficiency) destroys interalveolar septa and elastic recoil, causing permanent airspace enlargement distal to terminal bronchioles.
- ▶ **Infant respiratory distress syndrome/hyaline membrane disease:** failure of type II pneumocytes to differentiate and secrete adequate surfactant in preterm infants; managed with antenatal corticosteroids, exogenous surfactant and CPAP.
- ▶ **ARDS/diffuse alveolar damage:** injury to type I pneumocytes and capillary endothelium from infection, toxic-gas inhalation or hyperoxia, with exudation and hyaline membrane formation.

- ▶ **Pneumoconiosis/asbestosis:** macrophage-mediated chronic inflammation and fibrosis from indigestible particulates; asbestos additionally predisposes to pleural mesothelioma.
- ▶ **Heart-failure cells:** haemosiderin-laden alveolar macrophages in sputum/lung in pulmonary venous congestion (mitral stenosis, left ventricular failure).
- ▶ **Atelectasis:** airway obstruction (mucus plug/aspiration) causes gas absorption and collapse of the dependent lobule, reversible when relieved.
- ▶ Type II pneumocytes bear the ACE2 receptor and are the principal SARS-CoV-2 target, explaining surfactant loss and alveolar collapse in severe COVID-19 pneumonia.

High-yield exam pointers

- ▶ Conducting portion ends and respiratory portion begins at the **respiratory bronchiole**.
- ▶ Loss of cartilage and glands is the histological criterion distinguishing bronchiole from bronchus.
- ▶ **Terminal bronchiole** = last purely conducting airway; epithelium dominated by **Clara (club) cells**.
- ▶ **Acinus** = functional respiratory unit distal to a terminal bronchiole (3–4 orders of respiratory bronchioles → 3–8 orders of alveolar ducts).
- ▶ **Type I pneumocyte** ~90–95% of alveolar surface (gas exchange, non-dividing); **type II pneumocyte** the remainder (surfactant via lamellar bodies, alveolar stem cell).
- ▶ Blood–air barrier = type I pneumocyte + fused basal lamina + capillary endothelium (~25 nm–1.5 µm thick).
- ▶ Each lung: ~200 million alveoli; surface area ~75 m² (capillary surface ~125 m²).
- ▶ **Pores of Kohn** equalise inter-alveolar pressure and permit collateral air flow.
- ▶ **Alveoli have no lymphatic vessels** — lymphatics end at the alveolar ducts; the lung's principal functions are gas exchange, surfactant-mediated compliance, mucociliary/macrophage defence, and (secondarily) blood filtration and ACE-mediated metabolism.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e (figures).



Describe the microscopic structure of lung indicating its functional significance

Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction The lung is the principal organ of respiration, built as a progressively narrowing system of airways — the **bronchial tree** — that terminates in some 200 million thin-walled sacs, the **alveoli**, where gas exchange with pulmonary capillary blood occurs. Histologically the lung is divided into a **conducting portion** (bronchi → bronchioles → terminal bronchioles), which conditions and delivers air without permitting exchange, and a **respiratory portion** (respiratory bronchioles → alveolar ducts → alveolar sacs → alveoli), where O_2 and CO_2 actually cross the blood–air barrier. Every histological change along this pathway — falling epithelial height, disappearance of cartilage and glands, thinning of the wall to a single squamous layer — is graded precisely toward one functional end: maximal surface area with minimal diffusion distance.

1. General plan — conducting zone vs respiratory zone

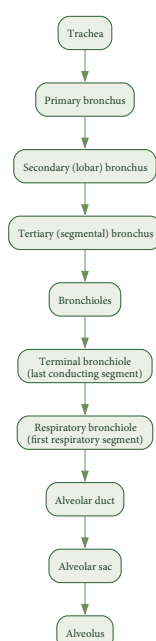
Feature	Conducting zone (bronchi → terminal bronchiole)	Respiratory zone (respiratory bronchiole → alveolus)
Epithelium	Pseudostratified ciliated columnar → simple cuboidal ciliated	Simple cuboidal (club cells) interrupted by simple squamous alveolar epithelium
Cartilage / glands	Present in bronchi, progressively lost	Absent
Smooth muscle	Present, proportionately increases as airways narrow	Confined to alveolar-duct openings
Function	Filters, warms, humidifies air; anatomical dead space	Site of gas exchange

- After entering the hilum, the principal bronchus divides into **secondary (lobar) bronchi** (3 right, 2 left, one per lobe), then **tertiary (segmental) bronchi** — each supplying a **bronchopulmonary segment**, about 10–12% of the lung with its own connective-tissue capsule and blood supply, which is why segments can be surgically resected individually.
- Each intrapulmonary bronchiole enters a pyramid-shaped **pulmonary lobule** (apex toward the hilum, base at the pleura, incompletely septated in adults) and gives off 5–7 **terminal bronchioles**, the last purely conducting segments.

2. Intrapulmonary airways — bronchus to alveolar duct

	Bronchus	Bronchiole	Terminal bronchiole	Respiratory bronchiole
Diameter	>1 mm	<1 mm	very small	very small, with scattered alveoli

	Bronchus	Bronchiole	Terminal bronchiole	Respiratory bronchiole
Epithelium	Pseudostratified ciliated columnar + goblet cells	Simple columnar, few cilia/goblet cells	Simple cuboidal, mainly club (Clara) cells	Simple cuboidal (club cells) with squamous patches at alveolar openings
Cartilage	Irregular plates	Absent	Absent	Absent
Glands	Seromucous, abundant	Absent	Absent	Absent
Smooth muscle	Spiral bundles, moderate	Well-defined layer, proportionately greater	1–2 layers	Thin layer around alveolar openings



Describe the microscopic structure of lung indicating its functional significance

□ Fig 15.15 — Photomicrograph. Histology of lung (low magnification, H&E stain), showing terminal bronchiole, respiratory bronchiole, alveolar duct and alveoli (Sontakke — *Textbook of Human Histology* — 2e, p.209)

Functional correlation:

- Loss of cartilage/glands and cilia distally reflects a shift from air-conditioning to gas exchange — a rigid, glandular wall would waste diffusion space.
- The relatively increasing muscle coat allows fine calibre control of the last conducting segments — the substrate for bronchospasm in **asthma**.
- **Club (Clara) cells** of terminal/respiratory bronchioles secrete surfactant lipoproteins and mucins, detoxify inhaled xenobiotics via smooth-ER P450 enzymes, secrete antimicrobial peptides/cytokines, and serve as the **stem cells** that regenerate bronchiolar epithelium.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A cross-section of lung parenchyma showing a respiratory bronchiole opening into alveoli, labelled with:

- ▶ **Terminal bronchiole** — simple cuboidal ciliated epithelium
- ▶ **Respiratory bronchiole** — cuboidal (club cell) epithelium interrupted by alveolar openings
- ▶ **Alveolar duct and alveolar sac**
- ▶ **Alveoli** — simple squamous lining
- ▶ **Interalveolar septum** with capillary network
- ▶ **Smooth muscle knobs** at alveolar-duct/alveolar openings

4. The alveolus and its cellular lining

- ▶ Each adult lung has **~200 million alveoli**, total gas-exchange surface **~75 m²** (capillary surface available for exchange **~125 m²**).
- ▶ **Type I alveolar cells (type I pneumocytes)**: extremely attenuated squamous cells; line **~90–95% of the alveolar surface**; cytoplasm thinned to 0.05–0.2 µm (as little as ~25 nm at the blood–air barrier itself); joined by tight junctions (prevent transudation of fluid into the alveolar space) and desmosomes; contain pinocytotic vesicles for surfactant turnover and particulate clearance; **cannot divide**.
- ▶ **Type II alveolar cells (type II pneumocytes, septal cells)**: cuboidal, bulge into the lumen, occur singly or in small groups (often at septal junctions); apical microvilli; cytoplasm rich in **lamellar bodies** (100–400 nm membrane-bound granules of stacked lamellae) that store and secrete **pulmonary surfactant**; also the **stem cell for both type I and II cells** (normal turnover ~1%/day, proliferate to replace either cell type after injury).
- ▶ **Type III alveolar cells (brush cells)**: occasional, function uncertain.
- ▶ **Alveolar macrophages ("dust cells")**: derived from circulating monocytes; phagocytose inhaled particulates, excess surfactant, and extravasated erythrocytes; in pulmonary venous congestion (mitral stenosis, left ventricular failure) they accumulate haemosiderin and are termed **heart-failure cells**; cleared via the mucociliary escalator, lymphatics, or remain in the septa for years.
- ▶ **Interalveolar septum**: sparse connective tissue of fibroblasts, **elastic fibres** (permit alveolar expansion on inspiration and passive recoil on expiration) and **reticular fibres** (prevent over-distension/rupture), supporting the densest capillary network in the body.
- ▶ **Pores of Kohn** (10–15 µm): perforate the septum, connecting adjacent alveoli of different bronchioles — equalize air pressure and permit **collateral ventilation** if a bronchiole is obstructed.

5. The blood–air barrier

□ Fig 15.18 — Blood–air barrier: surfactant, type I pneumocyte, fused basal laminae, endothelial cell, capillary and interalveolar interstitium (Sontakke — Textbook of Human Histology — 2e, p.213)

The respiratory membrane separating alveolar air from capillary blood has three components:

- ▶ The attenuated **type I alveolar cell** (and its basal lamina),

- ▶ The **fused basal laminae** of the type I cell and the capillary endothelium, and
- ▶ The thin, **continuous (non-fenestrated) capillary endothelial cell**.
- ▶ Total thickness varies **0.1–1.5 μm** , thinnest where the two basal laminae fuse.
- ▶ Endothelial cells show perinuclear clustering of organelles (mirroring type I cells) and numerous pinocytotic vesicles, maximising the thin diffusion surface.
- ▶ **Functional significance:** this minimal-thickness, maximal-area barrier allows O_2 to diffuse passively down its partial-pressure gradient into red-cell haemoglobin, while CO_2 (largely carried as bicarbonate, liberated by carbonic anhydrase in the erythrocyte) diffuses into alveolar air — the entire basis of pulmonary gas exchange (Fick's law of diffusion: $\text{rate} \propto \text{area} / \text{thickness}$).

6. Pulmonary surfactant

Component	Nature	Function
DPPC (dipalmitoylphosphatidylcholine)	Major phospholipid	Principal surface-tension-lowering agent
Cholesterol	Lipid	Maintains film fluidity
SP-A	Hydrophilic glycoprotein	Innate immune defence, surfactant homeostasis
SP-B	Hydrophobic protein	Orients DPPC in the surface film
SP-C	Hydrophobic protein	Orients DPPC in the surface film
SP-D	Hydrophilic glycoprotein	Innate immune defence

- ▶ Synthesised in type II cells (RER/Golgi), packaged in lamellar bodies, secreted by exocytosis onto the alveolar surface, and continuously recycled by pinocytosis in both alveolar-cell types and by macrophages.
- ▶ Appears only in the **last weeks of gestation** as type II cells differentiate — its deficiency in preterm infants causes **infant (neonatal) respiratory distress syndrome**, treated with antenatal steroids, exogenous surfactant and CPAP.
- ▶ **Functional significance:** by lowering alveolar surface tension it prevents alveolar collapse on expiration (Laplace's law), stabilises alveoli of unequal size, and reduces the muscular work of breathing.

Applied / Clinical anatomy

- ▶ **Bronchial asthma:** reversible bronchospasm from smooth-muscle contraction in bronchioles, whose muscle coat is proportionately thicker relative to the wall than in bronchi.
- ▶ **Bronchiolitis** (measles virus, adenovirus — mainly children): may progress to **obliterative bronchiolitis** with luminal fibrosis.
- ▶ **Emphysema:** chronic destruction of alveolar walls and elastic tissue distal to the terminal bronchiole (cigarette smoking) → irreversible airspace enlargement and loss of recoil.
- ▶ **Atelectasis:** lobular collapse following mucus/aspirate obstruction as trapped gas is absorbed into blood; reversible unless fibrosis supervenes.

- ▶ **Diffuse alveolar damage / ARDS:** injury to type I alveolar cells and capillary endothelium → pulmonary oedema and hyaline membranes.
- ▶ **Pneumonia:** infective consolidation of the respiratory zone.
- ▶ **Heart-failure cells:** haemosiderin-laden alveolar macrophages seen in pulmonary venous congestion.
- ▶ **Lung carcinoma histogenesis:** squamous cell carcinoma from bronchial epithelium (strongly smoking-linked); adenocarcinoma from peripheral bronchiolar/alveolar epithelium (commonest in non-smokers); small-cell carcinoma from neuroendocrine (Kulchitsky) cells of bronchial epithelium.
- ▶ **COVID-19:** type II pneumocytes are the principal pulmonary target via the ACE2 receptor; infection reduces surfactant production, causing alveolar collapse and hypoxia.
- ▶ **Pleura:** visceral and parietal layers are simple squamous mesothelium on elastic/collagenous connective tissue; the thin serous film between them lubricates lung movement — breach causes **pneumothorax**; inflammation/exudation causes **pleural effusion**.
- ▶ **Dual blood supply:** the low-pressure **pulmonary circulation** (systolic/diastolic $\approx 25/5$ mmHg) effects gas exchange at septal capillaries, while the systemic **bronchial circulation** nourishes the airway wall down to the respiratory bronchioles, anastomosing there with pulmonary vessels.
- ▶ **Lymphatics** run with bronchi and vessels to hilar nodes but are **absent beyond the alveolar ducts** — alveoli themselves have no lymphatics, relevant to patterns of interstitial fluid clearance and lymphatic tumour spread along the bronchovascular bundle.

High-yield exam pointers

- ▶ ~200 million alveoli per lung; total alveolar surface $\sim 75 \text{ m}^2$ (capillary surface $\sim 125 \text{ m}^2$).
- ▶ Blood–air barrier = type I pneumocyte + fused basal laminae + capillary endothelium; thickness **0.1–1.5 μm** .
- ▶ Type I pneumocytes: squamous, line $\sim 90\text{--}95\%$ of surface, cannot divide.
- ▶ Type II pneumocytes: cuboidal, contain lamellar bodies, secrete surfactant, act as stem cells for both cell types (turnover $\sim 1\%$ /day).
- ▶ Surfactant = DPPC + cholesterol + SP-A/B/C/D; deficiency causes neonatal RDS.
- ▶ Pores of Kohn provide collateral ventilation between alveoli.
- ▶ Cartilage and glands disappear, muscle proportion rises, cilia are lost by the terminal bronchiole — the conducting-to-respiratory gradient.
- ▶ Club (Clara) cells of terminal/respiratory bronchioles: surfactant/mucin secretion, xenobiotic detoxification, bronchiolar stem cells.
- ▶ Alveolar macrophages ("dust cells") become haemosiderin-laden "heart-failure cells" in pulmonary congestion.
- ▶ Pulmonary lymphatics stop at the alveolar duct — alveoli have none.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e (figures). Gray's Anatomy (Standring) 41e consulted for topographic/gross correlation.

Coverage note: Gray's Anatomy's thoracic chapter treats the lung mainly as a gross-anatomical/topographic organ and does not detail alveolar or bronchiolar histology-function correlation; this answer is therefore grounded primarily in the dedicated histology texts (Junqueira, IB Singh, Sontakke), as flagged in the topic spec.



Describe the structural and functional details of kidney

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction — The kidneys are a pair of bean-shaped, retroperitoneal excretory organs lying on the posterior abdominal wall, one on each side of the vertebral column, extending from the upper border of T12 to the body of L3 (right slightly lower than left). Each kidney is built of an outer cortex and inner medulla enclosing about one million microscopic functional units, the **nephrons**, together with a collecting-duct system. Functionally the kidney excretes nitrogenous waste and excess water/electrolytes, regulates acid–base and osmotic homeostasis, and acts as an endocrine organ (erythropoietin, renin, active vitamin D).

Gross anatomical structure

- ▶ **Shape/size/weight:** bean-shaped; ~11 cm long × 6 cm broad × 3 cm thick; left slightly longer/narrower than right; weighs ~150 g (male), 135 g (female); reddish-brown.
- ▶ **Location:** epigastric, hypochondriac, lumbar and umbilical regions; the **transpyloric plane** crosses the upper part of the right hilum and the lower part of the left hilum.
- ▶ **External features:** upper pole (broad, related to suprarenal gland) and lower pole (pointed, ~2.5 cm above the iliac crest); convex lateral border; medial border concave in its middle, showing the **hilum**.
- ▶ **Hilum contents (anterior → posterior):** renal vein, renal artery, renal pelvis ("VAP") — this order also permits identification of the side and orientation of an isolated kidney.
- ▶ **Coverings (deep to superficial):** fibrous capsule → perirenal (perinephric) fat → renal (perirenal) fascia (**Gerota's fascia**, anterior layer; **Zuckerkandl's fascia**, posterior layer) → pararenal (paranephric) fat.
- ▶ **Relations: posterior** (both sides) — diaphragm, psoas major, quadratus lumborum, transversus abdominis aponeurosis, subcostal vessels/nerve, iliohypogastric and ilioinguinal nerves; right kidney overlaps the 12th rib, left overlaps 11th–12th ribs. *Anterior, right* — suprarenal gland, liver, 2nd part of duodenum, hepatic flexure, small intestine. *Anterior, left* — suprarenal gland, stomach, spleen, pancreas, splenic vessels, splenic flexure/descending colon, jejunum.

Internal macroscopic structure

□ Fig 20.1 — Coronal section of kidney. (Sontakke — Textbook of Human Histology — 2e, p.272)

- ▶ A coronal section shows an outer reddish-brown **cortex**, an inner pale **medulla**, and a central space, the **renal sinus**.
- ▶ Medulla = ~10 conical **renal pyramids**; bases face the cortex, apices (**renal papillae**) project into the sinus and are perforated by the **area cribrosa** (openings of papillary ducts).
- ▶ Cortex = **cortical arches** (capping pyramid bases) + **renal columns** (dipping between pyramids); radial paler streaks in the cortex are **medullary rays** (extensions of pyramid tissue); a pyramid + its overlying cortical arch = one **renal lobe** (fetal kidney has ~12 lobules, which fuse postnatally).
- ▶ **Calyces and pelvis**: each minor calyx cups 1–3 papillae; minor calyces (7–13) unite to form 2–3 **major calyces**, which join to form the funnel-shaped **renal pelvis**, continuing as the ureter.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Coronal hemisection of kidney labelling: fibrous capsule → cortex (cortical arch, renal column) → medulla (renal pyramid, medullary ray) → renal papilla → area cribrosa → minor calyx → major calyx → renal pelvis → hilum (renal vein, renal artery, pelvis) → renal sinus → ureter.

Blood supply, lymphatics and nerve supply

- ▶ **Renal artery**: lateral branch of the abdominal aorta at the L1–L2 disc level; carries ~20% of cardiac output; single artery in ~70% of individuals (accessory in ~30%). Right artery is longer, passing posterior to the IVC.
- ▶ **Intrarenal branching**: renal artery → anterior + posterior divisions → **5 segmental arteries** (apical, superior, middle, inferior — anterior division; posterior — posterior division, all end-arteries) → **lobar** (one/pyramid) → **interlobar** → **arcuate** (arch at corticomedullary junction) → **interlobular arteries** → **afferent arterioles** → glomerulus → **efferent arterioles** → peritubular capillaries (cortex)/**vasa recta** (medulla). The avascular convex-border zone (**line of Brödel**) is the preferred surgical incision site.
- ▶ **Renal vein**: interlobular → arcuate → interlobar veins → renal vein → IVC (near right angles). Left vein (~7.5 cm) is 3× the right (~2.5 cm), crosses anterior to the aorta below the SMA (site of **nutcracker syndrome**) — hence left kidney preferred for donor nephrectomy.
- ▶ **Lymphatics** → lateral aortic (lumbar) nodes at L2. **Nerve supply**: renal plexus (from coeliac plexus), sympathetic T10–L1 (vasomotor); afferent pain fibres T10–T12, explaining loin-to-groin referred pain.

Microscopic structure — the nephron

Each kidney contains about **one million nephrons** (renal corpuscles), each ~50–55 mm long; no new nephrons form after birth. A nephron = **renal corpuscle** (filtration) + **renal tubule** (selective reabsorption/secretion), continuing into a **collecting duct** (final concentration).

Renal corpuscle (~0.2 mm diameter, in the cortex):

- ▶ **Glomerulus** — a tuft of anastomosing fenestrated capillaries between an afferent and efferent arteriole, supported by a mesangial matrix (mesangial cells: phagocytic, contractile, bear angiotensin-II receptors).
- ▶ **Bowman's (glomerular) capsule** — outer **parietal layer** (simple squamous epithelium) and inner **visceral layer** of **podocytes**, separated by the **urinary (Bowman's) space** continuous with the PCT lumen at the urinary pole.
- ▶ **Podocytes** send primary → secondary foot processes (pedicels) that interdigitate on the basal lamina, leaving 25 nm **filtration slits** bridged by a slit diaphragm (nephrin, podocin).
- ▶ **Glomerular filtration barrier** = fenestrated endothelium + fused glomerular basement membrane (~0.3 µm, type IV collagen + heparan sulphate — a size- and charge-selective filter) + podocyte filtration slits; retains plasma proteins >70 kDa and negatively charged/large (>~4 nm) molecules.

Renal tubule — comparison of its two convoluted segments:

Feature	Proximal convoluted tubule (PCT)	Distal convoluted tubule (DCT)
Origin	Bowman's capsule (urinary pole)	Ascending limb of loop of Henle
Length in cortex	Longer, more sections seen	Shorter, fewer sections seen
Continuity	With loop of Henle	With collecting duct
Epithelium	Cuboidal, tall microvilli (brush border)	Cuboidal, no brush border
Cytoplasm	Strongly acidophilic (mitochondria-rich)	Less acidophilic
Convolution	More convoluted	Less convoluted
Main function	Reabsorption (~80% of filtrate)	Secretion; Na ⁺ /K ⁺ exchange (aldosterone)

- ▶ **Loop of Henle:** U-shaped; thin segment (~15–30 µm, squamous epithelium, passive transport) forms most of the loop in juxtamedullary nephrons; thick ascending segment (~30–60 µm, cuboidal, mitochondria-rich, active Na⁺/Cl⁻ pump) is the source of Tamm–Horsfall protein.
- ▶ **Collecting ducts:** cortical → medullary → papillary (ducts of Bellini), lined by principal (clear) cells and intercalated (dark, H⁺-secreting) cells; open at the area cribrosa.
- ▶ **Two nephron populations:** cortical (superficial glomeruli, short loops) and juxtamedullary (glomeruli near the corticomedullary junction, long loops reaching the papilla, closely applied to vasa recta).

Juxtaglomerular apparatus (JGA)

□ Fig 20.7 — Structure of renal corpuscle and JG apparatus. (Sontakke — Textbook of Human Histology — 2e, p.276)

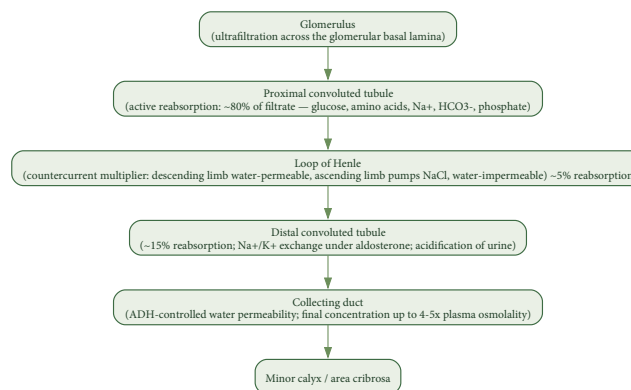
- ▶ **Macula densa** — columnar, closely packed cells of the DCT wall at the vascular pole, sensing NaCl content of tubular fluid.
- ▶ **Juxtaglomerular (granular) cells** — modified smooth-muscle/myoepithelioid cells in the afferent (and, to a lesser extent, efferent) arteriolar wall; act as baroreceptors and secrete **renin**.

- ▶ **Extraglomerular mesangial cells (lakis cells/cells of Polkissen)** — a lace-like network between macula densa and the arterioles, relaying the sensory signal.
- ▶ **Function:** tubuloglomerular feedback that maintains renal perfusion/GFR and systemic blood pressure via the renin–angiotensin–aldosterone axis (renin → angiotensin I → [ACE, lung] → angiotensin II → vasoconstriction + aldosterone/ADH release → Na^+ and water retention).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Renal corpuscle at the vascular pole labelling: afferent arteriole, efferent arteriole, glomerular capillary tuft, mesangial cells, parietal layer of Bowman's capsule, podocytes (visceral layer) with pedicels and filtration slits, Bowman's/urinary space, macula densa (DCT), juxtaglomerular cells, extraglomerular mesangial (lakis) cells, proximal convoluted tubule at the urinary pole.

Functional details — urine formation



Describe the structural and functional details of kidney

- ▶ **Glomerular filtration** — a protein-free, isotonic ultrafiltrate crosses the glomerular basal lamina under a hydrostatic pressure gradient (afferent arteriole wider than efferent); the rate is varied by afferent/efferent arteriolar tone.
- ▶ **Selective reabsorption:** PCT actively reabsorbs ~80% of the filtrate (glucose, amino acids, Na^+ , Cl^- , HCO_3^- , phosphate, Ca^{2+} , and small filtered proteins by endocytosis), with water following passively (isotonic).
- ▶ **Countercurrent multiplier (loop of Henle):** the water-impermeable ascending thick limb actively pumps Na^+/Cl^- into the interstitium while the descending limb remains water-permeable, building a corticomedullary osmotic gradient of 4–5× plasma osmolality at the papilla (~5% of filtrate reabsorbed here); the **countercurrent exchange** of the parallel vasa recta conserves this gradient rather than washing it out.
- ▶ **Distal tubule/collecting duct:** DCT reabsorbs a further ~15% of water and exchanges Na^+ for K^+ under aldosterone; collecting-duct water permeability is governed by ADH, giving final concentration/dilution of urine as needed. Acid–base balance is fine-tuned by intercalated cells (H^+ secretion, HCO_3^- reclamation); >90% of filtered K^+ is ultimately excreted via distal secretion.
- ▶ **Endocrine functions:** **erythropoietin** (cortical peritubular/interstitial cells) stimulates erythropoiesis; **renin** (JG cells) drives the renin–angiotensin–aldosterone axis; the kidney activates vitamin D to **1,25-dihydroxycholecalciferol** (calcium/phosphate metabolism); medullary interstitial cells secrete prostaglandins that modulate renal blood flow.

Applied / Clinical anatomy

- ▶ **Renal angle (costovertebral angle)** — between the 12th rib and erector spinae; tenderness here is elicited in pyelonephritis/renal calculi.
- ▶ **Congenital anomalies:** unilateral renal agenesis (compensatory hypertrophy of the solitary kidney); horseshoe kidney (isthmus trapped below the inferior mesenteric artery, may cause PUJ obstruction); pelvic/crossed ectopic kidney (failure of ascent).
- ▶ **Renal fascia:** perinephric abscess/haematoma is confined within Gerota's fascia and cannot cross the midline (vertebral attachment), but can track along the ureter into the pelvis.
- ▶ **Surgical vascular anatomy:** percutaneous nephrolithotomy/incisions exploit the avascular **line of Brödel**; the 5 vascular segments guide partial nephrectomy for renal cell carcinoma. Left kidney (longer renal vein) is preferred for live-donor nephrectomy; **nutcracker syndrome** (left renal vein compressed between aorta and SMA) causes haematuria/varicocele.
- ▶ **Imaging:** IVU/CT urography demonstrates calyces, pelvis and ureter; hydronephrosis (loss of calyceal cupping) follows PUJ obstruction, often from an aberrant lower-pole crossing vessel.
- ▶ **Glomerular/podocyte disease:** *NPHS1* (nephrin) mutation → congenital (Finnish-type) nephrotic syndrome; loss of GBM negative charge → selective proteinuria; *PKD1/PKD2* mutations → autosomal dominant polycystic kidney disease.
- ▶ **Renal biopsy** samples cortical tissue (glomeruli + PCT/DCT) for histological diagnosis of glomerulonephritis and other medical renal disease.

High-yield exam pointers

- ▶ Nephron = structural + functional unit; ~1 million/kidney; post-natally non-regenerating.
- ▶ Hilum order anteroposteriorly: **Vein–Artery–Pelvis** ("VAP").
- ▶ Renal artery = 20% of cardiac output; single artery in ~70%, accessory in ~30%; arises at L1–L2 disc level.
- ▶ 5 renal vascular segments (apical, superior, middle, inferior, posterior) — end-arteries; avascular "line of Brödel" used surgically.
- ▶ Glomerular filtration barrier = fenestrated endothelium + GBM (~0.3 µm) + podocyte filtration slits (25 nm); retains >70 kDa/negatively-charged molecules.
- ▶ JGA = macula densa (DCT) + JG cells (afferent arteriole, renin) + lacis cells → renin–angiotensin–aldosterone axis.
- ▶ PCT reabsorbs ~80% of filtrate isototically; loop of Henle countercurrent multiplier concentrates medullary interstitium 4–5×; collecting duct water permeability is ADH-dependent.
- ▶ Endocrine kidney: erythropoietin, renin, active vitamin D (1,25-dihydroxycholecalciferol).
- ▶ Left renal vein is 3× longer than right → preferred side for donor nephrectomy; crossed by aorta/SMA (nutcracker syndrome).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; BD Chaurasia Human Anatomy Vol 2 8e; IB Singh Textbook of Anatomy Vol 2 6e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e (figures).



Discuss the histology of renal tubules indicating the functional significance

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction

The renal tubule is the epithelial continuation of the nephron from the urinary pole of Bowman's capsule to its junction with a collecting duct. It comprises, in sequence, the **proximal convoluted tubule (PCT)**, the **loop of Henle** (proximal straight tubule/thick descending limb, thin segment, thick ascending limb/distal straight tubule), the **distal convoluted tubule (DCT)**, and the **connecting tubule**, which opens into the collecting-duct system. Each segment has a distinctive epithelium precisely matched to its transport function, so that together the tubule converts ~180 L/day of glomerular ultrafiltrate into ~1–1.5 L/day of concentrated urine through selective reabsorption, secretion, and countercurrent concentration.

1. Segments of the Renal Tubule — Overview

Segment	Epithelium	Diameter	Location
Neck	Simple squamous	—	Junction with Bowman's capsule
Proximal convoluted tubule (PCT)	Simple cuboidal/low columnar, prominent brush border	40–60 µm	Cortex
Proximal straight tubule (thick descending limb)	Cuboidal, reduced brush border	—	Medullary ray → outer medulla
Thin segment of loop of Henle	Simple squamous	~15 µm	Medulla (length varies with nephron type)
Thick ascending limb (distal straight tubule)	Simple cuboidal	~30 µm	Medulla → cortex
Macula densa	Tall, closely packed columnar cells	—	DCT wall at the vascular pole
Distal convoluted tubule (DCT)	Simple cuboidal, no brush border	20–50 µm	Cortex
Connecting tubule	Cuboidal (mixed DCT + principal cells)	—	Cortex

Segment	Epithelium	Diameter	Location
Collecting duct (cortical → medullary → papillary/duct of Bellini)	Simple cuboidal → columnar	40–50 µm up to 200 µm	Cortex to papilla

□ Fig 20.4 — Practice figure: Histology of kidney. PCT, proximal convoluted tubule; (Sontakke — Textbook of Human Histology — 2e, p.274)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label, on a longitudinal block of renal cortex + medulla: **Glomerulus** – **Bowman's** (parietal) **capsule** – **Bowman's/capsular space** – **Brush border** (microvilli) – **PCT** (simple cuboidal epithelium) – **Proximal straight tubule** (thick descending limb) – **Thin segment** (simple squamous epithelium) – **Thick ascending limb** (simple cuboidal epithelium) – **DCT** (simple cuboidal epithelium) – **Collecting tubules** (simple cuboidal to columnar epithelium).

2. Proximal Convoluted Tubule — Histology and Function

- ▶ Lines most of the cortical labyrinth; because it is longer and more coiled than the DCT, PCT profiles vastly outnumber DCT profiles in any cortical section — a key light-microscope identification point.
- ▶ Cells: cuboidal/low columnar, strongly **acidophilic** cytoplasm, central rounded nucleus, apex bears a tall **brush border** of long, closely packed microvilli that narrows the lumen and gives the tubule its characteristic "dark pink, indistinct-lumen" appearance in H&E.
- ▶ Ultrastructure: apical **tight junction** + zonula adherens seal the lumen; lateral **plicae** interdigitate with neighbouring cells; basal plasma membrane is thrown into deep infoldings packed with elongated, longitudinally oriented mitochondria — together producing the light-microscopic **basal striations**. Actin-filament bundles anchor the basal processes.
- ▶ Function — the major site of bulk, iso-osmotic reabsorption:
 - Reabsorbs ~80% of the glomerular filtrate (Na^+ , Cl^- , water) and virtually **100% of filtered glucose** (via apical Na^+ -glucose cotransporter **SGLT2**, basolateral **GLUT2**) and ~98% of **amino acids**.
 - Na^+/K^+ -ATPase on the lateral membrane drives active Na^+ reabsorption into the lateral intercellular space; water follows osmotically through **aquaporin-1 (AQP-1)** channels (obligatory, ADH-independent reabsorption).
 - Small proteins/peptides are recovered by receptor-mediated **endocytosis** and degraded in apical lysosomes; peptidases on the glycocalyx and **PepT1/PepT2** cotransporters handle di/tripeptides.
 - Reabsorbs filtered **bicarbonate** and secretes exogenous organic acids/bases into the lumen.

3. Loop of Henle — Histology and Function

- ▶ **Thin segment** (descending + ascending limbs): simple squamous epithelium, ~15 µm diameter, few organelles — a passive conduit for the countercurrent multiplier. Four electron-microscopic epithelial types are described:

Type	Location	Features
I	Thin descending + ascending limb, short-looped (cortical) nephrons	Very thin, few interdigitations, sparse organelles
II	Thin descending limb, long-looped nephrons (cortical part)	Taller, abundant organelles, short blunt microvilli
III	Thin descending limb, inner medulla	Thinner, simpler, fewer microvilli, no interdigitations
IV	Bend + entire thin ascending limb of long loops	Low, flat, no microvilli, few organelles

- **Thin descending limb** is highly permeable to water (AQP channels), poorly permeable to Na^+ /urea — water leaves passively into the hyperosmotic medullary interstitium, concentrating luminal fluid.
- **Thin ascending limb** is impermeable to water but permits passive Na^+/Cl^- efflux via apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters — hence called the "diluting segment"; contributes to the hyperosmotic medullary interstitium of the countercurrent exchange.
- **Thick ascending limb (distal straight tubule)**: simple cuboidal epithelium, $\sim 30\ \mu\text{m}$ diameter, pale-staining cells with indistinct lateral margins, extensive basolateral plications with numerous mitochondria; nucleus often apical, bulging into the lumen.
 - Actively transports $\text{Cl}^-/\text{Na}^+/\text{K}^+$ out via Na^+/K^+ -ATPase, generating a lumen-positive potential that drives paracellular reabsorption of Ca^{2+} and Mg^{2+} ; impermeable to water, so it separates solute from water reabsorption.
 - Secretes **uromodulin (Tamm–Horsfall protein)** — inhibits calcium-oxalate crystal aggregation (anti-lithogenic) and confers resistance to urinary tract infection; precipitated uromodulin forms the matrix of urinary casts.
 - Together, the loop of Henle reabsorbs about 5% of the filtered load and is the engine of the countercurrent multiplier that establishes the corticomedullary osmotic gradient.

4. Distal Convoluted Tubule — Histology and Function

Feature	Proximal convoluted tubule	Distal convoluted tubule
Origin	Bowman's capsule	Ascending limb of loop of Henle
Number of profiles per section	More (longer tubule)	Fewer (shorter, $\sim$ one-third the length of PCT)
Continuity	Continuous with loop of Henle	Continuous with connecting tubule/collecting duct
Epithelium	Cuboidal with microvilli (brush border)	Cuboidal without brush border
Cytoplasm	Strongly acidophilic	Less acidophilic/faintly basophilic
Lumen	Small, indistinct	Wide, distinct

Feature	Proximal convoluted tubule	Distal convoluted tubule
Convolutions	More convoluted	Less convoluted
Principal function	Reabsorption (bulk)	Secretion + fine regulation

- ▶ DCT epithelium (20–50 μm diameter) shows scattered short microvilli, prominent basal infoldings with mitochondria (basal striations, as in PCT but less developed), and the **highest Na^+/K^+ -ATPase activity of any nephron segment**.
- ▶ Begins beyond the **macula densa** and ends at the connecting tubule.
- ▶ Function: reabsorbs about 15% of filtered water; reabsorbs Na^+ (via thiazide-sensitive Na^+/Cl^- cotransporter) in exchange for K^+ secretion; the **early DCT is the principal site of parathyroid-hormone-regulated Ca^{2+} reabsorption**; participates in acidification of urine via bicarbonate reabsorption/ H^+ and ammonium secretion. Epithelium remains relatively impermeable to water.

5. Connecting Tubule and Collecting Duct — Histology and Function

- ▶ **Connecting tubule**: transitional segment where DCT cells intermingle with principal cells of the cortical collecting duct; important site of aldosterone-mediated K^+ secretion.
- ▶ **Collecting ducts** (cortical $\rightarrow$ medullary $\rightarrow$ papillary ducts of Bellini): simple epithelium, cuboidal in cortex becoming columnar toward the papilla; cell boundaries are distinct in H&E (unlike PCT/DCT), an important identification feature. Two cell types line the duct:

Cell type	Structure	Function
Principal (light) cells	Predominant; pale cytoplasm, true basal infoldings, single primary cilium, few short microvilli, small spherical mitochondria; abundant AQP-2 apically (AQP-3/4 basolaterally) and cytoplasmic mineralocorticoid receptors	ADH-regulated water reabsorption via AQP-2 insertion; primary target of aldosterone for Na^+ reabsorption/ K^+ secretion
Intercalated (dark) cells	Fewer; denser cytoplasm, many mitochondria, apical microplicae; α and β subtypes	α-intercalated : secretes H^+ (apical H^+ -ATPase), reclaims HCO_3^- basolaterally — acidifies urine; β-intercalated : opposite polarity, secretes HCO_3^- — alkalinises urine when needed. α -cells predominate (usual net acid excretion); dark cells progressively disappear toward the papilla

- ▶ Collecting ducts effect the final adjustment of urine volume and osmolality; under maximal ADH action, final urine volume is reduced to <1% of the original filtrate.

6. Juxtaglomerular Apparatus — Functional Link

A specialised complex at the vascular pole of every nephron, formed by three components, integrating tubular histology with systemic blood-pressure regulation:

- ▶ **Macula densa** — tall, densely packed columnar cells of the DCT wall abutting the afferent arteriole; senses luminal Na^+/Cl^- concentration and signals renin release/tubuloglomerular feedback.
- ▶ **Juxtaglomerular (JG) cells** — modified (epithelioid) smooth-muscle cells in the tunica media of the afferent arteriole, granule-laden with renin; act as intrarenal baroreceptors.
- ▶ **Lacis cells** (extraglomerular mesangial cells/cells of Polkissen) — polyhedral cells between macula densa and the two arterioles, forming a lace-like network that couples the other two components.
- ▶ Functional significance: renin $\rightarrow$ angiotensin I $\rightarrow$ (ACE, mainly pulmonary) $\rightarrow$ angiotensin II $\rightarrow$ vasoconstriction + aldosterone release from the adrenal zona glomerulosa $\rightarrow$ Na^+ /water retention at the DCT/collecting duct — the renin–angiotensin–aldosterone axis regulating blood pressure and volume.

Applied / Clinical anatomy

- ▶ **Acute tubular necrosis (ATN)**: PCT and thick ascending limb have the highest mitochondrial/ O_2 demand and are most vulnerable to ischaemic or nephrotoxic injury — the commonest histological correlate of intrinsic acute kidney injury; brush-border loss and epithelial cast formation are characteristic.
- ▶ **Fanconi syndrome**: generalised PCT transporter failure $\rightarrow$ glucosuria, aminoaciduria, phosphaturia, bicarbonate wasting (proximal/type II renal tubular acidosis).
- ▶ **Renal glucosuria**: isolated SGLT2 dysfunction in the PCT with normal blood glucose.
- ▶ **Distal (type I) renal tubular acidosis**: defective H^+ secretion by α -intercalated cells of the collecting duct $\rightarrow$ inability to acidify urine, nephrocalcinosis.
- ▶ **Bartter syndrome**: defect of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter of the thick ascending limb (mimics chronic loop-diuretic use); **Gitelman syndrome**: defect of the Na^+/Cl^- cotransporter of the DCT (mimics thiazide use).
- ▶ **Nephrogenic diabetes insipidus**: mutation of the V2 receptor or AQP-2 in collecting-duct principal cells $\rightarrow$ polyuria unresponsive to ADH.
- ▶ **Urinary casts**: precipitated Tamm–Horsfall protein (uromodulin) from the thick ascending limb forms the matrix of hyaline/granular casts seen in tubulointerstitial disease.

High-yield exam pointers

- ▶ PCT reabsorbs ~80% of the filtrate and is the only segment with a true brush border + prominent basal striations (mitochondria in basal infoldings).
- ▶ Thin segment of the loop of Henle shows four EM epithelial types (I–IV); the whole loop is responsible for the countercurrent multiplier and reabsorbs only ~5% of the filtrate.
- ▶ Thick ascending limb is water-impermeable ("diluting segment") and secretes uromodulin (Tamm–Horsfall protein).
- ▶ DCT has the highest Na^+/K^+ -ATPase activity in the nephron; its early part is the PTH-regulated site of Ca^{2+} reabsorption.
- ▶ Collecting duct has two cell types: principal/light cells (AQP-2, ADH and aldosterone target) and intercalated/dark cells ($\alpha = \text{H}^+$ secretion; $\beta = \text{HCO}_3^-$ secretion).

- ▶ Aldosterone's true histological target is the principal cells of the connecting tubule/collecting duct, not the DCT — a classic examiner trap based on current molecular evidence superseding older micropuncture data.
- ▶ Juxtaglomerular apparatus = macula densa (DCT) + JG cells (afferent arteriole) + lacis cells (Polkissen) — anatomical basis of the renin–angiotensin–aldosterone axis and tubuloglomerular feedback.
- ▶ PCT vs DCT distinguished in H&E by: number of profiles (PCT > DCT), lumen (PCT indistinct/small, DCT distinct/wide), brush border (PCT present, DCT absent), and staining (PCT more acidophilic).
- ▶ Clinically, PCT and thick ascending limb are the segments most susceptible to ischaemic acute tubular necrosis.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e (figure). Coverage note: this is a pure histology/ultrastructure topic; Gray's Anatomy (Standring) 41e provides only a brief overview of renal microanatomy, so depth here is drawn chiefly from the specialty histology texts above.



Functional histology of respiratory tract

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

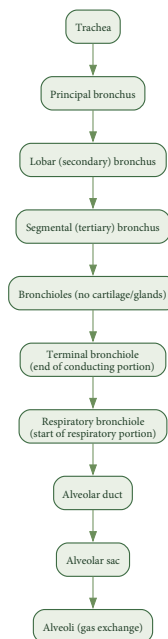
Definition / Introduction The respiratory tract is a branching system of tubes that conditions and conducts air and, at its terminal ramifications, provides the surface for gas exchange. Histologically it is divided into a **conducting portion** (nasal cavity to terminal bronchiole) that warms, humidifies and filters air but does not exchange gas, and a **respiratory portion** (respiratory bronchiole to alveoli) where actual gas exchange occurs. The epithelial lining changes progressively — from pseudostratified ciliated columnar in the upper conducting airways to simple squamous at the alveolus — and this graded transition underlies every functional property of the tract (mucociliary clearance, humidification, defence, and diffusion).

1. Organisation: conducting vs respiratory portion

Feature	Conducting portion	Respiratory portion
Components	Nasal cavity, pharynx, larynx, trachea, bronchi, bronchioles, terminal bronchiole	Respiratory bronchiole, alveolar duct, alveolar sac, alveoli
Function	Warming, humidifying, filtering air; no gas exchange	Gas exchange (O ₂ /CO ₂ diffusion)
Epithelium	Pseudostratified → simple ciliated columnar/cuboidal	Simple cuboidal (proximal) → simple squamous (alveoli)

Feature	Conducting portion	Respiratory portion
Wall support	Cartilage + smooth muscle (progressively less cartilage distally)	No cartilage; thin connective tissue + capillary net

- **Functional unit of the lung** = respiratory bronchiolar unit (or **pulmonary acinus**) — a single terminal bronchiole and all the respiratory bronchioles, alveolar ducts, sacs and alveoli it supplies.
- Sequence: Trachea → principal bronchus → lobar (secondary) bronchus → segmental (tertiary) bronchus → bronchioles → terminal bronchiole → respiratory bronchiole → alveolar duct → alveolar sac → alveoli.



Functional histology of respiratory tract

2. Nasal cavity, pharynx and larynx

- **Vestibule**: anterior dilated part; lined by **stratified squamous epithelium** with coarse hairs (**vibrissae**) that filter large particles; transitions posteriorly to respiratory epithelium.
- **Respiratory zone** (over middle/inferior conchae): lined by **ciliated pseudostratified columnar epithelium** (classic "respiratory epithelium") resting on an unusually **thick basement membrane**. Five cell types:
 - **Ciliated columnar cells** — most numerous; ~250–300 cilia/cell; drive the mucociliary escalator.
 - **Goblet cells** — secrete mucin glycoproteins.
 - **Brush cells** — chemosensory, sparse apical microvilli, synapse with sensory nerve endings.
 - **Small granule (Kulchitsky) cells** — part of the diffuse neuroendocrine system (DNES); dense-core granules.
 - **Basal cells** — mitotically active stem cells for all other types.
 - Lamina propria is highly vascular (warms air) with mucoserous glands and plasma cells secreting **IgA**.

- **Olfactory zone** (roof + superior concha, ~10 cm²): pseudostratified epithelium without goblet cells, containing bipolar **olfactory (receptor) neurons**, **sustentacular (supporting) cells**, and **basal cells**; **Bowman's (olfactory) glands** in the lamina propria wash odorants off the mucosa. Olfactory neurons are among the few CNS-related neurons that regenerate throughout life.
- **Pharynx**: nasopharynx — ciliated pseudostratified columnar (respiratory) epithelium; oro- and laryngopharynx — non-keratinised stratified squamous (food contact).
- **Larynx/epiglottis**: hyaline cartilages (thyroid, cricoid, arytenoids) and elastic cartilages (epiglottis, cuneiform, corniculate). Epiglottis has non-keratinised stratified squamous epithelium on its lingual surface and upper posterior surface (food contact), transitioning to ciliated pseudostratified columnar on the rest of its posterior surface. **Vocal folds (true cords)** are lined by **non-keratinised stratified squamous epithelium** (an adaptation to vibration/abrasion) over a dense elastic **vocal ligament** and the striated **vocalis muscle**; vestibular (false) folds retain respiratory epithelium.

□ Fig 4.6 — Ciliated pseudostratified columnar epithelium. Example, right: Respiratory epithelium (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.55)

3. Trachea

A cartilaginous, non-collapsible tube, 10–11 cm long, external diameter ~2 cm, extending from the larynx to the principal bronchi.

Layer	Composition
Mucosa	Ciliated pseudostratified columnar epithelium on a thick basement membrane; cells = ciliated columnar, goblet, brush, small granule (Kulchitsky), basal
Lamina propria	Loose connective tissue, elastic fibres condensed at its junction with submucosa (elastic membrane — seen with orcein, not H&E), diffuse lymphocytes/BALT
Submucosa	Seromucous glands with ducts opening into the lumen; blood vessels, lymphatics
Cartilage–muscle layer	16–20 C-shaped incomplete hyaline cartilage rings; posterior gap bridged by fibroelastic tissue and the trachealis (smooth) muscle
Adventitia	Fibroelastic connective tissue carrying vessels and nerves

- Functional correlate: the C-rings keep the lumen patent (anteriorly/laterally) while the flexible posterior trachealis muscle allows the oesophagus to bulge forward during swallowing, and contracts strongly during coughing to narrow the lumen and increase expiratory air velocity.

4. Bronchi and bronchioles

- **Extrapulmonary bronchi**: right bronchus is wider, shorter and more vertical than the left; right divides into 3 **secondary (lobar) bronchi**, left into 2; each further divides into 10 **tertiary (segmental) bronchi**, each supplying a bronchopulmonary segment.

- **Intrapulmonary bronchi:** mucosa similar to trachea (pseudostratified ciliated columnar with goblet cells); but cartilage is now **discontinuous irregular plates** (not complete rings), and a **complete circumferential layer of spirally arranged smooth muscle** appears between mucosa and cartilage — contraction of this muscle produces the characteristic mucosal folding seen in cross-section.
- **Bronchioles** (<1 mm diameter): no cartilage, no submucosal glands. Epithelium height decreases as calibre decreases — pseudostratified ciliated columnar (larger bronchioles) → simple ciliated columnar (medium) → simple cuboidal with **Clara (club) cells** replacing goblet cells (terminal bronchioles). Proportionately, the smooth muscle layer is thicker relative to wall thickness than in bronchi.

Feature	Bronchus	Bronchiole
Diameter	>1 mm	<1 mm
Epithelium	Pseudostratified ciliated columnar with goblet cells	Simple columnar → simple cuboidal, goblet cells reduce/absent, Clara cells appear
Cartilage	Irregular plates present	Absent
Seromucous glands	Present in submucosa	Absent
Smooth muscle	Complete layer between mucosa and cartilage	Prominent layer between mucosa and adventitia (proportionately thicker)

- **Clara (club) cells:** non-ciliated, dome-shaped, apical secretory granules; secrete **surfactant lipoprotein** and **Clara cell secretory protein (CC16)**, detoxify inhaled xenobiotics (smooth ER, cytochrome P450), and act as regional stem cells for the bronchiolar epithelium.

5. Respiratory portion: alveoli and the blood–air barrier

- **Respiratory bronchiole:** transitional segment — simple cuboidal (ciliated proximally, becoming non-ciliated with Clara cells distally) epithelium interrupted by scattered alveolar outpouchings; thin smooth muscle and elastic tissue in its wall.
- **Alveolar duct → alveolar sac → alveoli:** alveolar ducts are almost entirely lined by alveolar openings, each ringed by a knob of smooth muscle; alveolar sacs are terminal clusters of alveoli.
- **Alveoli:** thin-walled polyhedral sacs, ~200 µm diameter; each lung contains **300–500 million alveoli** with a combined surface area of **70–100 m²**.
- **Alveolar epithelial cells (pneumocytes):**
 - **Type I pneumocytes:** simple squamous cells forming **>90% of the alveolar surface**; extremely thin (0.05–0.2 µm), joined by tight junctions; cannot divide — replaced by differentiating type II cells when damaged.
 - **Type II pneumocytes** (septal/great alveolar cells): cuboidal, fewer in area (<10% of surface) but more numerous by cell count; contain **lamellar bodies** (concentric phospholipid whorls) that store and secrete **pulmonary surfactant** (dipalmitoylphosphatidylcholine + phosphatidylglycerol + surfactant proteins A–D); act as progenitors for type I cells.

- ▶ **Interalveolar septum:** thin connective tissue containing elastic and reticular fibres and a dense capillary network; **interalveolar pores of Kohn** (up to 7 per alveolus, 2–13 µm) allow collateral air flow between adjacent alveoli.
- ▶ **Blood–air (respiratory) barrier** — the thinnest gas-diffusion barrier in the body (as little as 0.2 µm): surfactant film → type I pneumocyte cytoplasm → fused basal laminae of epithelium and endothelium → continuous (non-fenestrated) capillary endothelium.
- ▶ **Alveolar macrophages ("dust cells"):** monocyte-derived; phagocytose particulates, carbon, extravasated erythrocytes (→ "**heart-failure cells**" with haemosiderin in congestive cardiac failure); migrate onto the mucociliary escalator or into lymphatics for clearance.

□ Fig 15.13 — *Histology of lung* (Sontakke — *Textbook of Human Histology* — 2e, p.208)

6. Functional correlations

- ▶ **Mucociliary escalator:** ciliated cells beat rhythmically to propel the overlying mucus blanket (produced by goblet cells + submucosal glands) toward the pharynx, clearing trapped particles and organisms — the first-line mechanical defence of the airway.
- ▶ **Air conditioning:** the vascular nasal/tracheal lamina propria warms and humidifies inspired air; vibrissae and mucus trap coarse and fine particulate matter respectively.
- ▶ **Mucosal immunity:** secretory IgA (from lamina propria plasma cells), lysozyme, and BALT/MALT nodules provide local defence; alveolar macrophages provide the deep-lung defence.
- ▶ **Surfactant physiology:** by lowering surface tension (Laplace's law: pressure $\propto 2 \times \text{tension} / \text{radius}$), surfactant prevents alveolar collapse on expiration and reduces the work of breathing; deficiency (premature type II pneumocyte immaturity) causes neonatal respiratory distress syndrome (hyaline membrane disease).
- ▶ **Progressive epithelial simplification** (pseudostratified → simple columnar → cuboidal → squamous) parallels the functional shift from conditioning/defence (needs cilia, glands, cartilage) to diffusion (needs minimal thickness).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a transverse section of **trachea**: (1) ciliated pseudostratified columnar epithelium, (2) goblet cell, (3) thick basement membrane, (4) lamina propria with BALT, (5) seromucous gland in submucosa, (6) C-shaped hyaline cartilage, (7) trachealis muscle (posterior gap), (8) adventitia.

Also label the **respiratory bronchiolar unit**: terminal bronchiole → respiratory bronchiole (with Clara cells) → alveolar duct → alveolar sac → alveoli, showing the surrounding capillary network and a type I/type II pneumocyte pair with the blood–air barrier.

Applied / Clinical anatomy

- ▶ **Squamous metaplasia:** chronic irritation (cigarette smoke, industrial pollutants) converts the normal pseudostratified ciliated columnar epithelium of bronchi to stratified squamous epithelium — a precancerous change that can progress to squamous cell carcinoma.

- ▶ **Primary ciliary dyskinesia (Kartagener syndrome):** autosomal recessive defect of ciliary dynein arms → immotile cilia → impaired mucociliary clearance, recurrent sinobronchial infections, bronchiectasis, situs inversus, and infertility.
- ▶ **Emphysema:** protease–antiprotease imbalance (e.g. α 1-antitrypsin deficiency, or protease release from macrophages activated by smoke) destroys alveolar septa, causing irreversible airspace dilatation.
- ▶ **Neonatal respiratory distress syndrome:** incomplete type II pneumocyte differentiation/surfactant deficiency in preterm infants; treated with exogenous surfactant and CPAP.
- ▶ **Asthma:** bronchiolar smooth muscle spasm (bronchospasm) with mast-cell-mediated inflammation, mucus hypersecretion and mucosal oedema, narrowing the bronchiolar lumen.
- ▶ **"Heart-failure cells":** haemosiderin-laden alveolar macrophages in sputum, seen in pulmonary venous congestion.
- ▶ **Type II pneumocytes** are the principal target cells for SARS-CoV-2 (via the ACE2 receptor), explaining surfactant loss and alveolar collapse in severe COVID-19 pneumonia.

High-yield exam pointers

- ▶ Conducting portion ends at the **terminal bronchiole**; respiratory portion begins at the **respiratory bronchiole**.
- ▶ Trachea: **16–20 C-shaped hyaline cartilage rings**; posterior gap = trachealis muscle.
- ▶ Bronchus vs bronchiole: cartilage + glands present vs absent; bronchiole diameter <1 mm.
- ▶ Terminal bronchiole epithelium: simple cuboidal with **Clara (club) cells** replacing goblet cells; no cilia distally.
- ▶ Alveolar epithelium: **Type I (>90% surface area, no mitosis) and Type II (surfactant-secreting, progenitor) pneumocytes**.
- ▶ Blood–air barrier components (list all four/five layers) — thinnest at ~0.2 μ m.
- ▶ **Pores of Kohn** allow collateral ventilation between alveoli.
- ▶ Vocal folds are the one site in the respiratory tract lined by **non-keratinised stratified squamous epithelium** away from the epiglottis.
- ▶ Olfactory epithelium cell types (olfactory neurons, sustentacular cells, basal cells) + Bowman's glands.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; IB Singh — Textbook of Human Histology 9e.



Histology of Lower Respiratory Tract

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction — The lower respiratory tract comprises the **trachea, bronchi, bronchioles and lung parenchyma (alveolar ducts, alveolar sacs, alveoli)**. Functionally it is divided into a **conducting portion** (trachea → terminal bronchioles), which warms, humidifies and filters air but performs no gas exchange, and a **respiratory portion** (respiratory bronchioles → alveoli), the site of actual O_2 - CO_2 exchange. A single design principle runs through the whole tract: as airways branch and narrow, cartilage, glands and pseudostratified epithelium are **progressively lost**, while smooth muscle becomes relatively more prominent, until the wall is reduced to the minimum compatible with gas exchange — a simple squamous epithelium apposed to a capillary.

General organisation — conducting vs respiratory portion

Portion	Structures	Chief function
Conducting	Trachea → primary bronchi → lobar (secondary) bronchi → segmental (tertiary) bronchi → bronchioles → terminal bronchioles	Conduct, warm, humidify and filter air; no gas exchange
Respiratory	Respiratory bronchioles → alveolar ducts → atria → alveolar sacs → alveoli	Gas exchange (O_2/CO_2) across the blood–air barrier

Histology of the trachea

The trachea extends from the lower border of the cricoid cartilage (C6) to its bifurcation into right and left principal bronchi at the level of T4 (the **carina**), and is about **10–12 cm long**. Its wall has four layers:

- ▶ **Mucosa** — lined by **pseudostratified ciliated columnar (respiratory) epithelium** resting on an unusually thick basement membrane. Five cell types are recognised:
 - **Ciliated columnar cells** — most numerous; ~250–300 cilia/cell; drive the mucociliary escalator.
 - **Goblet cells** — mucin-secreting, numerous, interspersed among ciliated cells.
 - **Brush cells** — sparse, blunt apical microvilli; chemosensory, synapse with afferent nerve endings.
 - **Small granule (Kulchitsky) cells** — part of the diffuse neuroendocrine system (DNES); secrete catecholamines/serotonin-like agents; ~3% of cells.
 - **Basal cells** — mitotically active stem cells that regenerate all other cell types.

Lamina propria contains lymphocytes, plasma cells, eosinophils and diffuse/nodular lymphoid aggregates (**bronchus-associated lymphoid tissue, BALT**).

- ▶ **Submucosa** — seromucous glands that keep the epithelium moist and trap dust in mucus; rich in elastic fibres.
- ▶ **Cartilage and smooth-muscle layer** — **16–20 C-shaped rings of hyaline cartilage**, open posteriorly against the oesophagus; the gap is bridged by the **trachealis muscle** (smooth muscle)

and fibroelastic tissue. The trachealis relaxes during swallowing (allows oesophageal bulge) and contracts strongly during the cough reflex.

- **Adventitia** — fibroelastic connective tissue carrying vessels and nerves.

□ Fig 15.9 — Photomicrograph. Histology of trachea (low magnification, H&E stain). (Sontakke — Textbook of Human Histology — 2e, p.205)

Bronchi, bronchioles and terminal bronchioles — progressive changes

The trachea bifurcates into **right and left principal (primary) bronchi**, which give rise to **lobar (secondary) bronchi** (3 right, 2 left — one per lobe) → **segmental (tertiary) bronchi**, each supplying a **bronchopulmonary segment** (an independent surgically-resectable unit with its own artery and segmental bronchus; 10 segments in the right lung, 8–10 in the left). Repeated division narrows the airway until, at about the tenth generation, bronchi become **bronchioles** (≤ 1 mm diameter). As the airway narrows, the following changes occur in sequence:

Feature	Bronchus (>1 mm)	Bronchiole (≤ 1 mm)	Terminal bronchiole
Epithelium	Pseudostratified ciliated columnar with goblet cells	Simple ciliated columnar → simple cuboidal; goblet cells diminish	Simple cuboidal, largely non-ciliated (club/Clara cells)
Cartilage	Present — complete rings → irregular plates as diameter falls	Absent (key distinguishing feature from bronchus)	Absent
Glands (seromucous)	Present, abundant	Absent	Absent
Smooth muscle	Present, as a spiral layer between mucosa and cartilage	Prominent, proportionately thicker than in bronchi	Prominent
Lymphoid tissue (BALT)	Present	Increases as bronchi/bronchioles narrow	Prominent

Club (Clara) cells dominate the terminal bronchiolar epithelium — non-ciliated, dome-shaped cells with apical secretory granules that (i) secrete surfactant lipoproteins and mucins that reduce surface tension and keep the small airway patent, (ii) detoxify inhaled xenobiotics via smooth-ER cytochrome P450 enzymes, and (iii) secrete antimicrobial peptides/cytokines; some also act as bronchiolar stem cells.

Respiratory portion — respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli

Each terminal bronchiole divides into **2 or more respiratory bronchioles** — the first respiratory structures, lined by simple cuboidal (club cell-rich) epithelium with a few scattered alveolar openings; as one moves distally, alveoli become progressively more numerous. Respiratory bronchioles branch into **alveolar ducts**, elongated tubes whose entire wall is a linear series of alveolar openings, each ringed by a knob of smooth muscle; each duct opens through a short **atrium** into two or more clusters of alveoli, the **alveolar sacs**. Alveoli themselves are thin-walled polyhedral outpouchings, each about 200

μm in diameter; an average adult lung contains ≈ 200 million alveoli, giving a total alveolar surface area of $\approx 75 \text{ m}^2$ (and an available capillary surface for gas exchange of $\approx 125 \text{ m}^2$).

Neighbouring alveoli are separated by thin **interalveolar septa** carrying the densest capillary network in the body, supported by elastic fibres (allow passive recoil on expiration) and reticular fibres (prevent over-distension). **Alveolar pores of Kohn** (10–15 μm) perforate the septa, equalising air pressure and permitting collateral ventilation if a bronchiole is obstructed.

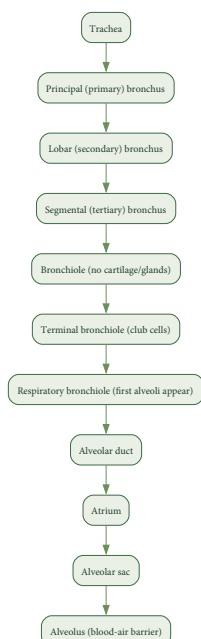
Cells of the alveolar wall

Cell	Proportion / feature	Function
Type I pneumocyte (type I alveolar cell)	Extremely attenuated squamous cell; lines $\sim 90\text{--}95\%$ of alveolar surface; cytoplasm as thin as 25 nm away from the nucleus	Forms the gas-exchange surface of the blood–air barrier
Type II pneumocyte (septal cell)	Cuboidal, bulges into lumen, apical microvilli; contains lamellar bodies (100–400 nm); often clustered at septal junctions	Synthesises and secretes pulmonary surfactant (dipalmitoylphosphatidylcholine + surfactant proteins A–D); replicates to regenerate both type I and II cells after injury (turnover $\sim 1\%$ /day)
Alveolar macrophage ("dust cell")	Free in alveolar lumen or septum; derived from circulating monocytes	Phagocytoses inhaled particulates, excess surfactant, and extravasated erythrocytes ($\rightarrow$ "heart-failure cells", haemosiderin-laden, in pulmonary venous congestion)

Blood–air barrier (respiratory membrane) — the minimal-diffusion pathway between alveolar air and capillary blood, made of:

- ▶ Type I alveolar epithelial cell (+ its basal lamina)
- ▶ Fused basal laminae of epithelial and endothelial cells
- ▶ Capillary endothelial cell (thin, continuous/non-fenestrated)

Total thickness varies from **0.1–1.5 μm** , allowing rapid diffusion of O_2 and CO_2 .



Histology of Lower Respiratory Tract

□ Fig 15.15 — Photomicrograph. Histology of lung (low magnification, H&E stain). (Sontakke — Textbook of Human Histology — 2e, p.209)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a labelled section through an interalveolar septum showing the blood-air barrier, and a small schematic of the terminal air passages:

- ▶ Alveolus (air space) on either side of the septum
- ▶ Type I pneumocyte (thin squamous lining cell)
- ▶ Type II pneumocyte (cuboidal, with lamellar bodies) at a septal junction
- ▶ Fused basal laminae of epithelium and endothelium
- ▶ Capillary endothelial cell (continuous, non-fenestrated) with an erythrocyte inside
- ▶ Alveolar macrophage ("dust cell") free in the septum/lumen
- ▶ Elastic and reticular fibres in the septum
- ▶ Alveolar pore of Kohn connecting adjacent alveoli
- ▶ A respiratory bronchiole opening into an alveolar duct → alveolar sac (label each)

Applied / Clinical anatomy

- ▶ **Squamous metaplasia** of the pseudostratified respiratory epithelium occurs in chronic smokers/industrial pollution; may progress to dysplasia — a precursor of bronchogenic carcinoma, which classically arises in larger bronchi (not bronchioles).
- ▶ **Kartagener syndrome (primary ciliary dyskinesia)** — defective ciliary ultrastructure impairs mucociliary clearance, causing recurrent sinusitis and bronchiectasis.
- ▶ **Bronchial asthma** — chronic bronchial inflammation with mast-cell-mediated smooth-muscle spasm (bronchospasm); the bronchiolar muscle layer is proportionately thicker than that of bronchi, explaining the severity of small-airway narrowing.

- ▶ **Bronchiolitis** (classically RSV in infants; also measles virus and adenovirus) predominantly affects children's bronchioles; may progress to **obliterative bronchiolitis** with luminal fibrosis.
- ▶ **Emphysema** — chronic destruction of alveolar septa (loss of elastic recoil) distal to terminal bronchioles, classically from smoking; irreversible airflow limitation.
- ▶ **Infant (neonatal) respiratory distress syndrome** — deficient type II pneumocyte surfactant production in prematurity (surfactant appears only in the last weeks of gestation); managed with antenatal corticosteroids, CPAP and exogenous surfactant.
- ▶ **COVID-19** — SARS-CoV-2 targets type II pneumocytes via the ACE2 receptor; loss of surfactant production contributes to alveolar collapse and diffuse alveolar damage/ARDS.
- ▶ **Congestive cardiac failure** — pulmonary venous congestion causes erythrocyte extravasation into alveoli; macrophages laden with haemosiderin become "heart-failure cells" in sputum.
- ▶ **Foreign-body aspiration** preferentially enters the **right principal bronchus**, which is wider, shorter (~2.5 cm) and more vertical than the left — a direct gross correlate of the same conducting-airway architecture described histologically above.
- ▶ **Bronchopulmonary segments** are surgically resectable units because each has its own segmental bronchus, artery and connective-tissue septum, sparing adjacent healthy lung in resections for localised disease (e.g., tuberculosis, tumour).

High-yield exam pointers

- ▶ Conducting portion = trachea → terminal bronchiole (no gas exchange); Respiratory portion = respiratory bronchiole → alveoli (gas exchange).
- ▶ Trachea: 10–12 cm, C6 to T4 (carina); 16–20 C-shaped hyaline cartilage rings, gap bridged posteriorly by trachealis muscle.
- ▶ Five cell types of respiratory epithelium: ciliated, goblet, brush, small granule (Kulchitsky/DNES), basal (stem) cells.
- ▶ Cartilage absent in bronchioles — the single feature that distinguishes a bronchiole from a bronchus.
- ▶ Club (Clara) cells (terminal bronchiole) secrete surfactant components, detoxify xenobiotics (P450), and are non-ciliated stem cells.
- ▶ Alveoli: ~200 million/lung, ~200 μm diameter, ~75 m^2 total surface area.
- ▶ Type I pneumocyte = thin squamous gas-exchange cell (~90–95% of surface); Type II pneumocyte = cuboidal surfactant-secreting cell with lamellar bodies, also the regenerative stem cell for both types.
- ▶ Blood–air barrier = type I cell + fused basal laminae + capillary endothelium; thickness 0.1–1.5 μm .
- ▶ Pores of Kohn interconnect alveoli, permitting collateral air flow if a bronchiole is blocked.
- ▶ Alveolar macrophages ("dust cells") → haemosiderin-laden "heart-failure cells" in pulmonary congestion.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e.



Histology of Urinary tract

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction. The urinary tract comprises the minor and major calyces, renal pelvis, ureter, urinary bladder and urethra — the excretory passages that collect, store and convey the urine formed in the nephron without modifying its composition. All of these passages, **except the urethra**, share an identical three-layered plan: a mucosa lined by **transitional epithelium (urothelium)**, a muscularis of smooth muscle, and an outer adventitia (or serosa where peritonealised). None possesses a muscularis mucosae or a true submucosa, so the epithelium rests directly on a vascular lamina propria. The urothelium is a unique stretch-tolerant stratified epithelium that also forms a blood–urine permeability barrier.

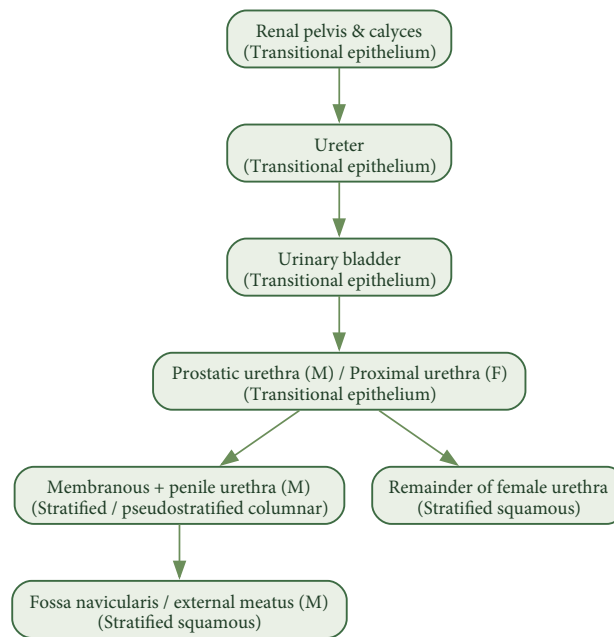
General plan of the excretory passages

Region	Mucosa (epithelium)	Muscularis	Outer coat
Minor/major calyces, renal pelvis	Transitional epithelium (urothelium), thin	Thin spiral smooth muscle (inner longitudinal, outer circular) — produces the "milking" action that moves urine from papilla to calyx	Adventitia, continuous with the renal capsule
Ureter	Transitional epithelium, 4–5 cells thick; mucosa thrown into longitudinal folds giving a star-shaped lumen	Inner longitudinal + outer circular layers (reverse of the gut); an additional outer longitudinal layer appears in the lower one-third	Adventitia (fibrofatty, carries vessels/nerves; blends with renal capsule above and bladder wall below)
Urinary bladder	Transitional epithelium (urothelium), 3 cells thick when distended to 6 or more when relaxed; surface umbrella/dome cells	Detrusor — three interlacing smooth-muscle layers (inner longitudinal, middle circular/oblique, outer longitudinal); thickens at the neck to form the internal (involuntary) sphincter	Serosa over the superior/posterosuperior surface (peritonealised); adventitia elsewhere

Region	Mucosa (epithelium)	Muscularis	Outer coat
Urethra	Epithelium varies segment to segment (see below)	Inner longitudinal + outer circular smooth muscle, better defined in the female urethra and in the male prostatic/membranous segments; a striated external urethral sphincter encircles the membranous part	Adventitia

Structure of the urothelium (transitional epithelium)

- ▶ A specialised stratified epithelium confined to the urinary passages, consisting of a **basal layer** (cuboidal stem cells), one or more **intermediate layers** (polyhedral, can slide over one another), and a **superficial layer** of large **umbrella (dome-shaped) cells** that bulge into the lumen; some umbrella cells are binucleate/polyploid.
- ▶ The apical membrane of umbrella cells is covered by rigid **urothelial plaques** separated by narrow hinge regions; the plaque membrane is thicker on its outer leaflet than its inner leaflet — the **asymmetric unit membrane (AUM)** — and is studded with a hexagonal crystalline array of **urolakins** (UPIa, UPIb, UPII, UPIIIa, UPIIIb), which render the surface impermeable to water, urea and protons.
- ▶ **Fusiform vesicles** in the apical cytoplasm store extra AUM-plaque membrane; on bladder distension these fuse with the surface by exocytosis (adding membrane and flattening the cells), and are recovered by endocytosis on emptying — the structural basis of the epithelium's ability to change from ~6 layers (relaxed) to ~3 layers (distended) without breaching the permeability barrier.
- ▶ Tight junctions between superficial cells complete the **urothelial permeability barrier**, protecting the deeper tissues from the hypertonic, acidic and cytotoxic content of urine.

*Histology of Urinary tract*

Histology of the ureter

embed: ![Fig 24.30 — Histology of the ureter (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.407)]
(C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/21072fd2bd47fd39.jpg)

- Each ureter is a muscular tube, ~25 cm long (range 25–30 cm) and 3–4 mm in luminal diameter, conducting urine from renal pelvis to bladder.
- **Mucosa:** transitional epithelium (urothelium), 4–5 cells thick, on a dense irregular lamina propria; longitudinal mucosal folds give the lumen its characteristic **star-shaped** outline on cross-section, which flattens on distension.
- **Muscle coat:** an inner longitudinal and outer circular layer of smooth muscle (the reverse arrangement to the gut); the muscle bundles interconnect and form a syncytium so that peristaltic waves propagate independently of extrinsic nerve supply. In the lower one-third of the ureter, an additional outer longitudinal layer appears; this becomes continuous with the detrusor muscle of the bladder, while the inner longitudinal layer contributes to the so-called **muscle of Bell** at the ureteric orifice.
- **Adventitia:** loose fibrofatty connective tissue carrying blood vessels, lymphatics and nerves; blends with the renal capsule proximally and the bladder wall/adventitia distally.
- Contemporary lineage studies (Gray's Anatomy 41e) show that eponymous peri-ureteric muscle bundles once thought to be ureteric in origin (**Bell's muscle**, **Mercier's bar**) are in fact derived from bladder detrusor, with only a limited contribution from ureteral smooth muscle.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label: star-shaped lumen; transitional epithelium (urothelium); lamina propria; inner longitudinal muscle layer; outer circular muscle layer; adventitia (with blood vessels/fat).

Histology of the urinary bladder

embed: ![Fig 20.14 — Histology of transverse section of urinary bladder (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.282)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/275c41bd1dddb856.jpg)

- ▶ **Mucosa:** urothelium thrown into folds (rugae) in the empty bladder, which disappear on distension; lamina propria is superficially dense and deeply loose connective tissue, carrying mucous glands near the internal urethral orifice.
- ▶ **Trigone:** the smooth, non-folded triangular area bounded by the two ureteric orifices and the internal urethral orifice; it remains constant in thickness whether the bladder is empty or full. Classical teaching attributed its distinct mucosa to a mesodermal contribution from the absorbed mesonephric ducts; current lineage-tracing evidence (Gray's Anatomy 41e) shows the trigonal urothelium is entirely **endodermal**, like the rest of the bladder, and its characteristic smooth muscle (**superficial trigonal muscle**) arises mainly from the **detrusor**, not from ureteric muscle.
- ▶ **Muscularis (detrusor):** three interlacing smooth-muscle layers — inner longitudinal, middle circular/oblique, outer longitudinal — irregularly arranged and intermixed with connective tissue (less regular than the tubular passages). At the bladder neck the circular fibres thicken to form the **internal (involuntary) urethral sphincter**.
- ▶ **Serosa/adventitia:** the superior and posterosuperior surfaces are covered by peritoneal mesothelium (serosa); the remainder has a fibroelastic adventitia carrying vessels, nerves and lymphatics.
- ▶ **Innervation:** sympathetic fibres form a plexus in the adventitia (mainly vasomotor); parasympathetic fibres from S2–S4 travel in the pelvic splanchnic nerves and are the motor (efferent) limb of the micturition reflex; visceral sensory fibres from the bladder wall form the afferent limb.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label: transitional epithelium (urothelium) with umbrella cells; folds of mucosa; lamina propria; detrusor (smooth muscle layer); blood vessels; serosa/adventitia. Add an inset marking the **trigone** as the smooth, non-folded zone between the two ureteric orifices and the internal urethral orifice.

Histology of the urethra

Segment	Approx. length	Epithelium
Male — Prostatic urethra	3–4 cm	Transitional epithelium (urothelium); ejaculatory ducts and prostatic ducts open here

Segment	Approx. length	Epithelium
Male — Membranous urethra	~1 cm	Stratified / pseudostratified columnar epithelium; surrounded by the striated external urethral sphincter in the deep perineal pouch
Male — Penile (spongy) urethra	~15 cm	Pseudostratified columnar (with patches of stratified columnar), becoming stratified squamous non-keratinised at the fossa navicularis/external meatus, continuous with penile skin; ducts of bulbourethral (Cowper's) glands and mucus-secreting glands of Littre open into it
Female urethra	3–5 cm	Transitional epithelium proximally (continuous with bladder urothelium), changing to stratified squamous non-keratinised over most of its length before the external meatus; some texts describe an intervening zone of stratified/pseudostratified columnar epithelium

- **Lamina propria** is highly vascular (resembling corpus spongiosum) and carries **urethral glands**; in the female, **paraurethral (Skene's) glands** — homologous to the male prostate — drain via paraurethral ducts beside the external urethral orifice and secrete an alkaline fluid.
- **Muscle coat**: inner longitudinal + outer circular smooth muscle, well defined in the female urethra and in the male prostatic/membranous segments (poorly defined around the penile urethra, which is instead surrounded by the erectile corpus spongiosum). A striated **external (voluntary) urethral sphincter** encircles the membranous urethra in the male and the corresponding segment in the female as each passes through the urogenital diaphragm.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label, male: prostatic urethra — transitional epithelium; membranous urethra — stratified/pseudostratified columnar + external sphincter; penile urethra — pseudostratified columnar → stratified squamous at fossa navicularis. Label, female: proximal urethra — transitional (continuous with bladder); remainder — stratified squamous; mark the paraurethral (Skene's) gland openings.

Applied / Clinical anatomy

- Ureteric calculi arrest most often at the three sites of physiological narrowing — the **pelviureteric junction**, the point where the ureter crosses the pelvic brim over the common iliac

vessels, and the **vesicoureteric junction** (the narrowest of the three, arresting stones as small as 2–3 mm).

- ▶ **Anti-reflux mechanism:** the terminal ureter runs obliquely through the bladder wall; detrusor contraction compresses this intramural segment, acting as a physiological (flap-valve) sphincter that prevents **vesicoureteric reflux (VUR)** — failure predisposes to ascending pyelonephritis and hydronephrosis.
- ▶ **Ureterocele:** cystic dilation of the intravesical/submucosal ureter with a thin covering membrane over the ureteric orifice; gives the classic "**cobra-head**" **halo** on intravenous urography.
- ▶ Approximately **85% of urinary tract infections** are caused by uropathogenic *E. coli*, whose **FimH adhesins** bind directly to uroplakins of the asymmetric unit membrane, allowing the organism to colonise the urothelium and resist being washed out during micturition.
- ▶ The **trigone** is the key cystoscopic landmark for locating the ureteric orifices during ureteric catheterisation/stenting and TURBT; its smooth, tumour- and stone-retentive floor also makes it a common site for **urothelial (transitional cell) carcinoma**, the commonest bladder neoplasm — a direct consequence of the urothelium's exposure to concentrated urinary carcinogens (e.g., aromatic amines, smoking).
- ▶ The involuntary **internal urethral sphincter** (detrusor thickening at the bladder neck) and the voluntary striated **external urethral sphincter** (around the membranous urethra) together maintain continence; damage to the latter (e.g., prostatectomy, childbirth) causes stress incontinence.
- ▶ Because the female urethra is short and its proximal urothelium is continuous with that of the bladder, ascending infection is far commoner in females than males.

High-yield exam pointers

- ▶ All excretory passages except the urethra follow the same mucosa (urothelium) – muscularis – adventitia/serosa plan; there is no muscularis mucosae or true submucosa.
- ▶ Urothelium: basal → intermediate → superficial umbrella-cell layers; apical **asymmetric unit membrane** studded with **uroplakins** (UPIa/Ib/II/IIIa/IIIb) forms the blood–urine barrier; fusiform vesicles allow layer number to fall from ~6 (relaxed) to ~3 (distended).
- ▶ Ureter: ~25 cm, star-shaped lumen, muscle layers reversed compared to the gut (inner longitudinal, outer circular), with an extra outer longitudinal layer in the lower third.
- ▶ Bladder detrusor = 3 interlacing smooth-muscle layers; thickens at the neck to form the internal sphincter.
- ▶ Trigone mucosa is smooth and non-distensible; current evidence shows it is entirely endodermal urothelium with detrusor-derived (not ureter-derived) musculature.
- ▶ Male urethra epithelium sequence: transitional (prostatic) → (pseudo)stratified columnar (membranous, penile) → stratified squamous (fossa navicularis).
- ▶ Female urethra: transitional proximally → stratified squamous distally; paraurethral (Skene's) glands are the prostate homologue.
- ▶ Three sites of physiological ureteric narrowing are the classic sites of calculus impaction; the vesicoureteric junction is the narrowest.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Gray's Anatomy (Standring) 41e; BD Chaurasia Human Anatomy Vol 2 8e (figure); Sontakke Textbook of Human Histology 2e (figure); Brijesh Kumar Histology Text & Atlas 3e.



Juxtaglomerular (J.G.) Complex

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction

The juxtaglomerular (J.G.) complex, also called the juxtaglomerular apparatus (JGA), is a specialized structure situated at the **vascular pole** of the renal corpuscle, where the initial (straight) part of the distal convoluted tubule of a nephron comes into close contact with the afferent and efferent arterioles of the **same** nephron's own glomerulus (*juxta* = near). It is built from three histologically distinct but functionally integrated cell populations — the **macula densa**, the **juxtaglomerular (granular) cells**, and the **extraglomerular mesangial (lakis) cells** — and forms the structural basis of tubuloglomerular feedback and the renin–angiotensin–aldosterone system (RAAS), the principal short-term regulator of glomerular filtration rate (GFR), renal blood flow and systemic blood pressure.

1. Location and relations

- Lies in the small triangular/wedge-shaped space enclosed by the **afferent arteriole**, the **efferent arteriole**, and the **macula densa** of the distal tubule, at the vascular pole of the renal corpuscle.
- The straight part of the distal tubule (terminal thick ascending limb of the loop of Henle) of a nephron travels back to lie against the vascular pole of **its own** glomerulus before continuing as the tortuous distal convoluted tubule (DCT) — the point of contact is the macula densa.
- Modifications of the arteriolar wall typical of the JGA begin about **30–50 µm proximal** to the glomerulus; the afferent arteriole shows more marked change than the efferent arteriole.
- Best appreciated in sections cut through the vascular pole (H&E or PAS); routine random sections of cortex often miss it because it is a small, focal structure.

2. Components of the JG apparatus

Component	Location	Structure (LM/EM)	Principal function
Macula densa	Wall of the terminal thick ascending limb/initial DCT, apposed to the afferent arteriole	Tall, narrow, columnar epithelial cells with crowded, closely-packed (overlapping) apical nuclei — unlike the cuboidal cells elsewhere in the DCT; apical nuclei, basal Golgi; no distinct basal lamina at this point	Chemoreceptor — senses luminal NaCl concentration; drives tubuloglomerular feedback and modulates renin release

Component	Location	Structure (LM/EM)	Principal function
Juxtaglomerular (granular/JG) cells	Tunica media of the afferent arteriole (occasionally the efferent arteriole too), just proximal to the glomerulus	Modified smooth-muscle cells that become large, rounded, epithelioid ("myoepithelioid"); spherical (not elongated) nuclei; abundant mitochondria, rough ER, Golgi and membrane-bound renin-containing secretory granules, 10–40 nm in diameter	Baroreceptor (afferent-arteriolar wall tension) + endocrine cell — synthesise, store and secrete renin
Extraglomerular mesangial cells (lakis cells / cells of Polkissen)	Triangular space between macula densa, afferent and efferent arterioles, continuous with the intraglomerular mesangium	Polyhedral/stellate cells forming a lace-like (<i>lakis</i> , Fr. lacework) network, joined by gap junctions	Relay/transmit the chemical signal from the macula densa to the JG cells and to intraglomerular mesangial/smooth-muscle cells; possibly contractile

3. Macula densa — detailed histology

- ▶ Each macula densa is a compact cluster of **up to ~40 tightly packed cells** in the tubular wall.
- ▶ Cells are taller and narrower than the surrounding cuboidal DCT cells, giving the crowded, "dense spot" (*macula densa*, L. thicker spot) appearance from which the name derives.
- ▶ Apical membrane carries **Na⁺/K⁺/2Cl⁻ co-transporters**, Na⁺/H⁺ exchangers and pH-/Ca²⁺-regulated K⁺ channels that allow the cell to sense luminal NaCl load.
- ▶ A rise in tubular NaCl concentration triggers release of ATP/adenosine (which acts via afferent-arteriolar vasoconstriction to reduce GFR — tubuloglomerular feedback), while nitric oxide release inhibits this feedback; the macula densa's role in stimulating renin release itself is comparatively less well defined.

4. Juxtaglomerular (granular) cells — detailed histology and function

- ▶ Represent smooth-muscle cells of the afferent arteriole that have undergone secretory (myoepithelioid) transformation: contractile filaments persist, but the cell also gains a well-developed rough ER and Golgi apparatus for protein (renin) synthesis.
- ▶ Renin is stored in membrane-bound secretory granules and released by exocytosis; the cells also act as intrinsic **baroreceptors**, responding directly to a fall in afferent-arteriolar wall tension/stretch (reduced renal perfusion pressure).
- ▶ Receive **unmyelinated sympathetic (adrenergic) innervation** — β₁-adrenergic stimulation is a further trigger for renin release, alongside low tubular Na⁺ sensed via the macula densa and reduced arteriolar stretch (the "three-signal" control of renin secretion).
- ▶ Some workers have proposed that JG cells also contribute to erythropoietin production, but the exact site of erythropoietin synthesis in the kidney remains uncertain.

5. Extraglomerular mesangial (lacis) cells

- ▶ Continuous with the intraglomerular mesangium and share several of its properties — phagocytic clearance of debris, contractility and possession of **angiotensin II receptors**.
- ▶ Bear angiotensin II receptors and are thought to help regulate glomerular filtration rate, and to transmit the macula densa's chemical signal to the JG cells (and to intraglomerular mesangial cells for vasoconstriction) via gap junctions.
- ▶ Also called **cells of Polkissen** ("polar cushion") — an older descriptive term for this polyhedral cell cluster at the vascular pole.

6. Development

- ▶ Mesangial and juxtaglomerular cells are derived embryologically from **smooth-muscle-cell precursors of the metanephric mesenchyme**, consistent with the myoepithelioid (contractile-cum-secretory) phenotype retained by mature JG cells.

□ Fig 20.7 — Structure of renal corpuscle and JG apparatus. (Sontakke — Textbook of Human Histology — 2e, p.276)

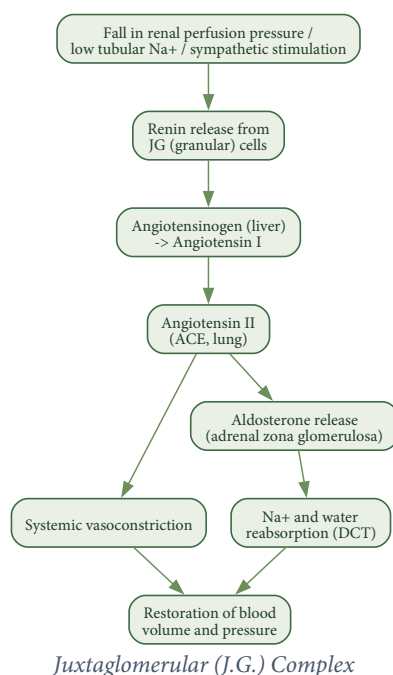
DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the vascular pole of a renal corpuscle in section and label:

- ▶ Glomerular capillaries; parietal layer and visceral layer (podocytes) of Bowman's/glomerular capsule
- ▶ Vascular pole and tubular pole
- ▶ Afferent arteriole (entering) and efferent arteriole (leaving)
- ▶ Proximal convoluted tubule (at tubular pole) and distal convoluted tubule (returning to vascular pole)
- ▶ **Macula densa** — columnar cells with crowded nuclei, on the DCT wall facing the arterioles
- ▶ **Juxtaglomerular (granular) cells** — modified smooth-muscle cells in the afferent arteriolar wall
- ▶ **Extraglomerular mesangial (lacis) cells** — triangular cell cluster between macula densa and the two arterioles
- ▶ Bracket the three components together and label the whole as the "**Juxtaglomerular apparatus**"

7. Function — tubuloglomerular feedback and the renin-angiotensin-aldosterone axis

- ▶ **Autoregulation of GFR (tubuloglomerular feedback):** a rise in GFR increases luminal NaCl delivered to the macula densa → macula densa releases ATP/adenosine → afferent-arteriolar vasoconstriction → GFR falls back towards normal (a negative-feedback loop intrinsic to each nephron).
- ▶ **Endocrine control of blood pressure (RAAS):** a fall in renal perfusion pressure, a fall in tubular NaCl, or sympathetic stimulation triggers JG cells to release renin.



- ▶ Angiotensin II also acts directly as a potent vasoconstrictor and stimulates ADH release, further conserving water; rising blood volume/stretch of JG cells closes the feedback loop and switches off further renin release.

Applied / Clinical anatomy

- ▶ **Juxtaglomerular cell tumour (reninoma):** a rare, benign renin-secreting tumour of JG cells presenting with severe secondary hypertension and hypokalaemia, cured by excision — a classic applied correlate of the JG cells' endocrine function.
- ▶ **Renal artery stenosis (Goldblatt phenomenon):** reduced renal perfusion pressure chronically activates the JGA, driving sustained renin–angiotensin activation and renovascular hypertension.
- ▶ **Bartter syndrome:** a tubular chloride-wasting disorder in which chronic volume depletion causes JG cell hyperplasia with hyperreninaemia, hyperaldosteronism and hypokalaemic alkalosis, without hypertension.
- ▶ **Pharmacological correlation:** the JGA is the anatomical site of action of ACE inhibitors, angiotensin-receptor blockers and direct renin inhibitors, all of which interrupt the cascade at different steps.
- ▶ **Diagnostic histology:** JGA morphology (hyperplasia/hypertrophy of granular cells) is assessed on renal biopsy in the work-up of secondary hypertension and in staging chronic glomerular disease.
- ▶ **Radiological/functional correlate:** captopril renography exploits JGA-mediated efferent-arteriolar tone to detect functionally significant renal artery stenosis.

High-yield exam pointers

- ▶ JG apparatus has **three components**: macula densa (DCT), juxtaglomerular/granular cells (afferent arteriole wall), and extraglomerular mesangial/lacis cells (cells of Polkissen).
- ▶ Macula densa = **tall columnar cells with crowded nuclei**, a chemoreceptor for luminal NaCl.

- ▶ JG cells = **modified smooth-muscle cells** of the afferent arteriole; myoepithelioid; store/secrete **renin** in 10–40 nm granules; act as baroreceptors; adrenergically innervated.
- ▶ Lacis cells = extraglomerular mesangial cells with a lace-like network; relay signals between macula densa and JG/mesangial cells; bear angiotensin II receptors.
- ▶ JGA cells derive embryologically from **metanephric mesenchyme** smooth-muscle precursors.
- ▶ Functions: **tubuloglomerular feedback** (autoregulates GFR) and initiation of the **renin–angiotensin–aldosterone system** (regulates systemic BP and blood volume).
- ▶ Clinical correlations: reninoma, Goldblatt/renal-artery-stenosis hypertension, Bartter syndrome, site of action of ACE inhibitors/ARBs/renin inhibitors.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e.



Micro anatomy of nephron – correlate the structure with function

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction The **nephron** is the structural and functional unit of the kidney, numbering approximately **1–2 million per kidney** (cited as ~1.25 million in Junqueira, ~2 million in Ross/Sontakke). Each nephron has two components: a **renal corpuscle** (filters plasma) and a long **renal tubule** — proximal convoluted tubule (PCT), loop of Henle, and distal convoluted tubule (DCT) — that selectively reabsorbs and secretes to convert ~180 L/day of filtrate into urine. The tubule drains into the **collecting duct**, which develops separately from the ureteric bud but is functionally continuous with the nephron. At every level, the epithelial ultrastructure is precisely tailored to the local transport function — the classic exam correlation.

1. Overview — nephron types and general plan

- ▶ **Cortical (subcapsular) nephrons** — the majority; renal corpuscle in the outer cortex; **short loop of Henle** confined to the outer medulla.
- ▶ **Juxtamedullary nephrons** — renal corpuscle near the corticomedullary junction; **long loop of Henle** dipping deep into the inner medulla/papilla — essential for the urine-concentrating mechanism.
- ▶ Efferent arterioles of cortical glomeruli branch as a **peritubular capillary plexus** around PCT/DCT; efferent arterioles of juxtamedullary glomeruli form the **vasa recta**, long hairpin vessels that run parallel to the loop of Henle and support the countercurrent mechanism.
- ▶ Renal corpuscles and the bulk of PCT/DCT lie in the **cortex**; loops of Henle and collecting ducts occupy the **medullary rays and pyramids**.

□ Fig 20.2 — *Histology of kidney (low magnification)*. (Sontakke — *Textbook of Human Histology* — 2e, p.273)

2. Renal corpuscle — structure of the filtration unit

- ▶ Has a **vascular pole** (afferent and efferent arterioles enter/leave) and an opposite **urinary/tubular pole** (continuous with the PCT).
- ▶ **Glomerulus** — a tuft of anastomosing **fenestrated** capillaries (fenestrae without diaphragms) fed by the afferent arteriole and drained by the efferent arteriole; the two-arteriole arrangement (glomerular capillaries lie in series between two resistance vessels, not draining into a venule) sustains a uniquely high intracapillary hydrostatic pressure that drives filtration.
- ▶ **Bowman's capsule** — double-layered: outer **parietal layer** of simple squamous epithelium, inner **visceral layer** of **podocytes** wrapping the capillaries; the **Bowman's (urinary) space** between them is continuous with the PCT lumen.
- ▶ **Mesangial cells** (intraglomerular) — lie between capillary loops in a matrix; **contractile** (modulate capillary loop diameter/filtration surface), **phagocytic** (clear trapped macromolecules/immune complexes from the basement membrane) and matrix/growth-factor secreting — mainly a supportive/regulatory role.

Glomerular filtration barrier — three layers, each a structural correlate of a distinct filtering function:

Layer	Structure	Functional correlate
Fenestrated endothelium	Capillary endothelial cells with pores, no diaphragm	Free passage of water/small solutes; excludes blood cells
Glomerular basement membrane (GBM)	Fused basal laminae; central lamina densa (type IV collagen network) + inner/outer lamina rara (heparan sulfate, anionic)	Lamina densa = physical size barrier; lamina rara = charge barrier repelling anionic plasma proteins
Podocyte filtration slit diaphragm	Interdigitating pedicels (foot processes) of podocytes; slit pores 25–30 nm wide bridged by a nephrin/podocin zipper diaphragm	Final size-selective sieve; retains plasma proteins above ~70 kDa and negatively-charged molecules

Net effect: the ultrafiltrate entering Bowman's space is virtually cell- and protein-free plasma.

3. Proximal convoluted tubule (PCT) — structure for bulk reabsorption

- ▶ Longest, most coiled segment; begins at the urinary pole, runs a convoluted course (**pars convoluta**) in the cortex, then a short straight part (**pars recta**) descending toward the medulla.
- ▶ Lined by **simple cuboidal to low columnar** epithelium with strongly **acidophilic** cytoplasm.
- ▶ Apical **brush border** — long, closely packed microvilli — vastly increases luminal surface area for transporters; makes the PCT lumen appear indistinct on H&E.
- ▶ **Basal striations** — deep basal plasma-membrane infoldings closely applied to numerous elongated **mitochondria**, providing the ATP for Na^+/K^+ -ATPase-driven active transport.
- ▶ Apical **tight junctions** and lateral cell interdigitations limit paracellular back-leak and add lateral surface area; abundant apical **endocytic vesicles** and **lysosomes** reclaim small filtered proteins by receptor-mediated endocytosis.
- ▶ **Function correlate:** the PCT reabsorbs about **80% of the glomerular filtrate** — obligatory, iso-osmotic reabsorption of Na^+ , water, glucose, amino acids, Cl^- , phosphate, calcium and

bicarbonate — directly reflecting its brush border (surface amplification), basal mitochondria (active transport) and endocytic apparatus (protein reclamation).

4. Loop of Henle — structure for the countercurrent multiplier

Continues from the PCT as the thick descending limb, narrows to a thin segment, then thickens again as the thick ascending limb before reaching the DCT.

Segment	Epithelium	Permeability / transport	Functional result
Thin descending limb	Simple squamous, few organelles	Highly water-permeable ; poorly permeable to NaCl	Water leaves passively into the hypertonic medullary interstitium → filtrate progressively concentrated
Thin ascending limb	Simple squamous	Impermeable to water ; passively permeable to NaCl	NaCl diffuses out passively → filtrate becomes dilute
Thick ascending limb	Cuboidal, few microvilli, deep basal infoldings, abundant mitochondria (like DCT)	Impermeable to water ; actively pumps NaCl out via $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport	Establishes/maintains medullary interstitial hypertonicity; tubular fluid leaves hypo-osmotic

- ▶ The descending and ascending limbs run in parallel but opposite directions (**countercurrent**); their differing permeabilities progressively **multiply** the corticomedullary osmotic gradient (dilute near the cortex, most concentrated at the papilla).
- ▶ Only the **long loops of juxtamedullary nephrons**, paired with the vasa recta, generate the deep medullary gradient essential for maximally concentrated urine.

5. Distal convoluted tubule and collecting duct — fine tuning

- ▶ DCT begins as the thick ascending limb reaches the vascular pole of its own glomerulus (forming the **macula densa**), then continues in the cortex as a shorter, less-coiled tubule.
- ▶ Lined by **simple cuboidal cells without a brush border** — fewer, shorter microvilli give the DCT a **distinct lumen** (unlike the PCT); cytoplasm is less acidophilic; basal infoldings with mitochondria are present but less prominent than in the PCT.
- ▶ **Function correlate:** fine regulation of $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}/\text{H}^+$ balance — aldosterone-driven Na^+ reabsorption coupled to K^+ secretion, PTH-driven Ca^{2+} reabsorption, and further urine acidification.
- ▶ **Collecting duct** (connecting tubule → cortical → medullary collecting duct → papillary ducts) is lined by two cell types:

Cell type	Structure	Function
Principal (light) cells	Pale-staining columnar cells, few short microvilli, a single central cilium, distinct lateral margins	Bear ADH (V2) receptors → apical insertion of aquaporin-2 channels → water reabsorption, concentrating urine; also mediate aldosterone-driven Na ⁺ reabsorption/K ⁺ secretion
Intercalated (dark) cells	Fewer, apical microvilli, numerous mitochondria	α-cells secrete H ⁺ (reabsorb HCO ₃ ⁻) — predominant, net acid excretion; β-cells have reversed polarity, secrete HCO ₃ ⁻

- ▶ As ducts descend through the medulla toward the papilla, the epithelium becomes taller and intercalated cells fewer — a structural gradient matching the duct's role as the terminal site of water and acid–base fine tuning.

6. Juxtaglomerular apparatus (JGA) — the kidney's pressure/flow sensor

Formed at the vascular pole, where the thick ascending limb/early DCT of a nephron contacts the afferent (and often efferent) arteriole of its own glomerulus. Three components:

- ▶ **Juxtaglomerular (JG) cells** — modified smooth muscle (myoepithelioid) cells of the afferent arteriolar wall, packed with **renin**-containing granules; act as mechanoreceptors for arteriolar wall stretch and release renin in response to reduced perfusion pressure or sympathetic stimulation.
- ▶ **Macula densa** — narrow, columnar, closely packed cells of the distal tubule with crowded, overlapping nuclei (hence the name); a chemoreceptor sensing luminal NaCl concentration.
- ▶ **Extraglomerular mesangial (laci) cells** — a lace-like network of cells in the triangular space between the arterioles and the macula densa, continuous with the intraglomerular mesangium; relay signals between the macula densa and the JG cells.
- ▶ **Function correlate:** falling NaCl delivery to the macula densa (as with reduced GFR) stimulates JG-cell renin release, activating the **renin–angiotensin–aldosterone system**; rising distal NaCl triggers **tubuloglomerular feedback** — afferent arteriolar constriction that reduces GFR — an intrinsic autoregulatory loop entirely built from local epithelial and vascular smooth-muscle specialisation.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a longitudinal schema of one nephron with its JGA:

- ▶ Bowman's capsule (parietal + visceral/podocyte layers), Bowman's space, glomerular capillary tuft
- ▶ Afferent arteriole (wider) and efferent arteriole (narrower) at the vascular pole
- ▶ Proximal convoluted tubule (brush border) → thin descending limb → thin ascending limb → thick ascending limb
- ▶ Distal convoluted tubule, with **macula densa** at the point of contact with the afferent arteriole
- ▶ **Juxtaglomerular cells** in the afferent arteriolar wall; **extraglomerular mesangial (lakis) cells** in the angle between the two arterioles
- ▶ Connecting tubule → collecting duct (principal + intercalated cells) → papillary duct

□ Fig 20.7 — Structure of renal corpuscle and JG apparatus. (Sontakke — Textbook of Human Histology — 2e, p.276)

Applied / Clinical anatomy

- ▶ **Diabetic nephropathy** — thickening of the GBM and mesangial matrix expansion (Kimmelstiel–Wilson nodules) is the earliest structural correlate of microalbuminuria.
- ▶ **Minimal change disease / nephrotic syndrome** — effacement (flattening) of podocyte foot processes with loss of slit-diaphragm nephrin causes heavy, selective proteinuria despite a normal light-microscopic appearance.
- ▶ **Congenital nephrotic syndrome (Finnish type)** — mutation of the **NPHS1** gene coding for nephrin disrupts the filtration slit diaphragm.
- ▶ **Acute tubular necrosis** — the PCT and thick ascending limb, with their high mitochondrial/active-transport demand, are most vulnerable to ischaemic or nephrotoxic (aminoglycoside, contrast) injury.
- ▶ **Diuretic sites of action** mirror tubular structure: loop diuretics (furosemide) block the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter of the thick ascending limb; thiazides act on the DCT Na^+/Cl^- cotransporter; K^+ -sparing diuretics act on collecting-duct principal cells.
- ▶ **Diabetes insipidus** — failure of ADH secretion (central) or of aquaporin-2 insertion (nephrogenic) leaves the collecting duct water-impermeable, producing dilute polyuria.
- ▶ **Renin-secreting tumour (reninoma)** and hyaline arteriolosclerosis of the afferent arteriole (hypertension/diabetes) both act at the JGA/vascular pole.
- ▶ Renal biopsy interpretation (light microscopy + electron microscopy + immunofluorescence) is built directly on this three-layer filtration-barrier and tubular-segment anatomy.

High-yield exam pointers

- ▶ Nephron = renal corpuscle (filtration) + renal tubule (PCT + loop of Henle + DCT); ~1–2 million per kidney; cortical (short-loop) vs juxtamedullary (long-loop, urine-concentrating) types.

- ▶ Filtration barrier = fenestrated endothelium + GBM (type IV collagen, heparan sulfate) + podocyte slit diaphragm (25–30 nm, nephrin) — size- and charge-selective; excludes proteins >70 kDa.
- ▶ PCT reabsorbs ~80% of filtrate; brush border + basal mitochondrial infoldings + endocytic apparatus = structural basis of bulk active reabsorption.
- ▶ Loop of Henle = countercurrent multiplier: water-permeable thin descending limb + NaCl-pumping (impermeable to water) ascending limbs → medullary hypertonicity → concentrated urine.
- ▶ DCT lacks a brush border (distinct lumen); site of aldosterone/PTH action.
- ▶ Collecting duct: principal cells (ADH → aquaporin-2; aldosterone → Na⁺/K⁺) and intercalated cells (α/β — acid–base regulation).
- ▶ JGA = JG cells (renin) + macula densa (NaCl sensor) + lacis cells — mediates tubuloglomerular feedback and the renin–angiotensin–aldosterone axis.
- ▶ Clinical correlates: podocyte effacement → nephrotic syndrome; GBM thickening → diabetic nephropathy; PCT/thick ascending limb → acute tubular necrosis.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



Microscopic structure of Kidney

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction The kidney is a compound tubular organ made up of countless tortuous, closely packed **uriniferous tubules** embedded in a delicate vascularised connective-tissue stroma. Each uriniferous tubule has two embryologically distinct parts — the **nephron** (which forms urine) and the **collecting duct** (which completes urine concentration and conducts it to the calyces). Each kidney contains about **1–1.3 million nephrons** (renal corpuscles), a number fixed before birth; none are added after birth, and their loss with age or disease is permanent. On section, the kidney shows an outer **cortex** and an inner **medulla**, whose architecture directly reflects the arrangement of nephron segments.

1. General organisation — cortex and medulla

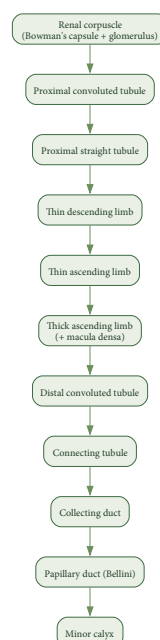
Feature	Cortex	Medulla
Colour/texture (fresh)	Granular, darker	Striated, paler
Contains	Renal corpuscles, PCT, DCT, cortical collecting ducts	Loops of Henle, medullary collecting ducts, vasa recta

Feature	Cortex	Medulla
Subdivision	Medullary rays (Ferrein's pyramids) of collecting ducts + straight tubules extend into cortex; renal columns (of Bertin) of cortical tissue dip between pyramids	8–15 conical renal pyramids , base at cortico-medullary junction, apex forms renal papilla projecting into a minor calyx
Blood flow	~90–95% of total renal blood flow	~5–10%
Functional unit grouping	A pyramid + the cortical tissue over its base and along its sides = one renal lobe ; a medullary ray + its associated cortex = one renal lobule	—

2. The nephron — parts and types

The nephron consists of a **renal corpuscle** (filtration) continuous with a long **renal tubule** (selective reabsorption/secretion), ending in a **connecting tubule** that joins a collecting duct.

- **Renal corpuscle** → **Proximal convoluted tubule (PCT)** → proximal straight tubule → **thin descending limb** → **thin ascending limb** → **thick ascending limb** (loop of Henle) → **macula densa** → **distal convoluted tubule (DCT)** → **connecting tubule** → collecting duct → papillary duct (of Bellini) → minor calyx.



Microscopic structure of Kidney

Two nephron types, based on corpuscle position and loop length:

Type	Renal corpuscle location	Loop of Henle	Proportion
Cortical (subcapsular) nephrons	Outer cortex	Short, barely enters medulla	Majority (~85–87%)

Type	Renal corpuscle location	Loop of Henle	Proportion
Juxtamedullary nephrons	Cortex adjoining the medulla	Long, penetrates deep into inner medulla; runs with vasa recta	~1/7–1/8 of total; establish the medullary concentration gradient
Midcortical (intermediate) nephrons	Mid-cortex	Intermediate length	Remainder

3. Renal corpuscle

- ▶ Small, spherical structure, ~0.2 mm in diameter; consists of a tuft of capillaries (**glomerulus**) invaginating a double-walled epithelial **glomerular (Bowman's) capsule**.
- ▶ **Vascular pole** — where the **afferent arteriole** enters and **efferent arteriole** leaves.
- ▶ **Urinary pole** — opposite pole, where the capsule continues as the PCT.
- ▶ **Parietal layer** of the capsule — simple squamous epithelium, forms the outer wall.
- ▶ **Visceral layer** — composed of **podocytes**, stellate epithelial cells whose primary processes give rise to interdigitating secondary processes ending in **pedicels (foot processes)** that wrap the glomerular capillaries.
- ▶ Between the pedicels of adjacent podocytes are **filtration slits** (~25–30 nm) bridged by a **slit diaphragm**; the differentiated podocyte phenotype depends on specific proteins — **nephrin** (mutated in the Finnish type of congenital nephrotic syndrome), **podocin**, **synaptopodin** and **GLEPP-1**. The luminal surface coat is rich in sialoglycoproteins (podocalyxin), giving a strong negative charge that is key to the perm-selectivity barrier.
- ▶ Between the two capsular layers is the flattened **urinary (Bowman's) space**, continuous with the PCT lumen.
- ▶ **Mesangial cells** (contractile, phagocytic; related to pericytes) and their matrix (**mesangium**) fill the spaces between capillary loops not covered by podocytes — provide structural support, clear immune complexes, and regulate capillary blood flow.

embed: ![Fig 20.3 — Photomicrograph. Histology of kidney (low magnification, cortex and medulla) (Sontakke — Textbook of Human Histology — 2e, p.274)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/61bebed2eb45fe10.jpg)

embed: ![Fig 20.7 — Structure of renal corpuscle and JG apparatus (Sontakke — Textbook of Human Histology — 2e, p.276)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/abc72b308ccce6b4.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a renal corpuscle in longitudinal section with these labels: Afferent arteriole · Efferent arteriole · Vascular pole · Urinary pole · Glomerular capillary tuft · Fenestrated endothelium · Glomerular basement membrane (GBM) · Podocyte (visceral layer) with pedicels · Filtration slit/slit diaphragm · Parietal layer (simple squamous) · Bowman's (urinary) space · Mesangial cell and mesangium · Macula densa (of DCT) · Juxtaglomerular (granular) cells in afferent arteriole wall · Extraglomerular mesangial (lakis) cells · Proximal convoluted tubule at urinary pole.

4. Glomerular filtration barrier

Filtration of plasma occurs through three layered components:

Layer	Structure	Function
Fenestrated capillary endothelium	Pores (~70–90 nm), no diaphragm	Excludes blood cells and platelets
Glomerular basement membrane (GBM)	Fused endothelial + podocyte basal laminae; ~0.3–0.36 µm thick; type IV collagen, laminin, heparan-sulphate proteoglycans	Restricts large plasma proteins and organic anions (size- and charge-selective)
Filtration slit diaphragm (between podocyte pedicels)	Nephrin, podocin, CD2AP-based zipper-like junction	Restricts remaining small proteins

Roughly 20% of plasma entering the glomerulus is filtered (GFR ≈ 125 mL/min ≈ 180 L/day); virtually no plasma protein > ~70 kDa normally crosses the filter.

5. Renal tubule — segment-by-segment histology

Segment	Epithelium	Key histological features	Principal function
Proximal convoluted tubule (PCT)	Simple cuboidal/low columnar (large cells; 3–5 nuclei/cross-section)	Tall brush border (long microvilli), strongly eosinophilic cytoplasm (abundant perpendicular basal mitochondria), extensive basal infoldings and lateral interdigitations, apical endocytotic vesicles	Reabsorbs ~2/3 of filtered water, Na ⁺ , and virtually all glucose/amino acids; degrades filtered small proteins; secretes organic anions/cations (drugs)
Thin segment of loop of Henle (descending + ascending limbs)	Simple squamous, ~30 µm diameter	Few organelles — passive role	Passive water movement (descending) as part of countercurrent multiplier

Segment	Epithelium	Key histological features	Principal function
Thick ascending limb	Simple cuboidal, ~60 µm diameter, mitochondria-rich	Deep basolateral infoldings; source of Tamm–Horsfall protein	Active NaCl transport into interstitium (impermeable to water) — builds medullary hyperosmolality
Macula densa	Tall, closely packed columnar cells at the tubule's contact with its own glomerulus	Apical nuclei, basal Golgi	Chemoreceptor — senses luminal NaCl for tubuloglomerular feedback
Distal convoluted tubule (DCT)	Simple cuboidal, smaller cells, more nuclei/section	No brush border, empty-looking lumen, fewer mitochondria than PCT	Aldosterone-regulated Na ⁺ reabsorption/K ⁺ secretion; less reabsorption overall than PCT
Connecting tubule & collecting duct	Simple cuboidal → columnar (principal cells)	Principal cells: pale, few organelles, sparse microvilli, rich in ADH-regulated aquaporins; Intercalated cells: darker, more mitochondria, apical folds (type A secretes H ⁺ , type B secretes HCO ₃ ⁻)	ADH-dependent water reabsorption; acid–base regulation
Papillary duct (of Bellini)	Tall columnar	Widest ducts (~200 µm), open at area cribrosa of papilla	Final conduit of urine into minor calyx

6. Juxtaglomerular apparatus (JGA)

- ▶ Located at the vascular pole, where the DCT of a nephron touches the afferent (and efferent) arteriole of its **own** parent glomerulus.
- ▶ Three components:
- ▶ **Macula densa** — modified, closely packed columnar cells of the DCT wall; chemosensor for luminal Na⁺/Cl⁻.
- ▶ **Juxtaglomerular (granular) cells** — modified smooth muscle cells of the afferent arteriole wall (tunica media), with a secretory phenotype (rough ER, Golgi, renin-containing granules).
- ▶ **Extraglomerular mesangial (laci) cells** — a cluster of cells in the triangular space between afferent and efferent arterioles and the macula densa; relay signals between the macula densa and JG cells.
- ▶ Function: **tubuloglomerular feedback** (autoregulation of GFR) and **renin secretion** → activation of the renin–angiotensin–aldosterone axis, regulating systemic blood pressure and Na⁺/water balance.

Applied / Clinical anatomy

- ▶ **Congenital nephrotic syndrome (Finnish type)** — mutation of the *NPHS1* gene coding for nephrin, disrupting the slit diaphragm → massive proteinuria in infancy.
- ▶ **Glomerulonephritis** — acute/chronic inflammation of glomeruli from immune-complex deposition on the GBM; diagnosis relies on renal biopsy studied by light, immunofluorescence and electron microscopy.
- ▶ **Diabetic nephropathy** — progressive GBM thickening and mesangial expansion (diabetic glomerulosclerosis); leading cause of end-stage renal disease.
- ▶ **Proteinuria** — loss of GBM/slit-diaphragm charge- and size-selectivity lets plasma proteins leak into urine; a key clinical marker of glomerular disease.
- ▶ **Nephrogenic diabetes insipidus** — defective aquaporin-2 response to ADH in principal cells of the collecting duct.
- ▶ **Renal calculi** — form in the pelvis/calyces (commonly calcium oxalate/phosphate or uric acid); obstruction can injure the nephron and cause severe loin-to-groin pain; treated by lithotripsy or surgical removal.
- ▶ **Polycystic kidney disease** — cystic dilatation of any nephron segment, effacing normal cortical architecture.
- ▶ **Renal biopsy correlation** — the number and structural integrity of glomeruli, tubular epithelial changes, and interstitial fibrosis on light/electron microscopy guide diagnosis and staging of most medical renal diseases.

High-yield exam pointers

- ▶ Uriniferous tubule = **nephron + collecting duct**; ~1–1.3 million nephrons/kidney, fixed at birth, non-regenerable.
- ▶ Cortex = corpuscles + convoluted tubules; medulla = 8–15 pyramids of loops of Henle + collecting ducts + vasa recta.
- ▶ Renal corpuscle (~0.2 mm) = glomerulus + Bowman's capsule; vascular pole (afferent/efferent arterioles) vs urinary pole (PCT origin).
- ▶ Filtration barrier = fenestrated endothelium + GBM (~0.3 µm) + podocyte slit diaphragm (nephrin/podocin); filtration slits ~25–30 nm.
- ▶ PCT: brush border, eosinophilic, basal mitochondria — bulk reabsorption. DCT: no brush border, fewer mitochondria, aldosterone target.
- ▶ Thick ascending limb reaches its own glomerulus as the **macula densa**, forming the sensory arm of the JGA.
- ▶ JGA = macula densa + juxtaglomerular (granular, renin-secreting) cells + extraglomerular mesangial (lakis) cells → tubuloglomerular feedback + RAAS.
- ▶ Collecting duct: principal cells (ADH/aquaporin-mediated water reabsorption) + intercalated cells (H^+/HCO_3^- secretion, acid–base balance).
- ▶ Cortical nephrons (short loop, majority) vs juxtamedullary nephrons (long loop, ~1/7–1/8, build medullary concentration gradient).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e.



Microstructure of Lung

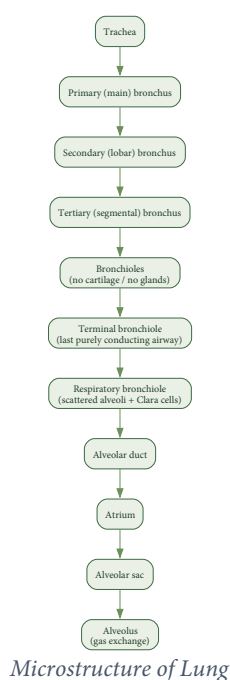
Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction — The lung parenchyma is organised into a **conducting portion** (bronchi → bronchioles → terminal bronchiole), which merely transmits air and takes no part in gas exchange, and a **respiratory portion** (respiratory bronchiole → alveolar duct → atrium → alveolar sac → alveolus), whose walls are interrupted by thin-walled alveolar outpouchings that are the actual site of gas exchange. Both lungs together contain **300–500 million alveoli**, giving a combined respiratory surface area of **70–100 m²** — the largest epithelial surface in the body packed into the volume of the thoracic cavity. Understanding this microarchitecture — the bronchiolar wall, the pulmonary acinus, the interalveolar septum and the blood–air barrier — is essential to interpret restrictive/obstructive lung disease and diffuse parenchymal imaging.

1. Bronchial tree — conducting vs respiratory portion

Level	Epithelium	Wall components	Cartilage / glands
Trachea → primary (main) bronchus	Ciliated pseudostratified columnar + goblet cells	Smooth muscle (trachealis posteriorly); seromucous glands	C-shaped hyaline cartilage rings
Secondary (lobar) → tertiary (segmental) bronchus	Ciliated columnar, fewer goblet cells	Complete muscle coat; glands present but fewer	Irregular cartilage plates (not rings)
Bronchiole (no cartilage)	Ciliated simple columnar → cuboidal; few/no goblet cells	Prominent circular smooth muscle; thin elastic network deep to muscle	Absent
Terminal bronchiole	Ciliated & non-ciliated cuboidal + Clara (club) cells	Smooth muscle coat; last airway with no alveoli in its wall	Absent
Respiratory bronchiole	Simple cuboidal (ciliated proximally → non-ciliated Clara cells distally)	Smooth muscle bundles; wall interrupted by scattered alveolar outpouchings	Absent

Level	Epithelium	Wall components	Cartilage / glands
Alveolar duct / atrium / alveolar sac / alveolus	Simple squamous (type I) + cuboidal (type II) pneumocytes	Rings of smooth muscle/elastic fibres at septal "knobs"; otherwise wall = confluent alveolar mouths	Absent



2. Structure of the bronchiole

- ▶ **Terminal bronchiole** (~1 mm diameter) — the most peripheral airway that still has **no alveoli in its wall**; lined by ciliated/non-ciliated cuboidal epithelium with scattered **Clara (club) cells**; mucosa thrown into folds when contracted; no cartilage, no submucosal glands, but a well-developed circular smooth-muscle coat (site of bronchospasm in asthma).
- ▶ **Respiratory bronchiole** (<1 mm) — epithelium continues as simple cuboidal, but its wall is now interrupted at intervals by a few thin-walled **alveoli**, so it participates partly in gas exchange; smooth muscle and a delicate connective-tissue layer still surround it.
- ▶ **Clara (club) cells** — non-ciliated, dome-shaped cells that bulge into the lumen; they have secretory (surfactant-like, protective lipoprotein) and detoxifying (cytochrome P450) functions, contribute to **innate immunity**, and act as regional progenitor cells for the bronchiolar epithelium.

3. Pulmonary acinus and lobule — the functional units

- ▶ **Acinus** — the lung tissue distal to one terminal bronchiole; the true **functional unit for gas exchange**; comprises 3–4 orders of respiratory bronchioles, each leading to 3–8 orders of alveolar ducts; ~6–10 mm in diameter and radiologically resolvable.
- ▶ **Primary lobule** — the lung distal to a single respiratory bronchiole.

- **Secondary (pulmonary) lobule** — the smallest macroscopic subdivision of peripheral lung, bounded by fibrous connective-tissue septa, of uneven size and shape, and containing roughly **six terminal bronchioles** (each supplying its own acinus); ~2.5 cm across — this is the anatomical basis of the HRCT **secondary-lobule pattern** used to localise interstitial and airway disease.

4. The alveolus — interalveolar septal architecture

Each alveolus is a thin-walled **polyhedral pouch** lined by simple squamous epithelium, its wall (the **interalveolar septum**) shared with the neighbouring alveolus and traversed by a dense capillary plexus.

Feature	Type I pneumocyte	Type II pneumocyte
Shape	Simple squamous, markedly attenuated	Cuboidal, rounded, protrude into lumen
Surface area covered	≈ 90–95% of alveolar surface	< 10% of alveolar surface
Cell number	Fewer	Often more numerous than type I (numerically)
Cytoplasm	Extremely thin (0.05–0.2 µm)	Contains numerous secretory lamellar bodies
Function	Form the epithelial side of the blood–air barrier; joined by tight junctions	Secrete pulmonary surfactant (phospholipid-rich); progenitor cells
Division	Post-mitotic — do not divide	Proliferate and differentiate into type I cells after injury
Junctions	Overlapping cell edges, tight junctions — seal the diffusion barrier	—

The septum has two histologically distinct zones:

- **Thin portion** — epithelial cytoplasm + fused basal lamina (epithelium/endothelium share one basal lamina) + continuous capillary endothelium; total air-to-blood diffusion distance may be as little as **0.2 µm**; virtually all gas exchange occurs here.
- **Thick portion** — contains genuine interstitial connective tissue: **elastic fibres and type III collagen fibres**, fibroblasts and migratory cells (macrophages); this side accommodates interstitial fluid and lymph drainage rather than gas exchange. The elastic-fibre network of the alveolar wall gives the lung its elastic recoil and is the structure destroyed in emphysema.

5. Blood–air (alveolocapillary) barrier — ultrastructure

□ Fig 15.18 — Blood–air barrier. (Sontakke — *Textbook of Human Histology* — 2e, p.213)

The barrier that O₂ and CO₂ must cross, from alveolar lumen to capillary blood, is built of:

- A thin film of **surfactant** lining the alveolar epithelium.
- **Type I pneumocyte** cytoplasm.
- **Fused basal laminae** of the type I pneumocyte and the capillary endothelial cell.
- **Capillary endothelial cell** — continuous (non-fenestrated), extremely thin, connected by tight junctions.

This arrangement, together with the endothelial barrier, limits movement of fluid from blood/interstitium into the alveolar lumen, keeping the alveolus dry for efficient gas exchange.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Ultrastructure of the blood–air barrier — label: Lumen of alveolus; Surfactant film; Type I pneumocyte; Basal lamina of type I pneumocyte; Basal lamina of endothelial cell (fused with the above); Capillary endothelial cell; Capillary lumen (with erythrocyte); Type II pneumocyte (adjacent); Interstitium — fibroblasts, collagen and elastic fibres, migratory cells/macrophages.

6. Alveolar macrophages and interalveolar pores of Kohn

- ▶ **Alveolar (dust) macrophages** — derived from circulating monocytes/bone-marrow precursors; migrate into the alveolar lumen and wander over the epithelial surface phagocytosing inhaled particulate matter and microorganisms; most are cleared via the **mucociliary escalator** after migrating to the bronchioles, others cross the epithelium to enter lymphatics draining the peribronchial connective tissue (**the alveoli themselves have no lymphatics**). They also participate in **surfactant turnover**.
- ▶ **Interalveolar pores of Kohn** — small pores (2–13 μm), up to **seven per alveolus**, usually lined by type II cells, that connect adjacent alveolar airspaces across the septum; they equalise pressure and provide **collateral ventilation** if a bronchiole is obstructed, but also allow macrophages, bacteria and viruses to spread between alveoli.

7. Vascular and lymphatic microarchitecture

- ▶ **Pulmonary capillaries** form a single-layered, exceedingly thin-walled plexus immediately deep to the alveolar epithelium, within the interalveolar septum — this dense, sheet-like network (rather than discrete individual capillaries) maximises the area of blood exposed to alveolar air.
- ▶ **Bronchial vessels** (systemic) supply only the conducting airways down to the respiratory bronchioles; **pulmonary vessels** supply the alveolar capillary bed for gas exchange.
- ▶ **Lymphatics** begin at the level of the bronchiole (deep plexus) and beneath the visceral pleura (superficial plexus); the alveolar wall proper has no lymphatic channels, so alveolar fluid must first traverse the septal interstitium before reaching a lymphatic.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Respiratory unit (TS from a terminal bronchiole to alveoli) — label: Terminal bronchiole; Respiratory bronchiole (with scattered alveolar outpouchings); Alveolar duct; Atrium; Alveolar sac; Alveolus; Interalveolar septum; Pore of Kohn; Capillary network in septum; Alveolar macrophage.

Applied / Clinical anatomy

- ▶ **Neonatal respiratory distress syndrome** — type II pneumocytes mature late (surfactant production adequate usually only after ~34–36 weeks); surfactant deficiency in preterm infants raises alveolar surface tension, causing progressive atelectasis.

- ▶ **Emphysema** — proteolytic destruction of the elastic-fibre network of the interalveolar septum (e.g. in $\alpha 1$ -antitrypsin deficiency, where unchecked macrophage/neutrophil proteases damage the wall) causes irreversible alveolar wall breakdown and airspace enlargement.
- ▶ **Pulmonary oedema** — fluid first accumulates in the **thick** (connective-tissue) side of the septum before flooding the alveolar lumen; the thin diffusion side is relatively protected until late.
- ▶ **Diffuse alveolar damage / ARDS** — injury to type I pneumocytes (non-dividing) is repaired by type II cell proliferation and differentiation; failure of this repair underlies fibrosing lung disease.
- ▶ **Interstitial lung fibrosis** — thickening of the interstitial (thick) portion of the septum with excess collagen widens the diffusion barrier and impairs gas exchange without necessarily affecting airway calibre.
- ▶ **"Heart-failure cells"** — alveolar macrophages laden with haemosiderin after phagocytosing extravasated erythrocytes (pulmonary venous congestion) appear brick-red in sputum — a diagnostic clue to left heart failure/mitral stenosis.
- ▶ **Secondary pulmonary lobule** — the anatomical unit visualised on HRCT; centrilobular, perilymphatic and random nodule patterns are localised with reference to it, and each lobule's ~6 terminal bronchioles/acini explain the "tree-in-bud" pattern of small-airway disease.
- ▶ **Pores of Kohn and collateral ventilation** — explain why obstruction of a single bronchiole may not always produce distal atelectasis, and why infection/tumour cells can spread alveolus-to-alveolus.
- ▶ **Bronchoscopic biopsy / BAL** — samples bronchiolar and alveolar macrophage populations for diagnosis of interstitial and infective lung disease; "tar bodies" in macrophages of smokers are a cytological marker of exposure.

High-yield exam pointers

- ▶ Conducting portion ends at the **terminal bronchiole** (last airway with no alveoli); respiratory portion begins at the **respiratory bronchiole**.
- ▶ **Acinus** = functional gas-exchange unit distal to one terminal bronchiole (3–4 orders of respiratory bronchioles + 3–8 orders of alveolar ducts).
- ▶ Type I pneumocytes: ~90–95% of alveolar **surface area**, but type II cells are **more numerous** — a classic reversal trap.
- ▶ Blood–air barrier (thin part) = surfactant + type I pneumocyte + fused basal lamina + capillary endothelium; may be as thin as **0.2 μm** .
- ▶ Type II pneumocytes secrete **surfactant** (lamellar bodies) and act as **stem cells** for type I cells after injury.
- ▶ **Pores of Kohn**: up to 7/alveolus, 2–13 μm , allow collateral air flow.
- ▶ 300–500 million alveoli per pair of lungs; total surface area 70–100 m^2 .
- ▶ Alveolar wall has **no lymphatics**; alveolar macrophages are cleared via mucociliary escalator or peribronchial lymphatics.
- ▶ Elastic fibres of the septum give lung recoil — lost in emphysema; thickened collagenous septum impairs diffusion in fibrosis.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; IB Singh — Textbook of Human Histology 9e / Textbook of Anatomy Vol 2 6e; Vishram Singh — Textbook of Anatomy Vol 1 4e / General Anatomy 4e.



Name the parts of a Nephron. Describe the light and ultra microscopic features and correlate with its functions

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — The **nephron** is the structural and functional unit of the kidney, numbering approximately 1–2 million per kidney (Junqueira: ~1.25 million; Ross: ~2 million). Each nephron is 30–55 mm long and consists of a filtering **renal corpuscle** continuous with a long **renal tubule** — the proximal convoluted tubule (PCT), the loop of Henle, and the distal convoluted tubule (DCT) — which drains via a connecting tubule into the collecting duct system. The nephron proper develops from the **metanephric blastema**, whereas the collecting duct develops from the **ureteric bud**; the collecting duct is therefore functionally continuous with, but embryologically and conventionally distinct from, the nephron.

1. Parts of a nephron and classification

Part	Location
Renal corpuscle (Bowman capsule + glomerulus)	Cortex
Proximal convoluted tubule (PCT)	Cortex
Proximal straight tubule (thick descending limb)	Medullary ray → outer medulla
Thin descending limb of loop of Henle	Medulla
Thin ascending limb of loop of Henle	Medulla
Thick ascending limb (distal straight tubule)	Inner/outer medulla → cortex
Macula densa	Cortex, at vascular pole
Distal convoluted tubule (DCT)	Cortex
Connecting tubule	Cortex (→ joins collecting duct, a separate ureteric-bud derivative)

Nephrons are classified by the depth of their renal corpuscle in the cortex, which determines loop length:

- ▶ **Cortical (subcapsular) nephrons** — corpuscle in the outer cortex; short loops of Henle confined to the outer medulla; the majority of nephrons.
- ▶ **Juxtamedullary nephrons** — corpuscle near the cortico-medullary junction; about **one-eighth** of all nephrons; long loops of Henle penetrating deep into the medulla, essential for the urine-concentrating countercurrent mechanism; accompanied by the vasa recta.

- ▶ **Midcortical (intermediate) nephrons** — corpuscle in the mid-cortex; loops of intermediate length.

2. Renal corpuscle — light and ultrastructural features, and functional correlation

The renal corpuscle is spherical, about **200 μm** in diameter, with a **vascular pole** (afferent/efferent arterioles) and a **tubular pole** (continuous with the PCT). It comprises the **glomerulus** (a tuft of fenestrated capillaries) enclosed by the double-layered **Bowman (glomerular) capsule**.

- ▶ **Parietal layer** — simple squamous epithelium resting on a basal lamina; continuous with the PCT epithelium at the tubular pole.
- ▶ **Visceral layer** — specialised epithelial cells called **podocytes**; each has primary processes giving rise to numerous interdigitating secondary processes (**pedicels**) that embrace the glomerular capillaries. Between adjacent pedicels lie **filtration slit pores** (25–30 nm wide), bridged by zipper-like **slit diaphragms** built of nephrin and podocalyxin.
- ▶ **Capsular (urinary) space** — between parietal and visceral layers; receives the ultrafiltrate.
- ▶ **Mesangial cells** — pericyte-like cells with contractile, phagocytic and secretory (cytokines, prostaglandins) functions; provide structural support where capillaries lack podocyte covering.

Glomerular filtration barrier (three layers, EM):

- ▶ Fenestrated capillary endothelium (fenestrae 70–90 nm, no diaphragm) — excludes blood cells.
- ▶ Glomerular basement membrane (GBM), 300–360 nm thick, fused podocyte + endothelial basal laminae, rich in type IV collagen and heparan-sulfate proteoglycans — excludes large/anionic plasma proteins.
- ▶ Filtration slit diaphragms between pedicels — restrict small proteins and organic anions.

Functional correlation: ~20% of plasma entering the glomerulus is filtered (GFR $\approx$ 125 mL/min or ~180 L/day; total filtration area $\approx$ 500 cm^2), so the entire plasma volume is filtered about 60 times a day. The negatively charged GBM/slit-diaphragm mesh normally excludes plasma proteins — loss of this fixed negative charge (e.g., minimal change disease) or GBM thickening (diabetic glomerulosclerosis) causes proteinuria.

! [Fig 19.2 — A nephron and its parts. (Junqueira's Basic Histology — Text & Atlas — 17e, p.950)]
(C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/82e844eb702c7f72.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labels: Renal corpuscle (Bowman capsule, glomerulus, afferent arteriole, efferent arteriole) → Proximal convoluted tubule → Proximal straight tubule → Thin descending limb → Loop of Henle (hairpin turn) → Thin ascending limb → Thick ascending limb → Macula densa (at vascular pole) → Distal convoluted tubule → Connecting tubule → Collecting duct → Papillary duct (of Bellini).

3. Proximal convoluted tubule (PCT)

Feature	Light microscopy	Ultrastructure (EM)	Function
Cells	Cuboidal/low columnar, acidophilic cytoplasm, central nucleus; 40–60 μm diameter; only 3–5 nuclei per cross-section (large cells)	Apical brush border (long microvilli); numerous apical endocytotic pits/vesicles near lysosomes; long basal membrane infoldings with long mitochondria; extensive lateral interdigitations	Reabsorbs ~65–80% of filtered water, Na^+ , Cl^- , glucose, amino acids, HCO_3^- ; endocytosis/proteolysis of filtered small proteins; secretion of organic anions/cations (drug clearance, e.g., creatinine, many antibiotics); 1α -hydroxylation of vitamin D
Lumen	Filled, "fuzzy" (microvilli + protein)	—	—

4. Loop of Henle (ansa nephroni)

- **Thin descending limb** — simple squamous epithelium, ~15 μm diameter, few organelles (passive transport); freely permeable to water.
- **Thin ascending limb** — simple squamous, continues after the hairpin turn; impermeable to water, passively permeable to NaCl .
- **Thick ascending limb (distal straight tubule)** — simple cuboidal, ~30 μm diameter, numerous mitochondria (basal striations); **actively transports Na^+** (and Cl^- passively) out into the interstitium.

Functional correlation: the countercurrent multiplier — active NaCl extrusion by the thick ascending limb renders the medullary interstitium hyperosmotic; water diffuses passively out of the water-permeable descending limb, progressively concentrating the tubular fluid. Vasa recta (countercurrent exchangers) preserve this gradient. Only about 5% of the filtrate is reabsorbed here, but the loop is essential for urinary concentration; juxtamedullary nephrons with long loops achieve the steepest gradient.

5. Distal convoluted tubule (DCT)

Feature	PCT	DCT
Origin	Bowman capsule	Ascending limb of loop of Henle

Feature	PCT	DCT
Length in section	Longer, more numerous	Shorter, fewer
Cells	Cuboidal, microvilli/brush border	Cuboidal, no brush border
Cytoplasm	More acidophilic (many mitochondria)	Less acidophilic
Lumen	Filled	Empty, distinct
Main function	Reabsorption (bulk)	Fine-tuning: Na^+ reabsorption (aldosterone-regulated), K^+/H^+ secretion

Ultrastructurally DCT cells show basal infoldings with mitochondria (basal striations) similar to but sparser than PCT, consistent with active but lesser transport. Na^+ reabsorption here is regulated by aldosterone.

6. Juxtaglomerular apparatus (JGA) — structure and functional correlation

Formed at the vascular pole where the thick ascending limb contacts its own glomerulus's afferent arteriole:

- ▶ **Macula densa** — modified tall, columnar DCT cells with apical nuclei and basal Golgi; chemoreceptors that sense luminal Na^+/Cl^- concentration.
- ▶ **Juxtaglomerular (granular) cells** — modified smooth muscle cells of the afferent arteriole tunica media; secretory phenotype (rough ER, Golgi, renin-containing granules); also act as baroreceptors.
- ▶ **Extraglomerular mesangial (laci) cells** — between macula densa and the two arterioles; relay signalling between the two.

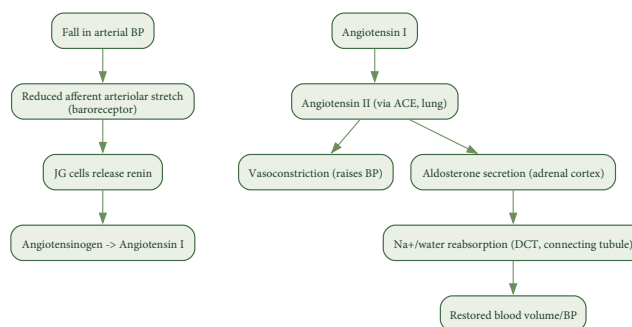
! [Fig 16.3 — Kidney Cortex: Juxtaglomerular Apparatus (diFiore's Atlas of Histology — Eroschenko, p.260)] (C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/90b898e97bac101d.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labels: Afferent arteriole → Juxtaglomerular (granular) cells → Macula densa (distal tubule cells) → Extraglomerular mesangial (laci) cells → Efferent arteriole → Glomerulus/vascular pole → Distal convoluted tubule.

Functional correlation — two feedback loops:

Tubuloglomerular feedback: a rise in GFR raises luminal Na^+/Cl^- at the macula densa → release of ATP/adenosine → afferent arteriolar constriction → GFR falls back toward normal, keeping GFR relatively constant despite fluctuating arterial pressure.



Name the parts of a Nephron. Describe the light and ultra microscopic features and correlate with its functions

Applied / Clinical anatomy

- ▶ **Diabetic glomerulosclerosis** — GBM thickening + mesangial expansion → progressive proteinuria and is a leading cause of end-stage renal disease.
- ▶ **Glomerulonephritis** — immune-complex deposition in the glomerular filter (circulating or in-situ antigen-antibody complexes) triggers inflammation and hematuria/proteinuria.
- ▶ **Minimal change disease / nephrotic syndrome** — effacement (flattening) of podocyte foot processes and loss of GBM negative charge → selective proteinuria without overt hematuria.
- ▶ **Sickle cell nephropathy** — sickling of erythrocytes in the low-oxygen vasa recta causes renal papillary/pyramidal infarcts.
- ▶ **PCT necrosis** — the PCT's high metabolic demand and rich mitochondrial content make it especially vulnerable to ischemic and nephrotoxic acute tubular necrosis (aminoglycosides, contrast agents, heavy metals).
- ▶ **Renal clearance/pharmacology** — PCT organic anion/cation transporters actively secrete creatinine, many drugs and their metabolites; this is the anatomical basis of tubular drug clearance (independent of GFR).
- ▶ **Renin-secreting JGA tumour (reninoma)** — rare cause of secondary hypertension; ACE inhibitors/ARBs act by blocking the RAAS cascade generated here.
- ▶ **Renal biopsy** correlates light-microscopic patterns (mesangial, membranous, crescentic) with the corresponding ultrastructural site of injury (podocyte, GBM, mesangium) seen on electron microscopy.

High-yield exam pointers

- ▶ Nephron = renal corpuscle + PCT + loop of Henle + DCT; the collecting duct is NOT part of the nephron (different embryological origin — ureteric bud).
- ▶ ~1–2 million nephrons/kidney; juxtamedullary nephrons (~1/8) have the longest loops and drive urine concentration.
- ▶ Filtration barrier = fenestrated endothelium + GBM (300–360 nm) + slit diaphragm between pedicels (25–30 nm slit pores).
- ▶ PCT: brush border + basal infoldings/long mitochondria → bulk reabsorption (~65–80%) and organic ion secretion.
- ▶ Thin limbs = simple squamous (passive transport); thick ascending limb = cuboidal with mitochondria (active NaCl pump) — basis of the countercurrent multiplier.

- ▶ DCT: no brush border, empty lumen, aldosterone-regulated Na^+ reabsorption.
- ▶ JGA = macula densa (DCT) + JG cells (modified afferent arteriolar smooth muscle, secrete renin) + extraglomerular mesangial (lakis) cells — mediates tubuloglomerular feedback and the renin-angiotensin-aldosterone system.
- ▶ GFR $\approx$ 125 mL/min ($\sim$ 180 L/day); $\sim$ 20% of renal plasma flow is filtered.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e.



Name the parts of respiratory systems; describe the microscopic structure of respiratory system and its functional correlation?

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction

The respiratory system provides the surface across which oxygen and carbon dioxide are exchanged between inspired air and pulmonary capillary blood. It is divided functionally into a **conducting portion**, which conditions (warms, humidifies, filters) and transports air without gas exchange, and a **respiratory portion**, where gas exchange occurs. From the nasal cavity to the alveolus the epithelium, glands, cartilage, and smooth muscle change in a graded, predictable fashion that directly explains the graded change in regional function — the essence of "functional correlation" here.

1. Parts of the Respiratory System

Conducting portion (air conduction and conditioning; no gas exchange):

- ▶ External nose and nasal cavity — vestibule, respiratory region, olfactory region
- ▶ Paranasal sinuses — frontal, maxillary, ethmoidal, sphenoidal
- ▶ Pharynx — naso-, oro-, and laryngo-pharynx
- ▶ Larynx
- ▶ Trachea
- ▶ Bronchi — primary $\rightarrow$ secondary (lobar: 3 right, 2 left) $\rightarrow$ tertiary (segmental, one per bronchopulmonary segment)
- ▶ Bronchioles $\rightarrow$ terminal bronchioles (last conducting segment; no alveoli, cartilage, or glands)

Respiratory portion (site of gas exchange):

- ▶ Respiratory bronchioles
- ▶ Alveolar ducts
- ▶ Alveolar sacs
- ▶ Alveoli

Each lung is invested by **visceral pleura**, continuous at the hilum with the **parietal pleura** lining the thoracic wall.

2. Nasal Cavity, Paranasal Sinuses and Pharynx — Microscopic Structure

- ▶ **Vestibule** — anterior dilated part; skin-lined with vibrissae, sebaceous and sweat glands filtering coarse particles; keratinised epithelium transitions to respiratory epithelium deeper in.
- ▶ **Respiratory region** (over middle and inferior conchae) — **respiratory epithelium**: ciliated pseudostratified columnar epithelium on an unusually thick basement membrane, with five cell types — **ciliated columnar cells** (most abundant, 250–300 cilia each, drive the mucociliary escalator), **goblet cells** (mucin secretion), **brush cells** (chemosensory, ~3%), **small granule/Kulchitsky cells** (DNES, dense-core granules, ~3%), and **basal cells** (stem cells for the other four types). The vascular lamina propria with seromucous glands warms and humidifies air; IgA-laden mucus traps particles; conchae create turbulence for air-mucosal contact.
- ▶ **Olfactory region** (superior concha and adjoining septum; ~10 cm², up to 100 µm thick) — pseudostratified epithelium of bipolar **olfactory neurons** (each with ~20 nonmotile cilia; axon crosses the cribriform plate as CN I), **sustentacular cells**, and **basal cells**; lamina propria has serous **glands of Bowman** (lysozyme/IgA-rich fluid with odorant-binding proteins).
- ▶ **Paranasal sinuses** — thin respiratory epithelium with goblet cells and sparse glands; drain into the nasal cavity by ciliary action.
- ▶ **Pharynx** — nasopharynx retains respiratory epithelium plus the pharyngeal/tubal tonsils; oro- and laryngo-pharynx are nonkeratinised stratified squamous, suited to the abrasion of swallowing.

□ Fig 4.6 — Ciliated pseudostratified columnar epithelium. Example, right: *Respira* (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.55)

3. Larynx — Microscopic Structure

- ▶ Cartilaginous skeleton, nine pieces: **hyaline** — thyroid, cricoid, arytenoids (may calcify with age); **elastic** — epiglottis, cuneiform, corniculate (do not calcify).
- ▶ **Epiglottis** — elastic-cartilage core; lingual surface and upper laryngeal surface are nonkeratinised stratified squamous; lower laryngeal surface transitions to respiratory epithelium; occasional taste buds; seromucous glands in lamina propria.
- ▶ **Vestibular (false) folds** — respiratory epithelium over seromucous glands and occasional lymphoid nodules (laryngeal MALT).
- ▶ **Vocal (true) folds** — nonkeratinised stratified squamous epithelium, a core **vocal ligament** (dense elastic tissue), and striated **vocalis muscle**. Adduction narrows the rima glottidis; expelled air vibrates the folds, tension altering pitch, producing phonation.

4. Trachea and Principal Bronchi — Microscopic Structure

The trachea extends from the lower border of the cricoid cartilage (C6) to its bifurcation at T4; 10–12 cm long, kept patent by 16–20 C-shaped **hyaline cartilage rings**. The posterior gap, against the oesophagus, is bridged by the **trachealis muscle** and fibroelastic tissue.

Layer	Histological features
Mucosa	Respiratory epithelium (ciliated columnar, goblet, brush, small-granule/Kulchitsky, basal cells) on a thick basement membrane; lamina propria with lymphocytes, eosinophils, plasma cells and diffuse/nodular BALT
Submucosa	Seromucous glands keeping the mucosa moist and trapping dust; numerous elastic fibres
Cartilage & muscle layer	16–20 C-shaped hyaline cartilages, open posteriorly; gap filled by the trachealis muscle and fibrous tissue
Adventitia	Fibroelastic connective tissue carrying blood vessels and nerves

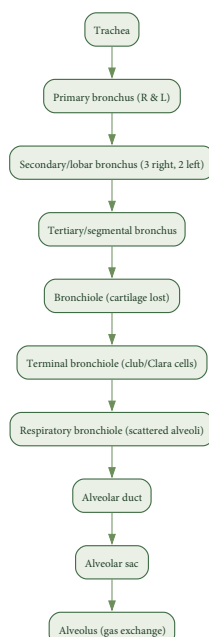
The principal bronchi have essentially the same four-layered structure as the trachea.

5. Intrapulmonary Bronchial Tree — Bronchi to Bronchioles

Sequence: principal bronchus → secondary (lobar) bronchus → tertiary (segmental) bronchus → smaller bronchi → bronchioles → terminal bronchioles. Moving distally, the epithelium simplifies (pseudostratified ciliated columnar → simple ciliated columnar → simple cuboidal); glands and cartilage progressively diminish and disappear; smooth muscle and BALT become proportionately more prominent. **Cartilage presence/absence is the criterion separating bronchus from bronchiole.**

Feature	Bronchus	Bronchiole
Diameter	> 1 mm	≤ 1 mm
Epithelium	Pseudostratified ciliated columnar with goblet cells	Simple columnar (few cilia/goblet cells), becoming simple cuboidal (no cilia/goblet cells) in the smallest branches
Smooth muscle	Present between mucosa and cartilage	Well-defined layer beneath the mucosa, proportionately more prominent
Cartilage	Present, as irregular plates	Absent
Submucosal glands	Present (serous and mucous)	Absent

The **terminal bronchiole**, the last conducting segment, is lined mainly by nonciliated, dome-shaped **club (Clara) cells**, which secrete surfactant lipoprotein/mucins, detoxify inhaled xenobiotics via smooth-ER P450 enzymes, and secrete antimicrobial peptides/IgA secretory component; a small stem-cell population regenerates the epithelium.



Name the parts of respiratory systems; describe the microscopic structure of respiratory system and its functional correlation?

6. Respiratory Portion — Where Gas Exchange Occurs

- ▶ **Respiratory bronchiole** — cuboidal, club-cell epithelium interrupted by scattered alveolar openings (squamous at the mouths); lamina propria retains smooth muscle and elastic tissue.
- ▶ **Alveolar duct** — wall formed entirely of a linear series of alveoli, each opening guarded by a knob of smooth muscle; ends in two or more **alveolar sacs**.
- ▶ **Alveolus** — saccular evagination ~200 μm in diameter; each adult lung has ~200 million alveoli, total alveolar surface ~75 m^2 (capillary surface ~125 m^2).
- ▶ **Interalveolar septum** — fibroblasts in a meshwork of elastic fibres (expansion/recoil) and reticular fibres (prevent over-distension/collapse), richly vascularised. **Pores of Kohn** (10–15 μm) equalise air pressure and permit collateral flow if a bronchiole is obstructed.
- ▶ **Pneumocytes: Type I (squamous)** cells line ~95% of the surface, extremely attenuated (as little as ~25 nm at the barrier), tight-junctioned, forming the alveolar side of the blood-air barrier. **Type II (cuboidal/"septal")** cells sit singly/in small clusters; cytoplasmic **lamellar bodies** (RER/Golgi-processed) are exocytosed as **surfactant** — DPPC, cholesterol, SP-A/B/C/D — lowering surface tension and preventing collapse on expiration; also mitotic progenitors for both cell types (~1%/day turnover). **Alveolar macrophages ("dust cells")** phagocytose particulates, excess surfactant and extravasated erythrocytes, becoming hemosiderin-laden "heart-failure cells" in venous congestion.
- ▶ **Blood-air barrier** — thin type I pneumocyte + fused epithelial-endothelial basal laminae + thin continuous (non-fenestrated) capillary endothelium; total thickness 0.1–1.5 μm .
- ▶ **Pleura** — visceral (lung) continuous at the hilum with parietal (thoracic wall) pleura; both simple squamous mesothelium on a thin collagen/elastic connective tissue layer; serous fluid film lubricates respiratory movement.

□ Fig 15.13 — Histology of lung (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.208)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled diagram of the alveolar wall/blood-air barrier — label: two adjacent alveoli; Type I pneumocyte (squamous lining); Type II pneumocyte (cuboidal, lamellar bodies); alveolar pore of Kohn; interalveolar septum (elastic/reticular fibres); capillary lumen with an erythrocyte; capillary endothelial cell (thin, continuous); fused basal laminae (the barrier); alveolar macrophage (dust cell); fibroblast in the septum.

7. Functional Correlation

Region	Key histological feature	Functional correlation
Vestibule	Vibrissae, sebaceous/sweat glands	Filters coarse particles at entry
Respiratory mucosa (nose to bronchi)	Ciliated epithelium + goblet/seromucous glands + vasculature	Mucociliary clearance; warms and humidifies air
Olfactory mucosa	Bipolar neurons, odorant-receptor cilia	Smell via CN I
Larynx (vocal folds)	Stratified squamous over vocal ligament + vocalis muscle	Withstands vibration/abrasion; variable tension → phonation
Trachea and bronchi	C-shaped/irregular hyaline cartilage	Keeps lumen patent; posterior gap allows oesophageal bulging
Bronchi → bronchioles	Cartilage/glands lost, muscle relatively increases	Shifts conduction to a muscularly-regulated, variable-calibre airway
Terminal bronchiole (club cells)	Nonciliated secretory dome cells	Surfactant component, detoxification, innate immunity at the conducting–respiratory junction
Resp. bronchiole → duct → alveolus	Cuboidal replaced by squamous epithelium	Progressive thinning maximises diffusion surface
Alveolar wall	Type I cells + fused basal laminae + endothelium (0.1–1.5 µm)	Minimal diffusion distance for O ₂ /CO ₂
Type II pneumocytes	Lamellar bodies → surfactant	Lowers surface tension, prevents collapse, eases breathing
Interalveolar septum	Elastic/reticular fibres; pores of Kohn	Elastic recoil; collateral ventilation
Pleura	Squamous mesothelium + serous fluid	Frictionless lung movement

Applied / Clinical anatomy

- **Squamous metaplasia** of bronchial epithelium (smoking/pollution) is precancerous; **squamous cell carcinoma** arises from it, peripheral **adenocarcinoma** from bronchiolar/alveolar epithelium, **small cell carcinoma** from Kulchitsky cells.

- ▶ **Kartagener syndrome** — defective ciliary dynein → sinusitis, bronchiectasis, situs inversus, male infertility.
- ▶ **Asthma** — mast-cell-triggered bronchiolar spasm; bronchiolar muscle is proportionately thicker than in bronchi.
- ▶ **Bronchiolitis** (measles/adenovirus, children) may progress to obliterative bronchiolitis.
- ▶ **Atelectasis** — bronchial obstruction causes distal lobular collapse, reversible if relieved.
- ▶ **Infant RDS** — incomplete type II pneumocyte differentiation causes surfactant deficiency; managed with CPAP and exogenous surfactant.
- ▶ **ARDS/hyaline membrane disease** — alveolar epithelial/endothelial injury; in **COVID-19**, SARS-CoV-2 targets type II pneumocytes via ACE2, reducing surfactant.
- ▶ **Congestive cardiac failure** — extravasated RBCs phagocytosed by macrophages produce "heart-failure cells" in sputum.
- ▶ **Emphysema** — smoking-related destruction of respiratory bronchiole–alveolar walls, irreversible loss of surface area.
- ▶ **Croup/acute epiglottitis/laryngitis** — laryngeal mucosal oedema narrows the airway in children.

High-yield exam pointers

- ▶ Conducting portion = nose to terminal bronchiole (no gas exchange); respiratory portion = respiratory bronchiole to alveolus.
- ▶ Respiratory epithelium: ciliated, goblet, brush, small-granule (Kulchitsky/DNES), and basal cells.
- ▶ Trachea: C6 to T4, 10–12 cm, 16–20 C-shaped hyaline cartilage rings, posterior gap bridged by trachealis muscle.
- ▶ Cartilage presence/absence separates bronchus (has it) from bronchiole (none, no glands either).
- ▶ Terminal bronchiole epithelium = club (Clara) cells — surfactant, detoxification, innate immune secretion.
- ▶ Blood-air barrier = type I pneumocyte + fused basal laminae + capillary endothelium; 0.1–1.5 µm thick.
- ▶ Type II pneumocytes synthesise surfactant (DPPC, cholesterol, SP-A/B/C/D) and are progenitors for both pneumocyte types.
- ▶ Pores of Kohn permit collateral air circulation between adjacent alveoli.
- ▶ Alveolar macrophages ("dust cells") become "heart-failure cells" in pulmonary venous congestion.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; BD Chaurasia — Human Anatomy Vol 1 8e (tracheal level and cartilage-ring count).



Systemic histology - endocrine & reproductive



Histology of Testis

Asked in: Paper III 02-Aug-2021 · Paper III May 2007 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The testis is a paired, encapsulated compound tubular gland with a dual function — **exocrine** (spermatogenesis, occurring in the seminiferous tubules) and **endocrine** (testosterone synthesis, by the interstitial Leydig cells). Each testis is invested by the tunica vaginalis, tunica albuginea and tunica vasculosa, and is subdivided by fibrous septa into lobules, each containing one to four highly coiled seminiferous tubules. The seminiferous epithelium is unique in being built from two cooperating cell populations — non-dividing supporting **Sertoli cells** and the proliferating, differentiating **spermatogenic cell line** — separated from the systemic circulation by the **blood–testis barrier**.

1. Capsule and lobulation

- ▶ **Tunica vaginalis** — a peritoneal remnant carried down during testicular descent; parietal layer lines the scrotum, visceral layer covers the tunica albuginea (except posteriorly at the mediastinum).
- ▶ **Tunica albuginea** — dense collagenous capsule; thickens on the posterior border to form the **mediastinum testis**, through which vessels, lymphatics and genital ducts enter/leave.
- ▶ **Tunica vasculosa** — thin vascular areolar layer lining each lobule, deep to the albuginea.
- ▶ Fibrous **septa** radiate from the mediastinum and incompletely partition the gland into ~200–300 **pyramidal lobules** (apex directed to the mediastinum), each lobule containing **1–4 seminiferous tubules** in sparse interstitial connective tissue.

2. Seminiferous tubule — general architecture

- ▶ Each testis contains **400–1000 highly coiled seminiferous tubules**; each tubule is **150–250 µm** in diameter and **30–80 cm** long; combined tubular length per testis is ~250 m.
- ▶ Each tubule is a closed loop — both ends converge at the mediastinum via a short, narrow **straight tubule (tubulus rectus)**.
- ▶ Tubule wall, outside-in: **tunica/lamina propria** (fibrous connective tissue with flattened, contractile **myoid cells** whose peristalsis moves sperm/fluid) → **basement membrane** → thick stratified **seminiferous (germinal) epithelium**.
- ▶ The germinal epithelium has two cell populations: **Sertoli cells** (supporting) and the **spermatogenic cell line** (spermatogonia → spermatocytes → spermatids → spermatozoa), arranged basal-to-luminal in that developmental order.
- ▶ Loose interstitial tissue between tubules carries blood vessels, lymphatics, macrophages, mast cells and clusters of **Leydig cells**.

Diagram (real plate)

- Fig 21.9 — *Histology of testis (low magnification on left) and seminiferous tubule (high magnification on right) (Sontakke — Textbook of Human Histology — 2e, p.290)*

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw (a) a longitudinal schematic of the testis and (b) a cross-section of one seminiferous tubule; label:

- ▶ Tunica vaginalis (parietal and visceral layers)
- ▶ Tunica albuginea
- ▶ Mediastinum testis
- ▶ Septa of testis → lobules of testis (1–4 tubules/lobule)
- ▶ Seminiferous tubule (convoluted part) → tubulus rectus (straight tubule) → rete testis → efferent ductules → head of epididymis
- ▶ Interstitial (Leydig) cells in the loose connective tissue between tubules
- ▶ Cross-section of one tubule (base→lumen): tunica propria with myoid cells → basement membrane → spermatogonia → primary spermatocytes → secondary spermatocytes → early/late spermatids → Sertoli cell body (base to lumen) → lumen with spermatozoa

3. Sertoli cells

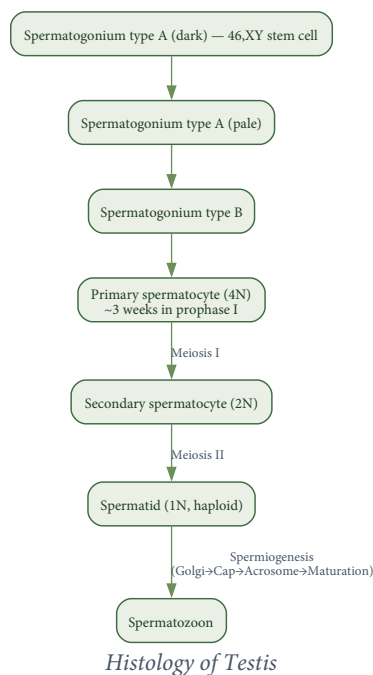
Feature	Description
Shape/nucleus	Tall, columnar, non-dividing; base on basement membrane, apex reaching lumen; nucleus euchromatic, irregular/triangular, with 1–2 prominent nucleoli
Cytoplasm	Abundant SER, some RER, well-developed Golgi, many mitochondria, lysosomes (phagocytic)
Junctions	Basolateral tight (occluding) junctions between adjacent Sertoli cells → blood–testis barrier ; also gap junctions between Sertoli cells
Support function	Physical/metabolic support, nutrition and protection of germ cells; each Sertoli cell supports ~30–50 germ cells
Exocrine secretion	Fructose- and protein-rich fluid that carries sperm out of the tubule
Endocrine secretion	Androgen-binding protein (ABP) and inhibin B (FSH-driven, inhibin B feeds back to suppress FSH); fetal Sertoli cells secrete Müllerian-inhibiting substance (MIS/AMH)
Phagocytosis	Engulfs and digests residual bodies shed during spermiogenesis
Hormonal control	Stimulated by FSH from anterior pituitary

4. Spermatogenesis and spermiogenesis

- ▶ **Spermatogonia** — small (~12 µm), diploid (46,XY) cells resting on the basement membrane; subtyped as **dark type-A (Ad)** (reserve stem cells), **pale type-A (Ap)** (transit-amplifying), and **type-B** (committed) — Ad → Ap → B is the standard sequence.
- ▶ Type-B spermatogonia undergo a final mitosis to form **primary spermatocytes** (diploid, 4N DNA) — the **largest** germ cells, seen in a prolonged first meiotic prophase lasting ~3 weeks; they cross the blood–testis barrier from the basal to the adluminal compartment.
- ▶ Meiosis I → **secondary spermatocytes** (haploid, 23 chromosomes, 2N DNA) — short-lived, rarely seen in sections.
- ▶ Meiosis II → haploid **spermatids** (1N DNA), which then undergo **spermiogenesis** (no further division) to become spermatozoa.
- ▶ **Duration:** a spermatogonium takes ~64 days to become a mature spermatozoon (Gray's Anatomy; IB Singh); Ross Histology derives a comparable figure (~74 days) from a 16-day human seminiferous-epithelial cycle × 4.6 cycles, with **6 recognisable cellular associations (stages)** described by Clermont (1963) recurring along the tubule. A further ~12 days is needed for epididymal transit/maturation.
- ▶ **Sperm output:** ~200–300 million sperm produced per testis per day (textbooks vary in the exact figure).

Spermiogenesis — 4 phases

Phase	Key event
Golgi phase	Proacrosomal vesicles from Golgi coalesce into an acrosomal cap; centrioles migrate to the pole opposite the acrosome and organise the flagellar axoneme
Cap phase	Acrosomal cap spreads over ~half the condensing nucleus; acrosome = a lysosome-like organelle containing hyaluronidase and acrosin
Acrosome phase	Nucleus elongates and condenses (histones replaced by protamines); flagellum elongates; mitochondria aggregate around the proximal flagellum forming the middle piece
Maturation phase	Excess cytoplasm shed as a residual body (phagocytosed by Sertoli cells); mature (but still non-motile) spermatozoon released into the lumen



5. Blood–testis barrier

- ▶ Formed by **tight junctions between adjacent Sertoli cells**, at a level just above the spermatogonia.
- ▶ Divides the seminiferous epithelium into a **basal compartment** (spermatogonia — freely exposed to interstitial tissue) and an **adluminal compartment** (spermatocytes, spermatids — sequestered from plasma/immune cells).
- ▶ Newly formed primary spermatocytes transiently open and re-seal the junctions to cross from basal to adluminal compartment without breaching barrier integrity.
- ▶ Function: creates an immunologically privileged environment for haploid germ cells (which express "foreign" surface antigens), and controls the local micro-environment (ionic/nutrient composition) via Sertoli cell transport.

6. Leydig (interstitial) cells and hormonal regulation

- ▶ Large, **polygonal/polyhedral cells** with an **eccentric nucleus** (1–3 nucleoli); **eosinophilic/pale, foamy cytoplasm** rich in smooth endoplasmic reticulum, mitochondria (tubular cristae), lipid droplets and lipofuscin pigment.
- ▶ Pathognomonic **crystals (crystalloids) of Reinke** — rod-shaped cytoplasmic inclusions.
- ▶ Secrete **testosterone**, using the same SER/mitochondrial steroidogenic enzyme system as the adrenal cortex.
- ▶ **Fetal Leydig cells** are active in the 2nd–4th month of gestation (driven by placental gonadotropin, masculinising the duct system), then become quiescent, fibroblast-like cells until they resume activity at puberty.

Hormonal axis (HPG)

Level	Hormone	Target/action
Hypothalamus	GnRH	Stimulates anterior pituitary

7. Intratesticular ducts (brief)

- ▶ **Tubuli recti (straight tubules)** — short, narrow segments lined only by **Sertoli cells** (no germ cells), connecting the seminiferous tubule loop to the rete testis.
- ▶ **Rete testis** — anastomosing channels within the mediastinum testis, lined by simple **cuboidal epithelium**; reabsorbs tubular fluid and concentrates sperm.
- ▶ **Efferent ductules** — several (quoted 7–20 across texts) ducts draining the rete testis into the caput epididymis; distinctive **scalloped epithelium** of alternating ciliated columnar and non-ciliated absorptive cuboidal cells, surrounded by a thin circular smooth-muscle coat.

Applied / Clinical anatomy

- ▶ **Cryptorchidism** — failure of testicular descent (~4% of male neonates; most descend within the first year); if uncorrected by 2–3 years causes progressive tubular hyalinisation/germinal epithelial loss → infertility, and carries a markedly increased risk of germ-cell tumour.
- ▶ **Hydrocele** — excess serous fluid within the tunica vaginalis; the commonest cause of painless scrotal swelling, easily correctable surgically.
- ▶ **Varicocele** — abnormal dilatation of the pampiniform plexus, classically left-sided (longer left testicular vein draining at right angles into the left renal vein); raises scrotal temperature and impairs spermatogenesis — a treatable cause of male infertility.
- ▶ **Testicular germ-cell tumours** (~95% of testicular cancers, e.g. seminoma) arise from spermatogenic-line cells, occur mainly after puberty, and are strongly associated with a history of cryptorchidism; Sertoli-cell and Leydig-cell tumours are rare (Leydig-cell tumours may show Reinke crystals histologically).
- ▶ **Lymphatic spread** of testicular tumours follows the gonadal (not inguinal) lymphatics — to the **para-aortic/lumbar and inter-aortocaval nodes** — reflecting the organ's retroperitoneal embryological origin; this dictates the surgical approach (radical orchidectomy via the inguinal canal, never trans-scrotal biopsy, to avoid disturbing this lymphatic drainage).
- ▶ **Blood–testis barrier breach** (trauma, infection, vasectomy) exposes sperm antigens to the immune system → anti-sperm antibodies → autoimmune subfertility.
- ▶ **Testicular biopsy in male infertility** is interpreted against the normal cellular associations/stages of the spermatogenic cycle; complete absence of germ cells with only Sertoli cells lining the tubule defines **Sertoli-cell-only syndrome**.
- ▶ **Orchitis/epididymitis** (commonly gonococcal/chlamydial, ascending from the epididymis) can damage the seminiferous epithelium; **mumps orchitis** is a classical post-pubertal cause of testicular atrophy and infertility.

High-yield exam pointers

- ▶ Tunica albuginea → thickens posteriorly as **mediastinum testis** → septa → ~200–300 lobules, each with 1–4 **seminiferous tubules**.
- ▶ Seminiferous epithelium = **Sertoli cells** (non-dividing, supportive) + **spermatogenic cell line** (spermatogonia → primary spermatocyte → secondary spermatocyte → spermatid → spermatozoon).

- ▶ **Blood–testis barrier** = tight junctions between adjacent Sertoli cells; separates **basal** (spermatogonia) from **adluminal** (spermatocytes/spermatids) compartments; confers immune privilege.
- ▶ **Leydig cells**: interstitial, testosterone-secreting, eosinophilic with **Reinke crystals**; driven by LH/ICSH.
- ▶ Duration of spermatogenesis ~**64 days** (spermatogonium → mature spermatozoon); spermiogenesis has **4 phases** — Golgi, Cap, Acrosome, Maturation.
- ▶ **FSH** → **Sertoli cell** (ABP, inhibin B); **LH** → **Leydig cell** (testosterone) — the essential hormonal loop.
- ▶ Duct sequence: seminiferous tubule → **tubulus rectus** → **rete testis** → **efferent ductules** → epididymis.
- ▶ Applied triad to remember: **cryptorchidism** (descent failure, malignancy risk), **varicocele** (impaired spermatogenesis), **testicular tumour lymphatic spread to para-aortic nodes** (not inguinal).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e (figure).



APUD Cells

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction APUD stands for **Amine Precursor Uptake and Decarboxylation** — a concept introduced by A.G.E. Pearse (1968, elaborated 1977–80) to group a large, widely dispersed family of unicellular endocrine cells found singly or in small clusters throughout the body (pituitary, thyroid C-cells, parathyroid, adrenal medulla, carotid body, gut, pancreatic islets, skin, and elsewhere). These cells share a common **functional/cytochemical phenotype** — they take up circulating amine precursors and decarboxylate them to synthesise biogenic amines and/or peptide hormones, which they store in dense-core secretory granules and release as **paracrine (local)** or **endocrine (distant)** messengers, and often also as neurotransmitters. Because of this dual nervous–endocrine character, the APUD system is now more accurately termed the **Diffuse Neuroendocrine System (DNES)**, a bridge between the autonomic nervous system and the classical endocrine organs.

1. Concept and defining criteria of the APUD/DNES cell

An APUD cell is recognised by a combination of the following shared properties:

- ▶ **Amine precursor uptake** — active uptake of precursors (e.g. 5-hydroxytryptophan, L-DOPA) from the circulation.
- ▶ **Decarboxylase activity** — presence of a cytoplasmic **decarboxylase enzyme** that converts the precursor to a biogenic amine (serotonin, dopamine, catecholamines) or, in many cells, to a regulatory **peptide** instead of/in addition to an amine.

- ▶ **Dense-core (neurosecretory) granules** — membrane-bound, spherical/oval cytoplasmic granules with an electron-dense core surrounded by an **electron-lucent halo**, seen on electron microscopy.
- ▶ **Histochemical reactivity** — many stain with silver salts (**argentaffin** cells reduce silver nitrate directly; **argyrophil** cells need an external reducing agent) or with chromium/dichromate salts (**chromaffin reaction**, classically the adrenal medulla).
- ▶ **Immunohistochemical markers** — general neuroendocrine differentiation markers such as **chromogranin A** (a granule-matrix protein that regulates dense-core granule biogenesis) and **synaptophysin** are used to identify APUD/DNES cells and their tumours.
- ▶ **Dual signalling role** — the same amine/peptide can act locally as a paracrine signal or neurotransmitter, and systemically as a classical hormone.

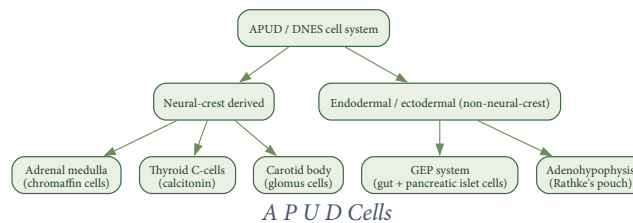
2. Classification — APUD/DNES cells by site and secretory product

Site	Cell type	Product(s)
Adenohypophysis	Acidophils, basophils	GH, prolactin, ACTH, TSH, FSH/LH
Hypothalamus	Neurosecretory neurons	Oxytocin, vasopressin (ADH); hypophysiotropic releasing/inhibiting factors
Parathyroid	Chief cells	Parathyroid hormone (PTH)
Thyroid	Parafollicular (C) cells	Calcitonin
Adrenal medulla	Chromaffin cells; SIF cells of sympathetic ganglia	Adrenaline, noradrenaline
Carotid body	Glomus (type I) cells	Dopamine, noradrenaline (chemoreceptor function)
Pancreatic islets	α , β , δ , PP cells	Glucagon, insulin, somatostatin, pancreatic polypeptide
Gastrointestinal mucosa (enteroendocrine/GEP cells)	G, S, I, D, EC, K, Mo, L cells	Gastrin, secretin, cholecystokinin, somatostatin, serotonin/substance P, GIP, motilin, enteroglucagon (GLP)
Skin	Melanocytes	Promelanin/melanin precursors
Pineal gland, placenta, heart (myoendocrine cells), kidney (juxtaglomerular cells)	Various	Melatonin-related products; placental peptides; ANP; renin

Collectively, the pancreatic-islet + gut-endocrine-cell component of this list is called the **gastroenteropancreatic (GEP) endocrine system** — the single largest and most clinically important subset of the APUD/DNES family.

3. Embryological origin — the neural-crest hypothesis and its revision

- ▶ Pearse originally proposed that **all** APUD cells share a common **neuroectodermal (neural crest)** origin, migrating out during development to colonise their definitive sites — the "APUD series" concept.
- ▶ This holds true for several members: **adrenal medullary chromaffin cells**, **thyroid parafollicular (C) cells**, and **carotid body glomus cells** are genuinely **neural-crest derivatives**.
- ▶ However, it is now established that the **GEP (gastrointestinal + pancreatic islet) endocrine cells** are **not** neural-crest migrants — they differentiate **in situ from the same multipotent endodermal stem cells** of the gut epithelium that also give rise to enterocytes and goblet cells. Similarly, **adenohypophyseal cells** arise from oral ectoderm (Rathke's pouch), not neural crest.
- ▶ Hence two histologically/functionally similar cells (e.g. an adrenal medullary chromaffin cell and a gut EC cell) may share the APUD phenotype **without** sharing a common embryonic lineage — the shared trait is a **convergent functional specialisation**, not a single origin. This revision is precisely why the broader, origin-neutral term "**diffuse neuroendocrine system**" has generally superseded "APUD system," although the acronym remains in wide clinical use (e.g. "apudoma").



4. Ultrastructural and histochemical features

- ▶ **Electron microscopy:** membrane-bound, spherical **dense-core granules**, usually concentrated at the **basal pole** of the cell (away from the lumen), with a dense electron-opaque core and a surrounding electron-lucent halo; well-developed rough ER and Golgi reflect active peptide/amine synthesis.
- ▶ "Closed" vs "open" type enteroendocrine (APUD) cells — a key distinction for the GEP cells:

Feature	Closed type	Open type
Apex	Does not reach the gland lumen	Reaches the lumen, bears microvilli
Stimulus sensed	Blood-borne/neural/paracrine signals	Luminal contents (chemoreceptor)
Example	Most gastric fundic EC cells	Antral/pyloric G cells
Secretion	Basal, paracrine/endocrine	Basal, after luminal sampling

- ▶ **Staining reactions:** **argentaffin** (direct silver reduction — enterochromaffin/EC cells), **argyrophil** (silver reduction needs an external reducer — most other GEP cells), **chromaffin reaction** with dichromate salts (adrenal medulla, hence "chromaffin cells"). Enterochromaffin cells are also eponymously called **Kulchitsky cells**.

- ▶ **Immunohistochemistry:** chromogranin A and synaptophysin positivity is the standard diagnostic confirmation of neuroendocrine (APUD) differentiation in both normal tissue and tumours.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a schematic "closed"-type gastric enteroendocrine (APUD) cell amid surrounding epithelium:

- ▶ Basal lamina of the gastric gland
- ▶ Basally-placed **dense-core secretory granules** (with electron-lucent halo)
- ▶ Central/basal **nucleus**
- ▶ Supranuclear **Golgi complex** and **rough endoplasmic reticulum**
- ▶ Adjacent parietal cell, chief cell, and mucous neck cell for orientation
- ▶ Capillary in the lamina propria receiving the basally-secreted product

□ Fig 17.12 — ▲ *Electron micrograph and diagrams of enteroendocrine cells. a. This e* (Ross Histology — A Text and Atlas — 7e, p.597)

Applied / Clinical anatomy

- ▶ **Apudoma** — generic term for a tumour arising from any APUD/DNES cell; the behaviour and secretory product reflect the cell of origin (e.g. insulinoma, glucagonoma, gastrinoma, VIPoma, pheochromocytoma, medullary carcinoma of thyroid).
- ▶ **Carcinoid tumour** — arises from GI EC cells; classically produces **serotonin**, causing the **carcinoid syndrome** (flushing, diarrhoea, bronchoconstriction, right-sided cardiac valve fibrosis). The **appendix** is the commonest GI site.
- ▶ **Zollinger–Ellison syndrome** — a **gastrinoma** (G-cell tumour, usually pancreatic/duodenal) causing recurrent, refractory peptic ulceration through unchecked gastrin-driven HCl secretion.
- ▶ **Pheochromocytoma** — a neuroendocrine tumour of **adrenal medullary chromaffin cells**, usually benign, secreting catecholamines.
- ▶ **Medullary carcinoma of thyroid** — arises from C-cells; secretes calcitonin; part of MEN 2A/2B syndromes.
- ▶ **Multiple Endocrine Neoplasia (MEN) syndromes** — link several APUD/DNES-derived organs (parathyroid, pituitary, pancreatic islets, thyroid C-cells, adrenal medulla) in a single patient, reflecting their shared secretory phenotype.
- ▶ **Carotid body tumour (chemodectoma/paraganglioma)** — arises from glomus (type I) cells of the carotid body.
- ▶ Diagnostically, **chromogranin A/synaptophysin immunopositivity** on biopsy confirms a neuroendocrine (APUD) tumour irrespective of its embryological origin.

High-yield exam pointers

- ▶ APUD = Amine Precursor Uptake and Decarboxylation; concept by A.G.E. Pearse; modern synonym = **Diffuse Neuroendocrine System (DNES)**.

- ▶ Defining features: amine-precursor uptake + decarboxylase activity + basal dense-core granules with electron-lucent halo; argentaffin/argyrophil/chromaffin reactivity; chromogranin A/synaptophysin IHC positivity.
- ▶ Not all APUD cells share one embryonic origin: adrenal medulla, thyroid C-cells, carotid body glomus cells = **neural crest**; GEP (gut + pancreatic islet) cells and adenohypophysis = **endodermal/ectodermal**, NOT neural crest.
- ▶ GEP (gastroenteropancreatic) system = the largest APUD subgroup — gastrin (G), secretin (S), CCK (I), somatostatin (D), serotonin (EC/Kulchitsky), GIP (K), motilin (Mo), enteroglucagon (L) cells, plus pancreatic islet $\alpha/\beta/\delta$ /PP cells.
- ▶ "Closed" enteroendocrine cells sense blood/neural signals basally; "open" cells sample luminal contents via apical microvilli (chemoreceptors).
- ▶ Clinically: apudoma, carcinoid syndrome (EC cells/serotonin), Zollinger–Ellison syndrome (gastrinoma), pheochromocytoma, MEN 2A/2B (C-cells).
- ▶ Enterochromaffin cells = **Kulchitsky cells** (eponym).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e. Coverage note: APUD cells are covered only briefly in Gray's Anatomy (mainly in reference lists); the depth of this answer is drawn from the specialty histology texts (IB Singh, Junqueira, Ross, Sontakke), as directed by the topic's specialty grounding.



APUD cells

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction APUD cells are a widely dispersed family of specialised cells that take up amine precursors from the circulation and decarboxylate them intracellularly to form biogenic amines and/or polypeptide hormones — hence **Amine Precursor Uptake and Decarboxylation**. The concept was proposed by **A.G.E. Pearse** (late 1960s–1970s) to unify a group of morphologically and cytochemically similar, hormone-secreting cells scattered through endocrine glands, the gut, respiratory tract and elsewhere. Because later work showed these cells do **not** share a single embryonic origin, the more embryologically neutral term **diffuse (or dispersed) neuroendocrine system (DNES)** is now preferred, though "APUD cell/APUD system" remains in universal clinical and examination use.

1. Historical concept and nomenclature

- ▶ Coined by **A.G.E. Pearse**, who cytochemically characterised a series of polypeptide-hormone-producing cells sharing a common set of enzymatic and staining properties (the "APUD series").
- ▶ Pearse's original **APUD concept** proposed that all these cells were programmed, common precursor cells of **neuroectodermal (neural crest)** origin — a **unitary hypothesis**.

- ▶ As evidence accumulated that many gut and pancreatic endocrine cells are in fact **endodermal**, the broader term **diffuse neuroendocrine system (DNES)** replaced "APUD cell series" as the preferred umbrella term — the two names are used interchangeably today.
- ▶ Synonyms encountered for individual members of the system (reflecting the histochemical methods by which they were first identified):
 - **Enterochromaffin cells** — give a positive chromaffin reaction with chromium salts (potassium dichromate).
 - **Argentaffin cells** — reduce silver salts directly; **argyrophil cells** — require an external reducing agent to stain with silver.
 - **Clear cells** — poor staining with routine (H&E) methods.
 - **Kulchitsky cells** — the corresponding cells of the bronchial/respiratory epithelium.
 - **Paraneurones** — a functional term (Fujita) for cells that share neurone-like secretory/electrophysiological properties but are not themselves neurones (e.g., chromaffin cells, taste-bud cells, carotid body glomus cells).

2. General cytochemical and ultrastructural characteristics

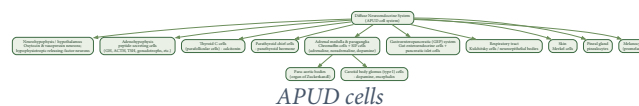
Feature	Detail
Secretory granules	Numerous membrane-bound, spherical/oval, dense-core granules with an electron-dense core and a surrounding electron-lucent halo — resemble neurotransmitter vesicles of neuronal terminals (seen by EM).
Special stains	Chromaffin reaction (chromium salts) → enterochromaffin cells; argentaffin/argyrophil (silver) reactions; some are functionally " clear " on routine staining.
Biochemical property	Uptake of amine precursors (e.g., 5-hydroxytryptophan, L-DOPA) from blood/interstitium and intracellular decarboxylation to the active amine (5-HT, catecholamines) and/or synthesis of a polypeptide hormone.
Secretory products	A biogenic amine (serotonin, catecholamines, dopamine, histamine) and/or a regulatory peptide (gastrin, secretin, CCK, VIP, somatostatin, calcitonin, ACTH, enkephalin, etc.).
Cell polarity / position	" Closed " type — cell does not reach the lumen (e.g., carotid body glomus cells, thyroid C-cells) — secretory pole faces a capillary/interstitium. " Open " type — apical microvilli reach the lumen and sample its contents (most gut enteroendocrine cells), basal granules discharge toward the lamina propria capillaries.

Feature	Detail
Signalling mode	May act as a classical endocrine cell (secretion into blood), a paracrine cell (local diffusion to neighbouring cells), or a neurocrine/neurotransmitter -like cell (synaptic release) — often several simultaneously.

3. Embryological origin

- Pearse's original unitary hypothesis held that **all** APUD cells arise from the **neural crest**, migrating widely to their definitive sites — this is genuinely true for several classic members.
- **Confirmed neural-crest derivatives**: chromaffin cells of the **adrenal medulla** and other paraganglia; **thyroid C-cells (parafollicular cells)**; melanocytes; the small intensely fluorescent (SIF) cells of sympathetic ganglia.
- **Revision of the concept**: many cells of the DNES — in particular, the **enteroendocrine cells of the gastrointestinal tract and the endocrine cells of the pancreatic islets** — are now known to be **endodermal**, arising locally from the gut/pancreatic epithelium rather than migrating in from the neural crest. Some enteroendocrine cells are only *functionally* classifiable as APUD cells and should not be equated embryologically with the true neural-crest-derived APUD cells found elsewhere.
- The dual origin is precisely why the neutral term "**diffuse neuroendocrine system**" (rather than an origin-implying name) is now favoured — it groups cells by shared cytochemical/functional behaviour, not by a single embryonic source.

4. Classification / distribution of the DNES (APUD) cell system



- Fits: adenohypophysis peptide cells and hypothalamic neurosecretory/releasing-factor neurons; **thyroid C-cells** (calcitonin); **parathyroid chief cells**; **adrenal medullary chromaffin cells** together with the SIF cells of sympathetic ganglia, the **para-aortic bodies (organ of Zuckerkandl)**, and other outlying chromaffin/paraganglion tissue; **carotid body glomus (type I) cells** (dopamine, noradrenaline, enkephalin — chemoreceptor + neuroendocrine function); the **gastroenteropancreatic (GEP) system** — enteroendocrine cells scattered in the gastric and intestinal epithelium (5-HT, gastrin, secretin, CCK, motilin, VIP, somatostatin, etc.) plus the endocrine cells of the **pancreatic islets** (insulin, glucagon, somatostatin, PP); **Kulchitsky cells / neuroepithelial bodies** of the bronchial mucosa; **Merkel cells** of the basal epidermis; **pinealocytes**; and **melanocytes**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 22.10 — Structure of the carotid body (schematic representation). (IB Singh — Textbook of Human Histology — 9e, p.651)

Also draw a simple schematic of a single "open-type" **enteroendocrine (APUD) cell** within gut epithelium, labelling:

- ▶ Adjacent absorptive/columnar epithelial cells and goblet cells
- ▶ The APUD cell — pyramidal shape, broad base, narrow apex
- ▶ **Apical microvilli** projecting into the lumen (luminal-content sampling)
- ▶ **Basal cytoplasmic dense-core secretory granules**
- ▶ Basement membrane
- ▶ Underlying **lamina propria capillary** receiving the secreted amine/peptide (paracrine/endocrine release)
- ▶ A nearby afferent nerve fibril (neurocrine link)

5. Functional significance

- ▶ Act as the anatomical **link between the autonomic nervous system and the classical endocrine glands** — hence "neuroendocrine".
- ▶ Individual cells may function simultaneously as **endocrine** (hormone into blood), **paracrine** (local diffusion to adjoining secretory/contractile cells), and **neurocrine/neurotransmitter-like** elements — the same molecules (5-HT, dopamine, VIP, somatostatin) act as both circulating hormones and CNS neurotransmitters.
- ▶ The "**paraneuron**" concept (Fujita) captures this dual nature: a non-neuronal cell that shares a neurone's secretory machinery, action-potential generation and (in some sites) even synaptic contacts with sensory nerve terminals (e.g., carotid body glomus cells, taste-bud cells).
- ▶ In the gut, DNES cells regulate motility and glandular secretion largely through **paracrine** signalling on neighbouring smooth muscle and secretory cells.

Applied / Clinical anatomy

- ▶ **Apudomas**: a generic term for tumours arising from any polypeptide/amine-secreting cell of the DNES/APUD system; many are functionally active and produce distinct hormonal syndromes.
- ▶ **Gastroenteropancreatic (GEP) neuroendocrine tumours**: uncommon GI/pancreatic neoplasms; the **appendix is the commonest GI site**. The classical example is the **carcinoid syndrome** — serotonin (and other vasoactive amine) release causing episodic **flushing, diarrhoea, bronchoconstriction and right-sided cardiac valvular disease**.
- ▶ **Pheochromocytoma**: a tumour of adrenal medullary chromaffin cells producing excess catecholamines; **paraganglioma** denotes an identical tumour arising from extra-adrenal chromaffin tissue (sympathetic chain, organ of Zuckerkandl, carotid body).
- ▶ **Medullary carcinoma of the thyroid**: arises from the C-cells and characteristically secretes excess **calcitonin**; may occur as part of the MEN 2 spectrum (with pheochromocytoma), reflecting the shared neural-crest/APUD lineage of C-cells and adrenal medullary cells.

- ▶ **Merkel cell carcinoma:** an aggressive cutaneous neuroendocrine tumour arising from epidermal Merkel cells.
- ▶ **Carotid body tumour (chemodectoma):** arises from the glomus (type I) cells at the carotid bifurcation.

High-yield exam pointers

- ▶ APUD = **Amine Precursor Uptake and Decarboxylation**; concept introduced by A.G.E. Pearse; modern preferred name — **diffuse neuroendocrine system (DNES)**.
- ▶ Ultrastructural hallmark: **membrane-bound, dense-core secretory granules**.
- ▶ Synonyms by staining method: **enterochromaffin** (chromium salts), **argentaffin/argyrophil** (silver salts); functional synonym — **paraneurones**.
- ▶ Original hypothesis = single **neural crest** origin for all APUD cells; **now revised** — GI/pancreatic enteroendocrine cells are **endodermal**, while chromaffin cells, C-cells and SIF cells remain genuinely **neural-crest-derived**.
- ▶ Major sites: pituitary/hypothalamus, thyroid C-cells, parathyroid, adrenal medulla + paraganglia + carotid body, GEP system (gut + pancreatic islets), bronchial Kulchitsky cells, Merkel cells of skin, pinealocytes, melanocytes.
- ▶ Cells may be "**open**" type (apical microvilli reach the gut lumen) or "**closed**" type (do not reach a lumen, e.g., glomus cells, C-cells).
- ▶ Functional triad: **endocrine + paracrine + neurocrine** signalling — the anatomical bridge between nervous and endocrine systems.
- ▶ Key applied correlations: **apudoma, carcinoid syndrome** (appendix commonest site), **pheochromocytoma/paraganglioma, medullary thyroid carcinoma, Merkel cell carcinoma**.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e.



Compare and contrast the microscopic structure of testis and ovary

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction The testis and ovary are the primary reproductive organs (gonads); each is a dual-function organ combining a gamete-producing (gametogenic) compartment with a hormone-secreting (steroidogenic) compartment. Both arise from a common indifferent gonad in the fifth week of intrauterine life and share the same basic architectural template — a fibrous capsule, a germ-cell-bearing parenchyma, and a steroidogenic stroma — yet they differ profoundly in the continuity, timing and cytology of gametogenesis. This answer compares the two organs system by system: general organisation and coverings; the germ-cell-bearing unit; gametogenesis; supporting/steroidogenic cells; duct/post-ovulatory structures; and hormonal regulation.

1. General organisation and coverings

Feature	Testis	Ovary
Shape/size	Ovoid, paired, ~4–5 cm long	Almond-shaped, ~3 cm long × 1.5 cm wide × 1 cm thick
Surface covering	Tunica vaginalis — a mesothelial serous sac (parietal + visceral layers) derived from peritoneum, covering the anterior/lateral surface	Surface (germinal) epithelium — simple cuboidal epithelium, continuous with the peritoneal mesothelium, covering the whole organ
Capsule	Tunica albuginea — thick, dense fibrous capsule; thickens posteriorly to form the mediastinum testis	Tunica albuginea — a thinner dense connective-tissue capsule immediately beneath the surface epithelium
Internal septa/zonation	Fibrous septa radiating from the mediastinum divide the organ into ~250 pyramidal lobules	No septa or lobulation; organ shows an outer cortex (follicles in cellular stroma) and inner medulla (loose connective tissue, vessels, nerves) with no sharp cortex–medulla boundary
Parenchymal unit per compartment	1–4 seminiferous tubules per lobule, in sparse interstitial connective tissue	Numerous follicles of every stage of maturation scattered through the cellular cortical stroma

2. The germ-cell-bearing unit — seminiferous tubule vs ovarian follicle

Feature	Seminiferous tubule	Ovarian follicle
Basic unit	A closed, highly convoluted tube , patent throughout	A solid ball (oocyte + investing cells); becomes cavitated only in later stages
Number/dimensions	250–1000 tubules/testis; 150–250 µm diameter; 30–70 cm long each; combined length ≈250 m/testis	~2–2.5 million primordial follicles at birth, ~400,000 at puberty; only ~450 ever ovulate in a lifetime

Feature	Seminiferous tubule	Ovarian follicle
Wall	Complex stratified germinal (spermatogenic) epithelium on a basement membrane, covered externally by contractile myoid cells	Follicular/granulosa cell layer(s) resting on a basal lamina; oocyte lies centrally, encircled by these cells
Barrier	Basal lamina + myoid layer; deeper, tight-junctional blood–testis barrier between Sertoli cells	Basal lamina forms a blood–follicle barrier — the granulosa layer itself stays avascular until luteinisation
Lumen	Present from the outset	Absent in primordial/primary stages; an antrum appears only in the secondary (antral) stage
Fate of the unit	Persists lifelong; reused for successive spermatogenic cycles	Each follicle is used only once — it either ovulates or undergoes atresia ; never reused

3. Gametogenesis — spermatogenesis vs oogenesis

Feature	Spermatogenesis	Oogenesis
Onset	Puberty	Fetal life — oogonial mitosis is complete by the 5th gestational month; all oocytes are already arrested in meiotic prophase I before birth
Continuity	Continuous throughout reproductive life; ~200 million sperm produced per day	Cyclical — one dominant follicle completes maturation roughly every 28 days
Stem-cell renewal	Spermatogonia are self-renewing stem cells throughout life	Oogonial mitosis ceases prenatally; there is no postnatal renewal of the follicular pool — a fixed, non-renewable "ovarian reserve"
Meiotic divisions	Two equal divisions; one primary spermatocyte yields 4 equivalent haploid spermatids	Two grossly unequal divisions: primary oocyte → secondary oocyte + tiny 1st polar body (at ovulation); secondary oocyte → ovum + 2nd polar body (only if fertilised) — nearly all cytoplasm is retained by the ovum
Meiotic arrest	None once initiated; spermatogonium-to-spermatid takes ~2 months, followed by spermiogenesis	Long arrests: prophase I from fetal life until just before ovulation (years to decades); metaphase II until fertilisation

Feature	Spermatogenesis	Oogenesis
Final cytodifferentiation	Spermiogenesis — acrosome formation, nuclear condensation/elongation, flagellum growth, shedding of cytoplasm	No comparable step — the definitive gamete is simply the larger product of the second meiotic division

4. Supporting (nurse) cells and steroidogenic cells

Feature	Testis	Ovary
Nurse/supporting cell	Sertoli cell — tall, columnar, non-dividing, base on the basement membrane, apex reaching the lumen; each supports 30–50 germ cells	Granulosa cell — cuboidal to columnar, proliferates as the follicle grows, surrounds the oocyte
Barrier formed	Tight junctions between adjacent Sertoli cells create the blood–testis barrier , dividing the tubule into basal and adluminal compartments	Basal lamina around the follicle forms the blood–follicle barrier ; granulosa layer is avascular until luteinisation
Key secretions	Androgen-binding protein (concentrates testosterone), inhibin (suppresses FSH), fetal Müllerian-inhibiting substance	Estrogen (by aromatisation of thecal androgen), follicular fluid, inhibin
Steroidogenic stromal cell	Interstitial (Leydig) cell — large, polygonal, eosinophilic, mitochondria- and SER-rich, lying singly/in groups in the sparse interstitial connective tissue	Theca interna cell — vacuolated, lipid-droplet-rich, forming a vascular layer just outside the granulosa/basal lamina
Steroid produced	Testosterone, driven by pituitary ICSH (LH)	Androstenedione (LH-driven) → diffuses across the basal lamina → aromatised to estradiol by granulosa cells (FSH-dependent)
Embryological homology	Sertoli cells and granulosa cells are homologous — both are supporting-cell derivatives of the sex cords; Leydig cells and theca interna cells are homologous — both derive from gonadal stroma (Larsen 6e)	—

5. Post-gametogenic and duct structures

- **Testis** possesses a built-in intragonadal excurrent duct system: seminiferous tubule → short **straight tubules** (lined initially only by Sertoli cells) → **rete testis** (an anastomosing labyrinth of cuboidal-lined channels within the mediastinum) → 10–20 **efferent ductules** (alternating ciliated and non-ciliated cuboidal cells giving a scalloped lumen) → epididymis. The gamete is carried out

of the organ inside a continuous tubular system, and the tubule wall is reused for each successive spermatogenic wave.

- **Ovary has no intragonadal duct.** At ovulation the oocyte–corona radiata complex ruptures through the surface at the ischaemic **stigma** and is swept directly into the fimbriated infundibulum of the uterine tube. The emptied follicle wall itself transforms *in situ* into the **corpus luteum** — granulosa lutein cells ($\approx 80\%$ of its mass, centrally placed, secreting progesterone) plus theca lutein cells (peripheral, secreting estrogen). Without pregnancy this regresses after 10–12 days into a fibrous scar, the **corpus albicans**; with pregnancy, hCG sustains it as the corpus luteum of pregnancy for 4–5 months. The testis has no equivalent post-gametogenic transformation of its parenchyma.

6. Hormonal regulation

Feature	Testis	Ovary
Trophic hormone → target	FSH → Sertoli cells; LH (ICSH) → Leydig cells	FSH → granulosa cells (aromatase activity); LH → theca interna (pre-ovulation) and corpus luteum (post-ovulation)
Feedback hormone	Inhibin (Sertoli) suppresses FSH	Inhibin (granulosa/luteal) suppresses FSH; estrogen and progesterone feed back on the hypothalamic–pituitary axis
Secretory pattern	Tonic, non-cyclical	Strongly cyclical, driving the ~28-day ovarian and endometrial cycles

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Testis — seminiferous tubule and interstitium

- Fig 21.9 — Histology of testis (low magnification on left) and seminiferous tubule (high magnification on right)
(Sontakke — Textbook of Human Histology — 2e, p.290)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a cross-section of one seminiferous tubule with surrounding interstitium and label:

- ▶ Tunica propria (with flattened myoid cells)
- ▶ Basement membrane
- ▶ Sertoli cell (basal nucleus, cytoplasm extending to the lumen)
- ▶ Spermatogonia (basal compartment, against basement membrane)
- ▶ Primary spermatocyte (largest cell, mid-epithelium)
- ▶ Secondary spermatocyte
- ▶ Early and late spermatids (near lumen)
- ▶ Lumen (with released spermatozoa)
- ▶ Interstitial (Leydig) cells with adjacent capillary, in the surrounding connective tissue

Ovary — follicular stages

 Fig 31.5 — Histology of ovary (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.476)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a sectioned ovary showing cortex and medulla with sequential follicular stages, and label:

- ▶ Surface (germinal) epithelium and tunica albuginea
- ▶ Cortex (cellular stroma) and medulla (vessels, loose connective tissue), hilum
- ▶ Primordial follicle (oocyte + single squamous follicular-cell layer)
- ▶ Primary follicle — unilaminar then multilaminar (granulosa cells, zona pellucida)
- ▶ Secondary (antral) follicle — antrum, cumulus oophorus, corona radiata, theca interna, theca externa
- ▶ Graafian (mature) follicle
- ▶ Corpus luteum (granulosa lutein cells centrally, theca lutein cells peripherally)
- ▶ Corpus albicans

Applied / Clinical anatomy

- ▶ **Cryptorchidism** — failure of testicular descent, ~4% of male neonates; most descend within the first year; uncorrected bilateral cryptorchidism by 2–3 years causes infertility and raises germ-cell tumour risk.
- ▶ **Hydrocele** — accumulation of serous fluid within the tunica vaginalis; the commonest cause of scrotal swelling.
- ▶ **Varicocele** — dilatation of the pampiniform plexus, impairing the countercurrent testicular cooling mechanism; a recognised cause of subfertility.
- ▶ Germ-cell tumours account for ~95% of testicular cancers and occur mainly after puberty; Sertoli-cell and Leydig-cell (sex-cord–stromal) tumours are rare.

- ▶ **Vasectomy** — surgical division of the ductus deferens for contraception; sperm continue to be produced but are phagocytosed within the epididymis.
- ▶ **Polycystic ovary syndrome (PCOS)** — multiple cystic follicles with anovulation, a common cause of female infertility.
- ▶ **Follicular cysts** — persistent, unruptured antral/mature follicles that can secrete excess estrogen and cause menstrual irregularity.
- ▶ Ovarian reserve falls below ~1000 follicles by the fourth decade of life, heralding the approach of menopause.
- ▶ Ovarian germ-cell and sex-cord–stromal tumours (teratoma, dysgerminoma, granulosa-cell tumour, thecoma) form a histogenetic spectrum analogous to testicular tumours.

High-yield exam pointers

- ▶ Both gonads share the same template — capsule (tunica albuginea) + germ-cell compartment + steroidogenic stromal cell — from a common indifferent gonad.
- ▶ Seminiferous tubule = tubular, patent, lifelong-reused structure; ovarian follicle = solid-to-antral, one-time-use structure.
- ▶ **Sertoli cell** ≈ **granulosa cell** (supporting/nurse cells); **Leydig cell** ≈ **theca interna cell** (steroidogenic stromal cells) — embryologically homologous pairs.
- ▶ Blood–testis barrier (Sertoli tight junctions) vs blood–follicle barrier (basal lamina).
- ▶ Spermatogenesis: continuous, equal divisions, self-renewing stem-cell pool. Oogenesis: cyclical, unequal divisions, fixed non-renewable pool established prenatally.
- ▶ Testis has a built-in excurrent duct system (straight tubules, rete testis, efferent ductules); the ovary has none — the oocyte is expelled directly onto the surface.
- ▶ Corpus luteum is a unique post-ovulatory transformation of the follicle wall *in situ* — the testis has no equivalent.
- ▶ Hormones: testosterone (Leydig cell, tonic secretion) vs estrogen (granulosa cell)/progesterone (corpus luteum), both cyclical.
- ▶ Clinical correlates: cryptorchidism/varicocele/germ-cell tumours (testis) vs PCOS/follicular cysts/ovarian tumours (ovary).

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas, 17e (primary source for both testicular and ovarian histology); Larsen's Human Embryology, 6e (for the Sertoli–granulosa / Leydig–theca cell homology); BD Chaurasia's Human Anatomy Vol 2, 8e and Sontakke's Textbook of Human Histology, 2e (figures).



Describe in detail the histological details in relation to different phases and ages in ovary

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction — The ovary is the paired female gonad performing gametogenesis (oogenesis) and steroidogenesis (estrogen and progesterone). Almond-shaped, about 3 cm long x 1.5 cm wide x 1 cm thick, it lies on the posterior layer of the broad ligament. Histologically it consists of a germinal-epithelium-covered cortex containing ovarian follicles in every stage of maturation, and an inner vascular medulla — but its microscopic picture is not static: it changes profoundly with **age** (fetal life -> birth -> childhood -> puberty/reproductive life -> menopause) and, within reproductive life, cycles through the **phases** of folliculogenesis, ovulation, and corpus luteum formation/regression every month.

1. General histological organisation of the ovary

- ▶ **Germinal epithelium** — single layer of cuboidal (occasionally flattened) mesothelial cells covering the surface; continuous with the peritoneal mesothelium over the mesovarium. It is a **misnomer** — it does NOT give rise to female gametes; primordial germ cells are of extragonadal (yolk sac) origin.
- ▶ **Tunica albuginea** — dense irregular collagenous connective-tissue capsule deep to the germinal epithelium; thinner and less dense than in the testis.
- ▶ **Cortex** — peripheral, highly cellular connective-tissue stroma (reticular fibres + spindle-shaped stromal/interstitial cells) housing ovarian follicles at all stages, corpora lutea, corpora albicantia, and atretic follicles.
- ▶ **Medulla** — central, loose connective tissue with large tortuous blood vessels, lymphatics and nerves entering at the **hilum**; hilum also contains **hilus cells** (androgen-secreting, resemble testicular Leydig cells, contain Reinke crystalloids) and mesonephric duct remnants. No sharp cortico-medullary boundary exists.

2. Histology of the ovary in relation to different AGES

Age / life period	Histological picture
Fetal life	Primordial germ cells migrate from yolk sac endoderm to the genital ridge (~4th–6th week); differentiate into oogonia , which multiply mitotically, peaking at about 7 million by the 5th gestational month. Most then enter meiotic prophase I, become primary oocytes , and each is enveloped by a single layer of flat follicular cells to form a primordial follicle ; the rest degenerate by atresia.
At birth	Cortex is packed with numerous dormant primordial follicles — about 6–8 lakh (600,000–800,000) primary oocytes , each arrested in the dictyotene (diplotene) stage of meiotic prophase I under oocyte-maturation-inhibitor (OMI). Ovarian surface is smooth; no growing or antral follicles are seen.

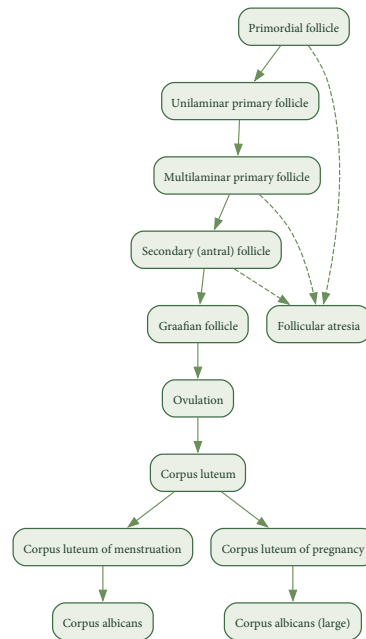
Age / life period	Histological picture
Childhood (infancy to prepuberty)	Follicles remain in a dormant/quiescent phase; no cyclical growth occurs. Ovarian surface stays smooth. Continued background atresia further reduces the oocyte pool, so that only about 4 lakh (400,000) primordial follicles remain by puberty.
Puberty and reproductive life (menarche 9–14 yrs onward)	With cyclical FSH/LH secretion, cohorts of primordial follicles are recruited each month; the cortex now shows follicles at every stage simultaneously — primary, secondary/antral, and Graafian follicles, together with corpora lutea, corpora albicantia, and atretic follicles. Repeated ovulation progressively scars the surface, which becomes irregular. Over ~30–35 reproductive years only about 400 oocytes are actually ovulated.
Menopause / postmenopausal ovary (climacterium, average age 45–55 yrs, mean ~51 yrs)	Ovarian reserve falls below ~1000 follicles; growing/antral follicles and corpora lutea disappear. Cortex becomes fibrotic and atrophic , containing only scattered corpora albicantia and interstitial/hilar cells (still androgen-secreting). Germinal epithelium flattens; the postmenopausal ovary shrinks to roughly one-fourth the size of the reproductive-period ovary.

3. Ovarian follicles — the different PHASES of folliculogenesis

Every follicle passes through primordial → primary (unilaminar → multilaminar) → secondary (antral) → Graafian stages; a mature Graafian follicle takes about 90 days to develop from a recruited primordial follicle.

Stage	Oocyte	Follicular / granulosa cells	Other features
Primordial follicle	Primary oocyte, ~25–30 µm, nucleus arrested in prophase I	Single layer of flat/squamous follicular cells	Lies just deep to tunica albuginea; present from fetal life; dormant till puberty
Unilaminar primary follicle	Oocyte enlarging	Single layer of cuboidal follicular cells	Earliest zona pellucida material begins to appear
Multilaminar primary follicle	Oocyte grows toward ~120–125 µm	Stratified granulosa (several cell layers), avascular, rests on a basal lamina	Well-formed zona pellucida (PAS-positive glycoprotein layer, ZP1–ZP3); surrounding stroma differentiates into theca interna (vascular, cuboidal, LH-receptor bearing) and theca externa (fibrous, smooth muscle)

Stage	Oocyte	Follicular / granulosa cells	Other features
Secondary (antral) follicle	Oocyte reaches maximum size (~125 µm), further growth arrested by oocyte-maturation inhibitor	Granulosa cells proliferate; small fluid-filled spaces (liquor folliculi) coalesce into a single antrum ; some granulosa cells form the cumulus oophorus	Theca interna secretes androgens (LH-driven); granulosa cells aromatise these to estrogen; Call-Exner bodies (PAS-positive, hyaluronic acid + proteoglycan) seen among granulosa cells
Graafian (mature) follicle	Secondary oocyte + first polar body (meiosis I completed just before ovulation)	Large antrum (up to ~2 cm); cumulus oophorus projects into antrum; innermost adherent granulosa layer around the oocyte forms the corona radiata	Follicle bulges the ovarian surface; an ischaemic stigma/macula pellucida appears at the point of rupture



Describe in detail the histological details in relation to different phases and ages in ovary

Fig 21.2 — Histological structure of ovary showing follicles at various stages of development (IB Singh — Textbook of Human Histology — 9e, p.582)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- ▶ (a) **Panoramic section of ovary** — label: Germinal epithelium, Tunica albuginea, Cortex, Medulla, Mesovarium/hilum, Primordial follicles (peripheral cortex), a Maturing (secondary) follicle, a Mature (Graafian) follicle, an early Corpus luteum, a Mature corpus luteum, a Corpus albicans, an Ovum near the surface.
- ▶ (b) **Wall of a Graafian follicle (high power)** — label: Oocyte, Zona pellucida, Corona radiata, Cumulus oophorus, Membrana granulosa, Antrum (liquor folliculi), Basement membrane, Theca interna, Theca externa.

4. Ovulation

- ▶ Occurs around day 14 of a 28-day cycle, triggered by the mid-cycle **LH surge**.
- ▶ The primary oocyte completes the **first meiotic division** -> secondary oocyte + first polar body.
- ▶ Follicular fluid, proteases and prostaglandins weaken the wall at the **stigma**; theca externa smooth-muscle contraction and rising intrafollicular pressure rupture the follicle.
- ▶ The secondary oocyte, corona radiata and follicular fluid are expelled; the oocyte is swept into the uterine tube. If unfertilised it degenerates within 24–48 hours; second meiotic division is completed only on fertilisation.

5. Corpus luteum and its fate

- ▶ Immediately after ovulation the collapsed, folded follicular wall fills with blood — **corpus hemorrhagicum**.
- ▶ Under LH influence, granulosa and theca interna cells hypertrophy and lutenise:
 - **Granulosa-lutein cells** — large, pale, centrally placed, ~80% of the mass; secrete **progesterone** (and convert androgens to estrogen).
 - **Theca-lutein cells** — smaller, darker-staining, peripherally placed, ~20% of the mass; secrete **androgens/estrogen**.
- ▶ **Corpus luteum of menstruation** — if no fertilisation, persists ~14 days, then regresses (invaded by fibroblasts) to form the **corpus albicans** (fibrous, collagenous scar).
- ▶ **Corpus luteum of pregnancy** — maintained and enlarged by hCG from the trophoblast, persists 4–5 months (until the placenta takes over progesterone secretion), then likewise regresses to a (larger) corpus albicans.

6. Follicular atresia and interstitial tissue

- ▶ Most follicles (at any stage — primordial to antral) never ovulate; they undergo **atresia**: apoptosis of granulosa cells, autolysis of the oocyte, phagocytic removal by macrophages, and thickening/hyalinisation of the follicular basement membrane into a wavy **glassy membrane** (a histological hallmark of an atretic follicle).
- ▶ Persisting theca interna cells of atretic follicles form cords — **interstitial glands (corpora atretica)** — which continue to secrete estrogens/androgens.
- ▶ **Hilar cells**, at the ovarian hilum, resemble testicular Leydig cells, contain **Reinke crystalloids**, and secrete androgens.

Applied / Clinical anatomy

- ▶ **Polycystic ovarian disease (Stein–Leventhal syndrome)** — multiple cystic anovulatory antral follicles with anovulation and oligo/amenorrhoea.
- ▶ **Follicular / corpus luteum cysts** — persistence of an unruptured antral follicle or corpus luteum; may cause menstrual irregularity or, if it bleeds, a haemorrhagic cyst.
- ▶ **Mittelschmerz** — mid-cycle ovulation pain (~45% of women), from stretching/rupture of the follicle wall at ovulation.
- ▶ Surface-epithelial (germinal-epithelial) tumours account for the great majority (~70%) of ovarian neoplasms, attributed to repeated disruption-and-repair of the germinal epithelium at each ovulation.
- ▶ **Urine pregnancy test** — based on detecting hCG, which is secreted by the trophoblast to rescue and enlarge the corpus luteum of pregnancy.
- ▶ **Premature ovarian failure / early menopause** — accelerated depletion of the primordial-follicle pool below the ~1000-follicle threshold at an earlier-than-average age.

High-yield exam pointers

- ▶ Germinal epithelium is a **misnomer**; ova arise from yolk-sac-derived primordial germ cells, not from this epithelium.
- ▶ Numbers to remember: ~7 million oogonia (5th gestational month) -> 6–8 lakh primordial follicles at birth -> ~4 lakh at puberty -> only ~400 ovulate in a reproductive lifetime.
- ▶ All primary oocytes are formed **before birth**; none are added postnatally.
- ▶ Sequence: Primordial -> Primary (unilaminar -> multilaminar) -> Secondary/antral -> Graafian -> Ovulation -> Corpus luteum -> Corpus albicans.
- ▶ In reproductive life, the ovarian cortex simultaneously shows follicles at **every stage** plus corpora lutea, corpora albicantia and atretic follicles — a key identification point in practicals.
- ▶ Two-cell mechanism: theca interna (LH-driven) makes androgens -> granulosa cells (FSH/aromatase-driven) convert these to estrogen.
- ▶ Corpus luteum: granulosa-lutein cells (central, 80%, progesterone) vs theca-lutein cells (peripheral, 20%, androgen/estrogen).
- ▶ Menarche 9–14 years; menopause (climacterium) 45–55 years (mean ~51 years); postmenopausal ovary is fibrotic and about one-fourth reproductive-period size.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e.



Describe the histology of the pituitary gland

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction — The pituitary gland (hypophysis cerebri) is a pea-sized endocrine gland, about $10 \times 13 \times 6$ mm, lying in the hypophyseal fossa of the sella turcica and suspended from the floor of the third ventricle by the infundibulum. It is called the "master endocrine gland" because its secretions regulate the activity of most other endocrine organs. Because of its dual embryological origin, the gland is histologically two glands fused into one capsule — a glandular **adenohypophysis** (anterior lobe) and a neural **neurohypophysis** (posterior lobe) — and its microanatomy is best understood lobe by lobe.

1. Development and General Subdivisions

- ▶ **Adenohypophysis** develops from an ectodermal evagination of the roof of the primitive oral cavity (stomodeum) called **Rathke's pouch**; its anterior wall thickens to form the bulk of the anterior lobe.
- ▶ **Neurohypophysis** develops as a downward neuroectodermal growth from the floor of the diencephalon (the infundibulum/neurohypophyseal bud), which is why it retains the histological character of nervous tissue.
- ▶ The two primordia meet and fuse in the fetal period; the residual lumen of Rathke's pouch persists as small colloid-filled cysts in the **pars intermedia**.
- ▶ Functional/structural subdivisions:
 - **Adenohypophysis** — pars distalis (pars anterior), pars intermedia, pars tuberalis.
 - **Neurohypophysis** — pars nervosa (pars posterior) and the infundibular stem/stalk.

2. Adenohypophysis — Pars Distalis

- ▶ Forms about 75% of the adenohypophysis and is enclosed by a thin fibrous capsule.
- ▶ Parenchyma is arranged as **cords/clumps of secretory epithelial cells** separated by an extensive network of **fenestrated sinusoidal capillaries** (the secondary capillary plexus of the hypophyseal portal system) supported by a fine reticular stroma.
- ▶ **Routine H&E staining** divides cells into two broad groups by their granule staining affinity:
 - **Chromophils** — brightly-granulated cells, further split into
 - **Acidophils** (~40%) — granules stain with acid dyes (eosin) → bright pink/orange (Gomori trichrome: orange).
 - **Basophils** (~10%) — granules stain with basic dyes (haematoxylin) and are PAS-positive → blue/violet.
 - **Chromophobes** (~50%) — poorly-stained, agranular or degranulated cells; a heterogeneous group including resting/degranulated chromophils and stem-like cells.
- ▶ **Immunohistochemical/functional reclassification** of chromophils (identifies the actual hormone-secreting cell, now the standard nomenclature):

Cell type	Staining group	Approx. % of pars distalis	Hormone secreted
Somatotrophs	Acidophil	~50% (commonest cell)	Growth hormone (GH/somatotropin)

Cell type	Staining group	Approx. % of pars distalis	Hormone secreted
Lactotrophs (mammotrophs)	Acidophil	~15–20% (hypertrophy in pregnancy/lactation)	Prolactin (PRL)
Corticotrophs	Basophil, strongly PAS+	~15–20%	ACTH, via cleavage of pro-opiomelanocortin (POMC) into ACTH, β -lipotropin, MSH, β -endorphin
Gonadotrophs (delta basophils)	Basophil	~10%	LH and FSH (same cell secretes both)
Thyrotrophs	Basophil (least abundant)	~5%	TSH

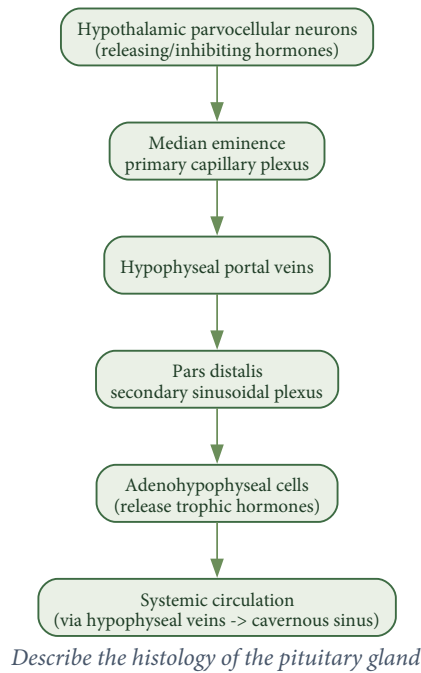
- ▶ Ultrastructurally, each chromophil is a typical polypeptide-secreting cell — abundant rough endoplasmic reticulum, prominent Golgi, and membrane-bound secretory granules whose size and electron density are cell-type specific.

3. Pars Intermedia and Pars Tuberalis

- ▶ **Pars intermedia** lies between pars distalis and pars nervosa; it is vestigial/poorly developed in humans.
- ▶ Composed of **basophils**, **chromophobes**, and small **colloid-filled follicles/vesicles** representing the residual lumen of Rathke's pouch.
- ▶ Its corticotroph-lineage cells process POMC preferentially to **melanocyte-stimulating hormone (MSH)**, which stimulates melanocyte pigment production; overall functional importance in the human adult is uncertain.
- ▶ **Pars tuberalis** is a thin, highly vascular collar of glandular tissue wrapping around the infundibular stalk.
- ▶ Contains small clusters/cords of cells (predominantly **gonadotrophs**, with some acidophils) and carries the **primary capillary plexus** of the hypophyseal portal system.

4. Hypothalamo-Hypophyseal Portal System (functional histology of the adenohypophysis)

- ▶ The adenohypophysis has **no direct secretomotor nerve supply**; it is controlled by hypothalamic releasing/inhibiting hormones delivered through a portal blood system.
- ▶ Superior hypophyseal arteries (from the internal carotid) form a **primary capillary plexus** in the median eminence/infundibular stem → drain as **hypophyseal portal veins** → break up into a **secondary sinusoidal capillary plexus** amid the cords of pars distalis.
- ▶ This portal circulation carries hypothalamic hormones (releasing/inhibiting hormones) directly to the adenohypophyseal cells and carries adenohypophyseal hormones into the systemic circulation via hypophyseal veins draining to the cavernous sinus.
- ▶ Inferior hypophyseal arteries chiefly supply the neurohypophysis.



5. Neurohypophysis (Pars Nervosa and Infundibulum)

- ▶ Histologically resembles **CNS/neural tissue**, not a true gland — it stores and releases, but does not synthesise, its hormones.
- ▶ Composed of about a lakh (10^5) of **non-myelinated axons** of neurosecretory neurons whose cell bodies lie in the **supraoptic and paraventricular nuclei** of the hypothalamus (the hypothalamo-hypophyseal tract); these axons do not synapse locally.
- ▶ **Pituicytes** — specialised, elongated glial cells (astrocyte-like) that ensheath the axons and their terminals; on H&E their cytoplasm blends with the surrounding unmyelinated fibres and is not sharply demarcated.
- ▶ **Herring bodies** — dilated axon terminals packed with neurosecretory granules; appear as eosinophilic (pink), irregular, granular swellings on H&E (dark on aniline blue) closely apposed to fenestrated capillaries.
- ▶ Axon terminals release their neurosecretory vesicles directly into an adjacent **fenestrated capillary bed** for uptake into the systemic circulation.
- ▶ **Hormones stored/released:** oxytocin (paraventricular nucleus predominant) and vasopressin/antidiuretic hormone, ADH (supraoptic nucleus predominant) — both nonapeptides synthesised in the hypothalamic perikarya and transported down the axons bound to their carrier protein, neurophysin.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 25.4 — Histology of pituitary gland (low magnification on left, high magnification on right, practice figure)
(Sontakke — Textbook of Human Histology — 2e, p.333)

Hand-drawn labelled diagram should carry these exact labels (low-power whole-gland sketch + high-power inset of pars distalis):

- ▶ Low power (whole gland): Capsule, Infundibulum, Pars tuberalis, Pars distalis, Pars intermedia, Neurohypophysis (pars nervosa), Herring bodies.
- ▶ High power (pars distalis inset): Sinusoidal capillaries, Acidophils (alpha cells), Basophils (beta cells), Chromophobes, Chromophils (bracket over acidophils + basophils), Colloid-filled follicle (pars intermedia), Pituicytes and Herring bodies (neurohypophysis side).

Applied / Clinical anatomy

- ▶ **Pituitary adenoma:** benign monoclonal proliferation of a single adenohypophyseal cell type (commonly somatotroph or lactotroph); large tumours cause **bitemporal hemianopia** by compressing the optic chiasma, which lies just above the diaphragma sellae covering the gland.
- ▶ **Somatotroph excess** — gigantism if before epiphyseal fusion; **acromegaly** (coarse facial features, prognathism, enlarged hands/feet) if after fusion.
- ▶ **Corticotroph excess** — Cushing's disease; **prolactinoma** — the commonest functioning pituitary adenoma, causing galactorrhoea/amenorrhoea by lactotroph hyperplasia (physiological correlate: lactotroph hypertrophy of pregnancy enlarges the gland).
- ▶ **Neurohypophyseal deficiency** — diabetes insipidus from reduced ADH secretion/action, causing polyuria and polydipsia.
- ▶ **Sheehan syndrome** — post-partum pituitary (mainly adenohypophyseal) infarction/necrosis following severe obstetric haemorrhage, exploiting the gland's dependence on the low-pressure portal circulation.
- ▶ **Rathke's pouch remnants** in the pars intermedia are the developmental basis of **craniopharyngioma**.
- ▶ **Surgical relevance:** the gland's relation to the sella turcica/diaphragma sellae, optic chiasma, and cavernous sinus (with the internal carotid artery and cranial nerves III, IV, VI, Va, Vb) is exploited in the **trans-sphenoidal approach** to pituitary surgery.

High-yield exam pointers

- ▶ Dual embryological origin: adenohypophysis from Rathke's pouch (oral ectoderm); neurohypophysis from neuroectoderm of the diencephalic floor.
- ▶ Adenohypophysis = cords of secretory cells + sinusoidal capillaries; Neurohypophysis = unmyelinated axons + pituicytes, no local hormone synthesis.
- ▶ Pars distalis cells: Acidophils (somatotrophs 50%, lactotrophs 15–20%) and Basophils (corticotrophs 15–20%, gonadotrophs 10%, thyrotrophs 5%); chromophobes ~50%, unstained/degranulated.
- ▶ Corticotrophs secrete POMC, the common precursor of ACTH, β -lipotropin, and MSH.

- ▶ Pars intermedia = colloid cysts (Rathke's pouch remnant) + basophils secreting MSH; poorly developed in adult humans.
- ▶ Pars tuberalis = vascular collar around the infundibulum, mainly gonadotrophs, site of the primary portal capillary plexus.
- ▶ Hypophyseal portal system (superior hypophyseal artery → primary plexus → portal veins → secondary plexus in pars distalis) is the sole route of hypothalamic control of the adenohypophysis.
- ▶ Neurohypophysis stores oxytocin and ADH synthesised in paraventricular/supraoptic nuclei; Herring bodies = dilated axon terminals with neurosecretory granules; pituicytes = supporting glial cells.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology 2e; Junqueira's Basic Histology — Text & Atlas 17e; IB Singh — Textbook of Human Histology 9e; Gray's Anatomy (Standring) 41e.

Coverage note: Gray's Anatomy (Standring 41e) covers the gross/topographic relations and hypothalamic control of the pituitary in the diencephalon chapter but does not detail anterior/posterior lobe cell histology; the cellular histology (cell types, staining, Herring bodies, pituicytes) is drawn from the dedicated histology texts (Sontakke, Junqueira, IB Singh), which is standard for this question.



Describe the micro-anatomy of ovary. Add a note on its age changes

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — The ovary is the paired female gonad, an almond-shaped organ about 3 cm long, 1.5 cm wide and 1 cm thick, lying on the posterior leaf of the broad ligament, attached to it by the mesovarium, to the pelvic wall by the suspensory (infundibulopelvic) ligament carrying the ovarian vessels, and to the uterus by the ligament of the ovary. It performs a dual function — **gametogenesis** (production and periodic release of oocytes) and **steroidogenesis** (estrogen and progesterone secretion) — and, unlike most glands, its histological picture changes continuously through life. Structurally it is organised into an outer **cortex** and inner **medulla**, without a sharp boundary between them.

General organisation

- ▶ **Surface (germinal) epithelium** — a single layer of simple cuboidal (mesothelial) cells covering the ovary, continuous with the peritoneal mesothelium; it is a *misnomer* since it does not give rise to the female gametes (oogonia arise from primordial germ cells that migrate in from the yolk sac in the first month of embryonic life).
- ▶ **Tunica albuginea** — a dense, irregular collagenous connective-tissue capsule immediately deep to the surface epithelium; thinner and less dense than the tunica albuginea of the testis.

- **Cortex** — the peripheral, highly cellular connective-tissue stroma (reticular fibres + fusiform stromal/interstitial cells resembling mesenchymal cells) containing ovarian follicles at all stages of development, corpora lutea, corpora albicantia and atretic follicles.
- **Medulla** — the central zone of loose connective tissue, large tortuous blood vessels, lymphatics, smooth muscle and nerves; continuous at the **hilum** with the mesovarium, where **hilar cells** (resembling testicular Leydig cells, containing Reinke crystalloids) and remnants of the mesonephric duct are found.
- Before puberty the ovarian surface is smooth; with repeated ovulation through reproductive life it becomes progressively scarred and irregular.

Ovarian follicles — stages of development

An ovarian follicle is an oocyte surrounded by one or more layers of follicular/granulosa cells within a basal lamina.

Stage	Oocyte	Follicular/ granulosa cells	Zona pellucida	Antrum	Theca
Primordial follicle	Primary oocyte, ~25–30 µm, arrested in meiotic prophase I (dictyotene)	Single layer of flat (squamous) cells	Absent	Absent	Absent
Unilaminar primary follicle	Enlarging (~120 µm)	Single layer of cuboidal cells	Beginning to appear	Absent	Absent
Multilaminar primary follicle	Large, growing	Stratified cuboidal layer = granulosa	Well formed (5–10 µm; glycoproteins ZP1, ZP2, ZP3)	Absent	Differentiating into theca interna/externa
Secondary (antral) follicle	~125 µm	Granulosa with fluid-filled spaces coalescing into an antrum (liquor folliculi)	Present	Present, enlarging	Theca interna (vascular, steroidogenic) + theca externa (fibromuscular)
Graafian (mature) follicle	Bulges at ovarian surface	Thinned granulosa; cumulus oophorus and corona radiata around oocyte	Present	Single large antrum, up to ~2 cm follicle diameter	Well developed theca interna/externa

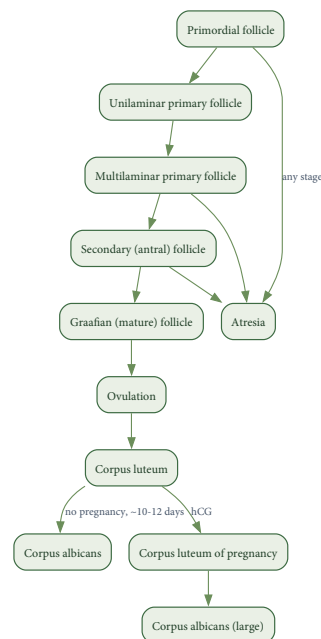
Key points on maturation:

- ▶ FSH drives recruitment of a cohort of primordial follicles each month; complex hormonal/receptor differences select the single **dominant follicle**.
- ▶ Theca interna cells secrete androstenedione (LH-driven); granulosa cells aromatise it to estradiol (FSH-dependent) — the classic **two-cell, two-gonadotropin** mechanism.
- ▶ A mature follicle develops from a primordial follicle over about 90 days.
- ▶ **Call-Exner bodies** (PAS-positive, hyaluronic acid/proteoglycan-rich extracellular bodies) appear among granulosa cells of the growing antral follicle.

□ Fig 31.5 — Histology of ovary (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.476)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a circular low-power ovary section and label: **surface epithelium** → **tunica albuginea** → **cortex** (containing, from superficial to deep: **primordial follicles**, **primary follicle**, **growing/secondary follicle** with antrum, a **corpus luteum**) → **medulla** with **blood vessels/capillaries**. Add a bracket marking "Cortex" along the periphery.



Describe the micro-anatomy of ovary. Add a note on its age changes

Follicular atresia

- ▶ Most follicles, at any stage (including late antral), undergo **atresia** rather than ovulation — apoptosis of granulosa cells, autolysis of the oocyte, and collapse/hyalinisation of the zona pellucida into a wavy glassy membrane.
- ▶ Macrophages invade to remove apoptotic debris; hypertrophied theca interna cells may persist as scattered cords contributing to the **interstitial gland of the ovary** (an estrogen-secreting remnant).
- ▶ Menopause supervenes once atresia and ovulation together have reduced the follicular reserve to fewer than about 1000 follicles.

- ▶ Although atretic oocytes never complete meiosis, developing follicles destined for atresia still secrete estrogen before degenerating.

Ovulation

- ▶ Occurs mid-cycle (~day 14 of a 28-day cycle) under the LH surge.
- ▶ The primary oocyte completes meiosis I just before ovulation, forming a **secondary oocyte** and the first polar body; the secondary oocyte arrests again at metaphase II.
- ▶ The dominant follicle bulges the tunica albuginea and germinal epithelium; local ischaemia produces the **stigma (macula pellucida)**, at which plasmin-mediated collagenolysis and prostaglandin-driven theca externa contraction rupture the follicle, expelling the oocyte with its corona radiata into the peritoneal cavity (to be caught by the fimbriae).

Corpus luteum and corpus albicans

- ▶ The collapsed follicle wall is invaded by capillaries; granulosa and theca interna cells hypertrophy (without further division) under LH to form the **corpus luteum**.
- ▶ **Granulosa lutein cells** — large, centrally placed, ~80% of the gland, secrete progesterone (and, via aromatase, some estrogen).
- ▶ **Theca lutein cells** — smaller, peripheral, in the folds, secrete androstenedione/estrogens.
- ▶ Without pregnancy the **corpus luteum of menstruation** secretes progesterone for only 10–12 days, then undergoes apoptosis and is replaced by fibrous scar tissue, the **corpus albicans**, which slowly shrinks and is lost in the stroma.
- ▶ If implantation occurs, trophoblastic **hCG** rescues and enlarges the structure as the **corpus luteum of pregnancy**, sustaining progesterone secretion for 4–5 months until the placenta takes over; it then also regresses to a (larger) corpus albicans.

Age changes in the ovary

Life stage	Ovarian histology
Fetal life	Primordial germ cells migrate from the yolk sac (1st month) and proliferate as oogonia; germ-cell number peaks at ~7 million around mid-gestation (5th month), after which mitosis ceases and surviving cells enter meiotic prophase I as primary oocytes , each enclosed in a primordial follicle.
At birth	About 2 million primordial follicles are present in the cortex (the "ovarian reserve"); the ovary is smooth-surfaced.
Childhood	Follicles remain dormant (arrested at the dictyotene stage); no follicular growth beyond the primordial stage.
Puberty (menarche, mean ~12–13 yr, range 9–14 yr)	~400,000 primordial follicles remain; cyclic FSH-driven recruitment and ovulation begin; interstitial cells (numerous in the first year of life and early puberty) involute at menarche.

Life stage	Ovarian histology
Reproductive/childbearing years	Monthly cohorts of follicles grow, one ovulates and forms a corpus luteum, the rest undergo atresia; the cortex shows follicles, corpora lutea, corpora albicantia and atretic follicles side by side; the surface becomes progressively scarred by repeated ovulation; only ~400–450 oocytes are ovulated in a lifetime.
Perimenopause/climacteric (mean onset ~51 yr, range 45–55 yr)	Follicular reserve falls below ~1000; cycles become irregular as atresia and ovulation decline.
Postmenopause/senile ovary	Ovulation and atresia cease; the ovary shrinks to about one-quarter of its reproductive-age size; the cortex becomes fibrotic , containing mainly stromal/interstitial cells and residual corpora albicantia, with virtual disappearance of follicles; any oocytes still present degenerate within a few years; hilar cells may respond to the altered hormonal milieu at menopause onset.

Applied / Clinical anatomy

- ▶ **Polycystic ovary syndrome (PCOS)** — enlarged ovaries with numerous unruptured cystic follicles and anovulation; a common cause of infertility.
- ▶ **Follicular cysts** — thin-walled, fluid-filled remnants of late primary/antral follicles; common and usually benign but can secrete excess estrogen causing menstrual irregularity, or become haemorrhagic if a vessel bleeds into the cavity.
- ▶ **Ovarian tumours** are classified by the cell of origin — **surface (coelomic) epithelium** (commonest), **germ cells**, and **sex-cord stroma**; correlating tumour type with the normal three-component histology (epithelium/germ cells/stroma) taught above is a favourite viva link.
- ▶ **Endometriosis** — ectopic endometrial tissue frequently implants on the ovary, forming an endometrioma ("chocolate cyst"); presents with dysmenorrhoea and infertility.
- ▶ **Hilar cell tumours** — arise from the Reinke-crystalloid-containing hilar cells and, by secreting androgens, cause virilisation/masculinisation.
- ▶ **Surgical relation** — the ureter runs immediately behind the ovary, forming the posterior boundary of the ovarian fossa, and is therefore at risk during oophorectomy or hysterectomy.
- ▶ The ovary is a recognised (rare) site for **ectopic implantation** of a fertilised ovum.

High-yield exam pointers

- ▶ Germinal epithelium is a *misnomer* — gametes derive from yolk-sac primordial germ cells, not from this mesothelium.
- ▶ Tunica albuginea of the ovary is thinner/less dense than that of the testis.
- ▶ Zona pellucida glycoproteins **ZP1, ZP2, ZP3** are synthesised by the oocyte and mediate sperm binding.
- ▶ Primary oocyte is arrested at **dictyotene (diplotene of prophase I)** from fetal life until just before ovulation.

- ▶ **Two-cell, two-gonadotropin theory:** LH → theca interna → androstenedione; FSH → granulosa aromatase → estradiol.
- ▶ Granulosa lutein cells (progesterone, ~80% of corpus luteum, central, larger) vs theca lutein cells (estrogen/androstenedione, peripheral, smaller).
- ▶ Corpus luteum of menstruation lasts 10–12 days; corpus luteum of pregnancy is maintained by hCG for 4–5 months.
- ▶ Follicular reserve trajectory: ~7 million (5th fetal month) → ~2 million (birth) → ~400,000 (puberty) → <1000 (menopause) → only ~400–450 oocytes actually ovulated in a lifetime.
- ▶ Postmenopausal cortex = fibrous stroma + corpora albicantia, no active follicles; ovary shrinks to ~¼ its reproductive-age size.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; BD Chaurasia Human Anatomy Vol 2 8e (figure); Gray's Anatomy (Standring) 41e (ovarian fossa/ureter relation).



Describe the microscopic anatomy of pituitary gland. Discuss the special stains used to differentiate the cells

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction The pituitary gland (hypophysis cerebri) is the "master endocrine gland" — a pea-sized structure (~500 mg; ~8 mm anteroposteriorly × 12 mm transversely) lodged in the hypophyseal fossa (sella turcica) of the sphenoid and suspended from the floor of the third ventricle by the infundibulum. It has a dual embryological origin: an ectodermal up-growth from the roof of the stomodeum (Rathke's pouch) forms the **adenohypophysis**, while a neural down-growth from the floor of the diencephalon forms the **neurohypophysis**. This dual origin is the key to its histology — the adenohypophysis is a true cord-and-sinusoid glandular epithelium, whereas the neurohypophysis is modified neuroglial tissue that stores, but does not synthesise, its hormones.

1. General Organisation

- ▶ The gland is invested by a thin fibrous capsule continuous with the dura; the diaphragma sellae covers it above, pierced centrally by the infundibulum.
- ▶ **Adenohypophysis** (from Rathke's pouch) = pars distalis (pars anterior, ~75% of the gland) + pars intermedia + pars tuberalis.
- ▶ **Neurohypophysis** (neural ectoderm) = pars nervosa (posterior lobe) + infundibular stem, continuous above with the median eminence of the tuber cinereum.
- ▶ A residual hypophyseal cleft (vestige of the lumen of Rathke's pouch) separates the pars distalis from the pars intermedia; its persistence as a craniopharyngeal canal, or as colloid-filled cysts, is of applied importance (below).

2. Pars Distalis (Anterior Lobe) — Histology

- ▶ Largest and most vascular part; parenchyma consists of **cords/clusters of polyhedral epithelial cells** separated by a delicate reticular stroma and an extensive network of thin-walled, fenestrated **sinusoidal capillaries** (the secondary plexus of the hypophyseal portal system).
- ▶ On routine H&E, cells are divided by their tinctorial (dye-affinity) reaction into:
 - **Chromophils** — granule-laden, hormone-storing cells, subdivided into **acidophils** (granules take up acid dyes such as eosin/orange G) and **basophils** (granules take up basic dyes such as haematoxylin, and are strongly PAS-positive because their hormones are glycoproteins).
 - **Chromophobes** — poorly granulated, weakly staining cells; represent degranulated chromophils and reserve/progenitor cells.
- ▶ With immunohistochemistry and electron microscopy, five functional cell types are recognised within the chromophil population:

Cell type	Tinctorial class	~% of cells	Hormone	Secretory granule (EM)
Somatotroph	Acidophil	~50%	Growth hormone (GH)	Dense, ~350 nm, closely packed
Lactotroph (mammotroph)	Acidophil	~15–20%	Prolactin (PRL)	Sparse; enlarges (up to 600 nm) with lactation
Corticotroph	Basophil	~15–20%	ACTH (via POMC cleavage)	100–300 nm; lipid droplets, perinuclear filaments
Gonadotroph	Basophil	~10%	FSH and LH	Dense, 200–250 nm; prominent Golgi
Thyrotroph	Basophil	~5% (least numerous)	TSH	Small, sparse granules

- ▶ Somatotrophs are concentrated in the lateral wings of the pars distalis; thyrotrophs in the antero-medial part; corticotrophs postero-medially; gonadotrophs and lactotrophs are scattered throughout.
- ▶ Chromophobes are now understood by immunocytochemistry to be largely **degranulated chromophils** rather than a truly separate resting population.

 Fig 23.12 — The pituitary gland (trichrome-stained). A, The endocrine cells of the adenohypophysis. Chromophils are distinguished as acidophils (yellow) and basophils (pink); chromophobes are pale-staining. A network of sinusoids lies between the cell clusters. (Gray's Anatomy — Standring — 41e, p.502)

Blood supply (integral to pars distalis histophysiology): the pars distalis has **no direct arterial supply**. Superior hypophyseal arteries (from the internal carotid) form a primary capillary plexus in the median eminence/upper infundibulum; this drains via long hypophyseal portal veins into a secondary capillary plexus (sinusoids) among the cell cords of the pars distalis, carrying hypothalamic releasing/inhibiting hormones to the gland — the **hypothalamo-hypophyseal portal system**. Inferior hypophyseal arteries supply mainly the neurohypophysis.

3. Pars Intermedia and Pars Tuberalis

- ▶ **Pars intermedia** — a narrow, rudimentary zone between the pars distalis and pars nervosa; shows **colloid-filled follicles/cysts**, remnants of the lumen of Rathke's pouch, lined by basophils and chromophobes. Its corticotrophs cleave POMC preferentially into γ -LPH, β -endorphin and melanocyte-stimulating hormone (MSH) rather than ACTH; functional significance in the adult human is uncertain.
- ▶ **Pars tuberalis** — a thin, highly vascular funnel-shaped collar wrapping the infundibular stalk; contains the primary capillary plexus of the portal system and cords of predominantly gonadotroph cells with some undifferentiated cells.

4. Neurohypophysis (Pars Nervosa + Infundibulum) — Histology

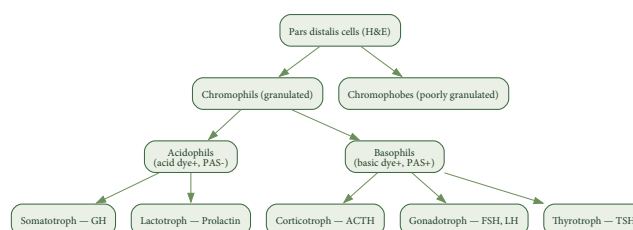
- ▶ Not a secretory gland but modified neural tissue: contains ~100,000 unmyelinated axons of the **magnocellular neurons** of the supraoptic (secreting ADH/vasopressin) and paraventricular (secreting oxytocin) nuclei of the hypothalamus, constituting the **hypothalamo-hypophyseal tract**.
- ▶ **Pituicytes** — branched, astrocyte-like glial cells with long processes running parallel to the axons and ending on perivascular spaces of fenestrated capillaries; they form the bulk of the non-neuronal tissue here.
- ▶ **Herring bodies** — eosinophilic, dilated axonal swellings in which neurosecretory granules (hormone bound to its carrier protein, neurophysin I or II) accumulate before release into the fenestrated capillary bed for uptake into the systemic circulation.

□ Fig 23.12 — B, The neurohypophysis (right) with nerve fibres and pituicytes; to the left, the pars intermedia (PI) with scattered deeper-staining secretory cells and a colloid-filled cyst (top left), a remnant of Rathke's pouch (RP). (Gray's Anatomy — Standring — 41e, p.502)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled sagittal-section diagram of the pituitary gland, with:

- ▶ Diaphragma sellae / sella turcica (orientation only)
- ▶ Infundibulum with the hypothalamo-hypophyseal tract (axons from supraoptic and paraventricular nuclei)
- ▶ **Pars distalis** — cords of acidophils, basophils and chromophobes around sinusoidal capillaries
- ▶ **Pars tuberalis** wrapped around the infundibular stalk
- ▶ **Pars intermedia** with colloid-filled follicles (Rathke's cleft remnant)
- ▶ **Pars nervosa** — unmyelinated axons, pituicytes, Herring bodies
- ▶ Superior and inferior hypophyseal arteries → primary capillary plexus (median eminence) → hypophyseal portal veins → secondary capillary plexus (pars distalis) → hypophyseal veins



Describe the microscopic anatomy of pituitary gland. Discuss the special stains used to differentiate the cells

5. Special Stains Used to Differentiate the Pituitary Cells

Routine H&E only separates cells into acidophils (pink), basophils (blue) and chromophobes (pale) — it cannot subtype them further. Classical histotechnique developed a battery of tinctorial (special) stains, later superseded by immunohistochemistry, to identify the individual hormone-producing cells:

Special stain	Cell(s) identified	Result
Trichrome/polychrome methods (Mallory–Heidenhain, Gomori trichrome; or brilliant crystal scarlet–aniline blue–Martius yellow)	All chromophil types together	Acidophils orange/cherry-red, basophils blue/violet, chromophobes unstained — used for the routine sections illustrated above
Orange G	Somatotrophs, lactotrophs (acidophils)	Orange-staining, PAS-negative cytoplasm
Herlant's erythrosin / Brooke's carmosine	Lactotrophs specifically	Differentiates lactotrophs from somatotrophs within the acidophil population
Lead haematoxylin (Halmi method)	Corticotrophs	Dark, PAS-positive granules
Aldehyde-fuchsin / aldehyde-thionine	Gonadotrophs and thyrotrophs	Deep violet/blue, PAS-positive granules
Periodic acid–Schiff (PAS) reaction	Basophils (glycoprotein-hormone cells) vs acidophils	Periodic acid oxidises the vicinal-glycol (adjacent –OH) groups on hexose sugars to aldehydes, which react with Schiff's (leuco-fuchsin) reagent to give a magenta colour. Corticotrophs, gonadotrophs and thyrotrophs (all glycoprotein-hormone secretors) are PAS-positive ; somatotrophs and lactotrophs (straight-chain protein hormones GH, PRL) are PAS-negative — this single reaction is the most useful practical discriminant between the two chromophil families

Special stain	Cell(s) identified	Result
Immunohistochemistry (IHC)	All five cell types individually	Specific antibodies against GH, PRL, ACTH, FSH/LH, and TSH localise each hormone precisely to its cell of origin; this is the current gold standard and has resolved the "chromophobe" category into mostly degranulated chromophils
Electron microscopy	All five cell types	Distinguishes cells by secretory-granule size, density and shape (see table in Section 2) when combined with immunogold labelling

Applied / Clinical anatomy

- ▶ **Pituitary adenoma** is the commonest pituitary tumour; the classic teaching is that it most often arises from **chromophobe cells** (non-functioning) — though functioning adenomas of any single cell type (commonly a prolactinoma) also occur. Symptoms are of two kinds:
 - **Pressure effects** — enlargement/"ballooning" of the sella turcica on plain lateral skull radiograph; **bitemporal hemianopia** from upward pressure of a suprasellar extension on the optic chiasma; exophthalmos/ophthalmoplegia from lateral extension into the cavernous sinus.
 - **Endocrine effects** — somatotroph adenoma → gigantism (before epiphyseal fusion) or acromegaly (after fusion); corticotroph adenoma → Cushing syndrome; lactotroph adenoma → amenorrhoea–galactorrhoea.
- ▶ **Craniopharyngioma** arises from persisting epithelial remnants of Rathke's pouch (often along a patent craniopharyngeal canal); typically cystic and calcified, and seen chiefly in children.
- ▶ **Pharyngeal hypophysis** — an accessory nest of adenohypophyseal tissue that may persist in the mucoperiosteum of the nasopharyngeal roof, a further remnant of Rathke's pouch migration.
- ▶ **Sheehan syndrome** — post-partum pituitary necrosis following severe obstetric haemorrhage/hypotension, because the anterior lobe depends entirely on the low-pressure hypophyseal portal circulation with no direct arterial supply.
- ▶ **Diabetes insipidus** — deficient ADH from destruction of the neurohypophysis/hypothalamo-hypophyseal tract (surgery, trauma, tumour), producing polyuria with dilute, low-specific-gravity urine.
- ▶ **Trans-sphenoidal (trans-nasal) surgical approach** exploits the gland's close relation to the sphenoidal air sinus for removal of pituitary adenomas, avoiding a transfrontal craniotomy.

High-yield exam pointers

- ▶ Dual embryological origin — Rathke's pouch → adenohypophysis; neural (diencephalic) down-growth → neurohypophysis — explains the entire histological picture.

- ▶ Pars distalis = ~75% of the gland; cords of chromophil (acidophil + basophil) and chromophobe cells around fenestrated sinusoids; **no direct arterial supply** — entirely dependent on the hypophyseal portal system.
- ▶ Five immunohistochemically-defined cell types: somatotroph (~50%), lactotroph (~15–20%), corticotroph (~15–20%), gonadotroph (~10%), thyrotroph (~5%, least numerous).
- ▶ **PAS-positive = glycoprotein hormone (basophils: ACTH, FSH/LH, TSH); PAS-negative = protein hormone (acidophils: GH, PRL)** — the key discriminant to remember.
- ▶ Special stains: Orange G/Herlant's erythrosin for acidophils; lead haematoxylin for corticotrophs; aldehyde-fuchsin/aldehyde-thionine for gonadotrophs and thyrotrophs — now largely superseded by immunohistochemistry.
- ▶ Neurohypophysis is not glandular — it stores (does not make) ADH and oxytocin, transported down axons of the supraoptic and paraventricular nuclei and released from Herring bodies; pituicytes are the supporting glial cells.
- ▶ Pars intermedia is rudimentary in humans, marked by colloid-filled follicles (Rathke's cleft remnants).
- ▶ Chromophobe adenoma is the classic "commonest pituitary tumour" teaching point; bitemporal hemianopia is the classic chiasmal-compression sign.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Vishram Singh Textbook of Anatomy Vol 3 4e.



Describe the microscopic structure of testis. Add a note on blood testis barrier

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction The testis is a paired male gonad that performs two functions — spermatogenesis (in the seminiferous tubules) and steroidogenesis (testosterone, by the interstitial Leydig cells). Each testis is invested by a capsule (tunica vaginalis, tunica albuginea, tunica vasculosa) and internally divided by fibrous septa into lobules, each containing highly coiled seminiferous tubules embedded in a vascular interstitial stroma. The seminiferous epithelium is unique in being renewed throughout reproductive life and in possessing a specialised Sertoli-cell tight-junctional barrier — the blood-testis barrier — that immunologically isolates the haploid germ cells from the systemic circulation.

General organisation of the testis

- ▶ **Capsule** (outside to in): visceral layer of **tunica vaginalis** (mesothelium) → **tunica albuginea** (thick, dense fibrous capsule) → **tunica vasculosa** (innermost vascular areolar coat lining each lobule).

- ▶ The tunica albuginea thickens posteriorly to form the **mediastinum testis**, from which fibrous **septa** radiate to divide the gland into ~200–300 **conical lobules** (apex directed toward the mediastinum).
- ▶ Each lobule contains 1–4 **highly coiled seminiferous tubules**, giving a total of roughly 400–600 **tubules per testis**. Each tubule, when uncoiled, measures about 30–80 cm in length and 150–200 µm in diameter; the combined length of all tubules in one testis is several hundred metres.
- ▶ Near the mediastinum the tubules lose their convolutions and become **straight tubules (tubuli recti)**, which unite in the mediastinum to form the anastomosing **rete testis**; this drains via 12–30 **efferent ductules** into the head of the epididymis.
- ▶ The interstitial (interlobular/intertubular) space between tubules contains loose vascular connective tissue, lymphatics, and clusters of **Leydig cells**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw (a) a low-power scheme of the testis — tunica albuginea, tunica vasculosa, mediastinum testis, radiating septa, conical lobules each with 1–4 seminiferous tubules, interstitial tissue with Leydig-cell clusters, straight tubules converging into the rete testis, efferent ductules leaving into the epididymal head; then (b) a high-power cross-section of one seminiferous tubule labelling, from base to lumen: basement membrane → peritubular myoid (contractile) cells of the lamina propria → Sertoli cell (base on basement membrane, apex at lumen) → spermatogonium → primary spermatocyte → secondary spermatocyte → spermatid → lumen with released spermatozoa → interstitial (Leydig) cells in the surrounding stroma.

□ Fig 17.12 — Histology of seminiferous tubule (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.294)

Seminiferous tubule — the seminiferous epithelium

- ▶ Each tubule is bounded externally by a fibrous **lamina (tunica) propria** containing flattened, contractile **myoid (peritubular) cells** external to the basal lamina; the lamina propria itself is devoid of fibroblasts, and rhythmic myoid contraction helps move tubular fluid and sperm toward the rete testis.
- ▶ Internal to the basal lamina lies the **seminiferous epithelium**, a tall, complex stratified epithelium of two cell populations: (1) non-dividing **Sertoli (sustentacular) cells**, and (2) the proliferating, differentiating **spermatogenic (germ) cell series**.
- ▶ On routine H&E sections the individual cell boundaries are indistinct; identification relies on **nuclear morphology, size, and position** within the epithelium.

Sertoli cells

- ▶ Tall, columnar-to-pyramidal cells whose broad base rests on the basement membrane and whose apex reaches the tubular lumen; they constitute the **true epithelium** of the tubule (the germ cells are transient "guests").
- ▶ **Nucleus**: pale-staining, euchromatic, ovoid/triangular with a prominent nucleolus — the feature that distinguishes them from the darker germ-cell nuclei.

- **Cytoplasm:** abundant smooth endoplasmic reticulum, some rough ER, well-developed Golgi, numerous mitochondria and lysosomes; contains characteristic **crystalloids of Charcot–Böttcher** (rod-shaped inclusion bodies) in the basal cytoplasm.
- **Do not divide after puberty;** are more conspicuous relative to germ cells before puberty and in old age.
- **Lateral surfaces of adjacent Sertoli cells are joined by an elaborate tight-junctional (occluding) complex** — this is the anatomical basis of the blood-testis barrier (detailed below).
- **Functions:** physical/mechanical support and nutrition of germ cells; phagocytosis of residual bodies shed during spermiogenesis; secretion of **androgen-binding protein (ABP)** (concentrates testosterone in the tubule), **inhibin** (feedback-inhibits pituitary FSH), **anti-Müllerian hormone/Müllerian-inhibiting factor** (regresses Müllerian ducts in the male fetus), transferrin, and plasminogen activator; bear **FSH receptors** on their basal membrane.

Spermatogenic (germ) cells

Arranged in ascending, stratified order from the basement membrane to the lumen, reflecting successive stages of spermatogenesis:

Cell	Position	Key feature
Spermatogonia (Type A dark, Type A pale, Type B)	Basal, resting on basement membrane	Type A dark = reserve stem cell; Type A pale renews itself and gives Type B; Type B (diploid, 46,XY) divides mitotically into primary spermatocytes
Primary spermatocytes	Basal → mid-epithelium	Largest germ cells, spherical nucleus, undergo meiosis I
Secondary spermatocytes	Adluminal	Short-lived (23,X or 23,Y), rapidly complete meiosis II
Spermatids	Adluminal, near lumen	Haploid (1n); undergo spermiogenesis (Golgi → cap → acrosome → maturation phases) without further division
Spermatozoa	Released into lumen	Head, neck, middle piece, tail; released by spermiation

- Not all stages are seen in any single cross-section; a **wave of maturation (spermatogenic cycle)** passes along the length of the tubule.
- Excess spermatid cytoplasm is shed as a **residual body**, phagocytosed by the Sertoli cell.

Interstitial (Leydig) cells

- Large, **polygonal, eosinophilic** cells lying in clusters within the vascular connective tissue between seminiferous tubules.
- Round nucleus; abundant **smooth endoplasmic reticulum** giving a foamy cytoplasm (steroidogenic cell morphology); contain **lipofuscin pigment** and diagnostic rod-shaped cytoplasmic **crystals of Reinke**.
- Bear **LH receptors**; LH stimulates testosterone synthesis — their principal secretory product.

- **Biphasic activity:** active in fetal life from about the 5th month (masculinising the fetus), quiescent through childhood, then reactivated by gonadotropins at puberty and active thereafter.

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

- **Structure:** formed by an elaborate belt of **tight (occluding) junctions** between the lateral membranes of adjacent Sertoli cells, located just above the basal lamina. This is described as the **tightest blood–tissue barrier in the body**.
- These junctions divide the seminiferous epithelium into two compartments:
 - **Basal compartment** — between the basal lamina and the Sertoli-junctional complex; contains **spermatogonia and early (preleptotene) primary spermatocytes**, which therefore remain in continuity with blood-borne nutrients and plasma proteins.
 - **Adluminal (luminal) compartment** — above the junctional complex, toward the tubular lumen; contains **later primary spermatocytes, secondary spermatocytes, and spermatids**, which are shielded from the systemic circulation.
- As a preleptotene primary spermatocyte matures and must cross the barrier, a **new junctional complex forms basal to it before the old one above it breaks down**, so epithelial continuity (and barrier integrity) is never lost even while the cell physically migrates from basal to adluminal compartment.
- **Functions:**
- Creates an immunologically privileged compartment that **isolates haploid germ cells (which express novel, "non-self" surface antigens) from the immune system**, preventing formation of anti-sperm antibodies and autoimmune orchitis.
- Maintains a distinct **ionic and hormonal microenvironment** (high androgen-binding-protein-bound testosterone, specific luminal fluid composition) within the adluminal compartment, essential for meiosis and spermiogenesis.
- Excludes blood-borne toxins, drugs, and pathogens from the maturing germ cells.
- The barrier matures at **puberty**, coinciding with the onset of meiosis, and is maintained by Sertoli cells throughout reproductive life.

Applied / Clinical anatomy

- **Cryptorchidism (undescended testis):** scrotal temperature (2–3°C below core) is required for spermatogenesis but not for testosterone synthesis; an undescended testis shows germ-cell arrest with normal Leydig-cell function — hence infertility with normal secondary sexual characters. Orchidopexy by 2 years of age is advised to prevent irreversible tubular changes.
- **Breach of the blood–testis barrier** (trauma, torsion, vasectomy, mumps orchitis, biopsy) exposes sequestered sperm antigens to the immune system, provoking **anti-sperm antibodies** and immune-mediated (autoimmune) infertility — the rationale for careful, minimally traumatic technique in testicular biopsy and for counselling before vasectomy.
- **Leydig cell tumour:** Reinke crystals, though present in only about one-third of tumours, are a useful diagnostic clue on histopathology; tumours may cause precocious puberty (androgen excess) in children.

- ▶ **Sertoli-cell-only syndrome (germ-cell aplasia):** tubules lined only by Sertoli cells, a cause of non-obstructive azoospermia diagnosed on testicular biopsy.
- ▶ **Testicular biopsy** for the infertility work-up is interpreted against this normal seminiferous-epithelial architecture and cell ratios.
- ▶ **Varicocele** and elevated intratesticular temperature can secondarily impair spermatogenesis by the same temperature-dependent mechanism as cryptorchidism.

High-yield exam pointers

- ▶ Capsule sequence: tunica vaginalis (visceral) → tunica albuginea → tunica vasculosa; septa from mediastinum testis create ~200–300 lobules, each with 1–4 seminiferous tubules.
- ▶ Duct pathway: seminiferous tubule → straight tubule (tubulus rectus) → rete testis → efferent ductules → epididymis.
- ▶ Seminiferous epithelium = Sertoli cells (non-dividing, supporting, pale nucleus, Charcot–Böttcher crystalloids) + spermatogenic cells (spermatogonia → primary/secondary spermatocytes → spermatids → spermatozoa).
- ▶ Leydig cells: eosinophilic, foamy (SER-rich) cytoplasm, Reinke crystals, LH-driven testosterone secretion, active in fetal life and after puberty.
- ▶ Blood-testis barrier = Sertoli–Sertoli tight junctions, divides epithelium into **basal** (spermatogonia, early primary spermatocytes) and **adluminal** (later spermatocytes, spermatids) compartments.
- ▶ Function of barrier: immune isolation of haploid germ cells + creation of a controlled luminal microenvironment for meiosis/spermiogenesis.
- ▶ Clinical hooks: cryptorchidism and temperature-dependent germ-cell arrest; anti-sperm antibodies after barrier breach; Reinke crystals in Leydig-cell tumours; Sertoli-cell-only syndrome.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; BD Chaurasia — Human Anatomy Vol 2 8e (figure). Coverage note: Gray's Anatomy (Standring) 41e covers testicular gross/vascular anatomy but not seminiferous-epithelial cytology or the blood-testis barrier in comparable histological detail, so the histology specialty texts above are the primary grounding for this answer, consistent with the topic spec's grounding note.



Describe the microscopic structure of the Mammary gland

Asked in: Paper III Nov 2016 — RGUHS MD/MS Anatomy

Definition / Introduction The mammary gland is a modified, apocrine-type cutaneous gland lying in the superficial fascia of the anterior chest wall, histologically classified as a **compound tubulo-alveolar gland**. Each gland is organised into 15–20 **lobes**, each lobe further subdivided into numerous **lobules** (**terminal duct lobular units, TDLUs**), all embedded in a fibro-fatty connective-tissue stroma. Its microscopic architecture is not static — the parenchyma-to-stroma ratio and the epithelial morphology change strikingly with age, the menstrual cycle, pregnancy, and lactation, so the gland is best described in its **resting (inactive)** and **lactating (active)** states.

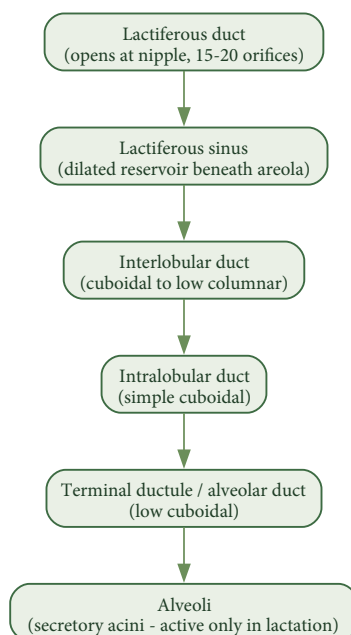
1. General organisation

- ▶ Each **lobe** is a complete secretory unit with its own excretory **lactiferous duct**, opening independently at the nipple — hence 15–20 separate ductal orifices at the nipple tip.
- ▶ Each lobe contains many **lobules (TDLUs)**, the basic structural and functional unit of the gland; each TDLU consists of a cluster of terminal ductules/alveoli plus the intralobular collecting duct, all invested by specialised intralobular stroma.
- ▶ Lobes are separated from each other by dense **interlobular connective tissue** and abundant adipose tissue; lobules within a lobe are separated by looser **intralobular connective tissue**.
- ▶ The whole gland is a modified apocrine sweat gland of ectodermal origin, developing from paired **mammary ridges (milk lines)** that extend from axilla to groin; in humans only the pectoral segment of the ridge persists and forms the breast.

2. Duct system — histology

Duct segment	Location	Epithelial lining
Lactiferous duct	Drains each lobe; opens at nipple tip (15–20 orifices)	Near orifice: keratinised stratified squamous (continuous with epidermis); further in: stratified/two-layered cuboidal
Lactiferous sinus	Dilated part of the duct beneath the areola (reservoir, further distended with milk during lactation)	Stratified cuboidal (two cell layers)
Interlobular duct	Between lobules, within a lobe	Cuboidal to low columnar
Intralobular duct	Within a lobule, leading to the TDLU	Simple cuboidal
Alveolar duct (terminal ductule)	Innermost segment draining the alveoli	Low cuboidal

- ▶ All ducts are surrounded by a discontinuous, basket-like layer of **myoepithelial cells** lying between the luminal epithelium and the basal lamina; they are of ectodermal origin and are most easily identified around the larger ducts on routine H&E.
- ▶ Sparse smooth-muscle fibres also encircle the larger ducts.



Describe the microscopic structure of the Mammary gland

3. Histology of the inactive (resting) gland

- Predominantly **ductal** — the lobule (TDLU) consists mainly of small, blind-ending, branched **terminal ductules**, which appear as solid cords or small clusters of cuboidal cells with little or no lumen; mature secretory alveoli are absent or rudimentary.
- **Intralobular stroma**: loose, cellular, vascular connective tissue with few fibres and no fat, surrounding the ductules.
- **Interlobular stroma**: dense fibrocollagenous tissue with abundant interlobular **adipose tissue** — this fat, not glandular tissue, accounts for most of the bulk of the resting adult breast.
- Minor cyclical change occurs with the menstrual cycle: in the follicular phase (oestrogen-driven) the intralobular stroma is sparse and ductules show little lumen; in the luteal phase (progesterone-driven) epithelial cells increase in height, small lumina appear, and the stroma becomes oedematous (clinical premenstrual breast fullness/tenderness); this is followed by abrupt regression/apoptosis just before menstruation.

4. Histology of the active (lactating) gland

- Under the combined action of oestrogen, progesterone, prolactin and placental lactogen during pregnancy, the terminal ductules proliferate and differentiate into fully formed, spherical **secretory alveoli** (acini).
- Alveoli are lined by **low columnar/cuboidal secretory epithelium** resting on a basal lamina, encircled by a basket of contractile **myoepithelial cells**.
- Secretory cells show **basal basophilia** (abundant rough endoplasmic reticulum) and **apical eosinophilia** (supranuclear Golgi complex) on H&E; ultrastructurally they contain numerous mitochondria, dense lysosomes, membrane-bound secretory vesicles, and large cytoplasmic lipid droplets.

- ▶ Alveolar lumina are dilated and filled with precipitated secretory product (milk); intralobular ducts carrying this secretion may themselves show a secretory epithelium, making them hard to distinguish from alveoli.
- ▶ The stroma is markedly reduced — less collagen and less adipose tissue than in the resting gland — and is infiltrated by **lymphocytes and plasma cells**, the latter secreting IgA that is transferred into colostrum/milk.
- ▶ Degree of glandular development is not uniform — it varies from lobule to lobule and even within a single lobule, reflecting asynchronous alveolar maturation.

□ Fig 24.2 — *Histology of mammary gland. (A) Inactive mammary gland; (B) Lactating* (Sontakke — *Textbook of Human Histology* — 2e, p.319)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power lobule (TDLU) in both states side by side, labelling:

- ▶ Lactiferous/intralobular duct
- ▶ Interlobular connective tissue (dense, with fat)
- ▶ Intralobular connective tissue (loose, cellular)
- ▶ (A) Inactive gland: solid/blind terminal ductules with narrow or absent lumen
- ▶ (B) Lactating gland: dilated secretory alveoli filled with milk, low cuboidal epithelium
- ▶ Myoepithelial cell layer around ducts/alveoli
- ▶ Basal lamina

5. Stroma / connective tissue framework

- ▶ **Interlobular stroma:** dense, collagenous, richly admixed with adipose tissue; forms fibrous septa — the **suspensory (Cooper's) ligaments** — that run from the deep fascia to the dermis and support the gland.
- ▶ **Intralobular stroma:** specialised, hormonally-sensitive, loose, vascular connective tissue surrounding the ductules/alveoli of each TDLU; contains little or no fat and is the site of the cyclical/pregnancy-related stromal changes described above.

6. Nipple and areola histology

- ▶ **Nipple:** covered by pigmented, wrinkled, keratinised **stratified squamous epithelium** with long dermal papillae; core is dense collagenous connective tissue containing bundles of **smooth muscle** arranged radially and circumferentially (and longitudinally along the ducts) — contraction (from cold, tactile stimuli, or suckling) produces nipple erection; richly supplied with **sensory nerve endings**.
- ▶ **Areola:** pigmented skin surrounding the nipple, containing sebaceous glands, sweat glands, and the **glands of Montgomery** (areolar tubercles) — structurally intermediate between sweat and mammary glands, producing a lubricating/protective secretion; fewer sensory endings than the nipple.
- ▶ Near the ductal orifices at the nipple tip, the ductal epithelium transitions from simple/stratified cuboidal to **keratinised stratified squamous**, continuous with the epidermis.

7. Mode of milk secretion (functional histology)

- ▶ **Merocrine secretion:** milk proteins (casein, α -lactalbumin, lactoferrin, lysozyme, IgA, epidermal growth factor) are synthesised on the rER, packaged in the Golgi into membrane-bound secretory vesicles, and released by exocytosis (vesicle membrane fusing with plasma membrane).
- ▶ **Apocrine secretion:** lipid droplets coalesce in the cytoplasm, migrate to the apex, and are pinched off enclosed in a rim of plasma membrane (with a thin film of cytoplasm) to form milk-fat globules.
- ▶ **Colostrum**, secreted for the first few days after delivery, is an alkaline, yellowish fluid richer in protein, IgA, vitamin A, sodium and chloride, but poorer in fat, carbohydrate and potassium than mature milk; the abundant plasma cells of the stromal infiltrate provide the secretory IgA that confers passive immunity on the neonate.
- ▶ Milk secretion (synthesis) is driven by **prolactin** (anterior pituitary); milk ejection is a neurohormonal reflex — suckling $\rightarrow$ hypothalamus $\rightarrow$ posterior pituitary releases **oxytocin** $\rightarrow$ contraction of myoepithelial cells around alveoli and ducts $\rightarrow$ milk expressed into the duct system.

Applied / Clinical anatomy

- ▶ **Breast carcinoma** arises from the ductal or alveolar (lobular) epithelium of the TDLU and is the commonest malignancy in women; hormonal exposure (early menarche, late menopause, late first full-term pregnancy) is the dominant risk factor, and roughly 5% are linked to autosomal-dominant **BRCA1/BRCA2** mutations. Early detection is possible by breast self-examination.
- ▶ **Fibroadenoma** is a benign tumour arising from the intralobular (glandular + stromal) tissue of the gland.
- ▶ **Suspensory (Cooper's) ligaments** and the retromammary space define surgical planes; their infiltration by tumour produces skin dimpling/tethering.
- ▶ **Polymastia (accessory breast)** and **polythelia (accessory nipple)** occur in ~1% of women (and occasionally men) due to persistence of parts of the milk line that normally involute; **amastia/athelia** result from failure of the mammary bud/pit to develop.
- ▶ **Lactational amenorrhoea:** high prolactin suppresses pulsatile GnRH $\rightarrow$ suppressed LH $\rightarrow$ anovulation/infertility during full breast-feeding — the physiological basis of lactational contraception.
- ▶ Involution/atrophy at **menopause:** withdrawal of ovarian hormones causes the secretory alveoli/ductules of the TDLUs to degenerate, leaving a duct-predominant pattern resembling the male breast, with fibro-fatty stromal replacement.
- ▶ Radiologically, the ductal/glandular pattern seen on mammography reflects this same lobar-lobular architecture.

High-yield exam pointers

- ▶ Compound **tubulo-alveolar**, apocrine-type gland; 15–20 lobes, each an independent unit draining via its own lactiferous duct.
- ▶ TDLU (terminal duct lobular unit) = the basic structural/functional unit; site of most benign and malignant breast disease.

- ▶ Duct epithelium lining changes progressively: stratified squamous (nipple) → stratified/2-layer cuboidal (sinus) → cuboidal/low columnar (interlobular/intralobular) → low cuboidal (alveolar duct).
- ▶ Myoepithelial cells (basket-shaped, ectodermal) surround ducts and alveoli — contract under oxytocin for milk ejection.
- ▶ Resting gland = duct-predominant + abundant interlobular fat; lactating gland = alveoli dilated with milk + reduced stroma + plasma-cell infiltrate (IgA).
- ▶ Secretory cell shows basal basophilia (rER) and apical eosinophilia (Golgi) — classic H&E correlate of merocrine + apocrine dual secretion.
- ▶ Oestrogen → duct proliferation; progesterone → alveolar growth; prolactin → milk synthesis; oxytocin → milk ejection.
- ▶ Colostrum precedes true milk — richer in protein/IgA, poorer in fat, confers passive immunity.
- ▶ Glands of Montgomery (areola) are structurally intermediate between sweat and mammary glands.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Gray's Anatomy (Standring) 41e.



Discuss the microscopic anatomy of hypophysis cerebri and the stains used to differentiate the different types of cells

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction The hypophysis cerebri (pituitary gland) is a pea-sized endocrine organ (≈ 0.5 g; $10 \times 13 \times 6$ mm) lodged in the sella turcica of the sphenoid bone and suspended from the floor of the third ventricle by the infundibulum. Called the "master endocrine gland" because its secretions regulate most peripheral endocrine glands, it is structurally a composite of two histologically dissimilar tissues — a glandular **adenohypophysis** derived from Rathke's pouch (oral ectoderm) and a neural **neurohypophysis** derived from a neuroectodermal downgrowth of the diencephalon. This dual origin is the key to its microscopic anatomy, and it is precisely because routine staining cannot fully resolve the several secretory cell types of the adenohypophysis that a range of special stains (and, today, immunohistochemistry) were developed to differentiate them.

1. Subdivisions and their embryological basis

- ▶ **Adenohypophysis** (anterior lobe) — three parts, all derived from the hypophyseal (Rathke) pouch: **pars distalis** (the bulk), **pars tuberalis** (a vascular collar around the infundibulum), and **pars intermedia** (a thin zone abutting the pars nervosa).
- ▶ **Neurohypophysis** (posterior lobe) — **pars nervosa** + **infundibular stalk**, derived from the neurohypophyseal bud growing down from the floor of the third ventricle; it remains continuous with the hypothalamus.

- By the end of the second month the hypophyseal pouch detaches from the pharyngeal roof and apposes the neurohypophyseal bud, after which the two components mature side by side but retain their distinct histological character throughout life.

2. Histology of the adenohypophysis

Pars distalis ($\approx 75\%$ of the adenohypophysis)

- Covered by a thin fibrous capsule; parenchyma is arranged as **cords/clumps of cells** separated by **fenestrated sinusoidal capillaries** and supported by a delicate reticular connective-tissue framework.
- On routine H&E the cells fall into two broad groups: **chromophils** (granules with dye affinity) and **chromophobes** ($\approx 50\%$, few granules, poorly stained — degranulated chromophils, resting cells, or progenitor/stem cells).
- Chromophils subdivide into **acidophils** ($\approx 40\%$, affinity for acid dyes such as eosin/orange G) and **basophils** ($\approx 10\%$, affinity for basic dyes such as haematoxylin, and PAS-positive).
- Immunocytochemistry and electron microscopy resolve five functional cell types within these two groups:

Cell type	% of pars distalis	Hormone secreted	Routine (H&E) reaction	EM secretory granule
Somatotroph	$\sim 50\%$	Growth hormone (GH)	Acidophil	Dense, ~ 350 nm, closely packed
Lactotroph (mammotroph)	$\sim 15\text{--}20\%$	Prolactin (PRL)	Acidophil	Inactive: 200 nm sparse; active: dense pleomorphic ~ 600 nm
Corticotroph	$\sim 15\text{--}20\%$	ACTH (via POMC), β -LPH	Basophil	$100\text{--}300$ nm, with lipid droplets and lysosomes
Gonadotroph	$\sim 10\%$	FSH and LH	Basophil	Dense, $200\text{--}250$ nm; prominent Golgi
Thyrotroph	$\sim 5\%$	TSH	Basophil (least abundant)	Dense, ~ 150 nm

Non-hormone-secreting **folliculo-stellate cells**, joined by gap junctions, ramify between the secretory cords and may coordinate paracrine signalling within the pars distalis.

Pars intermedia

- A narrow, poorly developed zone between the pars distalis and pars nervosa.
- Contains **colloid-filled cysts/vesicles**, the residual lumen of Rathke's pouch, lined by cells derived from folliculo-stellate or hormone-secreting precursors.
- Predominant cells are **basophils** (corticotroph-related, cleaving POMC to yield β -endorphin and melanocyte-stimulating hormone, MSH) and **chromophobes**; its functional significance in the adult human is uncertain.

Pars tuberalis

- ▶ A thin, highly vascular, funnel-shaped collar wrapping the infundibulum; contains the **primary capillary plexus** of the hypophyseal portal system.
- ▶ Cells are arranged in small cords/clusters around the vessels — predominantly **gonadotrophs**, with scattered acidophils and small squamous/cuboidal cell nests.

3. Histology of the neurohypophysis (pars nervosa + infundibulum)

- ▶ Not a true secretory gland but a **neurohaemal storage-and-release site** — it contains no hormone-synthesising cell bodies.
- ▶ Composed of ≈100,000 **unmyelinated axons** of neurosecretory neurons whose cell bodies lie in the **supraoptic** (→ antidiuretic hormone/vasopressin) and **paraventricular** (→ oxytocin) nuclei of the hypothalamus, together forming the **hypothalamo-hypophyseal tract**.
- ▶ Hormone (bound to its carrier protein, **neurophysin I or II**) accumulates in dilated axonal swellings called **Herring bodies** — faintly eosinophilic on H&E.
- ▶ **Pituicytes**, branched astrocyte-like glial cells, form the supporting stroma (≈25% of neurohypophyseal volume); on routine H&E their cytoplasm blends with the surrounding unmyelinated fibres.
- ▶ A rich fenestrated capillary bed permits uptake of released hormone directly into the systemic circulation.

4. Hypothalamo-hypophyseal portal system (functional correlate)

- ▶ **Superior hypophyseal arteries** → primary capillary plexus in the median eminence/infundibulum → **hypophyseal portal veins** → secondary capillary plexus (sinusoids) of the pars distalis → hypophyseal veins → cavernous sinus.
- ▶ **Inferior hypophyseal arteries** supply the neurohypophysis directly, largely bypassing the portal system.
- ▶ This arrangement lets hypothalamic releasing/inhibiting hormones reach the adenohypophyseal cells in high local concentration before dilution in the systemic circulation.

5. Stains used to differentiate the different pituitary cell types

- ▶ **Routine H&E** only separates chromophils (granular, stained) from chromophobes (pale); it cannot reliably subclassify chromophils into functional subtypes.
- ▶ **Classical mixed acid–basic dye (trichrome/polychrome) methods** resolve acidophils from basophils simultaneously in one section:
 - **Trichrome technique with brilliant crystal scarlet, aniline blue, and Martius yellow** — acidophils cherry-red, basophils blue, connective-tissue stroma blue, sinusoidal erythrocytes yellow.
 - **Azan stain** (azocarmine–aniline blue–orange G) — acidophil granules red/orange, basophil granules blue, nuclei orange-red, chromophobes pale and non-granular.
 - **Gomori trichrome** — acidophils orange, basophils violet, chromophobes poorly stained.
- ▶ **Cell-subtype-specific classical special stains** (pre-immunohistochemistry era), summarised below:

Cell type	General reaction	Specific differentiating stain	PAS reaction
Somatotroph	Acidophil	Orange G	Negative
Lactotroph	Acidophil	Orange G; Herlant's erythrosin; Brooke's carmosine	Negative
Corticotroph	Basophil	Lead haematoxylin	Positive
Gonadotroph	Basophil	Aldehyde-fuchsin / aldehyde-thionin	Positive
Thyrotroph	Basophil	Aldehyde-fuchsin / aldehyde-thionin	Positive

- **Periodic acid–Schiff (PAS)** reaction is itself a key discriminator: basophils (corticotroph, gonadotroph, thyrotroph) are **PAS-positive** because their hormones are glycoproteins (or, for POMC, carry carbohydrate moieties), whereas acidophils (somatotroph, lactotroph) are **PAS-negative**.
- **Immunocytochemistry/immunohistochemistry**, using antibodies against GH, PRL, ACTH, FSH/LH, and TSH, is the current gold standard and definitively identifies each of the five cell types, superseding classical dye methods.
- **Transmission electron microscopy**, correlated with immunogold labelling, differentiates the cell types further by granule size, shape and density (see table in Section 2).
- In the **neurohypophysis**, aniline blue renders Herring bodies as dense, dark granular islands, while H&E stains them faintly pink; pituicytes require special glial stains to be distinguished clearly from adjacent axons.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- Fig 12.12b — *Histology of hypophysis cerebri, 400X* (BD Chaurasia — *Human Anatomy Vol 3-4* — 8e, p.242)
- Fig 23.12 — *The pituitary gland (trichrome-stained). A, The endocrine cells of the adenohypophysis* (Gray's *Anatomy — Standring* — 41e, p.502)

For the examination, reproduce a low-power labelled sketch of the gland showing: **capsule** → **pars distalis** (cords of cells around sinusoids, with **acidophil**, **basophil**, and **chromophobe** cells individually labelled) → **pars intermedia** (colloid-filled cyst/cleft) → **pars nervosa** (unmyelinated fibres and pituicytes). Add a second inset at high power showing acidophils (pink, rounded), basophils (blue/violet, polygonal), and chromophobes (pale, clustered), each with an arrow to a sinusoid.

Applied / Clinical anatomy

- **Chromophobe adenoma** is the commonest pituitary tumour; typically non-functioning. It enlarges and "balloons" the sella turcica (visible on plain lateral skull radiograph) and, on suprasellar extension, compresses the optic chiasma to produce **bitemporal hemianopia**; pressure on the cavernous sinus can cause exophthalmos/ophthalmoplegia.

- ▶ **Acidophil (somatotroph) adenoma** — excess GH causes **gigantism** (before epiphyseal fusion) or **acromegaly** (after fusion).
- ▶ **Basophil (corticotroph) adenoma** — excess ACTH causes **Cushing's disease**.
- ▶ **Lactotroph adenoma (prolactinoma)** — hyperprolactinaemia with amenorrhoea-galactorrhoea.
- ▶ Hyposecretion of anterior lobe hormones causes **dwarfism** (GH deficiency in children) or panhypopituitarism; damage to the neurohypophysis/hypothalamic nuclei causes **diabetes insipidus** from ADH deficiency.
- ▶ Remnants of **Rathke's pouch** may give rise to a **craniopharyngioma** (commonly suprasellar) or persist as colloid-filled **Rathke's cleft cysts** in the pars intermedia; ectopic Rathke's-pouch tissue in the nasopharyngeal roof forms the **pharyngeal hypophysis**.
- ▶ Knowledge of the sella turcica's relation to the sphenoid sinus underlies the **trans-sphenoidal** surgical approach to hypophysectomy.

High-yield exam pointers

- ▶ Dual embryological origin: Rathke's pouch (oral ectoderm) → adenohypophysis; neurohypophyseal bud (neuroectoderm) → neurohypophysis.
- ▶ Adenohypophysis = pars distalis + pars tuberalis + pars intermedia; neurohypophysis = pars nervosa + infundibulum.
- ▶ Classical H&E ratio in pars distalis: chromophobes ~50% > acidophils ~40% > basophils ~10%.
- ▶ Five functional cell types: somatotroph (GH, acidophil, ~50%), lactotroph (PRL, acidophil, ~15–20%), corticotroph (ACTH, basophil, ~15–20%), gonadotroph (FSH/LH, basophil, ~10%), thyrotroph (TSH, basophil, ~5%).
- ▶ PAS positivity cleanly separates basophils (PAS+, glycoprotein/POMC hormones) from acidophils (PAS–).
- ▶ Classical differentiating stains: Orange G (acidophils), lead haematoxylin (corticotroph), aldehyde-fuchsin/aldehyde-thionin (gonadotroph, thyrotroph), Herlant's erythrosin and Brooke's carmosine (lactotroph); immunohistochemistry is now definitive.
- ▶ Neurohypophysis is not a true secretory gland: it stores/releases ADH and oxytocin synthesised in the supraoptic and paraventricular nuclei; Herring bodies are axonal hormone accumulations; pituicytes are the glial support cells.
- ▶ Hypothalamo-hypophyseal portal system delivers releasing/inhibiting hormones to the pars distalis; a separate inferior hypophyseal artery supplies the neurohypophysis directly.
- ▶ Commonest pituitary tumour is the chromophobe adenoma; classical presentation includes bitemporal hemianopia from chiasmal compression.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; diFiore's Atlas of Histology (Eroschenko) (azan-staining detail); Gray's Anatomy (Standring) 41e (hypothalamic-control context and Fig. 23.12); Vishram Singh Textbook of Anatomy Vol 3 4e and BD Chaurasia Human Anatomy Vol 3-4 8e (applied anatomy and labelled histology figure). Coverage note: Gray's Anatomy gives only hypothalamic-control and gross/regional context for the pituitary (its dedicated histology detail is thin), so the microscopic and staining detail above is drawn primarily from the specialty histology texts, cross-checked across all five.



Discuss the microscopic features of ovary in different phases and their effect on the uterus

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — The ovary shows continuously changing microscopic architecture through the reproductive years, driven by the **ovarian cycle** — a follicular (pre-ovulatory) phase dominated by growing follicles secreting oestrogen, and a luteal (post-ovulatory) phase dominated by the corpus luteum secreting progesterone. Each phase produces a corresponding, hormonally-timed change in the uterine endometrium — the **menstrual cycle** — so that the two cycles run strictly in parallel, the ovary acting as the endocrine driver and the endometrium as the target organ. A 28-day cycle is conventionally divided at ovulation (day 14) into these two ovarian phases, matched by three endometrial phases (proliferative, secretory, menstrual).

General Microscopic Organisation of the Ovary

- ▶ Surface covered by a single layer of cuboidal-to-squamous **germinal epithelium** (a modified mesothelium, continuous with the mesovarium; not the site of germ-cell origin despite the name).
- ▶ Deep to it, a dense connective tissue layer, the **tunica albuginea**.
- ▶ **Cortex** (peripheral) — cellular stroma containing ovarian follicles at all stages of development, corpora lutea, corpora albicantia and atretic follicles.
- ▶ **Medulla** (central) — loose connective tissue with large coiled blood vessels, lymphatics and nerves; the cortico-medullary junction is indistinct.
- ▶ At any point in reproductive life the cortex shows a mixed population of follicles at different stages — primordial follicles always predominate numerically.

Follicular (Pre-ovulatory) Phase — Microscopic Stages of the Growing Follicle

Of the ~600,000–800,000 primary oocytes present at birth (already reduced by atresia from as many as 5 million in the fetus), only about 400 ever reach full maturity and ovulation in a lifetime. Each cycle, a cohort of follicles is recruited and passes through the following histological stages:

Stage	Oocyte	Follicular/ granulosa cells	Key feature
Primordial follicle	~30 µm, eccentric nucleus, arrested in prophase (diplotene) of meiosis I	Single layer of flattened (squamous) follicular cells	Earliest stage; first appears in the 3rd fetal month; lies just beneath the tunica albuginea
Primary follicle (unilaminar)	Enlarging; oocyte 50–80 µm when zona begins	Follicular cells become cuboidal (single layer)	Zona pellucida first appears (PAS-positive glycoprotein coat, ZP-1/2/3)
Primary follicle (multilaminar / late primary)	Growing	Stratified granulosa layer (membrana granulosa) by mitosis	Theca folliculi differentiates outside the basal lamina into theca interna (vascular, steroidogenic) and theca externa (smooth muscle + collagen)
Secondary (antral) follicle	~125 µm, growth arrested by oocyte-maturation inhibitor	Granulosa 6–12 layers thick; Call-Exner bodies (PAS+ material) appear	Coalescing fluid spaces form a single antrum filled with liquor folliculi; follicle grows from ~0.2 mm to >10 mm
Mature (Graafian) follicle	Meiosis I resumes after the LH surge → secondary oocyte + 1st polar body	Cumulus oophorus projects into antrum; innermost layer around oocyte forms corona radiata	Diameter ≥10 mm; bulges the ovarian surface; theca interna secretes androgens (LH-driven), granulosa aromatises them to oestrogen (FSH-driven)

Diagram to draw

□ Fig 22.2 — Follicle development and changes within the ovary. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1101)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled panoramic section of the ovarian cortex showing, in sequence: **germinal epithelium** → **tunica albuginea** → **primordial follicles** → **unilaminar primary follicle** → **multilaminar primary follicle (with zona pellucida)** → **secondary/antral follicle (antrum, theca interna, theca externa, Call-Exner bodies)** → **Graafian follicle (cumulus oophorus, corona radiata)** → **ruptured follicle** → **corpus luteum (granulosa-lutein and theca-lutein cells)** → **corpus albicans** → **atretic follicles**.

Ovulation — Microscopic Events

- ▶ LH surge (~24 h pre-ovulation) → resumption of meiosis I → secondary oocyte + first polar body; second meiotic division begins and arrests at metaphase II until fertilisation.
- ▶ Blood flow ceases over a localised area of germinal epithelium overlying the bulge — the **macula pellucida/follicular stigma** — which ruptures.
- ▶ Contributory mechanisms: rising follicular fluid pressure, proteolysis of the follicular wall (activated plasminogen/collagenase), prostaglandin-mediated contraction of theca externa smooth muscle.
- ▶ Secondary oocyte, corona radiata and cumulus cells are extruded and swept into the fimbriated end of the uterine tube.

Luteal (Post-ovulatory) Phase — Corpus Luteum and Corpus Albicans

- ▶ Immediately after rupture, bleeding from theca-interna capillaries into the collapsed follicular cavity forms the **corpus hemorrhagicum**.
- ▶ Granulosa and theca-interna cells undergo **luteinisation** — hypertrophy, lipid accumulation, and yellow lipochrome pigmentation — to form the **corpus luteum**.

Feature	Granulosa-lutein cells	Theca-lutein cells
Proportion	~80% of corpus luteum	~20%
Location	Large, pale, centrally placed	Smaller, darker-staining, peripheral
Size	~30 µm	~15 µm
Secretion	Oestrogen, progesterone, inhibin	Androgens, progesterone

- ▶ If fertilisation does **not** occur: the **corpus luteum of menstruation** remains active for only ~14 days (degeneration begins 10–12 days post-ovulation, driven by falling LH/absence of hCG), then involutes into the **corpus albicans** (a hyaline, avascular scar formed by fibroblast invasion), which slowly sinks into the stroma and disappears over months.
- ▶ If fertilisation **does** occur: trophoblastic **hCG** rescues and maintains the **corpus luteum of pregnancy**, which is larger and persists 3–4 months (until the placenta takes over progesterone production).

Follicular Atresia

- ▶ Most follicles never complete maturation; they are lost by **atresia** — apoptosis of granulosa cells, oocyte degeneration, macrophage invasion — beginning as early as the 5th fetal month and continuing throughout reproductive life.
- ▶ A collapsed zona pellucida remnant is the reliable histological marker of an atretic follicle.
- ▶ Theca-interna cells of large atretic follicles may persist as steroidogenic **interstitial glands** (**corpora atretica**), eventually becoming scar tissue resembling corpus albicans.

Effect of the Ovarian Cycle on the Uterine Endometrium

The endometrium (functional layer over a stable basal layer) mirrors the ovarian cycle through three phases of a standard 28-day cycle, mediated by oestrogen (follicular phase) and progesterone (luteal phase):

Feature	Proliferative phase (Day 6–13)	Secretory phase (Day 15–28)	Menstrual phase (Day 1–4/5)
Driving ovarian phase/hormone	Follicular phase — oestrogen	Luteal phase — progesterone (+ oestrogen)	Corpus luteum regression — hormone withdrawal
Endometrial thickness	~1 → 3 mm	~5–6 mm (2–3× proliferative)	Shrinks/desquamates
Glands	Straight, narrow lumen, mitotically active	Coiled/corkscrew-shaped, sacculated, "saw-toothed"; secretory, glycogen-rich	Glands collapse, cease secretion
Stroma	Cellular, non-oedematous, proliferating	Oedematous, vascular; glycogen-laden stromal (decidual) cells appear	Ischaemic, necrotic, sloughs off
Spiral arteries	Lengthening, only slightly coiled, confined to lower 2/3	Markedly coiled, extend to surface	Rhythmic vasoconstriction → ischaemia → rupture
Event	Re-epithelialisation of the denuded surface from stratum basale	Endometrium primed for implantation	Desquamation of stratum functionale; stratum basale retained

- ▶ Menstrual flow lasts ~4–5 days with average blood loss of 35–50 mL; only the stratum basale (supplied by unchanging straight arteries) survives to regenerate the next cycle.
- ▶ In an **anovulatory cycle** (no corpus luteum, no progesterone), the endometrium remains arrested in the proliferative phase until it eventually breaks down — a key basis of dysfunctional uterine bleeding.

Diagram to draw

□ Fig 3.13 — Changes in the uterine mucosa correlated with those in the ovary (Langman's Medical Embryology — Sadler — 14e, p.66)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A single time-axis chart (Day 1–28) with three parallel tracks labelled: (1) **ovarian events** — primordial → growing follicles → Graafian follicle → ovulation (Day 14) → corpus luteum → corpus albicans; (2) **hormone levels** — oestrogen curve (peaking pre-ovulation), LH surge (Day 13–14), progesterone curve (peaking mid-luteal); (3) **endometrium** — menstrual → proliferative → ovulation line → secretory → premenstrual, with endometrial thickness rising against the same axis.

Applied / Clinical anatomy

- ▶ **Endometrial dating (biopsy)** — the characteristic gland/stroma morphology of each day of the secretory phase is used clinically to date the endometrium and diagnose luteal-phase defects/infertility.
- ▶ **Anovulatory cycles** — persistent oestrogen-only stimulation (no corpus luteum) causes endometrial hyperplasia and irregular bleeding; seen in **polycystic ovarian disease (Stein–Leventhal syndrome)**, where thickened tunica albuginea prevents ovulation and multiple atretic/cystic follicles accumulate.
- ▶ **Corpus luteal cyst / haemorrhagic cyst** — persistence or excessive bleeding of the corpus luteum can mimic an acute abdomen.
- ▶ **Ectopic implantation** — a fertilised ovum failing to enter the tube may implant on the ovarian surface or in the recto-uterine pouch (Douglas), an important cause of first-trimester maternal mortality.
- ▶ **Ovarian surface epithelial tumours** account for the majority of ovarian cancers; thought to relate to repeated disruption/repair of the germinal epithelium at each ovulation.
- ▶ **Corpus luteum rescue** by hCG is the physiological basis of the urine/serum pregnancy test and of early luteal support in assisted reproduction.
- ▶ **Zona pellucida** integrity (species-specific ZP-3 sperm receptor) underlies the biology of in-vitro fertilisation and the post-fertilisation "zona reaction" block to polyspermy.

High-yield exam pointers

- ▶ Three basic follicle categories: primordial → growing (primary [unilaminar/multilaminar] → secondary/antral) → Graafian (mature).
- ▶ Zona pellucida appears at the primary follicle stage; antrum defines the secondary follicle; corona radiata + cumulus oophorus define the Graafian follicle.
- ▶ Theca interna (LH → androgens) + granulosa (FSH/aromatase → oestrogen) = the two-cell, two-gonadotropin mechanism of follicular oestrogen synthesis.
- ▶ Corpus luteum: granulosa-lutein (80%, central, large) vs theca-lutein (20%, peripheral, small) cells.
- ▶ Corpus luteum of menstruation lasts ~14 days; corpus luteum of pregnancy lasts 3–4 months (rescued by hCG).

- ▶ Ovarian follicular phase → uterine proliferative phase (oestrogen-driven); ovarian luteal phase → uterine secretory phase (progesterone-driven); corpus luteum regression → menstrual phase.
- ▶ Endometrium has a stable stratum basale (straight arteries) and a shed stratum functionale (spiral arteries) — the anatomical basis of menstruation and its regeneration.
- ▶ Atresia is the fate of the vast majority of follicles, mediated by granulosa-cell apoptosis; the collapsed zona pellucida is its histological marker.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas, 7e (primary source for follicular stages, ovulation, corpus luteum, and endometrial phase histology); IB Singh — Textbook of Human Histology, 9e (corpus luteum cell details, Table of proliferative vs secretory phase differences, endometrial phase timings); Junqueira's Basic Histology — Text & Atlas, 17e (follicle-development overview figure); Langman's Medical Embryology — Sadler, 14e (ovarian–endometrial correlation figure). Gray's Anatomy (Standring) 41e was checked but covers only gross/embryological gonadal development for this region, not adult ovarian histology — the detailed microscopic and endometrial-correlation content above is grounded in the histology texts named.



Graafian follicle

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction The **Graafian follicle** (mature, tertiary or preovulatory/vesicular follicle) is the final, largest stage of ovarian **folliculogenesis**, reached by only one dominant follicle in a given menstrual cycle. It contains a fully grown oocyte eccentrically placed within a single large fluid-filled **antrum**, surrounded by concentric layers of granulosa cells and a well-vascularised, hormonally active theca. It is named after the 17th-century Dutch anatomist **Regnier de Graaf**, and its rupture at mid-cycle under the LH surge constitutes **ovulation**.

1. Formation, site and general features

- ▶ Located in the **ovarian cortex**, deep to the tunica albuginea and germinal (surface) epithelium.
- ▶ Represents the terminal stage of a developmental sequence: **primordial** → **unilaminar primary** → **multilaminar primary** → **secondary (antral)** → **Graafian (mature) follicle**.
- ▶ The follicle destined to ovulate is selected as the **dominant follicle** and begins its growth roughly **2–3 months (~90 days)** before the cycle in which it finally ovulates — most of this time is spent as a small growing/antral follicle; all other recruited follicles of that cohort undergo **atresia**.
- ▶ Diameter increases from **~0.2 mm** (early antral/secondary stage) to **10–25 mm** (commonly quoted as **~2 cm**) immediately before ovulation — the follicle now occupies the full thickness of the cortex and **bulges the ovarian surface**, a bulge that is visible on transvaginal ultrasound.
- ▶ As the antrum enlarges, mitotic activity of granulosa cells declines and the granulosa layer becomes relatively **thinner**, even as the thecal layers become thicker and more prominent.
- ▶ Typically **only one** Graafian follicle matures and ovulates per cycle (around **day 14 of a 28-day cycle**); the rest of the cohort becomes atretic.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 21.4 — Mature ovarian follicle (schematic representation) (IB Singh — Textbook of Human Histology — 9e, p.589)

From the periphery to the centre, label:

- ▶ **Ovarian stroma / tunica albuginea** and germinal epithelium (surface), with the **stigma** — the thinned, avascular bulge point.
- ▶ **Theca externa** — outer fibrous layer with fibroblasts, collagen, and smooth muscle, merging with ovarian stroma.
- ▶ **Theca interna** — inner, richly vascularised, steroidogenic layer of epithelioid cells with lipid droplets and LH receptors.
- ▶ **Basement membrane (basal lamina)** — separates the avascular granulosa from the theca interna.
- ▶ **Membrana granulosa (stratum granulosum)** — multilayered granulosa cells lining the antral wall; **Call-Exner bodies** (small PAS-positive, hyaluronan/proteoglycan-rich extracellular globules secreted by granulosa cells) may be seen between them.
- ▶ **Antrum** — single large fluid-filled cavity containing **liquor folliculi** (follicular fluid: hyaluronic acid, growth factors, plasminogen, heparan-sulphate proteoglycan, steroids with binding proteins).
- ▶ **Cumulus oophorus (discus proligerus)** — mound of granulosa cells projecting into the antrum, anchoring the oocyte to the wall.
- ▶ **Corona radiata** — innermost, radially arranged granulosa cells immediately investing the zona pellucida; sends microvilli through the zona to gap-junction with the oocyte.
- ▶ **Zona pellucida** — 5–10 µm thick glycoprotein coat (ZP1–ZP3, secreted by the oocyte), mediating species-specific sperm binding.
- ▶ **Primary oocyte** (~120–125 µm diameter) with its nucleus (germinal vesicle), arrested in prophase I of meiosis.

Layer (outside → in)	Composition	Key feature
Theca externa	Fibroblasts, collagen, smooth muscle	Merges with ovarian stroma; contracts at ovulation
Theca interna	Epithelioid, lipid-laden steroidogenic cells + capillaries	LH receptors; secretes androstenedione
Basement membrane	Acellular basal lamina	Avascular barrier — blocks vessels from entering granulosa
Membrana granulosa	Stratified granulosa cells	FSH receptors, aromatase; Call-Exner bodies
Antrum	Liquor folliculi	Single confluent fluid cavity
Cumulus oophorus/corona radiata	Granulosa cells around oocyte	Carried with oocyte at ovulation
Zona pellucida	Glycoprotein layer (ZP1–3)	Sperm receptor/binding site

3. The oocyte within the Graafian follicle

- ▶ The **primary oocyte** has been arrested in **prophase of meiosis I** since fetal life and remains so throughout follicular growth.
- ▶ **Just before ovulation**, under the influence of the **LH surge**, the primary oocyte **completes meiosis I**: chromosomes divide equally, but nearly all the cytoplasm goes to one daughter cell — the **secondary oocyte** — while the other becomes the small, non-viable **first polar body**.
- ▶ The secondary oocyte immediately enters the **second meiotic division** but arrests again, this time at **metaphase II**, and will complete meiosis only if fertilised.
- ▶ Growth of the oocyte itself has already ceased at the secondary-follicle stage; further increase in follicle size is due to antral fluid, not oocyte growth. This growth arrest is enforced by a small (1–2 kDa) granulosa-cell peptide, **oocyte maturation inhibitor (OMI)**, whose antral concentration is inversely related to follicle size (highest in small follicles, lowest in the mature Graafian follicle).

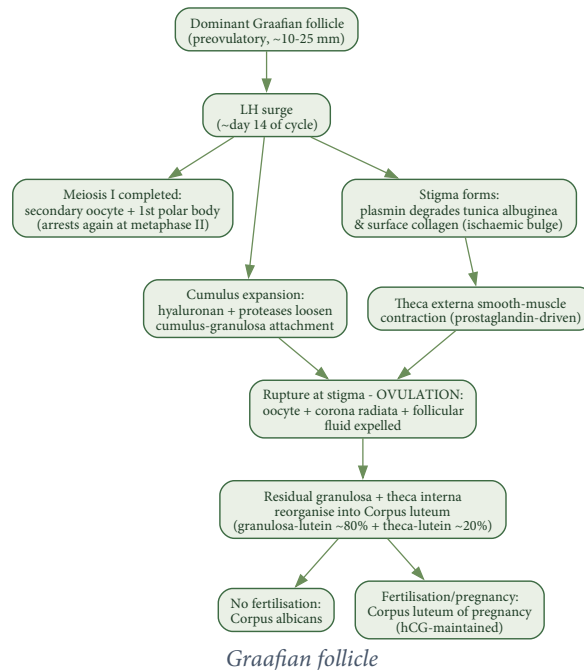
4. Hormonal regulation — the two-cell, two-gonadotropin mechanism

Hormone	Target cell	Receptor/enzyme	Action
FSH	Granulosa cells	FSH receptors; induces aromatase	Drives granulosa proliferation, antrum formation, and aromatisation of androgen → estradiol
LH	Theca interna cells	LH receptors	Stimulates steroidogenesis of androstenedione/androgens from cholesterol (theca interna itself lacks aromatase and cannot make estrogen)
Estrogen (from granulosa)	Hypothalamus–pituitary; endometrium; granulosa itself	—	Local: stimulates further granulosa proliferation. Systemic: endometrial proliferation; near mid-cycle, high sustained estradiol switches to a positive feedback on the hypothalamo-pituitary axis, triggering the LH surge
Inhibin (granulosa/luteal cells)	Hypothalamus, anterior pituitary	—	Reinforces negative feedback, selectively suppressing FSH

- ▶ Androgens diffuse from the theca interna across the basement membrane into the avascular granulosa layer, where **aromatase** (an FSH-dependent enzyme) converts them to **estradiol** — the essence of the **two-cell, two-gonadotropin theory**.

- ▶ As the follicle matures, increasing microvilli on the granulosa cells' free (antral) surface correlate with the appearance of **LH receptors on granulosa cells** as well — this priming is what allows the LH surge to trigger both ovulation and subsequent **luteinisation** of the granulosa.

5. Ovulation and fate of the Graafian follicle



- ▶ The **LH surge** (about 24 hours before rupture) simultaneously triggers: (i) resumption/completion of meiosis I in the oocyte; (ii) **cumulus expansion** — granulosa cells of the cumulus secrete hyaluronan, swelling the extracellular matrix and loosening the oocyte–cumulus mass from the follicle wall; (iii) **stigma** formation — an ischaemic, avascular point on the bulging follicle where activated **plasmin** degrades collagen of the tunica albuginea and surface epithelium; and (iv) **prostaglandin**-mediated smooth-muscle contraction of the theca externa.
- ▶ Rising intrafollicular pressure plus local wall weakening cause **rupture at the stigma**; the **oocyte, its corona radiata, and follicular fluid** are extruded and swept into the fimbriated end of the uterine tube.
- ▶ The collapsed, blood-filled follicular remnant reorganises into the **corpus luteum**: granulosa cells become large, pale **granulosa-lutein cells** (~80% of the cell population, producing progesterone and aromatising thecal androgens to estrogen), while theca interna cells become smaller, darker **theca-lutein cells** (~20%, producing progesterone, some estrogen and androgens).
- ▶ Absent fertilisation, the corpus luteum regresses into the fibrous **corpus albicans**; with fertilisation, it is maintained as the **corpus luteum of pregnancy** under hCG stimulation.

Applied / Clinical anatomy

- ▶ **Follicular monitoring/ART**: the Graafian follicle's size is directly tracked by transvaginal ultrasound during ovulation induction/IVF; the ovulatory (hCG) trigger is timed once the leading follicle approaches the mature ~18–20 mm range, and **transvaginal ultrasound-guided needle aspiration** of the Graafian follicle is the basis of oocyte retrieval in assisted reproduction.

- ▶ **Polycystic ovary syndrome (PCOS):** enlarged ovaries with numerous arrested antral follicles and an anovulatory state, associated with increased ovarian/adrenal androgen production — a common cause of infertility.
- ▶ **Follicular cysts:** a late antral/Graafian follicle that fails to rupture can persist as a thin-walled, fluid-filled cyst with both granulosa and thecal endocrine cells; these may raise estrogen levels enough to cause menstrual irregularity, and can become **haemorrhagic** if follicular vessels are disrupted.
- ▶ **Mittelschmerz:** transient mid-cycle pelvic pain attributed to peritoneal irritation by follicular fluid/blood released at rupture of the stigma.
- ▶ **Multiple ovulation:** simultaneous rupture of more than one Graafian follicle is the basis of **dizygotic (fraternal) twinning**.
- ▶ **Granulosa cell tumour:** Call-Exner bodies (rosette-like PAS-positive granulosa arrangements, structurally related to the normal antral follicle) are a characteristic diagnostic histological clue.
- ▶ **Ovarian reserve/menopause:** progressive depletion of the follicle pool by cyclical recruitment and atresia (and not primarily by ovulation) eventually reduces the reserve to under ~1000 follicles, marking the approach of menopause.

High-yield exam pointers

- ▶ Graafian follicle = mature/tertiary/preovulatory follicle, terminal stage of folliculogenesis; diameter **10–25 mm** (≈ 2 cm) just before ovulation; only **one** matures and ovulates per cycle.
- ▶ Layer sequence outside $\rightarrow$ in: **theca externa** $\rightarrow$ **theca interna** $\rightarrow$ **basement membrane** $\rightarrow$ **membrana granulosa** $\rightarrow$ **antrum (liquor folliculi)** $\rightarrow$ **cumulus oophorus** $\rightarrow$ **corona radiata** $\rightarrow$ **zona pellucida** $\rightarrow$ **oocyte**.
- ▶ **Two-cell, two-gonadotropin theory:** LH $\rightarrow$ theca interna $\rightarrow$ androgens (no aromatase there); FSH $\rightarrow$ granulosa cells $\rightarrow$ aromatase $\rightarrow$ estradiol.
- ▶ Oocyte arrested in **prophase I** since fetal life; completes meiosis I just before ovulation (LH surge) $\rightarrow$ secondary oocyte + first polar body; arrests again at **metaphase II** until fertilisation.
- ▶ Growth arrest of the oocyte maintained by **oocyte maturation inhibitor (OMI)** from granulosa cells.
- ▶ Ovulation (~day 14/28-day cycle) driven by the **LH surge**: cumulus expansion, plasmin-mediated stigma rupture, prostaglandin-driven theca-externa contraction.
- ▶ Post-ovulation remnant forms the **corpus luteum** (granulosa-lutein ~80% + theca-lutein ~20%) $\rightarrow$ corpus albicans (no pregnancy) or corpus luteum of pregnancy (hCG).
- ▶ Applied: PCOS, follicular/haemorrhagic cysts, ultrasound follicle tracking for ART, Call-Exner bodies in granulosa cell tumours, dizygotic twinning.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Langman's Medical Embryology (Sadler) 14e.



Histological structure of the uterine tube

Asked in: Paper III Nov 2016 — RGUHS MD/MS Anatomy

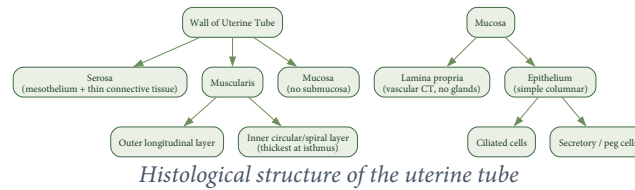
Definition / Introduction The uterine (Fallopian) tubes are paired mucomuscular tubes, about 10–12 cm long, extending from the cornu of the uterus to the region of the ovary; they capture the ovulated oocyte, provide the site and micro-environment for fertilisation, and transport the zygote/embryo to the uterine cavity. Histologically, each tube shows the typical three-layered organisation of a hollow viscus — an outer **serosa**, an intermediate **muscularis**, and an inner, highly folded **mucosa** — but, uniquely, it has **no submucosa and no muscularis mucosae**. The mucosal architecture and epithelial cell population vary along the length of the tube and across the menstrual cycle, closely reflecting its transport and secretory functions.

Gross parts (orientation for the histology)

Part	Location / dimensions	Salient feature
Infundibulum	Funnel-shaped, most lateral/distal part; opens into the peritoneal cavity at the abdominal ostium	Fringed by finger-like fimbriae (~1 mm wide); the longest, the ovarian fimbria , is applied to the tubal pole of the ovary
Ampulla	Widest part, ~5 cm long, luminal diameter up to 1 cm; thin-walled	Longest segment (about two-thirds of tube length); mucosal folds are maximal and labyrinthine here; usual site of fertilisation
Isthmus	Narrow, thick-walled, rounded and firm; ~3 cm long, 1–5 mm wide	Adjoins the uterus; circular muscle is thickest here, acting almost as a sphincter that regulates passage of the fertilised ovum into the uterus
Interstitial / intramural (uterine) part	~1 cm long, ~0.7 mm wide; traverses the myometrium	Mucosal folds are minimal/absent; opens into the uterine cavity at the uterine ostium

General plan of the wall

- ▶ **Serosa (peritoneal coat)** — outermost; a thin layer of connective tissue covered by mesothelium; covers the whole tube except along its lower (mesosalpingeal) border and the intramural part.
- ▶ **Muscularis** — smooth muscle arranged as an **inner circular (spiral)** layer and an **outer, thinner longitudinal** layer, the boundary between the two often indistinct; their coordinated contraction produces peristalsis.
- ▶ **Mucosa** — thrown into longitudinal folds throughout the length of the tube; lined by simple columnar epithelium resting on a vascular lamina propria.
- ▶ There is **no submucosa and no muscularis mucosae** — the lamina propria abuts the muscularis directly, a distinguishing feature from most other tubular viscera.



Histological structure of the uterine tube

□ Fig 31.7 — Histology of uterine tube (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.479)

Mucosa — folds, lamina propria and epithelial cell types

- **Mucosal folds:** thin, longitudinal, branching folds project into the lumen throughout the tube. They are **most numerous and complex in the ampulla**, where the cross-section resembles a labyrinth, become progressively fewer and simpler in the isthmus, and are minimal/absent in the intramural part.
- **Lamina propria:** a highly cellular, vascular connective-tissue core within each fold; it is richly supplied with capillaries and lymphatics but contains **no glands**.
- **Epithelium:** a single-layered, tall **simple columnar epithelium** composed of two principal, interconvertible functional cell types (regarded by several texts as different functional states of one progenitor cell):

Cell type	Distribution	Function
Ciliated cells	Most numerous in the infundibulum and ampulla	Cilia beat toward the uterus, sweeping the oocyte/zygote and the peg-cell secretions along the tube
Non-ciliated secretory (peg) cells	Relatively more numerous toward the isthmus; project further into the lumen than their ciliated neighbours (hence "peg")	Secrete a glycoprotein-rich, nutritive mucous film that bathes and nourishes the oocyte/sperm/zygote and supplies factors that assist capacitation of spermatozoa

- Some texts (e.g. Sontakke) additionally describe a third, **intercalary (basal) cell** population, sandwiched between the ciliated and secretory cells, regarded as a reserve/stem-cell pool for the two principal cell types.
- Occasional **intraepithelial lymphocytes** are also described within the epithelium.

Muscularis and serosa

- The **muscularis** consists of an inner circular (or spiral) and an outer, thinner longitudinal layer of smooth muscle, continuous with the myometrium at the intramural end. It is thickest in the **isthmus**, where it behaves in a sphincter-like fashion controlling the passage of the fertilised ovum into the uterine cavity.
- Coordinated peristaltic contraction of the muscularis, together with ciliary beating of the mucosa, produces the **bidirectional transport** required by the tube — spermatozoa moving toward the ampulla, and the oocyte/zygote moving toward the uterus.
- The **serosa** is a thin outer coat of loose connective tissue covered by mesothelium (visceral peritoneum), continuous with the broad ligament/mesosalpinx; it is deficient along the mesosalpingeal border and over the intramural part.

Cyclic (hormonal) histological changes

The height of the epithelium and the ciliated : secretory cell ratio change with the ovarian/menstrual cycle, driven by oestrogen and progesterone:

Phase	Epithelial height / ciliation	Peg-cell secretory activity
Proliferative (oestrogenic, follicular) phase	Epithelium hypertrophies; oestrogen stimulates ciliogenesis — cilia increase in number and cells become taller	Relatively quiescent
Around ovulation	Epithelium reaches maximal height ($\approx 30 \mu\text{m}$)	Peg cells at their most active/developed state , providing maximal nutritive secretion — progesterone increases secretory-cell numbers
Late secretory (luteal)/premenstrual phase	Epithelium atrophies to about half its peak height; ciliated cells reduced	Secretory activity declines
Postmenopause	Epithelium regresses further; ciliated cells markedly reduced	Minimal

Blood supply, innervation and tubal transport

- **Arterial supply:** the lateral third of the tube by the **ovarian artery** (via the mesosalpinx), the medial two-thirds by the **uterine artery**; the two anastomose within the mesosalpinx.
- **Innervation:** a dual autonomic supply running mainly with the ovarian and uterine vessels — preganglionic sympathetic fibres from T10–L2 spinal segments, and preganglionic parasympathetic fibres from the vagus (lateral half of the tube) and the pelvic splanchnic nerves (medial half). The **ampullary submucosa contains modified Pacinian corpuscles**.
- **Mechanism of transport:** propulsion of the gametes/zygote is a combined effect of ciliary beating (which sweeps fluid and the oocyte toward the uterus and is important for initial pick-up at the fimbrial end) and peristaltic contraction of the muscularis, which is the dominant force driving the ovum/zygote through the tube; fertilisation typically occurs in the ampulla near the ampullary–isthmic junction, and the ovum/zygote remains within the tube for about 3 days before entering the uterine cavity.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a circular cross-section of the uterine tube (ampullary level) and label, from lumen outward:

- ▶ **Fold of mucosa** projecting into the lumen (draw several branching folds)
- ▶ Inset of one fold at high power showing **ciliated and non-ciliated (peg) columnar cells** on the epithelial surface
- ▶ **Lamina propria** — cellular, vascular connective-tissue core of the fold
- ▶ **Inner circular coat** (muscularis)
- ▶ **Outer longitudinal coat** (muscularis)
- ▶ **Serosa** — outermost mesothelial covering

Add a side note: no submucosa/muscularis mucosae present between mucosa and muscularis.

Applied / Clinical anatomy

- ▶ **Ectopic (tubal) pregnancy**: the uterine tube is the commonest site of ectopic implantation (about 98% of ectopic pregnancies), most often in the ampulla; the thin ampullary wall and the sphincteric, thick-walled isthmus predispose to tubal rupture and haemoperitoneum if implantation occurs there.
- ▶ **Salpingitis / pelvic inflammatory disease**: infection (commonly ascending gonococcal/chlamydial) damages the ciliated mucosal folds, causing agglutination of folds, tubal blockage, hydrosalpinx and adhesions — a major cause of tubal-factor infertility and a predisposing factor for ectopic pregnancy.
- ▶ **Endometriosis**: retrograde menstrual flow through the uterine tubes can seed viable endometrial cells onto the ovaries, tubal serosa or peritoneum, producing ectopic endometrial deposits that respond cyclically to ovarian hormones.
- ▶ **Primary ciliary dyskinesia (Kartagener/immotile-cilia syndrome)**: impaired ciliary beating in the tubal epithelium is classically linked to sub-fertility from defective ovum transport; muscular peristalsis of the tube, however, is thought to be the dominant transport force, so tubal transport of the embryo may still proceed even when cilia are immotile.
- ▶ **Tubal sterilisation (ligation)**: commonly performed at or near the isthmus (e.g., Pomeroy technique, Falope ring), exploiting its narrow, accessible, reproducibly identifiable location.
- ▶ **Hysterosalpingography (HSG)**: radiographic assessment of tubal patency — contrast fills the narrow intramural and isthmic lumina before expanding into the wider ampulla, with free spill into the peritoneal cavity confirming patency.
- ▶ **Capacitation and nutrition**: peg-cell secretions provide the nutritive and capacitating micro-environment essential for sperm maturation and early embryonic (morula-stage) development before the conceptus reaches the uterus.

High-yield exam pointers

- ▶ Four gross parts: infundibulum (with fimbriae) → ampulla → isthmus → interstitial/intramural part.

- ▶ Three-layered wall — serosa, muscularis (inner circular + outer longitudinal), mucosa — but **no submucosa/muscularis mucosae**.
- ▶ Mucosal folds maximal in ampulla (labyrinthine), minimal/absent in intramural part.
- ▶ Two principal epithelial cell types: **ciliated cells** (most in infundibulum/ampulla, beat toward uterus) and **secretory/peg cells** (nutritive secretion, capacitation factors); some texts add a basal/intercalary reserve-cell type.
- ▶ Ampulla = commonest site of fertilisation and of tubal ectopic pregnancy; isthmus muscle is thickest and sphincteric.
- ▶ Epithelial height and ciliation are oestrogen/progesterone-dependent, peaking around ovulation (~30 µm) and regressing before menstruation and postmenopausally.
- ▶ Blood supply: ovarian artery (lateral 1/3) + uterine artery (medial 2/3); ampullary submucosa has modified Pacinian corpuscles.
- ▶ Transport is bidirectional — ciliary beating plus peristalsis of the muscularis.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; BD Chaurasia — Human Anatomy Vol 2 8e (figure).



Histology of active and passive Mammary gland

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition/Introduction The mammary gland is a modified, apocrine-type cutaneous gland derived embryologically from ectodermal thickenings of the milk line, and it exists in a spectrum of functional states — **inactive/resting (passive)** in the non-pregnant adult, and **active (lactating)** during pregnancy and lactation. Both states share the same basic architecture (15–20 lobes, each drained by a lactiferous duct, subdivided into lobules called terminal duct lobular units, TDLUs) but differ strikingly in the proportion of glandular parenchyma to fibrofatty stroma and in the secretory activity of the alveolar epithelium. This dichotomy — resting vs lactating histology — is the commonest way the topic is examined.

General Architecture (common to both states)

- ▶ Mammary gland = a modified, highly branched **compound tubulo-alveolar apocrine sweat gland** lying in the superficial fascia of the pectoral region.
- ▶ Each gland has **15–20 pyramidal lobes**, radiating from the nipple, separated by dense interlobular connective tissue and adipose tissue; each lobe has its own excretory **lactiferous duct** opening independently at the nipple.
- ▶ Each lobe → several **lobules**, each drained by a segmental/terminal duct; the parenchyma drained by one terminal duct = the **terminal duct lobular unit (TDLU)** — the functional unit of the breast, comprising terminal ductules/acini embedded in specialized, hormone-sensitive

intralobular stroma (loose, cellular, fat-poor), itself surrounded by denser, fat-rich **interlobular stroma**.

- ▶ Fibrous septa connecting the deep fascia to the dermis are the **suspensory (Cooper's) ligaments**.
- ▶ Two principal cell types line the duct–acinar system throughout: **glandular (luminal) epithelial cells** and, deep to them, a discontinuous basket-like layer of **myoepithelial cells** applied to the basal lamina; myoepithelial contraction (oxytocin-driven) helps eject secretion.
- ▶ Near the nipple, duct epithelium shows a **gradual transition** — simple/stratified cuboidal in the smaller ducts → two-layered cuboidal in the lactiferous sinus → **stratified squamous keratinized** at its nipple orifice, continuous with epidermis.

Histology of the Resting (Inactive/Passive) Mammary Gland

□ Fig 24.2 — Histology of mammary gland. (A) Inactive mammary gland; (B) Lactating mammary gland (Sontakke — *Textbook of Human Histology* — 2e, p.319)

- ▶ Represents the gland from puberty to the first pregnancy, and between successive lactations.
- ▶ **Glandular tissue is scanty**; the bulk of the breast is made up of dense fibrocollagenous connective tissue and abundant adipose tissue that widely separate the lobules.
- ▶ Secretory portions are mostly **terminal ductules** (rudimentary, poorly-developed acini) rather than true alveoli — often solid cords or small tubules with a **large, clear lumen**, lined by **simple cuboidal epithelium**.
- ▶ Myoepithelial cells are present but sparse and less conspicuous on routine H&E.
- ▶ Intralobular stroma is comparatively **dense and inconspicuous**; interlobular stroma is fat-rich.
- ▶ Mild cyclical change occurs with the menstrual cycle: in the follicular phase estrogen stimulates duct proliferation (ductules appear as solid cords with little lumen); in the luteal phase progesterone causes slight alveolar budding and stromal oedema (premenstrual breast fullness/tenderness), followed by regression before menstruation.

Histology of the Active (Lactating) Mammary Gland

- ▶ Represents the gland during late pregnancy and lactation, when TDLUs undergo full morphological and functional maturation.
- ▶ **Glandular parenchyma predominates**; interlobular connective tissue septa become thin and inconspicuous, and adipose tissue is markedly reduced.
- ▶ Terminal ductules differentiate into numerous, closely packed, distended **secretory alveoli**, giving each lobule a compact, "grape-cluster" appearance.
- ▶ Alveoli are lined by **low columnar to cuboidal secretory epithelium** (height varies with the phase of secretion — columnar when accumulating product, reduced toward cuboidal just after discharge, never returning to the small resting size); a basket network of **myoepithelial cells** surrounds each alveolus and the smaller ducts.
- ▶ Alveolar lumina are **distended with eosinophilic, vacuolated secretion** (milk) — the vacuolation reflecting extracted/dissolved lipid droplets.
- ▶ Secretory cells show marked ultrastructural activity: abundant rough endoplasmic reticulum, prominent supranuclear Golgi apparatus, numerous mitochondria and lysosomes, and apical lipid droplets/secretory vesicles.

- Late in pregnancy, alveoli and ducts are distended with **colostrum** (protein/IgA-rich, alkaline, low-fat premilk); plasma cells and lymphocytes infiltrate the intralobular stroma and secrete IgA, transferred into colostrum/milk conferring passive immunity to the neonate.

Comparative Summary: Resting vs Lactating Mammary Gland

Feature	Resting (passive) gland	Lactating (active) gland
Stroma	More connective tissue and fat; less glandular tissue	More glandular tissue; less connective tissue and fat
Secretory units	Few, poorly developed, duct-like acini	Numerous, well-developed alveoli, closely packed
Alveolar epithelium	Cuboidal, with a wide/large lumen	Low columnar to cuboidal, with a narrow lumen (distended by secretion)
Excretory ducts	Narrow and empty	Wider, and filled with milk
Interlobular septa	Thick, prominent	Thin, inconspicuous

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a comparative diagram (resting vs lactating gland, low power):

- **Resting/inactive half:** interlobular fibrous & adipose stroma (predominant), lobule with sparse duct-like alveoli, cuboidal epithelium, wide clear lumen, myoepithelial cell layer, intralobular stroma.
- **Lactating half:** thin interlobular septum, closely packed distended alveoli, cuboidal/columnar secretory epithelium, alveolar lumen filled with vacuolated eosinophilic milk, myoepithelial "basket" cells, intralobular collecting duct, larger lactiferous duct.
- Common labels: nipple, areola, lactiferous sinus, lactiferous duct, terminal duct lobular unit (TDLU).

Secretory Mechanism and Milk Composition

Milk components leave the alveolar cell by two distinct mechanisms:

Component	Mode of secretion	Key detail
Proteins (casein, α -lactalbumin, IgA, lactoferrin, lysozyme, EGF)	Merocrine	Synthesised on rER, packaged in Golgi into secretory vesicles, released by exocytosis (vesicle membrane fuses with plasma membrane)
Lipid	Apocrine	Cytoplasmic lipid droplets coalesce, migrate apically, and are pinched off enveloped in a rim of plasma membrane ($\pm$ a thin cytoplasmic layer)

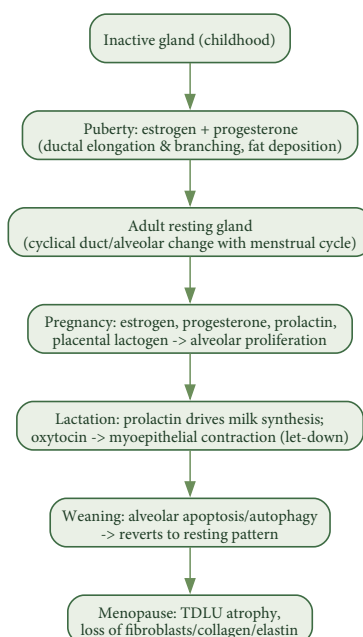
Component	Mode of secretion	Key detail
Lactose	Golgi-synthesised (α -lactalbumin-regulated enzyme)	Major carbohydrate/energy source; generates the osmotic gradient drawing water and Ca^{2+} into the alveolar lumen

Approximate content of mature human milk: protein ≈ 1 g/dL (44% casein), fat $\approx 4\text{--}5$ g/dL, lactose > 7 g/dL — lactose exceeding the combined protein + fat content.

Nipple, Areola and Duct System

- **Nipple:** conical projection pierced by 15–20 lactiferous duct openings; covered by pigmented, keratinized stratified squamous epithelium continuous with the duct lining at the tip; core has dense connective tissue with circular and longitudinal smooth muscle bundles (erection/retraction on suckling) and rich sensory innervation.
- **Areola:** pigmented skin around the nipple, darkens further and irreversibly with pregnancy; contains sebaceous glands, sweat glands, and modified/intermediate mammary glands — **glands of Montgomery (Montgomery's tubercles)** — which enlarge in pregnancy/lactation and secrete a lubricating, antimicrobial oily secretion.
- **Lactiferous sinus:** dilated portion of each duct beneath the areola acting as a milk reservoir; lined by stratified cuboidal epithelium.

Hormonal Regulation



Histology of active and passive Mammary gland

- **Puberty:** rising ovarian estrogen $\rightarrow$ adipose accumulation and ductal elongation/branching (mesenchymal proliferation); testosterone in males inhibits further growth, keeping the male gland rudimentary.
- **Menstrual cycle:** follicular-phase estrogen stimulates duct proliferation; luteal-phase progesterone stimulates alveolar budding and stromal oedema (cyclical breast fullness).

- ▶ **Pregnancy:** synergistic estrogen, progesterone, prolactin, human placental lactogen (and adrenal glucocorticoids) drive TDLU hyperplasia — 1st trimester (ductal elongation/branching), 2nd trimester (alveolar differentiation, plasma-cell/lymphocyte infiltration), 3rd trimester (alveolar maturation, colostrum accumulation); high progesterone keeps actual milk secretion suppressed until delivery.
- ▶ **Lactation:** withdrawal of placental estrogen/progesterone after parturition unmasks the lactogenic action of **prolactin** (anterior pituitary) → milk synthesis; suckling reflex → hypothalamic release of **oxytocin** (posterior pituitary) → myoepithelial contraction → milk-ejection ("let-down") reflex.
- ▶ **Weaning/involution:** withdrawal of suckling stimulus → alveolar epithelial apoptosis/autophagy, macrophage clearance of debris → duct system reverts to the resting pattern; **post-menopausal** involution shows further TDLU atrophy with loss of stromal fibroblasts, collagen and elastic fibres.

Applied / Clinical anatomy

- ▶ **Fibroadenoma** — most common benign breast tumour (fibrous + epithelial elements), typically age 15–30, presents as a solitary, discrete, freely mobile nodule.
- ▶ **Carcinoma of the breast** — the commonest malignancy of the breast; most (invasive ductal carcinoma) arises from epithelial cells of the TDLU/terminal ducts and invades the surrounding stroma as a fixed, painless, palpable mass; spreads via lymphatics (axillary/parasternal nodes) and blood to lung/brain. Risk rises with cumulative hormonal exposure (early menarche, late menopause, late first pregnancy); ~5% are linked to autosomal dominant **BRCA1/BRCA2** mutations.
- ▶ **Acute mastitis** — bacterial infection of the lactating (or involuting) breast, usually following ductal obstruction by retained milk.
- ▶ **Fibrocystic-type stromal/cyclical change** and premenstrual breast tenderness reflect the luteal-phase alveolar budding and stromal oedema described above.
- ▶ **Polymastia/polythelia** — accessory breast tissue/nipples along the persistent milk line (~1% incidence).
- ▶ **Gynaecomastia** — pathological ductal/stromal proliferation in the otherwise rudimentary male gland (e.g., hormonal imbalance, Klinefelter syndrome).
- ▶ Histological grading of breast carcinoma and core-needle/FNAC diagnosis both rely directly on recognising normal TDLU/duct histology described above.

High-yield exam pointers

- ▶ Mammary gland = modified apocrine sweat gland; 15–20 lobes, each with its own lactiferous duct opening at the nipple; TDLU is the functional lobular unit.
- ▶ Resting gland: stroma (fibrofatty) predominates over sparse, poorly-developed, cuboidal-lined, wide-lumen ducts/alveoli.
- ▶ Lactating gland: glandular tissue predominates over thin stroma; numerous, well-developed, columnar/cuboidal alveoli distended with milk; ducts wide and filled with milk.

- ▶ Myoepithelial cells (basket cells) lie between luminal epithelium and basal lamina throughout the duct–alveolar system; contract under oxytocin to cause milk ejection.
- ▶ Two secretory mechanisms: **merocrine** (proteins, by exocytosis) and **apocrine** (lipid, membrane-enveloped droplet extrusion).
- ▶ Nipple = keratinized stratified squamous epithelium + circular/longitudinal smooth muscle; areola contains glands of Montgomery.
- ▶ Hormonal axis: estrogen/progesterone (development) → prolactin (milk synthesis) → oxytocin (milk ejection reflex).
- ▶ Colostrum = IgA/protein-rich premilk secreted in the first few postpartum days, conferring passive immunity.
- ▶ Applied correlate: invasive ductal carcinoma arises from TDLU/terminal duct epithelium — the single most examined clinical link to this histology.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; BD Chaurasia — Human Anatomy Vol 1 8e (gynaecomastia/Klinefelter note); Gray's Anatomy (Standring) 41e (myoepithelial cell figure).



Histology of ovary

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction The **ovary** is the paired female gonad — an almond-shaped, dull pinkish-white organ (about 3 cm long, 1.5 cm wide, 1 cm thick) lying in the ovarian fossa attached to the posterior layer of the **broad ligament** by the **mesovarium**. It performs two linked functions: **gametogenesis** (oogenesis, i.e., production and periodic release of oocytes) and **endocrine secretion** (estrogen from follicular/granulosa cells, progesterone from luteal cells). Histologically it is organised into a surface germinal epithelium, an outer follicle-bearing cortex, and an inner vascular medulla, with no sharp boundary between the two.

General organisation — surface, capsule, cortex and medulla

- ▶ **Germinal epithelium**: a single layer of cuboidal (mesothelial) cells covering the ovarian surface, continuous with the peritoneal mesothelium. The name is a **misnomer** — it does *not* give rise to female gametes; oogonia instead arise from **primordial germ cells that migrate from the yolk sac** into the developing gonad.
- ▶ **Tunica albuginea**: a connective tissue capsule immediately deep to the germinal epithelium; unlike the testis, it is **thin and poorly demarcated** in the ovary.
- ▶ **Cortex**: the peripheral zone beneath the tunica albuginea. Consists of a highly cellular connective tissue **stroma** (reticular fibres and spindle-shaped fusiform/interstitial cells) housing **ovarian follicles at all stages of development, corpora lutea, corpora albicantia, and atretic follicles**.

- **Medulla:** the central zone — loose connective tissue with blood vessels, lymphatics and nerve fibres. The **hilum** is the site of entry of these vessels into the medulla; it also contains **hilar (hilus) cells**.
- Before puberty and regular ovulation the surface is smooth; thereafter repeated ovulation and scarring make it progressively irregular.

Ovarian follicles — stages of folliculogenesis

An ovarian follicle = one oocyte + its surrounding somatic (follicular/granulosa) cells within a basal lamina. Development passes through four morphological stages:

Stage	Oocyte	Surrounding cell layer(s)	Key features
Primordial follicle	~25–30 μm , arrested in prophase I (dictyotene stage)	Single layer of flattened (squamous) follicular cells	Smallest, most numerous; lie just beneath the tunica albuginea; dormant from birth to puberty
Primary follicle	Enlarging oocyte	Follicular cells become cuboidal (unilaminar), then proliferate into several layers = granulosa cells / stratum granulosum (multilaminar)	Zona pellucida appears — a PAS-positive glycoprotein layer (ZP1, ZP2, ZP3) secreted jointly by oocyte and granulosa cells; theca interna (inner, vascular, cuboidal) and theca externa (outer, fibrous/smooth muscle) differentiate from surrounding stroma
Secondary (antral) follicle	Oocyte reaches maximum size (~125 μm); growth then arrested by oocyte maturation inhibitor	Granulosa cells proliferate; small fluid-filled spaces coalesce into a single antrum filled with liquor folliculi ; some granulosa cells form the cumulus oophorus	Call-Exner bodies (PAS-positive, hyaluronic acid/proteoglycan) appear between granulosa cells
Graafian (mature) follicle	Completes 1st meiotic division just before ovulation → secondary oocyte + first polar body	Cumulus-oophorus cells immediately around the oocyte form the corona radiata ; theca interna secretes androgens, aromatised to estrogen by granulosa cells	~10 mm or more in diameter; bulges on the ovarian surface

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

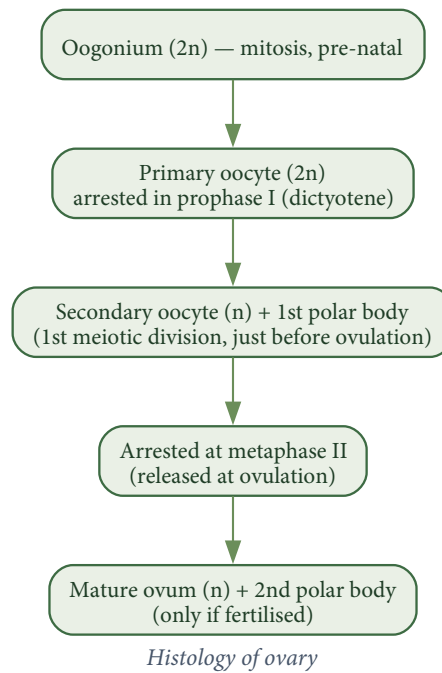
Draw a circular low-power outline of the ovarian cortex and label exactly:

- ▶ **Surface (germinal) epithelium** and **tunica albuginea** at the periphery.
- ▶ **Cortex** bracket containing, from superficial to deep: **primordial follicle**, **primary follicle**, **growing/secondary follicle** (with antrum), and a **corpus luteum** (cluster of large luteal cells).
- ▶ **Medulla** with **capillary/blood vessels** centrally.
- ▶ Optionally add a small **atretic follicle** and **corpus albicans** as extra labels for completeness.

□ Fig 31.5 — Histology of ovary (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.476)

Oogenesis (nuclear maturation of the oocyte)

- ▶ **Oogonia** are derived from primordial germ cells that migrate from the yolk sac to the gonadal cortex; they multiply mitotically and enlarge to form **primary oocytes**, all formed **before birth** (no post-natal renewal).
- ▶ Primary oocytes complete prophase of the first meiotic division and are arrested at the **dictyotene (diplotene) stage** by an oocyte maturation inhibitor (OMI) — this arrest lasts from before birth until just before ovulation, decades later.
- ▶ Of the roughly **6–8 lakh** ($\approx 6,00,000$ – $8,00,000$) **primary oocytes** present, only about **4 lakh** **persist to puberty**, and only about **400 are ever ovulated** across a woman's reproductive life; the rest are lost to atresia.
- ▶ With each menstrual cycle, the primary oocyte destined to ovulate completes the **first meiotic division** shortly before ovulation, producing a **secondary oocyte** (bulk of cytoplasm) and a **first polar body** (discarded).
- ▶ The secondary oocyte immediately enters the **second meiotic division** but is **arrested at metaphase II**; this division is completed — yielding the mature **ovum** and a **second polar body** — only if fertilisation occurs.



Ovulation

- ▶ Triggered by the mid-cycle **LH surge** (around day 14 of a 28-day cycle).
- ▶ Increased follicular fluid volume, plasminogen-mediated proteolysis of follicular wall cells, and prostaglandin-induced contraction of theca externa smooth muscle together rupture the follicle.
- ▶ A small avascular necrotic zone on the bulging surface — the **macula pellucida (stigma)** — is the point of rupture through which the oocyte is extruded.
- ▶ The oocyte released is a **secondary oocyte surrounded by zona pellucida and corona radiata**, not a fully mature ovum.
- ▶ If unfertilised, the secondary oocyte remains viable for about **24–48 hours** before degenerating.

Corpus luteum and follicular atresia

- ▶ After ovulation the collapsed follicle wall becomes folded; granulosa and theca interna cells hypertrophy, accumulate the yellow pigment **lipochrome**, and luteinise to form the **corpus luteum**.
- ▶ **Granulosa lutein cells** — large, centrally placed, majority population — secrete **progesterone** and convert theca-derived androgens to estrogen.
- ▶ **Theca lutein cells** — smaller, peripherally placed — secrete progesterone, some estrogen, and androgens.
- ▶ If fertilisation does not occur, the **corpus luteum of menstruation** persists about 14 days before regressing; if fertilisation occurs, the **corpus luteum of pregnancy** persists 3–4 months (its progesterone supports early pregnancy until the placenta takes over).
- ▶ Degeneration converts the corpus luteum into a hyalinised fibrous scar, the **corpus albicans**.
- ▶ **Follicular atresia**: most follicles, at any stage, undergo degeneration rather than ovulation — beginning with granulosa cell apoptosis and autolysis, followed by hypertrophy of theca interna cells and replacement by connective tissue; the basement membrane between granulosa and theca interna thickens into a characteristic wavy **hyaline (glassy) membrane**. Macrophages

phagocytose the degenerating oocyte, zona pellucida and granulosa cells. Some persisting theca-derived cells form **interstitial gland cells (corpora atretica)**, which continue to secrete estrogen.

- ▶ **Hilar (hilus) cells**, found at the ovarian hilum, resemble testicular Leydig cells, contain **Reinke crystalloids**, and secrete androgens.

Applied / Clinical anatomy

- ▶ **Polycystic ovarian disease (Stein–Leventhal syndrome)**: ovaries enlarged, studded with numerous fluid-filled follicular cysts arrested at the antral stage with a thickened tunica albuginea; associated with anovulation and irregular/scanty menstruation — a common histology-based diagnosis and cause of infertility.
- ▶ **Ovarian tumour classification follows the three principal cellular components of the histology described above** — surface (germinal) epithelium ($\approx 70\%$ of ovarian tumours — serous/mucinous cystadenomas etc.), germ cells (oocyte lineage), and sex-cord/stromal cells (granulosa, theca, hilar lineage — e.g., estrogen-secreting granulosa cell tumour, androgen-secreting hilar-cell/Sertoli–Leydig tumours).
- ▶ **Mittelschmerz**: mid-cycle ovulation pain, experienced by a significant proportion of women, correlating with follicular rupture and the resulting peritoneal irritation.
- ▶ **Pregnancy test basis**: hCG produced by the corpus luteum of pregnancy is detectable in urine within 10–14 days of conception.
- ▶ **Corpus luteal/haemorrhagic cyst**: excessive bleeding into the collapsed follicle (corpus hemorrhagicum) can persist as a symptomatic ovarian cyst.
- ▶ **Endometriosis**: ectopic endometrial glands and stroma most commonly implant on the ovary (followed by the pouch of Douglas), producing the classic "chocolate cyst."

High-yield exam pointers

- ▶ Ovary = germinal epithelium (misnomer) → tunica albuginea → cortex (follicles, corpus luteum, corpus albicans, atretic follicles) → medulla (vessels, hilum); no sharp cortico-medullary boundary.
- ▶ Follicle sequence: **primordial** → **primary** (zona pellucida appears) → **secondary/antral** (antrum, liquor folliculi, Call–Exner bodies) → **Graafian** (corona radiata, ~ 10 mm).
- ▶ Theca interna (vascular, secretes androgen) + theca externa (fibrous) differentiate from stroma around the primary follicle; granulosa cells aromatise androgen to estrogen.
- ▶ Oogenesis: primary oocyte arrested at **dictyotene** until ovulation → 1st meiosis gives secondary oocyte + 1st polar body → 2nd meiosis arrested at **metaphase II**, completed only on fertilisation.
- ▶ Ovulation = LH surge-driven rupture through the **macula pellucida (stigma)**; releases a secondary oocyte, not a mature ovum.
- ▶ Corpus luteum: granulosa-lutein cells (progesterone) + theca-lutein cells (estrogen/androgen) → regresses to corpus albicans if no pregnancy; persists as corpus luteum of pregnancy (hCG-dependent) if fertilisation occurs.
- ▶ Atresia is the fate of the vast majority of follicles; hallmark = thick wavy **hyaline (glassy) membrane**.
- ▶ Hilar cells (Reinke crystalloids) = ovarian analogue of testicular Leydig cells.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology (2e); IB Singh — Textbook of Human Histology (9e); Junqueira's Basic Histology — Text & Atlas (17e); BD Chaurasia's Human Anatomy Vol 2 (8e); Gray's Anatomy (Standing) 41e.



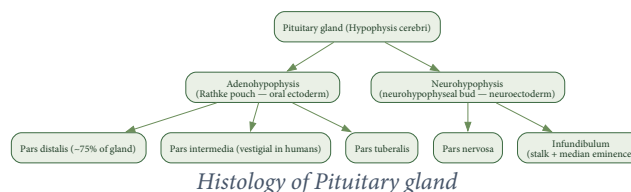
Histology of Pituitary gland

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction — The pituitary gland (hypophysis cerebri) is a reddish-grey, ovoid endocrine organ, about 12 mm transversely and 8 mm antero-posteriorly, lodged in the hypophyseal (pituitary) fossa of the sphenoid bone and suspended from the tuber cinereum of the hypothalamus by the infundibulum (pituitary stalk). It is enclosed by a thin connective-tissue capsule and has a dual embryological origin — an ectodermal outgrowth of the roof of the stomodeum (Rathke pouch) forming the **adenohypophysis**, and a neuroectodermal downgrowth of the floor of the diencephalon (neurohypophyseal bud) forming the **neurohypophysis**. This dual origin explains why one part is a true glandular epithelium and the other a modified neural (glial) tissue, and it is the histological basis of the gland's role as the "master endocrine gland."

1. Subdivisions of the gland

Division	Embryonic origin	Parts
Adenohypophysis	Hypophyseal (Rathke) pouch — oral ectoderm	Pars distalis (pars anterior), Pars intermedia, Pars tuberalis
Neurohypophysis	Neurohypophyseal bud — neuroectoderm of diencephalon	Pars nervosa (pars posterior), Infundibulum (stalk + median eminence)



- The whole gland is invested by a thin fibrous **capsule**; delicate reticular connective tissue carries the rich sinusoidal capillary bed into the parenchyma.
- The **pars distalis** is the largest subdivision, forming about 75% of the adenohypophysis.

2. Pars distalis — cell types

Cells are arranged in anastomosing **corde**s and **nest**s separated by a delicate reticular stroma and fenestrated **sinusoidal capillaries**. Classically, three cell types are recognised by tinctorial (H&E / trichrome) staining; immunocytochemistry further resolves five functional cell types, each secreting a defined hormone.

Classical (tinctorial) type	% of cells	Functional cell type	% of cells	Hormone secreted	Key stain
Acidophil (eosin/orange-G +ve)	~40%	Somatotroph (GH cell)	~50%	Growth hormone (GH)	Orange G, eosin
		Lactotroph/ Mammothroph (PRL cell)	15–20%	Prolactin (PRL)	Erythrosine/ carmoisine
Basophil (haematoxylin /PAS +ve)	~10%	Corticotroph (ACTH cell)	15–20%	ACTH (from POMC)	PAS +, lead haematoxylin
		Gonadotroph (FSH/LH cell)	~10%	FSH, LH	PAS +
		Thyrotroph (TSH cell)	~5% (least numerous)	TSH	PAS +
Chromophobe (poorly staining)	~50%	Degranulated chromophils / resting reserve cells / folliculo-stellate cells	—	Non-secretory (mostly)	Unstained/ pale

- ▶ **Acidophils** are rounded cells with eosinophilic, orange-G-positive granules; **basophils** stain with haematoxylin and are **PAS-positive** because their hormones (ACTH via POMC, FSH, LH, TSH) are glycoproteins. **Chromophobes** lack conspicuous granules and appear pale — many represent degranulated chromophils rather than a distinct resting cell population.
- ▶ **Folliculo-stellate cells** are non-hormone-secreting, star-shaped supportive cells interconnected by gap junctions; they form an intercommunicating network thought to mediate paracrine signalling within the pars distalis.
- ▶ Ultrastructurally each of the five functional types has a characteristic secretory-granule size/density, permitting identification by electron microscopy in addition to immunocytochemistry.

3. Pars tuberalis and pars intermedia

- ▶ **Pars tuberalis**: a thin, funnel-shaped, highly vascular collar of cells that wraps around the infundibulum; it is composed predominantly of **gonadotrophs**, arranged as low cuboidal cells around blood vessels of the hypophyseal portal system.
- ▶ **Pars intermedia**: a narrow zone between the pars distalis and pars nervosa; in humans it is **poorly developed (vestigial)**. It contains a mixture of **basophils** (chiefly corticotrophs producing POMC, cleaved here into γ -LPH, β -endorphin, MSH-related peptides rather than ACTH) and chromophobes, together with small **colloid-filled cysts/follicles** that are remnants of the lumen of the embryonic Rathke pouch.

4. Neurohypophysis (pars nervosa + infundibulum)

- ▶ Histologically this is **not a true gland** but a neural structure composed of:
 - Unmyelinated axons of the **hypothalamo-hypophyseal tract**, arising from neurosecretory neurons of the **supraoptic and paraventricular nuclei** of the hypothalamus.
 - **Herring bodies** — irregular, eosinophilic dilatations of these axon terminals, packed with accumulated neurosecretory granules containing **oxytocin or antidiuretic hormone (ADH/vasopressin)** bound to their respective carrier proteins (neurophysins).
 - **Pituicytes** — modified, glial (astrocyte-like) supporting cells with elongated processes; they do not themselves synthesise hormone but support and ensheath the axons and their terminal endings.
 - A rich network of **fenestrated capillaries** onto which the axon terminals/Herring bodies abut, permitting release of neurohormones directly into the blood.

5. Blood supply and the hypophyseal portal system (functional histology)

- ▶ **Superior hypophyseal artery** (from internal carotid) → **primary capillary plexus** in the median eminence/infundibulum → **hypophyseal portal veins** running along the pars tuberalis → **secondary (sinusoidal, fenestrated) capillary plexus** in the pars distalis.
- ▶ This **hypothalamo-hypophyseal portal system** is the histological basis by which hypothalamic releasing/inhibiting hormones (GHRH, somatostatin, TRH, CRH, GnRH, dopamine) reach the pars distalis and regulate each hormone-secreting cell type without passing through the systemic circulation.
- ▶ **Inferior hypophyseal artery** supplies the pars nervosa directly and has little connection with the portal system.
- ▶ Venous drainage from both plexuses is into the **cavernous sinus**, then to the internal jugular vein.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 25.4 — Histology of pituitary gland (low magnification whole-gland section on left; high magnification of pars distalis / pars intermedia / neurohypophysis junction on right, showing acidophils, basophils, chromophobes, sinusoidal capillaries, colloid, follicles, Herring bodies and pituicytes) (Sontakke — Textbook of Human Histology — 2e, p.333)

Reproduce a labelled low-power + high-power diagram of the pituitary with these exact labels:

- ▶ Capsule; Infundibulum; Pars tuberalis
- ▶ Pars distalis — cords/nests of cells, sinusoidal capillaries, acidophils (alpha cells), basophils (beta cells), chromophobes, chromophils
- ▶ Pars intermedia — colloid, follicles (Rathke-pouch remnant cysts)
- ▶ Neurohypophysis (pars nervosa) — pituicytes, Herring bodies, unmyelinated nerve fibres

Applied / Clinical anatomy

- ▶ **Pituitary adenomas** are classified by their cell-of-origin tinctorial/functional type: **acidophil (somatotroph) adenoma** → gigantism (children) / acromegaly (adults); **basophil (corticotroph) adenoma** → Cushing disease; **chromophobe adenoma** → usually non-functioning but large

enough to compress the optic chiasm, causing **bitemporal hemianopia**, and to erode the sella turcica (radiologically visible on MRI/CT as sellar enlargement).

- ▶ **Prolactinoma** (lactotroph adenoma) is the commonest functioning pituitary tumour, presenting with galactorrhoea–amenorrhoea; physiological lactotroph hyperplasia during pregnancy/lactation likewise enlarges the gland.
- ▶ **Rathke cleft cyst / craniopharyngioma**: arise from remnants of the Rathke pouch lumen (the same colloid-filled cysts normally seen in the pars intermedia).
- ▶ **Sheehan syndrome**: post-partum hypotension/haemorrhage causes ischaemic necrosis of the pars distalis because its sinusoidal supply is entirely portal (low-pressure), while the pars nervosa (direct arterial supply via the inferior hypophyseal artery) is relatively spared.
- ▶ **Pituitary stalk section/compression** interrupts the hypophyseal portal flow and hypothalamic axonal input, causing loss of anterior hormone regulation and diabetes insipidus (loss of ADH delivery from the neurohypophysis).
- ▶ **Surgical relevance**: the trans-sphenoidal route to the pituitary fossa exploits its position immediately above the sphenoid sinus, in relation to the optic chiasm superiorly and the cavernous sinuses (with III, IV, VI nerves and internal carotid artery) laterally.

High-yield exam pointers

- ▶ Adenohypophysis (glandular, from Rathke pouch) + Neurohypophysis (neural, from diencephalon) — dual origin is the key concept.
- ▶ Pars distalis = 75% of adenohypophysis; classical tinctorial cells: acidophils 40%, basophils 10%, chromophobes 50%.
- ▶ Five functional cell types (immunocytochemistry): somatotrophs (~50%, GH), lactotrophs (15–20%, PRL), corticotrophs (15–20%, ACTH), gonadotrophs (~10%, FSH/LH), thyrotrophs (~5%, TSH, least numerous).
- ▶ Basophils are PAS-positive (glycoprotein hormones); acidophils are PAS-negative.
- ▶ Pars intermedia is vestigial in humans; contains colloid-filled cysts, remnants of Rathke pouch lumen.
- ▶ Neurohypophysis: unmyelinated axons of supraoptic/paraventricular nuclei, Herring bodies (ADH/oxytocin + neurophysin), pituicytes (glial support cells) — it makes no hormone of its own.
- ▶ Hypophyseal portal system links median eminence (primary plexus) to pars distalis (secondary plexus) — the histological basis of hypothalamic control.
- ▶ Clinical correlation: acidophil adenoma → acromegaly/gigantism; basophil adenoma → Cushing disease; chromophobe adenoma → mass effect/bitemporal hemianopia; Sheehan syndrome → anterior lobe infarction.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Vishram Singh — Textbook of Anatomy Vol 3 4e; Brijesh Kumar — Histology Text & Atlas 3e; Gray's Anatomy (Standing) 41e.

**Histology of prostate**

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction — The prostate is the largest accessory sex gland of the male reproductive system, shaped and sized like a walnut, lying below the bladder neck and surrounding the prostatic urethra. It is a compound tubuloalveolar (tubuloacinar) gland — 30–50 individual glands embedded in a dense fibromuscular stroma — with a parenchyma : stroma ratio of roughly 70 : 30 by volume. Its microscopic architecture is best understood through McNeal's zonal classification, which correlates directly with the two commonest prostatic diseases: benign prostatic hyperplasia (BPH) and adenocarcinoma.

Capsule and gross-to-microscopic framework

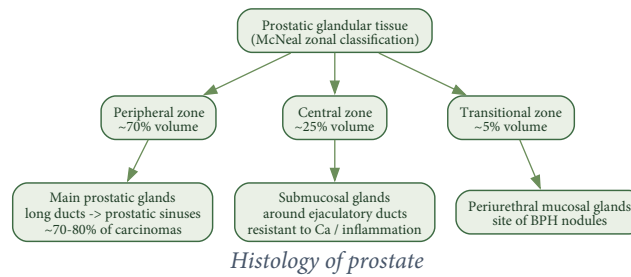
- ▶ The gland is invested by a thin fibroelastic **true capsule**, continuous with the stroma, from which septa penetrate the parenchyma and incompletely divide it into indistinct lobules.
- ▶ Outside this lies a **false capsule** derived from endopelvic fascia, with the prostatic venous plexus sandwiched between the two capsules.
- ▶ Normal adult weight is about **20 g** (Junqueira, Ross); Gray's records the young, non-hyperplastic gland at **~8 g**, enlarging to **~40 g** (occasionally >150 g) once BPH develops after the fifth decade.
- ▶ Overall composition: **glandular tissue (parenchyma) ~70%, fibromuscular tissue (stroma) ~30%** of the organ.

Zonal (McNeal) classification of the glandular parenchyma

Histological sections do not show the classical "five-lobe" pattern (only demonstrable in the fetal gland before 20 weeks' gestation); instead the adult glandular tissue is organised into concentric **zones**, each with a distinct duct group, embryological origin and disease association.

Zone	Share of glandular volume	Location / duct pattern	Origin	Clinical association
Peripheral zone	~70%	Cup-shaped; occupies posterior and postero-lateral parts, encircling the central zone; main prostatic glands with the longest ducts, opening into the prostatic sinuses; palpable on digital rectal examination (DRE)	Urogenital-sinus buds	Site of ~70–80% of adenocarcinomas ; most susceptible to inflammation
Central zone	~25%	Cone-shaped, apex at the verumontanum; surrounds the ejaculatory ducts; submucosal glands	Cells thought to be incorporated from the mesonephric (Wolffian) duct system	Relatively resistant to carcinoma and inflammation; distinctive cytology (prominent, slightly basophilic cytoplasm, nuclei at different levels)
Transitional zone	~5%	Surrounds the pre-prostatic urethra just proximal to the central zone/ejaculatory ducts	Urogenital sinus	Site of origin of BPH nodules
Periurethral (mucosal) gland zone	<1%	Simple mucus-secreting glands immediately around the pre-prostatic urethra, resembling female urethral glands (unlike true prostatic glands)	Urogenital sinus	Also implicated in later stages of BPH

- **Anterior fibromuscular stroma** — a non-glandular fibromuscular mass anterior to the pre-prostatic urethra, continuous with the bladder-neck detrusor apron; devoid of glands.
- On MRI (T2-weighted), the peripheral zone shows high signal intensity; the central and transitional zones together form the low-signal "central gland."
- Note: BD Chaurasia and Gray's describe the classical **three-zone** McNeal scheme (peripheral, central, transitional); Ross and Sontakke additionally separate out a fourth, periurethral mucosal-gland zone — both descriptions are used interchangeably in the literature.



Microscopic architecture of the parenchyma (acini and ducts)

- ▶ Each of the 30–50 tubuloalveolar glands is arranged in **three concentric groups**: an inner **mucosal layer** (opens directly into the urethra), an intermediate **submucosal layer**, and an outer **main/peripheral layer** — the latter two draining via ducts into the **prostatic sinuses** on either side of the urethral crest.
- ▶ **Acinar/follicular epithelium**: highly variable — ranges from simple cuboidal/columnar to **pseudostratified columnar**, often with a folded or papillary luminal surface that gives the acini serrated, irregular outlines. A distinct **basal cell layer** (round nuclei, close to the basement membrane) underlies the taller luminal secretory cells and serves as the reserve/regenerative layer for the epithelium.
- ▶ Three functional cell types are recognised in the glandular epithelium: **basal cells**, **exocrine secretory (columnar) cells** — including a transient mucous-cell population lost as the gland matures — and scattered **neuroendocrine cells** (containing neurone-specific enolase, chromogranin and serotonin), whose numbers decline after middle age.
- ▶ **Ducts** (12–20 in number) are lined by **stratified columnar epithelium**, with a superficial columnar layer and a deeper cuboidal layer.
- ▶ The **prostatic urethra** traverses the gland vertically at the junction of its anterior one-third and posterior two-thirds; it is lined by transitional epithelium proximally and stratified columnar epithelium distally.

Fibromuscular stroma

- ▶ Forms the non-glandular framework and septa dividing the parenchyma; comprises **smooth muscle**, **collagen** and **elastic fibres** running in different directions, together with blood vessels, nerve fibres and lymphatics.
- ▶ Smooth muscle within the stroma contracts at ejaculation, expelling glandular secretions into the prostatic urethra.
- ▶ The relative proportion of stroma to gland changes with age — abundant at birth, condensing as glandular tissue proliferates through puberty and the third decade.

Corpora amylacea (prostatic concretions)

- ▶ Rounded, **concentric lamellated eosinophilic bodies** lying free within the acinar lumina, up to 2 mm in diameter.
- ▶ Composed of precipitated glycoproteins and keratan sulfate around cell debris; may become partially calcified with time.

- ▶ Increase progressively in number with advancing age and are a useful **histological identification feature** of the prostate (along with glands set in prominent fibromuscular stroma); they have no known pathological significance.

Functional histology — secretion and hormonal control

- ▶ Prostatic fluid is thin, milky and slightly acidic, contributing roughly **10–30% of seminal fluid volume**.
- ▶ Secretory products include **acid phosphatase**, **prostate-specific antigen (PSA, a kallikrein-family serine protease of ~33–34 kDa)**, **fibrinolysin**, **citric acid**, **amylase** and **zinc**. PSA and fibrinolysin liquefy the coagulated seminal clot to permit sperm release; acid phosphatase levels rise with metastatic disease.
- ▶ Secretion and expulsion are under combined **sympathetic and parasympathetic** control via the prostatic plexus (from the inferior hypogastric plexus).
- ▶ Epithelial growth and secretory activity are androgen-dependent: testosterone and adrenal androgens are converted intracellularly to **dihydrotestosterone (DHT)** by **5 α -reductase**; DHT (~30 $\times$ more potent than testosterone) binds the androgen receptor, driving nuclear translocation and glandular growth — the basis of 5 α -reductase-inhibitor therapy in BPH.

Development and age changes

- ▶ The prostate develops from a series of **endodermal outgrowths (buds) from the urethral epithelium and adjacent urogenital sinus** during the first three months of intrauterine life; surrounding mesenchyme condenses to form the stroma. The prostatic utricle develops from the Müllerian tubercle region (homologous to the vaginal primordium). Cells of the central zone are believed to be derived from the mesonephric (Wolffian) duct system.
- ▶ **Birth**: gland small, mainly stroma with a simple duct system and small end-buds (future follicles); ducts, seminal colliculus and utricle epithelium show transient hyperplasia/squamous metaplasia from maternal oestrogens, subsiding within weeks, followed by relative quiescence to about 12–14 years.
- ▶ **Puberty (~14–18 years)**: testosterone-driven maturation — the gland more than doubles in size through follicular development from ductal end-buds and stromal condensation.
- ▶ **Third decade (20–30 years)**: marked epithelial proliferation with irregular infolding of the glandular epithelium into the follicle lumen.
- ▶ **30–45 years**: size remains essentially constant; involution begins, infoldings start to regress.
- ▶ **After 45–50 years**: epithelial foldings disappear, follicle outlines become regular, and corpora amylacea increase in number — signs of prostatic involution; beyond this age the gland almost invariably develops BPH.

Applied / Clinical anatomy

- ▶ **Benign prostatic hyperplasia (BPH)**: nodular hyperplasia of glandular and/or stromal elements arising in the **transitional and periurethral zones**, driven by DHT; compresses the prostatic urethra causing outflow obstruction. Managed medically (α 1-blockers, 5 α -reductase inhibitors) or surgically (TURP).

- ▶ **Adenocarcinoma of prostate:** arises predominantly in the **peripheral zone**, palpable as a hard nodule on DRE; associated with elevated serum PSA and acid phosphatase; may metastasise to the vertebral column/skull via valveless communications between prostatic and vertebral venous plexuses.
- ▶ **Prostatitis:** inflammation of the gland, most often affecting the peripheral zone.
- ▶ **PSA and acid phosphatase** assays, together with DRE, are used in screening/monitoring prostate cancer, though PSA screening's mortality benefit remains debated.
- ▶ **Radical prostatectomy** exploits the true/false capsular planes and the neurovascular bundles (cavernous nerves) postero-lateral to the gland; their injury causes erectile dysfunction.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Low-power section through the prostate showing:

- ▶ **Capsule** (thin fibroelastic true capsule)
- ▶ **Fibromuscular stroma / septa** dividing the gland into indistinct lobules
- ▶ **Tubuloalveolar glands (acini)** with folded/papillary luminal epithelium giving serrated outlines
- ▶ **Glandular (pseudostratified columnar) epithelium** with a basal cell layer at its base
- ▶ **Corpora amylacea** — concentric lamellated eosinophilic bodies within acinar lumina
- ▶ **Duct** draining into a prostatic sinus
- ▶ Concentric **zones** around the prostatic urethra — peripheral, central and transitional — with the anterior fibromuscular stroma labelled anteriorly

□ Fig 32.12 — *Histology of prostate* (BD Chaurasia — *Human Anatomy Vol 2* — 8e, p.500)

High-yield exam pointers

- ▶ 30–50 tubuloalveolar glands embedded in fibromuscular stroma; parenchyma : stroma $\approx$ 70 : 30.
- ▶ McNeal's zones: **peripheral (70%, carcinoma-prone)**, **central (25%, disease-resistant)**, **transitional (5%, BPH-prone)**; four-zone descriptions add a periurethral mucosal-gland zone.
- ▶ Acinar epithelium: cuboidal to pseudostratified columnar with a basal (reserve) cell layer; ducts lined by stratified columnar epithelium (12–20 ducts).
- ▶ **Corpora amylacea** — concentric lamellated eosinophilic concretions, increase with age, key identification feature.
- ▶ Secretions: PSA, acid phosphatase, fibrinolysin, citric acid, zinc, amylase — androgen (DHT)-dependent via 5α -reductase.
- ▶ BPH $\rightarrow$ transitional/periurethral zone; carcinoma $\rightarrow$ peripheral zone — the single most tested clinical correlation.
- ▶ Age changes: pubertal doubling in size, third-decade epithelial infolding, post-45–50-year involution with rising corpora amylacea and BPH.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; IB Singh — Textbook of Human Histology 9e (Practical/Atlas plate); BD Chaurasia — Human Anatomy Vol 2 8e; Vishram Singh — Textbook of Anatomy Vol 2 4e.



Histology of Suprarenal gland

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction — The suprarenal (adrenal) glands are paired retroperitoneal endocrine organs capping the superior pole of each kidney. Each is a flattened, crescentic body about 4–6 cm long and a few mm thick, weighing approximately 4–5 g. Histologically each gland is really **two glands united within one capsule**: an outer **cortex** (mesodermal origin, steroid-secreting, ~90% of gland volume) and an inner **medulla** (neural-crest origin, catecholamine-secreting, ~one-tenth of the gland), each with distinct embryology, function and microstructure.

1. General microscopic organisation

- ▶ A thick collagenous **capsule** sends thin **trabeculae/septa** into the cortex; the stroma is a **reticular-fibre network** supporting the secretory cords and a rich microvasculature — there is no true hilum, but arteries pierce the capsule at multiple points and a single vein exits at a hilar point.
- ▶ Both cortex and medulla are organised as **cords/clusters of epithelial cells running alongside wide, fenestrated sinusoidal capillaries** — the typical arrangement of a ductless (endocrine) gland.
- ▶ Cortical cells show the classic ultrastructure of **steroid-secreting cells**: acidophilic/basophilic cytoplasm rich in lipid droplets, abundant smooth endoplasmic reticulum (cholesterol → pregnenolone → steroid conversion), and mitochondria with **tubular/vesicular cristae** (not the usual shelf-like cristae) — steroids are not stored in granules but diffuse out as synthesised.

2. Suprarenal cortex — the three zones

The cortex is divided, from outside in, into zona glomerulosa, zona fasciculata and zona reticularis; boundaries between zones are indistinct and variable.

Zone	Approx. share of cortex	Cell arrangement	Cell morphology	Principal secretion	Regulation
Zona glomerulosa	~15% (outer, subcapsular)	Closely packed rounded/arched, inverted-U-shaped clusters or acinus-like groups	Small polyhedral/columnar cells; basophilic cytoplasm, deeply staining nuclei, few lipid droplets; elongated mitochondria	Mineralocorticoids — aldosterone, deoxycorticosterone	Renin–angiotensin II and plasma K^+ ; largely ACTH-independent
Zona fasciculata	~65–80% (thickest layer)	Long, straight parallel cords, 1–2 cells thick, separated by straight sinusoids	Large polyhedral cells, heavily lipid-laden → "empty"/vacuolated in routine sections, hence spongiocytes; rich in vitamin C; spherical mitochondria; best-developed Golgi	Glucocorticoids — cortisol, cortisone; small amounts of weak androgens (DHEA)	ACTH-dependent, with cortisol negative feedback
Zona reticularis	~10% (innermost, next to medulla)	Branching, anastomosing cords forming a network ("reticulum")	Smaller cells, more acidophilic/eosinophilic, fewer lipid droplets, accumulate brown lipofuscin pigment with age; mitochondria with unusual tubular cisternae	Weak androgens (DHEA, DHEAS) — precursor of testosterone; some cortisol	ACTH-dependent

- **Enzymatic basis of zonation** (explains why each zone makes only its own hormone class): the zona glomerulosa lacks **17 α -hydroxylase** (so cannot make cortisol or sex steroids) but uniquely expresses **aldosterone synthase**; the zona fasciculata/reticularis have 17 α -hydroxylase but lack aldosterone synthase.
- Some authors describe a "**migration/zonal**" theory — zona glomerulosa acts as a germinative layer whose daughter cells migrate centripetally through fasciculata into reticularis, the reticularis thus representing a "graveyard" of ageing cortical cells; this is not universally accepted.
- **Clinical correlate to remember while writing**: Addison disease may damage any/all layers with loss of the corresponding hormone class.

3. Suprarenal medulla

- ▶ Composed of large, pale-staining **chromaffin cells (pheochromocytes)** arranged in **cords/clumps**, separated by wide venous sinusoids and supported by reticular fibres; a few **sympathetic (parasympathetic) ganglion cells** are scattered among them.
- ▶ Each chromaffin cell is tall columnar/polyhedral, basophilic, and classically described as **sandwiched between an arterial capillary at one pole and a venous sinusoid at the other**.
- ▶ **Chromaffin cells are derived from neural-crest cells** (as are postganglionic sympathetic neurons) and are functionally **modified postganglionic sympathetic neurons that have lost their axons/dendrites and become purely secretory** — hence the medulla behaves as a "giant sympathetic ganglion."
- ▶ **Chromaffin reaction**: the cytoplasmic granules stain **brown on treatment with potassium dichromate/bichromate salts**, due to oxidation-polymerisation of stored catecholamines — the basis of the name "chromaffin."
- ▶ EM: granules (150–350 nm) contain catecholamines bound to **chromogranin** proteins together with Ca^{2+} and ATP. **Epinephrine-granules** are smaller and less electron-dense; **norepinephrine-granules** are larger with an eccentric, denser core. Roughly **80% of cells secrete epinephrine and 20% norepinephrine** — epinephrine is made *only* in the adrenal medulla.
- ▶ Innervated by **preganglionic sympathetic fibres** (splanchnic nerves via the coeliac plexus, arising from lower thoracic/upper lumbar cord segments) that pierce the cortex and synapse directly on chromaffin cells, triggering catecholamine release during stress ("fight-or-flight").
- ▶ Unlike the cortex, the medulla is **not essential for life** (its functions can be taken over by other chromaffin/paraganglion tissue).

4. Vascular architecture — the corticomedullary portal system

- ▶ Each gland is supplied by **superior, middle and inferior suprarenal arteries** (from inferior phrenic artery, aorta, and renal artery respectively), which ramify to form a **subcapsular arterial plexus**.
- ▶ From this plexus, two separate arteriolar systems arise: (a) branches that traverse the cortex as **fenestrated sinusoidal capillaries irrigating all three zones**, then drain into the medullary sinusoids as "**suprarenal portal vessels**"; and (b) **medullary arterioles** that supply the medulla directly. The medulla thus has a genuine **dual blood supply**.
- ▶ Functional significance: cortisol-rich portal blood bathing the medulla **induces the enzyme PNMT (phenylethanolamine-N-methyltransferase)**, which methylates norepinephrine to epinephrine — explaining the anatomical logic of why the medulla is enclosed by, and drained through, the cortex.
- ▶ Venous drainage: medullary sinusoids unite into a **central medullary vein**, which continues as the single **suprarenal vein** emerging at the hilum — **short on the right (drains directly into the IVC)**, longer on the left (drains into the left renal vein). Because venous drainage is via a single vein, gland infarction is more often due to venous than arterial compromise.

5. Development (brief, for context)

- ▶ **Cortex:** from proliferating **coelomic mesothelium** of the "suprarenal ridge" beside the root of the dorsal mesentery (T6–T12 level) — mesodermal in origin. An initial **fetal cortex** (secreting DHEA, which the placenta converts to oestrogen — the feto-placental unit) is later covered by the **definitive cortex**; the fetal zone regresses within 2–3 weeks after birth, after which the definitive cortex differentiates into the three adult zones.
- ▶ **Medulla:** neural-crest cells migrate ventrally from the primitive sympathetic ganglia into the suprarenal ridge and differentiate into **chromaffin cells**, while adjacent neural-crest derivatives form sympathetic ganglia (sympathoblasts) — hence cortex and medulla, though united by birth, have entirely separate origins.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

embed: ![Fig 25.15 — Histology of suprarenal gland (practice figure). (Sontakke — Textbook of Human Histology — 2e, p.342)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/2cb5b8f5d424fe14.jpg)

For hand reproduction in the exam, draw a low-power radial section and label, from outside in:

- ▶ **Capsule** (thick collagenous, with trabeculae)
- ▶ **Zona glomerulosa** — rounded/arched (inverted-U) clusters of columnar cells
- ▶ **Zona fasciculata** — long parallel two-cell-thick cords ("spongiocytes"), sinusoids between
- ▶ **Zona reticularis** — anastomosing network of cords
- ▶ **Medulla** — chromaffin cells in cords around wide sinusoids + an occasional sympathetic ganglion cell
- ▶ **Central medullary vein**
- ▶ Arrows for subcapsular arterial plexus → cortical sinusoids → medullary sinusoids ("portal vessels") + separate medullary arteriole

Applied / Clinical anatomy

- ▶ **Addison disease** — autoimmune or tuberculous destruction of the cortex → deficient mineralo-/glucocorticoids → hyperpigmentation, muscle weakness, weight loss, hypotension.
- ▶ **Conn syndrome** — adrenal hyperplasia/adenoma of zona glomerulosa → excess aldosterone → hypertension with hypokalaemia.
- ▶ **Cushing syndrome** — zona fasciculata hyperplasia/excess cortisol → truncal obesity, hypertension, hirsutism; when driven by a pituitary ACTH-secreting adenoma this is termed Cushing disease.
- ▶ **Adrenogenital (congenital adrenal hyperplasia) syndrome** — excess zona reticularis androgens → masculinisation in females, precocious puberty in males.
- ▶ **Pheochromocytoma** — tumour of medullary chromaffin cells → paroxysmal hypertension from catecholamine excess; obeys the "**rule of 10s**" (10% extra-adrenal/paraganglioma, 10% bilateral, 10% in children, 10% malignant, 10% not hypertensive); extra-adrenal chromaffin tumours are called **paragangliomas**.

- ▶ **Accessory suprarenal (cortical rest) tissue** — small nodules of cortex-only tissue may persist near the kidney, or ectopically in the **broad ligament, spermatic cord/testis, or mesenteries**, reflecting the separate embryological origin of cortex and medulla; rarely a site of neoplastic change.
- ▶ **Surgical anatomy** — the gland's retroperitoneal position on the posterior abdominal wall (related to IVC/liver on the right, stomach/spleen on the left, both crura of diaphragm posteriorly) underlies the **posterior retroperitoneoscopic approach** to adrenalectomy; the **short right suprarenal vein** draining directly into the IVC is vulnerable to avulsion during dissection.

High-yield exam pointers

- ▶ Cortex = mesoderm (coelomic epithelium, suprarenal ridge); medulla = neural crest — two glands in one capsule.
- ▶ Three cortical zones, outer to inner: **glomerulosa (aldosterone)** → **fasciculata (cortisol, thickest)** → **reticularis (androgens)**; mnemonic "salt, sugar, sex."
- ▶ Zona glomerulosa lacks 17 α -hydroxylase but has aldosterone synthase; is regulated by renin–angiotensin, largely ACTH-independent, unlike the other two zones.
- ▶ Medulla = chromaffin cells = modified postganglionic sympathetic neurons; give a positive **chromaffin (potassium dichromate) reaction**; secrete ~80% epinephrine : 20% norepinephrine.
- ▶ PNMT, induced by cortisol delivered via the corticomedullary portal circulation, converts noradrenaline to adrenaline — only in the adrenal medulla.
- ▶ Medulla has a **dual blood supply** (direct medullary arterioles + cortical sinusoidal "portal" drainage); single suprarenal vein — short and direct-to-IVC on the right.
- ▶ Fetal cortex secretes DHEA for the feto-placental oestrogen unit and regresses in early neonatal life.
- ▶ Clinical correlates by zone: Addison (whole cortex), Conn (glomerulosa), Cushing (fasciculata), adrenogenital syndrome (reticularis), pheochromocytoma (medulla).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e (figure); AK Datta Human Anatomy Vol 1 (Thorax & Abdomen) — for applied/development detail and the corticomedullary portal (PNMT) mechanism.



Histology of thyroid

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction — The thyroid is the largest pure endocrine gland in the body, a bilobed organ lying anterior to the trachea in the neck, its two lobes joined by an isthmus. It develops from the thyroglossal duct, an endodermal downgrowth from the foregut (tongue base), and is unique among endocrine glands in storing its secretory product **extracellularly** as colloid within spherical follicles. The parenchyma comprises two functionally distinct cell populations — **follicular cells (thyrocytes)**, which synthesise the iodinated hormones T4 and T3, and **parafollicular (C) cells**, which secrete calcitonin.

1. General organisation — capsule, septa and vascularity

- ▶ The gland is covered by a thin **fibrous (true) capsule**; loose areolar tissue outside this forms a surgical "false capsule" continuous with the pretracheal fascia, allowing the gland to move with deglutition.
- ▶ Thin **connective tissue septa** extend from the capsule into the parenchyma, dividing it into incompletely demarcated **lobules**.
- ▶ Each lobule contains numerous **follicles** packed closely together, separated only by a sparse reticular stroma.
- ▶ The interfollicular connective tissue carries an extremely rich capillary plexus (fenestrated capillaries) around every follicle, blood vessels, lymphatics and nerves — the thyroid has one of the highest blood flows per gram of any organ, reflecting its high secretory and iodide-trapping demand.

2. The thyroid follicle — structure and the colloid

- ▶ The **follicle** is the structural and functional unit — a hollow spherical-to-ovoid sac, diameter varying widely (about **0.2–1.0 mm**), lined by a **single layer (simple epithelium)** of follicular cells resting on a basement membrane.
- ▶ The **lumen** is filled with **colloid**, a gel-like, homogeneous, acidophilic (pink on H&E), PAS-positive material composed mainly of **thyroglobulin** (a 660 kDa iodinated glycoprotein) — the inactive storage form of thyroid hormone.
- ▶ The thyroid is the *only* endocrine gland that stores a large quantity of its hormone precursor outside the cell, in the follicular lumen; there is enough stored colloid to supply the body for up to ~3 months without fresh synthesis.
- ▶ Follicles sectioned tangentially may show no visible lumen; adjacent follicles vary considerably in size and colloid content in the same field.

□ Fig 8.10 — Histology of thyroid gland (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.177)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a low-power section of thyroid gland with:

- ▶ Fibrous capsule
- ▶ Connective tissue septum (with blood vessel)
- ▶ Lobule (group of follicles)
- ▶ Follicle wall — simple cuboidal follicular cells
- ▶ Colloid (acidophilic, filling lumen)
- ▶ Basement membrane of follicle
- ▶ Parafollicular (C) cell — within follicular basal lamina/between follicles
- ▶ Interfollicular connective tissue with capillary plexus

3. Follicular cells (thyrocytes) — light and electron microscopic features

- ▶ Shape varies with functional activity, controlled by TSH: squamous in hypoactive glands, low-to-tall columnar in hyperactive glands.

Feature	Resting / inactive follicle	Active / secretory follicle
Epithelial height	Squamous to low cuboidal, flattened nuclei	Low columnar to tall columnar, rounded euchromatic nuclei
Colloid quantity	Abundant, densely fills lumen	Reduced, being actively resorbed
Colloid margin	Smooth, even edge	Scalloped / "moth-eaten" edge (resorption vacuoles at the apical border)
Cytoplasmic organelles	Sparse RER and Golgi	Prominent RER (basal), well-developed Golgi (supranuclear), numerous apical microvilli, secretory and colloid-resorption vesicles

- ▶ **Ultrastructure** (active cell): basal rough endoplasmic reticulum and nucleus; supranuclear Golgi complex; apical microvilli and pseudopodia projecting into the colloid; apical secretory (exocytotic) vesicles carrying thyroglobulin out, and colloid-resorption (endocytotic) vesicles/phagolysosomes carrying colloid back in; basolateral cell membrane bears the Na^+/I^- symporter (NIS); tight junctions seal the apical intercellular space, separating colloid from the basal extracellular fluid.

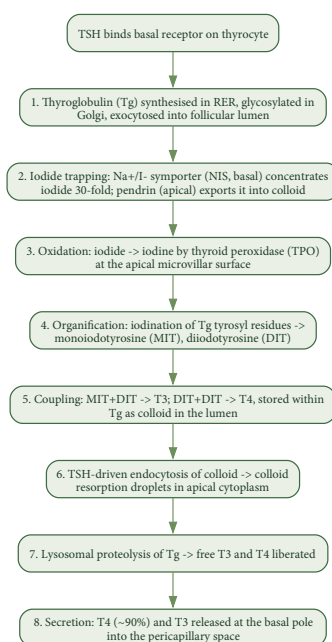
4. Parafollicular cells (C cells)

Feature	Follicular cells (thyrocytes)	Parafollicular / C cells
Location	Line the follicle wall, apical surface faces colloid	Lie within the follicular basal lamina or singly/in small clusters in the interfollicular connective tissue; not exposed to the follicular lumen

Feature	Follicular cells (thyrocytes)	Parafollicular / C cells
Embryological origin	Endoderm (thyroglossal duct)	Neural crest, via the ultimobranchial body
Morphology	Cuboidal–columnar, basophilic cytoplasm, apical microvilli	Larger, polyhedral, pale/clear cytoplasm, eccentric oval nucleus; occur singly or in small clusters
Secretory product	Thyroxine (T ₄) and triiodothyronine (T ₃)	Calcitonin
Principal action	Raise basal metabolic rate; essential for growth and CNS development	Lowers blood Ca ²⁺ by inhibiting osteoclastic bone resorption (antagonises PTH)
Regulation	TRH–TSH axis (hypothalamo-pituitary)	Directly by rising serum Ca ²⁺ (independent of hypothalamus/pituitary)
Associated tumour	Papillary / follicular carcinoma	Medullary carcinoma of thyroid — calcitonin used as a tumour marker

5. Synthesis and release of thyroid hormone

Both an exocrine phase (thyroglobulin synthesis/iodination in the colloid) and an endocrine phase (hormone release into blood) occur in the same follicular cell, all driven by TSH:



Histology of thyroid

- Nearly all circulating T₄ and T₃ travel bound to **thyroxine-binding globulin (TBG)**/albumin; T₄ is quantitatively dominant (~90%) but **T₃ is 2–10-fold more biologically active** with a shorter half-life (~1.5 days vs ~1 week for T₄).
- Both hormones increase mitochondrial number/size and protein synthesis, raising the basal metabolic rate of target tissues.

6. Regulation — hypothalamo-pituitary-thyroid axis

- ▶ **Hypothalamus** secretes TRH → stimulates **thyrotrophs** of the anterior pituitary to secrete TSH (**thyrotropin**) → TSH acts on basal TSH-receptors of follicular cells, increasing cell height and stimulating every step of hormone synthesis/release described above.
- ▶ Circulating T3/T4 exert **negative feedback** on both the hypothalamus (↓TRH) and the anterior pituitary (↓TSH), keeping circulating hormone levels within a narrow physiological range.
- ▶ Loss of dietary iodine reduces hormone output, removing feedback inhibition; the resulting rise in TSH drives follicular hyperplasia/hypertrophy — the histological basis of **goitre**.

Applied / Clinical anatomy

- ▶ **Endemic (iodine-deficiency) goitre**: chronic iodine deficiency → sustained TSH drive → follicular hyperplasia and colloid accumulation, producing diffuse or multinodular enlargement.
- ▶ **Graves' disease** (commonest cause of hyperthyroidism): autoantibodies (long-acting thyroid stimulator, TSH-receptor antibodies) chronically stimulate follicular cells → tall columnar epithelium with scalloped, peripherally-vacuolated colloid, hypervascularity, and clinical hyperthyroidism (weight loss, heat intolerance, tremor, exophthalmos). Treated by antithyroid drugs, subtotal thyroidectomy, or radioactive ^{131}I ablation, which selectively destroys the actively iodine-trapping follicular cells.
- ▶ **Hashimoto's thyroiditis** (commonest cause of primary hypothyroidism in iodine-sufficient areas): autoantibodies against thyroglobulin (TgAb), thyroid peroxidase (TPOAb) and the TSH receptor cause follicular cell apoptosis/destruction; histology shows dense **lymphocytic infiltration with germinal centres** replacing follicular parenchyma.
- ▶ **Medullary carcinoma of thyroid** arises from parafollicular (C) cells; serum **calcitonin** is used as a **tumour marker** to detect residual/recurrent disease after surgical resection.
- ▶ **Surface/applied anatomy**: the gland is supplied by the superior thyroid artery (external carotid) and inferior thyroid artery (thyrocervical trunk), explaining its high perfusion; the recurrent laryngeal nerve's close relation to the inferior thyroid artery at the lower pole is the classical operative landmark protected during thyroidectomy.
- ▶ The two parathyroid glands lie embedded in the posterior capsule of each lobe and are at operative risk during thyroid surgery — accidental removal produces hypoparathyroidism (hypocalcaemic tetany).

High-yield exam pointers

- ▶ Thyroid is the **only endocrine gland storing hormone extracellularly** — colloid supplies the body for ~3 months without fresh synthesis.
- ▶ Follicle diameter is variable, roughly **0.2–1.0 mm**; wall = simple epithelium ranging squamous (inactive) to columnar (active).
- ▶ Colloid = **thyroglobulin** (660 kDa glycoprotein), acidophilic, PAS-positive; **scalloped/moth-eaten margins = active resorption**.
- ▶ **Follicular cells** → T3/T4 (from endoderm); **parafollicular/C cells** → calcitonin (from neural crest via ultimobranchial body) and are never exposed to the follicular lumen.

- ▶ Synthesis sequence: Tg synthesis → NIS iodide trapping → TPO oxidation/organification (MIT, DIT) → coupling to T3/T4 → TSH-driven endocytosis → lysosomal proteolysis → secretion.
- ▶ **TSH** is the master regulator of thyroid histoarchitecture (cell height) and every synthetic step; axis = TRH → TSH → T3/T4 with negative feedback.
- ▶ T4 is 90% of secreted hormone but **T3 is the more potent, shorter half-life hormone**.
- ▶ Graves' disease → tall active epithelium with scalloped colloid; Hashimoto's → lymphocytic infiltrate with germinal centres.
- ▶ Calcitonin is the tumour marker for **medullary carcinoma** (C-cell derived).

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas, 17e; Ross Histology — A Text and Atlas, 7e; Sontakke — Textbook of Human Histology, 2e; IB Singh — Textbook of Human Histology, 9e; Brijesh Kumar — Histology Text & Atlas, 3e; Gray's Anatomy (Standring), 41e (gross relations, capsule, blood supply and applied/operative anatomy).



Micro anatomy of supra renal gland

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction — The suprarenal (adrenal) glands are paired, retroperitoneal endocrine organs capping the superior pole of each kidney at the T12–L3 level. Each is a flattened body about 50 mm long, 30 mm broad and 10 mm thick, weighing roughly 5 g — the right gland pyramidal ("top hat"-shaped), the left crescentic ("cocked hat"-shaped). Histologically each gland is really two separate endocrine organs fused within one capsule: an outer **cortex** (mesodermal origin, steroid-secreting, about 90% of the gland by weight) and an inner **medulla** (neural-crest origin, catecholamine-secreting, about 10%) — different embryology, different histology, different hormones, yet functionally integrated.

1. General microscopic organisation

- ▶ Each gland is enclosed by a thick **fibrous/collagenous capsule** that sends thin **trabeculae (septa)** into the substance of the gland, carrying blood vessels and nerves.
- ▶ The **stroma** is a delicate network of **reticular fibres** supporting the secretory cells and a rich sinusoidal microvasculature; there is no true anatomical hilum for arteries (which pierce the capsule at many points), though a single vein does leave at a hilar point.
- ▶ Both cortex and medulla show the typical arrangement of a **ductless (endocrine) gland**: cords or clusters of secretory epithelial cells applied to wide, fenestrated **sinusoidal capillaries**.
- ▶ Cortical parenchymal cells show the classic ultrastructure of **steroid-secreting cells**: lipid droplets, abundant smooth endoplasmic reticulum (for cholesterol → steroid conversion), well-developed Golgi, and mitochondria with **tubulo-vesicular cristae** rather than the usual shelf-like (lamellar) cristae.

2. Suprarenal cortex — the three zones

The cortex is arranged, from outside inward, into the zona glomerulosa, zona fasciculata and zona reticularis; the boundaries between adjacent zones are gradual rather than sharp.

Zone	Share of cortex	Cell arrangement	Cell morphology	Principal secretion	Regulation
Zona glomerulosa	~15% (thin, subcapsular)	Small, closely packed cells in rounded/arched clusters or inverted-U (acinus-like) groups , separated by thin connective tissue and capillaries	Columnar/polyhedral cells; spherical nuclei; relatively few lipid droplets; basophilic cytoplasm	Mineralocorticoids — aldosterone and deoxycorticosterone	Renin–angiotensin system and plasma K^+ level; feedback loop that is largely independent of ACTH
Zona fasciculata	~80% (thickest layer, two cells wide)	Long straight parallel cords, two cells thick , separated by straight radial sinusoids	Large polyhedral cells, heavily laden with lipid droplets → appear vacuolated/"empty" in routine H&E, hence called spongiocytes	Glucocorticoids — cortisol and corticosterone; small quantities of adrenal androgens	ACTH-dependent ; cortisol exerts negative feedback on the hypothalamo-pituitary axis
Zona reticularis	~5–7% (innermost, abutting the medulla)	Branching, anastomosing cords forming a network ("reticulum"), separated by fenestrated capillaries	Smaller, more acidophilic cells with less lipid; accumulate brown lipofuscin pigment with advancing age	Gonadocorticoids (weak androgens — DHEA, DHEAS, androstenedione)	ACTH-dependent

- ▶ Together the three zones behave as one physiological unit but each secretes a distinct hormone class — a useful mnemonic is "**salt** (glomerulosa) — **sugar** (fasciculata) — **sex** (reticularis)."
- ▶ The zona glomerulosa is discontinuous, forming clusters rather than a complete ring, and is thinnest of the three.

3. Suprarenal medulla

- ▶ Occupies the central one-tenth of the gland and is completely enclosed by cortex except at the hilum.
- ▶ Composed of large, pale-staining, ovoid **chromaffin cells (pheochromocytes)** arranged in short, interconnecting **cords/clumps** around numerous wide sinusoidal capillaries, supported by

reticular fibres; scattered **sympathetic (parasympathetic-type) ganglion cells** are also present among them.

- ▶ Chromaffin cells give a positive **chromaffin reaction** — their cytoplasmic secretory granules stain brown on treatment with potassium dichromate (chromium salts), due to oxidation of stored catecholamines; this reaction gives the cell type its name.
- ▶ **Embryological identity:** chromaffin cells arise from **neural-crest cells**, exactly as do the postganglionic neurons of sympathetic ganglia, and are best regarded as **modified postganglionic sympathetic neurons that have lost their axons and dendrites** and become purely secretory — hence the medulla is often described as a "giant sympathetic ganglion."
- ▶ They secrete the **catecholamines** epinephrine (adrenaline) and norepinephrine (noradrenaline); about 80% of chromaffin cells secrete epinephrine and 20% norepinephrine. Conversion of norepinephrine to epinephrine occurs **only in the chromaffin cells of the adrenal medulla**, and this conversion is stimulated by the glucocorticoids reaching the medulla directly through the continuity of cortical and medullary sinusoids (the enzyme classically named is phenylethanolamine-N-methyltransferase).
- ▶ Innervation is unusual for an endocrine gland: chromaffin cells receive **direct synapses from preganglionic sympathetic fibres** (via the splanchnic nerves) rather than the postganglionic innervation typical of other viscera — consistent with the cells being homologous to postganglionic neurons themselves.

4. Vascular architecture — the corticomedullary relationship

- ▶ Grossly, each gland is supplied by three arteries — the **superior suprarenal artery** (from the inferior phrenic artery), the **middle suprarenal artery** (directly from the abdominal aorta) and the **inferior suprarenal artery** (from the renal artery) — which pierce the capsule at multiple points.
- ▶ Inside the capsule these vessels form a **subcapsular arterial plexus**, from which three intraglandular capillary sets arise in series: **capsular, cortical and medullary** capillaries.
- ▶ Cortical sinusoidal capillaries run radially through all three zones and drain into the medullary sinusoids as so-called "**suprarenal portal vessels**"; in addition, separate **medullary arterioles** bypass the cortex and supply the medulla directly. The medulla thus receives a genuine **dual (arterial + portal) blood supply**.
- ▶ Medullary sinusoids converge on a **central medullary vein**, which continues as the single **suprarenal vein** emerging at the hilum. Venous drainage is asymmetrical: the short **right suprarenal vein drains directly into the inferior vena cava**, while the longer **left suprarenal vein drains into the left renal vein**.
- ▶ Because each gland has only a single venous outflow channel (unlike the multiple arterial inflows), the gland is more vulnerable to venous than arterial compromise, and the short right vein is a recognised surgical hazard during adrenalectomy.

5. Nerve supply and lymphatic drainage

- ▶ Nerve supply is exceedingly rich — predominantly myelinated **preganglionic sympathetic fibres derived from the splanchnic nerves**, distributed mainly to the medullary chromaffin cells (as noted above); a smaller postganglionic component supplies the cortical vasculature.
- ▶ **Lymphatic drainage** is via capsular lymphatic channels that communicate with subserous lymphatics, ultimately draining to the **para-aortic (lateral aortic) lymph nodes**.

6. Development (brief, for context)

- ▶ **Cortex:** arises from proliferating **coelomic mesothelium** near the root of the dorsal mesentery ("suprarenal ridge") — hence mesodermal in origin. An initial, large **fetal cortex** (secreting DHEA that the placenta converts to oestrogens — the feto-placental unit) is progressively covered by the thin **definitive cortex**; the fetal cortex regresses rapidly after birth, after which the definitive cortex differentiates into the adult zona glomerulosa, fasciculata and reticularis.
- ▶ **Medulla: neural-crest cells (sympathogonia)** migrate from the adjacent developing sympathetic ganglia, invade the fetal cortex, and differentiate into chromaffin cells; adjacent neural-crest derivatives instead differentiate into sympathetic ganglion cells. Chromaffin reaction and catecholamine secretion are demonstrable from around the second trimester onward.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

embed: ![Fig 21.26 — Photomicrographs of the cortex and medulla of the human adrenal gland (Ross Histology — A Text and Atlas — 7e, p.788)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/2f3fa7a830ad0a32.jpg)

For hand reproduction in the exam, draw a low-power radial (wedge) section from capsule to medulla and label, outside in:

- ▶ **Capsule** (dense collagenous, with trabeculae carrying vessels/nerves)
- ▶ **Zona glomerulosa** — arched/inverted-U clusters of columnar cells
- ▶ **Zona fasciculata** — long parallel two-cell-thick cords ("spongiocytes"), radial sinusoids between cords
- ▶ **Zona reticularis** — anastomosing network of smaller, pigmented cords
- ▶ **Medulla** — chromaffin cells in cords/clumps around wide sinusoids, with an occasional sympathetic ganglion cell
- ▶ **Central medullary vein**
- ▶ Arrows showing: subcapsular arterial plexus → capsular → cortical sinusoids → medullary sinusoids ("portal" pathway), plus a separate direct medullary arteriole

Applied / Clinical anatomy

- ▶ **Addison disease** — autoimmune or tuberculous destruction of the cortex → combined deficiency of mineralo- and glucocorticoids → hyperpigmentation, muscular weakness, hypotension, weight loss.
- ▶ **Conn syndrome** — adenoma/hyperplasia of the zona glomerulosa → aldosterone excess → hypertension with hypokalaemia.

- ▶ **Cushing syndrome** — hyperplasia/adenoma of the zona fasciculata with excess cortisol → truncal obesity, moon facies, hypertension, hirsutism (pituitary ACTH-driven cases are termed Cushing disease).
- ▶ **Congenital adrenal hyperplasia / adrenogenital syndrome** — excess zona reticularis androgens → masculinisation of female infants (may be mistaken for males), precocious puberty in males.
- ▶ **Pheochromocytoma** — a benign chromaffin-cell tumour of the medulla causing paroxysmal hypertension, headache and palpitations from catecholamine excess; extra-adrenal chromaffin tumours are termed **paragangliomas**.
- ▶ **Neuroblastoma** — a malignant tumour of primitive medullary (neural-crest) cells, occurring in infants and children below 15 years of age.
- ▶ **Accessory suprarenal (cortical rest) tissue** — isolated nodules of cortex-only tissue, reflecting its separate embryological origin from the medulla, may be found near the kidney or ectopically in the **spermatic cord, epididymis or broad ligament of the uterus**.
- ▶ **Radiological demonstration** — the gland can be visualised by CT scan, useful in localising cortical or medullary tumours pre-operatively.

High-yield exam pointers

- ▶ Cortex (mesoderm, coelomic epithelium) + medulla (neural crest) = two glands, one capsule; cortex ≈ 90%, medulla ≈ 10% of gland weight.
- ▶ Three cortical zones outer→inner: **glomerulosa (aldosterone, ~15%) → fasciculata (cortisol, ~80%, spongiocytes) → reticularis (androgens, ~5–7%, lipofuscin-laden)**.
- ▶ Zona glomerulosa is regulated by the renin–angiotensin system/ K^+ (largely ACTH-independent); zona fasciculata and reticularis are ACTH-dependent.
- ▶ Medulla = chromaffin cells (positive potassium-dichromate/chromaffin reaction) = modified postganglionic sympathetic neurons directly synapsed by preganglionic splanchnic fibres; ~80% secrete epinephrine, ~20% norepinephrine.
- ▶ Medulla has a **dual blood supply**: direct medullary arterioles plus a corticomedullary "portal" sinusoidal pathway; glucocorticoids in this portal blood promote NE→epinephrine conversion.
- ▶ Venous drainage is via a single vein per gland: **right → IVC directly, left → left renal vein**; lymph drains to para-aortic nodes.
- ▶ Development: fetal cortex (large, DHEA-secreting) regresses after birth; definitive cortex then differentiates into the three adult zones; medulla forms from invading neural-crest sympathogonia.
- ▶ Clinical correlation by zone/region: Addison (whole cortex), Conn (glomerulosa), Cushing (fasciculata), congenital adrenal hyperplasia (reticularis), pheochromocytoma/neuroblastoma (medulla).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e (figure); IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; BD Chaurasia Human Anatomy Vol 2 8e; Vishram Singh Textbook of Anatomy Vol 2 4e and General Anatomy 4e — for gross essentials, vascular/nerve supply and applied anatomy.



Microanatomy of uterus in different phases of menstrual cycle

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction The uterus is a thick-walled, pear-shaped muscular organ whose wall has three concentric coats — **endometrium** (mucosa), **myometrium** (muscularis) and **perimetrium** (serosa). Of these, only the endometrium undergoes profound, reproducible histological change every menstrual cycle (~28 days), passing through **proliferative**, **secretory** and **menstrual** phases under the sequential drive of ovarian **estrogen** and **progesterone**. This cyclicity prepares the endometrium to receive a blastocyst and, failing fertilisation, sheds itself as menstrual flow — a process that repeats from menarche to menopause.

1. General Histology of the Uterine Wall

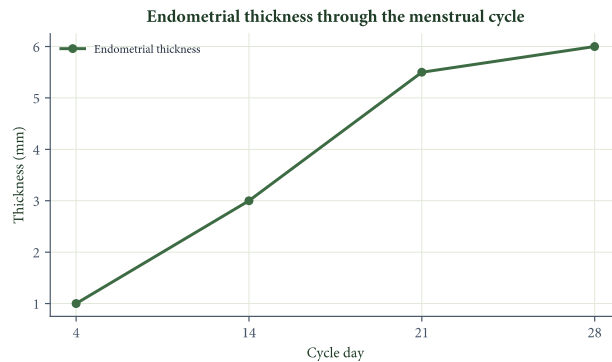
- ▶ **Endometrium** — mucosa lined by simple columnar epithelium (ciliated cells + non-ciliated secretory/peg cells) that invaginates into the underlying stroma (lamina propria) to form simple tubular **uterine glands**; there is **no submucosa** separating endometrium from myometrium.
 - Functionally it has two zones: **stratum functionale** (superficial, shed every cycle) and **stratum basale** (deep, adjacent to myometrium, retained — the regenerative reservoir). Some texts further split the functionale into an outer **stratum compactum** (gland necks) and a middle **stratum spongiosum** (loose, edematous stroma with gland bodies).
- ▶ **Myometrium** — the thickest coat; smooth muscle arranged in three ill-defined layers: inner longitudinal, middle circular/oblique (richly vascular — "stratum vasculare"), and outer longitudinal. Undergoes hypertrophy and hyperplasia in pregnancy and contracts under oxytocin to expel the fetus.
- ▶ **Perimetrium** — outer serous coat continuous with pelvic peritoneum; outer mesothelium over a layer of vascular connective tissue.

2. Hormonal Basis of the Cyclical Change

- ▶ Anterior-pituitary gonadotropins (FSH, LH) drive the **ovarian cycle** (follicular growth → ovulation → corpus luteum); the ovarian steroid output in turn drives the parallel **endometrial cycle**.
- ▶ Three phases (28-day cycle, day 1 = onset of bleeding): **proliferative** (≈ days 5–14, estrogen-driven, concurrent with follicular maturation), **secretory** (≈ days 15–28, progesterone-driven,

coincides with corpus luteal activity), **menstrual** ($\approx$ days 1–4/5, follows withdrawal of both hormones on corpus luteal regression).

- Only the stratum functionale responds to this hormonal swing; the stratum basale, supplied by hormone-insensitive straight arteries, is preserved and re-epithelialises the surface each cycle.



Approximate endometrial thickness at end-menstrual (day 4), end-proliferative/ovulation (day 14) and secretory-phase peak (days 21–28).

3. Proliferative (Follicular/Preovulatory) Phase — Estrogen-driven

- **Repair and regeneration:** epithelial cells from the basal stumps of glands broken during menses proliferate and migrate to re-cover the denuded surface; stromal fibroblasts proliferate and lay down collagen and ground substance.
- **Glands:** simple columnar epithelium, straight, with narrow, nearly empty lumina and scanty secretion.
- **Stroma:** highly cellular, mitotically active, minimal edema.
- **Spiral arteries:** lengthen but remain only slightly coiled and do not extend into the upper third of the endometrium.
- **Thickness:** increases from a thin ~ 1 mm post-menstrual remnant to ≈ 3 mm by the time of ovulation (day 14).

□ Fig 31.21 — Histology of proliferative phase of uterine endometrium (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.486)

4. Secretory (Progestational/Luteal) Phase — Progesterone-driven

- Begins about a day after ovulation under progesterone (plus estrogen) from the corpus luteum.
- **Glands:** become coiled and tortuous, corkscrew/saw-tooth in profile, with sacculated lumina distended by glycogen-rich mucoid secretion; growth is now by epithelial **hypertrophy** rather than mitosis (mitoses become rare).
- **Stroma:** edematous; stromal cells accumulate glycogen and lipid and transform into large, pale **decidual cells** (decidual reaction) — priming the tissue for possible implantation.
- **Spiral arteries:** lengthen further, become markedly coiled, and extend almost to the surface; superficial thin-walled dilated vascular **lacunae** appear.
- **Thickness:** maximal, reaching 5–6 mm (up to 6–7 mm in some accounts).
- **Implantation** of the blastocyst, when fertilisation has occurred, takes place in this phase — the mid-secretory endometrium is optimal for implantation and embryonic nutrition.

□ Fig 31.22 — Histology of secretory phase of uterine endometrium (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.486)

5. Menstrual Phase — Hormone Withdrawal

- ▶ **Trigger:** without fertilisation the corpus luteum regresses 8–10 days after ovulation, and progesterone/estrogen levels fall sharply.
- ▶ **Vascular event:** spiral arteries undergo periodic contraction and relaxation causing ischemia of the stratum functionale; local release of prostaglandins produces further vasoconstriction; hypoxic cells release cytokines that recruit leukocytes releasing collagenase/matrix metalloproteinases, degrading the stromal matrix.
- ▶ **Shedding:** the surface epithelium, most of each gland, the stroma and blood-filled lacunae of the stratum functionale detach and slough away, mixed with escaping blood, as the **menstrual flow**; the stratum basale (supplied by hormone-independent straight arteries) remains intact.
- ▶ **Duration/loss:** menstrual flow lasts about 4–5 days with an average blood loss of 35–50 mL.
- ▶ By the end of this phase the endometrium is reduced to a thin (~1 mm) basal remnant, ready to re-enter the proliferative phase.

6. Endometrial Vascular Architecture — Anatomical Basis of Cyclical Shedding

- ▶ Uterine artery → 6–10 arcuate arteries (anastomose within the myometrium) → radial arteries penetrate perpendicularly into the endometrium and give off two sets of vessels:
 - **Straight (basal) arteries** — short, supply only the stratum basale; do **not** undergo cyclical change and are unaffected by hormone withdrawal.
 - **Spiral (coiled) arteries** — long, traverse the full stratum functionale nearly to the surface; uniquely **progesterone-sensitive**, elongating and coiling through the secretory phase and constricting premenstrually — the proximate cause of ischemic sloughing.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Longitudinal schematic of the endometrium–myometrium junction, labelling:

- ▶ Myometrium (base)
- ▶ Arcuate artery (within myometrium)
- ▶ Radial artery (perpendicular branch)
- ▶ Straight (basal) artery → stratum basale
- ▶ Spiral (coiled) artery → stratum functionale, reaching near the surface
- ▶ Stratum basale (deep, retained layer)
- ▶ Stratum functionale — stratum compactum (superficial, gland necks) and stratum spongiosum (middle, loose stroma)
- ▶ Surface simple columnar epithelium with uterine gland openings
- ▶ Superficial capillary/venous lacunae

7. Comparative Table — Endometrium in the Three Phases

Feature	Proliferative phase	Secretory phase	Menstrual phase
Dominant hormone	Estrogen	Progesterone (+ estrogen)	Withdrawal of both
Approx. days	5–14	15–28	1–4/5
Thickness	~1 → 3 mm	5–6 mm (max)	Reduced to ~1 mm
Glands	Straight, narrow lumen, sparse secretion	Coiled/corkscrew, sacculated, glycogen-rich secretion	Glands disintegrating/collapsing
Stroma	Highly cellular, mitotic, little edema	Edematous; decidual reaction (glycogen/lipid-laden cells)	Necrotic, infiltrated by leukocytes, disintegrating
Spiral arteries	Slightly coiled, confined to lower 2/3	Markedly coiled, reach near surface	Constrict intermittently → ischemia
Strata present	Basale regenerating functionale	Both strata, functionale maximal	Functionale sloughed; only basale remains

Applied / Clinical anatomy

- ▶ **Endometrial dating/biopsy:** the histological appearance of glands and stroma (gland shape, secretory content, decidual change) is used clinically to "date" the cycle and assess luteal-phase adequacy in the infertility work-up.
- ▶ **Dysfunctional uterine bleeding (DUB):** excessive or irregular bleeding without an organic uterine lesion (tumour, polyp, infection); commonly due to anovulatory cycles in which unopposed, persistent estrogen sustains a proliferative-type endometrium without secretory transformation, predisposing to hyperplasia.

- ▶ **Asherman syndrome:** over-vigorous curettage that damages the stratum basale destroys the endometrium's regenerative reservoir, producing intrauterine adhesions and amenorrhoea — a direct clinical correlate of basal-layer physiology.
- ▶ **Endometriosis:** presence of endometrial glands and stroma at ectopic sites (commonest — ovary, then pouch of Douglas); these ectopic deposits respond to the same hormonal cycle, bleeding cyclically and causing pain, adhesions and infertility.
- ▶ **Adenomyosis:** benign endometrial glands/stroma within the myometrium, with myometrial hypertrophy.
- ▶ **Intrauterine contraceptive device (IUCD):** acts partly through a local sterile endometrial inflammatory reaction hostile to implantation.
- ▶ **Pregnancy test basis:** on fertilisation, trophoblastic **hCG** rescues and maintains the corpus luteum (sustaining progesterone and the secretory/decidualised endometrium); hCG is detectable in urine from about 10–14 days of pregnancy, the basis of urine pregnancy-test kits.
- ▶ **Ectopic pregnancy:** the commonest site of tubal (ectopic) implantation is the ampulla of the uterine tube, with interstitial implantation being least common.

High-yield exam pointers

- ▶ Uterine wall = **endometrium, myometrium, perimetrium**; only the endometrium shows cyclical histological change.
- ▶ Endometrium functionally = **stratum functionale** (shed each cycle) + **stratum basale** (retained, regenerates the functionale).
- ▶ Three phases per 28-day cycle: **proliferative** (estrogen, days ~5–14) → **secretory** (progesterone, days ~15–28) → **menstrual** (hormone withdrawal, days ~1–4/5).
- ▶ Best single light-microscopic discriminator: **gland shape** — straight/narrow (proliferative) vs corkscrew/sacculated with secretion (secretory).
- ▶ **Straight arteries** supply stratum basale (hormone-independent); **spiral arteries** supply stratum functionale (progesterone-sensitive) and their constriction causes menstrual ischemia.
- ▶ Endometrial thickness roughly: ~1 mm post-menstrual → ~3 mm end-proliferative → 5–6 mm secretory peak.
- ▶ **Decidual reaction** of stromal cells is a secretory-phase, progesterone-driven change preparing for implantation.
- ▶ Average menstrual blood loss ≈ 35–50 mL over ~4–5 days.
- ▶ Key clinical correlates: DUB (anovulatory, unopposed estrogen), Asherman syndrome (basal-layer destruction), endometriosis/adenomyosis.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Brijesh Kumar Histology Text & Atlas 3e; BD Chaurasia Human Anatomy Vol 2 8e; Gray's Anatomy (Standing) 41e.



Microanatomy of Uterus

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction The uterus is a hollow, pyriform muscular organ of the female pelvis, differentiated grossly into fundus, body, isthmus and cervix. Like other hollow viscera it has a three-layered wall — **endometrium** (mucosa), **myometrium** (muscularis) and **perimetrium** (serosa) — but its mucosa is unique in the body: the endometrium undergoes cyclical monthly proliferation, secretion and shedding under ovarian hormonal control, and is specialised to receive and nourish the implanting blastocyst. This cyclical microanatomy, and its distinct blood supply, are the areas most heavily tested.

1. General organisation of the uterine wall

Layer	Nature	Key microscopic features
Endometrium	Mucosa	Simple columnar epithelium + cellular lamina propria (stroma) with tubular uterine glands; two functional zones (basalis, functionalis); undergoes cyclical change
Myometrium	Muscularis	Thick bundles of smooth muscle in three ill-defined layers, richly vascular, hormone-responsive (hypertrophy in pregnancy)
Perimetrium	Serosa/adventitia	Mesothelium-covered connective tissue continuous with the broad ligament peritoneum; incomplete anteriorly

The wall is thickest in the fundus and body, thins at the **isthmus** (a short transitional zone with sparser glands and thinner myometrium, corresponding to the lower uterine segment obstetrically), and is histologically distinct in the **cervix** (see section 7). This answer concentrates on the body/fundus, where the classical three-layer architecture and cyclical change are best seen.

2. Endometrium — epithelium and stroma

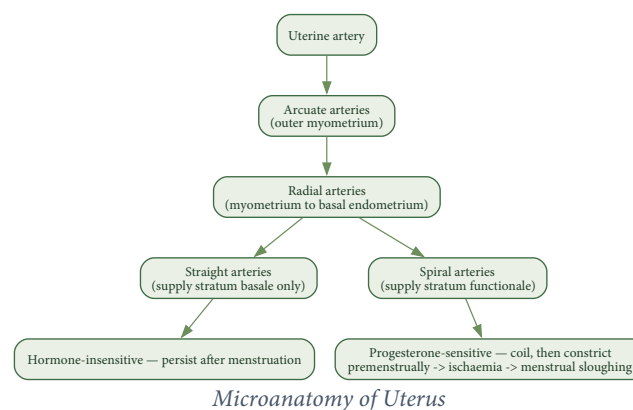
- **Lining epithelium:** simple columnar, made up of **ciliated cells** and **secretory (non-ciliated) cells**; cilia are most conspicuous before menarche and their proportion diminishes in reproductive life because of repeated shedding of the superficial epithelium.

- ▶ **Uterine glands:** simple tubular glands lined by the same secretory columnar epithelium; they arise from the surface and penetrate the full thickness of the endometrium, with their basal ends embedded in the stroma next to the myometrium.
- ▶ **Lamina propria / stroma:** a distinctive cellular connective tissue rich in fibroblasts, ground substance and fine (type III) reticular collagen, containing an extensive microvasculature, lymphatics and nerves; unlike most connective tissues it is **devoid of adipocytes**.
- ▶ **Two concentric functional zones:**
 - **Stratum basale (basal layer)** — deep, adjacent to the myometrium; densely cellular stroma; contains the basal (deep) ends of the uterine glands and straight arteries; relatively insensitive to hormone withdrawal; **persists through menstruation** and regenerates the functionalis.
 - **Stratum functionale (functional layer)** — superficial, forms most of the endometrial thickness; spongier, oedematous stroma; contains most of the gland length and the surface epithelium; undergoes the full cyclical growth, secretion and **shedding at menstruation**.

□ Fig 22.16 — Uterine myometrium and endometrium. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1129)

3. Blood supply of the endometrium

The vascular pattern is the structural basis of menstruation and is a favourite short-answer point.



- ▶ **Straight arteries** are short, do not coil, and supply only the basal layer — largely independent of hormonal fluctuation, so the basalis survives menstruation.
- ▶ **Spiral arteries** are long, markedly coiled, progesterone-sensitive vessels that lengthen through the secretory phase and extend into the superficial functionalis, ending in a rich subepithelial capillary/lacunar bed.
- ▶ Premenstrually, falling progesterone causes intense, intermittent constriction of the spiral arteries, producing ischaemic necrosis of the functionalis; its subsequent shedding, with intermittent bleeding from ruptured vessels, constitutes menstrual flow. Alternating constriction –relaxation of the basal segments of these arteries limits blood loss.

4. Myometrium

- ▶ The thickest coat of the uterine wall; composed of interlacing bundles of smooth muscle separated by connective tissue carrying **venous plexuses**, lymphatics and nerves.

- Functions as a **structural and functional syncytium** (gap-junction coupled) so that contraction is coordinated during labour.
- Classically described as **three ill-defined layers** (boundaries are not sharp, and bundles interweave):
- **Outer longitudinal layer** — fibres chiefly parallel to the long axis of the uterus; continuous with the longitudinal muscle of the uterine tubes; helps anchor the uterus and expel its contents.
- **Middle circular/oblique layer (stratum vasculare)** — the thickest of the three; contains the **large arcuate blood vessels**; its circularly disposed fibres act as a physiological sphincter around vessels, limiting bleeding at menstruation and after placental separation.
- **Inner layer** — mixed, chiefly longitudinal/reticular fibres continuous with the endometrial stroma; endometrial vessels thread through its interstices, so on contraction it functions as a "living ligature".
- Hormonally responsive: oestrogen (and mechanical stretch) drives smooth-muscle **hyperplasia and hypertrophy** during pregnancy (myocytes lengthen many-fold); after parturition, involution occurs by cellular shrinkage and apoptosis with collagenolysis, returning the uterus nearly to its pre-pregnant size.

5. Perimetrium

- The outer serous coat — mesothelium on a thin layer of connective tissue, continuous with the peritoneum of the broad ligament.
- **Covers completely:** the fundus and the entire posterior surface of the body.
- **Covers incompletely:** on the anterior surface it covers only the upper part (adherent to myometrium); over the lower part it is separated from the myometrium by loose areolar tissue and reflects onto the bladder, leaving the supravaginal cervix and vaginal part of the cervix uncovered — these regions instead have a fibrous **adventitia**. This anterior reflection creates the **utero-vesical pouch**.

6. Cyclical (menstrual) changes in the endometrium

Feature	Proliferative phase (day 6–13/14)	Secretory phase (day 15–28)	Menstrual phase (day 1–4/5)
Driving hormone	Oestrogen (ovarian follicle)	Progesterone (+ oestrogen; corpus luteum)	Withdrawal of progesterone (corpus luteum regression)
Endometrial thickness	~2–3 mm, progressively increasing	~5–6 mm (2–3× proliferative)	Reduced; only basalis remains
Lining epithelium	Simple columnar, regenerating by mitosis from gland stumps	Simple columnar	Denuded/exfoliated over functionalis
Stroma	Highly cellular, non-oedematous	Oedematous, spongy; stromal cells enlarge with glycogen (pre-decidual/decidual cells)	Disrupted, infiltrated with blood

Feature	Proliferative phase (day 6–13/14)	Secretory phase (day 15–28)	Menstrual phase (day 1–4/5)
Uterine glands	Narrow, straight, tubular, no secretion	Dilated, tortuous, saw-toothed outline, active secretion	Basal stumps of glands only
Spiral arteries	Short, less coiled, do not reach surface	Long, markedly coiled, reach superficial functionalis	Constrict intermittently, then rupture

- ▶ **Pre-menstrual phase** (1–2 days before bleeding): regression of the corpus luteum → spiral-artery constriction → ischaemia and shrinkage of the functionalis.
- ▶ **Regenerative phase** (first 1–3 days after bleeding stops): epithelial cells from the residual gland stumps in the basalis proliferate under oestrogen to re-cover the denuded surface.
- ▶ Implantation, when it occurs, takes place in the secretory-phase endometrium; if the blastocyst implants, the endometrium is transformed into the **decidua** (basalis, capsularis, parietalis).

7. Regional note — isthmus and cervix

- ▶ **Isthmus**: transition zone with fewer, less-developed glands and a thinner myometrium than the body; corresponds to the surgical/obstetric "lower uterine segment."
- ▶ **Cervix** is histologically distinct from the body and shows little cyclical change:
 - **Endocervix** — mucosa thrown into oblique palmate folds (*plicae palmatae*) with deep branching mucus-secreting glands; lined by tall non-ciliated columnar epithelium in its lower two-thirds becoming ciliated columnar above; gland-duct obstruction produces retention cysts (**Nabothian cysts**).
 - **Ectocervix (portio vaginalis)** — covered by stratified squamous non-keratinised epithelium continuous with the vagina.
 - The **squamocolumnar (transformation) zone** near the external os is the site of most cervical dysplasia/carcinoma and is the target of the Pap smear.
 - The cervical wall is mostly (~85–90%) dense fibrocollagenous connective tissue with only a small admixture of smooth muscle — unlike the muscle-dominant body — so it does **not desquamate** during menstruation but dilates enormously in labour.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled longitudinal (sagittal) section through the uterine wall, body region, showing (from lumen outward):

- ▶ Uterine cavity/lumen
- ▶ Surface epithelium — simple columnar (ciliated + secretory cells)
- ▶ Uterine gland (cut in cross- and longitudinal section, penetrating full thickness)
- ▶ **Stratum functionale** (superficial endometrium) with a coiled **spiral artery**
- ▶ **Stratum basale** (deep endometrium) with a **straight artery** and basal gland stumps
- ▶ Endometrial–myometrial junction (no submucosa)
- ▶ **Myometrium** — label its three layers: outer longitudinal, middle circular (**stratum vasculare**, with an **arcuate artery** shown), inner mixed/longitudinal
- ▶ **Perimetrium** — outer serosa (mesothelium + thin connective tissue)
- ▶ Arrow/inset showing the vascular tree: uterine artery → arcuate artery → radial artery → straight artery / spiral artery

Applied / Clinical anatomy

- ▶ **Endometrial dating (Noyes' criteria)**: an endometrial biopsy can be histologically dated to a day of the cycle from gland tortuosity, secretion and stromal change — used in the infertility workup to confirm ovulation and adequate luteal-phase development.
- ▶ **Asherman syndrome**: aggressive curettage that damages the stratum basale destroys the regenerative source of the endometrium, producing intrauterine adhesions, secondary amenorrhoea and infertility — underlines why the basalis, not the functionalis, is the regenerative layer.
- ▶ **Dysfunctional uterine bleeding (DUB)**: commonly due to anovulatory cycles with unopposed oestrogen causing endometrial hyperplasia and irregular, incomplete shedding.
- ▶ **Endometriosis**: retrograde menstruation deposits viable functional-layer fragments on the ovaries, pouch of Douglas or elsewhere; ectopic endometrium responds cyclically to hormones, producing cyclical pelvic pain, adhesions and infertility.
- ▶ **Adenomyosis**: endometrial glands and stroma extend abnormally into the myometrium, causing a diffusely enlarged, tender, boggy uterus.
- ▶ **Intrauterine contraceptive device (IUCD)**: acts largely through a sterile inflammatory reaction confined to the endometrium (functionalis), which is hostile to implantation, without disturbing the myometrium.
- ▶ **Postpartum involution**: rapid reduction in uterine size after delivery reflects myometrial smooth-muscle apoptosis and collagenolysis rather than cell loss alone; can be tracked clinically/on ultrasound by fundal height and myometrial echotexture.
- ▶ **Surgical/ureteric relation**: the uterine artery, given off from the internal iliac artery, runs in the broad ligament and crosses **anterior and superior to the ureter** about 2–2.5 cm lateral to the supravaginal cervix before turning medially to ascend alongside the uterus — the classical "**water**

under the bridge" relation — placing the ureter at risk of injury or ligation during hysterectomy or when the uterine artery is clamped.

- ▶ **Cervical cytology:** the Pap smear samples the squamocolumnar/transformation zone of the cervix (endocervix/ectocervix junction) for early detection of dysplastic change.

High-yield exam pointers

- ▶ Uterine wall = **endometrium (mucosa) + myometrium (muscularis) + perimetrium (serosa, incomplete anteriorly)** — no submucosa between endometrium and myometrium.
- ▶ Endometrium has **two zones**: stratum basale (permanent, regenerative, straight arteries) vs stratum functionale (cyclical, shed at menstruation, spiral arteries).
- ▶ Vascular hierarchy: **uterine artery → arcuate → radial → straight (basalis) / spiral (functionalis)**.
- ▶ Myometrium = **3 ill-defined layers**; middle layer = **stratum vasculare**, thickest, carries the arcuate vessels, acts as a physiological sphincter.
- ▶ **Proliferative phase** (oestrogen, day 6–14): straight tubular glands, non-oedematous stroma, 2–3 mm thickness.
- ▶ **Secretory phase** (progesterone, day 15–28): tortuous saw-toothed glands, oedematous/decidual stroma, 5–6 mm thickness.
- ▶ Menstruation = ischaemic necrosis of the functionalis from spiral-artery constriction after corpus luteum regression; basalis survives and regenerates the lining.
- ▶ Perimetrium is incomplete: absent over the supravaginal and vaginal cervix and over the lower anterior body (utero-vesical pouch).
- ▶ Cervix is histologically distinct — mucus-secreting endocervical glands + stratified squamous ectocervix, little smooth muscle, no cyclical desquamation; transformation zone is clinically vital (Pap smear/cervical cancer).
- ▶ Surgical landmark: uterine artery crosses **over** the ureter ~2 cm lateral to the cervix ("water under the bridge").

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas (17e); Ross Histology — A Text and Atlas (7e); IB Singh — Textbook of Human Histology (9e); BD Chaurasia — Human Anatomy Vol 2 (8e); Gray's Anatomy (Standring) 41e — used for the gross/applied uterine artery–ureter relation.



Microscopic Features of Testis

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction The testis is a paired, encapsulated **mixed exocrine–endocrine gland**. Its exocrine (spermatogenic) component is the coiled **seminiferous tubule**, whose stratified germinal epithelium produces spermatozoa under the physical and metabolic support of tall **Sertoli cells**; its endocrine component is the **interstitial (Leydig) cell**, which synthesises testosterone in the loose connective tissue between tubules. On low power the testis therefore shows numerous cross-sections of seminiferous tubules separated by scanty vascular stroma, all enclosed by a dense fibrous capsule, the **tunica albuginea**. This microscopic organisation is the structural basis for both fertility and androgen production, and is a favourite RGUHS spot/essay topic.

1. Capsule, mediastinum and lobulation

- ▶ **Tunica vaginalis** — a peritoneal remnant carried down with testicular descent; parietal layer lines the scrotal wall, visceral layer invests the tunica albuginea except posteriorly.
- ▶ **Tunica albuginea** — thick, dense collagenous capsule; thickens along the posterior border to form the **mediastinum testis**, a wedge of fibrous tissue through which vessels, lymphatics and the rete testis traverse.
- ▶ **Tunica vasculosa** — a thin, areolar, vascular layer lining the inner aspect of each lobule, deep to the albuginea.
- ▶ Fibrous **septa** radiate from the mediastinum and incompletely divide the gland into ~200–300 **pyramidal lobules** (apex directed towards the mediastinum), each containing 1–4 **highly convoluted seminiferous tubules** embedded in sparse interstitial connective tissue.

2. Seminiferous tubule — wall and epithelial architecture

- ▶ Each testis contains roughly 400–1000 **seminiferous tubules**; each tubule is 150–250 µm in diameter, with a length variously cited as 30–80 cm, giving a combined tubular length of the order of several hundred metres per testis.
- ▶ Each tubule is a closed loop; both limbs converge towards the mediastinum via a short, narrow **straight tubule (tubulus rectus)**.
- ▶ Wall, from outside inward: a thin fibrous **lamina/tunica propria** containing flattened, contractile **peritubular myoid cells** (weak peristalsis moves luminal fluid and sperm) → **basement membrane** → thick stratified **seminiferous (germinal) epithelium**.
- ▶ The germinal epithelium is built from two co-operating populations: non-dividing, supporting **Sertoli cells**, and the proliferating **spermatogenic cell line** (spermatogonia → spermatocytes → spermatids), arranged basal-to-luminal in that developmental order — classically described as **three ill-defined zones**: an outer zone of spermatogonia, an intermediate zone of primary and secondary spermatocytes, and an inner zone of spermatids.
- ▶ Cell boundaries within the epithelium are indistinct on H&E; the identifying low-power feature is the many-layered, heterogeneous nuclear population with an ill-defined luminal margin.
- ▶ Loose interstitial tissue between tubules carries fenestrated capillaries, lymphatics, fibroblasts, mast cells/macrophages and clusters of **Leydig cells**.

Diagram (real plate)

□ Fig 17.12 — Histology of seminiferous tubule (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.294)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw (a) a low-power schematic of testicular architecture and (b) a high-power cross-section of one seminiferous tubule; label:

- ▶ Tunica vaginalis (parietal + visceral layers) → tunica albuginea → mediastinum testis → septa → lobules (1–4 tubules/lobule)
- ▶ Seminiferous tubule (convoluted loop) → tubulus rectus (straight tubule) → rete testis (in mediastinum) → efferent ductules → head of epididymis
- ▶ Interstitial (Leydig) cells clustered in the vascular connective tissue between tubules
- ▶ Tubule cross-section, base → lumen: lamina propria with myoid cells → basement membrane → spermatogonia (outer zone) → primary and secondary spermatocytes (intermediate zone) → spermatids (inner zone) → Sertoli cell body spanning base to lumen → lumen with released spermatozoa

3. Sertoli (sustentacular) cells

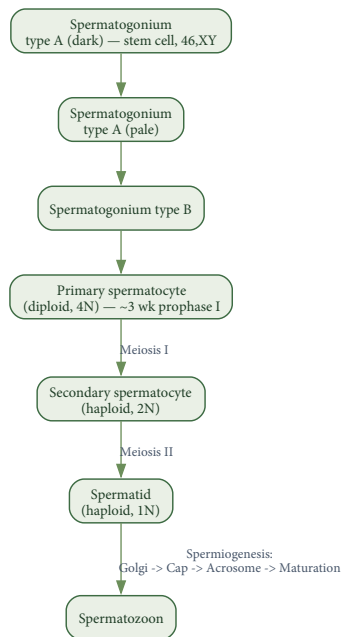
Feature	Description
Shape/nucleus	Tall, columnar/pyramidal, non-dividing; base fixed to the basement membrane, apex extending to the lumen; nucleus oval/triangular, euchromatic, with a prominent nucleolus
Cytoplasm	Abundant smooth ER, some rough ER, well-developed Golgi, numerous mitochondria and lysosomes
Junctions	Basolateral tight (occluding) junctions between adjacent Sertoli cells form the blood–testis barrier ; gap junctions couple neighbouring Sertoli cells
Support	Physical, metabolic and nutritional support of every germ cell embedded in its lateral/apical cytoplasm; each Sertoli cell supports ~30–50 germ cells
Secretion (exocrine)	Fructose- and protein-rich fluid that carries sperm passively along the tubule toward the rete testis
Secretion (endocrine)	FSH-driven androgen-binding protein (ABP) — concentrates testosterone intratubularly — and inhibin , which feeds back to suppress pituitary FSH; fetal Sertoli cells secrete Müllerian-inhibiting substance (anti-Müllerian hormone)
Phagocytosis	Engulfs and digests residual bodies shed during spermiogenesis, and degenerating germ cells
Hormonal control	Stimulated by FSH acting on the Sertoli cell (Leydig cells are the target of LH/ICSH)

4. Spermatogenic cell series and spermatogenesis

- ▶ **Spermatogonia** — small (~12 µm), diploid (46, XY) stem/progenitor cells resting on the basement membrane, sub-typed as **dark type-A** (reserve stem cells, divide infrequently), **pale type-A** (transit-amplifying) and **type-B** (committed) spermatogonia.
- ▶ Type-B spermatogonia undergo a final mitosis to form **primary spermatocytes** — diploid, 4N DNA, the **largest** cells of the series — which enter a prolonged first meiotic prophase lasting ~3 **weeks**, during which synapsis and recombination occur; most spermatocytes seen in a section are at this stage.
- ▶ **Meiosis I** → **secondary spermatocytes** (haploid, 23 chromosomes, 2N DNA) — short-lived and therefore rarely captured in sections, as they proceed almost immediately to **meiosis II** → haploid **spermatids** (1N DNA).
- ▶ Newly formed primary spermatocytes must cross from the basal to the adluminal compartment by transiently opening the Sertoli–Sertoli tight junctions.
- ▶ **Spermiogenesis** — the differentiation of a spermatid into a spermatozoon without further division — proceeds in four phases:

Phase	Key event
Golgi phase	Proacrosomal vesicles from the Golgi coalesce into a single acrosomal cap; centrioles migrate to the pole opposite the acrosome and organise the flagellar axoneme
Cap phase	Acrosomal cap spreads over roughly half the condensing nucleus; the acrosome is a lysosome-like organelle rich in hyaluronidase and acrosin
Acrosome phase	Nucleus elongates and condenses (histones replaced by protamines); the flagellum elongates and mitochondria aggregate around its proximal segment, forming the middle piece
Maturation phase	Excess cytoplasm is shed as a residual body (phagocytosed by the Sertoli cell); a mature but still non-motile spermatozoon is released into the tubular lumen

- ▶ **Duration** — spermatogonium to mature spermatozoon takes on the order of ~64–74 days (texts vary: ~64 days is the commonly quoted Indian-text figure; a ~74-day figure derives from a 16-day human seminiferous-epithelial cycle recurring ~4.6 times); a further period of epididymal transit is needed before full motility.

*Microscopic Features of Testis*

5. Blood–testis barrier

- ▶ Formed by the belt of **tight junctions between adjacent Sertoli cells**, situated just above the level of the spermatogonia.
- ▶ Divides the seminiferous epithelium into a **basal compartment** (spermatogonia — freely continuous with the vascularised interstitium) and an **adluminal compartment** (spermatocytes and spermatids — sequestered from plasma proteins and circulating immune cells).
- ▶ Newly formed primary spermatocytes cross the barrier by transient, coordinated opening and resealing of the junctional complex, so barrier integrity is never breached.
- ▶ Function: creates an immunologically privileged compartment for haploid germ cells (which express antigens foreign to the mature immune system) and allows the Sertoli cell to control the local ionic/nutrient micro-environment.

6. Interstitial cells of Leydig

- ▶ Large, **polygonal/round** cells lying singly or in small clusters in the loose vascular connective tissue between seminiferous tubules, typically near capillaries.
- ▶ **Eccentric nucleus**; abundant **eosinophilic, foamy cytoplasm** rich in smooth endoplasmic reticulum, mitochondria (tubular cristae) and lipid droplets — the classic steroid-secreting-cell morphology; yellow-brown **lipofuscin** pigment may accumulate.
- ▶ Pathognomonic cytoplasmic **crystalloids of Reinke** (rod-shaped inclusions), useful in identifying Leydig-cell tumours histologically.
- ▶ Secrete **testosterone**, using the same steroidogenic SER/mitochondrial enzyme system employed by the adrenal cortex; secretion is driven by pituitary LH (= **interstitial cell-stimulating hormone, ICSH**).
- ▶ **Fetal Leydig cells** are active from early gestation (driven by placental gonadotropin, masculinising the developing duct system and external genitalia), then regress to quiescent, fibroblast-like cells that resume testosterone synthesis only at puberty.

7. Intratesticular ducts

- ▶ **Tubuli recti (straight tubules)** — short, narrow segments connecting each seminiferous-tubule loop to the rete testis; lined only by **Sertoli cells**, with no germ cells.
- ▶ **Rete testis** — an anastomosing labyrinth of channels embedded in the mediastinum testis, lined by simple cuboidal/low columnar epithelium bearing microvilli; reabsorbs some tubular fluid and concentrates the sperm suspension.
- ▶ **Efferent ductules** — roughly 10–20 ducts draining the rete testis into the head (caput) of the epididymis; lined by a characteristic **scalloped epithelium** of alternating tall ciliated columnar cells and shorter non-ciliated absorptive cuboidal cells, surrounded by a thin circular smooth-muscle coat — ciliary beat plus fluid absorption together create the flow that carries sperm passively out of the testis.

Applied / Clinical anatomy

- ▶ **Cryptorchidism** — failure of testicular descent; if uncorrected, progressive germinal epithelial hyalinisation leads to infertility, and undescended/maldeveloped testes carry a markedly increased risk of germ-cell tumour.
- ▶ **Hydrocele** — excess serous fluid within the tunica vaginalis; the commonest cause of painless scrotal swelling.
- ▶ **Varicocele** — abnormal dilatation of the pampiniform plexus, more often left-sided because of the longer left testicular vein entering the left renal vein at a right angle; raises scrotal temperature and can impair spermatogenesis.
- ▶ **Testicular tumours** — germ-cell tumours (seminoma, embryonal carcinoma, teratoma, choriocarcinoma) make up the great majority of testicular cancers and occur mainly after puberty; sex cord–stromal tumours (Leydig-cell, Sertoli-cell, granulosa-cell tumours) are rare, and combined germ-cell/sex-cord-stromal tumours (gonadoblastoma) also occur.
- ▶ **Lymphatic spread of testicular tumours** follows the gonadal vessels (not the inguinal/superficial lymphatics) to the **para-aortic and inter-aortocaval nodes**, reflecting the organ's retroperitoneal embryological origin — this dictates radical orchidectomy via the inguinal canal rather than trans-scrotal biopsy, to avoid disturbing this drainage.
- ▶ **Orchitis/epididymitis**, commonly gonococcal or chlamydial and ascending from the epididymis, can damage the seminiferous epithelium and cause infertility.
- ▶ **Blood–testis-barrier breach** (trauma, torsion, vasectomy) exposes normally sequestered sperm antigens to the immune system, provoking anti-sperm antibodies and autoimmune subfertility.
- ▶ **Testicular biopsy in male infertility** is read against the expected proportions of Sertoli cells and successive germ-cell stages; complete absence of germ cells with tubules lined only by Sertoli cells defines the **Sertoli-cell-only syndrome**.

High-yield exam pointers

- ▶ Capsule sequence: tunica vaginalis → **tunica albuginea** → thickens posteriorly as **mediastinum testis** → septa → ~200–300 lobules, each with 1–4 **seminiferous tubules**.

- ▶ Seminiferous epithelium = **Sertoli cells** (supportive, non-dividing) + **spermatogenic series** (spermatogonia → primary spermatocyte → secondary spermatocyte → spermatid → spermatozoon), classically in **three zones** (outer/intermediate/inner).
- ▶ **Blood–testis barrier** = tight junctions between adjacent Sertoli cells; separates a **basal** (spermatogonia) from an **adluminal** (spermatocytes/spermatids) compartment; confers immune privilege.
- ▶ **Leydig cells**: interstitial, testosterone-secreting, eosinophilic/foamy cytoplasm with **Reinke crystalloids**; secretion driven by **LH/ICSH**.
- ▶ Duration of spermatogenesis ~**64–74 days**; spermiogenesis has **4 phases** — Golgi, Cap, Acrosome, Maturation.
- ▶ Hormonal loop: **FSH** → **Sertoli cell** (ABP, inhibin); **LH** → **Leydig cell** (testosterone).
- ▶ Duct sequence out of the testis: seminiferous tubule → **tubulus rectus** → **rete testis** → **efferent ductules** → epididymis.
- ▶ Applied triad: **cryptorchidism** (malignancy risk), **varicocele** (impaired spermatogenesis), **lymphatic spread of testicular tumours to para-aortic nodes** (not inguinal).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; BD Chaurasia — Human Anatomy Vol 2 8e (figure).



Microscopic structure of ovary

Asked in: Paper III Nov 2017 — RGUHS MD/MS Anatomy

Definition / Introduction — The ovary is the paired female gonad, an oval body about 3 cm long, attached to the posterior layer of the broad ligament below the uterine tube. Histologically it is organised into a surface **germinal epithelium**, a subjacent **tunica albuginea**, an outer **cortex** containing ovarian follicles at every stage of development, and an inner **medulla** of loose vascular connective tissue continuous with the **hilum**. The cortex is the site of oogenesis, folliculogenesis, ovulation and corpus luteum formation, making the ovary both a gametogenic and a steroidogenic (estrogen/progesterone/androgen) organ.

General architecture

Layer	Composition	Key points
Germinal epithelium	Single layer of cuboidal (mesothelial) cells	Continuous with peritoneal mesothelium; cells bear microvilli; misnomer — does NOT give rise to germ cells

Layer	Composition	Key points
Tunica albuginea	Dense, irregular collagenous connective tissue capsule beneath the epithelium	Thinner and less dense than the testicular tunica albuginea; collagen fibres run parallel to the surface
Cortex	Stroma of reticular fibres + fusiform stromal/interstitial cells , housing follicles	Site of primordial, primary, secondary and Graafian follicles, corpora lutea, corpora albicantia and atretic follicles at different reproductive ages
Medulla	Loose connective tissue, smooth muscle, coiled blood vessels, lymphatics, nerves	Continuous with the hilum
Hilum	Entry point of vessels/lymphatics; contains hilus cells	Hilus cells resemble testicular Leydig cells, contain Reinke crystalloids , and secrete androgens

□ Fig 31.5 — Histology of ovary (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.476)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

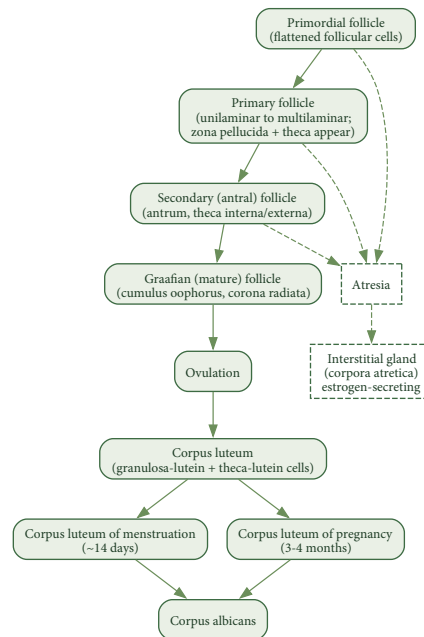
Draw a low-power section of the ovary and label:

- ▶ Germinal (surface) epithelium
- ▶ Tunica albuginea
- ▶ Cortex (outer zone)
- ▶ Medulla (inner zone) with coiled blood vessels
- ▶ Primordial follicles (subcapsular)
- ▶ Unilaminar and multilaminar primary follicles
- ▶ Secondary (antral) follicle — antrum, membrana granulosa, theca interna/externa
- ▶ Graafian follicle — cumulus oophorus, corona radiata, zona pellucida, oocyte
- ▶ Corpus luteum (with granulosa-lutein and theca-lutein zones)
- ▶ Corpus albicans and an atretic follicle
- ▶ Hilum with hilus cells

Folliculogenesis — the four stages of the ovarian follicle

Stage	Oocyte	Surrounding follicular (granulosa) cells	Distinguishing features
Primordial follicle	Primary oocyte (arrested in prophase I)	Single layer of flattened cells	Smallest, simplest; sub-tunical in position; present from birth; the "ovarian reserve"

Stage	Oocyte	Surrounding follicular (granulosa) cells	Distinguishing features
Primary follicle	Primary oocyte enlarging	Cuboidal cells — first a single layer (unilaminar), then several layers (multilaminar)	Zona pellucida first appears here (glycoprotein coat between oocyte and follicular cells, crossed by follicular-cell microvilli forming gap junctions with the oocyte); stroma organises into inner theca interna and outer theca externa around the multilaminar follicle
Secondary (antral) follicle	Oocyte reaches near-maximum size (~125 μm)	Multiple layers = membrana granulosa ; fluid-filled spaces (liquor folliculi) coalesce into a single antrum	Theca interna acquires LH receptors and produces androstenedione, aromatised to estrogen by granulosa cells; PAS-positive Call-Exner bodies (hyaluronan/proteoglycan) appear between granulosa cells
Graafian (mature) follicle	Oocyte lies eccentrically	Oocyte attached to the wall by the discus proligerus ; granulosa cells immediately around the oocyte = cumulus oophorus ; the innermost layer next to the zona pellucida = corona radiata	Single large antrum; may expand to ~2 cm and bulge the ovarian surface (visible on ultrasound); develops from a primordial follicle over ~90 days

*Microscopic structure of ovary*

Oogenesis — numerical milestones

Time point	Number of germ cells
5th month of gestation (peak)	~7 million primary oocytes
Birth	~1 million
Puberty	~40,000
Ovulated over reproductive life	~400

- ▶ All oogonia destined for use in life are formed **before birth**; no post-natal renewal.
- ▶ The **primary oocyte** is arrested in **prophase of meiosis I** from fetal life until just before ovulation.
- ▶ Just before ovulation, meiosis I is completed unequally: a large **secondary oocyte** + a small **first polar body**.
- ▶ The secondary oocyte immediately enters meiosis II and arrests in **metaphase II**; this is the cell actually shed at ovulation (surrounded by zona pellucida + corona radiata).
- ▶ Meiosis II is completed only on fertilisation, yielding the mature ovum + second polar body.

Ovulation

- ▶ Rising estrogen at mid-cycle triggers an **LH surge** (~day 14).
- ▶ The mature follicle bulges the ovarian surface; the stroma/theca over the most convex point becomes thin, forming an avascular **stigma**.
- ▶ Cumulus cells loosen; the follicle ruptures and releases the secondary oocyte (with zona pellucida and corona radiata) into the peritoneal cavity, picked up by the fimbriated infundibulum.

Corpus luteum and its fate

- ▶ After rupture, bleeding into the collapsed follicle forms the transient **corpus hemorrhagicum**.

- ▶ Granulosa cells enlarge into **granulosa-lutein cells** (~80% of the mass, large, pale, centrally placed) — secrete progesterone and convert theca-derived androgens to estrogen.
- ▶ Theca interna cells enlarge into **theca-lutein cells** (~20%, smaller, darker, peripherally placed) — secrete progesterone, some estrogen and androgens.
- ▶ **Corpus luteum of menstruation**: persists ~14 days if no fertilisation, then degenerates.
- ▶ **Corpus luteum of pregnancy**: rescued by hCG, persists 3-4 months, enlarges further, secretes progesterone until the placenta takes over.
- ▶ Both regress by fibrosis into the **corpus albicans** (white scar).

Atresia and interstitial glands

- ▶ Follicles that begin to grow but do not ovulate in a given cycle undergo **atresia** (apoptosis of the oocyte and granulosa cells, collapse of the zona pellucida, macrophage clearance).
- ▶ The theca interna of an atretic follicle may persist and proliferate to form the **interstitial gland** (**corpora atretica**) — estrogen/androgen-secreting cell clusters in the stroma — which eventually also scars down like a corpus albicans.

Applied / Clinical anatomy

- ▶ **Follicular and corpus-luteal cysts** — an unruptured Graafian follicle or a persistent corpus luteum can enlarge into a functional ovarian cyst; luteal cyst rupture can mimic a ruptured ectopic pregnancy (haemoperitoneum).
- ▶ **Polycystic ovary syndrome (PCOS)** — multiple subcapsular cystic follicles arrested at the antral stage, thickened tunica albuginea, hyperandrogenic stroma/theca — classic clinico-histological correlate.
- ▶ **Antral follicle count** on transvaginal ultrasound is used clinically as a surrogate marker of **ovarian reserve** (correlates with the shrinking primordial-follicle pool).
- ▶ **Premature ovarian insufficiency** — accelerated follicular atresia depleting the cortex before age 40.
- ▶ **Turner syndrome (45,X)** — germ cells fail to be maintained; ovaries become fibrous **streak gonads** devoid of follicles.
- ▶ **Ovarian neoplasms** classified by cell of origin, mirroring the histology above: surface epithelial tumours (serous/mucinous cystadenoma–cystadenocarcinoma) from the germinal epithelium — the commonest group; germ cell tumours (dysgerminoma, teratoma) from the oocyte lineage — commoner in younger women; sex cord-stromal tumours (granulosa cell tumour — estrogenic; thecoma; Sertoli-Leydig cell tumour — androgenic/virilising) from granulosa/theca lineage.
- ▶ **Hilus (Leydig) cell tumour** — arises from hilar hilus cells, virilising, with diagnostic Reinke crystalloids — same cytological marker as testicular Leydig-cell tumours.
- ▶ Post-menopausally the cortex becomes fibrotic, follicles are exhausted, and only interstitial/hilus cells and corpora albicantia remain — the histological basis of ovarian atrophy.

High-yield exam pointers

- ▶ Four stages of folliculogenesis, in order: **primordial** → **primary** (unilaminar → multilaminar) → **secondary/antral** → **Graafian**.

- ▶ **Zona pellucida** appears first at the primary follicle stage; **antrum** appears first at the secondary follicle stage.
- ▶ **Corona radiata** = innermost cumulus cell layer touching the zona pellucida; extruded with the oocyte at ovulation.
- ▶ **Theca interna** (LH receptors, androgen precursor) + **theca externa** (fibrous) = theca folliculi, differentiating from stroma around the multilaminar primary follicle.
- ▶ Corpus luteum has two cell types: **granulosa-lutein** (80%, central, progesterone + aromatisation) and **theca-lutein** (20%, peripheral, progesterone/androgen/estrogen).
- ▶ Germ-cell numbers to recall: **7 million (5-month fetus) → 1 million (birth) → 40,000 (puberty) → ~400 ovulated in a lifetime.**
- ▶ **Germinal epithelium is a misnomer** — it is continuous with peritoneal mesothelium and does not generate germ cells.
- ▶ **Hilus cells** = ovarian homologue of testicular Leydig cells; contain Reinke crystalloids; secrete androgens.
- ▶ Corpus luteum of menstruation lasts ~14 days; corpus luteum of pregnancy is rescued by hCG and persists 3-4 months.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; BD Chaurasia — Human Anatomy Vol 2, 8e (figure).



Microscopic structure of suprarenal gland

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction The paired suprarenal (adrenal) glands are retroperitoneal endocrine organs capping the superior pole of each kidney — the right gland flattened and triangular, the left semilunar. In section, the gland shows an outer **cortex** (yellowish, forming the main mass) and an inner **medulla** (dark red/greyish, about one-tenth of the gland, completely enclosed by cortex except at the hilum). The cortex and medulla are, in effect, two different endocrine organs of separate embryological origin — the cortex from coelomic (mesodermal) epithelium and the medulla from neural-crest cells — united within a single connective-tissue capsule during development. This dual identity underlies the sharply distinct microscopic pictures of the two regions.

General architecture

- ▶ Each gland is invested by a thick, dense collagenous **capsule**, from which thin fibrous **septa/trabeculae** carry blood vessels and nerves into the parenchyma.
- ▶ The **stroma** throughout the gland is a delicate network of **reticular fibres** supporting cords/clumps of secretory cells, all closely applied to a rich sinusoidal capillary bed — the typical arrangement of a steroid/amine-secreting endocrine gland.

- Combined weight of both glands is about 8 g; each gland measures roughly 4–6 cm long, 1–2 cm wide and 4–6 mm thick in the adult, the **cortex constituting close to 90% of the gland by weight**.
- The gland has **no hilum for its arteries** — superior, middle and inferior suprarenal arteries pierce the capsule independently (see Vascular architecture below).

Suprarenal cortex — zonation

The cortex is made up of cords/columns of cells separated by sinusoids, arranged into three concentric zones that differ in cell arrangement, staining and secretion. All cortical cells share the ultrastructural signature of steroid-secreting cells — abundant smooth endoplasmic reticulum (sER) and mitochondria with tubular/vesicular (rather than shelf-like) cristae, since both organelles cooperate in converting cholesterol to active steroid.

Zone	Position / proportion of cortex	Cell arrangement (LM)	Cytological features	Hormone secreted	Principal control
Zona glomerulosa	Outermost, just deep to capsule; ~15% of cortex	Closely packed, rounded/ovoid clusters or inverted-U ("arched") cords, continuous with zona fasciculata; may be discontinuous in places	Small polyhedral/columnar cells, basophilic cytoplasm, densely-staining nuclei; sparse lipid; mitochondria with shelf-like cristae	Mineralocorticoids — mainly aldosterone	Renin–angiotensin II (chiefly) and plasma K ⁺ ; only weak ACTH effect
Zona fasciculata	Middle, thickest layer; ~65–80% of cortex	Long straight cords, 1–2 cells thick, at right angles to the surface; fenestrated sinusoids between cords	Large polyhedral cells, lipid-laden and "vacuolated"/spongy in routine sections (spongiocytes); vesicular nuclei; best-developed Golgi of the cortex	Glucocorticoids — cortisol , corticosterone; small amounts of adrenal androgens (DHEA)	ACTH , with negative feedback by circulating glucocorticoids
Zona reticularis	Innermost, next to medulla; ~5–10% of cortex	Branching, anastomosing cords forming a network ("reticulum"); wide capillaries	Smaller, more acidophilic cells than fasciculata; fewer lipid droplets, more brown lipofuscin pigment	Weak androgens — DHEA/DHEAS ; some cortisol	ACTH

Suprarenal medulla

- ▶ Forms the centre of the gland, composed of large, pale-staining polyhedral **chromaffin cells** arranged in cords/clumps around wide sinusoidal capillaries, supported by a reticular-fibre network; scattered **sympathetic ganglion cells** are also present.
- ▶ Chromaffin cells are **neural-crest derived**, essentially modified postganglionic sympathetic neurons that have lost their axons/dendrites and become purely secretory; they belong to the **APUD** (amine precursor uptake and decarboxylation) system.
- ▶ Name derives from the **chromaffin reaction** — the cell granules oxidise and polymerise on exposure to chromate/dichromate fixatives, staining the tissue brown-black (classic histochemical identification test).
- ▶ With EM, cells show abundant granular (rough) ER, a prominent Golgi, and numerous membrane-bound, electron-dense secretory granules (150–350 nm). Granules of **norepinephrine**-secreting cells are larger/more electron-dense than those of **epinephrine**-secreting cells; both catecholamines are stored bound to Ca^{2+} , ATP and the acidic protein **chromogranin A** (also a precursor of regulatory peptides — vasostatin, pancreastatin, catestatin, parastatin).
- ▶ About 80% of catecholamine output is epinephrine; conversion of norepinephrine to epinephrine (via PNMT) occurs **only** in adrenal medullary chromaffin cells, and requires the locally high cortisol delivered by the cortex's venous drainage (see below).

Vascular architecture

- ▶ Because the gland lacks a true hilum, the **superior** (from the inferior phrenic artery), **middle** (direct branch of the aorta) and **inferior** (from the renal artery) **suprarenal arteries** pierce the capsule independently and branch immediately into a **subcapsular arterial plexus**.
- ▶ From this plexus, three patterns of flow supply the gland: (i) **capsular capillaries** for the capsule itself; (ii) **fenestrated cortical sinusoidal capillaries** that traverse the cords of the cortex and then drain onward into (iii) the **medullary sinusoids**; and separately, **medullary arterioles** run within the trabeculae straight through the cortex to supply the medullary sinusoids directly. The medulla thus has a **dual blood supply** — arterial blood via medullary arterioles, and hormone-laden venous blood arriving from the cortical sinusoids.
- ▶ Venules from cortex and medulla unite into small **adrenomedullary collecting veins**, which converge on a **central adrenomedullary vein** — unusual in having longitudinal bundles of smooth muscle in its tunica media, whose synchronous contraction reduces gland volume and helps express hormone into the circulation. This vein continues as the **suprarenal vein**, draining directly into the **inferior vena cava on the right** and into the **left renal vein on the left**.
- ▶ Lymphatics are present in the capsule, around the larger vessels, and within the medullary parenchyma (a route for distributing chromogranin A).

Nerve supply

Chromaffin cells of the medulla are innervated **directly** by myelinated **preganglionic (presynaptic) sympathetic fibres**, chiefly via the splanchnic nerves — functionally the medulla behaves as a single, greatly modified sympathetic ganglion whose "postganglionic neurons" have become secretory cells.

The cortex, in contrast, has no significant direct secretomotor innervation and is regulated almost entirely by ACTH, the renin–angiotensin system and plasma electrolytes.

□ Fig 71.5 — A cross-section through an adult suprarenal gland showing the cortex (capsule, zona glomerulosa and zona fasciculata) and its vascularity, H&E (Gray's Anatomy — Standring — 41e, p.1668)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power radial "wedge" of the gland (capsule to medulla) and label:

- ▶ Fibrous **capsule** with a septum/trabecula carrying a vessel
- ▶ **Zona glomerulosa** — arched/rounded clusters just under the capsule
- ▶ **Zona fasciculata** — long straight two-cell-thick cords ("spongiocytes"), the thickest layer
- ▶ **Zona reticularis** — branching anastomosing network, darker cells
- ▶ **Medulla** — clumps of pale chromaffin cells around wide sinusoids; a sympathetic ganglion cell
- ▶ **Central (adrenomedullary) vein** with muscular wall
- ▶ Subcapsular arterial plexus / medullary arteriole running through a trabecula
- ▶ Sinusoidal capillaries between cortical cords

Applied / Clinical anatomy

- ▶ **Addison disease** (adrenocortical insufficiency): usually autoimmune destruction of the cortex, causing combined loss of mineralocorticoid, glucocorticoid and androgen secretion — hypotension, hyponatraemia/hyperkalaemia, hyperpigmentation.
- ▶ **Cushing syndrome/disease**: hyperplasia or adenoma of the zona fasciculata (or an ACTH-driven basophil adenoma of the pituitary in Cushing disease) causes hypercortisolism.
- ▶ **Conn syndrome** (primary hyperaldosteronism): adenoma/hyperplasia of the zona glomerulosa → hypertension with hypokalaemia.
- ▶ **Congenital adrenal hyperplasia**: autosomal-recessive enzyme defects in cortisol/aldosterone synthesis, over 90% due to **21-hydroxylase deficiency**, with compensatory ACTH-driven cortical hyperplasia and virilisation.
- ▶ **Phaeochromocytoma**: a chromaffin-cell tumour of the medulla causing episodic catecholamine excess (paroxysmal hypertension); classically summarised by the "rule of 10s" (about 10% extra-adrenal/bilateral/malignant/familial). Extra-adrenal chromaffin tissue (e.g. the para-aortic organ of Zuckerkandl) can give rise to an extra-adrenal **paraganglioma**.
- ▶ **Neuroblastoma**: the commonest extracranial solid tumour of childhood, arising from primitive neural-crest-derived cells of the adrenal medulla/sympathetic chain.

High-yield exam pointers

- ▶ Cortex = mesoderm (coelomic epithelium); medulla = neural crest — "two glands, one capsule."
- ▶ Three cortical zones (outer→inner): **glomerulosa** (aldosterone, ~15%) → **fasciculata** (cortisol, ~65–80%, spongiocytes) → **reticularis** (androgens, ~5–10%, lipofuscin).
- ▶ Gland has **no hilum**; three suprarenal arteries pierce the capsule independently to form a subcapsular plexus.

- ▶ Medulla has a **dual blood supply** — arterial (medullary arterioles) + venous (cortical sinusoidal blood, which supplies the cortisol needed to convert NE→Epi).
- ▶ Chromaffin cells are modified postganglionic sympathetic neurons, directly innervated by **preganglionic splanchnic fibres**; they give a positive **chromaffin reaction** with chromate/dichromate fixatives.
- ▶ Central adrenomedullary vein uniquely carries smooth muscle in its tunica media; right suprarenal vein drains directly to the IVC, left to the left renal vein.
- ▶ Zone-specific disease correlations: Addison (whole cortex), Conn (glomerulosa), Cushing (fasciculata), CAH/21-hydroxylase (biosynthetic block), pheochromocytoma/neuroblastoma (medulla).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e.



Microscopic structure of testis

Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction The testis is a paired, encapsulated compound tubular gland with a dual histological function — an **exocrine** function (spermatogenesis, carried out in the seminiferous tubules) and an **endocrine** function (testosterone synthesis, by the interstitial Leydig cells lying between the tubules). Externally it is invested by three concentric coats — tunica vaginalis, tunica albuginea and tunica vasculosa — and internally partitioned by fibrous septa into lobules, each containing one to four highly coiled seminiferous tubules. The seminiferous epithelium itself is built from two co-operating cell populations — non-dividing, supportive **Sertoli cells** and the proliferating, differentiating **spermatogenic cell series** — kept apart from the systemic circulation by the **blood–testis barrier**, a feature that underlies both normal fertility and several of its disorders.

1. Capsule and lobulation

- ▶ **Tunica vaginalis** — a peritoneal remnant carried down during testicular descent; its parietal layer lines the scrotal wall and its visceral layer covers the tunica albuginea everywhere except along the posterior border.
- ▶ **Tunica albuginea** — a tough, dense collagenous capsule covering the whole gland; it thickens along the posterior border to form the **mediastinum testis**, through which the genital ducts, blood vessels and lymphatics enter or leave.
- ▶ **Tunica vasculosa** — a thin, loose, vascular areolar layer lining the interior of each lobule, deep to the albuginea.
- ▶ Fibrous **septa** radiate from the mediastinum and incompletely divide the gland into ~200–300 **pyramidal lobules** (apex directed towards the mediastinum), each lobule containing 1–4 **convoluted seminiferous tubules** set in sparse, vascular interstitial connective tissue.

2. Seminiferous tubule — architecture and duct continuation

- ▶ Each testis contains several hundred (~600–1200, by different estimates) highly coiled seminiferous tubules, each **150–250 µm** in diameter and, uncoiled, **30–80 cm** long (average ~50 cm); the combined tubular length per testis is of the order of **250 m**.
- ▶ Every tubule forms a closed loop whose two ends converge near the mediastinum, losing their convolutions to become a short, narrow **straight tubule (tubulus rectus)** — a segment lined only by Sertoli-type cuboidal cells and devoid of germ cells.
- ▶ Wall of the tubule, outside to in: an outer **tunica/lamina propria** (fibrous connective tissue containing flattened, contractile **myoid cells** whose rhythmic peristalsis helps propel non-motile sperm and tubular fluid) → **basement membrane** → thick, stratified **seminiferous (germinal) epithelium**.
- ▶ The germinal epithelium comprises two cell lines arranged basal-to-luminal in developmental order — **Sertoli cells** (supporting) interposed with the **spermatogenic cell series** (spermatogonia → spermatocytes → spermatids → spermatozoa).
- ▶ Within the mediastinum the straight tubules anastomose to form the **rete testis** (flattened-to-cuboidal, microvillous epithelium), which drains via **7–15 efferent ductules** — lined by an alternating ciliated-columnar and non-ciliated absorptive epithelium, giving a characteristic scalloped luminal outline — into the head of the epididymis.

□ Fig 17.12 — Histology of seminiferous tubule (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.294)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A low-power schematic of the testis alongside a high-power cross-section of one seminiferous tubule, labelled with:

- ▶ Tunica vaginalis (parietal and visceral layers), tunica albuginea, mediastinum testis
- ▶ Septa of testis → lobules of testis (1–4 tubules per lobule)
- ▶ Seminiferous tubule (convoluted part) → tubulus rectus → rete testis → efferent ductules → head of epididymis
- ▶ Interstitial (Leydig) cells in the loose connective tissue between tubules
- ▶ Cross-section of one tubule, base to lumen: tunica/lamina propria with myoid cells → basement membrane → spermatogonia → primary spermatocytes → secondary spermatocytes → early/late spermatids → Sertoli cell nucleus and cytoplasm spanning base to lumen → lumen containing spermatozoa

3. Sertoli cells

Feature	Description
Shape / nucleus	Tall, columnar, non-dividing cells; base rests on the basement membrane, apex reaches the lumen; nucleus euchromatic, irregular/triangular, with one or two prominent nucleoli

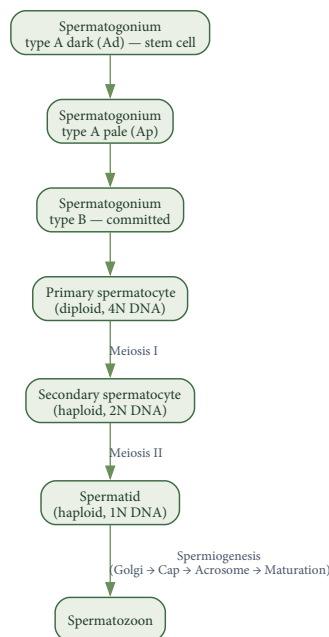
Feature	Description
Cytoplasm	Abundant smooth ER, some rough ER, well-developed Golgi, numerous mitochondria, and lysosomes reflecting a phagocytic function
Junctions	Basolateral tight (occluding) junctions between adjacent Sertoli cells constitute the blood–testis barrier ; gap junctions also link neighbouring Sertoli cells
Physical support	Deep lateral recesses envelop spermatogonia, spermatocytes and spermatids, providing mechanical support and a nutritive/metabolic exchange route to the vasculature
Phagocytosis	Engulfs and digests residual bodies shed by spermatids during spermiogenesis, and any germ cells that fail to differentiate
Exocrine secretion	A fructose- and protein-rich fluid that facilitates transport of released sperm along the tubule
Endocrine secretion	Androgen-binding protein (ABP) , which concentrates testosterone within the tubule lumen, and inhibin B , which feeds back to suppress pituitary FSH; the fetal Sertoli cell additionally secretes Müllerian-inhibiting substance (MIS/AMH)
Hormonal control	Stimulated by FSH acting through membrane receptors on the Sertoli cell

4. Spermatogenesis and spermiogenesis

- **Spermatogonia** are small, diploid (46,XY) stem cells resting on the basement membrane, sub-typed as **dark type-A (Ad)** — the reserve stem-cell pool that divides mitotically to maintain its own numbers — **pale type-A (Ap)** — a transit-amplifying stage — and **type-B**, which are committed to entering meiosis.
- Type-B spermatogonia undergo a final mitotic division to give **primary spermatocytes** (diploid, 4N DNA content) — the largest germ cells, recognised by their large, darkly-staining nuclei; they enter a markedly prolonged prophase of the first meiotic division and, in doing so, cross the blood–testis barrier from the basal into the adluminal compartment.
- Meiosis I yields **secondary spermatocytes** (haploid, 23 chromosomes, 2N DNA) — short-lived cells rarely captured in sections because they proceed almost immediately to meiosis II.
- Meiosis II yields haploid **spermatids** (1N DNA); no further division occurs. Spermatids then remodel into spermatozoa by **spermiogenesis**, while remaining attached to the Sertoli cell surface.
- The generation of a mature spermatozoon from a spermatogonium takes **approximately 64 days**; an alternative derivation (based on a **16-day** human cycle of the seminiferous epithelium, of which **six recognisable cellular associations/stages** were described by Clermont, occurring over

~4.6 cycles) gives a comparable figure of ~74 days. A further ~12 days are needed for maturation during epididymal transit. Roughly 200–300 million sperm are produced per testis per day.

- **Spermiogenesis** proceeds through four phases: in the **Golgi phase**, PAS-positive proacrosomal granules from the Golgi complex coalesce to form the acrosomal vesicle, while the centriole migrates to the pole opposite it and organises the flagellar axoneme; in the **Cap phase**, the acrosomal vesicle spreads as a cap over the anterior half of the condensing nucleus; in the **Acrosome phase**, the nucleus elongates and condenses further (histones being replaced by protamines) while the flagellum lengthens and mitochondria aggregate around its proximal part to form the middle piece; in the **Maturation phase**, excess cytoplasm is shed as a residual body (phagocytosed by the Sertoli cell) and the structurally mature, still non-motile spermatozoon is released into the lumen — a step called **spermiation**.



Microscopic structure of testis

5. Blood–testis barrier and interstitial (Leydig) cells

- The barrier is formed by **tight junctions between adjacent Sertoli cells**, sited just above the level of the spermatogonia, and divides the seminiferous epithelium into a **basal compartment** (containing spermatogonia, freely exposed to the interstitial tissue) and an **adluminal compartment** (containing spermatocytes and spermatids, sequestered from the plasma and immune cells). It creates an immunologically privileged environment for the antigenically "foreign" haploid germ cells and permits the Sertoli cell to control the local ionic and nutrient microenvironment.
- **Leydig (interstitial) cells** are large, polyhedral cells with an eccentric nucleus (one to three nucleoli) and pale, eosinophilic, foamy cytoplasm rich in smooth endoplasmic reticulum, mitochondria with tubulovesicular cristae, lipid droplets and lipofuscin pigment; they contain characteristic rod-shaped cytoplasmic inclusions, the **crystals (crystalloids) of Reinke**, of uncertain function but regarded as a hallmark of the mature human Leydig cell.

- ▶ They synthesise and secrete **testosterone** from cholesterol using the same steroidogenic enzyme complement as the adrenal cortex, and in the embryo additionally secrete **insulin-like protein 3 (INSL3)**, which contributes to testicular descent.
- ▶ **Fetal Leydig cells** are active from early gestation (driven by placental gonadotropin, masculinising the duct system), then become quiescent, fibroblast-like cells until stimulated to resume androgen secretion at puberty, remaining active thereafter throughout reproductive life.

Hormonal axis (hypothalamo–pituitary–gonadal)

Level	Hormone	Target / action
Hypothalamus	GnRH	Stimulates anterior pituitary gonadotrophs
Anterior pituitary	FSH	Acts on Sertoli cells → secretion of ABP and inhibin B; supports spermatogenesis
Anterior pituitary	LH (= ICSH)	Acts on Leydig cells → testosterone synthesis
Sertoli cell	Inhibin B	Negative feedback on pituitary FSH
Leydig cell	Testosterone (+ ABP)	Maintains the high intratubular androgen concentration essential for spermiogenesis; negative feedback on LH

Applied / Clinical anatomy

- ▶ **Cryptorchidism** (undescended testis) causes progressive tubular hyalinisation and germinal-epithelial loss if uncorrected, leading to infertility and a markedly increased risk of germ-cell malignancy.
- ▶ **Testicular biopsy in male infertility** is interpreted against the normal cellular associations of the seminiferous epithelium; complete absence of germ cells with tubules lined only by Sertoli cells defines the **Sertoli-cell-only syndrome**.
- ▶ **Breach of the blood–testis barrier** (trauma, mumps orchitis, vasectomy) exposes previously sequestered sperm antigens to the immune system, generating anti-sperm antibodies and autoimmune subfertility.
- ▶ **Varicocele** — dilatation of the pampiniform plexus, classically left-sided — raises intrascrotal temperature and impairs the temperature-sensitive process of spermatogenesis; a common, treatable cause of male infertility.
- ▶ **Germ-cell tumours** (the great majority of testicular cancers, e.g. seminoma) arise from the spermatogenic cell line and are strongly associated with a history of cryptorchidism; **Leydig-cell tumours** are rare, may be hormonally active (causing precocious puberty in children or gynaecomastia in adults), and characteristically retain Reinke crystals on histology.
- ▶ **Lymphatic spread** of testicular tumours follows the **para-aortic/lumbar nodes** along the gonadal vessels (reflecting the retroperitoneal embryological origin of the testis), never the inguinal nodes — the reason biopsy is always via radical inguinal orchidectomy, never trans-scrotal.

High-yield exam pointers

- ▶ Coats outside-in: **tunica vaginalis** → **tunica albuginea** → **tunica vasculosa**; posterior thickening of the albuginea = **mediastinum testis**.
- ▶ ~200–300 **lobules**, each with 1–4 highly coiled seminiferous tubules embedded in interstitial (Leydig cell) tissue.
- ▶ Germinal epithelium = **Sertoli cells** (non-dividing, supportive) + **spermatogenic series** (spermatogonia → primary spermatocyte → secondary spermatocyte → spermatid → spermatozoon).
- ▶ **Blood–testis barrier** = tight junctions between adjacent Sertoli cells; separates basal (spermatogonia) from adluminal (spermatocytes/spermatids) compartments; confers immune privilege.
- ▶ **Leydig cells**: interstitial, testosterone-secreting, eosinophilic, foamy cytoplasm with pathognomonic **Reinke crystals**; driven by LH/ICSH.
- ▶ Duration of spermatogenesis ~64 days (spermatogonium to mature spermatozoon); spermiogenesis has 4 phases — **Golgi, Cap, Acrosome, Maturation**.
- ▶ Hormonal loop: FSH → **Sertoli cell** (ABP, inhibin B); LH → **Leydig cell** (testosterone).
- ▶ Duct sequence: seminiferous tubule → **tubulus rectus** → **rete testis** → **efferent ductules** → epididymis.
- ▶ Applied triad: **cryptorchidism** (descent failure, malignancy risk), **varicocele** (impaired spermatogenesis), **germ-cell tumour lymphatic spread to para-aortic nodes** (not inguinal).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; BD Chaurasia — Human Anatomy Vol 2, 8e (figure); Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e.



Microscopy of uterine endometrium

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

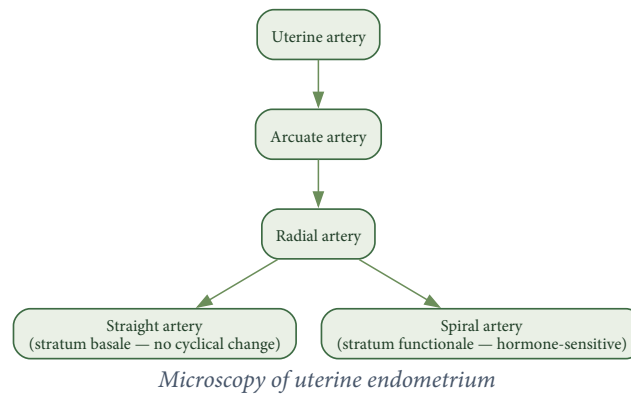
Definition / Introduction The endometrium is the innermost, mucosal layer of the uterine wall, lying internal to the myometrium (thick smooth-muscle coat) and perimetrium (peritoneal serosa/adventitia). It is composed of a simple columnar surface epithelium, numerous simple tubular uterine glands that dip from the surface into the lamina propria, and a highly cellular, vascular endometrial stroma. Unique among body mucosae, the endometrium undergoes cyclical proliferation, secretory transformation and partial shedding every menstrual cycle under the sequential influence of ovarian oestrogen and progesterone — a cycle intimately linked to its dual (straight and spiral) arterial supply.

General architecture

- ▶ **Surface epithelium:** simple columnar; a mixture of non-ciliated secretory (peg) cells and ciliated cells; continuous with the epithelium lining the uterine glands.
- ▶ **Uterine glands:** simple tubular glands lined by columnar cells that secrete glycoproteins/glycogen; oriented perpendicular to the luminal surface; their basal portions penetrate deep into the endometrium, close to the myometrium.
- ▶ **Endometrial stroma (lamina propria):** resembles mesenchyme — highly cellular, abundant ground substance, fine (type III) collagen fibrils, fibroblasts and a rich microvasculature; no muscularis mucosae or submucosa separates endometrium from myometrium.
- ▶ **Two functional zones** (from the reproductive-age endometrium onward):
 - **Stratum basale** — deep layer next to the myometrium; contains the basal ends of the uterine glands and the terminations of the straight arteries; not shed at menstruation; regenerates the functionalis each cycle.
 - **Stratum functionale** — superficial, hormone-responsive layer; supplied by spiral arteries; shed at menstruation.
- ▶ Just before menstruation, the functionalis itself can be subdivided into a superficial **stratum compactum** (gland necks only, decidualised stroma) and a deeper **stratum spongiosum** (dilated, tortuous gland bodies separated by scant, oedematous stroma).

Blood supply and its role in cyclical change

- ▶ **Uterine artery** → **arcuate arteries** (circumferential, within the outer/middle myometrium) → **radial arteries** (pass inward through the myometrium toward the endometrium) →, at the endometrial–myometrial junction, each radial artery gives two types of terminal vessels:
 - **Straight (basal) arteries** — short, do not coil, supply only the stratum basale; hormone-insensitive; persist unchanged through the cycle.
 - **Spiral arteries** — long, muscular, highly coiled; supply the stratum functionale; exquisitely sensitive to oestrogen and progesterone; elongate and coil progressively through the proliferative and secretory phases and constrict just before menstruation.
- ▶ This dual supply explains why the basalis survives menstrual shedding while the functionalis does not.



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a vertical block schematic of the endometrium (uterine cavity at top, myometrium at bottom) and label:

- ▶ Surface (luminal) epithelium — simple columnar
- ▶ Uterine gland (cut in cross/longitudinal section)
- ▶ Endometrial stroma / lamina propria
- ▶ Stratum functionale (superficial ~2/3)
- ▶ Stratum basale (deep ~1/3, adjoining myometrium)
- ▶ Myometrium (smooth muscle)
- ▶ Uterine artery → Arcuate artery → Radial artery
- ▶ Straight artery (terminating in stratum basale)
- ▶ Spiral artery (coiled, ascending through stratum functionale to just below the surface)

□ Fig 31.21 — *Histology of proliferative phase of uterine endometrium* (BD Chaurasia — *Human Anatomy Vol 2* — 8e, p.486)

□ Fig 31.22 — *Histology of secretory phase of uterine endometrium* (BD Chaurasia — *Human Anatomy Vol 2* — 8e, p.486)

Proliferative (follicular/oestrogenic) phase — day 4/5 to day 14

- ▶ Begins as menstrual flow ceases; driven by rising **oestrogen** from the growing ovarian follicles.
- ▶ **Re-epithelialisation**: surviving glandular epithelium in the stratum basale grows outward to cover the denuded surface; complete within 5–6 days of the onset of menstruation.
- ▶ **Glands**: straight, narrow, tubular, with scanty secretion; widely spaced early in the phase.
- ▶ **Stroma**: dense and highly cellular; mitotic figures prominent in both epithelial and stromal cells; scattered lymphocytes.
- ▶ **Spiral arteries**: lengthen but remain only slightly coiled, and extend only into the basal two-thirds of the endometrium.
- ▶ By the end of the phase (around day 14), the endometrium has thickened from ~1–2 mm to roughly 3–4 mm.

Secretory (luteal/progestational) phase — day 14 to day 28

- ▶ Begins after ovulation, driven by **progesterone** (with oestrogen) from the corpus luteum; first morphological effects appear 24–36 hours after ovulation.
- ▶ **Early secretory**: glycogen accumulates in the **basal cytoplasm** of the glandular epithelial cells (basal glycogen vacuolation), often with associated lipid.
- ▶ **Mid-secretory**: glands become tortuous, dilated and closely packed, producing the classic "**saw-tooth**"/**cork-screw** profile in section; lumens fill with mucus- and glycogen-rich secretion. Endometrial thickness reaches its **maximum**, ~6–7 mm.
- ▶ **Stroma**: progressively oedematous; stromal cells enlarge and accumulate glycogen/lipid — the **decidual reaction**, most marked around blood vessels just beneath the surface epithelium.
- ▶ **Spiral arteries**: continue to elongate and coil, extending almost to the surface epithelium by the late secretory phase.
- ▶ **Late secretory (premenstrual)**: glandular secretory activity declines; the compacted, decidualised stroma near the surface and the spongy, gland-rich zone beneath it correspond to the (re-emerging) stratum compactum and stratum spongiosum.

Menstrual phase — day 1 to day 4/5

- ▶ Triggered by falling progesterone and oestrogen as the corpus luteum regresses (in the absence of fertilisation).
- ▶ Intermittent, then sustained, constriction of the **spiral arteries** produces ischaemia of the stratum functionale; glands stop secreting and the epithelium and stroma undergo necrosis and apoptosis; prostaglandin release and proteolytic breakdown of the stromal matrix follow.
- ▶ During brief periods of vessel relaxation, blood escapes from the devitalised capillaries and open venule ends, producing sub-epithelial haematomas and then frank bleeding.
- ▶ The **stratum functionale** is **shed piecemeal** (roughly 65–75% of endometrial thickness), discharged with blood through the vagina as the menstrual flow, which usually lasts 3–6 days.
- ▶ The **stratum basale**, supplied by the unaffected straight arteries, is retained and becomes the source of re-epithelialisation for the next proliferative phase.

Comparative summary — proliferative vs secretory phase

Feature	Proliferative phase	Secretory phase
Dominant hormone	Oestrogen	Progesterone
Endometrial thickness	~3–4 mm	~6–7 mm
Strata present	Stratum basale prominent; functionale re-forming	All strata (compactum, spongiosum, basale) well developed
Uterine glands	Straight, narrow lumen, widely spaced	Coiled ("saw-tooth"/cork-screw), wide lumen, closely packed
Glandular secretion	Scanty	Abundant, glycogen/mucus-rich

Feature	Proliferative phase	Secretory phase
Decidual reaction	Absent	Present (peri-vascular stromal cells)
Stromal glycogen	Minimal	Prominent
Spiral arteries	Minimally coiled, confined to basal two-thirds	Markedly coiled, extend to near the surface

Applied / Clinical anatomy

- ▶ **Endometrial dating:** the characteristic day-by-day glandular and stromal appearance (gland shape, secretory content, stromal oedema/decidual change) allows histological "dating" of an endometrial biopsy against the expected day of the cycle — used in the work-up of infertility and luteal-phase defects.
- ▶ **Anovulatory cycles:** in the absence of ovulation, no corpus luteum forms, progesterone is not secreted, and the endometrium remains in an oestrogen-driven proliferative pattern (with eventual irregular/"breakthrough" bleeding) rather than transforming into a secretory pattern.
- ▶ **Implantation site:** the blastocyst normally implants in the functionalis of the fundus/body; because the tubal mucosa lacks a comparable functionalis, ectopic tubal implantation cannot support a normal decidual/placental reaction and predisposes to rupture.
- ▶ **Endometrial ablation/curettage:** because regeneration depends on the stratum basale, procedures that spare the basalis (e.g., superficial curettage) preserve the capacity for cyclical regeneration; deep basalis injury (over-vigorous curettage, post-partum infection) can cause intrauterine adhesions (Asherman's syndrome) with impaired regeneration.
- ▶ **Endometrial pathology:** unopposed oestrogenic stimulation (anovulation, oestrogen-secreting tumours, exogenous oestrogen) can drive endometrial hyperplasia, a recognised precursor of endometrioid endometrial carcinoma — reflecting the same proliferative-phase mechanism operating without the counter-balancing effect of progesterone.
- ▶ **Imaging correlate:** on transvaginal ultrasound and T2-weighted MRI, the endometrium shows a "triple-line" appearance in the late proliferative phase (echogenic central line, hypoechoic functional layer, echogenic basal layer) that becomes uniformly hyperechoic in the secretory phase — used clinically to estimate cycle day and endometrial receptivity.

High-yield exam pointers

- ▶ Endometrium = surface epithelium + uterine glands + vascular stroma; two functional zones — stratum basale (permanent) and stratum functionale (cyclically shed).
- ▶ Blood supply: uterine → arcuate → radial → straight arteries (basale, no cyclical change) / spiral arteries (functionale, hormone-sensitive).
- ▶ Proliferative phase (oestrogen, day 4–14): straight glands, narrow lumen, ~3–4 mm thick, spiral arteries confined to basal two-thirds.
- ▶ Secretory phase (progesterone, day 14–28): coiled "saw-tooth" glands with abundant secretion, decidual reaction, ~6–7 mm thick, spiral arteries reach near the surface.

- ▶ Menstrual phase: spiral artery constriction → ischaemia → shedding of ~65–75% of endometrial thickness as menses (lasts 3–6 days); basalis (straight-artery supply) is preserved.
- ▶ Three pre-menstrual strata: compactum (superficial, gland necks) — spongiosum (middle, dilated glands) — basale (deep).
- ▶ Endometrial biopsy dating and detection of anovulatory (persistently proliferative) cycles are classic applied-anatomy correlates.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; BD Chaurasia — Human Anatomy Vol 2 8e (figures).



Ultra microscopic anatomy of pituitary gland

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction — The pituitary gland (hypophysis cerebri) has a dual embryological origin: an ectodermal **adenohypophysis** (from Rathke's pouch) and a neuroectodermal **neurohypophysis** (from the neurohypophyseal bud of the diencephalic floor). At the ultrastructural (electron-microscopic) level this dual origin is unmistakable — the adenohypophysis shows the classical fine structure of a true polypeptide/glycoprotein-secreting epithelium (abundant rough endoplasmic reticulum, Golgi complexes and membrane-bound secretory granules), while the neurohypophysis shows the fine structure of modified nervous tissue (unmyelinated axons, neurosecretory vesicles and astrocyte-like glia) and synthesises no hormone of its own. Because the pituitary regulates most other endocrine glands, it is often termed the "master endocrine gland."

1. General ultrastructural organisation

- ▶ The whole gland is invested by a thin fibrous **capsule**, from which fine reticular septa carry a rich **fenestrated sinusoidal capillary bed** into the parenchyma — the fenestrations (with an attenuated diaphragm and an intact basal lamina) permit rapid two-way exchange of hormones and releasing factors between the parenchymal cells and blood.
- ▶ The **hypothalamo-hypophyseal portal system** is itself an ultrastructural adaptation: a **primary capillary plexus** (fenestrated) in the median eminence/infundibulum, drained by portal veins along the pars tuberalis, re-forms as a **secondary sinusoidal plexus** in the pars distalis — the low-pressure, purely venous nature of this supply is the structural basis of the pars distalis's vulnerability to ischaemia.
- ▶ Parenchymal cells of the adenohypophysis are arranged in **cords and nests**, each cell abutting a sinusoid across a basal lamina — the typical EM arrangement of an endocrine (ductless) gland.

2. Ultrastructure of the pars distalis — the five chromophil cell types

By light microscopy the pars distalis shows **acidophils** (~40%, eosin/orange-G positive), **basophils** (~10%, haematoxylin- and PAS-positive, since their hormones are glycoproteins) and **chromophobes**

(~50%, poorly staining). Transmission electron microscopy (TEM) resolves these into five hormonally distinct, granule-secreting cell types, each identifiable by the **size, density and packing of its secretory granules**:

Cell type	% of cells	Tinctorial class	Granule size (TEM)	Nucleus	Other EM features	Hormone
Somatotroph	~50%	Acidophil	Dense, ~350 nm, closely packed	Round, central, prominent nucleolus	Abundant rER; well-developed Golgi	Growth hormone (GH)
Lactotroph (mammotroph)	15–20%	Acidophil	Inactive: ~200 nm, sparse; Active: pleomorphic, dense, ~600 nm, sparse	Oval, central	Lysosomes increase after lactation; cell hypertrophy/hyperplasia in pregnancy	Prolactin (PRL)
Corticotroph	15–20%	Basophil (PAS+)	100–300 nm	Round, eccentric	Lipid droplets, large lysosomes, perinuclear bundles of intermediate filaments	ACTH (via POMC)
Gonadotroph	~10%	Basophil (PAS+)	Dense, 200–250 nm	Round, eccentric	Prominent Golgi; distended rER cisternae	FSH and LH
Thyrotroph	~5% (least numerous)	Basophil (PAS+)	Dense, ~150 nm	Round, eccentric	Prominent Golgi with numerous vesicles	TSH

□ Fig 20.7 — Ultrastructure and immunohistochemistry of somatotrophic cells: TEM ($\times 10,000$) shows a somatotroph with a well-developed Golgi complex, euchromatic nucleus and cytoplasm packed with dense secretory granules — the EM criteria shared by all chromophil cell types, refined further by immunocytochemistry. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1008)

- **Somatotrophs** are the most numerous and largest single population — GH is regulated by GHRH, somatostatin and ghrelin.
- With two exceptions (gonadotrophs make both FSH and LH; corticotrophs cleave the single precursor **POMC** into ACTH, β -lipotropin, MSH-related peptides and β -endorphin), each cell type makes only one hormone.
- Granule diameter is the principal EM discriminant — somatotroph (350 nm) > lactotroph active phase (600 nm, but sparse) > gonadotroph (200–250 nm) > corticotroph (100–300 nm) > thyrotroph (150 nm, smallest and least numerous).

3. Chromophobes and folliculo-stellate cells

- ▶ **Chromophobes** show few or no secretory granules on EM and are a **heterogeneous** population: many are **degranulated (depleted) acidophils or basophils** that have discharged their stored hormone, while others are genuine **stem/undifferentiated progenitor cells** capable of replenishing the chromophil populations.
- ▶ **Folliculo-stellate cells** are star-shaped, non-hormone-secreting cells with long cytoplasmic processes that encircle the hormone-producing cells and interconnect with each other (and, on newer evidence, with hormone-producing cells) through **gap junctions containing connexin-43**. They are believed to form a paracrine signalling network relaying regulatory information from the pars tuberalis to the pars distalis.

4. Pars intermedia and pars tuberalis — fine structure

- ▶ **Pars intermedia**: a narrow, vestigial zone (in humans) between pars distalis and pars nervosa. TEM shows cells forming **apical junctional complexes** around small cystic cavities — remnants of the lumen of Rathke's pouch — filled with **colloid** (often with cell debris); these cells have **larger secretory vesicles** than the pars distalis. The parenchyma is chiefly **basophils** (corticotroph-like cells, whose POMC here is cleaved preferentially to γ -LPH, β -endorphin and MSH rather than ACTH) together with chromophobes.
- ▶ **Pars tuberalis**: a thin, highly vascular funnel of tissue wrapping the infundibulum, composed predominantly of **gonadotrophs** arranged as cords/clusters closely applied to the portal vessels, with scattered squamous-cell nests and small colloid-containing follicles.

5. Ultrastructure of the neurohypophysis (pars nervosa)

The neurohypophysis is **not a secretory gland** — it is modified CNS tissue that stores and releases neurohormones synthesised elsewhere. It contains the unmyelinated axons and terminals of **~100,000 neurosecretory neurons** whose cell bodies lie in the **supraoptic and paraventricular nuclei** of the hypothalamus (the hypothalamo-hypophyseal tract), together with specialised glial cells.

- ▶ **Three morphologically distinct, membrane-bound vesicle populations** are seen by EM in the axon terminals:
- ▶ **Neurosecretory vesicles, 10–30 nm** — the most numerous; they accumulate in terminals and dilate the axon to form **Herring bodies**. Each contains **oxytocin or antidiuretic hormone (ADH/vasopressin)** (a 9-amino-acid peptide) bound non-covalently to its specific carrier protein, **neurophysin I or II**, plus ATP; hormone and neurophysin are cleaved from a single precursor molecule during axonal transport.
- ▶ **~30 nm vesicles containing acetylcholine**, thought to help trigger release of the neurosecretory vesicles.
- ▶ **Larger, 50–80 nm dense-core vesicles**, resembling the dense-core vesicles of the adrenal medulla and adrenergic nerve terminals.
- ▶ **Herring bodies** — dilated axonal swellings visible even by light microscopy as eosinophilic globules — on EM additionally contain **mitochondria**, a few **microtubules** and profiles of **smooth endoplasmic reticulum (SER)**.

- ▶ **Pituicytes** — the most abundant cell type in the pars nervosa — are elongated, astrocyte-like glial cells whose processes ensheath and support the unmyelinated axons and their terminals, exactly as astrocytes support neurons in the CNS; they do not themselves synthesise hormone.
- ▶ Axon terminals/Herring bodies abut directly onto a rich **fenestrated capillary network** (no diffusion barrier), permitting oxytocin and ADH to enter the systemic circulation directly upon nerve-impulse-triggered release.

□ Fig 20.11 — *Pars nervosa: neurosecretory (Herring) bodies and pituicytes. LM correlate (×400, H&E) of the EM findings above — unmyelinated axons ensheathed by pituicytes (P), with axonal swellings (neurosecretory/Herring bodies, NB) abutting capillaries (C) for hormone release. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1016)*

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Reproduce a two-panel labelled EM-schematic with these exact labels:

- ▶ **Panel A — adenohypophyseal secretory cell:** cell membrane; euchromatic nucleus with nucleolus; rough endoplasmic reticulum (rER); Golgi complex; membrane-bound secretory granules (size proportional to cell type — largest in somatotroph, smallest in thyrotroph); adjacent fenestrated sinusoidal capillary with basal lamina.
- ▶ **Panel B — neurohypophyseal axon terminal:** axon terminal/Herring body; neurosecretory vesicles (10–30 nm, oxytocin/ADH + neurophysin); small 30-nm cholinergic vesicles; large 50–80-nm dense-core vesicles; mitochondria; SER profiles; microtubules; surrounding pituicyte process; adjacent fenestrated capillary.

Applied / Clinical anatomy

- ▶ **Somatotroph adenoma** (excess GH) — **gigantism** if it occurs before epiphyseal fusion in children, **acromegaly** in adults; GH deficiency in childhood causes pituitary **dwarfism**.
- ▶ **Corticotroph adenoma** (excess ACTH via POMC) — **Cushing syndrome/disease**.
- ▶ **Lactotroph adenoma (prolactinoma)** — the commonest functioning pituitary tumour; physiological lactotroph hypertrophy/hyperplasia also enlarges the gland in pregnancy and lactation.
- ▶ **Chromophobe adenoma** — the commonest pituitary tumour overall; usually non-functioning, but grows large enough to produce mass effect: **ballooning of the sella turcica** on lateral skull radiograph, **bitemporal hemianopia** from pressure on the optic chiasma, and **exophthalmos/ophthalmoplegia** from cavernous sinus involvement.
- ▶ **Rathke cleft cyst / craniopharyngioma** — arise from the colloid-filled remnants of Rathke's pouch normally seen in the pars intermedia; ectopic Rathke-pouch cell rests in the nasopharyngeal roof form the accessory **pharyngeal hypophysis**.
- ▶ **Diabetes insipidus** — results from loss of ADH delivery to the circulation, whether from a gene mutation affecting the vasopressin–neurophysin precursor, or from a lesion of the pituitary stalk/posterior lobe.
- ▶ **Sheehan syndrome** — post-partum hypotension/haemorrhage causes ischaemic necrosis chiefly of the **pars distalis**, because its entire supply is the low-pressure hypophyseal **portal** system, while the pars nervosa (direct arterial supply via the inferior hypophyseal artery) is relatively spared.

- ▶ **Surgical relevance:** pituitary adenomas are approached by the **trans-sphenoidal** route (through the sphenoid air sinus) or, less often, the **transfrontal** route.
- ▶ Correlation of granule size/density with immunocytochemistry (and, in unusual tumours, EM) remains the ultrastructural basis for sub-typing pituitary adenomas by cell of origin.

High-yield exam pointers

- ▶ Adenohypophysis (Rathke pouch, oral ectoderm) = true secretory ultrastructure (rER + Golgi + granules); neurohypophysis (neuroectoderm) = modified CNS/glial ultrastructure, makes no hormone of its own.
- ▶ Five EM-distinguishable pars distalis cell types + chromophobes + folliculo-stellate cells; granule size is the key EM discriminant (somatotroph 350 nm > lactotroph active 600 nm > gonadotroph 200–250 nm > corticotroph 100–300 nm > thyrotroph 150 nm).
- ▶ Approximate percentages: somatotroph 50%, lactotroph & corticotroph 15–20% each, gonadotroph 10%, thyrotroph 5% (fewest, largest cells).
- ▶ Chromophobes = degranulated chromophils + stem/progenitor cells; folliculo-stellate cells are non-secretory and linked by connexin-43 gap junctions.
- ▶ Neurohypophysis: three vesicle populations (10–30 nm neurosecretory, 30 nm cholinergic, 50–80 nm dense-core); Herring bodies contain neurosecretory vesicles, mitochondria, SER and microtubules; pituicytes are astrocyte-like glia.
- ▶ Fenestrated capillaries throughout (portal plexuses + pars nervosa capillaries) are the EM basis of rapid, barrier-free hormone entry into blood.
- ▶ Clinical correlation: somatotroph adenoma → gigantism/acromegaly; corticotroph adenoma → Cushing disease; chromophobe adenoma → commonest, mass effect/bitemporal hemianopia; Sheehan syndrome → selective anterior-lobe infarction due to its portal (not arterial) supply.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh — Textbook of Human Histology 9e; Vishram Singh — Textbook of Anatomy Vol 3 4e; Gray's Anatomy (Standing) 41e.

Coverage note: the spine text (Gray's Anatomy) and this topic's assigned specialty texts (Bancroft's Histological Techniques; Pranab Dey — Lab Techniques in Histopathology & Cytology) cover electron-microscopic principles and tissue-processing technique generally but carry no pituitary-specific ultrastructural description; the gland-specific EM detail above is grounded instead in the histology corpus (Ross, Junqueira, IB Singh, Vishram Singh) via targeted retrieval.

♦ ♦ ♦

Systemic histology - nervous tissue & special senses

♦ ♦ ♦

Histology of Cerebellum

Asked in: Paper III 15-Apr-2026 · Paper III 02-Aug-2021 · Paper III May 2018 (3×) — RGUHS MD/MS Anatomy

Definition / Introduction — The cerebellum is the largest part of the hindbrain, lying in the posterior cranial fossa dorsal to the pons and medulla and separated from the occipital lobes by the tentorium cerebelli; the weight ratio of cerebellum to cerebrum is approximately 1:10 in adults and 1:20 in infants. It consists of two hemispheres joined by a midline vermis, with a surface thrown into numerous transverse folds (folia) separated by fissures. Internally it has a superficial, highly convoluted cortex of gray matter overlying a core of white matter that branches in a characteristic tree-like pattern, the **arbor vitae**, within which four paired **deep cerebellar nuclei** are embedded. Unlike the cerebral cortex, which has diverse cell types arranged differently region to region, the cerebellar cortex is remarkably uniform, with only a small number of cell types connected in a highly stereotyped circuit throughout.

1. General organisation and deep nuclei

- ▶ Cortex (gray matter) is superficial; white matter (medullary core) is deep — the reverse arrangement is never seen in the cerebellum.
- ▶ The cortex, if unfolded, has roughly half the surface area of the cerebral cortex, yet because of the enormous number of densely packed granule cells it contains **more neurons than the cerebral cortex**.
- ▶ Four pairs of deep cerebellar nuclei lie embedded in the white matter, named from medial to lateral:

Nucleus	Position	Notes
Fastigial	Most medial, near midline in roof of 4th ventricle	Efferents mainly via inferior cerebellar peduncle
Globose	Lateral to fastigial	Together with emboliform = posterior/anterior interposed nuclei
Emboliform	Lateral to globose	
Dentate	Most lateral, largest, "crumpled-purse" shape	Main efferent pathway (brachium conjunctivum) emerges from its hilus

- ▶ The white matter of the two sides is connected by a thin lamina related to the 4th ventricle roof, whose upper part forms the **superior medullary velum** and lower part the **inferior medullary velum**.

2. The three layers of the cerebellar cortex

The cerebellar cortex shows a constant, three-layered architecture from without inwards — this is the single most important identification feature on any section.

Layer	Location	Principal contents	Staining (H&E)
Molecular layer	Outermost, just deep to pia mater	Sparse cell population: outer stellate cells , deeper basket cells ; dendritic arborisation of Purkinje cells; unmyelinated parallel fibres (axons of granule cells); climbing-fibre branches; radial processes of Bergmann glia	Lightly/palely stained (eosinophilic), cell-poor
Purkinje cell layer	Single row between molecular and granular layers	Large, flask/pear-shaped Purkinje cell somata in a strict monolayer, interspersed with somata of Bergmann glia	Large pink cell bodies, unmistakable on LM
Granular layer	Innermost, resting on white matter	Densely packed small granule cells ; scattered larger Golgi cells ; mossy-fibre rosettes forming cerebellar glomeruli (islands)	Deeply basophilic/hematoxyphilic — densely packed nuclei

Real histology plate:

□ Fig 26.7 — Photomicrograph. Histology of cerebellum (low magnification on left, high magnification on right) (Sontakke — *Textbook of Human Histology* — 2e, p.352)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a single cerebellar folium in longitudinal section and label:

- ▶ Pia mater (covering the folium)
- ▶ Molecular layer (outer, pale)
- ▶ Purkinje cell layer (single row of flask-shaped cells at the molecular–granular junction)
- ▶ Granular layer (inner, densely basophilic)
- ▶ White matter core (arbor vitae) with an afferent fibre entering
- ▶ A Purkinje cell with its flattened, fan-shaped dendritic tree spreading into the molecular layer
- ▶ A cerebellar glomerulus in the granular layer (mossy-fibre rosette + granule-cell dendritic claws)
- ▶ Parallel fibres running along the long axis of the folium in the molecular layer

3. Neurons of the cerebellar cortex

The cerebellar cortex has **five principal neuron types** — Purkinje, granule, Golgi, basket and stellate cells. Of these, only the granule cell is excitatory; all others (Purkinje, Golgi, basket, stellate) are inhibitory.

Cell	Layer	Morphology	Transmitter / role
Purkinje cell	Purkinje cell layer	Large, flask-shaped body; flattened, fan-like dendritic tree oriented transverse to the long axis of the folium, in a single geometric plane; up to ~180,000 dendritic spines per cell receive synapses from parallel fibres	GABAergic, inhibitory — the sole output neurone of the cerebellar cortex; axon crosses the granular layer to end in the deep cerebellar/vestibular nuclei
Granule cell	Granular layer	Smallest neuron in the body (4–5 μm), round nucleus, 4–5 claw-like dendrites forming glomeruli with mossy-fibre rosettes; axon ascends into the molecular layer and bifurcates in a "T" to form two long parallel fibres	Glutamatergic, the only excitatory cortical neuron
Golgi cell	Outer part of granular layer, beneath Purkinje cells	Largest interneuron; dendrites extend into both molecular and granular layers	Mostly glycinergic; inhibitory — gives feedback inhibition to granule cells
Basket cell	Deep molecular layer	Axon forms basket-like plexuses around Purkinje cell somata and initial axon segments	GABAergic; inhibitory — feed-forward inhibition of Purkinje cells
Stellate cell	Superficial molecular layer	Small, star-shaped; axon ends on Purkinje cell dendrites	GABAergic; inhibitory

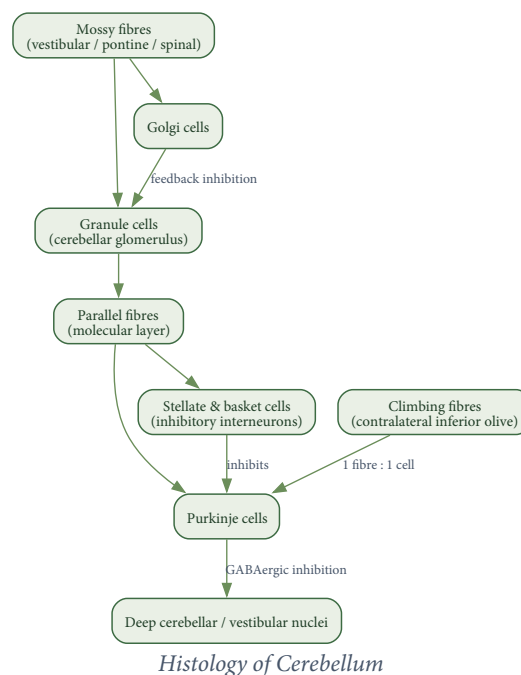
Two additional interneurons (Ramón y Cajal): **Lugaro cells** (cigar-shaped, at the level of the Purkinje layer, glycinergic, innervate stellate and basket cells) and **unipolar (monopolar) brush cells** (excitatory interneurons concentrated in vestibular-related cortex, receiving a giant mossy-fibre synapse and relaying it onward as a second-order mossy input to granule cells). Fine dendritic/axonal detail of all these neurons is best demonstrated with **silver-impregnation (Golgi/Cajal) staining**, since routine H&E shows only cell bodies and layering.

4. Afferent fibres and the cerebellar glomerulus

The cerebellar cortex receives only two types of afferent fibre, both excitatory (glutamatergic):

- **Climbing fibres** — arise exclusively from the contralateral inferior olivary nucleus (olivocerebellar fibres); each fibre passes through the granular and Purkinje layers to the molecular layer, where it "climbs" over and makes a direct, powerful, **one-fibre-to-one-Purkinje-cell** synaptic contact on the smooth proximal dendrites; collaterals also reach the deep cerebellar nuclei.

- **Mossy fibres** — the more numerous afferent, arising from the **vestibular nuclei** (vestibulocerebellar), **pontine nuclei** (pontocerebellar) and the **spinal cord** (spinocerebellar), among other sources; each fibre ends in the granular layer as 30–40 terminal dilatations called **rosettes**.
- **Cerebellar glomerulus (island)** — the functional synaptic unit of the granular layer: a central mossy-fibre rosette (presynaptic) is enveloped by the claw-like dendrites of about 15 granule cells (postsynaptic) and by axon terminals of Golgi cells, the whole complex wrapped in a capsule of **Bergmann glia**. A single mossy fibre, via its rosettes, activates roughly 450 granule cells.
- Granule-cell axons ascend into the molecular layer and bifurcate into **parallel fibres**, which run parallel to the folium's long axis and synapse on the spiny dendritic branchlets of Purkinje cells as well as on stellate, basket and Golgi cell dendrites — thereby also recruiting the inhibitory interneurons.



5. Efferent (output) pathway

- The **Purkinje cell** is the sole output neuron of the cerebellar cortex; its axon crosses the granular layer into the white matter and terminates, with an inhibitory (GABAergic) action, on neurones of the deep cerebellar nuclei or, in the vestibulocerebellum, directly on vestibular nuclei.
- Neurones of the deep nuclei are themselves mainly glutamatergic relay cells; their output leaves chiefly through the **superior cerebellar peduncle** (from dentate, emboliform, globose, and part of fastigial), decussating in the caudal midbrain to reach the contralateral red nucleus and thalamus, while fastigial fibres also leave via the inferior cerebellar peduncle.
- Small GABAergic **nucleo-olivary neurones** feed back to the contralateral inferior olive, closing a cortico-nucleo-olivary loop that regulates climbing-fibre input.

Applied / Clinical anatomy

- **Cerebellar ataxia**: damage to or loss of Purkinje cells removes the sole inhibitory output of the cortex, producing incoordination, dysmetria, intention tremor and a broad-based, unsteady gait.

- ▶ Selective, chronic loss of Purkinje cells in the anterior lobe/vermis is classically described in long-standing alcoholism, producing predominantly truncal and gait ataxia.
- ▶ **Medulloblastoma**, the commonest posterior-fossa tumour of childhood, is believed to arise from residual primitive precursor cells of the transient **external granular layer** (the fetal proliferative zone that gives rise to granule cells) persisting in the vermis/roof of the fourth ventricle.
- ▶ Because routine H&E preparations show cell bodies and layering but not fine neuronal processes, **silver-impregnation (Golgi/Cajal) techniques** are required to demonstrate the full dendritic and axonal architecture of cerebellar neurons — relevant to the histotechnique component of this paper.
- ▶ Damage to the white matter/peduncles (e.g., demyelinating disease) interrupts afferent-efferent traffic and produces ataxia and nystagmus similar to cortical lesions.

High-yield exam pointers

- ▶ Cortex = 3 constant layers, outer to inner: **molecular** → **Purkinje** → **granular**.
- ▶ **Purkinje cell** = only output neurone of the cortex; GABAergic, inhibitory.
- ▶ **Granule cell** = only excitatory cortical neurone; smallest neurone in the body.
- ▶ **Climbing fibre** (inferior olive) = 1 fibre : 1 Purkinje cell; **mossy fibre** (vestibular/pontine/spinal) = many : many via the glomerulus.
- ▶ Deep nuclei, medial to lateral: **fastigial** → **globose** → **emboliform** → **dentate**.
- ▶ Interneurons stellate, basket and Golgi are all **inhibitory**; only Golgi cells act on granule cells (feedback), while stellate/basket act on Purkinje cells (feed-forward).
- ▶ Cerebellar glomerulus = mossy-fibre rosette + granule-cell dendritic claws + Golgi axon terminals, glial-capsuled.
- ▶ Purkinje cell layer is a strict single-cell-thick row — the key low-power identification feature.
- ▶ Silver/Golgi staining, not routine H&E, is needed to show full dendritic/axonal detail (Cajal's classical descriptions).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Brijesh Kumar — Histology Text & Atlas 3e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e.



Cells of cerebral cortex

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction The **cerebral cortex (neocortex)** is a sheet of gray matter, **1.5–4 mm thick** (thicker over gyri, thinner over sulci), covering the cerebral hemispheres and made up of nerve cells, neuroglia and blood vessels. Its neurons show two principal morphological types — **pyramidal cells** and **non-pyramidal (stellate/granule) cells** — together with several accessory, eponymously-named cells (Betz, Martinotti, horizontal cells of Cajal, fusiform, basket, chandelier, neurogliaform). These neurons are arranged in **six horizontal laminae** and, cutting across the layers, in **vertical functional columns**; the composition of the layers varies regionally and forms the basis of cytoarchitectonic (Brodmann-type) mapping and of important clinical correlations.

1. Principal neuronal cell types

Cell type	Proportion	Morphology	Processes
Pyramidal cells	~two-thirds of all cortical neurons (~5.5 billion)	Pyramidal/triangular cell body, apex directed toward the cortical surface	One large apical dendrite from the apex (ascends, branches, ends in the molecular layer) + several basal dendrites spreading horizontally from the base; dendrites studded with spines. A single axon arises from the base and gives projection, association or commissural fibres
Non-pyramidal (stellate/granule) cells	~one-third of cortical neurons	Small, star-shaped, multipolar bodies (~6–10 µm) that resemble granules in Nissl stains, hence "granule cells"	Short axons that mostly remain within the cortex (Golgi type II/short-axon cells); processes chiefly vertical, occasionally horizontal

- Pyramidal cells are the **projection (output/efferent)** neurons of the cortex — larger cells give long projection fibres (to basal ganglia, brainstem, spinal cord), smaller ones give association/commissural fibres; axon collaterals feed back onto stellate cells and other interneurons.
- Non-pyramidal cells are subdivided into **spiny stellate cells** (glutamatergic, excitatory, concentrated in lamina IV, receive thalamocortical input) and a heterogeneous group of **non-spiny/GABAergic interneurons** — **basket cells**, **chandelier (axo-axonic) cells**, **double bouquet (bitufted) cells**, **neurogliaform (spiderweb) cells**, and **bipolar/fusiform cells** — all confined to gray matter and inhibitory, some co-expressing neuropeptides (VIP, CCK, somatostatin).

Named/special cortical cell types

- ▶ **Cells of Martinotti** — small, multipolar, triangular/polygonal neurons found in almost every layer (most numerous in layer VI); short dendrites, long axons that run **vertically toward the surface** to ramify in the molecular layer.
- ▶ **Horizontal cells of Cajal** — small, fusiform cells confined to **layer I**; axons run tangentially, parallel to the pial surface, linking adjacent cortical columns.
- ▶ **Fusiform cells** — spindle-shaped neurons oriented perpendicular to the cortical surface, chiefly in **layer VI**; axon and dendrites arise from opposite poles.
- ▶ **Betz cells (giant pyramidal cells of Betz)** — the largest pyramidal cells (cell bodies approach 80 μm), confined to **layer V of the motor cortex (precentral gyrus, area 4)**; give rise to corticospinal/corticonuclear (pyramidal tract) fibres and account for only a small fraction (~3%) of the pyramidal tract fibres. Named after **Vladimir Betz (1834–1894)**, Ukrainian anatomist/histologist.
- ▶ **Neurogliaform (spiderweb) cells** — small (10–12 μm) spherical cells with radiating dendrites, found mainly in laminae II–IV.

□ Fig 26.10 — Cells of cerebral cortex. H-horizontal cell of Cajal, G-granule cell, (Sontakke — Textbook of Human Histology — 2e, p.355)

2. Six-layered lamination (cytoarchitecture)

Lamina	Name	Principal cells	Special feature
I	Molecular / plexiform layer	Few neurons; mainly horizontal cells of Cajal and tangential fibres (apical dendrites of pyramidal cells, axons of stellate/Martinotti cells)	Lies just beneath pia mater
II	External granular layer	Small pyramidal cells + stellate/granule cells	Also called "small pyramidal cell layer"
III	External pyramidal layer	Small-to-medium pyramidal cells (size increases with depth), few stellate and Martinotti cells; often subdivided IIIa–IIIc	Axons form association and commissural fibres
IV	Internal granular layer	Densely packed spiny stellate/granule cells , few small pyramidal cells	Traversed by the external (outer) band of Baillarger ; maximally developed in granular (koniocortex) type
V	Internal pyramidal (ganglionic) layer	Medium and large pyramidal cells ; Betz cells in the motor cortex; scattered Martinotti, fusiform and granule cells	Traversed by the internal (inner) band of Baillarger ; major output/efferent layer

Lamina	Name	Principal cells	Special feature
VI	Multiform / polymorphic layer	Neurones of varied shape — pyramidal, fusiform, spindle, ovoid; cells of Martinotti most numerous here	Blends gradually with underlying white matter

□ Fig 12.10 — The six layers of the cerebral cortex. (Vishram Singh — Clinical Neuroanatomy — 4e, p.454)

- ▶ The **bands of Baillarger** (named after **Jules Baillarger**, 1809–1890) are tangential myelinated fibre bands — an **outer band** in lamina IV and an **inner band** in lamina V.
- ▶ In the **striate (primary visual) cortex**, the outer band of Baillarger is exceptionally thick and visible to the naked eye as the **stria of Gennari** (after **Francesco Gennari**, 1750–1797), giving this area its alternate name "striate cortex."
- ▶ Cells are also organised into **vertical functional columns**, within which pyramidal cells, stellate cells and interneurons form short excitatory/inhibitory loops (the structural basis of Mountcastle's columnar hypothesis).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a vertical strip of cerebral cortex from pia mater to white matter and label:

- ▶ **Lamina I — Molecular/plexiform layer:** few cells; horizontal cells of Cajal; tangential fibre plexus.
- ▶ **Lamina II — External granular layer:** small pyramidal cells + granule/stellate cells.
- ▶ **Lamina III — External pyramidal layer:** small-to-medium pyramidal cells (increasing size with depth); axons → association/commissural fibres.
- ▶ **Lamina IV — Internal granular layer:** densely packed spiny stellate cells; **outer band of Baillarger** running through it (label "**stria of Gennari**" as its exaggerated form in visual cortex).
- ▶ **Lamina V — Internal pyramidal (ganglionic) layer:** large pyramidal cells; **Betz cells** (label as present only in motor cortex, area 4); **inner band of Baillarger**; axons → corticospinal/corticobulbar/corticostriate fibres.
- ▶ **Lamina VI — Multiform/polymorphic layer:** fusiform cells, cells of Martinotti; axons → corticothalamic fibres; blends into white matter.
- ▶ Show a **pyramidal cell** in full with apical dendrite (to layer I), basal dendrites, dendritic spines, and a basal axon; show a **cell of Martinotti** in layer VI with its axon ascending to layer I; show a **horizontal cell of Cajal** confined to layer I.

3. Regional (cytoarchitectonic) variation

- ▶ **Homotypical cortex** (all six laminae identifiable) — subdivided (somewhat loosely) into **frontal**, **parietal** and **polar** types, differing in the relative prominence of granular vs pyramidal laminae.
- ▶ **Heterotypical cortex** (certain laminae diminished/absent):

- **Agranular type** — granular laminae (II, IV) diminished/absent, large pyramidal neurones dense; typifies **motor areas** (precentral gyrus, area 4; also areas 6, 8, 44) with numerous efferent (pyramidal tract) projections.
- **Granular type (konio cortex)** — granular layers maximally developed, laminae III/V poorly developed; typifies **sensory/receptive areas** — postcentral gyrus (somatosensory), striate area (visual, with the stria of Gennari), and superior temporal gyrus (auditory) — receiving the bulk of afferent (thalamocortical) input.

4. Functional (connectional) lamination

- ▶ **Lamina IV** receives the largest share of **thalamocortical afferents** (also laminae I and VI receive major afferents).
- ▶ **Lamina V** (Betz cells in motor cortex) is the principal source of **efferent projection fibres** — corticospinal, corticobulbar (incl. corticopontine), and corticostriate.
- ▶ **Lamina VI** is the major source of **corticothalamic** (feedback) fibres.
- ▶ **Supragranular laminae (II, III)** give rise mainly to **association** (intrahemispheric) and **commissural** (interhemispheric, via corpus callosum) corticocortical fibres.
- ▶ Golgi/Nissl/silver impregnation methods (Ramón y Cajal-type staining) are the classical histotechniques by which these layers and cell types were first delineated.

Applied / Clinical anatomy

- ▶ **Amyotrophic lateral sclerosis / motor neuron disease** — degeneration of upper motor neurons, including **Betz cells** of the precentral gyrus (layer V) and their corticospinal axons, together with lower motor neurons of the ventral horn, producing combined upper + lower motor neuron signs.
- ▶ **Alzheimer's disease** — neurofibrillary tangles and neuritic plaques accumulate preferentially within **pyramidal cells** (notably layers III and V), producing cortical (especially hippocampal/temporal) atrophy and progressive dementia.
- ▶ **Stria of Gennari / striate cortex** — a naked-eye landmark used to identify the **primary visual cortex** (area 17) on gross/histological section and on high-resolution MRI (line of Gennari), important in radiological localisation of the calcarine cortex.
- ▶ **Focal cortical dysplasia** — disruption of normal six-layered lamination and abnormal (giant/balloon) neurones is a recognised structural substrate of **medically refractory epilepsy**.
- ▶ **Laminar (pseudolaminar) necrosis** — after global cerebral hypoxia–ischaemia, the metabolically vulnerable pyramidal-rich layers (III, V, VI) are selectively damaged, producing a characteristic laminar pattern on imaging/histology.
- ▶ Cytoarchitectonic differences between agranular (motor) and granular (sensory) cortex underpin the **Brodman area** classification used clinically to localise cortical lesions/strokes to specific functions.

High-yield exam pointers

- ▶ Two principal neocortical cell types: **pyramidal** (~2/3, projection/efferent, Golgi type I) and **non-pyramidal/stellate-granule** (~1/3, mostly interneurons, Golgi type II).

- ▶ Named cells to recall: **Betz** (giant pyramidal, layer V, motor cortex, up to ~80 µm), **Martinotti** (axon ascends to layer I), **horizontal cells of Cajal** (layer I only, tangential axons), **fusiform cells** (layer VI).
- ▶ Six laminae, superficial to deep: **Molecular** → **External granular** → **External pyramidal** → **Internal granular** → **Internal pyramidal (ganglionic)** → **Multiform**.
- ▶ **Bands of Baillarger**: outer (lamina IV) and inner (lamina V); outer band exaggerated as the **stria of Gennari** in the visual cortex.
- ▶ **Agranular cortex** = motor (area 4) — thick pyramidal layers, thin granular layers; **granular cortex (koniocortex)** = sensory (postcentral, striate, superior temporal) — thick granular layers.
- ▶ Functional lamination: **layer IV** = major input; **layer V** = major output (corticospinal/corticostriate); **layer VI** = corticothalamic; **layers II/III** = association/commissural.
- ▶ Betz cells degenerate in **ALS**; pyramidal cells bear neurofibrillary tangles in **Alzheimer's disease**.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e; IB Singh — Textbook of Human Histology 9e; Snell — Clinical Neuroanatomy 7e; Vishram Singh — Clinical Neuroanatomy 4e (figure).



Compare and contrast histological appearance of spinal and sympathetic ganglion

Asked in: Paper III Nov 2017 — RGUHS MD/MS Anatomy

Definition / Introduction A **ganglion** is an aggregation of neuronal cell bodies lying outside the central nervous system (CNS), enclosed within a connective-tissue capsule and permeated by nerve fibre bundles. Ganglia are of two functional kinds — **sensory (craniospinal) ganglia**, of which the **spinal or dorsal root ganglion** is the prototype, and **autonomic ganglia**, of which the **sympathetic ganglion** is the prototype used for practical demonstration. Although both share the same general plan (capsule, neuron-cell-bodies, satellite glial cells, nerve-fibre bundles, neural-crest origin), their neurons differ fundamentally in polarity, arrangement, synaptic behaviour and function, giving each a distinctive histological signature.

General plan of a ganglion (features common to both)

- ▶ Each ganglion is covered by a **connective-tissue capsule** continuous with the endo-/peri-/epineurium of the nerve root or trunk.
- ▶ Contains **neuronal cell bodies** together with **satellite (capsular) glial cells** and **nerve fibre bundles** passing through or arising from it.
- ▶ The connective tissue within the ganglion is **highly vascularised**; the **blood–nerve barrier** is less efficient here than along peripheral nerve trunks.

- ▶ **Satellite glial cells** are the principal PNS glia investing neuronal somata in both types of ganglion (functionally analogous to CNS astrocytes — express glutamine synthetase, coupled by gap junctions).
- ▶ **Neurons and satellite cells of all peripheral ganglia are derived from the neural crest.**
- ▶ Enteric ganglia are the exception to the capsule rule — they lack a connective-tissue capsule and resemble CNS tissue in organisation.

Histological structure of spinal (dorsal root) ganglion

- ▶ **Location:** on the dorsal (posterior) root of each spinal nerve just proximal to its union with the ventral root; the same basic architecture is seen in sensory ganglia of the trigeminal, facial (geniculate), vestibulocochlear, glossopharyngeal and vagus nerves.
- ▶ **Neurons: pseudounipolar (unipolar)** — a single stem process arises from the soma and bifurcates (T-junction) into a peripheral process (functions as a sensory dendrite conducting toward the soma but structurally an axon) and a central process (axon proper entering the cord). *Exception:* the vestibulocochlear (spiral/cochlear) ganglion retains **bipolar** neurons.
- ▶ **Cell body:** large, spherical to oval, of variable size; nucleus **large, vesicular (euchromatic, pale-staining) and centrally placed**, with a prominent nucleolus.
- ▶ **Arrangement:** neuronal somata are aggregated in **tight clusters/groups, chiefly at the periphery** of the ganglion, leaving the centre occupied by fascicles of nerve fibres.
- ▶ **Nerve fibres:** predominantly **myelinated**, arranged in discrete bundles that separate and surround the clusters of cell bodies.
- ▶ **Satellite cells:** flattened capsular cells form a **complete, continuous ring** around each neuronal soma (since sensory neurons receive no synapses, complete glial insulation is the rule); satellite cells are continuous with the Schwann cells investing the peripheral/central processes.
- ▶ **Synapses: absent** — sensory ganglia are purely relay stations for afferent impulses, not sites of synaptic transmission.
- ▶ **Capsule:** a well-defined, relatively thick connective-tissue capsule.

Histological structure of sympathetic ganglion

- ▶ **Location:** the paired **paravertebral (sympathetic chain) ganglia** flanking the vertebral column, and the **prevertebral (collateral) ganglia** on the anterior aorta — coeliac, superior and inferior mesenteric, and aorticorenal ganglia; the **suprarenal medulla** is a modified sympathetic ganglion.
- ▶ **Neurons: multipolar** postganglionic neurons with elaborate dendritic trees on which preganglionic axons synapse (axodendritic > axosomatic synapses).
- ▶ **Cell body:** smaller than sensory neurons, **irregular in shape**; nucleus large, pale-staining (vesicular) with a prominent nucleolus but **placed eccentrically**; cytoplasm shows **well-developed Nissl granules** (reflecting active catecholamine synthesis) and may contain **lipofuscin pigment**.
- ▶ **Arrangement:** neuronal somata are **scattered randomly** throughout the ganglion, not clustered, interspersed with nerve fibre bundles running between them.
- ▶ **Nerve fibres:** a **mixture of myelinated preganglionic (B) fibres and thin unmyelinated postganglionic (C) fibres**; thinner and less conspicuous than in sensory ganglia.

- ▶ **Satellite cells: few and incomplete** — because the multipolar neuron's numerous processes break the continuity of the glial sheath, the capsule around each cell body is never complete.
- ▶ **Synapses: present** — the ganglion is a genuine relay/integrative station where preganglionic fibres synapse on postganglionic neurons (with considerable divergence, one preganglionic axon contacting many postganglionic neurons, aiding mass sympathetic discharge).
- ▶ **Additional cell type: small numbers of SIF cells (small intensely fluorescent cells)** — smaller than principal neurons, packed with catecholamine (noradrenaline/dopamine/serotonin)-containing granules, thought to act as interneurons/paracrine modulators of ganglionic transmission.
- ▶ **Capsule: thin connective-tissue capsule** (absent in intramural/terminal parasympathetic ganglia).
- ▶ **Special stain: silver impregnation** is preferred to demonstrate the fine processes of these multipolar neurons; H&E readily shows the pale nucleus, prominent nucleolus and Nissl-rich cytoplasm.

Comparative table — spinal (sensory) vs sympathetic (autonomic) ganglion

Feature	Spinal / dorsal root ganglion	Sympathetic ganglion
Functional type	Sensory (craniospinal)	Autonomic (motor/efferent)
Neuron type	Pseudounipolar (bipolar in vestibulocochlear ganglion)	Multipolar
Cell body shape/size	Large, spherical/oval	Smaller, irregular
Nucleus	Large, vesicular, centrally placed	Large, vesicular, eccentrically placed
Nissl substance	Present, less prominent	Well-developed, prominent (catecholamine synthesis)
Arrangement of somata	In clusters , chiefly at the periphery	Scattered randomly throughout
Nerve fibres	Myelinated, in bundles separating clusters	Mixed myelinated (pre-) + unmyelinated (post-ganglionic), thinner
Satellite cells	Complete , continuous ring around each neuron	Few , incomplete ring
Synapses in ganglion	Absent (pure relay)	Present (pre- to post-ganglionic synapse)
Capsule	Well-defined, thicker	Thin (absent in intramural ganglia)
Special cells	None	SIF cells (catecholaminergic interneurons)
Direction of conduction	Toward CNS (afferent)	Away from CNS, to viscera (efferent)

Feature	Spinal / dorsal root ganglion	Sympathetic ganglion
Embryological origin	Neural crest	Neural crest

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

- Fig 12.14 — Photomicrograph. Histology of dorsal root/sensory ganglion (low magnification on left, high magnification on right, H&E stain) (Sontakke — Textbook of Human Histology — 2e, p.160)
- Fig 12.16 — Photomicrograph. Histology of autonomic/sympathetic ganglion (low magnification on left, high magnification on right, H&E stain) (Sontakke — Textbook of Human Histology — 2e, p.162)

Draw two labelled low-power fields side by side for the exam sketch:

- ▶ **Spinal (dorsal root) ganglion:** connective-tissue capsule → clusters of large, spherical neurons at periphery → centrally placed vesicular nucleus with nucleolus → complete satellite-cell ring around each neuron → intervening bundles of myelinated nerve fibres in the centre.
- ▶ **Sympathetic ganglion:** thin capsule → scattered smaller, irregular multipolar neurons → eccentric vesicular nucleus with prominent nucleolus and Nissl-rich cytoplasm → few, incomplete satellite cells → fine bundles of thin (mixed myelinated/unmyelinated) nerve fibres running between neurons.

Applied / Clinical anatomy

- ▶ **Herpes zoster (shingles):** the varicella-zoster virus lies dormant in dorsal root/trigeminal ganglion neurons after chickenpox; reactivation causes dermatomal pain, erythema and blistering, and its most important complication is **post-herpetic neuralgia**. Involvement of the **geniculate ganglion** produces facial paralysis with vesicles (Ramsay Hunt syndrome).
- ▶ **Diagnostic/surgical relevance:** dorsal rhizotomy or ganglionectomy is used for intractable neuralgia; the dorsal root ganglion is spared in anterior (ventral) root lesions, which helps localise a lesion clinically (preserved sensation with motor loss).
- ▶ **Tumours of sympathetic ganglion cells:** **neuroblastoma** (malignant tumour of primitive neuroblasts, arising in the suprarenal medulla or upper abdominal sympathetic ganglia, seen in infants/children, metastasises early) and **ganglioneuroma** (benign tumour of mature sympathetic ganglion cells, in suprarenal medulla or sympathetic chain) both reflect the neural-crest origin of sympathetic ganglion cells; **pheochromocytoma** arises from the closely related chromaffin cells of the adrenal medulla.
- ▶ **Surgical sympathectomy** (cervical/lumbar) interrupts transmission at paravertebral ganglia and is used for palmar hyperhidrosis, Raynaud's phenomenon and some vascular disorders.
- ▶ **Referred pain:** convergence of visceral and somatic afferents at the level of the dorsal root ganglion/posterior horn underlies the clinical phenomenon of referred pain along a dermatome.

High-yield exam pointers

- ▶ Spinal ganglion = **pseudounipolar** neurons; sympathetic ganglion = **multipolar** neurons — the single most important discriminator.

- ▶ Satellite cells form a **complete ring** in sensory ganglia; an **incomplete ring** in sympathetic ganglia.
- ▶ **No synapse** occurs in a sensory ganglion; synapse (pre- to postganglionic) **does occur** in a sympathetic ganglion.
- ▶ Nucleus is **central** in spinal ganglion neurons, **eccentric** in sympathetic ganglion neurons.
- ▶ Neurons are **clustered at the periphery** in spinal ganglion; **scattered throughout** in sympathetic ganglion.
- ▶ Both ganglia share a connective-tissue capsule, neural-crest-derived satellite glial cells, and rich vascularity with a comparatively weak blood–nerve barrier.
- ▶ Exception to remember: the **vestibulocochlear (spiral) ganglion** has bipolar, not pseudounipolar, neurons.
- ▶ **SIF cells** (catecholaminergic interneurons) are found only in autonomic (sympathetic) ganglia, never in sensory ganglia.
- ▶ Clinical hook: sensory ganglion pathology → **herpes zoster**; sympathetic ganglion pathology → **neuroblastoma/ganglioneuroma**.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; Gray's Anatomy (Standring) 41e; Snell — Clinical Neuroanatomy 7e (applied correlations).



Histology of Cerebrum

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction The cerebrum comprises two cerebral hemispheres joined by the corpus callosum. Each hemisphere presents an outer mantle of gray matter, the **cerebral cortex**, overlying a core of **white matter** within which subcortical gray masses (basal ganglia) lie embedded. The surface is thrown into gyri and sulci to accommodate a large surface area within the rigid cranial cavity. The cortex is divided into the phylogenetically older **allocortex** (~10%, three- to five-layered — hippocampus, olfactory cortex) and the **isocortex/neocortex** (~90%), which shows a remarkably constant six-layered laminar pattern with well-defined regional cytoarchitectonic variation.

1. General Organization of the Cerebral Hemisphere

- ▶ **Gray matter (cortex)**: mantle of neuronal cell bodies, neuroglia, unmyelinated/synaptic processes and blood vessels; thickness 1.5–4 mm, thicker over gyri and thinner in the depths of sulci.
- ▶ **White matter (medullary centre)**: myelinated axons with their accompanying glia and vessels; contains no neuronal cell bodies; carries association, commissural and projection fibres.
- ▶ **Subcortical gray matter**: basal ganglia (caudate nucleus, lentiform nucleus, claustrum, amygdala) embedded within the white matter.

- Neurons establish extremely intricate connections with each other and with axons reaching the cortex from other gray masses; cells are arranged both in **horizontal laminae** and in **vertical (columnar) units**, the latter being the functional processing unit of the cortex.

2. Neuronal Cell Types of the Neocortex

Cell type	Proportion / location	Morphology	Functional role
Pyramidal cells	~two-thirds of cortical neurons (~5.5 billion)	Pyramid-shaped soma, apex directed to the surface; single large apical dendrite ascending to the molecular layer; multiple horizontal basal dendrites; single axon	Principal efferent (projection) neuron — axon leaves the cortex as a projection, association or commissural fibre
Stellate cells / granule cells	~one-third of cortical neurons	Small, multipolar; short axons ending within the cortex	Interneurons — synapse with afferent fibres and with collaterals of pyramidal axons; fusiform cells are modified stellate cells
Cells of Martinotti	Present in all layers, most numerous in layer VI	Small multipolar, short dendrites, long axon	Axon runs vertically outward to ramify in the molecular layer — feedback/association
Horizontal cells of Cajal	Confined to lamina I (molecular layer)	Small, fusiform	Neurites run tangentially, integrating several cortical columns

3. Six-Layered Lamination of the Neocortex

Lamina	Name	Composition
I	Molecular / plexiform layer	Few cell bodies (occasional horizontal cells of Cajal); mainly tangential fibres — apical dendrites of pyramidal cells, axons of stellate and Martinotti cells
II	External (outer) granular layer	Densely packed small stellate cells with a few small pyramidal cells
III	External (outer) pyramidal layer	Small pyramidal cells superficially, medium-sized cells deeper, with a few stellate cells; gives rise to association and commissural efferents

Lamina	Name	Composition
IV	Internal (inner) granular layer	Densely packed (spiny) stellate cells with scattered medium pyramidal cells; traversed by the external (outer) band of Baillarger — maximally developed in the visual cortex as the naked-eye stria of Gennari
V	Ganglionic / internal pyramidal layer	Medium- and large-sized pyramidal cells; giant Betz cells in the deeper zone (especially prominent in the motor cortex); traversed by the internal (inner) band of Baillarger ; origin of corticospinal, corticobulbar, corticopontine and corticostriate efferents
VI	Multiform / polymorphic (fusiform) layer	Heterogeneous, mostly spindle-shaped cells with numerous Martinotti cells; origin of corticothalamic fibres

Functionally, laminae **II and IV** receive thalamic sensory afferents; laminae **III and V** are chiefly somatomotor/association efferent layers; laminae **I and VI** integrate sensory and motor activity.

□ Fig 26.12 — Photomicrograph. Histology of cerebral cortex (Neocortex, low power, H&E stain). (Sontakke — Textbook of Human Histology — 2e, p.356)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A vertical section through the full thickness of the neocortex (pia to white matter), labelled:

- ▶ Pia mater with a penetrating blood vessel
- ▶ Lamina I — Molecular/plexiform layer
- ▶ Lamina II — External granular layer
- ▶ Lamina III — External pyramidal layer
- ▶ Lamina IV — Internal granular layer (mark the external band of Baillarger/stria of Gennari)
- ▶ Lamina V — Ganglionic layer with a labelled giant **Betz cell**
- ▶ Lamina VI — Multiform (polymorphic) layer
- ▶ Underlying white matter
- ▶ One each of a pyramidal cell, a stellate cell, a cell of Martinotti and a horizontal cell of Cajal, positioned in their respective layers

4. Regional (Cytoarchitectonic) Variations

All regions of neocortex develop from the same basic six-layered pattern, but the relative prominence of the pyramidal and granular laminae varies regionally — the basis of **Brodmann's cytoarchitectonic areas**.

Type	Granular laminae (II, IV)	Pyramidal laminae (III, V)	Typical location
Agranular (heterotypical)	Diminished/absent	Large pyramidal neurons in high density (efferent-dominant)	Precentral gyrus (area 4, "motor" cortex), areas 6, 8, 44, parts of limbic system
Granular / koniocortex (heterotypical)	Maximally developed, densely packed stellate cells	Poorly developed/unidentifiable (afferent-dominant); thinnest of all types	Postcentral gyrus (somatosensory), striate area (visual, stria of Gennari), superior temporal gyrus (auditory), parts of parahippocampal gyrus
Frontal (homotypical)	Less prominent	Numerous small/medium pyramidal cells in III & V	Frontal, parietal and temporal lobes
Parietal (homotypical)	Wide, more stellate cells	Pyramidal cells smaller than frontal type	Large areas of parietal and temporal lobes
Polar (homotypical)	All six laminae present	Layer III reduced, less stellate invasion	Small areas near frontal and occipital poles

5. White Matter and Supporting Elements

- ▶ **Association fibres** — connect cortical areas within the same hemisphere (short arcuate/U-fibres; long fasciculi — superior/inferior longitudinal, uncinate, cingulum).
- ▶ **Commissural fibres** — link corresponding areas of the two hemispheres (corpus callosum, anterior commissure, fornix).
- ▶ **Projection fibres** — connect the cortex with subcortical structures, brainstem and spinal cord (corona radiata, internal capsule).
- ▶ **Neuroglia**: astrocytes (protoplasmic in gray matter, fibrous in white matter), oligodendrocytes (myelination), microglia (resident immune cells), ependymal cells (line the ventricles).

Applied / Clinical anatomy

- ▶ Disordered synchronous discharge within a cortical column underlies **epileptogenic foci**; failure of normal six-layer migration/lamination (**cortical dysplasia, lissencephaly**) is a well-recognised cause of intractable epilepsy.
- ▶ The **stria of Gennari** (external band of Baillarger, lamina IV) is visible to the naked eye in the primary visual (striate) cortex, area 17 — hence "striate cortex."
- ▶ Selective vulnerability of the pyramidal laminae (III, V, VI) to global hypoxic-ischaemic injury produces **laminar cortical necrosis**, seen as the "cortical ribbon sign" on CT/MRI.
- ▶ Occipital (striate) cortex lesions cause **cortical blindness with macular sparing**, attributed to the dual blood supply (posterior cerebral artery plus middle cerebral artery collaterals) of the occipital pole.

- ▶ Progressive loss of layer V pyramidal (Betz) cells occurs in **amyotrophic lateral sclerosis** and cortical neurodegenerative disease, correlating with upper-motor-neuron signs.
- ▶ **Corpus callosotomy** interrupts commissural fibres and is used surgically in refractory epilepsy ("split-brain" procedure).
- ▶ Classical demonstration of cortical neurons uses **Nissl stain** (cell bodies/Nissl granules) and the **Golgi silver-impregnation method** (dendritic and axonal arborisation).

High-yield exam pointers

- ▶ Cerebral cortex = **isocortex/neocortex** (90%, six layers) + **allocortex** (10%, three–five layers — hippocampus, olfactory cortex).
- ▶ Four basic neuron types: **pyramidal**, **stellate/granule**, **Martinotti**, **horizontal cells of Cajal**.
- ▶ Six layers, superficial to deep: **Molecular (I)** → **External granular (II)** → **External pyramidal (III)** → **Internal granular (IV)** → **Ganglionic/internal pyramidal (V, Betz cells)** → **Multiform (VI)**.
- ▶ Laminae **II & IV** = sensory input; **III & V** = motor/association output; **I & VI** = integration.
- ▶ External (outer) band of Baillarger lies in lamina IV (stria of Gennari in area 17); internal (inner) band of Baillarger lies in lamina V.
- ▶ Five cytoarchitectonic types: **agranular**, **granular (koniocortex)**, **frontal**, **parietal**, **polar** — the basis of Brodmann's areas.
- ▶ White matter fibres: **association** (same hemisphere), **commissural** (corpus callosum — both hemispheres), **projection** (cortex ↔ subcortex/brainstem/cord).
- ▶ Pyramidal cells are the principal excitatory projection neurons (glutamatergic); stellate and Martinotti cells are inhibitory/modulatory interneurons.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh's Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e; Sontakke's Textbook of Human Histology 2e (figure).



Histology of Retina

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction. The retina is the innermost, light-sensitive coat of the eyeball, lining its posterior three-fourths from the **optic disc** to the **ora serrata**. Embryologically it develops from the two layers of the optic cup (an outgrowth of the diencephalon) and therefore retains a **two-part structure throughout life** — an outer non-neural **retinal pigment epithelium (RPE)** and an inner **neural retina**, the latter itself organised into ten discrete histological layers. As an outpost of the CNS, the neural retina contains photoreceptors (rods and cones), three orders of interneurons (bipolar, horizontal, amacrine), ganglion cells, and specialised radial glia (Müller cells), all precisely stacked so that light must traverse the inner layers before striking the photoreceptors.

1. Development and basic two-layered plan

- ▶ The optic vesicle (from the diencephalon) invaginates to form the double-walled **optic cup**.

- ▶ **Outer layer** of the optic cup → thin, pigmented RPE.
- ▶ **Inner layer** → thick **neural retina**, with photoreceptors developing next to the RPE (outermost) and ganglion cells innermost — hence light must pass through the ganglion cell and inner layers before reaching the rods and cones.
- ▶ The two layers are separated in the embryo by a cavity (**intraretinal/optic-cup space**) that is normally obliterated by fusion in fetal life, but the plane remains **only apposed, not truly adherent** (no junctional complexes, only interdigitating microvilli) — this residual potential space is the anatomical basis of **retinal detachment**.
- ▶ The pigment layer continues anteriorly beyond the ora serrata as the pigmented epithelium of the ciliary body (**pars ciliaris retinae**) and iris (**pars iridica retinae**), i.e. the retina's pigment layer is continuous with the whole uveal epithelial lining.

2. Gross topography

- ▶ **Ora serrata** — the scalloped anterior limit of the sensory (neural) retina, marking the ciliary body–choroid junction; the neural retina is here replaced by unpigmented ciliary epithelium while the RPE continues as the pigmented ciliary/iridial epithelium.
- ▶ **Optic disc (optic papilla)** — the site of exit of ganglion cell axons as the optic nerve and entry of the central retinal vessels; devoid of photoreceptors, hence the "**blind spot**."
- ▶ **Macula lutea** — a yellow-pigmented area ~5.5 mm across, temporal to the disc.
- ▶ **Fovea centralis** — a shallow depression in the centre of the macula, ~2.5 mm lateral to the disc, containing **cones only** (no rods), with peripherally-displaced ganglion cells and inner nuclear layer neurons so light reaches the cones with minimal scatter — the site of **greatest visual acuity** and of the visual axis.

3. The ten histological layers

Light passes from the vitreous (layer 10) to the choroid (layer 1) before phototransduction occurs in layer 2 — i.e. the retina is "inverted."

#	Layer	Composition / key feature
1	Retinal pigment epithelium (RPE)	Simple cuboidal, melanin-laden cells; separated from choroid by Bruch's membrane
2	Layer of rods and cones	Outer and inner segments of photoreceptors
3	External (outer) limiting membrane	Not a true membrane — a row of zonulae adherentes between Müller cells and photoreceptors
4	Outer nuclear layer	Cell bodies/nuclei of rods and cones
5	Outer plexiform layer	Synapses of rod spherules/cone pedicles with bipolar and horizontal cell dendrites

#	Layer	Composition / key feature
6	Inner nuclear layer	Cell bodies of bipolar, horizontal, amacrine and Müller cells
7	Inner plexiform layer	Synapses between bipolar/amacrine cells and ganglion cell dendrites
8	Ganglion cell layer	Multipolar ganglion cell bodies; several layers thick near the macula, single layer at the periphery
9	Nerve fibre layer	Unmyelinated axons of ganglion cells converging on the optic disc
10	Internal limiting membrane	Basal lamina formed by expanded Müller cell footplates, separating retina from vitreous

4. Retinal pigment epithelium (RPE)

- ▶ Single layer of low cuboidal cells resting on **Bruch's membrane** (which separates it from the choriocapillaris of the choroid); apical microvilli interdigitate with, but do not junctionally attach to, the tips of the photoreceptor outer segments.
- ▶ **Functions:** (i) absorbs scattered light (with melanin), reducing glare; (ii) forms the outer **blood–retina barrier** via extensive tight junctions, regulating ion/metabolite exchange with the choroid; (iii) **regenerates 11-cis-retinal from all-trans-retinal (visual cycle), recycling it to photoreceptors;* (iv) *phagocytoses and degrades shed photoreceptor outer-segment discs**; (v) secretes trophic factors (growth factors, ATP) supporting the neural retina.

5. Photoreceptor cells — rods and cones

Each photoreceptor is a modified bipolar neuron with three parts: an **outer segment** (stacked membranous discs bearing visual pigment — a modified cilium arising from a basal body), a **connecting stalk**, and an **inner segment** (mitochondria- and Golgi-rich, site of protein synthesis). Discs are shed at the tip and phagocytosed by the RPE, with continual renewal from the base.

Feature	Rods	Cones
Outer segment shape	Cylindrical	Conical
Approx. number	~120 million	~6–7 million
Distribution	Throughout retina, absent at fovea and disc	Sparse peripherally, concentrated at (only) the fovea
Visual pigment	Rhodopsin (visual purple)	Iodopsin (three types: erythrolabe ~red, chlorolabe ~green, cyanolabe ~blue)
Sensitivity	High — scotopic (dim-light, night) vision	Lower — photopic (bright-light, day) vision

Feature	Rods	Cones
Colour discrimination	None (achromatic)	Present (trichromatic colour vision)
Bipolar convergence	Many rods : one bipolar cell (high sensitivity, low acuity)	~1 cone : 1 bipolar cell at fovea (low convergence, high acuity)
Disc membrane	Discs are free-floating, not continuous with plasma membrane	Discs remain continuous with the plasma membrane

Functional correlate (phototransduction, brief): light bleaches rhodopsin → activates **transducin** → activates phosphodiesterase → hydrolyses **cGMP** → closes cGMP-gated Na^+ channels → hyperpolarises the photoreceptor → reduces glutamate release onto bipolar cells. Vitamin A is essential for regeneration of the retinal chromophore.

6. Interneurons, Müller cells and the limiting membranes

- ▶ **Bipolar cells** (inner nuclear layer) relay signals from photoreceptors (outer plexiform layer) to ganglion cells (inner plexiform layer).
- ▶ **Horizontal cells** synapse laterally in the outer plexiform layer, integrating signals across adjacent photoreceptors (lateral inhibition).
- ▶ **Amacrine cells** synapse in the inner plexiform layer between bipolar and ganglion cells, modulating the ganglion cell output.
- ▶ **Ganglion cells** are multipolar neurons whose axons form the nerve fibre layer and converge at the optic disc to form the optic nerve.
- ▶ **Müller cells** are the principal glial cells of the retina, spanning its entire thickness from the external to the internal limiting membrane; they provide structural scaffolding, metabolic/trophic support, K^+ and water homeostasis, waste removal, and form both limiting membranes — making them central to the **inner blood–retina barrier**.

7. Blood supply and venous drainage

- ▶ **Central artery of the retina**, the first branch of the ophthalmic artery, enters the optic nerve and supplies the **inner two-thirds of the retina** (up to and including the bipolar neurons/inner nuclear layer) via capillary plexuses in the nerve fibre/ganglion cell layer and the inner nuclear layer.
- ▶ Its branches are **functional end arteries** with no anastomoses — a key basis for the catastrophic effect of occlusion.
- ▶ The **outer one-third** (photoreceptors and RPE) is **avascular** and nourished purely by **diffusion from the choriocapillaris** of the choroid across Bruch's membrane.
- ▶ **Venous drainage** is via the **central vein of the retina**, which drains into the **cavernous sinus** (directly or via the superior ophthalmic vein).

□ Fig 23.15 — Layers of the retina. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1202)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a vertical (light-to-choroid) schematic section through the retina, labelling from the vitreous side downward:

- ▶ **Internal limiting membrane** (Müller cell footplates)
- ▶ **Nerve fibre layer** (ganglion cell axons)
- ▶ **Ganglion cell layer**
- ▶ **Inner plexiform layer**
- ▶ **Inner nuclear layer** (bipolar, horizontal, amacrine, Müller cell bodies)
- ▶ **Outer plexiform layer**
- ▶ **Outer nuclear layer** (rod/cone nuclei)
- ▶ **External limiting membrane**
- ▶ **Layer of rods and cones** (outer + inner segments)
- ▶ **Retinal pigment epithelium**, with **Bruch's membrane** and **choriocapillaris of choroid** beneath it
- ▶ Mark a **Müller cell** running the full height of the diagram, and an arrow showing the **direction of incident light** (through the inner layers first)

Applied / Clinical anatomy

- ▶ **Retinal detachment** — separation of the neural retina from the RPE along the embryonic (unfused) cleavage plane; since the two layers are only apposed (interdigitating microvilli, no junctions), trauma or vitreous traction allows fluid to track between them, causing painless field loss ("curtain"), photopsia and floaters.
- ▶ **Central retinal artery occlusion** — because retinal arterial branches are end arteries, occlusion causes sudden, painless, profound monocular blindness with infarction of the inner retina; the fovea/perifoveal area may show relative pallor around a "**cherry-red spot**" because the choroid continues to nourish it.
- ▶ **Papilloedema** — raised intracranial/CSF pressure is transmitted along the optic nerve sheath (continuous with the intracranial subarachnoid space), impairing axoplasmic flow and venous return at the disc, producing bilateral disc swelling with early preservation of visual acuity.
- ▶ **Night blindness (nyctalopia)** — vitamin A deficiency impairs RPE-mediated regeneration of rhodopsin.
- ▶ **Age-related macular degeneration** — thickening/drusen formation in Bruch's membrane with RPE atrophy and choriocapillaris obliteration at the macula, causing central visual loss.
- ▶ **Retinoblastoma** — malignant tumour arising from primitive retinal (neuroepithelial) cells, typically presenting in early childhood.
- ▶ **Colour vision defects** — deficiency/absence of one or more cone photopigments (commonly red–green) causes congenital colour blindness.

High-yield exam pointers

- ▶ Retina develops from the **two layers of the optic cup** — outer → RPE, inner → neural retina; the unfused potential space between them is the plane of **retinal detachment**.
- ▶ **Ten layers**, from RPE (1) to internal limiting membrane (10); light traverses layers 10→2 before striking rods/cones.
- ▶ **Fovea centralis** = cones only, avascular, thinnest retina, greatest acuity; **optic disc** = no photoreceptors, the "blind spot."
- ▶ **Rods** (~120 million, rhodopsin, peripheral, scotopic, achromatic) vs **cones** (~6–7 million, iodopsin, foveal, photopic, colour vision).
- ▶ **RPE**: blood–retina barrier + visual-pigment regeneration + phagocytosis of shed discs.
- ▶ **Müller cells** span the full retinal thickness and form both limiting membranes — key retinal glia.
- ▶ **Inner two-thirds** supplied by the **central retinal artery** (end artery, branch of ophthalmic artery); **outer one-third** nourished by **diffusion from the choriocapillaris**.
- ▶ Venous drainage → **central retinal vein** → **cavernous sinus**.
- ▶ Applied triad to remember: **retinal detachment** (cleavage plane), **CRAO** (end-artery, cherry-red spot), **papilloedema** (CSF pressure via optic nerve sheath).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Sontakke's Textbook of Human Histology 2e; IB Singh's Textbook of Anatomy Vol 3 6e (Orbit and Eye); Vishram Singh's Textbook of Anatomy Vol 3 4e; Brijesh Kumar's Histology Text & Atlas 3e.



Histology of the cerebellum

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction The cerebellum ("little brain") is a homotypical structure — its microscopic architecture is uniform throughout all lobules and both hemispheres. It consists of an outer cortex (gray matter, thrown into leaf-like folds called **folia**, separated by fissures) and an inner core of **white matter** which branches in a tree-like pattern called the **arbor vitae**. Embedded within the white matter are four paired masses of gray matter, the **deep cerebellar nuclei**. The cerebellar cortex itself is arranged into three concentric layers, each with a characteristic cell population, and functions as a highly stereotyped, repeating synaptic circuit that receives two distinct classes of afferent fibre and delivers a single inhibitory output via the Purkinje cell.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 26.7 — Photomicrograph. Histology of cerebellum (low magnification on left, high magnification on right) (Sontakke — Textbook of Human Histology — 2e, p.352)

Diagram to draw (labelled, low power → high power):

- ▶ Outline of a **folium** with its core of white matter and covering cortex
- ▶ **Pia mater** — thin covering
- ▶ **Molecular layer** (outer, lightly stained) — label a **stellate cell** (superficial) and a **basket cell** (deep), with fine parallel-fibre and climbing-fibre lines
- ▶ **Purkinje cell layer** — single row of large flask-shaped **Purkinje cells** with an apical dendritic tree entering the molecular layer and a basal axon leaving into white matter
- ▶ **Granular layer** (inner, densely basophilic) — numerous small **granule cells**, a few large **Golgi cells**, and pale-staining **glomeruli**
- ▶ **White matter** — myelinated fibres and neuroglia
- ▶ Label incoming **mossy fibre** (ending in granular layer) and **climbing fibre** (ascending to molecular layer)

The three layers of the cerebellar cortex

Layer	Position	H&E staining	Principal cells	Fibres present
Molecular layer	Outermost, just deep to pia mater	Lightly stained (few cells, many fibres)	Stellate cells (superficial part), basket cells (deeper part)	Purkinje-cell dendrites, granule-cell axons (parallel fibres), climbing fibres
Purkinje cell layer	Single row between molecular and granular layers	—	Purkinje cells — large, flask-shaped	Dendrites arborise into molecular layer; single axon descends through granular layer to white matter

Layer	Position	H&E staining	Principal cells	Fibres present
Granular layer	Innermost, between Purkinje layer and white matter	Deeply basophilic (densely packed small nuclei)	Granule cells (small, round) and Golgi cells (large, stellate — largest neurons of the cortex, lying in its outer part)	Mossy-fibre rosettes, climbing fibres and Purkinje axons in transit, cerebellar glomeruli

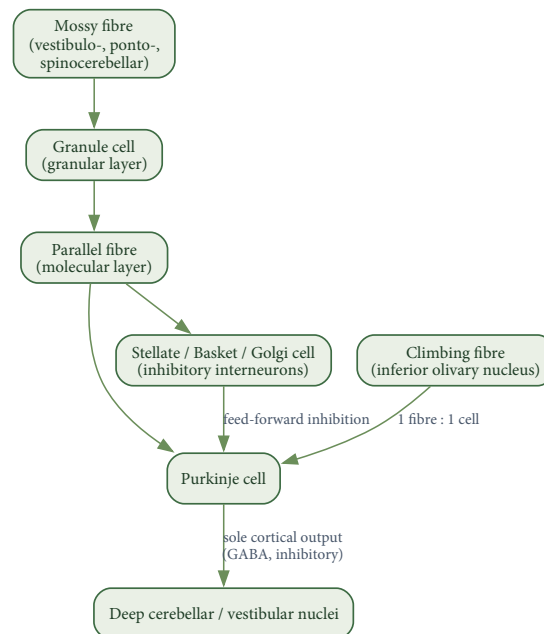
Cell types of the cerebellar cortex

Cell	Location	Dendrites	Axon / synaptic connections	Nature
Purkinje cell	Purkinje cell layer	Extensive, flattened, fan-shaped tree in molecular layer (~180,000 spines per cell — one of the richest of any neuron), oriented at right angles to the folium	Single axon → deep cerebellar/vestibular nuclei; collaterals to other Purkinje, Golgi, basket and stellate cells	Sole output neuron of the cortex; GABAergic, inhibitory
Granule cell	Granular layer	4–5 short dendrites forming claw-like endings in glomeruli	Axon ascends, bifurcates T-shaped into parallel fibres running along the folium, exciting Purkinje, stellate, basket and Golgi cells (one parallel fibre run contacts several hundred Purkinje cells)	Excitatory, glutamatergic — the only excitatory cortical interneuron
Golgi cell	Outer part of granular layer, beneath Purkinje cells	Enter both molecular layer (synapse with parallel/climbing fibres) and granular layer (synapse with mossy fibres)	Axon terminates on granule-cell dendrites within glomeruli	Inhibitory (mainly glycinergic)
Basket cell	Deep molecular layer	Ramify in molecular layer, contacted by parallel fibres	Axon runs transversely, covers ~10–12 Purkinje cells, forms a "basket" around each Purkinje soma/axon hillock	Inhibitory, GABAergic

Cell	Location	Dendrites	Axon / synaptic connections	Nature
Stellate cell	Superficial molecular layer	Confined to molecular layer	Synapses on Purkinje dendritic spines near their origin	Inhibitory, GABAergic

Afferent fibres of the cerebellar cortex

Feature	Mossy fibres	Climbing fibres
Origin	Extra-olivary: vestibulocerebellar, pontocerebellar, spinocerebellar fibres	Olivocerebellar — arise solely from the inferior olivary nucleus
Termination	Granular layer — each fibre gives 30–40 terminal swellings (rosettes), each rosette contacted by dendrites of ~15 granule cells	Ascend through granular and Purkinje layers to end in the molecular layer directly on Purkinje-cell dendrites
Ratio	One mossy fibre activates ~450 granule cells; their parallel fibres in turn excite several hundred Purkinje cells (divergent)	One climbing fibre : one Purkinje cell (highly specific, 1:1 relationship)
Neurotransmitter / effect	Glutamatergic, excitatory — acts indirectly on Purkinje cells via the granule-cell/parallel-fibre relay	Glutamatergic, excitatory — powerful, direct monosynaptic excitation of a single Purkinje cell
Collaterals	—	Collateral branches also synapse on deep cerebellar nuclei and, in the molecular layer, on stellate, basket and Golgi cells

*Histology of the cerebellum*

The cerebellar glomerulus

- ▶ A discrete, pale-staining synaptic complex situated in the granular layer, forming the "cerebellar islands" seen on light microscopy.
- ▶ **Composition:**
- ▶ A central **mossy-fibre rosette** (presynaptic element)
- ▶ **Claw-like dendritic expansions of granule cells** enveloping the rosette (postsynaptic element)
- ▶ **Axon terminals of Golgi cells**, also presynaptic to the granule-cell dendrites
- ▶ The whole complex is ensheathed by a glial capsule formed by **Bergmann glia** (specialised radial astrocytes whose somata also lie in the Purkinje cell layer).
- ▶ Functionally, the glomerulus is the site where excitatory mossy-fibre input to granule cells is modulated by Golgi-cell inhibitory feedback.

White matter and deep cerebellar nuclei

- ▶ Composed of myelinated afferent/efferent fibres (arbor vitae) and neuroglia.
- ▶ Four paired masses of gray matter lie within the white matter: **dentate** (largest, most lateral), **emboliform**, **globose** (emboliform + globose together = nucleus interpositus), and **fastigial** (most medial).
- ▶ Purkinje-cell axons are the only fibres leaving the cortex; they end on these nuclei (and on the lateral vestibular nucleus) and are inhibitory (GABAergic).
- ▶ Efferents from dentate, emboliform and globose nuclei leave via the **superior cerebellar peduncle**; fastigial-nucleus efferents leave via the **inferior cerebellar peduncle**.

Applied / Clinical anatomy

- ▶ **Purkinje cell as the vulnerable element:** because it is the sole output neuron, selective Purkinje cell loss (e.g., chronic alcoholism, phenytoin toxicity, paraneoplastic cerebellar degeneration)

produces disproportionate cerebellar dysfunction — ataxia, dysmetria, intention tremor, nystagmus.

- ▶ **Developmental origin — the external granular layer (EGL):** during development, granule-cell precursors proliferate transiently in a superficial external granular layer before migrating inward past the Purkinje layer to form the definitive (internal) granular layer; persistent EGL remnants are the classical origin ascribed to childhood **medulloblastoma** of the vermis.
- ▶ **Uniform histology, non-localising biopsy:** since the cortical architecture is homotypical, a cerebellar biopsy or imaging finding of cortical atrophy does not itself localise a lesion — clinical correlation with the vermis (midline, truncal ataxia) versus hemisphere (limb ataxia) is required.
- ▶ **Cerebellar ataxia:** damage to Purkinje cells disrupts the inhibitory output to the deep nuclei, producing poor coordination, past-pointing, and rebound phenomenon.
- ▶ **MRI/cross-sectional correlation:** the folial pattern and cortical ribbon (gray matter) versus arbor vitae (white matter) seen histologically correspond directly to the cerebellar cortex/white-matter contrast on MRI, useful in assessing cerebellar atrophy.

High-yield exam pointers

- ▶ Cerebellar cortex = **3 layers** — molecular (outer), Purkinje cell (middle, single row), granular (inner, deeply basophilic).
- ▶ **Purkinje cell = only output neuron** of the cortex; GABAergic and inhibitory to deep cerebellar/vestibular nuclei.
- ▶ **Two afferent systems:** mossy fibres (indirect, via granule cells/parallel fibres, divergent) and climbing fibres (direct, 1 fibre : 1 Purkinje cell, from inferior olive).
- ▶ **Cerebellar glomerulus** = mossy-fibre rosette + granule-cell dendrites + Golgi-cell axon terminals, capped by Bergmann glia.
- ▶ All cortical interneurons (stellate, basket, Golgi) are **inhibitory**.
- ▶ **Four deep nuclei** (lateral→medial): dentate, emboliform, globose, fastigial.
- ▶ Purkinje cell dendritic spines number roughly 180,000 per cell — reflects massive parallel-fibre convergence.
- ▶ Developmental **external granular layer** is the origin ascribed to medulloblastoma.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e; Ross Histology — A Text and Atlas 7e.



Irido-corneal Angle. Sclero-corneal Junction

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction The **sclero-corneal junction (limbus)** is the narrow (~1 mm) annular zone where the transparent, avascular cornea merges with the opaque, vascular sclera at the anterior one-sixth/posterior five-sixths boundary of the fibrous coat of the eyeball. The **irido-corneal angle** (filtration angle / anterior chamber angle) is the recess formed, at this same circumference, between the posterior surface of the peripheral cornea and the root of the iris; it houses the **trabecular meshwork** and the **canal of Schlemm (scleral venous sinus)**, the principal drainage route for aqueous humour. The two structures are anatomically inseparable — the limbus is the surface landmark, and the angle with its trabecular apparatus lies immediately deep to it — and together they determine intraocular pressure regulation.

1. Sclero-corneal junction (limbus) — gross features

- ▶ Marks the transition between the cornea (radius of curvature ≈ 7.8 mm) and the more gently curved sclera (radius ≈ 11.5 mm); this mismatch produces a shallow external groove, the **sulcus sclerae**, on the outer surface at the junction.
- ▶ The cornea is thickest at its periphery near the limbus (~670 μm) compared with its centre (~520 μm).
- ▶ Nasally and temporally the cornea-to-sclera transition is nearly perpendicular; superiorly and inferiorly it is more oblique, with the sclera overlapping the cornea more anteriorly — hence the cornea appears elliptical from the front (horizontal diameter 11.7 mm > vertical 10.6 mm) but circular from behind.
- ▶ Clinically and surgically the limbus is a key surface landmark: it demarcates the sites for cataract/glaucoma incisions, and a point ~4 mm posterior to it corresponds to the **pars plana** of the ciliary body, the safe entry site for posterior-segment surgery.

2. Histological transition at the limbus

Corneal layer	Change occurring at the limbus
Corneal epithelium (5–6 layers, non-keratinised stratified squamous)	Becomes thicker (up to ~12 cell layers) and continuous with the bulbar conjunctival epithelium ; contains the limbal stem cells , arranged in radial epithelial ridges called the palisades of Vogt , which are the sole source of corneal epithelial renewal (centripetal migration)
Anterior limiting lamina (Bowman's membrane)	Terminates abruptly at the limbus
Substantia propria (corneal stroma)	Collagen bundles lose their strict lamellar regularity and become continuous with (and indistinguishable from) scleral collagen; the stroma becomes vascularised here — capillary loops from the conjunctiva and episclera end at the limbus, since the central cornea itself is avascular

Corneal layer	Change occurring at the limbus
Posterior limiting lamina (Descemet's membrane)	Disperses peripherally into the connective-tissue beams of the trabecular meshwork ; classically described as continuing onto the iris root as the pectinate ligament , with the intervening gaps termed the spaces of the irido-corneal angle
Corneal endothelium	Continuous with the specialised endothelial lining that covers the trabecular beams

3. Irido-corneal (filtration) angle — boundaries and contents

- ▶ **Location:** the angle recess between the posterior corneal surface and the anterior surface of the iris root, at the circumference of the anterior chamber.
- ▶ **Anterior/roof boundary:** the corneoscleral junction, marked internally by the **line (ring) of Schwalbe** — the peripheral termination of Descemet's membrane/corneal endothelium.
- ▶ **Contents of the angle, antero-posteriorly:** line of Schwalbe → trabecular meshwork → canal of Schlemm (embedded in the outer wall) → scleral spur → ciliary muscle band → iris root.
- ▶ **Posterior/floor boundary:** the anterior surface of the ciliary body and the root of the iris; a single **iris process** commonly bridges the angle, connecting the iris root to the trabecular meshwork.
- ▶ **Clinical importance:** the width/depth of this angle (open vs narrow) determines susceptibility to angle-closure glaucoma; angle morphology also shows ethnic variation, correlating with differing population prevalence of primary angle-closure glaucoma.

4. Trabecular meshwork — structure and function

- ▶ A loose, collagenous **annulus of tissue** spanning the angle, composed of interconnected perforated beams/lamellae covered by specialised (modified) endothelial cells, forming small irregular intercommunicating channels.
- ▶ Continuous anteriorly with Descemet's membrane and the corneal endothelium; most of its fibres attach anteriorly to the external aspect of the **scleral spur**, while the remainder continue with the longitudinal fibres of the ciliary muscle and attach to the posterior internal aspect of the scleral spur.
- ▶ Traditionally subdivided, antero-posteriorly/inside-out, into three continuous zones (standard descriptive subdivision used across ophthalmic anatomy teaching):

Zone	Location/composition
Uveal meshwork	Innermost, facing the anterior chamber; widest spaces, cord-like trabeculae
Corneoscleral meshwork	Middle, bulk of the meshwork; sheet-like perforated trabecular lamellae, progressively narrower spaces
Juxtacanalicular (cribriform) tissue	Outermost, abutting the endothelium of the canal of Schlemm; loose connective tissue with the greatest resistance to aqueous outflow

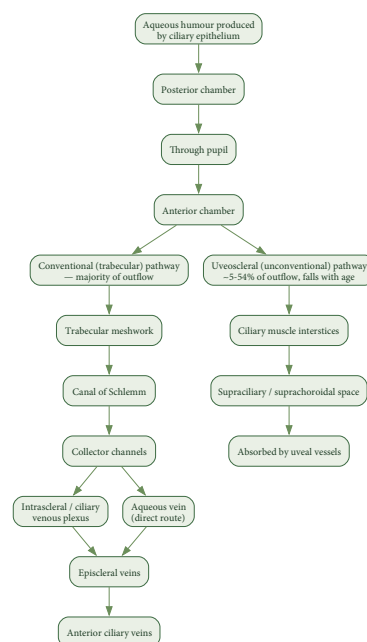
- ▶ **Function:** provides the principal physiological resistance to aqueous humour outflow, thereby helping generate a normal intraocular pressure of ~15 mmHg; it also acts as a coarse filter and its

lining cells can phagocytose particulate debris — overload of this phagocytic capacity contributes to some forms of secondary (obstructive) glaucoma.

5. Canal of Schlemm (scleral venous sinus)

- ▶ An **annular endothelium-lined channel** embedded in the sclera close to (just posterior to) the limbus, encircling the entire irido-corneal angle.
- ▶ In cross-section it appears as an oval cleft with an outer wall that grooves the sclera; posteriorly it relates to the **scleral spur**, a triangular projection of scleral tissue (apex directed forward) that forms its posterior/internal boundary and gives attachment to trabecular fibres and the ciliary muscle.
- ▶ Its wall is a single continuous thin endothelial layer; the canal may be double or plexiform over parts of its circumference.
- ▶ Aqueous humour crosses the inner endothelial face via giant pinocytotic vacuoles and transient transcellular pores that discharge into the canal at the outer face — a mechanism unique among endothelia.
- ▶ Drains via a plexus of fine intrascleral collector channels into the **episcleral veins**, either after mixing with blood in an intrascleral/ciliary venous plexus, or directly as a distinct, non-blood-mixed **aqueous vein** visible at the surface joining an episcleral vein.
- ▶ Normally contains no blood — the pressure gradient (IOP > episcleral venous pressure) prevents reflux even though the connecting channels are valveless; in venous congestion, blood can enter the canal transiently, but the continuous outer endothelial wall prevents further reflux.
- ▶ **Emerging concept:** the canal's endothelium expresses lymphatic-specific markers (PROX1, VEGFR3, CCL21), leading some authors to reclassify it as a specialised hybrid blood-lymphatic channel rather than a purely venous sinus — a refinement of the older "sinus venosus sclerae" concept. (Eponym: Friedrich Schlemm, German anatomist, 1795–1858.)

6. Pathways of aqueous humour outflow



Irido-corneal Angle. Sclero-corneal Junction

- ▶ **Conventional (trabecular) route** carries the bulk of outflow: anterior chamber → trabecular meshwork → canal of Schlemm → collector channels → intrascleral/ciliary venous plexus or a direct aqueous vein → episcleral veins → anterior ciliary veins.
- ▶ **Uveoscleral (unconventional) route:** since no epithelial barrier separates the anterior chamber from the ciliary body, aqueous can seep into the loose connective tissue anterior to the ciliary muscle, pass between its fibres into the supraciliary and suprachoroidal spaces, and be absorbed by uveal vessels. Estimated to account for under 5% to as much as ~54% of total outflow, and it declines with age; it can be pharmacologically augmented by topical prostaglandin analogues, first-line therapy in open-angle glaucoma.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Junqueira Fig 23.4 (below) is a real histology plate; for the exam, additionally draw a labelled schematic coronal section through the anterior chamber angle with these exact labels (after Gray's Fig. 42.3–42.4):

- ▶ Cornea, corneal stroma, conjunctival epithelium, conjunctiva
- ▶ Limbus (sclero-corneal junction)
- ▶ Line/ring of Schwalbe
- ▶ Trabecular meshwork (uveal / corneoscleral / juxtacanalicular zones)
- ▶ Canal of Schlemm
- ▶ Scleral spur
- ▶ Sclera
- ▶ Ciliary muscle, ciliary body
- ▶ Iris process, root of iris
- ▶ Anterior chamber, posterior chamber
- ▶ Filtration (irido-corneal) angle
- ▶ Collector channels → intrascleral venous plexus → aqueous vein → episcleral vein

□ Fig 23.4 — Corneoscleral junction (limbus) and ciliary body. (Junqueira's Basic Histology — Text & Atlas — 17e, p.1179)

Applied / Clinical anatomy

- ▶ **Primary angle-closure glaucoma:** in an anatomically narrow angle, pupillary dilatation thickens the peripheral iris, which apposes and occludes the trabecular meshwork, causing sudden severe rise in intraocular pressure with ocular pain, blurred vision and headache — an ophthalmic emergency; angle morphology shows ethnic variation that parallels differing disease prevalence.
- ▶ **Primary open-angle glaucoma:** the angle itself remains anatomically open, but increased resistance within the trabecular meshwork/juxtacanalicular tissue chronically raises intraocular pressure, with insidious optic nerve damage.
- ▶ **Gonioscopy:** the irido-corneal angle cannot be seen directly through an intact cornea (total internal reflection at the tear film–air interface); a gonioscopy contact lens with mirrors/prisms is used clinically to visualise the angle structures described above.

- ▶ **Limbal stem cell deficiency:** damage to the palisades of Vogt (chemical burns, prolonged contact lens wear, aniridia) removes the source of corneal epithelial renewal, leading to conjunctivalisation (vascular ingrowth) of the cornea and loss of transparency.
- ▶ **Trabeculectomy/trabeculotomy and canaloplasty:** surgical procedures that create an alternative drainage channel through, or bypass, a diseased trabecular meshwork/canal of Schlemm to control intraocular pressure.
- ▶ The limbus is the standard incision site for cataract extraction and is the reference point (4 mm posterior) for safe pars plana entry in vitreoretinal surgery.

High-yield exam pointers

- ▶ Limbus = sclero-corneal (corneoscleral) junction; irido-corneal angle = filtration angle between peripheral cornea and root of iris.
- ▶ Order of contents in the angle: Schwalbe's line → trabecular meshwork → canal of Schlemm/scleral spur → ciliary body band → iris root.
- ▶ Trabecular meshwork has three zones: uveal, corneoscleral, juxtacanalicular (cribriform) — greatest outflow resistance is juxtacanalicular.
- ▶ Canal of Schlemm = annular endothelial channel (sinus venosus sclerae) near the limbus, drains via collector channels/aqueous veins into episcleral veins; endothelium shows lymphatic-marker expression (PROX1, VEGFR3).
- ▶ Two outflow pathways: conventional trabecular (majority) and uveoscleral/unconventional (~5–54%, prostaglandin-responsive).
- ▶ Trabecular resistance generates normal IOP (~15 mmHg); loss of this resistance → glaucoma (open-angle) or angle occlusion → glaucoma (angle-closure).
- ▶ Limbal epithelium harbours corneal stem cells in the palisades of Vogt — clinically vital for corneal regeneration.
- ▶ Descemet's membrane disperses into the trabecular beams at the angle; corneal endothelium is continuous with trabecular endothelium.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology (Text & Atlas) 17e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Brijesh Kumar — Histology Text & Atlas 3e. Coverage note: the irido-corneal angle/trabecular apparatus is described in unusual depth in Gray's 41e (Ch. 42, Eye) despite the spec's hint that Gray's coverage is thin; the classic three-zone subdivision of the trabecular meshwork (uveal/corneoscleral/juxtacanalicular) is standard ophthalmic-anatomy teaching used for completeness, as it was not found verbatim zoned in the grepped chapters.



Microscopic features of ganglia

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction A **ganglion** is an aggregation of nerve cell bodies (perikarya) situated outside the central nervous system (CNS), along the course of peripheral nerves. Ganglia are of two principal types — **sensory (afferent) ganglia**, containing pseudounipolar/bipolar sensory neurons, and **autonomic (motor) ganglia**, containing multipolar postganglionic neurons of the sympathetic and parasympathetic outflow. Each ganglion cell body is intimately invested by a layer of glial **satellite cells**, and the whole ganglion is enclosed by a connective tissue capsule continuous with the epineurium/endoneurium of the entering and leaving nerves. (Note: the term "ganglion" is reserved for PNS neuronal aggregates; equivalent collections in the CNS are called **nuclei** — the "basal ganglia" of the cerebrum are a historical misnomer.)

1. General/gross features common to all ganglia

- ▶ Ganglia appear as **fusiform or oval swellings** along a peripheral nerve or nerve root; at least one nerve enters and another leaves each ganglion, since ganglia function as relay stations.
- ▶ Enclosed by a **connective tissue capsule**, continuous with the epineurium of the nerve; an internal connective tissue framework (continuous with perineurium/endoneurium) carries blood vessels and separates groups of neurons.
- ▶ **No blood–nerve barrier** exists in ganglia (unlike the perineurium-bound compartment of peripheral nerve trunks), so ganglion capillaries are relatively permeable.
- ▶ Each neuronal soma is surrounded by a single layer of flattened glial cells, the **satellite cells** (capsular cells), themselves derived from the **neural crest**.
- ▶ Nerve fibres (myelinated or unmyelinated, according to type) run between the groups of cell bodies within the ganglion.

2. Satellite cells

- ▶ Small, flat, cuboidal glial cells that completely envelop each neuronal cell body; only their small, rounded nuclei are usually visible in routine H&E sections, since their thin cytoplasmic sheets are not resolved by light microscopy.
- ▶ Derived embryologically from the **neural crest** (same origin as Schwann cells); their cytoplasm resembles Schwann-cell cytoplasm, and they are continuous with the Schwann cell sheath investing the neuronal processes.
- ▶ Functionally analogous to Schwann cells but do **not form myelin** — they provide **electrical insulation, metabolic/nutritive support**, and help regulate the ionic microenvironment (K^+ buffering) around the perikaryon.
- ▶ More prominent and well-defined around sensory ganglion neurons; less well-defined/thinner around autonomic ganglion neurons; **absent** around enteric ganglion neurons, which are instead supported by enteric glial cells resembling astrocytes.

3. Sensory (spinal/cranial) ganglia

- ▶ **Location:** on the dorsal (posterior) roots of spinal nerves (dorsal root/spinal ganglia), and on the sensory roots/trunks of cranial nerves V, VII, VIII, IX and X.

- ▶ **Neuron type:** unipolar/pseudounipolar neurons (except in the vestibulocochlear (VIII) nerve ganglia, where neurons are **bipolar**). The single process bifurcates a short distance from the soma into a peripheral (dendritic, sensory-receptor-directed) process and a central (axonal) process entering the spinal cord/brainstem; there are **no synapses on the cell bodies** within sensory ganglia.
- ▶ **Histological identification points:**
 - Large, spherical/oval neuronal cell bodies with a **large, pale-staining, centrally placed nucleus** and a prominent nucleolus (Nissl substance present but less conspicuous than in autonomic ganglion neurons).
 - Neurons are arranged in **discrete clusters/groups**, chiefly at the **periphery** of the ganglion.
 - Groups of cell bodies are separated by bundles of predominantly **myelinated** nerve fibres running through the centre of the ganglion.
 - Each soma is surrounded by a well-defined, prominent layer of **satellite cells**.
 - A well-defined **connective tissue capsule** surrounds the whole ganglion.
 - Ageing neurons accumulate golden-brown **lipofuscin** (wear-and-tear) pigment granules.

4. Autonomic (sympathetic and parasympathetic) ganglia

- ▶ **Location:** sympathetic ganglia — paravertebral (sympathetic chain) and prevertebral/collateral (e.g. coeliac, superior and inferior mesenteric); parasympathetic ganglia — small, close to or within the wall of the effector organ (e.g. intramural/enteric ganglia of the gut, cardiac and pelvic ganglia); cranial parasympathetic ganglia (ciliary, pterygopalatine, submandibular, otic) are intermediate in position.
- ▶ **Two-neuron relay:** the ganglion contains the cell bodies of **postganglionic** neurons; **preganglionic** neuron cell bodies lie within the CNS (spinal cord/brainstem) and their axons synapse here — i.e., a true synapse occurs within autonomic ganglia (unlike sensory ganglia).
- ▶ **Neuron type:** **multipolar**, smaller than sensory ganglion neurons.
- ▶ **Histological identification points:**
 - Neuronal cell bodies are **smaller** than sensory ganglion neurons, with an **eccentrically placed**, pale, vesicular nucleus and prominent nucleolus.
 - Neurons are **scattered irregularly** throughout the ganglion (not arranged in discrete peripheral groups as in sensory ganglia).
 - Nerve fibres between the cell bodies are predominantly thin and **unmyelinated**, hence much less conspicuous than the myelinated bundles of sensory ganglia.
 - Nissl substance is often **better defined** (more granular/coarse) than in sensory ganglion neurons.
 - Satellite cells are present but **less well-defined/thinner**, and are often incomplete or inconspicuous around intramural (enteric) ganglion neurons.
 - The connective tissue **capsule is thin or poorly defined**, and may be entirely absent around intramural ganglia embedded in the organ wall.
 - Cytoplasm shows the neurotransmitter phenotype: sympathetic ganglion neurons synthesise **catecholamines** (noradrenaline — mostly cholinergic input, adrenergic output);

parasympathetic ganglion neurons are cholinergic (synthesise **acetylcholine**) both pre- and postsynaptically.

- Some sympathetic ganglion neurons also show lipofuscin accumulation with age.

5. Sensory vs autonomic ganglia — comparative table

Feature	Sensory ganglion	Autonomic ganglion
Neuron type	Unipolar/pseudounipolar (bipolar in CN VIII)	Multipolar
Cell body size	Large	Smaller
Nucleus	Large, pale, centrally placed	Pale, eccentrically placed
Arrangement of cell bodies	In discrete groups , chiefly at periphery	Scattered , no definite grouping
Nerve fibres between groups	Bundled, mostly myelinated	Scattered, thin, unmyelinated
Satellite cells	Prominent, well-defined	Present but less well-defined
Connective tissue capsule	Well-defined	Thin/poorly defined; absent in intramural ganglia
Synapse within ganglion	Absent (relay only)	Present (preganglionic → postganglionic synapse)
Neurotransmitter synthesised	—	Catecholamines (sympathetic) / acetylcholine (parasympathetic)

6. Enteric ganglia (special variant)

- ▶ Intramural ganglia of the myenteric (Auerbach) and submucosal (Meissner) plexuses of the gut wall constitute the **enteric nervous system**, capable of independent local reflex activity.
- ▶ Neurons here are **not** supported by satellite cells but by **enteric glial cells** resembling CNS astrocytes; capsules are absent, the ganglia lying loosely within the connective tissue of the gut wall.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a low-power + high-power drawing of a **sensory (dorsal root) ganglion**: (1) connective tissue capsule, (2) peripheral clusters of large unipolar neuronal cell bodies, (3) centrally placed vesicular nucleus with nucleolus, (4) satellite cell layer around each neuron, (5) intervening bundles of myelinated nerve fibres, (6) blood vessels in the connective tissue septa. Alongside, draw a contrasting **autonomic (sympathetic) ganglion**: (1) thin/poorly defined capsule, (2) smaller multipolar neurons scattered irregularly, (3) eccentric nucleus, (4) thin satellite cell layer, (5) fine unmyelinated nerve fibre bundles between neurons.

□ Fig 12.14 — Photomicrograph. Histology of dorsal root/sensory ganglion (low magnification on left, high magnification on right, H&E stain) (Sontakke — Textbook of Human Histology — 2e, p.160)

□ Fig 12.15 — Histology of autonomic/sympathetic ganglion (low magnification on left, high magnification on right) (Sontakke — Textbook of Human Histology — 2e, p.161)

Applied / Clinical anatomy

- ▶ **Herpes zoster (shingles)**: reactivation of latent Varicella-zoster virus within sensory (dorsal root or trigeminal) ganglion neurons causes ganglionitis with a painful vesicular rash in the corresponding dermatome.
- ▶ **Tabes dorsalis (neurosyphilis)**: degeneration of dorsal root ganglion neurons and their central processes in the posterior columns produces loss of proprioception and sensory ataxia.
- ▶ **Hirschsprung disease**: congenital absence of enteric ganglion cells (aganglionosis) in the myenteric and submucosal plexuses of the rectosigmoid, due to failure of neural crest cell migration, leading to functional bowel obstruction.
- ▶ **Ganglion cell tumours**: neuroblastoma and ganglioneuroma arise from sympathetic ganglion precursor cells (neural-crest derived), typically in the adrenal medulla or paravertebral sympathetic chain.
- ▶ **Ganglionectomy/sympathectomy**: surgical excision of sympathetic ganglia (e.g. cervicothoracic/stellate ganglion, lumbar sympathetic chain) is used to relieve vascular spasm, hyperhidrosis and some forms of intractable pain.
- ▶ **Diagnostic/research value**: dorsal root ganglion biopsy or imaging is used in evaluating peripheral neuropathies and as a target site for spinal cord stimulation/DRG stimulation devices in chronic pain management.
- ▶ **Trigeminal (Gasserian) ganglion**: site of pathology in trigeminal neuralgia and target for percutaneous radiofrequency ablation/rhizotomy.

High-yield exam pointers

- ▶ Ganglion = collection of neuronal cell bodies **outside the CNS**; CNS equivalents are called nuclei.
- ▶ Two types: **sensory** (pseudounipolar, except bipolar in CN VIII) and **autonomic** (multipolar, postganglionic).
- ▶ **Satellite cells** — neural crest derived, surround each soma, function like Schwann cells but do not myelinate.

- ▶ Sensory ganglia: neurons in **peripheral groups**, **central** nucleus, prominent satellite cells, well-defined capsule, myelinated fibre bundles between groups, **no synapse**.
- ▶ Autonomic ganglia: neurons **scattered**, **eccentric** nucleus, thinner satellite layer, poor/absent capsule, unmyelinated fibres, **synapse present** (preganglionic → postganglionic).
- ▶ Enteric ganglia lack satellite cells (enteric glia instead) and lack a capsule.
- ▶ Clinical: herpes zoster (sensory ganglia), Hirschsprung disease (enteric ganglion aplasia), neuroblastoma/ganglioneuroma (sympathetic ganglion cells).

SOURCE & COVERAGE

Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; Gray's Anatomy (Standing) 41e.



Microscopic structure of Circumvallate papillae

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction — Circumvallate (vallate) papillae are the largest of the four types of lingual papillae, 8–12 in number, arranged in a V-shaped row immediately anterior to the sulcus terminalis on the dorsal surface of the tongue. Unlike fungiform papillae, each circumvallate papilla does not project above the mucosal surface but is flush with it, and is encircled by a deep circular trench (sulcus) bounded externally by a raised mucosal wall (vallum). Their lateral walls carry the highest density of taste buds anywhere on the tongue, and their trench floor receives the ducts of the purely serous glands of von Ebner — making this papilla the classic histology spot-diagram/practical exhibit for both lingual papillae and taste buds.

1. Location and gross arrangement

- ▶ **Number and site:** 8–12 papillae, situated just in front of the sulcus terminalis, forming a V whose apex points to the foramen caecum.
- ▶ **Size:** each papilla is about 1–2 mm in diameter (some texts describe up to 1–3 mm) — the largest lingual papilla.
- ▶ **Shape:** dome-shaped/cylindrical, but the free (dorsal) surface lies at the same level as the surrounding mucosa rather than standing proud of it, distinguishing it from the mushroom-shaped fungiform papilla.
- ▶ **Trench (sulcus/moat):** a deep circular cleft separates the papilla from a slightly raised circular mucosal elevation called the vallum (wall).

2. Histological layers of the papilla

Layer	Microscopic feature
Epithelium	Stratified squamous epithelium; lightly keratinized on the exposed dorsal (free) surface, thin and non-keratinized over the lateral walls of the trench where taste buds sit
Core	Connective tissue (lamina propria) continuous with the general lingual lamina propria; carries blood vessels and nerve fibres that supply the epithelium and taste buds
Secondary papillae	Connective-tissue projections indent the undersurface of the epithelium, increasing the surface for nutrient/gas exchange
Deep relation	Skeletal (lingual) muscle fibres extend up from the tongue substance into the connective-tissue core of the papilla

- ▶ The dorsal surface epithelium does **not** bear taste buds — these are restricted almost entirely to the two facing (lateral) walls of the surrounding trench.
- ▶ The trench wall epithelium is thin, allowing tastants dissolved in saliva easy access to the taste pores studding it.

3. The circular trench and serous glands of von Ebner

- ▶ The floor of the trench receives the ducts of several small, **purely serous salivary glands of von Ebner**, situated in the connective tissue deep to and around the papilla.
- ▶ Their watery secretion continuously **flushes the trench**, washing away food debris and dissolving tastants so that taste buds can register fresh gustatory stimuli.
- ▶ The secretion also contains a **lipase** that prevents a hydrophobic lipid film from coating the taste receptors, which would otherwise blunt gustation.
- ▶ This continuous-flushing arrangement is unique to circumvallate (and foliate) papillae and is a key applied/functional point examiners look for.

4. Microscopic structure of the taste bud

- ▶ Taste buds are numerous on the lateral walls of the trench (not on filiform papillae, which bear none) — on average about **250 taste buds per circumvallate papilla** (compare: ~53 per fungiform papilla), making circumvallate papillae the site of highest taste-bud density in the mouth.
- ▶ Each taste bud is a **barrel/oval-shaped intraepithelial structure**, about 70 µm in height and 40 µm across, composed of a cluster of roughly 50–150 spindle-shaped (fusiform) cells.
- ▶ The bud lies within an oval cavity in the stratified epithelium and is separated from the underlying lamina propria by a **basal lamina**.
- ▶ Apically, the cells converge on a **gustatory (taste) pore**, a ~2 µm wide opening onto the epithelial surface, through which apical microvilli of the sensory cells project to contact dissolved tastants.

Cell types within the taste bud

Cell type	Location / morphology	Function
Gustatory (neuroepithelial/receptor) cells	Elongated, spindle-shaped cells occupying the central bulk of the bud, extending from the basal lamina to the taste pore; apical microvilli; ~7–10-day turnover	Chemoreceptors bearing taste receptor proteins; depolarize on tastant binding and form basal synapses with afferent gustatory nerve fibres
Supporting (sustentacular) cells	Barrel-shaped cells at the periphery, forming an investing envelope around the gustatory cells	Structural support; function otherwise poorly understood
Basal cells	Small cells at the base of the bud, near the basal lamina	Slowly dividing stem cells that give rise to both gustatory and supporting cells

- ▶ A small fasciculus of **afferent nerve fibres** penetrates the basal lamina and spirals around the sensory cells, forming two plexuses: a **perigemmal plexus** (peripheral, containing neuropeptides such as CGRP and substance P, largely free sensory endings) and an **intragemmal plexus** (within the bud, synapsing on the base of the receptor cells).
- ▶ Tastants (sodium ions – salty; hydrogen ions – sour; sugars – sweet; alkaloids – bitter; glutamate/aspartate – umami) diffuse through the pore in saliva and act via ion channels (salt, sour) or G-protein-coupled receptors (sweet, bitter, umami) to depolarize the gustatory cells; circumvallate papillae are particularly associated with detection of **bitter and umami** stimuli.

5. Innervation and central connections

- ▶ Taste buds on the circumvallate papillae, the post-sulcal (posterior one-third) tongue, and the palatoglossal arches/oropharynx are supplied by the **glossopharyngeal nerve (CN IX)** — this is the key exception to the rule that the chorda tympani (facial nerve, CN VII) carries taste from the rest of the anterior two-thirds of the tongue.
- ▶ Peripheral gustatory fibres reach the **inferior (petrosal) ganglion of CN IX**; central processes join the tractus solitarius and terminate in the **rostral part of the nucleus solitarius** in the medulla oblongata, alongside taste afferents from CN VII and CN X.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Real plate for reference (embed from the library):

embed: ![Fig 31.13 — A section through a circumvallate papilla. Serous glands (of von Ebner (Gray's Anatomy — Standring — 41e, p.699)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/0ad9e80c60251b24.jpg)

For the practical spot-diagram, label a low-power section through a circumvallate papilla and a high-power inset of one taste bud with:

- ▶ Sulcus terminalis / foramen caecum (orientation landmark)
- ▶ Dome of the circumvallate papilla (flush with mucosal surface)
- ▶ Circular trench (sulcus/moat)
- ▶ Vallum (surrounding mucosal elevation)
- ▶ Stratified squamous epithelium — lightly keratinized (dorsal surface) vs non-keratinized (lateral trench wall)
- ▶ Taste buds studding the lateral epithelial walls
- ▶ Gustatory (taste) pore
- ▶ Connective-tissue core (lamina propria) with secondary papillae
- ▶ Duct of von Ebner's gland opening at the trench floor
- ▶ Serous acini of von Ebner's gland in the underlying connective tissue
- ▶ Skeletal (lingual) muscle fibres entering the papilla core
- ▶ High-power taste-bud inset: gustatory cell, supporting (sustentacular) cell, basal cell, basal lamina, afferent nerve fibres (perigemmal and intragemmal plexuses)

Applied / Clinical anatomy

- ▶ The sulcus terminalis, just behind the circumvallate row, marks the developmental and clinical boundary between the anterior two-thirds (branchial arch 1, general sensation via lingual/trigeminal, taste via chorda tympani) and posterior one-third (arches 3–4, both general sensation and taste via glossopharyngeal) of the tongue.
- ▶ **Glossopharyngeal nerve injury** (e.g. skull-base trauma, glomus jugulare tumour, iatrogenic injury during tonsillectomy or neck dissection) abolishes taste sensation from the circumvallate papillae and posterior one-third of the tongue, along with loss of general sensation from that region, pharynx and tonsil, and secretomotor loss to the parotid gland.
- ▶ Lymphatics from the circumvallate-papilla region and posterior one-third of the tongue drain bilaterally, which is clinically important in tongue carcinoma staging — posterior tongue tumours near the circumvallate row have a higher risk of contralateral/bilateral nodal metastasis than anterior tongue lesions.
- ▶ Continuous flushing by von Ebner's glands is functionally important for maintaining taste sensitivity; xerostomia (e.g. Sjögren syndrome, radiotherapy, anticholinergic drugs) reduces this flushing and contributes to dysgeusia.

- ▶ Circumvallate/foliate papillae are the preferred histological/biopsy sites for studying taste-bud density and regeneration because of their high taste-bud concentration.
- ▶ This papilla is the standard histology spot/practical exhibit for "taste bud" and "lingual papillae" questions, making its layered structure (epithelium – trench – gland openings – taste buds) a frequently tested applied-histology diagram.

High-yield exam pointers

- ▶ 8–12 circumvallate papillae, V-shaped row just anterior to the sulcus terminalis; largest lingual papilla, ~1–2 mm diameter, flush with the mucosal surface.
- ▶ Surrounded by a circular trench (sulcus) bounded by the vallum; trench floor receives ducts of the purely serous glands of von Ebner.
- ▶ Taste buds are confined to the lateral (trench) walls, not the dorsal free surface; average ~250 taste buds per papilla — the highest density on the tongue.
- ▶ Each taste bud: barrel-shaped, ~70 μm $\times$ 40 μm , 50–150 cells, opening via a ~2 μm gustatory pore.
- ▶ Three cell types: gustatory (receptor), supporting (sustentacular), and basal (stem) cells; gustatory cells turn over every 7–10 days.
- ▶ Nerve supply of circumvallate taste buds = glossopharyngeal nerve (CN IX) — the key exception to chorda tympani supply of the anterior two-thirds.
- ▶ Central pathway: inferior ganglion of CN IX $\rightarrow$ tractus solitarius $\rightarrow$ rostral nucleus solitarius (medulla).
- ▶ Von Ebner's gland secretion (serous + lipase) continuously flushes the trench, clearing tastants and preventing a lipid film over taste receptors.
- ▶ Circumvallate papillae are especially linked with bitter and umami taste detection.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; Ross Histology — A Text and Atlas 7e; IB Singh Textbook of Human Histology 9e; Sontakke Textbook of Human Histology 2e; BD Chaurasia Human Anatomy Vol 3–4 8e.



Microstructure of Tongue

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction — The tongue is a mucosa-covered muscular organ whose microstructure differs strikingly between its anterior two-thirds (papillated, mobile, oral part) and posterior one-third (nodular, lymphoid, pharyngeal part). Histologically it consists of a core of interlacing striated (skeletal) muscle fascicles running in three mutually perpendicular planes, invested by an oral mucosa (stratified squamous epithelium + lamina propria) that is thrown into specialised projections called **lingual papillae** on the dorsum. The papillae and their associated **taste buds** constitute the "gustatory" or specialised mucosa, making the tongue the principal peripheral organ of the special sense of taste as well as a key organ of mastication, deglutition and speech.

1. General architecture of the lingual mucosa

Feature	Description
Epithelium	Stratified squamous — keratinised over filiform papillae and hard-working dorsal areas; non-keratinised elsewhere (ventral surface, fungiform/foillate/vallate papilla walls, floor of mouth)
Lamina propria	Dense, highly vascular connective tissue that projects secondary papillae into the overlying epithelium; directly adherent to the underlying muscle on the dorsum
Submucosa	Absent under the dorsum of the anterior two-thirds — lamina propria is fused to the perimysium of the muscle, limiting mobility of the mucosa there; a loose submucous coat reappears in the posterior one-third, separating the mucosa from the muscle
Muscularis	Core of skeletal muscle bundles (intrinsic + extrinsic) crossing in three planes — this tri-planar interlacing arrangement, unique to the tongue, is itself a histological identifying feature
Glands	Numerous minor salivary glands (serous, mucous and mixed) lie between and around the muscle bundles and open onto the surface or into papillary sulci

2. Sulcus terminalis and regional division

- ▶ A shallow V-shaped **sulcus terminalis** on the dorsum divides the tongue into an **anterior two-thirds (oral/papillary part)** and a **posterior one-third (pharyngeal part)**.
- ▶ The apex of the V points backward to the **foramen caecum**, the vestigial opening marking the site of origin of the thyroid diverticulum (thyroglossal duct) in development.
- ▶ Anterior two-thirds: dorsal mucosa bears the four types of lingual papillae; ventral mucosa is smooth, thin, non-keratinised.
- ▶ Posterior one-third: mucosa is devoid of papillae, thick and freely movable, and shows an irregular "cobblestone" surface produced by underlying lymphoid nodules — the **lingual tonsil**.

3. Lingual papillae — types and microstructure

Four types of papillae are recognised on the anterior two-thirds of the dorsum, each a projection of lamina propria capped by epithelium:

Type	Number/ distribution	Epithelium	Taste buds	Distinguishing microstructure
Filiform	Most numerous; cover the entire anterior dorsum, arranged in rows parallel to the arms of the sulcus terminalis	Heavily keratinised stratified squamous, conical, pointed backward	Absent	Purely mechanical (rasping/holding food); tip may show a tuft of keratin
Fungiform	Scattered singly among filiform papillae, more numerous near the tip and margins	Thin, non-keratinised; highly vascular connective tissue core gives a red-dot appearance on gross inspection	Present , on the flattened upper surface	Mushroom-shaped, with deep secondary papillae of connective tissue indenting the epithelium
Circumvallate (vallate)	8–12 in number, arranged in a V-row immediately in front of and parallel to the sulcus terminalis	Non-keratinised (may be focally keratinised)	Numerous , on the lateral walls of the surrounding sulcus (moat)	Largest papilla; surrounded by a circular trench (sulcus); serous (von Ebner's) glands drain into the trench floor
Foliate	Present as parallel ridges on the postero-lateral margin of the tongue, just anterior to the sulcus terminalis; rudimentary/poorly developed in adult humans (best seen in children)	Non-keratinised	Present , in the epithelium of facing walls of adjoining ridges	Deep clefts between ridges; small serous glands empty into the clefts

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 15.4 — Tongue, lingual papillae, and taste buds. (Junqueira's Basic Histology — Text & Atlas — 17e, p.738)

Reproduce a longitudinal (sagittal) section of the tongue and label:

- ▶ Sulcus terminalis and foramen caecum (dividing line, anterior 2/3 vs posterior 1/3)
- ▶ Filiform papillae (keratinised, no taste buds) — anterior dorsum
- ▶ Fungiform papillae (taste buds on top) — scattered among filiform
- ▶ Circumvallate papillae (8–12, V-row) with surrounding trench, taste buds on lateral walls, and von Ebner's (serous) gland ducts opening into the trench
- ▶ Foliate papillae — lateral margin ridges
- ▶ Core of criss-crossing skeletal muscle bundles
- ▶ Minor salivary (serous + mucous) glands between muscle bundles
- ▶ Lingual tonsil (lymphoid nodules) in the posterior one-third mucosa
- ▶ A single taste bud shown in cross-section within the papilla epithelium (taste pore, sensory/supporting/basal cells)

4. Circumvallate papilla — structure in detail

- ▶ Each papilla is a truncated, dome-shaped elevation whose broad base merges with the tongue surface; it is surrounded by a deep circular **sulcus (moat/trench)**.
- ▶ The papilla itself is covered by non-keratinised stratified squamous epithelium; taste buds line the **lateral walls of the trench**, not the flattened dorsal surface of the papilla.
- ▶ **Von Ebner's glands** — purely serous minor salivary glands situated in the lamina propria/muscle beneath the papilla — send ducts that open into the floor of the trench. Their thin, watery secretion continuously flushes the trench, dissolving tastants and clearing debris so that taste buds can respond rapidly to changing stimuli.

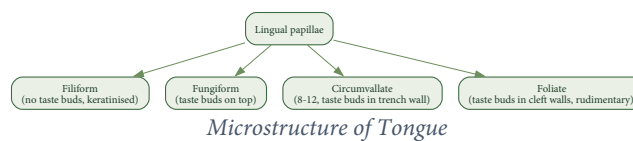
5. Taste bud — ultrastructure

Taste buds are oval, intra-epithelial neuroepithelial end-organs, pale-staining in H&E sections, extending from the basal lamina almost to the epithelial surface where they open by a **taste pore**. Three cell types are described:

Cell type	Location/shape	Features
Neuroepithelial (sensory/gustatory) cells	Most numerous; elongated, extend from base to taste pore	Apical microvilli project into the taste pore; tight junctions with neighbours near the apex; base synapses with afferent fibres of cranial nerves VII (chorda tympani), IX (glossopharyngeal) or X (vagus) ; turnover ≈ 10 days
Supporting (sustentacular) cells	Elongated, similar shape to sensory cells	Apical microvilli and tight junctions present, but do not synapse with nerve fibres; turnover ≈ 10 days

Cell type	Location/shape	Features
Basal cells	Small, situated near the basal lamina	Undifferentiated stem cells that divide and differentiate into new sensory and supporting cells

- **Taste transduction:** tastants act on the apical microvilli of sensory cells. Salty and sour stimuli act largely through ion-channel mechanisms (Na^+ channels for salt; H^+ -gated channels for sour), while sweet, bitter and umami are detected by **G-protein-coupled taste receptors** of the **T1R** and **T2R** receptor-gene families (about 30 distinct T2R receptors mediate bitter taste alone).
- Besides the papillae, scattered taste buds are also found in the epithelium of the soft palate, the glossopalatine (palatoglossal) arch, the posterior surface of the epiglottis, and the posterior pharyngeal wall down to the cricoid cartilage level.



6. Posterior one-third — lingual tonsil histology

- The mucosa here is smooth on its free surface but shows a nodular, cobblestone contour due to underlying aggregations of lymphoid tissue — the **lingual tonsil** — which contributes the antero-inferior part of **Waldeyer's ring**.
- Each lymphoid follicle possesses a **single, funnel-shaped crypt** (unlike the branching crypts of the palatine tonsil) into which the ducts of the underlying mucous glands drain and keep it self-cleansing.
- The mucosa is separated from the muscle core here by a loose submucous coat containing mixed mucous and serous glands and the lymphoid follicles; no papillae or taste buds are present in this region.

Applied / Clinical anatomy

- **Median rhomboid glossitis** — a smooth, papilla-free, diamond-shaped patch just anterior to the foramen caecum, classically attributed to failure of retraction of the tuberculum impar/persistent midline mesenchymal remnant, often colonised by *Candida*.
- **Geographic tongue (benign migratory glossitis)** — patchy, migrating loss of filiform papillae with a serpiginous keratotic margin, exposing red fungiform papillae; a benign, self-limiting inflammatory condition.
- **Glossitis/atrophic (bald) tongue** — loss/atrophy of filiform (and later fungiform) papillae in iron-deficiency, vitamin B12/folate deficiency and pellagra, giving a smooth, red, shiny dorsum with reduced/altered taste sensation.
- **Lingual thyroid** — ectopic thyroid tissue at or near the foramen caecum from failure of the thyroglossal duct/thyroid primordium to descend; presents as a midline swelling at the tongue base.
- **Squamous cell carcinoma** — commonest tongue malignancy; the ventral/lateral surfaces (an area lined by thin non-keratinised epithelium exposed to tobacco/alcohol) are classic high-risk sites.

- ▶ **Taste nerve mapping (clinically relevant for surgery/anaesthesia)** — anterior two-thirds: general sensation via the lingual nerve (V3), taste via the chorda tympani (facial nerve, VII); posterior one-third: both general sensation and taste via the glossopharyngeal nerve (IX), which also supplies the vallate papillae; extreme posterior tongue/epiglottic taste buds via the internal laryngeal branch of the vagus (X). A chorda tympani lesion (e.g., middle-ear surgery) selectively abolishes taste in the anterior two-thirds.
- ▶ **Ageusia/hypogeusia** — loss/reduction of taste can follow damage to any of the three "taste" cranial nerves, to the taste buds themselves (radiotherapy, zinc deficiency), or centrally along the gustatory pathway.
- ▶ **Ranula and mucocele** — retention cysts of minor lingual/sublingual salivary glands, reflecting the rich minor-gland population described above.
- ▶ **Von Ebner's gland secretion** — its washing action is the histological basis for why circumvallate/foliate taste buds recover rapidly between different tastants, relevant to clinical taste-testing technique.

High-yield exam pointers

- ▶ Anterior 2/3 = papillated, no submucosa, general sensation (lingual n., V3) + taste (chorda tympani, VII); posterior 1/3 = lymphoid (lingual tonsil), has submucosa, both general sensation and taste via glossopharyngeal (IX).
- ▶ Four papillae: filiform (no taste buds, keratinised, purely mechanical), fungiform (taste buds on top), circumvallate (8–12, V-row, taste buds in trench wall, von Ebner's glands), foliate (rudimentary in adult humans).
- ▶ Circumvallate papillae are the largest and are separated from the tongue surface by a circular sulcus/trench — taste buds are on the trench walls, NOT the papilla's dorsal surface.
- ▶ Von Ebner's glands are purely **serous**, drain into the circumvallate/foliate trench, and flush the moat.
- ▶ Taste bud = 3 cell types — neuroepithelial (sensory, synapse with VII/IX/X), supporting (no synapse), basal (stem cell); turnover ~10 days; opens via a taste pore.
- ▶ Taste transduction: ion-channel mechanism for salty/sour; GPCR (T1R/T2R families) for sweet/bitter/umami.
- ▶ Foramen caecum marks the site of the thyroglossal duct origin — clinically the site of lingual thyroid.
- ▶ Lingual tonsil has a single non-branching crypt per follicle (vs branching crypts of palatine tonsil) — a key histological differentiator.
- ▶ Muscle core runs in three mutually perpendicular planes — unique to the tongue and used to identify it on an unknown slide.

SOURCE & COVERAGE

Ross Histology — A Text and Atlas, 7e; Brijesh Kumar Histology Text & Atlas, 3e; Junqueira's Basic Histology — Text & Atlas, 17e (figure); Gray's Anatomy (Standring) 41e; AK Datta — Human Anatomy Vol 2 (Head & Neck); BD Chaurasia — Human Anatomy Vol 3–4, 8e; Moore's Clinically Oriented Anatomy, 9e.

♦ ♦ ♦

Organ of Corti

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction The organ of Corti (spiral organ), named after the Italian anatomist **Alfonso Corti (1822–1876)**, is the specialized neuroepithelium that serves as the receptor organ of hearing. It is a complex, spirally arranged epithelial ridge that rests throughout on the basilar membrane and runs the entire length of the cochlear duct (scala media), following the 2.75 turns of the bony cochlea over a basilar-membrane length of about 35 mm. It is built of two fundamentally different cell populations — sensory **hair cells** and several morphologically distinct **supporting cells** — which together transduce sound-induced vibrations of the basilar membrane into afferent nerve impulses carried by the cochlear (auditory) division of the vestibulocochlear nerve (CN VIII).

Location and gross relations

- ▶ The cochlear part of the membranous labyrinth is the **cochlear duct (scala media)**, a spirally coiled tube, triangular in cross-section, lying between the scala vestibuli and scala tympani within the 2.75 turns of the cochlea.
- ▶ The cochlear duct has **three walls**:
 - **Floor** — the **basilar membrane**, which separates scala media from the perilymph-filled scala tympani and carries the organ of Corti on its inner (zona arcuata) part.
 - **Roof** — the **vestibular (Reissner's) membrane**, separating scala media from the perilymph-filled scala vestibuli.
 - **Outer wall** — the **spiral ligament** and, applied to its endolymphatic face, the **stria vascularis**, the capillary-rich epithelium that secretes endolymph and maintains the endocochlear potential.
- ▶ The **medial margin** is formed by the **osseous spiral lamina**; the thickened periosteum on its upper surface forms the **spiral limbus**, which gives origin to the tectorial membrane.
- ▶ The basilar membrane widens from about 0.21 mm at the base to 0.36 mm at the apex, becoming less stiff apically — the structural basis of the cochlea's tonotopic (frequency) map.

□ Fig 28.5 — Section of cochlea. (Sontakke — Textbook of Human Histology — 2e, p.375)

Cellular architecture of the organ of Corti

The organ of Corti is a mosaic of sensory hair cells and named supporting cells resting on the zona arcuata of the basilar membrane.

Cell type	Nature	Key relations
Inner hair cells (IHC)	Sensory	Single row, medial to inner pillar cells
Outer hair cells (OHC)	Sensory	3–4 rows (up to 5 at the apex), lateral to outer pillar cells
Inner and outer pillar (rod) cells	Supporting	Enclose the triangular tunnel of Corti between them; bases rest on basilar membrane
Inner phalangeal cells	Supporting	Completely envelop each inner hair cell
Outer phalangeal cells (Deiters' cells , Otto Deiters, 1834–1863)	Supporting	Cup the base of each outer hair cell; a slender phalangeal process ascends to the reticular lamina
Border cells	Supporting	Line the internal spiral sulcus medial to the inner hair cells
Cells of Hensen	Supporting (tall columnar)	Lateral to the outer phalangeal cells
Cells of Claudius (Friedrich Claudius, 1822–1869)	Supporting (cuboidal)	Lateral to Hensen's cells, line the outer spiral sulcus
Cells of Böttcher (Jakob Ernst Arthur Böttcher, 1831–1889)	Supporting (polygonal)	Deep to Claudius's cells, resting on the basilar membrane

- There are about **6000 inner pillar cells** and **4000 outer pillar cells**; the inner pillar bodies make an angle of $\sim 60^\circ$ with the basilar membrane, the outer pillars $\sim 40^\circ$ — the angles diminish and the tunnel widens from base to apex.
- The apices of all these cells (hair cells and supporting cells alike) are bound by tight junctions, adherens junctions and desmosomes to form the **reticular lamina**.

□ Fig 28.6 — Cells in the organ of Corti. (Sontakke — Textbook of Human Histology — 2e, p.376)

Hair cells — inner versus outer

Feature	Inner hair cells (IHC)	Outer hair cells (OHC)
Number	~3500	~12,000
Arrangement	Single row along the whole cochlear coil	3–4 rows (up to 5 near the apex)
Shape	Flask/pear-shaped, curved	Long, cylindrical, nearly twice the height of an IHC
Stereocilia	50–60, in ascending rows, gently curved/linear pattern	Up to 100, arranged in a V or W, graded height
Support	Fully surrounded by inner phalangeal cells	Cupped basally only by Deiters' cells; middle third of the cell is free

Feature	Inner hair cells (IHC)	Outer hair cells (OHC)
Tectorial membrane contact	Stereocilia free in the sub-tectorial space	Tallest stereocilia embedded in the undersurface of the tectorial membrane
Predominant innervation	Afferent — each cell forms ≥ 10 ribbon-synapse contacts with dendrites of type I spiral ganglion cells	Efferent — dominant cluster of olivocochlear boutons at the base; afferent contacts fewer and smaller
Functional role	Principal transducer generating the afferent auditory signal	"Cochlear amplifier" — prestin-mediated electromotility sharpens frequency tuning and boosts sensitivity

- **Outer hair cell electromotility:** OHC depolarization triggers very rapid shortening mediated by **prestin**, an 80-kDa voltage-dependent anion-transporter motor protein in the lateral membrane; hyperpolarization elongates the cell again. These piston-like changes pull the tectorial membrane against the inner hair cell stereocilia, amplifying and sharpening the stimulus reaching them.

Tectorial membrane and reticular lamina

- The **tectorial membrane** is a stiff, gelatinous, acellular plate composed of collagen types II, V and IX embedded in glycoproteins (tectorins); it is attached medially to the vestibular labium of the spiral limbus and overhangs the whole organ of Corti.
- Only the tallest stereocilia of the **outer** hair cells are embedded in its under-surface; inner hair cell stereocilia remain free, bathed by endolymph in the sub-tectorial space.
- The **reticular lamina** is the mosaic plate formed by the apical surfaces of hair cells together with the apical (phalangeal) processes of the pillar and phalangeal cells, sealed by tight junctions.
- Functionally, the reticular lamina is an ionic diffusion barrier: it keeps the stereocilia-bearing apices of the hair cells bathed in endolymph (high K^+ , +80 mV endocochlear potential) while their basolateral membranes and synaptic poles remain bathed in perilymph/cortilymph — a gradient essential for mechanotransduction.

Fluid compartments — tunnel of Corti and spaces of Nuel

- The **tunnel (canal) of Corti** is the triangular space enclosed between the inner and outer pillar cells and the basilar membrane.
- The **spaces of Nuel** are additional intercommunicating spaces surrounding the outer hair cells.
- Both are filled with **cortilymph**, a fluid of composition similar to perilymph, which diffuses in through the matrix of the basilar membrane.

Innervation

- **Afferent:** peripheral processes of bipolar neurons whose cell bodies lie in the **spiral ganglion** (within Rosenthal's canal in the modiolus) pass through the **habenula perforata** of the osseous spiral lamina to reach the organ of Corti.

- ~90–95% are large **type I** ganglion cells, each of which contacts only a single inner hair cell (though each IHC receives ≥ 10 such afferent terminals) — these form the principal auditory pathway.
- The remaining smaller **type II** cells form the outer spiral bundle, running basally while branching to supply several outer hair cells each.
- ▶ **Efferent: the olivocochlear bundle**, with two components —
 - **Lateral olivocochlear efferents**, from neurons near the lateral superior olivary nucleus, mainly ipsilateral, unmyelinated; they run in the inner spiral bundle and terminate on the afferent (type I) dendrites beneath the inner hair cells.
 - **Medial olivocochlear efferents**, from neurons near the medial superior olivary nucleus, mainly contralateral, myelinated; they cross the tunnel of Corti to synapse directly on outer hair cell bases, inhibiting OHC electromotility and thereby adjusting cochlear-amplifier gain.
 - Afferent neurotransmitter: glutamate; efferent neurotransmitters: acetylcholine and/or GABA.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

(a) **Low-power cross-section of one cochlear turn** — label: Scala vestibuli (perilymph), Scala media/cochlear duct (endolymph), Scala tympani (perilymph), Vestibular (Reissner's) membrane, Basilar membrane, Stria vascularis, Spiral ligament, Osseous spiral lamina, Spiral limbus, Tectorial membrane, Organ of Corti, Spiral ganglion, Cochlear nerve.

(b) **High-power organ of Corti** — label: Tectorial membrane, Inner hair cell, Outer hair cells (3 rows), Reticular lamina, Inner pillar cell, Outer pillar cell, Tunnel of Corti, Inner phalangeal cell, Outer phalangeal (Deiters') cells, Cells of Hensen, Cells of Claudius, Cells of Böttcher, Basilar membrane, Habenula perforata, Cochlear nerve fibres.

Applied / Clinical anatomy

- ▶ **Sensorineural vs conductive hearing loss** — sensorineural hearing loss (~90% of all hearing loss) follows damage to hair cells, the cochlear nerve, or central auditory pathways; conductive hearing loss involves the external or middle ear (e.g., wax, otitis media, otosclerosis) with an intact organ of Corti.
- ▶ **Presbycusis** — age-related sensorineural loss; hair-cell loss begins at the basal turn of the cochlea and progresses apically, producing the characteristic early **high-frequency** hearing loss.
- ▶ **Noise-induced and ototoxic hearing loss** — excessive noise exposure or ototoxic drugs (aminoglycoside antibiotics, loop diuretics) destroy hair cells; the neighbouring supporting cells expand to reseal the reticular lamina, leaving "phalangeal scars" that restore the ionic barrier but not hearing, since human cochlear hair cells are **not regenerated**.
- ▶ **DFN3 (X-linked deafness)** — degeneration of spiral-ligament fibrocytes disrupts the gap-junctional potassium-recycling pathway (via Deiters' cells) from perilymph back into endolymph, producing late-onset sensorineural loss.
- ▶ **Usher syndrome** — mutations in the tip-link proteins cadherin-23/protocadherin-15 disrupt mechanotransduction, causing combined deafness with retinitis pigmentosa (deaf-blindness).

- ▶ **Cochlear implant** — a multi-electrode array is inserted along the scala tympani to directly stimulate spiral ganglion cells/cochlear nerve fibres, bypassing a non-functional organ of Corti in patients with an intact cochlear nerve.
- ▶ **Otoacoustic emissions (OAE)** — prestin-driven outer-hair-cell electromotility generates faint sounds detectable in the external canal; absent OAEs form the basis of universal neonatal hearing-screening programmes.
- ▶ **Tonotopic organization** — the narrow, stiff basal basilar membrane responds maximally to high-frequency sound; the wide, floppy apical membrane responds to low frequency — the anatomical basis of pure-tone audiometry and of the base-to-apex progression seen in noise-induced and age-related hearing loss.

High-yield exam pointers

- ▶ Organ of Corti = spiral organ, the receptor organ of hearing; named after **Alfonso Corti (1822–1876)**; rests on the basilar membrane throughout the cochlear duct's 2.75 turns (~35 mm long).
- ▶ Two receptor populations: **inner hair cells** (single row, ~3500, predominantly afferent) and **outer hair cells** (3–4 rows, ~12,000, predominantly efferent; electromotile via prestin).
- ▶ Named supporting cells: inner/outer **pillar (rod) cells** (enclose the tunnel of Corti), inner/outer **phalangeal (Deiters') cells**, **Hensen's**, **Claudius's**, and **Böttcher's** cells.
- ▶ Tectorial membrane arises from the spiral limbus; only outer hair cell stereocilia are embedded in it — inner hair cell stereocilia are free.
- ▶ Reticular lamina = the tight-junctional seal separating the endolymph bathing hair-cell apices from the cortilymph/perilymph bathing their bases.
- ▶ Afferent: ~90–95% type I spiral ganglion cells → inner hair cells. Efferent: medial olivocochlear → outer hair cells; lateral olivocochlear → inner hair cell afferent dendrites.
- ▶ Tonotopy: base = high frequency, apex = low frequency — the anatomical basis of the presbycusis pattern and of pure-tone audiometry.
- ▶ Human cochlear hair cells do **not regenerate** once lost — the basis of permanent noise-induced, ototoxic, and presbycusis hearing loss, and the rationale for cochlear implantation.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology — Text & Atlas 17e; Sontakke — Textbook of Human Histology 2e; IB Singh — Textbook of Human Histology 9e.



Sclerocorneal function

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction The **sclerocorneal junction**, also called the **limbus** or **corneoscleral junction** (CSJ), is the annular transitional zone (~1–2 mm wide) where the transparent, avascular **cornea** merges with the opaque, vascular **sclera**, the two components of the fibrous outer coat (tunica fibrosa) of the eyeball. It is not a passive seam — it is the site of a coordinated set of structural transitions that together subserve several distinct functions: corneal epithelial renewal, aqueous humour drainage and IOP regulation, corneal nutrition, an immune/vascular boundary, and a surgical landmark. Each is considered below.

Structural transition that underlies function

Layer	Cornea	At the limbus	Sclera
Epithelium	5–6 layers, stratified squamous, non-keratinised, avascular	Thicker; contains corneolimbal stem cells ; becomes continuous with conjunctiva	Continuous with bulbar conjunctiva
Anterior basement membrane	Bowman's membrane (8–10 μm)	Bowman's membrane ends abruptly	Absent
Stroma	~90% of thickness; ~60 regular orthogonal lamellae of type I collagen; avascular; transparent	Collagen bundles become irregular and vascularised as they merge with scleral fibres	Densely packed but irregularly arranged, interlacing collagen bundles → opaque
Posterior basement membrane / endothelium	Descemet's membrane + simple squamous endothelium	Descemet's membrane and endothelium are replaced by the trabecular meshwork	Absent
Curvature	$r \approx 7.8 \text{ mm}$	A slight sulcus sclerae marks the change in curvature	$r \approx 11.5 \text{ mm}$
Thickness	~520 μm centrally	~670 μm close to the junction	~1 mm posteriorly, thinning to ~0.3–0.4 mm at the equator, ~0.7 mm again near the limbus

 Fig 23.3 — Section of sclerocorneal junction. Low magnification (H&E pencil draw) (Brijesh Kumar — Histology Text & Atlas — 3e, p.377)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

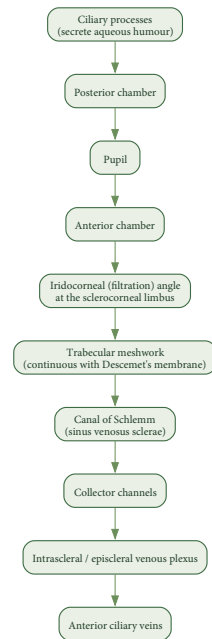
Label a low-power section through the sclerocorneal junction with: **Bulbar conjunctiva**, **Cornea** (anterior epithelium, Bowman's membrane, substantia propria, Descemet's membrane, posterior epithelium), **Sclera**, **Canal of Schlemm**, **Ciliary body**, **Ciliary process**, **Fibres of the suspensory ligament**, **Lens**, **Anterior chamber**, **Iris**.

Corneal epithelial stem-cell niche (regenerative function)

- ▶ The basal layer of the limbal epithelium contains **corneolimbal (limbal) stem cells** — the sole source of renewal for the corneal epithelium.
- ▶ These cells proliferate, differentiate, and migrate **centripetally** (limbus → periphery → centre of the cornea) to replace damaged or desquamated corneal epithelial cells; corneal epithelial turnover time is about 7 days.
- ▶ This centripetal migration also constitutes a **barrier function**: it prevents conjunctival epithelium from migrating onto the transparent cornea. Depletion of the stem-cell pool (chemical burns, extensive injury, disease) causes **conjunctivalisation of the cornea** — vascularised, unstable epithelium with reduced vision.

Aqueous humour drainage and IOP regulation

- ▶ Aqueous humour, secreted by the ciliary epithelium, passes from the posterior chamber through the pupil into the anterior chamber and drains chiefly through the **iridocorneal (filtration) angle**, formed between the posterior aspect of the limbus and the periphery of the iris.
- ▶ At the limbus, Descemet's membrane and the corneal endothelium are continued as the **trabecular meshwork**, a loose collagenous network of channels that filters the aqueous humour.
- ▶ The meshwork drains into the **canal of Schlemm (sinus venosus sclerae)**, an annular endothelial channel in the sclera close to the limbus, bounded posteriorly by the **scleral spur** (which also gives attachment to the ciliary muscle).
- ▶ From the canal, aqueous humour passes via collector channels into the intrascleral and episcleral venous plexuses and thence into the **anterior ciliary veins**.
- ▶ This outflow resistance normally maintains an **intraocular pressure of ~15 mmHg**. Obstruction to outflow at the limbal drainage angle raises IOP, producing **glaucoma** — cupping of the optic disc and progressive retinal/optic-nerve damage; drainage-angle morphology differences underlie ethnic variation in angle-closure glaucoma.

*Sclerocorneal function*

Nutritional and vascular-immune boundary function

- ▶ The cornea proper is **avascular**; it is nourished from three sources — the tear film on its anterior surface, the aqueous humour behind it, and **blood vessels at the sclerocorneal junction** diffusing nutrients into the peripheral stroma.
- ▶ The limbus is the point at which the **avascular** cornea gives way to the **vascular** sclera/conjunctiva; it therefore forms the effective boundary of corneal **immune privilege**. Corneal grafts (keratoplasty) succeed with a low rejection rate largely because the graft bed is avascular and lacks lymphatics — a property lost once vessels cross the limbus (as in an inflamed or vascularised cornea).
- ▶ During corneal inflammation, leukocytes reach the cornea by migrating in from limbal blood vessels, i.e. the limbus is the route of the inflammatory/immune response into an otherwise privileged tissue.
- ▶ **Anterior ciliary arteries** (from the muscular branches of the ophthalmic artery, running on the recti tendons) pierce the sclera near the sclerocorneal junction to form the **major arterial circle of the iris**, contributing to the vascular supply of the anterior uvea.

Surgical and applied landmark function

- ▶ The **fascia bulbi (Tenon's capsule)** extends from the optic nerve to the limbus and is most firmly attached to the sclera just behind the limbus — relevant to the plane used in **sub-Tenon (peribulbar) local anaesthesia**.
- ▶ The four recti are inserted into the sclera behind the limbus at characteristic distances (medial rectus ≈ 5.5 mm, inferior rectus ≈ 6.5 mm, lateral rectus ≈ 7 mm, superior rectus ≈ 8 mm) — joining these points clockwise traces the **spiral of Tillaux**, a surgical guide during strabismus surgery and an external marker of the ora serrata.

- ▶ The limbus is the standard site of the **corneoscleral incision** in cataract extraction and of the **trabeculectomy** flap in glaucoma filtration surgery, because it overlies the trabecular meshwork/canal of Schlemm.

Applied / Clinical anatomy

- ▶ **Glaucoma:** trabecular meshwork/Schlemm's canal obstruction at the limbus → raised IOP → optic disc cupping, field loss; measured clinically by tonometry.
- ▶ **Cataract surgery:** a limbal (corneoscleral) incision is a common access route to the anterior chamber and lens.
- ▶ **Trabeculectomy:** fashions a guarded filtration channel through the limbal trabecular meshwork to lower IOP in medically uncontrolled glaucoma.
- ▶ **Limbal stem cell deficiency:** chemical/thermal burns or chronic inflammation destroy corneolimbal stem cells, causing conjunctivalisation, vascularisation and opacification of the cornea; treated by **limbal stem cell transplantation**.
- ▶ **Pterygium:** a fibrovascular wing of degenerated conjunctiva that crosses the limbus onto the cornea, disrupting the stem-cell barrier.
- ▶ **Keratoplasty (corneal graft):** high success rate exploits corneal avascularity bounded by the limbus; vascularisation reaching the limbus increases rejection risk.
- ▶ **LASIK:** the corneal flap and stromal ablation are confined centrally, away from the limbal stem-cell zone, to preserve epithelial renewal.
- ▶ **Sub-Tenon block:** uses the firm attachment of fascia bulbi just behind the limbus as an anaesthetic landmark for ocular surgery.

High-yield exam pointers

- ▶ Limbus = **sclerocorneal/corneoscleral junction**; ~1–2 mm transition zone of the tunica fibrosa.
- ▶ Four coordinated changes at the limbus: epithelium thickens & meets conjunctiva; Bowman's membrane ends; stroma becomes irregular/vascularised; Descemet's/endothelium is replaced by trabecular meshwork.
- ▶ Houses **corneolimbal stem cells** — sole source of corneal epithelial renewal (turnover ~7 days) and a barrier against conjunctival overgrowth.
- ▶ Site of the **iridocorneal (filtration) angle** — trabecular meshwork → canal of Schlemm → collector channels → episcleral veins; regulates IOP (~15 mmHg); obstruction → glaucoma.
- ▶ Contributes to **corneal nutrition** (with tear film and aqueous humour) via limbal vessels.
- ▶ Marks the **avascular–vascular / immune-privilege boundary** of the cornea; relevant to graft success.
- ▶ Surgical landmark: corneoscleral incision (cataract), trabeculectomy (glaucoma), recti insertion distances/spiral of Tillaux, sub-Tenon anaesthesia plane.

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; Brijesh Kumar — Histology Text & Atlas 3e; IB Singh — Textbook of Human Histology 9e; Gray's Anatomy (Standring) 41e; BD Chaurasia — Human Anatomy Vol 3–4 8e; Vishram Singh — Textbook of Anatomy Vol 3 4e; IB Singh — Textbook of Anatomy Vol 3 6e (spiral of Tillaux).



Systemic histology - skin & musculoskeletal



Dermis of skin

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction

The dermis is the deep, irregular, moderately dense connective-tissue layer of the skin lying immediately beneath the epidermis, from which it is separated by a basement membrane at the dermo-epidermal junction. It is composed of an interwoven collagenous and elastic fibre network embedded in an amorphous ground substance of glycosaminoglycans, glycoproteins and bound water, together with nerves, blood vessels, lymphatics and epidermal appendages (hair follicles, sebaceous and sweat glands). Embryologically it arises from somatopleuric mesenchyme in the trunk and limbs, from somitic mesenchyme over the epaxial musculature of the back, and from neural crest mesenchyme in the head; its blood vessels are derived from angiogenic mesenchyme. The dermis is divided into a narrow superficial **papillary layer** and a deeper **reticular layer**, the boundary between them being indistinct. Overall skin thickness (epidermis + dermis) ranges from 1.5 mm (thinnest, e.g. eyelid) to 5.0 mm (thickest, e.g. back, palms, soles), the dermis contributing the bulk of this measurement.

1. Layers of the dermis

Feature	Papillary layer	Reticular layer
Position	Superficial, immediately subjacent to epidermis	Deep, merges with the deep aspect of the papillary layer
Connective tissue	Loose connective tissue	Dense, irregular connective tissue
Collagen	Fine, densely interwoven bundles of type I and III collagen	Thicker bundles, mainly type I collagen , in a predominantly parallel orientation
Elastic fibres	Fine elastic fibrils	Coarser elastic fibres forming a strong three-dimensional lattice
Cellularity	More cellular	Less cellular

Feature	Papillary layer	Reticular layer
Special features	Contains dermal papillae with a capillary loop per papilla (one loop/papilla) and, in thick hairless skin, Meissner's corpuscles	Contains the bulk of skin appendages (hair follicles, sweat and sebaceous glands); rests on hypodermis
Function	Mechanical anchorage, trophic/metabolic support and sensory supply to epidermis	Tensile strength; its collagen orientation determines skin tension lines

The superficial surface of the dermis is thrown into numerous **dermal papillae** (interdigitating with epidermal rete pegs/ridges) that form the dermo-epidermal junction. Papillae are small and few in thin skin but large, closely aggregated and arranged in parallel rows (following surface ridges) in the thick skin of the palm and sole — the basis of **dermatoglyphics** (fingerprint patterns), a stable, individual-specific pattern used forensically.

□ Fig 7.4 — The epidermis and papillary dermis (P) of human thick skin, showing the thick cornified layer, keratinocytes, and dermal papillae with capillary loops (Gray's Anatomy — Standring — 41e, p.206)

□ Fig 5.12 — Section of the reticular layer of the dermis showing dense irregular connective tissue with thick collagen bundles (Brijesh Kumar — Histology Text & Atlas — 3e, p.81)

2. Cells of the dermis

Two categories of cell are present, as in all general connective tissues:

► Permanent (resident) cells

- **Fibroblasts** — synthesize all components of the dermal extracellular matrix (collagen, elastin, ground substance).
- Cells of organized structures — endothelial cells of vessels, Schwann cells of nerves, and smooth-muscle cells of the arrector pili.

► Migrant (transient) cells, all bone-marrow derived

- Macrophages, mast cells, eosinophils, neutrophils.
- T and B lymphocytes, including antibody-secreting plasma cells.
- Dermal interstitial **dendritic cells**, capable of immune surveillance and antigen presentation.

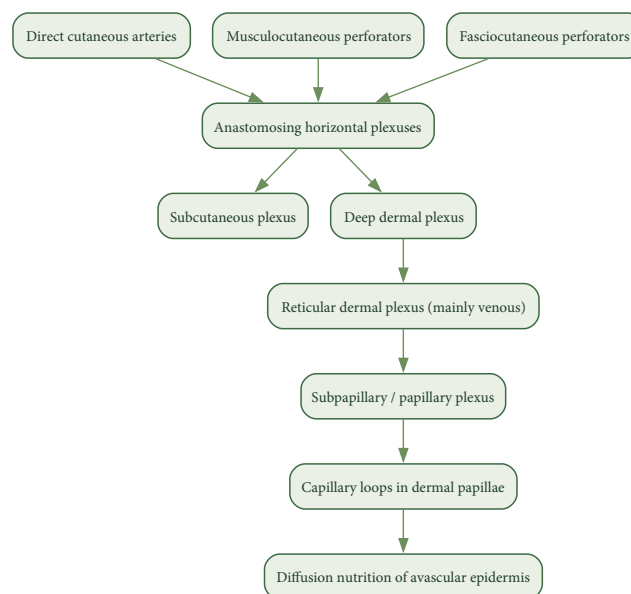
3. Extracellular matrix — fibres and ground substance

- **Collagen**: adult dermal collagen is mainly **type I** (80–85%) and **type III** (15–20%). Coarse type I fibres predominate in the deeper reticular dermis; finer type III fibres are found in the papillary dermis and around blood vessels. **Type IV collagen** lies in the basal lamina between epidermis and dermis and around Schwann cells/endothelial cells. **Types V, VI and VII** are minor components — type VII forms the **anchoring fibrils** linking the basal lamina to the papillary dermis. Deficiency of type XVII collagen (COL17A1 mutation) causes junctional epidermolysis bullosa, and deficiency of type VII collagen (COL7A1 mutation) causes dystrophic epidermolysis bullosa — both manifesting as trauma-induced skin fragility.
- **Elastic fibres**: form a fibrous network interwoven between the collagen bundles throughout the dermis, more prominent in certain regions (e.g. the axilla), permitting stretch and elastic recoil.

- **Ground substance:** an amorphous gel of glycosaminoglycans, glycoproteins and bound water that surrounds the fibres and cells and provides a medium for diffusion of nutrients, gases and metabolites (important since the epidermis itself is avascular).

4. Blood supply of the dermis

Cutaneous blood flow is about 5% of cardiac output and its capacity can vary up to 20-fold for thermoregulation. Skin is supplied by three arterial systems — **direct cutaneous** (from main arterial trunks, running parallel to the surface in subcutaneous fat, e.g. supraorbital, superficial circumflex iliac, dorsalis pedis arteries), **musculocutaneous perforators** (arising from intramuscular vessels and piercing the deep fascia), and **fasciocutaneous perforators** (from vessels deep to the deep fascia running along intermuscular septa, e.g. from radial/ulnar arteries). These feed six anastomosing horizontal plexuses; three lie within the skin itself.



Dermis of skin

- **Subpapillary (papillary) plexus** — at the junction of papillary and reticular layers; gives capillary loops into each dermal papilla.
- **Reticular dermal plexus** — mid-dermis, primarily venous.
- **Deep dermal plexus** — deepest reticular dermis, on the undersurface of the dermis; closely apposed arteriolar/venous plexi permit countercurrent heat exchange.
- Blood vessels never enter the epidermis; it is nourished entirely by diffusion from the papillary capillary loops.
- **Arteriovenous anastomoses** (glomus bodies) are numerous in the deep dermis of the extremities (hands, feet, ears, lips, nose) and are important in temperature regulation.
- Dermal lymphatics begin as blind-ended loops just below the papillary dermis and drain via a superficial plexus into a deeper plexus at the reticular dermis–subcutis junction.

5. Nerve supply of the dermis

Receptor / fibre	Location	Modality
Free nerve endings	Throughout dermis; some penetrate into epidermis	Pain, temperature, light touch (nociceptive)
Meissner's corpuscles	Dermal papillae, close to dermo-epidermal junction, especially thick hairless (glabrous) skin and finger pads	Rapidly-adapting light touch, texture
Pacinian corpuscles	Deep dermis/hypodermis, especially of the digits	Deep pressure and vibration
Ruffini corpuscles	Deep dermis	Sustained pressure/stretch
Merkel cell–neurite complexes	Basal epidermis/dermal papillae junction	Slowly-adapting light touch, shape, texture

- Sensory (afferent) fibres, with cell bodies in spinal/cranial ganglia, enter the dermis and branch to form a **deep reticular plexus** innervating most sweat glands, hair follicles and larger arterioles; smaller fasciculi pass to a more superficial **papillary plexus** at the reticular–papillary junction, from which branches terminate as free endings, or in relation to encapsulated receptors, or reach the epidermal basal lamina.
- **Autonomic (efferent) fibres** are unmyelinated, noradrenergic or cholinergic; they supply arterioles, arrector pili muscles and the myoepithelial cells of sweat/apocrine glands (secretomotor and vasomotor/pilomotor control); in the nipple, scrotum, labia minora and perineal skin they additionally supply smooth muscle fasciculi within the dermis.
- The segmental cutaneous sensory supply of a single spinal nerve (through its dorsal and ventral rami) defines a **dermatome**; adjacent dermatomes overlap considerably.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a vertical section through thick skin:

- ▶ Epidermis (with basal layer)
- ▶ Basement membrane / dermo-epidermal junction
- ▶ **Papillary layer** with dermal papillae
- ▶ Capillary loop within a dermal papilla
- ▶ Meissner's corpuscle (in a papilla)
- ▶ Free nerve ending (entering epidermis)
- ▶ **Reticular layer** with thick, parallel type I collagen bundles
- ▶ Elastic fibres interwoven between collagen bundles
- ▶ Subpapillary (papillary) plexus
- ▶ Deep dermal plexus
- ▶ Pacinian corpuscle (deep dermis/hypodermis)
- ▶ Hair follicle with arrector pili muscle
- ▶ Sebaceous gland opening into the follicle
- ▶ Sweat gland (secretory coil + duct)
- ▶ Hypodermis (adipose tissue)

Applied / Clinical anatomy

- ▶ **Skin grafts:** a **full-thickness graft** takes the epidermis plus the entire dermis, leaving a donor defect with no epidermal elements for regeneration (must be primarily closed). A **split-thickness graft** takes the epidermis and only part of the dermis; the donor site retains adnexal remnants (hair follicles) from which new epidermis can regenerate.
- ▶ **Skin tension lines:** the parallel orientation of reticular-layer collagen underlies the natural tension lines of skin — relaxed skin tension lines (RSTLs), Langer's lines and Kraissl's lines — used by surgeons to plan elective incisions for the least conspicuous scar.
- ▶ **Striae (stretch marks):** when mechanical demand on the skin exceeds what the dermis can accommodate, dermal collagen cohesion is disrupted with haemorrhage and poorly vascularized scar formation, seen as striae rubrae (pink/purple, early) maturing to striae albae (pale); on the abdomen in pregnancy these are striae gravidarum.
- ▶ **Epidermolysis bullosa:** mutation of anchoring-fibril collagen (type VII, COL7A1) or type XVII collagen (COL17A1) at the dermo-epidermal junction produces trauma-induced blistering (dystrophic and junctional forms respectively).
- ▶ **Dermatoglyphics:** the fixed pattern of dermal papillae/epidermal ridges in thick skin is used for identification (fingerprinting) and is disturbed in conditions such as Down syndrome; extremely rare absence occurs in adermatoglyphia (SMARCAD1 mutation).
- ▶ **Ageing of the dermis:** reduced fibroblast population and collagen synthesis (relative rise in type III collagen), senile elastosis, diminished vascularity of dermal papillary capillary loops (causing

easy bruising/senile purpura), and loss of extensibility/elasticity, together producing wrinkling and sagging.

- ▶ **Arteriovenous anastomoses (glomus bodies)** in the dermis of the digits are the site of glomus tumours, and their dysfunction contributes to conditions of abnormal peripheral thermoregulation (e.g. Raynaud phenomenon).
- ▶ Deep dermal/dermo-hypodermal infection tracks along the vascular plexuses and appendageal structures, underlying the spread pattern of cellulitis and abscess formation.

High-yield exam pointers

- ▶ Dermis = papillary layer (superficial, loose, type I+III collagen, capillary loops, Meissner's corpuscles) + reticular layer (deep, dense, thick type I collagen, elastic fibre lattice) — boundary indistinct.
- ▶ Adult dermal collagen: type I 80–85%, type III 15–20%; type IV in basal lamina; type VII forms anchoring fibrils (COL7A1 → dystrophic epidermolysis bullosa).
- ▶ Dermis develops from somatopleuric mesenchyme (trunk/limbs), somitic mesenchyme (back) and neural crest (head); vessels from angiogenic mesenchyme.
- ▶ Cells: permanent (fibroblasts, vessel/nerve/muscle-associated cells) vs migrant/bone-marrow-derived (macrophages, mast cells, lymphocytes, dermal dendritic cells).
- ▶ Vascular plexuses: subpapillary/papillary (capillary loops to papillae), reticular dermal (venous), deep dermal — fed by direct cutaneous, musculocutaneous and fasciocutaneous vessels; epidermis nourished purely by diffusion.
- ▶ Sensory receptors: free nerve endings, Meissner's (light touch), Pacinian (deep pressure/vibration), Ruffini (stretch), Merkel cell–neurite complexes (sustained touch); autonomic fibres control vessels, arrector pili and sweat myoepithelium.
- ▶ Dermal papillae pattern in thick skin → dermatoglyphics (fingerprints); collagen orientation of reticular layer → Langer's/RSTL skin tension lines guiding surgical incisions.
- ▶ Applied correlates: full- vs split-thickness skin grafts (defined by depth of dermis taken), striae, epidermolysis bullosa, senile skin changes.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh Human Histology 9e; Ross Histology Text & Atlas 7e; Brijesh Kumar Histology Text & Atlas 3e.



Haversian lamellae

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Haversian lamellae are the concentric, thin (3–7 μm thick) sheets of mineralized bone matrix that are wrapped, ring-within-ring, around a central neurovascular channel — the **Haversian (osteonal) canal** — to build the **Haversian system or osteon**, the basic structural and functional unit of mature compact (cortical) bone. Each lamella is a plywood-like layer of parallel type I collagen fibres embedded in a hydroxyapatite-mineralized ground substance, and successive lamellae have their fibres oriented roughly 90° to one another, which is what gives compact bone its characteristic laminated appearance and its great mechanical strength. Haversian lamellae are one of three lamellar systems recognised in compact bone (the others being circumferential and interstitial lamellae) and are the type most often asked about because they define the osteon itself.

Types of lamellae in compact bone

Type	Location	Key features
Outer circumferential lamellae	Immediately beneath the periosteum, encircling the whole shaft	A few broad layers running parallel to the periosteal surface; pierced obliquely by collagenous Sharpey's (perforating) fibres that anchor the periosteum and tendo-ligamentous attachments to the cortex
Concentric (Haversian) lamellae	Around each Haversian canal, forming the osteon	4–20 concentric rings per osteon (figures vary between textbooks); collagen fibre direction alternates by $\sim 90^\circ$ between successive lamellae
Interstitial lamellae	Irregular, angular areas between complete osteons	Remnants of older osteons and circumferential lamellae that were partially eroded during bone remodelling; lack a Haversian canal of their own
Inner circumferential lamellae	Bordering the medullary cavity, deep to the endosteum	Fewer, thinner rings than the outer set, lining the marrow cavity

The Haversian system (osteon) — overall architecture

- The **osteon** is a cylindrical unit, 100–400 μm in diameter, running mainly **parallel to the long axis of the bone**; it may be gently spiral, may branch, or end blindly, and appears round-to-oval in cross-section.
- At its core lies the **Haversian (osteonal) canal**, mean diameter $\sim 50 \mu\text{m}$, which contains one or two small blood vessels (usually a capillary and venule, occasionally an arteriole), a few unmyelinated (occasionally myelinated) nerve fibres, loose perivascular connective tissue, and is lined by an endosteal layer of osteoprogenitor/bone-lining cells.

- ▶ Surrounding the canal are the **concentric Haversian lamellae**; between successive lamellae lie flattened **osteocyte lacunae**, and the whole cylinder is demarcated from its neighbours by a hypermineralized, collagen-poor **cement (reversal) line**.
- ▶ It has been estimated that a typical adult skeleton contains on the order of **21 million osteons**, continuously being resorbed and rebuilt throughout life.

□ Fig 6.7 — Haversian system in compact bone (schematic representation). (IB Singh — *Textbook of Human Histology* — 9e, p.183)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A transverse (ground) section through compact bone showing one complete osteon, labelled with:

- ▶ **Haversian (osteonal) canal** — centre, containing blood vessel(s)
- ▶ **Concentric (Haversian) lamellae** — rings of matrix around the canal
- ▶ **Osteocyte lacunae** — small dark spaces between lamellae, arranged in concentric rows
- ▶ **Canaliculi** — fine radiating channels linking lacunae to each other and to the Haversian canal
- ▶ **Cement (reversal) line** — the outer boundary of the osteon
- ▶ **Interstitial lamellae** — irregular remnants filling the gaps between osteons
- ▶ **Volkman's (perforating) canal** — a transverse channel connecting two Haversian canals
- ▶ **Outer and inner circumferential lamellae** — at the periosteal and endosteal margins respectively, with the periosteum and Sharpey's fibres shown at the outer margin

Ultrastructure of an individual lamella

- ▶ Each lamella is built of bundles of **type I collagen fibrils**, 2–3 μm thick, running parallel to one another within that lamella but shifted by about 90° relative to the fibres of the adjacent lamella — an arrangement directly comparable to the grain of plywood.
- ▶ This alternating-ply organisation is best demonstrated under **polarized light microscopy**, where each osteon in transverse section typically shows two bright (birefringent) bands crossing one another — a direct consequence of the regularly alternating collagen fibre orientation between successive lamellae.
- ▶ Mineralization proceeds from the outermost (oldest) lamella of an osteon inward, so the peripheral lamellae of an osteon are typically **more heavily mineralized** than the newest, most centrally deposited ones; a thin (0.2–2 μm) unmineralized layer of osteoid persists at the mineralization front.
- ▶ Collagen fibre direction within an osteon also varies regionally with mechanical loading — more **longitudinal** fibres at sites under tension, more **oblique/transverse** fibres at sites under compression, with the outermost (peripheral) lamellae of an osteon containing more transverse fibres.

Osteocytes, lacunae and canaliculi — the nutritional pathway

- ▶ **Osteocytes**, the mature, entombed descendants of osteoblasts, occupy flattened **lacunae** located at the interface between successive lamellae.

- ▶ Each osteocyte sends out numerous fine cytoplasmic (dendritic) processes into narrow **canaliculi** (~0.25–0.5 µm wide) that radiate from its lacuna, making gap-junctional contact with the processes of neighbouring osteocytes and ultimately reaching the Haversian canal.
- ▶ This creates a continuous, three-dimensional **lacuno-canalicular network**, the sole route by which nutrients, gases, ions and waste products diffuse to and from osteocytes buried deep in calcified matrix, since no osteocyte lies more than ~200 µm from a blood vessel — the functional limit set by diffusion.
- ▶ Canaliculi of one osteon usually loop back within the same system and do **not**, as a rule, communicate across the cement line with canaliculi of a neighbouring osteon or with interstitial lamellae — the cement line is a diffusion barrier crossed by only a few canaliculi.
- ▶ Osteocytes also participate in **mechanosensation** and in perilacunar/pericanalicular matrix remodelling ("osteocytic osteolysis"), helping to regulate mineral homeostasis.

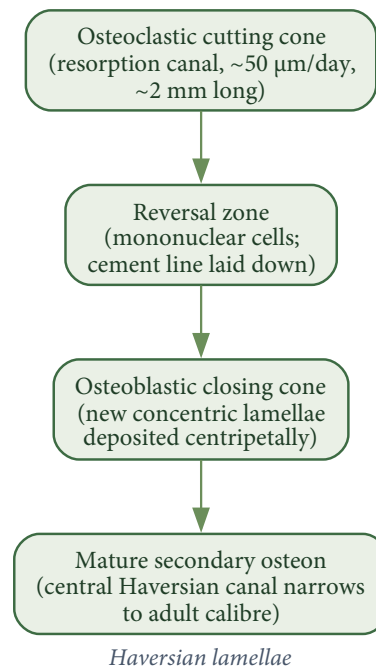
Vascular and nerve supply — Haversian and Volkmann's canals

Channel	Course	Lamellae around it?
Haversian (osteonal) canal	Runs parallel to the long axis of the bone/osteon	Yes — surrounded by concentric lamellae
Volkmann's (perforating) canal	Runs obliquely or transversely, connecting adjacent Haversian canals to each other and to the periosteal and medullary circulations	No — passes through, but is not itself ringed by, concentric lamellae

- ▶ Volkmann's canals thus provide the transverse cross-links that make the vascular supply of compact bone a continuous, interconnected network rather than a set of isolated parallel tubes.
- ▶ Periosteal and nutrient-artery-derived vessels enter via Volkmann's canals to reach the Haversian canals; the accompanying unmyelinated nerve fibres travel in the perivascular space.

Formation and remodelling of osteons

- ▶ Early (fetal/immature) compact bone is built of **primary osteons** — irregular, first-formed Haversian systems with woven (non-lamellar), less-organised matrix around a relatively large primary vascular space.
- ▶ Throughout life these are progressively replaced by **secondary osteons** (true, lamellar Haversian systems) via internal remodelling, which renews roughly 10% of adult bone each year.



- ▶ A **bone-remodelling unit** has an advancing **cutting cone** (osteoclasts boring a cylindrical resorption tunnel, ~2 mm long, advancing at ~50 µm/day, taking 1–3 months) followed by a **closing cone** in which osteoblasts line the tunnel wall and deposit new lamellae centripetally, narrowing the space until only the adult-calibre Haversian canal remains — a process also taking roughly 1–3 months.
- ▶ The reversal (cement) line marks the boundary between the cutting cone's resorptive front and the newly deposited osteon, and remains as a permanent, low-collagen scar.
- ▶ Older, partially eroded osteons left over from repeated remodelling cycles become the **interstitial lamellae** described above — living evidence of the bone's remodelling history.

Applied / Clinical anatomy

- ▶ **Forensic age estimation:** because the rate of remodelling and the accumulation of osteons and osteon fragments (interstitial lamellae) increase with age, quantitative osteon counts/osteon population density on a ground section are used to estimate age at death from skeletal remains.
- ▶ **Osteoporosis:** accelerated remodelling with resorption outpacing formation widens Haversian canals, thins concentric lamellae and increases cortical porosity, reducing mechanical strength and predisposing to fragility fractures.
- ▶ **Osteomalacia/rickets:** defective mineralization leaves wide, persistent osteoid seams around lamellae with poorly defined lacunae and canaliculi, seen as broad unmineralized borders on undecalcified sections.
- ▶ **Paget's disease (osteitis deformans):** grossly excessive, disordered remodelling produces a chaotic "mosaic" pattern of irregular, haphazardly-oriented cement lines joining lamellar fragments of different ages — the classic histological hallmark of the disease.
- ▶ **Fracture healing and graft incorporation:** creeping substitution of a bone graft, and remodelling of a fracture callus into mature cortical bone, both proceed by the same cutting-cone/closing-cone (Haversian remodelling) mechanism described above.

- ▶ **Polarized light microscopy:** the alternating collagen orientation of Haversian lamellae, producing bright/dark extinction bands, is used routinely to identify lamellar bone and distinguish it from woven bone in both diagnostic histopathology and forensic bone identification.
- ▶ **Ground-section histology practicals:** the Haversian system, its canal, concentric and interstitial lamellae, lacunae and canaliculi are the standard identification points tested in undecalcified ground-section spotters.

High-yield exam pointers

- ▶ Haversian (concentric) lamellae are one of **three types of lamellae** in compact bone — circumferential (outer/inner), concentric (Haversian), and interstitial.
- ▶ Concentric lamellae + central Haversian canal = **osteon (Haversian system)**, the structural unit of compact bone; osteon diameter 100–400 µm, roughly 4–20 lamellae per osteon.
- ▶ Collagen fibres in successive lamellae alternate direction by ~90° — the "plywood" arrangement — giving strength and a bright/dark banding pattern under polarized light.
- ▶ Osteocytes lie in **lacunae between lamellae**; their processes occupy **canaliculi**, forming a lacuno-canalicular network for nutrient exchange (no osteocyte >200 µm from a vessel).
- ▶ **Haversian canals** run parallel to the bone's long axis and are ringed by concentric lamellae; **Volkman's canals** run transversely/obliquely, connect Haversian canals, and are NOT ringed by concentric lamellae.
- ▶ The osteon is bounded by a **cement (reversal) line**, largely impermeable to canaliculi.
- ▶ Osteons remodel via a **cutting cone (osteoclastic resorption)** followed by a **closing cone (osteoblastic deposition)**; old remnants persist as **interstitial lamellae**.
- ▶ Primary (woven) osteons of immature bone are replaced by **secondary (lamellar) osteons** during growth and remodelling.
- ▶ Applied relevance: forensic age estimation by osteon counting, osteoporosis (cortical porosity), and Paget's disease (mosaic cement lines).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; IB Singh — Textbook of Human Histology, 9e; Junqueira's Basic Histology (Text & Atlas), 17e; Ross Histology — A Text and Atlas, 7e; Sontakke — Textbook of Human Histology, 2e; Vishram Singh — General Anatomy, 4e.



Micro anatomy of skin

Asked in: Paper III Apr 2006 — RGUHS MD/MS Anatomy

Definition / Introduction Skin (the integument) is the largest organ of the body, forming 15–20% of total body mass and covering roughly 1.5–2 m² of surface area. Histologically it is composed of two principal layers — a superficial, avascular **epidermis** (keratinized stratified squamous epithelium of surface ectodermal origin) and a deeper, vascular **dermis** (dense irregular connective tissue of mesodermal origin) — resting on the **hypodermis/subcutis** (superficial fascia), sometimes described as a third layer. The skin bears epidermal appendages (hair follicles, sebaceous glands, sweat glands, nails) and functions in protection, thermoregulation, sensation, vitamin D synthesis, excretion and immune surveillance.

Classification of skin: thick versus thin

Feature	Thin (hairy) skin	Thick (glabrous) skin
Distribution	Covers entire body except palms/soles	Palms of hands, soles of feet, flexor aspect of digits
Epidermal layers	4 layers — stratum lucidum absent ; stratum corneum thin	All 5 layers present; stratum corneum and lucidum very thick
Dermal papillae/ridges	Fewer, shallower — no dermatoglyphics	Numerous, tall, closely packed — form dermatoglyphic ridges (fingerprints)
Hair follicles, sebaceous & apocrine glands	Present	Absent
Eccrine sweat glands	Present, fewer	Present, very numerous
Arrector pili muscle	Present	Absent

Epidermis

The epidermis is a self-renewing, avascular, stratified squamous keratinized epithelium made up mainly of **keratinocytes**, with three resident **non-keratinocyte** populations — melanocytes, Langerhans cells and Merkel cells — interspersed among them. From deep to superficial it has five layers (from within outward):

Layer	Composition / key feature
Stratum basale (germinativum)	Single row of columnar/cuboidal mitotically active stem cells resting on the basal lamina, attached to it by hemidesmosomes; source of continuous keratinocyte renewal
Stratum spinosum (prickle cell layer)	Several layers of polyhedral cells joined by numerous desmosomes giving a "prickly" appearance; site of tonofilament (keratin) accumulation; contains Langerhans cells

Layer	Composition / key feature
Stratum granulosum	1–5 flattened layers containing basophilic keratohyalin granules (profilaggrin) and lamellar (Odland) bodies that extrude a lipid barrier into the intercellular space
Stratum lucidum	Thin, translucent, eosinophilic layer of dead cells (only in thick skin)
Stratum corneum	Multiple layers of dead, anucleate, flattened squames packed with keratin (cornification); shed by desquamation

Non-keratinocyte cells of the epidermis

Cell	Origin	Location	Function
Melanocyte	Neural crest	Basal layer; dendrites contact ~35–40 basal keratinocytes (density ~800–2300/mm ²)	Synthesizes melanin in melanosomes via tyrosinase; transferred to keratinocytes; protects against UV
Langerhans cell	Bone marrow (monocyte lineage)	Basal and prickle-cell layers	Antigen-presenting dendritic cell; contains Birbeck granules ; migrates to regional lymph nodes after antigen capture
Merkel cell	Epidermal/neural crest	Basal layer, especially in palms/soles and outer root sheath of hair follicles	Slow-adapting mechanoreceptor for light touch; forms Merkel cell–neurite complex with an afferent axon terminal

Cornification (keratinization) takes basal stem-cell progeny through the above layers over roughly 4 weeks, converting living polygonal cells into dead, keratin-filled squames.

Dermis

The dermis is a dense, irregular connective tissue of collagen and elastic fibres in a ground substance of glycosaminoglycans, glycoproteins and bound water; it houses blood vessels, lymphatics, nerves and epidermal appendages. It has two indistinctly demarcated zones:

- **Papillary layer** — superficial, thin, loose connective tissue forming the **dermal papillae** that interdigitate with epidermal (rete) ridges; contains finer **type III collagen**, capillary loops (one per papilla) and Meissner's corpuscles.
- **Reticular layer** — deeper, thicker, dense connective tissue with coarse **type I collagen** (types I:III in an 80–85% : 15–20% ratio) and elastic fibres giving tensile strength and recoil; houses appendages, the deeper vascular plexuses and Pacinian corpuscles.

Type IV collagen forms the basal lamina at the dermo-epidermal junction; type VII collagen anchoring fibrils tether this junction to the papillary dermis.

Skin appendages

1. **Pilosebaceous unit** — hair follicle + arrector pili muscle + sebaceous gland ± apocrine gland.

- ▶ **Hair follicle:** a tubular downgrowth of epidermis into the dermis/hypodermis, terminating in a bulb that caps a vascular **dermal papilla**; consists of inner and outer root sheaths surrounding the hair shaft (cuticle, cortex, medulla); matrix keratinocytes and melanocytes at the bulb produce the pigmented shaft. Growth is cyclical — **anagen** (growth), **catagen** (regression), **telogen** (rest).
- ▶ **Sebaceous gland:** branched acinar holocrine gland opening into the pilary canal (or directly onto skin surface where hairless, e.g. lips, glans penis, areola); secretes **sebum** by disintegration of whole cells.
- ▶ **Arrector pili muscle:** bundle of smooth muscle running obliquely from the hair follicle's bulge to the papillary dermis; sympathetic (noradrenergic) innervation; contraction erects the hair and produces "goose bumps"; absent from facial, axillary, pubic hair, eyelashes/eyebrows and nostril/meatal hairs.

2. **Sweat glands**

Feature	Eccrine gland	Apocrine gland
Distribution	Widespread (palms, soles, forehead — most numerous)	Axilla, areola, perianal region, mons pubis, scrotum, labia minora
Duct opening	Directly onto skin surface (spirals through epidermis)	Into the pilary canal above the sebaceous duct
Secretory mechanism	Merocrine	Merocrine/apocrine, historically termed "apocrine"
Onset of activity	Functional from birth; thermoregulatory	Minimal before puberty; androgen-dependent
Innervation	Sympathetic cholinergic	Sympathetic adrenergic
Structure	Simple coiled tubular gland — secretory coil (clear + dark cells) + duct (two layers of cuboidal cells)	Larger coiled gland, secretory coil up to 2 mm wide, cuboidal cells with apical decapitation, myoepithelial cell layer

3. **Nail** — the nail plate (compacted, hard keratin) arises from the **nail matrix** (dorsal, intermediate/germinal, and ventral/sterile parts); the proximal 50% of the matrix contributes ~80% of the plate. It is bordered by proximal and lateral nail folds, with the eponychium (cuticle) sealing the proximal fold and the hyponychium beneath the free edge.

Blood supply and nerve supply

Cutaneous circulation is derived from direct cutaneous, musculocutaneous perforating and fasciocutaneous perforating vessels, which anastomose into **six horizontal plexuses**: two plexuses of the deep fascia, a subcutaneous plexus, and three dermal plexuses — the **deep dermal plexus** (base of reticular dermis), the **reticular dermal plexus** (mid-dermis, mainly venous) and the **subpapillary plexus**

(papillary–reticular junction), which sends capillary loops into individual dermal papillae. Arteriovenous anastomoses in the extremities allow thermoregulatory shunting.

Cutaneous sensory nerve endings include:

- ▶ **Free nerve endings** — pain, temperature, crude touch (widely distributed, epidermis and dermis)
- ▶ **Meissner's corpuscles** — light touch/discriminative touch; dermal papillae of glabrous skin (palms, soles, fingertips)
- ▶ **Pacinian corpuscles** — deep pressure and vibration; deep dermis/hypodermis, especially digits
- ▶ **Ruffini's corpuscles** — sustained pressure and stretch; reticular dermis
- ▶ **Merkel cell–neurite complexes** — sustained light touch (basal epidermis)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a vertical section of thick (glabrous) skin: **Epidermis** (stratum basale, spinosum, granulosum, lucidum, corneum) → **dermal papilla** with capillary loop → **papillary dermis** → **reticular dermis** → **subpapillary plexus** → **hair follicle** with **arrector pili muscle** and **sebaceous gland** (pilosebaceous unit) → **eccrine sweat gland** (coiled secretory portion + duct) → **hypodermis/subcutaneous fat**. Mark Meissner's corpuscle (papillary dermis) and Pacinian corpuscle (deep dermis/hypodermis).

□ Fig 18.1 — Layers and appendages of skin. (Junqueira's Basic Histology — Text & Atlas — 17e, p.901)

Applied / Clinical anatomy

- ▶ **Skin biopsy/incisions:** elective incisions are planned along **relaxed skin tension lines** (RSTLs)/Kraissl's lines (not Langer's lines, which are cadaveric rigor-mortis cleavage lines and often run at right angles to RSTLs on the face) to minimize scarring.
- ▶ **Skin grafts:** **split-thickness grafts** (epidermis + variable dermis) leave adnexal remnants (hair follicles) at the donor site that allow re-epithelialization; **full-thickness grafts** (epidermis + entire dermis) remove all epidermal elements, so the donor site must be closed primarily.
- ▶ **Wound healing:** re-epithelialization is driven by keratinocyte migration/proliferation from wound edges under EGF, KGF/FGF-7 and TGF- β influence; loss of rete-ridge interdigitation at the dermo-epidermal junction is why scar skin lacks normal anchorage strength.
- ▶ **Dermatoglyphics:** the ridge pattern of palmar/plantar epidermis (determined by dermal papillae) is unique to each individual — used in forensic identification and correlates with certain chromosomal disorders (e.g., simian crease in Down syndrome).
- ▶ **Pigmentary disorders:** albinism (tyrosinase deficiency — melanocytes present but inactive); vitiligo (melanocyte destruction); ACTH-driven hyperpigmentation in Addison's disease; melasma of pregnancy (oestrogen/progesterone-driven).
- ▶ **Merkel cell carcinoma:** rare, aggressive tumour of Merkel cells, linked to Merkel cell polyomavirus, presents as a rapidly growing nodule on sun-exposed skin.
- ▶ **Apocrine gland disease:** obstruction underlies Fox–Fordyce disease; chronic apocrine-bearing skin inflammation causes hidradenitis suppurativa.

- ▶ **Ageing skin:** flattening of dermal papillae (fewer capillary loops), a 10–20% fall in melanocytes and sparser Langerhans cells with age — explains easy bruising and reduced immune surveillance in the elderly.

High-yield exam pointers

- ▶ Skin = epidermis (ectoderm) + dermis (mesoderm), resting on hypodermis; largest organ, 15–20% of body mass.
- ▶ Thick (glabrous) skin has all 5 epidermal layers (stratum lucidum present); thin (hairy) skin lacks stratum lucidum and has 4 layers.
- ▶ Epidermal layers, deep to superficial: basale → spinosum → granulosum → lucidum → corneum ("Come, Let's Get Sun Burnt").
- ▶ Three non-keratinocyte cells: melanocyte (neural crest, melanin), Langerhans cell (bone marrow, antigen presentation, Birbeck granules), Merkel cell (mechanoreceptor).
- ▶ Dermis = papillary (thin, type III collagen) + reticular (thick, type I collagen) layers; collagen I:III ≈ 80–85%:15–20%.
- ▶ Pilosebaceous unit = hair follicle + arrector pili + sebaceous (holocrine) gland ± apocrine gland.
- ▶ Eccrine (merocrine, cholinergic, thermoregulatory, everywhere) vs apocrine (androgen-dependent, adrenergic, axilla/areola/perianal) sweat glands.
- ▶ Six cutaneous vascular plexuses; three dermal — subpapillary, reticular, deep dermal.
- ▶ Receptors: Meissner (light touch), Pacinian (pressure/vibration), Ruffini (stretch), Merkel (sustained touch), free nerve endings (pain/temperature).
- ▶ Applied: incisions along RSTLs, not Langer's lines; split- vs full-thickness grafts differ in donor-site re-epithelialization capacity.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e — Chapter 7, Skin and its appendages; IB Singh — Textbook of Human Histology 9e (Skin and its Appendages); Ross Histology — A Text and Atlas 7e (Integumentary System); Junqueira's Basic Histology — Text & Atlas 17e (Skin); Sontakke — Textbook of Human Histology 2e (Skin and its Appendages).



Microanatomy of skin

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction — Skin (integument) is the largest organ of the body, constituting approximately 15% of total body weight, with a surface area of about 2 m² and a thickness ranging from 1.5 to 5.0 mm depending on region. It consists of two principal layers — a superficial, avascular **epidermis** (stratified squamous keratinized epithelium derived from surface ectoderm) and a deeper **dermis** (dense irregular connective tissue derived from mesenchyme) — resting on the **hypodermis** (subcutaneous tissue). The skin and its appendages (pilosebaceous units, sweat glands, nails) together form the integumentary system, subserving protection, thermoregulation, sensation, immune surveillance and vitamin D synthesis.

Types of skin — thick versus thin

Feature	Thick (glabrous) skin	Thin (hairy) skin
Location	Palms, soles, flexor surface of digits	Rest of the body
Epidermal layers	All five layers present; thick stratum corneum, stratum lucidum well formed	Only four layers; stratum lucidum absent
Dermis	Relatively thinner	Relatively thicker
Hair follicles, sebaceous glands	Absent	Present
Sweat glands (eccrine)	Numerous	Fewer
Arrector pili muscle	Absent	Present
Dermal papillae	Large, closely aggregated, arranged in ridges (fingerprints/dermatoglyphics)	Few, small

Epidermis — layers and cell populations

The epidermis is a self-renewing stratified epithelium composed mainly of **keratinocytes**, organised (deep to superficial) into five layers:

Layer	Key features
Stratum basale (stratum germinativum)	Single layer of columnar/cuboidal cells on the basal lamina; contains keratinocyte stem cells expressing keratins K5/K14 ; site of mitosis
Stratum spinosum (prickle cell layer)	Several layers of polygonal cells linked by numerous desmosomes (retraction during processing gives a "prickly" look); expresses K1/K10 ; basale + spinosum together are often termed the Malpighian layer

Layer	Key features
Stratum granulosum	3–5 flattened layers with basophilic keratohyalin granules (profilaggrin) and lamellar (Odland) bodies that discharge glycolipid into the intercellular space, forming the epidermal permeability barrier
Stratum lucidum	Thin, translucent layer, present only in thick (palmar/plantar) skin
Stratum corneum	Outermost, anucleate, cornified squames (corneocytes) with a cross-linked involucrin envelope and filaggrin-aggregated keratin

Basal and prickle-cell keratinocytes proliferate as **epidermal proliferation units** (a central stem cell with a ring of transit-amplifying cells, overlain by 6–8 basal cells); normal epidermal turnover time is 52–75 days, shortened to as little as 8 days in psoriasis.

Non-keratinocyte cells interspersed among keratinocytes:

- ▶ **Melanocytes** — dendritic, neural-crest-derived pigment cells in the basal layer; each melanocyte forms an "epidermal-melanin unit," supplying melanin (via tyrosinase-mediated melanosome synthesis) to about 35–40 **neighbouring basal keratinocytes**; lack desmosomal contacts.
- ▶ **Langerhans cells** — bone-marrow-derived antigen-presenting dendritic cells (2–4% of epidermal cells), located mainly in the stratum spinosum; contain characteristic rod-shaped **Birbeck granules**; migrate to regional lymph nodes on antigen stimulation.
- ▶ **Merkel cells** — clear oval cells in the basal layer (also in outer root sheath of large hair follicles); function as slowly-adapting mechanoreceptors, forming a synapse-like "tactile disc" with an afferent nerve terminal; embryonic origin debated (epidermal vs neural crest).

Diagram (Junqueira's Basic Histology, Fig 18.1 — layers and appendages of skin):

□ Fig 18.1 — Layers and appendages of skin. (Junqueira's Basic Histology — Text & Atlas — 17e, p.901)

Dermis and hypodermis

The dermis is an interwoven collagenous/elastic connective tissue in a ground substance of glycosaminoglycans, divided into two indistinctly bounded zones:

- ▶ **Papillary layer** — thin, superficial, fine type I and III collagen, capillary loop and (in thick, hairless skin) Meissner's corpuscles in each dermal papilla; anchored to the epidermis via anchoring fibrils of **type VII collagen** running from the basal lamina.
- ▶ **Reticular layer** — thicker, coarse type I collagen (80–85% of dermal collagen; type III forms 15–20%) arranged in a three-dimensional lattice with interwoven elastic fibres, giving tensile strength and recoil.

The **dermo-epidermal junction / basement membrane zone (BMZ)** comprises the basal keratinocyte plasma membrane, hemidesmosomes (containing $\alpha 6 \beta 4$ integrin, plectin, the 230-kDa bullous pemphigoid antigen and 180-kDa collagen XVII), the lamina lucida and lamina densa, and anchoring fibrils of type VII collagen — clinically important as the target of autoimmune blistering disease.

The **hypodermis** (superficial fascia) is loose, often adipose, connective tissue merging with the dermis; it provides thermal insulation, mechanical cushioning and an energy store, and carries the main subcutaneous nerve and vessel trunks.

Skin appendages

Pilosebaceous unit — hair + follicle + arrector pili + sebaceous gland ($\pm$ apocrine gland):

- ▶ **Hair shaft:** three concentric zones — cuticle (outer, overlapping cornified squames), cortex (bulk of shaft, pigmented), medulla (present only in thick hairs).
- ▶ **Hair follicle wall**, inner to outer: inner root sheath (its own cuticle, then Huxley's layer, then Henle's layer, all keratohyalin-containing), outer root sheath (continuous with stratum spinosum), and a glassy (basal lamina) membrane separating it from the connective tissue sheath.
- ▶ **Hair bulb** encloses the mesenchymal **dermal hair papilla**; its lower germinal matrix (mitotically active keratinocytes with interspersed melanocytes) generates hair and inner root sheath.
- ▶ **Hair cycle:** anagen (active growth) $\rightarrow$ catagen (involution) $\rightarrow$ telogen (rest), recurring asynchronously; scalp hair grows at $\sim 0.2\text{--}0.44$ mm/24 h.
- ▶ **Arrector pili** — smooth muscle bundles from the follicular bulge to the papillary dermis; sympathetic (noradrenergic) innervation; contraction elevates hair and expresses sebum; absent over face, axilla, pubis, eyelashes/brows.
- ▶ **Sebaceous glands** — holocrine acinar glands opening into the hair follicle (or directly onto skin in lip, buccal mucosa, Montgomery's tubercles of areola, Meibomian/tarsal glands of eyelid); sebocytes disintegrate completely to release sebum; androgen-dependent, most numerous on face/scalp ($400\text{--}900/\text{cm}^2$); absent from palms/soles.

Sweat glands:

- ▶ **Eccrine (merocrine)** — simple coiled tubular glands, secretory coil deep in dermis/hypodermis with clear cells (fluid), dark cells (glycoprotein) and myoepithelial cells; duct spirals through the epidermis (acrosyringium) to a surface pore; cholinergic sympathetic innervation; total $1.6\text{--}4.5$ million glands, absent from nail bed, lip margins, glans penis/clitoris.
- ▶ **Apocrine** — larger, confined to axilla, areola, perianal, periumbilical and anogenital skin; duct opens into the pilary canal above the sebaceous duct; active only after puberty, adrenergic innervation; implicated in Fox–Fordyce disease and hidradenitis suppurativa.

Nail apparatus — nail plate (compacted cornified squames from dorsal, intermediate and ventral matrices), proximal and lateral nail folds, eponychium (cuticle), nail matrix (dorsal/intermediate/ventral), pale **lunula**, nail bed (rich in longitudinally arranged vessels and **glomus bodies**), and **hyponychium**. Growth rate ≈ 0.1 mm/day (fastest in the middle finger); a fingernail regrows in ~ 6 months, a toenail in ~ 18 months. Germline mutations in **RSPO4** cause recessive anonychia; **FZD6** mutations underlie autosomal recessive nail dysplasia/twenty-nail dystrophy.

Blood supply, lymphatics and cutaneous sensory receptors

Cutaneous blood flow is $\sim 5\%$ of cardiac output and is chiefly thermoregulatory. Three feeder systems (direct cutaneous, musculocutaneous perforator, fasciocutaneous perforator) supply **six anastomosing**

horizontal plexuses: subpapillary, reticular (mid-dermal, mainly venous) and deep dermal plexuses within the skin, plus a subcutaneous plexus and two fascial plexuses. Capillary loops from the subpapillary plexus enter each dermal papilla; numerous **arteriovenous anastomoses** in the extremities permit rapid shunting for heat conservation/loss. Lymphatics begin as blind capillaries below the papillary dermis and drain via superficial and deep plexuses.

Sensory innervation supplies both unencapsulated and encapsulated receptors:

- ▶ Free nerve endings (pain, temperature, itch) and Merkel-cell "tactile discs" (sustained light touch/texture) — epidermal/basal.
- ▶ Root hair plexus around follicles — detects hair movement.
- ▶ **Meissner's corpuscles** ($30\text{--}75 \times 50\text{--}150\ \mu\text{m}$, dermal papillae of glabrous skin) — rapidly adapting, light touch.
- ▶ **Pacinian corpuscles** (up to $\sim 1\text{ mm}$, 15–50 concentric lamellae, deep dermis/hypodermis, especially digits) — deep pressure and vibration.
- ▶ **Ruffini corpuscles** (stretch/torque) and **Krause end bulbs** (low-frequency vibration; genital skin) — less numerous.

Autonomic (sympathetic) fibres are cholinergic to sweat glands and adrenergic/noradrenergic to arrector pili and blood vessels.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Vertical block diagram of skin (label each): **Epidermis** — stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, stratum basale; **dermo-epidermal junction** (dermal papilla/rete peg); **Dermis** — papillary layer (capillary loop, Meissner's corpuscle) and reticular layer; **Hypodermis** (adipose tissue); appendages — hair shaft & follicle, sebaceous gland, arrector pili muscle, sweat gland (coiled secretory part + duct), Pacinian corpuscle, free nerve ending; vasculature — subpapillary plexus and dermal capillary loop.

Applied / Clinical anatomy

- ▶ **Psoriasis** — markedly shortened epidermal turnover (~ 8 days), parakeratosis, thickened stratum spinosum/basale.
- ▶ **Ichthyosis vulgaris / atopic eczema** — loss-of-function mutations in the **filaggrin (FLG)** gene impair the cornified barrier.
- ▶ **Epidermolysis bullosa** — junctional type from **COL17A1** (collagen XVII) mutation, dystrophic type from **COL7A1** (anchoring fibril collagen VII) mutation; both cause trauma-induced blistering at the BMZ.
- ▶ **Bullous pemphigoid** (autoantibodies to hemidesmosomal BP180/BP230) versus **pemphigus** (autoantibodies to interkeratinocyte desmosomal proteins) — the two classic autoimmune blistering diseases, distinguished by the level of the split (sub-epidermal vs intra-epidermal).
- ▶ **Vitiligo** — autoimmune destruction of melanocytes causing depigmented patches; **Albinism** — tyrosinase deficiency with quiescent but present melanocytes, increased skin-cancer risk.

- ▶ **Tumours:** basal cell carcinoma (stratum basale), squamous cell carcinoma (stratum spinosum, sun-exposed sites), malignant melanoma (melanocytes), Merkel cell carcinoma (aggressive, linked to Merkel cell polyomavirus).
- ▶ **Acne vulgaris** — androgen-driven sebaceous hyperactivity with comedone formation; **hidradenitis suppurativa / Fox-Fordyce disease** — apocrine duct obstruction.
- ▶ **Nail disorders:** koilonychia (iron/B12 deficiency, spoon nails), paronychia (peri-ungual bacterial/fungal infection), onychia (nail-fold inflammation).
- ▶ **Surgical/forensic relevance:** incisions along Langer's/cleavage lines (parallel to reticular dermal collagen) gape less; dermatoglyphics (epidermal ridge pattern over dermal papillae) used for identification; split- versus full-thickness skin grafts are defined by how much dermis is harvested.

High-yield exam pointers

- ▶ Epidermis = 5 layers (basale, spinosum, granulosum, lucidum, corneum); stratum lucidum only in thick (palmar/plantar) skin.
- ▶ Epidermal turnover time 52–75 days normally; falls to ~8 days in psoriasis.
- ▶ Four epidermal cell types: keratinocytes (K5/K14 basal → K1/K10 suprabasal), melanocytes (neural crest, 1 melanocyte : ~35–40 keratinocytes), Langerhans cells (bone marrow, Birbeck granules), Merkel cells (basal, mechanoreceptor).
- ▶ Dermis = papillary (thin, type III collagen, capillary loops) + reticular (thick, type I collagen, elastic fibres); BMZ = lamina lucida + lamina densa + type VII collagen anchoring fibrils.
- ▶ Sebaceous secretion is holocrine; eccrine sweat is merocrine; apocrine sweat glands mature only after puberty and open above the sebaceous duct.
- ▶ Six horizontal vascular plexuses supply skin; sensory receptors — Meissner (light touch, dermal papillae), Pacinian (pressure/vibration, deep dermis), Merkel disc (sustained touch), Ruffini (stretch), Krause (genital skin).
- ▶ Nail growth ≈ 0.1 mm/day; fingernail regrows in ~6 months, toenail in ~18 months.
- ▶ Genetic correlates worth naming: FLG (ichthyosis/eczema), COL17A1/COL7A1 (epidermolysis bullosa), RSPO4/FZD6 (nail dysplasias).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology 17e; IB Singh — Textbook of Human Histology 9e.



Microscopy & histotechniques



Electron microscope

Asked in: Paper III Nov 2016 · Paper III May 2011 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction The electron microscope (EM) is an instrument that uses a beam of high-energy electrons, instead of visible light, as the imaging probe to visualise specimens at ultrastructural (subcellular/molecular) resolution. Because an accelerated electron has a far shorter wavelength than visible light, the resolving power of the EM is several thousand-fold greater than that of the light microscope (LM). The two basic forms used in biology are the **transmission electron microscope (TEM)**, which images thin sections by transmitted electrons to reveal internal ultrastructure, and the **scanning electron microscope (SEM)**, which images the surface topography of specimens using secondary/backscattered electrons.

1. Principle of electron microscopy

- ▶ Electrons behave as waves (de Broglie principle): $\lambda = h/mv$, where λ = wavelength, h = Planck's constant (6.626×10^{-34} J·s), m = mass and v = velocity of the electron.
- ▶ Accelerating the electron to high speed shortens its wavelength markedly (to a fraction of a nanometre), which is the physical basis of the EM's very high resolving power.
- ▶ Advantages of the electron beam as an imaging probe:
 - ▶ Very short wavelength → high resolving power.
 - ▶ Easily manipulated/focused by electromagnetic fields.
 - ▶ High brightness.
 - ▶ Strong interaction with matter (used to generate image contrast).

Feature	Light microscope	Electron microscope
Probe	Visible light (~400–700 nm)	High-energy electron beam (very short wavelength)
Maximum resolution	~0.5 μm	~0.1 nm
Maximum useful magnification	~1000×	up to ~500,000×
Focusing lens	Glass lens	Electromagnetic coil
Medium in optical path	Air	Vacuum
Image formed on	Eye (retina)	Fluorescent screen / CCD camera

2. Components of the transmission electron microscope

Component	Description / function
Electron gun	V-shaped tungsten filament (cathode) heated to ~2700 K generates electrons by thermionic emission; the Wehnelt cylinder (cathode shield) focuses them into a beam, which is accelerated toward the anode plate . Alternative high-brightness sources: lanthanum hexaboride (LaB_6) crystal or field-emission gun (FEG).

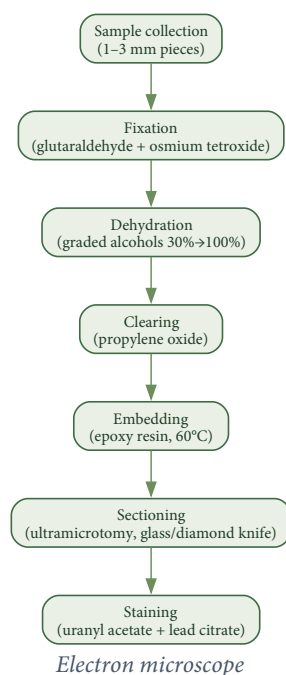
Component	Description / function
Condenser lens system	Two or more electromagnetic coils that focus the electron beam onto the specimen; current through the coil controls focal length.
Objective lens	The strongest electromagnetic lens; produces the first magnified ("intermediate") image of the specimen.
Intermediate & projector lenses	Further magnify and project the final image onto the viewing screen/camera.
Vacuum system	Rotary, diffusion, turbomolecular or ion pumps evacuate the column (to as low as 10^{-8} Pa) — needed because residual gas molecules would scatter the electron beam and because vacuum prevents oxidation (and hence prolongs the life) of the tungsten filament.
Detector	A fluorescent screen converts the electron pattern into a visible image, permanently recorded by a charge-coupled device (CCD) camera.

3. Electron–specimen interaction

- ▶ **Elastic scattering** — beam electrons interact with the atomic nucleus; their path is deflected but kinetic energy/velocity is unchanged. Heavier (higher atomic number) atoms scatter more strongly, producing greater contrast.
- ▶ **Inelastic scattering** — beam electrons interact with orbital electrons of the specimen atoms, lose energy, and are deflected.
- ▶ **Secondary electrons** — low-energy electrons ejected from the specimen surface; their detection forms the basis of the SEM image.
- ▶ **Backscattered electrons** — reflected incident electrons; more abundant from higher-atomic-number regions.
- ▶ **Excited/Auger electrons** — ejection of an orbital electron excites the atom; the excess energy released on return to the ground state appears as Auger electrons, X-rays or cathodoluminescence.
- ▶ In the TEM, three simultaneous interactions occur: unscattered electrons pass straight through thin/light areas (appear bright); elastically scattered electrons are deflected by heavier atoms (appear dark); inelastically scattered electrons lose energy passing through the specimen.

4. Specimen processing for TEM

Ultrathin, electron-transparent, mechanically robust sections are essential. The steps are:



- **Fixation** — aim: preserve tissue close to its living state and prevent disintegration during processing. **Glutaraldehyde** cross-links and stabilises protein but fixes lipid poorly and causes shrinkage; **osmium tetroxide** fixes lipid well and causes slight swelling — the two are therefore used together as "**double/combined fixation**" (primary fixation in buffered glutaraldehyde, 2–4%, for a few hours, followed by post-fixation in 1% osmium tetroxide for about an hour). Fixative volume should be roughly 10–15 times the tissue volume; prolonged fixation (>12 h) is avoided as it extracts protein, while under-fixation causes mitochondrial swelling and organelle disruption.
- **Dehydration** — graded series of ethanol (30% → 100%) removes tissue water, which is not miscible with resin.
- **Clearing** — an intermediate solvent (e.g. propylene oxide) bridges alcohol and resin.
- **Embedding** — **epoxy resin** is the standard medium (stable at high temperature, polymerises evenly, no shrinkage; disadvantages — high viscosity, slow infiltration, causes dermatitis). Alternatives: **Araldite**, **Epon** (aliphatic, low viscosity), acrylic resins (methacrylates — cause shrinkage) and polyester resin (e.g. Vestapol W).
- **Sectioning** — ordinary microtomy cannot cut thin enough; an **ultramicrotome** with a glass or diamond knife is required. **Semithin sections** (~1 µm), stained with toluidine blue, are first screened by light microscopy to select a representative area; **ultrathin sections** (optimum ~80 nm; interference colour grey <60 nm, silver 60–90 nm, gold 90–120 nm) are then cut and picked up on a copper grid.
- **Staining** — heavy-metal salts impart electron density/contrast: **uranyl acetate** (binds protein/lipid/nucleic acid) and **Reynold's lead citrate** are the routine "double stain."

5. Scanning electron microscope (SEM) — principle and preparation

- SEM images the **surface** of a specimen. The electron beam is scanned point-by-point across the surface in a raster pattern (unlike the static TEM beam); emitted secondary and backscattered electrons are collected to build the image, giving a three-dimensional topographic appearance.

- Its spatial resolution, while lower than TEM, is roughly ten times better than the light microscope.
- Specimen fixation, dehydration and processing for SEM largely mirror TEM protocols; specimens are dried (e.g. critical-point drying) and sputter-coated with a thin conductive metal layer to prevent surface charging under the beam.

Feature	TEM	SEM
Electron beam	Static, transmitted through thin section	Dynamic — scans the surface in two perpendicular directions
Image formation	Instantaneous, from transmitted/scattered electrons	Built point-by-point, like a scanned image
Information obtained	Internal ultrastructure (2-D projection through the section)	Surface topography (3-D appearance)
Specimen requirement	Ultrathin section (~60–90 nm) on a grid	Whole/surface specimen, conductively coated

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 1.7 — Working principle of a transmission electron microscope. (Sontakke — Textbook of Human Histology — 2e, p.26)

Reproduce a labelled vertical column diagram of the TEM with, top to bottom: **Electron gun** → **Magnetic (condenser) lens** → **Sample** → **Transmitted electrons** → **Screen** (fluorescent screen/CCD). Alongside, sketch the SEM as a scanning beam striking a solid specimen surface with a detector collecting **secondary/backscattered electrons**, contrasted with the TEM's straight-through transmitted beam.

Applied / Clinical anatomy

- **Diagnostic renal pathology** — evaluation of a renal biopsy is considered incomplete without EM; it demonstrates glomerular basement membrane thickening/splitting, immune-complex deposits, and **foot-process effacement** of podocytes, essential in diagnosing glomerular diseases.
- **Primary ciliary dyskinesia** — EM of the respiratory/sperm-tail ciliary axoneme (normally a 9+2 microtubule arrangement with dynein arms) is the diagnostic gold standard for ultrastructural ciliary defects.
- **Dermatopathology/connective tissue disease** — EM aids diagnosis of inherited bullous skin disorders and connective-tissue diseases such as Ehlers–Danlos syndrome, and is used in specialised ophthalmic pathology.
- **Study of cell organelle ultrastructure** — mitochondrial cristae, rough/smooth endoplasmic reticulum, Golgi apparatus, lysosomes, cell junctions and the sperm tail/acrosome are classically studied and illustrated by TEM in histology teaching.
- **Virology** — negative staining/TEM permits direct visualisation of virus morphology and assembly.

- ▶ Recent advances (Gray's Anatomy, Commentary 1.5 — "Electron microscopy in the twenty-first century") — **cryo-fixation/cryo-EM** (rapid vitrification, including high-pressure freezing, avoids chemical-fixation artefact and preserves near-native, hydrated ultrastructure); **field-emission gun (FEG)** sources and **direct detection device (DDD)** cameras that markedly improve signal-to-noise and resolution; **correlative light and electron microscopy (CLEM)**, which co-localises light-microscopic events with EM ultrastructure; and volume/3-D EM techniques — **electron tomography**, **focused ion beam-SEM (FIB-SEM)**, **serial block-face SEM (SBFSEM)** and **array tomography** — that reconstruct three-dimensional cellular/tissue nanostructure.

High-yield exam pointers

- ▶ Resolution of EM (~0.1 nm) vastly exceeds that of the LM (~0.5 µm) because electron wavelength ($\lambda = h/mv$) is far shorter than that of visible light.
- ▶ Electromagnetic lenses (not glass) focus the beam; the entire optical column is under vacuum.
- ▶ TEM = internal ultrastructure via a **static, transmitted** beam through an ultrathin section; SEM = surface topography via a **scanning** beam and secondary/backscattered electrons.
- ▶ Standard TEM fixation is combined/double fixation: **glutaraldehyde** (protein) followed by **osmium tetroxide** (lipid).
- ▶ Embedding medium: **epoxy resin**; sectioning: **ultramicrotome** with glass/diamond knife; ultrathin sections ~60–90 nm (optimum 80 nm).
- ▶ Routine EM stains: **uranyl acetate** and **lead citrate**.
- ▶ Diagnostic uses: renal biopsy (glomerular disease), primary ciliary dyskinesia, skin/connective-tissue disorders.
- ▶ Recent advances: cryo-EM, FEG source, CLEM, and 3-D volume-EM techniques (FIB-SEM, SBFSEM, array tomography).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e — Commentary 1.5 "Electron microscopy in the twenty-first century"; Pranab Dey, Basic and Advanced Laboratory Techniques in Histopathology and Cytology 2e (Chapter 28, Electron Microscopy: Principle, Components, Optics and Specimen Processing); Bancroft's Theory and Practice of Histological Techniques (chapter on specimen preparation for transmission electron microscopy).



Fixatives

Asked in: Paper III Oct 2010 · Paper III Apr 2008 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction

Fixation is the first and most critical step of any histological or cytological technique. It is the process by which cells and tissues are converted to a chemically and physically stable state — all autolytic (enzymatic) and putrefactive (bacterial) activity is arrested — so that the tissue resists distortion or decomposition during subsequent processing, sectioning and staining, while remaining as close as

possible to its living morphology. A **fixative** is any physical or chemical agent that brings about this change. No single universally-accepted "ideal" fixative exists; the fixative of choice is always a compromise dictated by the tissue, the stain, and the downstream technique (routine H&E, special stains, immunohistochemistry, electron microscopy, or enzyme histochemistry).

Aims of fixation and the ideal fixative

- ▶ To preserve the tissue nearest to its living state.
- ▶ To prevent autolysis (self-digestion by cellular enzymes) and putrefaction (bacterial decomposition).
- ▶ To prevent change in the shape, size and volume of the tissue during processing.
- ▶ To make the tissue firm/hard enough for infiltration, embedding and sectioning.
- ▶ To permit consistently good, reproducible staining (Haematoxylin & Eosin, special stains, Papanicolaou stain).
- ▶ To give good optical differentiation of cellular and nuclear detail.

An **ideal fixative** should: penetrate and act rapidly; prevent autolysis and bacterial decomposition; maintain cell volume/shape; give consistently high-quality H&E/Pap staining; be cheap, non-toxic and stable; and preserve antigenicity/nucleic acids for immunohistochemistry and molecular studies. In practice no fixative satisfies every criterion — hence several are kept in a histology laboratory.

Classification of fixatives

Basis	Types	Examples
Nature of fixation	Immersion · Coating (spray) · Vapour · Perfusion · Freeze-drying · Microwave	Immersion in 10% NBF; perfusion (whole-animal research); freeze-drying (soluble substances, enzymes)
Method	Physical (heat, microwave, freeze-drying) vs Chemical (liquid/vapour reagents)	Physical fixation is rarely used routinely, except heat-fixed smears for Gram staining
Chemical nature	Aldehydes · Oxidising agents · Protein-denaturing alcohols · Heavy-metal salts · Acids	Formaldehyde, glutaraldehyde; osmium tetroxide; ethanol, methanol; mercuric chloride, zinc salts; picric acid, trichloroacetic acid
Number of components	Simple (one chemical) vs Compound (mixture)	Simple: formalin, ethanol, glutaraldehyde, osmium tetroxide, picric acid. Compound: Bouin's, Zenker's, Helly's, B5, Carnoy's
Action on protein	Coagulant (denaturing) vs Non-coagulant (cross-linking)	Coagulant: alcohol, acetone, picric acid. Non-coagulant: formaldehyde, glutaraldehyde, osmium tetroxide



Mechanism of fixation

- **Dehydration and coagulation** (ethanol, methanol, acetone) — these remove free water from the tissue, destabilising hydrophobic and hydrogen bonding and disrupting the tertiary (but not secondary) structure of protein, producing an insoluble precipitate. Ethanol begins coagulating protein from about 50–60% concentration, methanol only above ~80%; disadvantages are cell shrinkage and loss of soluble substances.
- **Cross-linking / covalent addition** (formaldehyde, glutaraldehyde) — formaldehyde in water forms methylene glycol ($\text{CH}_2\text{O} + \text{H}_2\text{O} \rightleftharpoons \text{HOCH}_2\text{OH}$), which reacts with reactive protein side chains (e.g. lysine) to form a hydroxymethyl group; these then form intermolecular and intramolecular **methylene bridges** (cross-links), converting soluble protein to an insoluble product. This reaction is initially slow and washable, but once the methylene bridge forms it is stable and formalin is hard to remove. Formaldehyde also cross-links nucleic acids via nucleotide amino groups. Glutaraldehyde's aldehyde group reacts with protein amino groups to give rapid, irreversible cross-linking — hence its superior ultrastructure preservation for electron microscopy.
- **Oxidation** (osmium tetroxide) — reacts with the unsaturated ($\text{C}=\text{C}$) bonds of lipid molecules, cross-linking them; it fixes lipid and preserves organelles (Golgi apparatus, mitochondria) but has poor tissue penetration and does not fix protein or carbohydrate.
- **Salt formation / acid coagulation** (picric acid, trichloroacetic acid, mercuric chloride) — act through pH change and ionic interaction with charged protein side chains ($-\text{NH}_2 \rightarrow -\text{NH}_3^+$; $-\text{COO}^- \rightarrow -\text{COOH}$).

Factors affecting fixation

Factor	Effect
pH	Neutral pH (6–8) gives best morphology; too acidic/alkaline interferes with fixation
Temperature	Room temperature suitable for routine work (0–45 °C); higher temperature (60–65 °C) speeds fixation but may destroy antigens; 0–4 °C best for enzyme histochemistry
Duration	Depth of penetration (D) is proportional to the square root of time ($D = k\sqrt{t}$); formalin penetrates ~1 mm/hour — small tissue needs ~6 h, large tissue ~24 h
Osmolarity	Hypertonic fixative → cell shrinkage; hypotonic → cell swelling; mild hypertonic (400–450 mOsm) is ideal

Factor	Effect
Concentration	Very low concentration prolongs fixation; higher concentration speeds it but risks hardening/shrinkage/artefact; 10% is the optimal formaldehyde concentration
Agitation	Gentle agitation increases penetration rate; vigorous agitation damages delicate tissue
Volume & thickness	Fixative volume should be ≥ 20 times tissue volume; tissue should be sliced 3–5 mm thick and be blood-free before immersion

Commonly used fixatives

Fixative	Composition	Key properties	Use
10% neutral buffered formalin (NBF)	40% formaldehyde 100 ml + distilled water 900 ml + NaH_2PO_4 4 g + Na_2HPO_4 6.5 g	Penetrates ~1 mm/h; morphology well preserved; cheap, stable; but fails to preserve acid mucopolysaccharides, causes formalin pigment in vascular tissue, and formaldehyde is an irritant/carcinogen	Fixative of choice for routine histopathology and immunohistochemistry
10% formal saline	40% formaldehyde 100 ml + NaCl 9 g + water 900 ml	Isotonic, good for delicate/neural tissue	Brain/CNS specimens (fixation ~1 week)
Glutaraldehyde (2.5% in phosphate buffer, pH 7.0)	—	Extensive cross-linking → excellent ultrastructure preservation, less shrinkage; but poor penetration (tissue < 0.5 mm), unstable, no lipid fixation, costly	Electron microscopy
Osmium tetroxide (2–4% in buffer, pH 7.2)	—	Excellent lipid fixative, preserves Golgi/mitochondria; but fails to fix protein/carbohydrate, causes DNA clumping, toxic and expensive	Electron microscopy (often combined with glutaraldehyde)
95% / absolute ethanol	950 ml absolute alcohol + 50 ml water	Rapid dehydrating-coagulant fixative; preserves glycogen; flammable, causes shrinkage	Cytology smears (Pap smear)

Fixative	Composition	Key properties	Use
Bouin's fluid	Picric acid (1% aq.) 15 ml + formaldehyde (40%) 5 ml + glacial acetic acid 1 ml	Rapid penetration (small biopsy fixed in 3–4 h); excellent for glycogen, connective tissue and trichrome stains; produces a yellow stain (removed with 70% ethanol or lithium carbonate)	Testis, bone marrow biopsies
Zenker's fluid	Mercuric chloride 50 g + glacial acetic acid 50 g + potassium dichromate 25 g + distilled water 950 ml	Rapid, even penetration; excellent for nuclear chromatin and collagen; mercury is poisonous, produces pigment, poorly preserves RBCs	Highly vascular organs (e.g. spleen)
Helly's fluid / B5 fixative	Mercuric chloride + potassium dichromate (or sodium acetate) + formaldehyde, mixed as two stock solutions just before use	Excellent cytoplasmic and nuclear/lymphoid detail; mercury-based, needs de-mercuration	Helly's — bone marrow, pituitary (~12 h); B5 — lymph node (~18 h)
Carnoy's fluid	95% ethanol 60 ml + chloroform 30 ml + glacial acetic acid 10 ml (6:3:1)	Rapid fixation; acetic acid lyses RBCs (useful for haemorrhagic/bloody smears); preserves nucleic acids well; but causes cell shrinkage and nuclear overstaining	Bloody cytology smears, nucleic-acid studies

Fixative of choice by target substance (a favourite viva table): protein — 10% buffered formalin; lipid — frozen section or osmium tetroxide; glycogen — alcohol-based fixative; mucopolysaccharide and enzymes — frozen section; DNA/RNA — alcohol-based fixative.

Fixation artefacts

- **Formalin pigment** — brownish-black, granular, birefringent, extracellular pigment from formic acid reacting with haemoglobin derivatives; prevented by buffered formalin; removed with 1.8% picric acid in absolute alcohol.
- **Mercury pigment** — dark brown deposits after mercury-containing fixatives (Zenker's/Helly's/B5); removed by iodine (→ mercuric iodide) then sodium thiosulphate ("de-mercuration").
- **Fuzzy staining** — obscured nuclear/cytoplasmic detail from incomplete fixation.
- **Tissue separation/empty spaces** — from prolonged fixation causing shrinkage.
- **Dichromate deposit** — yellow-brown precipitate if not washed after dichromate fixatives; removed with 1% HCl in 70% alcohol.

Applied / Clinical anatomy

- ▶ **Cadaver embalming** for the dissection hall uses far stronger formalin-based fluids than diagnostic histology — arterial/cavity fluids combine formalin, phenol, glycerin and methylated spirit (e.g. a cavity fluid of formalin 60% + methanol 25% + phenol 10%) for deep, long-term preservation and disinfection of the donated body, unlike the dilute 10% NBF used for a biopsy.
- ▶ **Museum "wet" specimens** are fixed and stored long-term in formalin or glycerin-formalin mixtures; the Kaiserling technique restores near-natural colour to formalin-fixed museum specimens for teaching — directly relevant to the Paper III theme of organ donation and body preservation.
- ▶ **Occupational hazard** — formaldehyde vapour is a recognised carcinogen and irritant of eyes/respiratory tract and causes contact dermatitis; anatomy/pathology labs need ventilation, fume hoods and PPE.
- ▶ **Immunohistochemistry/molecular pathology** — prolonged fixation cross-links epitopes, needing heat-induced epitope retrieval, and degrades DNA/RNA yield, so tissue-banking protocols specify strict fixation times.
- ▶ **Electron microscopy** specimens must be cut very small (< 1 mm) and fixed immediately in glutaraldehyde (often followed by osmium tetroxide, "double fixation") because of poor penetration.
- ▶ **Frozen section (cryostat)** bypasses chemical fixation for rapid intra-operative diagnosis and for enzymes/lipids/antigens destroyed by conventional fixatives.

High-yield exam pointers

- ▶ Fixation is the first and most critical step in tissue processing — it arrests autolysis and putrefaction and stabilises tissue near its living state.
- ▶ No universally "ideal" fixative exists; the choice is always a compromise between rapid penetration, morphology preservation, and compatibility with the intended stain/technique.
- ▶ **10% neutral buffered formalin** is the routine fixative of choice — penetration rate ~1 mm/hour, optimum concentration 10%, pH 6–8, volume 20× tissue volume, tissue sliced 3–5 mm thick.
- ▶ Mechanistically: aldehydes (formaldehyde, glutaraldehyde) are **cross-linking/non-coagulant** fixatives; alcohols/acetone are **dehydrating-coagulant** fixatives; osmium tetroxide is an **oxidising** fixative acting on lipid double bonds.
- ▶ Glutaraldehyde and osmium tetroxide are the standard **electron microscopy** fixatives — excellent ultrastructure preservation but very poor tissue penetration.
- ▶ Mercury-based compound fixatives (Zenker's, Helly's, B5) give superior nuclear/cytoplasmic detail but require de-mercuration (iodine + sodium thiosulphate) before use.
- ▶ Bouin's fluid is the fixative of choice for glycogen, connective tissue and trichrome staining, but produces a yellow (picric acid) staining artefact.
- ▶ $D = k\sqrt{T}$ — the depth of fixative penetration is proportional to the square root of time; a classic viva/numerical point.
- ▶ Formalin pigment is a birefringent, extracellular, brownish-black artefact, prevented by buffering and removed with picric acid.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e; Bancroft's *Theory & Practice of Histological Techniques*; Halder — *Notes on Embalming & Museum Techniques*.

Coverage note: "Fixatives" is a histotechnique topic not covered in Gray's Anatomy (the gross-anatomy spine text); the core content above is grounded in the microscopy/histotechnique manuals as directed by the topic spec, with applied cadaver-embalming/museum-preservation correlations drawn from the preservation specialty text.

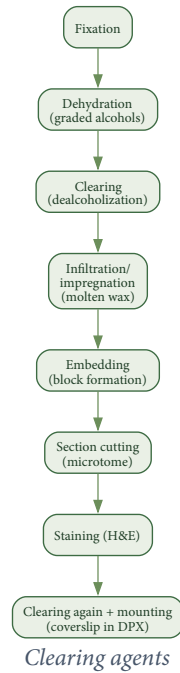
**Clearing agents**

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Clearing is the third step of routine paraffin tissue processing, performed after fixation and dehydration and before wax infiltration. A clearing agent (also called a "dealcoholizing agent") is a reagent that is miscible with both the anhydrous dehydrating agent (usually absolute alcohol) and the molten embedding medium (paraffin wax), and whose refractive index closely matches that of tissue protein. By replacing the alcohol, the clearing agent renders the anhydrous tissue translucent/transparent — hence the name "clearing" — and prepares it to accept wax. The same principle is exploited a second time in the H&E staining protocol, where a clearing step (xylene) follows post-stain dehydration and immediately precedes mounting of the coverslip in a resinous mountant such as DPX.

Position in tissue processing and underlying principle

- ▶ Sequence: Fixation → Dehydration (graded alcohols) → Clearing (dealcoholization) → Infiltration/Impregnation (molten paraffin) → Embedding → Section cutting → Staining → Mounting.
- ▶ Clearing removes the dehydrating agent, which is itself immiscible with paraffin wax and must be eliminated before wax can penetrate the tissue.
- ▶ A good clearant also dissolves residual lipid, which would otherwise impede wax penetration.
- ▶ **Refractive-index matching:** the clearing agent's refractive index approximates that of tissue protein, so the dehydrated (anhydrous) tissue becomes optically transparent — complete transparency is the visual endpoint of adequate clearing, while residual opacity signals incomplete dehydration.



Aims of clearing

- ▶ Remove the dehydrating agent (alcohol) so that it does not block infiltration by paraffin wax.
- ▶ Render the tissue transparent/translucent, permitting easy microscopic assessment and gross recognition of adequate processing.
- ▶ Prepare the tissue for smooth infiltration and, later (in stained sections), for mounting under a resinous medium.

Ideal properties of a clearing agent

Property	Requirement
Viscosity/penetration	Low viscosity, high penetration rate
Melting point	Low, so it is easily displaced by molten wax
Miscibility	Miscible with both the dehydrating alcohol and the molten wax
Tissue effect	Causes no shrinkage, hardening, or other damage
Safety	Less toxic, less inflammable
Cost	Cheap and readily available
Optical property	Refractive index close to tissue, giving a clear transparent appearance at the endpoint

Classification and properties of individual clearing agents

Clearing agents fall into hydrocarbons, halogenated solvents, esters, oils, and terpene-based "xylene substitutes."



- ▶ **Xylene (dimethylbenzene):** the most commonly used clearing agent; a clear, colourless, inflammable liquid. Clears small tissue pieces in 30–60 minutes. It is "practically insoluble" in water and hence draws out residual bound water, but prolonged exposure makes tissue hard and brittle. It also gives cytoplasm a transparent look during slide mounting, where its refractive index must match that of the mounting medium and coverslip.
- ▶ **Toluene:** properties almost identical to xylene but does **not** harden tissue even on prolonged exposure; its clearing action is slightly slower than xylene. Both flammable and toxic.
- ▶ **Chloroform:** highly volatile, non-inflammable, expensive, and toxic; penetrates more slowly than xylene but causes **no tissue shrinkage**, making it preferable for dense tissues such as uterus and muscle. It liberates dangerous toxic gas on heating and is now rarely used; it is also carcinogenic and can dissolve the plastic components of automated processors.
- ▶ **Esters** (e.g., amyl nitrate, methyl salicylate, methyl benzoate): less toxic, "safe" per comparative grading, and cause no tissue hardening even with prolonged exposure; suitable for manual processing.
- ▶ **Cedarwood oil:** an expensive but rapid clearing agent, mainly reserved for dense tissue.
- ▶ **Limonene:** a clear liquid that does not harden tissue, but the wax finds it difficult to displace from the tissue during infiltration.
- ▶ **Carbon tetrachloride:** a historical clearing agent, now obsolete owing to its hazardous toxicity.
- ▶ **Xylene substitutes:** introduced to reduce occupational hazard — citrus-based clearants (made from d-limonene) have a characteristic citrus odour and oily consistency but are skin sensitizers/irritants; aliphatic (straight-chain) hydrocarbon blends are largely odourless, non-sensitizing, and non-irritating, with oiliness/flammability depending on chain length.

Table — comparison of the principal clearing agents

Property	Xylene	Toluene	Chloroform	Esters
Tissue shrinkage	Yes	Yes	Minimal	No
Tissue hardening	Yes	No	No	No
Inflammable	Yes	Yes	No	Yes
Harmful effect	Irritant, less harmful	Irritant	Dangerous toxic gas on heating	Safe
Cost	Cheap	Cheap	Very expensive	High cost

Selection criteria, technique parameters, and endpoint

- ▶ **Selection of the appropriate agent** depends on: type/size of tissue, type of processor (manual vs automated), processing conditions (heat, vacuum), speed of removal of the dehydrant, ease of subsequent wax replacement, safety (flammability, toxicity), and cost.
- ▶ **Volume:** approximately 40 times the volume of the tissue specimen.

- ▶ **Duration:** a small biopsy clears in about 1 hour; larger tissue requires three changes of xylene or toluene, 60 minutes each.
- ▶ **Endpoint:** the tissue becomes fully transparent — this is the visual signal that clearing (and antecedent dehydration) is complete; residual opacity means dehydration was incomplete.
- ▶ **Ease of wax displacement** later during infiltration also depends on the clearant chosen — xylene and toluene are removed by molten wax more easily than cedarwood oil.
- ▶ Clearing recurs in the staining protocol itself: after H&E staining and re-dehydration, sections are cleared in xylene (e.g., two changes, 5 minutes each) before mounting, because the resinous mountant (DPX, Canada balsam/Euparal) must be miscible with the clearing agent used.

Precautions, artifacts, and safety

- ▶ **Under-clearing:** residual opacity, poor wax infiltration, and poor subsequent sectioning.
- ▶ **Over-clearing/prolonged exposure:** tissue becomes hard, brittle, and friable, complicating microtomy — hence a controlled, monitored clearing time is essential.
- ▶ **Faulty processing artifacts:** inadequate/incorrect processing (including clearing) can produce holes, tissue distortion, or excessive shrinkage that mimic pathological change on the slide, so correct timing and agent selection matter for diagnostic and teaching quality alike.
- ▶ **Occupational hazard:** xylene and toluene are irritant and neurotoxic on chronic exposure; chloroform and carbon tetrachloride are carcinogenic/hepatotoxic, prompting the shift toward safer xylene substitutes (citrus-based or aliphatic-hydrocarbon clearants) in modern laboratories, some processors even running "xylene-free" schedules using agents such as isopropanol as the clearing step.

Applied / Clinical anatomy

- ▶ Every routine H&E-stained slide used for gross and microscopic anatomy teaching, and every diagnostic histopathology section, passes through this clearing step; its quality directly determines whether wax infiltrates cleanly, whether sections cut without tearing, and whether the final teaching/diagnostic slide is artifact-free.
- ▶ Faulty clearing (wrong agent, wrong duration) is a recognised cause of processing artifacts (holes, brittleness, shrinkage) that can be mistaken for genuine tissue pathology by a trainee at the microscope — a common viva pitfall.
- ▶ Selection of clearant matters clinically for dense/fibrous specimens (uterus, muscle) where shrinkage must be minimised (chloroform historically preferred) versus routine soft tissue where xylene/toluene suffice.
- ▶ The same clearing principle underlies the final "clear-and-mount" step of cytology smears (e.g., Pap smears) before applying a synthetic resin mountant, linking histotechnique to cytopathology practice.
- ▶ Laboratory safety planning (ventilation, PPE, choice of xylene substitute) in the anatomy/pathology museum and histology laboratory is directly informed by the toxicity profile of the chosen clearing agent.

High-yield exam pointers

- ▶ Clearing is the step **between dehydration and infiltration**; principle = miscibility (with alcohol and wax) + refractive-index matching → tissue becomes transparent.
- ▶ Endpoint of clearing = tissue turns **fully transparent**; opacity = incomplete dehydration.
- ▶ **Xylene** — commonest, hardens tissue on prolonged exposure, cheap, flammable, irritant.
- ▶ **Toluene** — like xylene but does not harden tissue.
- ▶ **Chloroform** — no shrinkage, best for dense tissue (uterus/muscle), but toxic, expensive, carcinogenic — now rarely used.
- ▶ **Esters** (amyl nitrate, methyl salicylate, methyl benzoate) — safe, no hardening.
- ▶ **Cedarwood oil** — expensive, rapid, for dense tissue; **Limonene** — no hardening but hard to remove by wax.
- ▶ Volume of clearant ≈ **40× tissue volume**; small biopsy clears in ~1 hour.
- ▶ Xylene substitutes (d-limonene citrus clearants, aliphatic hydrocarbons) reduce occupational toxicity versus xylene/chloroform.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (Processing of Tissue in the Histopathology Laboratory; Haematoxylin and Eosin Stain of the Tissue Section chapters); Bancroft's *Theory & Practice of Histological Techniques* (Tissue Processing chapter). **Coverage note:** this is a laboratory/histotechnique topic (Paper III — Principles of Microscopy) and is not addressed in Gray's Anatomy (Standring), which does not cover routine tissue-processing methodology; grounding is drawn entirely from the specialty histotechnique texts above.



Connective tissue and a stain for demonstration of each of its components

Asked in: Paper III Apr 2008 — RGUHS MD/MS Anatomy

Definition / Introduction Connective tissue is a mesenchyme-derived tissue that binds, supports and packs the other basic tissues of the body. Unlike epithelium, it is characterised by widely-spaced cells embedded in an abundant **extracellular matrix (ECM)**, which itself has two components — **fibres** (collagen, reticular, elastic) and **amorphous ground substance** (glycosaminoglycans, proteoglycans, glycoproteins). Because most of these components are colourless or poorly differentiated by routine haematoxylin and eosin (H&E), each requires a specific **special/histochemical stain** for its selective demonstration — the basis of this question.

1. Components of connective tissue — overview

Category	Components
Cells — fixed	Fibroblasts, adipocytes, macrophages (histiocytes), pericytes, mast cells

Category	Components
Cells — transient/wandering	Plasma cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils
Fibres	Collagen fibres, reticular fibres, elastic fibres
Ground substance (matrix)	Glycosaminoglycans (hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate) bound to core proteins as proteoglycans ; structural glycoproteins (fibronectin, laminin)

2. Master key — stain of choice for each component

Component	Recommended special stain	Colour result
Collagen fibres	Van Gieson's stain (picro-fuchsin); Masson's trichrome	Van Gieson: collagen red , nuclei black, muscle/cytoplasm yellow. Masson: collagen blue/green , muscle red
Reticular fibres	Silver impregnation — Gordon & Sweet's method	Reticular fibres black , nuclei red
Elastic fibres	Verhoeff's stain (Verhoeff–Van Gieson); Weigert's resorcin-fuchsin; orcein	Verhoeff: elastic fibres black , collagen red. Weigert: blue-black. Orcein: brown
Ground substance (GAGs/proteoglycans)	Alcian blue (pH 2.5); colloidal iron; PAS (for neutral glycoproteins)	Alcian blue-positive material stains blue
Mast cell granules	Toluidine blue (metachromatic stain); also PAS-positive	Granules purple/red-violet against a blue background (metachromasia)

3. Collagen fibres — Van Gieson's stain

Collagen is the most abundant fibre, secreted by fibroblasts as thick, wavy, eosinophilic bundles that give tensile strength to tendon, ligament, dermis and bone.

- **Principle:** Van Gieson solution combines 1% aqueous acid fuchsin with saturated aqueous picric acid. The small acid-fuchsin molecule and the larger picric-acid molecule compete for tissue binding sites; the loosely-packed collagen fibres retain the smaller acid-fuchsin dye (turning red), while the densely-packed muscle/cytoplasm is penetrated by the larger picric acid-bound dye (turning yellow).
- **Procedure (brief):** deparaffinise → Weigert's iron haematoxylin (nuclear stain resistant to decolourisation by picric acid) → differentiate in acid alcohol → van Gieson solution 2–5 min → rapid dehydration directly into alcohol (aqueous washing removes the red stain) → clear → mount.
- **Result:** collagen fibres red; nuclei black; muscle, cytoplasm and fibrin yellow.

- **Alternative — Masson's trichrome:** sequential dyes of increasing molecular size (acid fuchsin → phosphomolybdic acid → aniline blue) differentiate collagen (blue/green) from muscle (red) and is preferred for quantifying fibrosis on liver and kidney biopsies.

4. Reticular fibres — silver impregnation (Gordon & Sweet's method)

Reticular fibres are fine, branching type-III collagen fibres forming the supporting meshwork of liver, spleen, lymph node and bone marrow.

- **Principle:** reticulin carries an abundant carbohydrate coat. Oxidation (acidified potassium permanganate, then bleached with oxalic acid, sensitised with iron alum) generates aldehyde groups on this coat. Ammoniacal silver nitrate is reduced by these aldehydes to black metallic silver (the fibres are said to be **argyrophilic**); the image is toned with gold chloride and fixed with sodium thiosulfate to remove unreacted silver.
- **Result:** reticular fibres black; nuclei red (neutral-red counterstain).
- **Uses:** demonstrating early cirrhosis and marrow fibrosis, lymph-node architecture, and glomerular basement-membrane changes.

5. Elastic fibres — Verhoeff's stain

Elastic fibres are thin, branching fibres composed of an amorphous elastin core wrapped by fibrillin microfibrils, providing recoil to arteries, lungs and skin.

- **Principle (Verhoeff):** an alcoholic haematoxylin–ferric chloride–iodine complex binds elastin with high affinity; differentiation in 2% ferric chloride selectively removes the stain from collagen and other tissue, leaving elastic fibres and nuclei black, and the section is then counterstained with van Gieson's solution.
- **Result:** elastic fibres and nuclei black; collagen red; other tissue yellow.
- **Alternatives:** **Weigert's resorcin-fuchsin** (basic fuchsin–resorcin–ferric chloride complex; blue-black fibres) and **orcein/Taenzer–Unna stain** (natural dye with affinity for elastin; brown fibres) — both are used interchangeably as demonstration stains, particularly on arterial wall and lung sections.

6. Ground substance and mast cells — Alcian blue and toluidine blue

- **Ground substance (GAGs/proteoglycans):** chondroitin sulfate, dermatan sulfate, heparan sulfate and hyaluronic acid carry ionisable sulfate/carboxylate groups. **Alcian blue** is a large cationic phthalocyanine dye whose isothiuronium groups form electrostatic (ionic) bonds with these anionic groups when the solution is buffered to **pH 2.5**; this stains the proteoglycan/hyaluronic-acid ground substance blue. **Colloidal iron** works similarly via electrostatic adsorption of iron colloid onto acid mucopolysaccharides, and **PAS** demonstrates the neutral glycoprotein component.
- **Mast cells:** their cytoplasmic granules contain heparin (a strongly-sulfated, polyanionic proteoglycan) and histamine. **Toluidine blue**, a basic dye, is used in concentrated solution — the closely-packed dye molecules stack on the polyanionic heparin to form dimers/polymers whose absorption spectrum shifts from blue to purple/red, a phenomenon called **metachromasia**. Mast-

cell granules are thus selectively coloured purple against the blue-stained background tissue. Mast-cell granules are also PAS-positive.

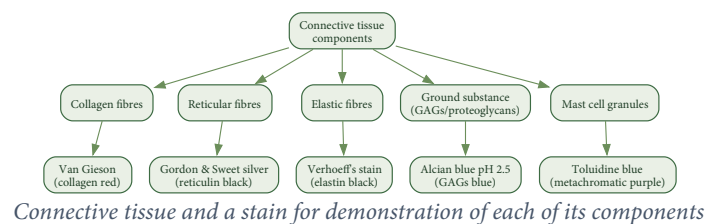
DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

□ Fig 19.7 — Liver showing reticular fibers, high magnification, Gomori's silver impregnation (Sontakke — *Textbook of Human Histology* — 2e, p.263)

□ Fig 5.13 — Elastic fibers (Junqueira's *Basic Histology* — Text & Atlas — 17e, p.284)

Diagram to draw — a labelled low-power sketch of loose areolar connective tissue (e.g. mesentery spread or subcutaneous tissue), showing:

- ▶ Wavy, eosinophilic **collagen fibre bundles**
- ▶ Fine, branching **reticular fibres** around a capillary
- ▶ Thin, refractile **elastic fibres**
- ▶ Homogeneous **ground substance** filling the background
- ▶ Spindle-shaped **fibroblast** with elongated nucleus
- ▶ **Macrophage/histiocyte** with irregular outline and granular cytoplasm
- ▶ **Mast cell** with dense basophilic cytoplasmic granules
- ▶ **Plasma cell** with eccentric "cart-wheel" nucleus
- ▶ A small blood **capillary**



Applied / Clinical anatomy

- ▶ **Liver biopsy:** reticulin stain demonstrates early cirrhosis (disruption of the normal reticulin framework) before it is evident on H&E; Masson's trichrome quantifies established fibrosis.
- ▶ **Renal biopsy:** PAS and silver stains outline the glomerular basement membrane and mesangium — thickened, PAS-positive nodules constitute the Kimmelstiel–Wilson lesion of diabetic nephropathy.
- ▶ **Bone marrow:** reticulin (Gordon & Sweet) staining grades marrow fibrosis in myelofibrosis.
- ▶ **Aneurysm/dissection and Marfan syndrome:** loss/fragmentation of elastic laminae, best shown by Verhoeff's or orcein stain, underlies aortic wall weakness.
- ▶ **Soft-tissue tumours:** reticulin pattern helps distinguish tumour types (e.g. "chicken-wire" pattern in myxoid liposarcoma, "Zellballen" pattern in paraganglioma highlighted by surrounding reticulin).
- ▶ **Mast cell/allergic disease:** toluidine blue metachromasia and tryptase immunostaining identify mast-cell hyperplasia in mastocytosis and allergic mucosal biopsies.

- ▶ **Keloid and scar tissue:** excess, densely-packed collagen is well demonstrated with van Gieson or Masson's trichrome.

High-yield exam pointers

- ▶ Two structural components of ECM: **fibres** (collagen, reticular, elastic) and **ground substance**; plus resident/wandering **cells**.
- ▶ **Van Gieson** = collagen (red); mnemonic: picric acid (yellow) + acid fuchsin (red) → collagen red, rest yellow.
- ▶ **Reticular fibres** = **collagen type III**, argyrophilic (silver-loving, not argentaffin), demonstrated by Gordon & Sweet's/Wilder's silver impregnation.
- ▶ **Verhoeff's stain** / **Weigert's resorcin-fuchsin** / **orcein** — all three demonstrate elastic fibres.
- ▶ **Alcian blue at pH 2.5** stains acidic ground substance (GAGs); pH 1.0 restricts staining to sulfated GAGs only (a useful viva point).
- ▶ **Metachromasia** = a basic dye (toluidine blue) changing colour (blue→purple) on binding polyanionic heparin in mast-cell granules.
- ▶ Masson's trichrome differentiates collagen from smooth muscle better than van Gieson — useful in fibrosarcoma vs leiomyosarcoma.
- ▶ PAS additionally stains basement membrane, neutral mucins and mast-cell granules.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; Sontakke — Textbook of Human Histology 2e; Pranab Dey — Basic and Advanced Laboratory Techniques in Histopathology and Cytology 2e (Chapter 10, "Connective Tissue Stain"); Bancroft's Theory and Practice of Histological Techniques (Chapters 12–13, "Connective and other mesenchymal tissues with their stains" and "Carbohydrates").



Describe in detail the procedure you will follow for procuring a tissue from a cadaver upto the preparation of a histological slide

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction

Tissue processing is the sequence of steps by which a small block of tissue, procured from a cadaver, autopsy, surgical specimen or animal source, is converted into a thin, stained section mounted on a glass slide fit for microscopic study. The pathway comprises **procurement/grossing** → **fixation** → **dehydration** → **clearing** → **infiltration** → **embedding** → **section cutting (microtomy)** → **staining** → **mounting**, each step designed to preserve the tissue's life-like architecture while rendering it thin enough (3–10 µm) to transmit light and stainable enough to reveal cellular detail.

1. Selection and procurement of tissue from the cadaver

- ▶ **Source and consent:** tissue is procured from a cadaver made available for teaching/research under the Anatomy Act (willed-body donation or unclaimed body handed over with due legal formalities); use requires institutional/ethics committee permission, as for any autopsy, surgical or animal-derived specimen.
- ▶ **Timing:** procurement should be done **as soon as possible after death**, before significant post-mortem autolysis and bacterial putrefaction set in, since delay degrades cellular and nuclear detail.
- ▶ **Site selection:** a representative area of the organ/structure of interest is chosen, avoiding obviously crushed, cauterised or artefactually damaged tissue.
- ▶ **Grossing:** the selected region is inspected for gross pathological change and cut into thin slices of about **5 mm thickness** — thin enough to allow rapid, uniform fixative penetration.
- ▶ **Labelling and orientation:** the block is labelled (case/cadaver identifier, site, date) and, where orientation matters (e.g. a tubular structure), a suture or ink mark is used to preserve orientation through processing.
- ▶ **Note:** cadavers in the dissection hall are usually already embalmed with a formalin-containing preservative; tissue taken from such a cadaver is thus already partly fixed, whereas tissue from a fresh (unembalmed) autopsy or surgical specimen must be fixed afresh immediately after procurement.

2. Fixation

- ▶ **Purpose:** to preserve the tissue's biological structure by halting autolysis (enzymatic self-digestion) and bacterial putrefaction, and to harden the tissue for subsequent handling.
- ▶ **Properties of an ideal fixative:** acts rapidly, produces immediate death of cells, preserves life-like appearance, prevents autolysis/putrefaction, neither shrinks nor swells the tissue, is cheap, non-toxic, and permits a wide range of subsequent staining.
- ▶ **Mechanism:** formalin (and related aldehydes) cross-link tissue proteins (enzymes and structural proteins), rendering them insoluble and inactivating degradative enzymes.
- ▶ **Commonly used fixatives:**

Fixative	Typical use
10% formalin (formalin is a 37–40% formaldehyde stock, diluted to ~10% i.e. ~4% formaldehyde, neutral buffered)	Routine light microscopy — the most widely used fixative
Glutaraldehyde (± osmium tetroxide)	Electron microscopy — preserves fine ultrastructure
Absolute alcohol, acetone	Enzyme histochemistry (avoids denaturing certain enzymes)
Zenker's fluid (mercuric chloride-based)	Special staining of connective tissue/blood cells

- ▶ Fixative volume must considerably exceed tissue volume and be given adequate time to fully diffuse through the block before processing continues.

- **Decalcification:** if the procured tissue contains bone or calcified cartilage, it cannot be sectioned on a routine microtome until the deposited calcium salts are removed using strong mineral acids (nitric/hydrochloric), weak organic acids (formic acid) or a chelating agent (EDTA).

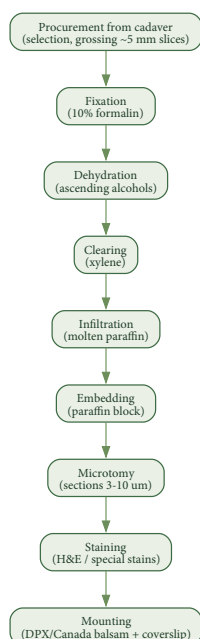
3. Tissue processing (dehydration → clearing → infiltration → embedding)

Step	Agent used	Purpose
Dehydration	Ascending grades of alcohol (50% → 70% → 90% → absolute/100%)	Gradual, graded removal of water, which is not miscible with paraffin wax
Clearing (dealcoholization)	Xylene (or cedar-wood oil/chloroform)	Removes alcohol; renders tissue translucent by altering its refractive index; xylene is miscible with both alcohol and paraffin
Infiltration/impregnation	Melted paraffin wax (oven, ~52–60°C)	Replaces the clearing agent within the tissue, providing internal support
Embedding (block-making)	Melted paraffin poured into a mould	Tissue is oriented and allowed to solidify into a firm block on cooling, giving external support for cutting

- For electron microscopy, epoxy resin (a harder plastic medium) is substituted for paraffin to permit ultrathin sectioning.

4. Microtomy (section cutting)

- The paraffin block is trimmed and mounted on a **microtome** (commonly a rotary microtome), which advances the block a controlled distance with each turn of the wheel before it passes over a steel knife edge.
- Sections are cut at a thickness of **3–10 µm for routine light microscopy** (5–7 µm is the thickness generally used for H&E preparations); ultrathin sections for electron microscopy (<1 µm) require a diamond/glass-knife **ultramicrotome**.
- Cut ribbons/sections are floated on a warm water bath (~45°C) to flatten out wax-induced wrinkles, then picked up onto a clean, adhesive-coated glass slide and dried.



Describe in detail the procedure you will follow for procuring a tissue from a cadaver upto the preparation of a histological slide

5. Staining

- ▶ Deparaffinized sections are rehydrated (descending alcohols to water) before staining, since most tissue components are colourless and require differential dyes for visualisation.
- ▶ **Haematoxylin and eosin (H&E)** is the routine stain of choice.
 - *Principle:* chemical attraction between an ionic dye and charged tissue components.
Haematoxylin is a basic dye (from *Haematoxylon campechianum* wood) that stains **basophilic** structures (nuclei, ribosomes, cartilage matrix) blue-purple; **eosin** is an acidic dye that stains **acidophilic/eosinophilic** structures (cytoplasm, collagen, muscle, red blood cells) shades of pink/red.
 - *Procedure sequence:* deparaffinize in xylene → rehydrate through descending alcohols to water → stain in haematoxylin → differentiate excess stain in acid-alcohol and blue in running water → counterstain in eosin → dehydrate through ascending alcohols → clear in xylene.
- ▶ **Special stains** are used when a specific tissue component must be selectively demonstrated:

Structure	Special stain
Collagen fibres	Van Gieson; Masson's trichrome
Elastic fibres	Verhoeff–Van Gieson; Weigert's resorcin-fuchsin; orcein
Reticular fibres	Gordon–Sweet's / Gomori's silver impregnation
Basement membrane, carbohydrates (glycogen)	Periodic acid–Schiff (PAS)
Lipids/fat	Oil red-O; Sudan black B
Calcium	Von Kossa method
Melanin	Masson–Fontana
Nissl substance (neurons)	Cresyl fast violet

Structure	Special stain
Myelin	Luxol fast blue

6. Mounting and labelling

- ▶ After staining and final dehydration/clearing, a drop of mounting medium — DPX (distrene-plasticiser-xylene) or **Canada balsam** — is placed over the section and a coverslip applied, excluding air bubbles.
- ▶ The mounting medium hardens, fixing the coverslip permanently and rendering the slide suitable for long-term storage and microscopic examination.
- ▶ The finished slide is labelled with the case/tissue identity for archival and teaching use.

Applied / Clinical anatomy

- ▶ **Frozen section technique:** for rapid intra-operative diagnosis, fresh unfixed tissue is snap-frozen and cut on a **cryostat** (a refrigerated cabinet housing a microtome); H&E staining can be completed within minutes, avoiding the many hours needed for paraffin processing, though morphological detail is inferior to a paraffin section.
- ▶ **Electron microscopy** requires smaller tissue fragments, glutaraldehyde ± osmium tetroxide fixation (to preserve membranes/lipids), resin embedding and ultramicrotomy with diamond/glass knives — used to study ultrastructure beyond the resolution of light microscopy.
- ▶ **Decalcification** is mandatory before microtomy for any cadaveric bone, joint or heavily calcified specimen; omitting it damages the microtome knife and tears the section.
- ▶ **Processing artefacts:** delayed fixation causes autolytic change; inadequate fixative volume/time, over-hard or over-soft wax infiltration, and blunt knives produce holes, wrinkles, shrinkage or uneven-thickness sections — all reduce diagnostic/teaching value of the slide.
- ▶ **Biosafety:** cadaveric/autopsy tissue must be handled with standard precautions (gloves, appropriate disposal of fixative-soaked waste) given the possibility of transmissible infection.

High-yield exam pointers

- ▶ Sequence to remember: **procurement (grossing) → fixation → dehydration → clearing → infiltration → embedding → microtomy → staining → mounting.**
- ▶ 10% formalin is the commonest fixative; it acts by cross-linking proteins.
- ▶ Dehydration uses ascending alcohols (50→100%); clearing uses xylene (miscible with both alcohol and paraffin).
- ▶ Paraffin infiltration/embedding is done at ~52–60°C; routine section thickness is 3–10 µm (usually 5–7 µm).
- ▶ H&E: haematoxylin (basic dye) stains basophilic structures blue-purple; eosin (acidic dye) stains acidophilic structures pink.
- ▶ DPX/Canada balsam is used for permanent mounting.
- ▶ Decalcification (EDTA/acids) is needed before sectioning calcified/bony tissue.
- ▶ Frozen section (cryostat) is the rapid alternative to paraffin processing for intra-operative diagnosis.

- Electron microscopy substitutes glutaraldehyde-osmium fixation and resin embedding for ultrathin sectioning.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology (2e); Brijesh Kumar — Histology Text & Atlas (3e); Junqueira's Basic Histology — Text & Atlas (17e); Ross Histology — A Text and Atlas (7e); IB Singh — Textbook of Human Histology (9e).



Electron microscopy

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction Electron microscopy (EM) is a technique that uses a focused high-energy **electron beam**, instead of visible light, as the imaging probe. Because an accelerated electron has a wavelength several orders of magnitude shorter than that of visible light, the electron microscope achieves a resolving power roughly **1000-fold greater** than the light microscope, permitting visualisation of subcellular ultrastructure that is otherwise below the limit of resolution of light microscopy. The two principal forms used in biomedical work are the **transmission electron microscope (TEM)**, which images a thin section through which the beam passes, and the **scanning electron microscope (SEM)**, which images the surface topography of a specimen. Both use electromagnetic (not glass) lenses and require a high-vacuum optical column.

Principle and resolution

- **Physical basis:** an accelerated electron behaves as a wave whose wavelength (λ) is given by the de Broglie relation, $\lambda = h / (mv)$, where h = Planck's constant, m = electron mass, v = electron velocity. Increasing the accelerating voltage increases electron speed and shortens λ , which improves resolving power.
- Electrons are chosen as the imaging probe because they have a very short wavelength, are easily manipulated by electromagnetic fields, and interact strongly with matter, giving high contrast and brightness.
- **Resolution comparison** (distance between two just-resolvable points):

Instrument	Resolution	Approx. maximum useful magnification
Human eye	~0.2 mm	—
Bright-field light microscope	~0.2 μm (0.5 μm practical)	~1000 $\times$
Scanning electron microscope (SEM)	~2.5 nm	high (surface only)
Transmission electron microscope (TEM)	~0.05–0.1 nm theoretical ($\approx$ 1–2 nm on routine biological sections)	up to ~500,000 $\times$
Atomic force microscope	~50 pm	—

- ▶ In practice, biological tissue prepared by standard chemical fixation and staining cannot be examined at the theoretical limit; resolution is constrained by specimen preparation artefact, not by the instrument alone. Cryo-techniques narrow this gap and allow examination closer to the molecular level.

Instrumentation — the TEM column

The TEM column consists of, in sequence: electron source → condenser lens system → specimen stage → objective lens → intermediate/projector lenses → detector, all housed in a vacuum.

- ▶ **Electron gun:** a V-shaped **tungsten filament** (heated to ~2700 K, generating electrons by thermionic emission) surrounded by a negatively charged **Wehnelt cylinder (cathode shield)** that focuses the electrons into a narrow beam emerging toward a positively charged **anode plate**. Lanthanum hexaboride (LaB₆) crystals or a field-emission gun (FEG) are used as brighter, longer-lived alternative electron sources.
- ▶ **Condenser lenses:** two or more electromagnetic coils that focus the beam onto the specimen; changing coil current alters focal length (unlike glass lenses, which are fixed).
- ▶ **Specimen stage:** holds the grid bearing the ultrathin section below the condenser system.
- ▶ **Objective lens:** the strongest electromagnetic lens; produces the first magnified (intermediate) image.
- ▶ **Intermediate and projector lenses:** further magnify the image and project it onto the viewing screen/detector.
- ▶ **Detector:** a fluorescent screen (visualised directly or via a charge-coupled device camera) converts the electron image to a visible/digital image.
- ▶ **Vacuum system:** essential because residual gas molecules would scatter the electron beam and oxidise the filament. A combination of rotary, diffusion/turbomolecular, and ion pumps progressively reduces column pressure (coarse vacuum near the gun down to very high vacuum near the source).

□ Fig 1.3 — Electron microscope. (a) Scanning electron microscope and (b) transmission electron microscope (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.32)

□ Fig 1.3 (contd.) — Electron microscope schematic showing electron gun, condenser/objective/projector lenses and detector/monitor (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.32)

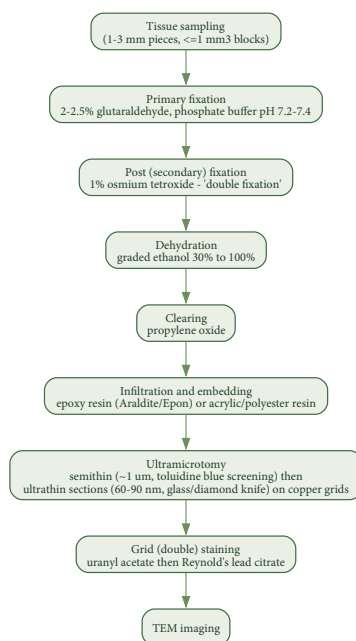
DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw the electron-microscope column (TEM on one side, SEM alongside for comparison) and label:

- ▶ Electron gun — tungsten filament (cathode), Wehnelt cylinder, anode plate
- ▶ Condenser lens(es)
- ▶ Specimen — held on a grid (TEM) / mounted stub (SEM)
- ▶ Objective lens
- ▶ Intermediate lens
- ▶ Projector lens
- ▶ Fluorescent screen / CCD camera / monitor (TEM output)
- ▶ Scanning coils, secondary-electron detector, amplifier, CRT/monitor (SEM output)
- ▶ Vacuum pumps (rotary, diffusion/turbomolecular, ion pump) maintaining the vacuum column

Specimen preparation for TEM

Good ultrastructural preservation requires the tissue to be very small, fixed within minutes of devascularisation, and processed through the following sequence:



Electron microscopy

- ▶ **Fixation:** the aim is to preserve ultrastructure as close to the living state as possible and to prevent tissue disintegration during processing. **Glutaraldehyde** cross-links and stabilises proteins but fixes lipids poorly and causes some shrinkage; **osmium tetroxide** fixes lipids well and counteracts this by causing slight swelling — hence the two are used in combination ("double fixation"). Fixative volume should be several-fold the tissue volume; prolonged fixation (>12 h) is avoided as it leaches protein and hampers sectioning, while under-fixation causes mitochondrial swelling and organelle disruption.

- **Embedding media** must cut cleanly, tolerate the heat and vacuum of the microscope, and polymerise evenly: **epoxy resins** (Araldite, Epon) are the most widely used (stable, no shrinkage, but viscous and can cause dermatitis); **acrylic resins** (methacrylates) infiltrate faster but shrink tissue by up to ~20%; **polyester resin** (Vestopal) causes no shrinkage.
- **Sectioning** is done on an **ultramicrotome** with glass or diamond knives; interference colour of the floating section (grey <60 nm, silver 60–90 nm, gold 90–120 nm) indicates thickness, the silver range being optimal.
- **Staining** uses electron-dense heavy metal salts rather than dyes: **uranyl acetate** binds nucleic acids, proteins and lipids (photosensitive, precipitates in light), followed by **lead citrate**, which further enhances membrane contrast.

Scanning electron microscopy (SEM) and TEM vs SEM

- In SEM the electron beam does **not** pass through the specimen; instead a fine probe is scanned point-by-point across the (gold- or gold-palladium-coated) surface, and **secondary and backscattered electrons** emitted from the surface are collected by a detector, amplified, and reconstructed into a point-by-point image on a monitor — giving a striking three-dimensional, shadowed appearance of surface topography.
- Fixation and initial processing for SEM follow the same principles as for TEM; the specimen is subsequently dried (critical-point drying to avoid surface-tension distortion) and sputter-coated with a thin conductive metal layer before imaging.

Feature	TEM	SEM
Incident beam	Static, passes through thin section	Dynamic, scans surface in two directions
Image formation	Formed instantly from transmitted/scattered electrons	Built point-by-point like a scanned image
Interaction imaged	Electrons transmitted through / elastically & inelastically scattered by the specimen	Secondary and backscattered electrons emitted from the surface
Information yielded	Internal ultrastructure (organelles, membranes)	Surface topography, 3-D contour
Specimen requirement	Ultrathin section (60–90 nm) on a grid	Whole/dried, conductive-coated specimen on a stub

Related and newer EM-based techniques

- **Freeze-fracture/freeze-etch**: tissue is rapidly frozen (liquid nitrogen) and fractured with a knife; a platinum/metal replica of the exposed (often intramembranous) surface is made in vacuum and examined by TEM after removal of the underlying tissue — particularly useful for studying membrane protein architecture, since the fracture plane tends to split the lipid bilayer.
- **Immuno-electron microscopy (immunogold labelling)**: the TEM equivalent of immunofluorescence — antibodies (direct, or indirect via protein-A) tagged with electron-dense colloidal gold particles localise a specific antigen with high spatial precision.

- ▶ **Cryo-electron microscopy (cryo-EM):** specimens are vitrified (plunge-frozen in liquid ethane) to trap them in an amorphous, near-native ice film and imaged without chemical fixation or heavy-metal staining, giving near-atomic resolution structural information on macromolecules and viruses; related approaches (CEMOVIS, correlative light-and-electron microscopy/CLEM) extend this to intact cells and tissue.
- ▶ **Serial block-face SEM / array tomography / focused-ion-beam SEM:** newer volume-EM methods that combine an in-chamber microtome or ion-beam mill with SEM imaging to reconstruct 3-D ultrastructure over larger volumes than a single TEM section.

Applied / Clinical anatomy

- ▶ **Renal biopsy pathology:** evaluation of a native or transplant renal biopsy is considered incomplete without EM alongside light microscopy and immunofluorescence — TEM demonstrates glomerular basement membrane thickening/splitting, podocyte foot-process effacement, and the size/location of electron-dense immune-complex deposits, which are often diagnostic.
- ▶ **Primary ciliary dyskinesia:** TEM of the respiratory ciliary axoneme (normally a 9+2 microtubule arrangement with inner/outer dynein arms) is used to demonstrate dynein-arm or other axonemal defects underlying the disease.
- ▶ **Connective tissue and bullous skin disease:** TEM helps localise the level of skin cleavage in inherited bullous disorders (e.g. epidermolysis bullosa) and demonstrates collagen/elastin ultrastructural abnormalities in conditions such as Ehlers–Danlos syndrome.
- ▶ **CADASIL:** TEM of a skin/vessel biopsy shows characteristic granular osmiophilic material within vascular smooth muscle, supporting the diagnosis.
- ▶ **Microbial/viral diagnosis:** negative-contrast/thin-section EM remains used for rapid identification of viruses and for typing microsporidia in tissue.
- ▶ **Reproductive and neural ultrastructure (teaching relevance):** EM of sperm demonstrates acrosome, mid-piece mitochondrial sheath and axonemal (9+2) organisation; EM of the neuron demonstrates organelle detail (rough endoplasmic reticulum/Nissl substance, synaptic vesicles) not resolvable by light microscopy.

High-yield exam pointers

- ▶ Electron probe gives ~1000-fold better resolution than light microscopy (TEM theoretical ~0.05–0.1 nm vs LM ~0.2 µm); practical biological sections resolve to ~1–2 nm.
- ▶ Electromagnetic (coil) lenses, not glass; entire optical column under high vacuum.
- ▶ TEM images a transmitted-electron pattern through an ultrathin (60–90 nm) resin section; SEM images secondary/backscattered electrons from a coated surface — TEM shows internal ultrastructure, SEM shows 3-D surface topography.
- ▶ Routine TEM processing = **double fixation** (glutaraldehyde then osmium tetroxide) → dehydration → resin (epoxy) embedding → ultramicrotomy → double staining (uranyl acetate + lead citrate).
- ▶ Freeze-fracture reveals intramembranous protein architecture; immunogold labelling is the EM analogue of immunofluorescence.

- ▶ Cryo-EM (vitrified, unstained specimens) now permits near-atomic resolution structural biology without conventional chemical fixation.
- ▶ Clinically indispensable in renal biopsy triad (LM + IF + EM), ciliary ultrastructure in primary ciliary dyskinesia, and CADASIL diagnosis.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology 2e; Bancroft's Theory & Practice of Histological Techniques; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; Gray's Anatomy (Standring) 41e (Commentary 1.5 — clinical/research applications); Brijesh Kumar — Histology Text & Atlas 3e (figure).



Fluorescent microscopy

Asked in: Paper III 30-May-2025 — RGUHS MD/MS Anatomy

Definition / Introduction Fluorescent (fluorescence) microscopy is a form of light microscopy that exploits the phenomenon of **fluorescence** — the property by which certain substances, when illuminated with light of a specific (shorter) wavelength, instantaneously re-emit light of a longer wavelength. A high-intensity beam of short-wavelength (exciting) light is directed on to an object stained with a fluorescent dye; the dye absorbs this high-energy light and releases low-energy photons, which are visualised by the observer as a brightly glowing image against a dark, high-contrast background. Emission ceases as soon as the exciting illumination is withdrawn, which distinguishes fluorescence from phosphorescence, in which afterglow persists. The technique is central to modern histopathology, immunodiagnostics and cell biology because it permits specific molecules to be localised with a sensitivity and contrast unattainable by conventional staining.

1. Principle and types of fluorescence

- ▶ **Basic steps:** high-energy (short-wavelength) light → passed through a selective filter → strikes the fluorochrome-stained object → the dye absorbs this energy and instantly emits light of longer wavelength → emitted light passes through a barrier filter → detected against a dark background.
- ▶ The exciting radiation is usually **ultraviolet (~360 nm)** or **blue light (~400 nm)**, though longer wavelengths are used with some modern dyes.
- ▶ **Primary fluorescence (autofluorescence)** — a substance possessing a natural fluorophore fluoresces without any added dye, e.g. vitamin A, porphyrins and chlorophyll; UV excitation gives optimum results for these.
- ▶ **Secondary fluorescence** — dyes, chemicals or certain antibiotics added to the tissue produce fluorescence; these agents are called **fluorochromes**. Most fluorochromes require only blue-light excitation, making this the commonest use of fluorescence in microscopy.
- ▶ **Induced fluorescence** — a substance that is not normally fluorescent is chemically converted into one; e.g. **catecholamines**, after treatment with **formaldehyde vapour**, are converted into fluorescent quinoline compounds.

2. Instrumentation — light source and filters

- ▶ **Light source:** traditionally a high-pressure **mercury vapour** or **xenon arc lamp** housed in a quartz capsule. Output diminishes with use (blackening of the quartz, shift in spectral emission); high-pressure capsules are explosive, the risk increasing with hours of use, so the capsule is changed every **200–300 hours**, and field uniformity is checked regularly. Halogen filament lamps were used for weaker fluorescence but were toxic and needed warm-up/cool-down time. Modern instruments increasingly use **LED** sources, which avoid these hazards and give stable output.
- ▶ **Selective (excitation) filter** — allows only the desired excitation wavelength through, chosen close to the absorption maximum of the fluorochrome in use, so that maximum fluorescence is produced.
- ▶ **Barrier (emission/suppression) filter** — placed between objective and eyepiece; blocks the short-wavelength excitation light (protecting the observer's retina) while transmitting the longer-wavelength emitted fluorescence.
- ▶ **Dichroic mirror** — a mirror that reflects light of some wavelengths and transmits light of others; it diverts the excitation beam on to the specimen and, on the return path, transmits the (longer-wavelength) emitted fluorescence to the eyepiece while reflecting residual excitation light away.
- ▶ Modern systems capture images digitally as monochrome (black-and-white); the vividly coloured composite images seen in publications are the result of pseudo-colouring.

3. Types of fluorescence microscope

Feature	Transmitted (diascopic) fluorescent microscope	Incident (epi-illumination) fluorescent microscope
Light path	Illumination from below, through condenser and specimen, to the objective	Illumination from above, through the objective itself (which also acts as the condenser)
Condenser	A dark-field condenser is preferred — gives a dark contrasting background and avoids stray light entering the objective/causing autofluorescence	Not required — objective and illumination share the same numerical aperture, giving optically efficient, brighter fluorescence
Key optical element	Excitation filter + barrier filter in the light path	Dichroic mirror gathers most of the excitation beam and reflects most of the emitted fluorescence
Image brightness	Comparatively dimmer	Brighter — fluorescence is not masked by mounting medium or section thickness
Modern usage	Largely historical	Standard design in current fluorescence and immunofluorescence microscopes

□ Fig 1.6 — Light path for fluorescence microscopy. (Sontakke — *Textbook of Human Histology* — 2e, p.25)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a vertical light-path column, top to bottom (incident/epi-fluorescence design): **White light source** → **Excitation (selective) filter** → **Dichroic mirror** (angled at 45°) → **Objective lens** (doubles as condenser) → **Fluorophore/specimen on slide**; emitted light returns up through the objective and the dichroic mirror → **Emission (barrier) filter** → **Observer/eyepiece**. Label the dichroic mirror clearly as the component that reflects the excitation beam down and transmits the emitted fluorescence up.

4. Common fluorochrome dyes

Fluorochrome	Excitation maximum (nm)	Emission maximum (nm)
Fluorescein isothiocyanate (FITC)	494	520 (apple-green)
Hoechst 33342	346	460
DAPI	359	461
Rhodamine	570	590 (orange-red)
Cy3	548	561
Texas red	595	613
R-Phycoerythrin (PE)	565	575 (orange-red)

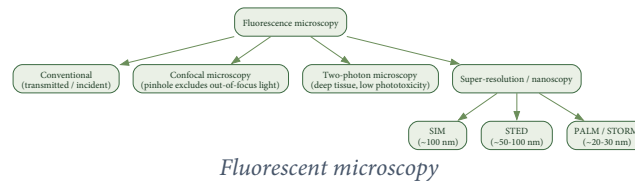
- ▶ Each fluorochrome is tagged (conjugated) to a specific antibody or molecular probe so that the target it binds can be localised.
- ▶ FITC is the most widely used fluorochrome for immunofluorescence — it has a broad UV-to-blue absorption spectrum, a characteristic apple-green emission, and this colour is rarely mimicked by tissue autofluorescence.
- ▶ Rhodamine (orange-red emission) is commonly combined with FITC in a **two-colour (double-labelling) technique**, allowing two different antigens to be localised on the same section using antibodies conjugated to the two dyes.

5. Applications

- ▶ **Immunofluorescence** — a fluorochrome-conjugated antibody is used to localise a specific antigen in tissue.
 - **Direct technique** — the primary antibody itself is fluorochrome-conjugated.
 - **Indirect technique** — an unlabelled primary antibody is followed by a fluorochrome-conjugated secondary antibody, giving signal amplification.
 - Performed on fresh-frozen sections; a standard clinical application is **direct immunofluorescence (DIF)** on renal and skin biopsies.
- ▶ **Fluorescent in situ hybridisation (FISH)** — fluorochrome-labelled DNA probes are hybridised to chromosomal/nuclear DNA to study chromosomal abnormalities.

- ▶ **DNA and RNA studies/sequencing and biomolecular assays.**
- ▶ **Green fluorescent protein (GFP)** — a protein of interest is genetically fused to GFP so that its expression, location and trafficking can be tracked within a living cell; unlike antibody-based immunostaining, GFP is genetically encoded and therefore compatible with live-cell imaging under physiological conditions.
- ▶ Study of specific cellular/subcellular components of interest, tagged with organelle-specific fluorescent probes.

6. Derivative and advanced fluorescence-based microscopes



- ▶ **Confocal microscopy** — a pinhole stop excludes out-of-focus fluorescence, improving contrast and resolution; permits **optical sectioning** and 3-dimensional (and, with time as the fourth dimension, 4-D) reconstruction. Components: high-power laser/mercury source, X-Y-Z scanning stage, **photomultiplier tube (PMT)** detector, and computer for image processing/reconstruction. Advantages — live (unfixed) tissue can be imaged, high-resolution/high-contrast, artefact-free, thick sections can be studied. Limitations — expensive, poorer photobleaching performance than conventional microscopy, and limited depth of penetration (~100 μm).
- ▶ **Two-photon microscopy** — two low-energy (red/infrared) photons combine at the focal point to excite the fluorophore; better tissue penetration and less photo-damage, but requires costly pulsed lasers.
- ▶ **Super-resolution microscopy (nanoscopy)** — circumvents the diffraction-limited resolution (~200–300 nm) of conventional fluorescence microscopy; recognised by the 2014 Nobel Prize in Chemistry. Three broad approaches: **structured illumination microscopy (SIM)** — a grid pattern of excitation light is projected and multiple acquisitions are computationally combined (~100 nm resolution, compatible with live cells); **stimulated emission depletion (STED)** — a doughnut-shaped depletion beam narrows the effective excitation spot (~50–100 nm resolution); and **single-molecule localisation microscopy (PALM/STORM)** — individual switchable fluorophores are activated, imaged and localised in sequence over many frames (highest resolution, ~20–30 nm, but slow, mainly used on fixed cells).

Applied / Clinical anatomy

- ▶ **Direct immunofluorescence (DIF)** of a fresh-frozen renal biopsy is a standard diagnostic step in glomerular disease, demonstrating immunoglobulin/complement deposits; DIF of a skin biopsy is similarly used in diagnosing immunobullous disorders.
- ▶ **FISH** is applied in clinical cytogenetics for the detection of chromosomal microdeletions/aneuploidies.
- ▶ Confocal fluorescence microscopy has clinical/in-vivo applications such as **corneal thickness measurement** and adjunctive **screening for colorectal cancer**.

- ▶ **GFP-tagging** underlies modern research into intracellular trafficking, gene expression and cell-signalling pathways.
- ▶ **Photobleaching** (progressive, irreversible loss of fluorescence on prolonged excitation) and **false-positive/high background staining** (from inadequate washing or excess unconjugated fluorochrome) are important practical limitations that must be controlled for reliable interpretation.

High-yield exam pointers

- ▶ Fluorescence: absorption of shorter-wavelength (high-energy) light with instantaneous emission of longer-wavelength (lower-energy) light; ceases as soon as excitation stops (unlike phosphorescence).
- ▶ Three types: **primary/autofluorescence** (vitamin A, porphyrins, chlorophyll — UV excited), **secondary** (fluorochrome-induced — usually blue-light excited), **induced** (e.g. formaldehyde-treated catecholamines → fluorescent quinolines).
- ▶ Key optical elements: **selective/excitation filter**, **barrier/emission filter**, and — in the modern **incident (epi-illumination)** design — a **dichroic mirror**, which gives a brighter image than the older transmitted design.
- ▶ Common fluorochromes: **FITC** (494/520 nm, apple-green), **DAPI** (359/461 nm, nuclear stain), **Rhodamine** (570/590 nm, orange-red — paired with FITC for double labelling).
- ▶ Applications: **direct/indirect immunofluorescence**, **FISH**, **GFP-tagged live-cell imaging**, DNA/RNA studies.
- ▶ **Confocal microscopy** uses a pinhole to exclude out-of-focus light, enabling optical sectioning and 3-D reconstruction of live, unfixed tissue.
- ▶ **Super-resolution (nanoscopy)** techniques — SIM, STED, PALM/STORM — break the ~200–300 nm diffraction limit (2014 Nobel Prize in Chemistry).
- ▶ Direct immunofluorescence on renal/skin biopsy is a clinical gold-standard diagnostic test for immune-mediated disease.
- ▶ Light source: mercury vapour/xenon arc lamp (capsule life 200–300 h) — increasingly replaced by LEDs.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e — Commentary 1.1, "Fluorescence microscopy in cell biology today"; Pranab Dey, *Basic and Advanced Laboratory Techniques in Histopathology and Cytology* 2e (Chapter 27, Fluorescence Microscope, Confocal Microscope and Other Advanced Microscopes); Bancroft's Theory and Practice of Histological Techniques (Light microscopy chapter; Immunohistochemical and immunofluorescent techniques chapter); diagram from Sontakke, *Textbook of Human Histology* 2e.



Hematoxylin and Eosin staining procedure

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction — Hematoxylin and Eosin (H&E) is the **most widely used routine histological stain**, applied to every paraffin (and frozen) tissue section for diagnostic and teaching purposes. It is a **combination stain**: hematoxylin (a basic/nuclear dye, used after mordanting) stains cell nuclei blue-black, while eosin (an acidic/cytoplasmic counterstain) stains cytoplasm and connective-tissue fibres varying shades of pink. Together the two dyes reveal general tissue architecture with sufficient contrast for routine morphological diagnosis, and the technique is simple, reproducible and easily automated.

1. Principle of H&E staining

- ▶ **Hematoxylin** is extracted from the heartwood ("logwood") of *Haematoxylon campechianum*, a tree native to the Campeche state of Mexico and now cultivated mainly in the West Indies. Fresh wood is chopped, boiled in water, and hematoxylin is precipitated from the aqueous extract using **urea** (or ether), yielding a purified brown powder.
- ▶ Hematoxylin itself has **no staining property**. It must first be oxidised to **hematein**, its major oxidation product and the actual dye. Oxidation ("ripening") occurs by two routes:
 - **Natural ripening** — exposure to light and air for 2-4 months (e.g. Ehrlich's, Delafield's hematoxylin). Slower, but the ripened stain retains its staining power for a long time.
 - **Chemical oxidation** — using an oxidising agent such as **sodium iodate** (Mayer's) or **mercuric oxide** (traditional Harris's); conversion is instantaneous and the stain is usable at once, but its useful life is shorter because oxidation continues after preparation. Mercuric oxide is now largely replaced by sodium/potassium iodate because mercury is toxic and corrodes automated staining machines.
- ▶ **Hematein is anionic** and has poor affinity for tissue on its own — it is an inadequate nuclear stain without a **mordant**. The metal cation of the mordant combines with hematein to form a **dye-mordant (hematein-lake) complex** that carries a net **positive charge**, enabling it to bind the anionic sites of **nuclear chromatin** (phosphate groups of DNA), staining nuclei **blue-black**.
- ▶ Hematoxylin is classified by the **mordant** used: **alum** (aluminium potassium/ammonium sulfate — the routine H&E mordant), **iron** (ferric chloride/ferric ammonium sulfate — e.g. Heidenhain's, Weigert's, Verhoeff's), **tungsten** (phosphotungstic acid — PTAH), **molybdenum**, **lead**, and hematoxylin used **without a mordant**.
- ▶ **Progressive vs regressive staining**:
 - *Progressive* — the section is stained for a predetermined time so that nuclei are stained adequately while the background remains relatively unstained (no separate differentiation step); Mayer's hematoxylin is the classic example and is well suited to automated staining.
 - *Regressive* — the section is deliberately **over-stained**, then excess dye is removed ("differentiated") in **acid-alcohol**, followed by bluing; Harris's and Ehrlich's hematoxylin are traditionally used this way in routine H&E.
- ▶ **Bluing ("blueing")**: alum hematoxylin first stains nuclei a reddish colour; washing in a weak alkali (raising pH to about 8.5) converts the soluble red hemalum to an insoluble blue-black form — the familiar crisp blue nuclear colour of a well-stained H&E section.

- **Eosin** is an acidic (anionic) **xanthene dye** (chemical class: tetrabromofluorescein) that stains basic/eosinophilic tissue components — cytoplasm, most connective-tissue fibres and matrices, and red blood cells — shades of pink, red and orange. Three types exist (Eosin Y, Eosin B, ethyl eosin); **Eosin Y** ("Y" = yellowish tinge) is by far the most commonly used, and is soluble in both water and alcohol.

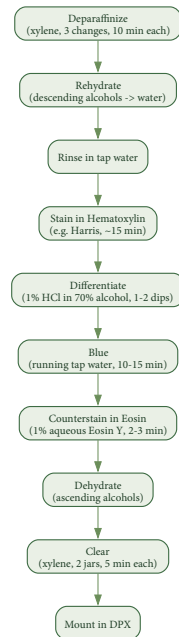
2. Reagents required

Reagent	Common example	Purpose
Alum hematoxylin	Harris's / Mayer's / Ehrlich's / Gill's / Cole's	Nuclear stain
Acid-alcohol	1% HCl in 70% alcohol	Differentiation (regressive staining)
Bluing agent	Running tap water / Scott's tap water / 5% ammonium hydroxide / ammonia vapour	Converts red hemalum → blue-black form
Counterstain	1% aqueous Eosin Y	Cytoplasmic/connective-tissue counterstain
Graded alcohols	Absolute, 95%, 80%, 60%	Deparaffinized-section rehydration and post-stain dehydration
Clearing agent	Xylene (dimethyl benzene)	Removes alcohol before mounting; renders section transparent
Mounting medium	DPX (Distyrene-Plasticiser-Xylene)	Permanent, colourless mounting; refractive index close to glass

Alum hematoxylin commonly used for routine H&E (Table):

Stain	Mordant	Oxidant	Typical staining time	Comment
Ehrlich's	Potassium alum	Natural ripening (light/air, ~2 months)	20-30 min	Long-lasting; good for mucin, bone, cartilage
Mayer's	Potassium/ ammonium alum	Sodium iodate	5-10 min (progressive)	Best for automation; used as nuclear counterstain
Harris's	Ammonium/ potassium alum	Mercuric oxide (traditionally)	5-15 min (regressive)	Good general-purpose and cytology stain
Gill's	Aluminium sulfate	Sodium iodate	~5-15 min (regressive)	Stable, most resistant to auto-oxidation
Cole's	Potassium alum	Iodine in alcohol	~30 min (regressive)	Stable for ~3 months

3. Procedure — step-by-step routine H&E staining of a paraffin section



Hematoxylin and Eosin staining procedure

Step	Details
1. Deparaffinization	Xylene, 3 changes, 10 min each
2. Rehydration	Descending grades of alcohol — absolute → 95% → 80% → 60% — 1-2 min each, then water
3. Rinse	Tap water
4. Nuclear stain	Hematoxylin (e.g. Harris's, ~15 min; time varies with type of hematoxylin used)
5. Differentiation	For regressive staining: 1-2 dips in acid-alcohol (1% HCl in 70% alcohol); check microscopically for adequate nuclear staining
6. Rinse	Water
7. Bluing	Running tap water 10-15 min (or Scott's tap water/ammonium hydroxide for a few min)
8. Counterstain	1% aqueous Eosin Y, 2-3 min
9. Dehydration	Ascending alcohols — 95% (3 min), absolute (3 min), absolute (5 min)
10. Clearing	Xylene, 2 jars, 5 min each
11. Mounting	Mount in DPX under a coverslip

4. Colour reactions (results)

Structure	Colour
Nuclei / chromatin	Blue to bluish-black

Structure	Colour
Cytoplasm	Pink/pinkish
Collagen and connective-tissue matrix	Pale pink
Muscle fibres	Deeper pink/red
Red blood cells	Bright red-orange (strongly eosinophilic)

□ Fig 1.1 — Hematoxylin and eosin (H&E) staining. This series of specimens from the pancreas are serial (adjacent) sections that demonstrate staining with hematoxylin alone, eosin alone, and combined hematoxylin-eosin. (Ross Histology — A Text and Atlas — 7e, p.20)

5. Troubleshooting and quality control

Problem	Common cause	Remedy
Pale nuclei	Over-differentiation; too little time in hematoxylin; over-oxidised stain	Re-stain; increase hematoxylin time; change stain solution
Darkly stained nuclei	Too little differentiation; too much time in hematoxylin; thick section	Decolorise and re-differentiate; recut thinner section
Reddish-brown nuclei	Insufficient bluing; degenerating hematoxylin	Prolong bluing step; check oxidation status of stain
Pale/washed-out cytoplasm (eosin)	Thin section; eosin pH > 5 (diluted by carry-over bluing solution); over-dehydration	Recut section; adjust eosin pH with acetic acid; limit alcohol time
Overly dark cytoplasm	Prolonged/over-concentrated eosin; too-rapid dehydration	Shorten eosin time; dilute eosin optimally
Bluish-black precipitate	Precipitated hematoxylin	Filter the hematoxylin solution before use
Irregular/spotty staining	Incomplete deparaffinization	Prolong xylene step
Milky sections before mounting	Incomplete dehydration	Re-dehydrate through fresh alcohols before xylene

- **Quality control:** automated staining machines give consistent step timings, but batch-to-batch variation in commercial hematoxylin, and prolonged/acidic formalin fixation of tissue (common with unbuffered fixative or hot-climate storage), can still cause a **uniformly muddy purple** section — nuclear and cytoplasmic staining must be re-optimised whenever the stain batch, fixation schedule, section thickness or hot-plate temperature changes.

Applied / Clinical anatomy

- H&E remains the **gold-standard, first-line stain in diagnostic surgical pathology** — essentially every histopathology biopsy and museum/teaching section is examined as an H&E slide before any special stain is used.
- **Rapid (frozen-section) H&E** allows intraoperative diagnosis (e.g. tumour margin/lymph node assessment) within minutes, using a shortened staining schedule.

- ▶ **Correct fixation** (usually 10% neutral buffered formalin) must precede staining — under- or over-fixed, or acidic/deteriorated formalin, produces poor differential nuclear/cytoplasmic staining and can mimic technical "pale nuclei" artefacts on diagnostic sections.
- ▶ In **decalcified bone/hard-tissue sections**, over-decalcification classically produces **loss of nuclear (hematoxylin) staining** with only a uniform eosin pink remaining — an important, commonly examined artefact in bone histotechnique.
- ▶ H&E-stained sections are the substrate on which special stains (PAS, reticulin, Masson's trichrome, immunohistochemistry) are subsequently correlated for a complete histopathological diagnosis.

High-yield exam pointers

- ▶ Hematoxylin (the dye precursor) is oxidised to **hematein**, the true dye; hematein needs a **mordant** (usually alum) to bind anionic nuclear chromatin.
- ▶ **Natural ripening** (light/air, months) vs **chemical oxidation** (sodium iodate/mercuric oxide, instant but shorter shelf-life).
- ▶ **Progressive staining** (Mayer's — timed, no differentiation) vs **regressive staining** (Harris's/Ehrlich's — over-stain then differentiate in acid-alcohol).
- ▶ **Bluing** converts red hemalum → insoluble blue-black form by raising pH to ~8.5 (tap water, Scott's tap water, ammonia).
- ▶ Eosin Y is an acidic xanthene dye (tetrabromofluorescein) used as 1% aqueous or 0.5% alcoholic solution; it counterstains cytoplasm/connective tissue pink.
- ▶ Standard sequence: **deparaffinize → rehydrate → hematoxylin → differentiate → blue → eosin → dehydrate → clear → mount (DPX)**.
- ▶ Result: **nuclei blue-black, cytoplasm pink**.
- ▶ Common artefacts: pale nuclei (over-differentiation/weak stain), reddish-brown nuclei (insufficient bluing), muddy sections (acidic/old fixative), precipitate (unfiltered hematoxylin).
- ▶ H&E is the universal, first-line diagnostic stain in histopathology; frozen-section H&E permits rapid intraoperative reporting.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology 2e; Bancroft's Theory & Practice of Histological Techniques; Ross Histology — A Text and Atlas 7e; Junqueira's Basic Histology (Text & Atlas) 17e; Sontakke — Textbook of Human Histology 2e.

Coverage note: this is a routine laboratory-methodology topic (not covered in Gray's Anatomy); grounding is drawn entirely from the specialty histotechnique texts (Bancroft's, Pranab Dey) as the spec's note anticipated.



Honing

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Honing is the manual (or mechanical) process of sharpening a blunted microtome knife by rubbing its cutting edge over an abrasive stone until a fine, uniform cutting edge is restored. It is one of the basic histotechnique skills of the anatomy/histology laboratory, because the quality of a paraffin or frozen section — its thinness, absence of chatter, tears and compression artefacts — depends directly on how sharp and even the microtome knife edge is. Honing is always followed by **stropping**, a finishing step that polishes the honed edge and removes the microscopic "burr" raised during honing. Together, honing and stropping constitute the manual method of microtome-knife sharpening, now increasingly supplemented or replaced by automatic knife sharpeners and disposable blades.

1. The microtome knife — types and materials

A microtome knife is classified by the shape of its cross-section and by the material of which it is made.

Profile (shape)	Feature	Use
Plano-concave (Profile A)	One side plain, other side concave; very sharp	Cutting soft tissues ; now used less frequently
Bi-concave (Profile B)	Concave on both sides; concavity hard to judge	Used on rocking microtomes ; vibrates during cutting
Wedge (Profile C)	Plain on both sides	Most widely used knife ; compatible with most microtomes; easiest to sharpen
Tool-edge (Profile D)	Both sides plain, steep cutting edge, resembles a carpenter's chisel	Hard tissue (decalcified bone); difficult to sharpen — not recommended now

□ Fig 5.3 — Different types of microtomy knives based on shape. Profile C is the most commonly used knife (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.67)

Materials of the knife

- ▶ **Conventional (steel) knife** — high-quality carbon steel, tempered from the edge to the inner one-third of its width; desirable hardness **400–900 on the Vickers scale**. This is the knife that requires periodic manual honing.
- ▶ **Tungsten-carbide-tipped knife** — used for undecalcified bone cryotomy and plastic-embedded tissue; needs infrequent but expensive reconditioning, best done on a multi-plate automatic sharpener.
- ▶ **Diamond knife** — costly; used to cut epoxy-resin blocks for electron microscopy; requires specialised sharpening.
- ▶ **Glass knife** — used in ultramicrotomy for very hard/resin-embedded tissue; its cutting edge is freshly fractured parallel to one glass surface and is generally used once, not honed.
- ▶ **Disposable blades** (low-profile for small biopsies/soft tissue; high-profile for firm tissue such as uterus, heart) need no honing at all — a fresh, factory-sharpened edge is simply clicked into a

blade holder for each block, saving time and eliminating sharpening costs, though they are less rigid and may vibrate more than a solid steel knife.

2. Angles of the knife — the basis for correct honing

Honing aims to restore the correct geometry of the knife edge, defined by three angles:

Angle	Definition	Typical value / significance
Rake angle	Angle between the upper bevel of the knife and the perpendicular drawn from the block surface	A higher rake angle eases section cutting and gives smoother ribbon flow; a lower rake angle is used for hard tissue
Angle of clearance	Angle between the lower bevel of the knife and the block surface	Usually 2°–5°; governs friction between block and knife — the lower the clearance angle, the less the compression on the block
Bevel (knife-edge) angle	Angle between the two planes of the knife that meet at the cutting edge	Ranges 18°–30°; the smaller the bevel angle, the sharper the knife
Cutting edge	The straight line formed by the intersection of the two bevel planes	The actual working edge produced/restored by honing and refined by stropping

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled schematic of the knife-in-section, to be reproduced by hand exactly as asked in the exam:

- ▶ Horizontal line = **surface of the block**, with the embedded tissue block resting on it.
- ▶ A **perpendicular line** dropped from the block surface.
- ▶ The knife shown obliquely above the block, with its **upper bevel** and **lower bevel** meeting at the **cutting edge**.
- ▶ Label the **rake angle** between the upper bevel and the perpendicular line.
- ▶ Label the **angle of clearance** between the lower bevel and the block surface.
- ▶ Label the **bevel (knife-edge) angle** between the two bevel planes at the cutting edge.
- ▶ Show the direction of the knife stroke relative to the block.

3. Purpose and timing of honing

- ▶ With repeated use a steel knife edge develops microscopic nicks, dullness and unevenness, producing thick, torn or chattered sections.
- ▶ **Honing removes this gross irregularity and restores the primary cutting edge** by grinding away a controlled amount of metal; it is the coarser of the two manual steps.
- ▶ Honing is required periodically for **permanent (reusable) steel knives**; it is **not needed** for disposable blades, which are simply discarded and replaced.

- ▶ Honing must always be completed by stropping before the knife is used, because honing itself leaves a fine burr and micro-serrations that would otherwise tear the section.

4. Method of honing (manual)

- ▶ **Abrasive:** fine abrasive grindings — commonly **iron oxide, aluminium oxide or silicon carbide** — are used on a flat sharpening stone (hone).
- ▶ **Lubrication:** the stone is lubricated with a **light oil** before use, to float away metal debris and reduce heat/friction.
- ▶ **Grip and stroke:** the knife handle is held in one hand and the front (tip) end of the knife in the other; the knife is **pushed forward and diagonally across the stone** repeatedly.
- ▶ **Coverage:** the same forward-diagonal stroke is repeated over the **other surface(s) of the knife** so that both bevels are ground evenly and symmetrically.
- ▶ **End-point:** honing is continued until a fine, continuous burr can be felt along the full length of the edge, indicating that both facets have been ground to meet at a true edge; this burr is then removed by stropping, not by further honing.

5. Stropping

- ▶ Stropping **polishes the cutting edge that has already been sharpened by honing** and removes any burr thrown up during honing.
- ▶ The **strop is made of leather** and must be kept scrupulously **free of dust or grit**, since any abrasive contamination on the strop would blunt or nick the very edge it is meant to refine.
- ▶ Stropping is the fine-finishing step; a knife may be honed only occasionally but is stropped before every session or whenever the edge begins to drag.

6. Automatic knife sharpener

- ▶ Modern laboratories increasingly use an **automatic knife sharpener**, in which the knife is clamped horizontally against a **rotating plate (glass or copper) coated with abrasive agents**.
- ▶ This standardises the sharpening angle, saves technician time, and is cost-effective for knives that are sharpened routinely.
- ▶ A **multiple-plate automatic sharpener** can also recondition the harder **tungsten-carbide knives** used for undecalcified bone/plastic-embedded tissue, for which manual honing on a stone is impractical and repeated professional sharpening is expensive.

7. Factors that influence the quality of the cut section

Factor	Effect
Temperature	Lowering the temperature of the block (chilling) facilitates easier, cleaner section cutting
Rake angle	Higher rake angle → smoother ribbon formation; lower rake angle is preferred for hard/dense tissue
Consistency of the tissue	Soft tissue is cut at a slower rate than hard tissue to avoid tearing/compression

Applied / Clinical anatomy

- ▶ A blunt or unevenly honed knife is one of the commonest causes of section artefacts on the microtome bench: **chatter marks** (fine parallel ridges from vibration of a dull/loose edge), **compression**, and frank **tears in the tissue** wherever the edge is nicked or uneven.

□ Fig 5.9 — Tear in the tissue due to uneven cutting edge of the knife (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.72)

- ▶ Such artefacts obscure nuclear and cytoplasmic detail and can mimic or mask genuine pathology, so a histopathology/histology quality-assurance protocol always lists "**check/re-hone the knife**" among the first corrective steps for poor sections.
- ▶ **Disposable low/high-profile blades** have largely replaced manual honing and stropping in routine diagnostic laboratories, because they give a consistently sharp, factory-set edge for every block, eliminate the recurring cost of abrasive stones and knife-sharpener servicing, and remove the repetitive hand-wheel/sharpening motions that were an occupational (repetitive-strain) hazard for histotechnologists.
- ▶ **Hard-tissue knives** (tungsten-carbide-tipped, for undecalcified bone) and **electron-microscopy knives** (glass, used once and discarded; diamond, professionally reconditioned) follow a different sharpening regime from the routine steel knife, and this distinction is a favourite viva point.
- ▶ Correct honing/stropping technique is directly examinable practical knowledge for the PG anatomy museum/histology laboratory posting, since it underlies the quality of every teaching slide and demonstration section prepared in the department.

High-yield exam pointers

- ▶ **Honing** = coarse manual sharpening of a blunted knife edge on an abrasive stone (iron oxide/aluminium oxide/silicon carbide), lubricated with light oil.
- ▶ **Stropping always follows honing** — it polishes the edge and removes the "burr" raised during honing; the strop is leather and must be free of dust/grit.
- ▶ Three knife angles to remember: **rake angle** (higher = easier cutting), **clearance angle** (2° – 5° ; lower = less block compression), **bevel/knife-edge angle** (18° – 30° ; smaller = sharper).
- ▶ **Wedge (Profile C)** knife is the most widely used and easiest to sharpen; **tool-edge (Profile D)** is for hard tissue and is now rarely recommended.
- ▶ Disposable blades (low-profile/high-profile) require **no honing**.
- ▶ **Automatic knife sharpener** = rotating abrasive-coated glass/copper plate; a multi-plate design can also recondition tungsten-carbide knives.
- ▶ A poorly honed knife produces **chatter, compression and tears** — a common cause of section artefact.
- ▶ Diamond and glass knives (electron microscopy/ultramicrotomy) are sharpened/handled differently from the routine steel microtome knife.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology (2e); Bancroft's Theory & Practice of Histological Techniques.

Coverage note: Honing is a narrow histotechnique topic that standard gross-anatomy/embryology/genetics spine texts (Gray's Anatomy, BD Chaurasia, etc.) do not cover; this answer is grounded entirely in the two dedicated histotechnique manuals available in the corpus.

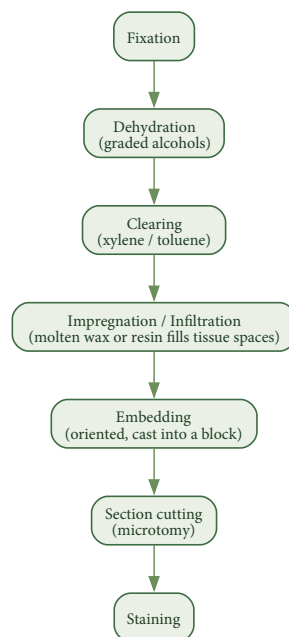


Impregnation and embedding media

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction **Impregnation** (infiltration) is the tissue-processing step in which the clearing agent occupying the dehydrated tissue spaces is replaced, by diffusion, with a molten support medium — classically paraffin wax — so that every space in the tissue is filled with a substance capable of solidifying. **Embedding** is the subsequent step in which the impregnated tissue is correctly oriented and cast within a mould of the same (or a compatible) medium, which is then allowed to solidify into a firm **block**. Together these two steps convert soft, fixed tissue into a rigid block that can be cut into uniformly thin (3–5 μm) sections on a microtome without tearing, folding or distortion. They are the fourth and fifth stages of routine tissue processing, following fixation, dehydration and clearing, and directly determine the quality of subsequent sectioning and staining.

1. Position in tissue processing



Impregnation and embedding media

2. Aims and ideal properties of the medium

- Aims of the embedding medium (three crucial functions):

- ▶ To provide **support/rigidity** to the tissue.
- ▶ To **prevent distortion** of the tissue during cutting.
- ▶ To **preserve the tissue** for archival use.
- ▶ **Ideal impregnating/embedding medium** should be:

Property	Reason
Miscible with the clearing agent	Ensures complete replacement/removal of the clearing agent
Liquid at 30–60 °C, solid at room temperature	Allows infiltration when molten and rigidity for cutting once cooled
Homogeneous and stable	Uniform support throughout the block
Non-toxic and cheap	Safe and practical for routine daily use
Transparent	Does not obscure microscopic detail
Fit for sectioning the tissue	Correct hardness to allow thin, ribbon-like sections

- ▶ **Factors governing time/number of changes of medium:** size of the tissue (larger blocks need longer impregnation and more changes), type of tissue (hard tissue such as bone/cartilage needs longer than soft tissue), type of clearing agent used (cedarwood oil is harder to remove than xylene/toluene), and type of processing (vacuum accelerates impregnation).

3. Paraffin wax — the standard impregnating/embedding medium

- ▶ **Nature:** a solid, polycrystalline **hydrocarbon** obtained as a by-product of refining crude petroleum; the most popular, universally accepted embedding medium.
- ▶ **Melting point:** ranges from 39 °C to 70 °C; wax is sold according to its melting point. For routine laboratory use in the Indian subcontinent, wax of melting point ~56–62 °C is preferred, as it gives a good ribbon on sectioning. Low-melting-point wax is soft; higher-melting-point wax gives a harder block.
- ▶ **Duration:** about 3–4 hours in molten wax is sufficient for impregnation of an average-sized biopsy; the embedding wax itself should be kept at 2–4 °C **above its own melting point** — overheating causes hardening/distortion and can break down blended paraffinic additives.
- ▶ **Advantages:** tissue blocks can be **stored long-term**; non-toxic; cheap; safe; easily adaptable; compatible with routine and special stains, including immunohistochemistry.
- ▶ **Disadvantages:** prolonged impregnation causes **shrinkage and hardening**; wax infiltrates very hard tissues such as **bone and the eye** slowly.
- ▶ **Additives/modifications to alter its physical properties:**

Additive	Effect
Stearic acid	Increases hardness of the wax
Phenanthrene	Reduces the melting point
Ceresin (0.5%)	Improves adhesiveness between wax and tissue

Additive	Effect
Dimethyl sulphoxide (DMSO)	Reduces infiltration time, removes residual clearing agent, gives a homogeneous matrix and better support

- **Vacuum impregnation:** molten wax is impregnated into the tissue under **negative pressure**. This removes entrapped air from the tissue (enhancing fluid penetration), shortens the time the tissue is exposed to hot wax (reducing hardening), and removes residual clearing agent completely (prolonging the wax's usable life). It is particularly used for **air-containing, spongy organs** — **lung and spleen**.
- **Automated tissue processors:** transfer tissue through the dehydration–clearing–impregnation sequence automatically; two types exist —

Feature	Tissue-transfer processor	Fluid-transfer processor
Method	Tissue basket is transferred between buckets of reagent	Reagents are pumped through stationary tissue cassettes
Speed	Relatively slow	Fast
Risk of tissue drying	Common problem during transfer	Essentially none (closed system)
Flexibility of reagents	Flexible	Limited
Infiltration	Adequate	Better

4. Alternative / special embedding media

Paraffin wax is unsuitable when: the tissue component under study is destroyed by processing reagents (e.g. **lipids**), heat would damage the target (e.g. **enzymes**), thinner sections than paraffin permits are needed (e.g. **electron microscopy**), or the tissue is too hard/dense for wax to support (e.g. **undecalcified bone**).

Medium	Typical use	Key feature
Epoxy/acrylic resin	Electron microscopy; ultra-thin sectioning; undecalcified bone	Polymerised (hardened) by heat, UV light or a chemical catalyst; gives superior resolution and fine tissue detail
Methacrylate (acrylic) monomer	Hard, clear blocks for high-resolution light microscopy	Polymerised in the presence of a catalyst (2% benzoyl peroxide); hydroquinone inhibitor must first be removed by weak alkali wash
Agar (agar gel)	Cohesive agent for small, friable tissue — cytology samples, endometrial curettings, small endoscopic biopsies	Does not itself give adequate support for cutting; used as the first stage of double embedding , with paraffin as the second stage

Medium	Typical use	Key feature
Gelatin	Small friable tissues; frozen sections; whole-organ sectioning (Gough–Wentworth technique)	Low melting point (35–40 °C) makes it unsuitable as a stand-alone routine embedding medium
Celloidin (nitrocellulose)	Dense/hard tissues (classically whole brain, eye)	Requires special equipment and non-conventional (sliding) microtomy; largely obsolete in routine laboratories today
Carbowax (water-soluble wax)	Occasions requiring a water-miscible, low-heat medium	Alternative infiltration medium listed alongside paraffin, resin and methacrylate

5. Double embedding and tissue orientation

- ▶ **Double embedding** = tissue is embedded twice in two different media — first in **agar or nitrocellulose**, then in **paraffin wax**. It prevents loss/fragmentation of very small pieces of tissue and is specially used for **endoscopic biopsies and curettage specimens**; a nitrocellulose-then-paraffin technique is used for **brain tissue**.
 - *Agar–paraffin method*: tissue is taken up in filter paper → molten agar is poured over it → allowed to solidify → margins trimmed → the agar block is processed and embedded again in paraffin.
- ▶ **Tissue orientation rules** at embedding (critical for correct plane of section):
 - **Tubular structures** (fallopian tube, vas deferens, artery) are placed to yield a **transverse section** showing all layers.
 - Tissue with an **epithelial surface** is placed vertically, at right angles to the surface, to display all layers.
 - **Multiple fragments** (e.g. endometrial curettings) are placed centrally together.
 - **Linear, long tissue** is placed diagonally in the mould.
 - **Muscle biopsy** is oriented in both longitudinal and transverse planes.
 - **Long, membranous tissue** (e.g. amniotic membrane) is rolled into a **Swiss roll**.

6. Embedding technique (Tissue-Tek system)

- ▶ **Equipment**: a **dispenser of molten paraffin** held at constant temperature, a **metal or plastic mould**, and a **cold plate**.
- ▶ **Steps**: the tissue is placed on the lower (floor) surface of the mould with its cutting surface facing down, using forceps → molten paraffin is poured into the mould → a labelled cassette is placed on top and the mould is completely filled with wax → the whole assembly is transferred to the **cold plate** to solidify → once firm, the metallic mould is detached from the cassette/plastic ring, leaving a finished **block** ready for microtomy.
- ▶ **Moulds in use**: **L-shaped Leuckhart mould**, stainless-steel mould, and chemical-resistant plastic mould.

embed: ![Fig 3.5 — Illustrated view of the whole embedding process: the Tissue-Tek system is set up, tissue removed from the processing cassette, molten paraffin poured into the metal mould, tissue embedded and pressed into position with forceps, the mould covered with a labelled plastic ring, and the assembly placed on the cold plate to solidify. (Pranab Dey — Lab Techniques in Histopathology & Cytology 2e, p.56)](C:/Users/Harshad/Documents/anatomy-notes/answer-bank/rguhs-anatomy/answers/figures/plates/c1932c430945ad6b.jpg)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple schematic of the paraffin embedding set-up and process:

- ▶ Dispenser nozzle delivering molten paraffin wax, at constant temperature
- ▶ Metal (or plastic) base mould, with the tissue placed cut-surface down using forceps
- ▶ Labelled tissue cassette placed on top of the mould and filled with wax
- ▶ Cold plate beneath the mould, allowing the wax to solidify
- ▶ The finished, labelled paraffin block after removal of the mould/ring

Applied / Clinical anatomy

- ▶ **Sectioning artefacts from faulty impregnation:** inadequate dehydration/clearing leaves defective wax infiltration, so tissue is soft and falls out of the block during microtomy; conversely, overheating or prolonged impregnation makes tissue **hard, brittle and shrunken**, distorting histological detail and impairing immunohistochemical staining.
- ▶ **Electron microscopy and undecalcified bone** cannot use paraffin because ultra-thin (nanometre-range) sections and very hard matrices are required — **resin (epoxy/acrylic) embedding** is mandatory here, hardened by heat, UV light or a chemical catalyst.
- ▶ **Vacuum impregnation** is specifically applied to **lung and spleen**, air-containing/spongy organs where trapped air would otherwise impede infiltration and cause poor block quality.
- ▶ **Double embedding** is of direct diagnostic importance in **endoscopic biopsies, cervical/endometrial curettings and brain tissue**, where fragmentation or loss of small, friable pieces could compromise histopathological (including margin/malignancy) assessment.
- ▶ **Frozen/cryostat sections**, used for rapid intra-operative diagnosis, avoid the paraffin route altogether — the tissue is embedded in OCT compound and frozen rather than impregnated with heated wax, precisely because heat and organic solvents would destroy enzymes, lipids or antigens under study.
- ▶ **Tissue orientation errors** (e.g. an epithelial surface embedded tangentially rather than perpendicularly) are a common, entirely preventable cause of a surgical margin or dysplasia being missed on the slide — hence the strict orientation rules followed at embedding.

High-yield exam pointers

- ▶ Impregnation = infiltration of the clearing-agent-vacated tissue spaces with the support medium; embedding = orienting and casting that impregnated tissue in a mould to form a solid, sectionable block.

- ▶ Sequence: Fixation → Dehydration → Clearing → **Impregnation** → **Embedding** → Section cutting → Staining.
- ▶ Paraffin wax: hydrocarbon by-product of petroleum refining; melting point 39–70 °C (routine Indian use ~56–62 °C); embedding temperature kept 2–4 °C above the wax's melting point.
- ▶ Ideal medium: miscible with clearing agent, liquid 30–60 °C/solid at room temperature, homogeneous, non-toxic, cheap, transparent, fit for sectioning.
- ▶ Paraffin additives: stearic acid (hardness↑), phenanthrene (melting point↓), ceresin (adhesiveness↑), DMSO (faster infiltration, better matrix).
- ▶ Vacuum impregnation → used for lung and spleen; reduces hardening, removes residual clearant, prolongs wax life.
- ▶ Non-paraffin media: resin/epoxy (EM, undecalcified bone), methacrylate (catalysed by benzoyl peroxide), celloidin/nitrocellulose (dense tissue, now largely obsolete), gelatin (friable/frozen tissue, low melting point), agar (double embedding of friable biopsies).
- ▶ Double embedding = two sequential media (agar or nitrocellulose, then paraffin) — used for endoscopic biopsies, curettings and brain.
- ▶ Tissue-transfer processors are slower and risk drying; fluid-transfer processors are faster with no drying risk but less reagent flexibility.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology 2e; Bancroft's Theory & Practice of Histological Techniques. Coverage note: this is a histotechnique (tissue-processing) topic; Gray's Anatomy (Standring) does not cover laboratory histological methods, so grounding is drawn entirely from the microscopy/histotechnique specialty texts as specified.



Magnification

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction Magnification is the ratio by which the linear dimension of the image of an object, as seen through an optical (or electron-optical) instrument, is enlarged in comparison with the true dimension of the object itself. Together with **resolution** (resolving power) and **contrast**, it is one of the three fundamental optical properties of a microscope, but by itself it is of little diagnostic value — an image can be magnified indefinitely without revealing any new detail once the resolving power of the objective has been exhausted ("**empty magnification**"). In a compound microscope, magnification is built up in two stages, first by the objective and then by the eyepiece, and the achievable *useful* magnification of any instrument (light or electron) is ultimately set by its resolving power.

1. Definition and types of magnification

- ▶ **Linear (lateral) magnification** — the ratio of the size of the image to the size of the object, measured perpendicular to the optical axis; this is what is meant when a microscope is said to give "×400" or "×1000".

- ▶ **Angular magnification** — the ratio of the angle subtended at the eye by the virtual image to the angle subtended by the object placed at the near point (25 cm); relevant to simple (single-lens) magnifiers.
- ▶ **Empty magnification** — magnification carried beyond the resolving power of the objective; the image becomes larger but no additional detail is revealed (e.g. digitally enlarging a light-microscope photograph on a screen).
- ▶ **Useful magnification** — magnification that is genuinely accompanied by an increase in perceptible detail; for the light microscope this ceiling is about $\times 1000$ – 1500 .

2. Total magnification of the compound microscope

- ▶ The object lies between the **condenser** and the **objective**. The objective (short focal length) forms a **real, inverted, magnified image** within the body tube; the **eyepiece (ocular)** re-magnifies this real image into a **virtual image** viewed about 25 cm from the eye.
- ▶ **Total (final) magnification**, $M = M_1 \times M_2$, where M_1 = linear magnification by the objective and M_2 = linear magnification by the eyepiece.
- ▶ In microscopes with a non-standard optical tube length (standard = 160 mm), an additional **tube-length/draw-tube factor** is multiplied in — e.g. objective $\times 40 \times$ tube factor $\times 1.25 \times$ eyepiece $\times 10$ gives a total magnification of $\times 500$.
- ▶ Ordinary biological light-microscope objectives range from about $\times 4$ to $\times 100$ (oil-immersion); the eyepiece is conventionally $\times 10$.

Objective	Eyepiece	Final (total) magnification
2×	10×	20×
4×	10×	40×
10×	10×	100×
20×	10×	200×
40×	10×	400×
100× (oil-immersion)	10×	1000×

3. Resolving power — the true limit on useful magnification

- ▶ **Resolution (D)** = the smallest distance between two points at which they can still be perceived as two separate entities rather than one; it — not magnification — is the true determinant of image quality, since the objective lens fixes the finest detail that can ever be shown, and the eyepiece can only enlarge (never sharpen) that image.
- ▶ Governed by the classical relation: $D = 0.61 \sigma / NA$, where σ = wavelength of the illuminating light and NA = numerical aperture of the objective.
- ▶ **Numerical aperture**, $NA = n \cdot \sin \mu$, where n = refractive index of the medium between the object and the front lens of the objective, and μ = half the angle of the cone of light entering the objective.

- Maximum attainable NA: **dry objective** ≈ 0.95 ; **water-immersion** ≈ 1.3 ; **oil-immersion** ≈ 1.5 . Immersion oil, with a refractive index close to that of glass, reduces refraction and light loss at the slide–objective interface, so it gives the highest NA and therefore the best resolution — hence the routine use of the $\times 100$ oil-immersion objective for critical fine detail.
- The maximal resolving power of a bright-field light microscope is about **$0.2\ \mu\text{m}$** , which is what sets its practical ceiling of $\times 1000$ – 1500 useful magnification; objectives of higher magnifying power are always designed to also carry a correspondingly higher NA/resolving power, otherwise the extra magnification would be "empty".

4. Magnification and resolution across microscope types

Electron microscopes use an electron beam whose wavelength is far shorter than that of visible light, giving roughly a **1000-fold gain in resolving power** over the light microscope — this is why they can be used at very much higher *useful* magnification.

Instrument	Illuminating source	Best resolving power	Useful/attainable magnification
Unaided human eye	Visible light	$\sim 0.2\ \text{mm}$	$1\times$
Bright-field light microscope	Visible light	$\sim 0.2\ \mu\text{m}$	up to $\times 1000$ – 1500
Transmission electron microscope (TEM)	Electron beam	~ 1 – $3\ \text{nm}$ on tissue sections (theoretical $\sim 0.05\ \text{nm}$)	up to $\times 400,000$ (isolated particles)
Scanning electron microscope (SEM)	Electron beam	$\sim 2.5\ \text{nm}$	high, surface detail only

□ Fig 1.3 — Components and light path of a bright-field microscope. (Junqueira's Basic Histology — Text & Atlas — 17e, p.30)

□ Fig 1.9 — Diagram of the optical path in a transmission electron microscope. (Ross Histology — A Text and Atlas — 7e, p.30)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A. Light path through a bright-field compound microscope (build-up of total magnification) — label, from light source to eye: Tungsten-halogen lamp → Collector lens → Field diaphragm → Sub-stage condenser → Specimen on mechanical stage → Objective lens (forms real, inverted, magnified image in body tube) → Revolving nosepiece → Eyepiece/ocular (forms magnified virtual image ~25 cm from eye) → Eye. Mark M_1 (objective magnification) and M_2 (eyepiece magnification) at the respective stages, and show total $M = M_1 \times M_2$.

B. Optical path of a transmission electron microscope (for comparison) — label: Electron gun (cathode) → Anode → Condenser (electromagnetic) lens → Specimen (ultrathin resin section) → Objective (electromagnetic) lens → Projection lens → Fluorescent screen / CCD detector, all enclosed within a high-vacuum column.

Applied / Clinical anatomy

- ▶ **Oil-immersion ($\times 1000$) magnification** is the diagnostic standard wherever fine cytological/microbial detail must be resolved — e.g. malarial parasites and AFB on a Ziehl-Neelsen smear, sperm morphology assessment — because only the oil-immersion objective's high NA (~1.5) delivers adequate resolving power at this magnification.
- ▶ **Empty magnification is a real pitfall** in practice — merely zooming into a digital/virtual-microscopy image, or photographing a light-microscope field and enlarging it, adds no genuine detail once the objective's resolving power has been exhausted; only a higher-NA objective (or a shorter-wavelength source, as in EM) genuinely improves resolution.
- ▶ **Determining true (actual) size of a structure:** since the observed image is enlarged by a known factor, actual size = measured/observed size $\div$ total magnification; in practice this is done by calibrating an eyepiece (ocular) micrometer against a stage micrometer of known scale for each objective in use, since the calibration factor changes with every objective-eyepiece combination.
- ▶ **Electron microscopy** is essential for ultrastructural diagnosis where light-microscope resolution (~0.2 μm) is inadequate — e.g. TEM evaluation of the glomerular basement membrane and podocyte foot processes in renal biopsies, and assessment of ciliary axonemal (9+2) ultrastructure in primary ciliary dyskinesia; SEM is used where three-dimensional surface topography (rather than internal ultrastructure) is required.
- ▶ **Virtual (digital slide) microscopy** displays a whole slide scanned at high resolution and viewed at any convenient on-screen magnification — but the true diagnostic resolution is still fixed at the scanning step (i.e. by the objective NA used during scanning), not by the on-screen zoom factor, another practical instance of the magnification-vs-resolution distinction.

High-yield exam pointers

- ▶ Magnification = ratio of image size to object size; total magnification of a compound microscope, $M = M_1$ (objective) $\times M_2$ (eyepiece).
- ▶ **Resolving power, not magnification, is the true limiting factor** of a microscope's image quality; the eyepiece enlarges but never sharpens the image formed by the objective.

- ▶ Formula: $D = 0.61\sigma/NA$ (D = resolution, σ = wavelength, NA = numerical aperture); $NA = n \cdot \sin \mu$.
- ▶ Maximum NA: dry objective ~ 0.95 , water-immersion ~ 1.3 , oil-immersion ~ 1.5 — hence oil-immersion gives the best resolution and is used at $\times 1000$.
- ▶ Light microscope resolving power $\approx 0.2 \mu\text{m}$, ceiling of useful magnification $\approx \times 1000$ – 1500 ; magnification beyond this is "empty magnification".
- ▶ Electron beam wavelength is far shorter than visible light $\rightarrow \sim 1000$ -fold better resolution $\rightarrow$ TEM resolution ~ 1 – 3 nm (isolated particles magnified up to $\times 400,000$), SEM $\sim 2.5 \text{ nm}$ (surface only).
- ▶ Actual object size = observed/measured size $\div$ total magnification; measured practically with a calibrated ocular micrometer against a stage micrometer.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology 2e; Bancroft's Theory & Practice of Histological Techniques; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e.

Coverage note: this is a discrete optics/microscopy-technique concept with no dedicated Gray's Anatomy chapter; grounding is drawn entirely from the specialty microscopy/histotechnique texts above, each of which treats magnification and resolving power in full quantitative detail.



Method of decalcification

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Decalcification is the histotechnical process of removing calcium salts from a hard tissue (bone, tooth, or a pathologically calcified soft tissue) so that it becomes soft enough to be cut on a microtome without damaging the knife or the tissue morphology. It is an essential pre-processing step, performed after fixation and before dehydration/paraffin (or resin) embedding, whenever the specimen contains hydroxyapatite crystals that would otherwise fracture the blade and tear the section. A correctly decalcified block preserves cellular and matrix architecture with minimal impairment of subsequent staining.

Aim and calcium-containing tissues

- ▶ **Aim of decalcification** (three-fold):
- ▶ Complete removal of calcium salt from the tissue.
- ▶ No damage to tissue morphology.
- ▶ No significant impairment of subsequent staining.
- ▶ **Tissues requiring decalcification:**
- ▶ Bone (biopsy, resection, femoral/knee replacement specimens).
- ▶ Tooth (for ground/ paraffin sections).
- ▶ Pathologically calcified soft tissue — e.g., a tuberculous lymph node, dystrophic calcification, calcified tumours such as teratomas.

Requisites for successful decalcification

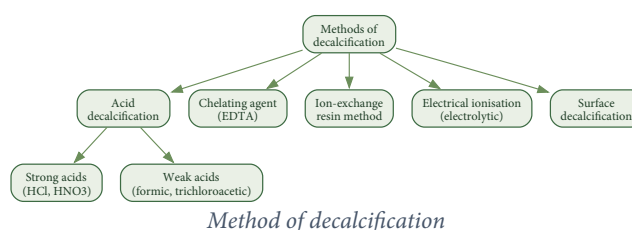
- ▶ **Adequate prior fixation** — bone must be *completely* fixed (10% neutral buffered formalin) before any acid decalcifying agent is applied; incomplete fixation lets the acid macerate the tissue. Alcohol-containing fixatives are avoided before acid decalcification as alcohol slows/prevents the reaction, and chloroform- or mercury-containing fixatives (Carnoy's, B5, Zenker's, Susa's) are avoided if the specimen is to be radiographed, since they render bone radio-opaque.
- ▶ **Small tissue pieces** — ideal slab thickness ~3–5 mm (up to 6 mm); thicker pieces prolong both decalcification and later wax infiltration, while pieces <2 mm become brittle.
- ▶ **Thorough washing** of the fixed tissue before immersion in the decalcifying fluid.
- ▶ **Adequate volume of decalcifying agent** — a large fluid : tissue ratio (about 20 : 1, or ~100 mL of fluid per gram of tissue) prevents exhaustion of the acid/chelator; the fluid should be changed periodically (ideally daily with endpoint testing) if a smaller volume is used.
- ▶ **Suitable choice of decalcifying agent**, governed by four inter-dependent factors: urgency of the case, degree of mineralisation of the bone, extent of the investigation required, and the staining/IHC techniques planned.
- ▶ **Correct end-point detection** to avoid under- or over-decalcification.

Factors controlling the rate of decalcification

Factor	Effect on rate
Concentration of decalcifying agent	Higher concentration → faster decalcification, but more tissue damage
Temperature	Higher temperature → faster reaction, but more damage to tissue/antigens
Agitation of the solution	Mild agitation increases the rate
Thickness/size of tissue	Thinner tissue decalcifies faster
Density/maturity of bone	Dense, mature cortical bone decalcifies slower than immature/trabecular bone
Volume of fluid used	Large volume (20 : 1) prevents depletion and maintains rate

Methods of decalcification

Five standard methods are recognised:



1. Acid decalcification — the commonest routine method; the acid converts insoluble calcium salts to soluble ones.

Agent	Concentration	Advantages	Disadvantages
Aqueous nitric acid	5–10%	Rapid action; good nuclear stain	Gives tissue a yellow colour (removable with urea); old acid is especially damaging
Nitric acid–formaldehyde	10% HNO ₃ + formalin	Rapid (1–3 days); less tissue damage/swelling; long water-washing not needed	Nuclear staining less good
Hydrochloric acid (Von Ebner's fluid: saturated NaCl 175 g + conc. HCl 15 mL, made up to 1000 mL)	8% (simple aqueous)	Rapid; ideal for tooth	Poor nuclear staining
Perenyi's fluid (10% HNO ₃ 40 mL + 0.5% chromic acid 30 mL + absolute alcohol 30 mL)	—	Excellent morphology; softens fibrous tissue	Slow; endpoint detection difficult
Formic acid (Gooding & Stewart: 5 mL formic acid + 5 mL 40% formalin + 90 mL water)	5–10% (aqueous)	Decalcifying agent of choice for routine work; gentler than mineral acids	Slow, may take many days
Trichloroacetic acid (5 g in 95 mL 10% formal saline)	—	Good for small biopsies; good nuclear stain	Unsuitable for hard bone; slow (4–5 days)

Strong (inorganic) acids act rapidly but damage tissue antigens/enzymes if used beyond 24–48 hours; weak (organic) acids, chiefly formic acid, are gentler and preferred when immunohistochemistry is planned. As a rule, 1 N/1 M solutions of HCl, nitric or formic acid are equivalent in strength; concentrated formic acid (~4 M) decalcifies about twice as fast as a 1 M solution without added tissue damage.

2. Chelating agent — EDTA (ethylenediaminetetraacetic acid): the standard chelating decalcifier. It binds calcium of the hydroxyapatite crystal surface to form a non-ionised soluble complex, so the crystal shrinks progressively rather than being dissolved by acid. Action is slow and gentle (pH-dependent, works best at pH 7–7.6) — dense cortical bone may take 6–8 weeks, though small spicules decalcify within a week. *Solution:* EDTA 5.5 g + formalin 100 mL + distilled water 900 mL (aqueous/buffered EDTA disodium 10% or tetrasodium 14% preparations are also used).

- Advantages: best morphological preservation; permits ancillary techniques (immunohistochemistry, FISH, enzyme histochemistry, electron microscopy); ideal for **bone marrow trephine biopsy** (preserves glycogen).
- Disadvantages: very slow; pH ~7 must be maintained; requires thin tissue.

3. Ion-exchange resin method — a sulfonated polystyrene cation-exchange resin is combined with a weak organic acid decalcifying fluid. The resin (~1 cm layer) is placed at the bottom of the container and the specimen laid over it, submerged in the weak acid; the resin continuously replaces Ca²⁺ with H⁺,

keeping the acid from becoming saturated. Faster decalcification with morphology preserved (weak acid used); resin activity falls after 3 uses and is regenerated by washing twice in 10 N HCl.

4. Electrical ionisation (electrolytic) method — the specimen is suspended in an electrolytic solution (distilled water 820 mL + 88% formic acid 80 mL + HCl 100 mL) between a carbon cathode and a platinum anode connected to a power unit; the electric current accelerates ionisation and migration of Ca^{2+} . Very rapid — bone can be decalcified in 5–6 hours — but heat generated by the current can damage delicate tissue/architecture, and only one specimen can be processed at a time.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Electrolytic (electrical ionisation) method of decalcification of bone — label:

- ▶ Power unit (DC source)
- ▶ Platinum anode
- ▶ Carbon cathode
- ▶ Bone specimen (suspended between electrodes)
- ▶ Electrolytic decalcifying solution (formic acid + HCl in water) in the container

5. Surface decalcification — the surface of a paraffin block (containing unrecognised residual calcium) is inverted into 1% HCl for 1 hour; only the exposed top ~30 micron layer is decalcified, so only the first few cut sections are free of calcium. The block must be washed thoroughly before sectioning.

Endpoint determination of decalcification

Four methods are used to judge completeness:

- ▶ **Radiographic examination** — the most accurate method; a pre-decalcification radiograph is compared with serial radiographs during the process (e.g., using a cabinet X-ray unit such as a Faxitron) until no residual calcium density is seen. Accurate but costly, and requires a baseline film.
- ▶ **Microradiography** — a high-resolution radiographic technique that maps bone mineral density and distribution; de-mineralised areas appear yellowish-grey to black.
- ▶ **Chemical test** (used with weak-acid decalcifiers such as formic acid): 5 mL of the used decalcifying fluid is mixed with 5 mL of an ammonium hydroxide–ammonium oxalate reagent and left overnight; a calcium-oxalate precipitate indicates residual calcium (decalcification incomplete), while a clear solution on two successive tests indicates the endpoint has been reached.
- ▶ **Physical test** — the tissue is bent, or a pin is pushed into it; adequate decalcification allows easy bending/penetration. Crude and damaging (may itself distort the tissue), so it is used only when other methods are unavailable.

□ Fig 17.5 — An example of a Faxitron X-ray (a) before decalcification and (b) after decalcification is completed. The bright white area is calcium; as it is removed the bone becomes more translucent. (Bancroft's Theory & Practice of Histological Techniques, p.300)

Effects of faulty decalcification

Under-decalcification	Over-decalcification
Incomplete paraffin impregnation	Loss of nuclear detail
Section difficult to cut	Damage to the cell membrane
Bony dust seen on the knife/section	Loss of cell glycogen
—	Tissue swelling

Applied / Clinical anatomy

- ▶ **Bone biopsy reporting** (marrow, tumour, infection): acid decalcification is used for urgent diagnostic turnaround, while EDTA is preferred when immunohistochemistry or molecular studies are needed afterwards.
- ▶ **Bone marrow trephine biopsy** is the classic indication for EDTA decalcification because it preserves cell glycogen and antigenicity for panels such as CD markers.
- ▶ **Dental/tooth histology and ground sections** commonly use Von Ebner's fluid (rapid, ideal for tooth) despite its poorer nuclear staining.
- ▶ **Forensic/museum bone specimens** and teaching wet/dry preparations rely on the same acid-decalcification principles used in the museum-preservation curriculum (Paper III).
- ▶ **Faxitron/radiographic endpoint testing** is routine in busy orthopaedic-pathology laboratories to standardise decalcification time and avoid both under- and over-decalcification artefacts that can mimic pathology on H&E.
- ▶ **Excess acid decalcification** impairs special stains and immunostaining (nuclear chromatin fails to take up haematoxylin; eosin gives an unpleasant brick-red tinge) — a reason many labs prefer buffered or weak-acid/EDTA regimens for panels requiring immunohistochemistry.

High-yield exam pointers

- ▶ Decalcification = removal of calcium salt from bone/tooth/calcified tissue **without damaging morphology or staining**.
- ▶ Five methods: **acid, chelating (EDTA), ion-exchange resin, electrical ionisation, surface decalcification**.
- ▶ Acid decalcification is commonest; strong acids (HNO₃, HCl, 5–10%) are rapid but damaging; weak acids (formic acid) are the routine agent of choice — gentler, slower.
- ▶ **EDTA** is the standard chelating agent — slow (weeks), pH 7–7.6, but gives the best morphology/antigen preservation; ideal for bone marrow trephine biopsy.
- ▶ Electrolytic method is fastest (5–6 h) but heat can damage tissue; only one specimen at a time.
- ▶ Fluid : tissue ratio ~20 : 1 (or 100 mL/g); complete fixation must precede acid decalcification.
- ▶ Endpoint tests: radiographic (most accurate), microradiography, chemical (ammonium oxalate test), physical (bending/pin — crude).
- ▶ Under-decalcification → hard/bony dust, poor sectioning; over-decalcification → loss of nuclear detail, swelling, loss of glycogen.

SOURCE & COVERAGE

Pranab Dey — *Basic and Advanced Laboratory Techniques in Histopathology and Cytology*, 2e (Ch. 4, "Decalcification of Bony and Hard Tissue for Histopathology Processing"); Bancroft's *Theory & Practice of Histological Techniques* (Ch. 17, "Bone" — Decalcification, Decalcifying agents, Fine-detail specimen radiography).



Microscopy

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction Microscopy is the branch of technique that uses instruments (microscopes) to visualise structures too small to be resolved by the unaided human eye, and it is the fundamental tool of histology and microscopic anatomy. Microscopes are broadly of two types depending on the illuminating source — **light (photon-based) microscopes** and **electron microscopes** — each with several specialised variants suited to different tissue and research needs. The performance of any microscope is judged not by magnification alone but chiefly by its **resolving power**, i.e. its ability to display fine detail as separate entities.

Basic optical principles

- ▶ **Magnification** — the ratio by which the image is enlarged over the real object; in a compound microscope it is the product of the objective and ocular (eyepiece) magnifying powers (e.g. $\times 40$ objective $\times \times 10$ eyepiece = $\times 400$ total).
- ▶ **Resolving power (resolution)** — the smallest distance between two points at which they can still be seen as separate objects; it depends on the **wavelength (λ) of the illuminating source** and the **numerical aperture (NA)** of the objective, following the classical relation $d = 0.61\lambda/\text{NA}$ (smaller d = better resolution).
- ▶ **Numerical aperture (NA)** = refractive index of the medium between lens and object $\times$ sine of the half-angle of the cone of light entering the objective. Maximal attainable NA is about **0.95 for a dry objective**, **1.3 with water**, and **1.5 with oil immersion** — hence oil-immersion objectives give the best resolution.
- ▶ The maximal resolving power of the light microscope is about **0.2 μm** , permitting clear images up to $\times 1000$ – 1500 ; magnification beyond this ("empty magnification") adds no further detail because resolution, not enlargement, is the limiting factor.
- ▶ **Köhler illumination** (even, centred illumination with the field diaphragm image focused on the object plane) is used for photomicrography and critical work; simple **critical illumination** is used in basic instruments but gives uneven lighting.

Preparation of tissue for microscopic study (brief)

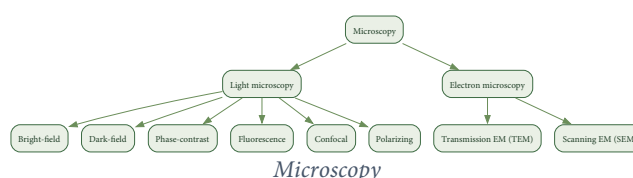
Step	Purpose
Fixation	Cross-links/denatures proteins, inactivates autolytic enzymes (e.g. formalin for LM; glutaraldehyde ± osmium tetroxide for EM)
Dehydration	Graded alcohols remove water
Clearing	Organic solvent miscible with both alcohol and embedding medium
Infiltration & embedding	Paraffin (LM) at 52–60 °C, or epoxy resin (EM)
Sectioning	Microtome — 3–10 µm sections for LM; ultramicrotome with glass/diamond knife — 40–90 nm sections for TEM
Staining & mounting	Dyes render otherwise colourless tissue visible; coverslip applied

(Fixatives, staining and microtomy are each examined separately in detail elsewhere in this bank; only the essentials relevant to microscopy are given here.)

Types of light microscopy

Type	Principle	Chief use
Bright-field	Ordinary transmitted light through a stained section, via condenser–objective–eyepiece	Routine H&E histology
Dark-field	Direct light blocked; only light scattered/diffracted by the specimen enters the objective, giving a bright image on a dark background	Unstained/living material, spirochaetes, autoradiographic grain counting
Phase-contrast	Exploits differences in refractive index; the specimen retards light by $\frac{1}{8}$ – $\frac{1}{4}$ λ , converted by a phase plate into visible contrast without staining	Living, unfixed cultured cells
Differential interference contrast (Nomarski)	Modification of phase-contrast using polarised beams	Gives a pseudo-3-D image of live cells
Fluorescence	UV/short-wavelength excitation of a fluorochrome (DAPI, FITC, acridine orange) causes emission of longer-wavelength visible light	Immunofluorescence, nucleic-acid probes
Confocal	A pinhole aperture placed confocal with a scanning laser point-source excludes out-of-focus light, allowing thin "optical sections"	3-D reconstruction, precise subcellular localisation

Type	Principle	Chief use
Polarizing	Specimen placed between two crossed polarising filters (polarizer + analyzer); only birefringent (regularly-oriented) structures appear bright on a dark field	Collagen, amyloid, crystals (see Applied anatomy)



□ Fig 1.3 — Components and light path of a bright-field microscope. (Junqueira's Basic Histology — Text & Atlas — 17e, p.30)

Electron microscopy

Electron microscopes exploit the much shorter wavelength of an electron beam (accelerated by **60–120 kV** between a tungsten cathode and anode) to give roughly a **1000-fold gain in resolution** over light microscopy.

- **Transmission electron microscopy (TEM)** — the beam is focused by electromagnetic "lenses" and passes *through* an ultrathin (40–90 nm), resin-embedded section; electrons that pass freely produce electron-lucent (bright) areas, while those absorbed/scattered by heavy-metal stains (osmium tetroxide, lead citrate, uranyl salts) produce electron-dense (dark) areas. Resolution is about **3 nm**; isolated particles can be magnified up to $\times 400,000$, and tissue sections are typically studied up to about $\times 120,000$.
- **Scanning electron microscopy (SEM)** — the beam does not traverse the specimen but is scanned point-by-point across its metal(usually gold)-coated surface; reflected and secondary electrons are captured to build a black-and-white image with a striking three-dimensional, shadowed surface appearance. SEM gives excellent depth of field for surface topography but does not reveal internal ultrastructure.

Feature	Light microscope	TEM	SEM
Source	Visible light	Electron beam (60–120 kV)	Electron beam
Best resolution	$\sim 0.2\ \mu\text{m}$	$\sim 3\ \text{nm}$	lower than TEM, surface only
Useful magnification	$\times 1000\text{--}1500$	up to $\times 400,000$ (particles)	high (surface)
Specimen	Paraffin section, 3–10 μm , stained with dyes	Resin section, 40–90 nm, heavy-metal "stained"	Whole surface, metal-coated
Image	Coloured	Black/white/grey (electron-dense vs lucent)	Black/white, 3-D shadowed

Feature	Light microscope	TEM	SEM
Vacuum needed	No	Yes	Yes
Living cells	Yes (phase-contrast)	No	No

□ Fig 1.7 — Working principle of a transmission electron microscope. (Sontakke — Textbook of Human Histology — 2e, p.26)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A. Bright-field compound light microscope — label: Eyepiece → Binocular tube → Revolving nosepiece → Objective lenses (×4, ×10, ×40, ×100-oil) → Mechanical stage with specimen holder → Sub-stage condenser → Field/aperture diaphragm → Collector lens → Tungsten-halogen lamp → Base/stand → Coarse & fine focus knobs → Illumination-intensity control.

B. Transmission electron microscope (working principle) — label: Electron gun (cathode) → Magnetic (condenser) lens → Sample → Transmitted electrons → Magnetic (objective) lens → Fluorescent screen/CCD detector.

Newer/special visualisation techniques

- ▶ **Autoradiography** — radiolabelled precursors (e.g. ^3H -thymidine) incorporated into macromolecules are localised by silver grains produced in a photographic emulsion coating the section; used to study DNA replication and secretory pathways, by LM or TEM.
- ▶ **Enzyme histochemistry** — cryostat sections (unfixed/lightly fixed, to preserve enzyme activity) are reacted with a substrate; an insoluble coloured end-product marks the enzyme's site (phosphatases, dehydrogenases, peroxidase).
- ▶ **Immunohistochemistry / in-situ hybridisation** — labelled antibodies (direct or indirect method) or labelled cDNA probes localise specific antigens or nucleic-acid sequences respectively; indirect methods amplify the signal and are more widely used.
- ▶ **Virtual (digital) microscopy** — a slide-scanner captures the whole stained slide as a tiled high-resolution digital image navigable on a computer, increasingly replacing glass slides for teaching.
- ▶ **Newer-generation EM (Gray's Anatomy 41e)** — cryofixation/vitrification preserves near-native ultrastructure without chemical fixation artefact; focused-ion-beam SEM and serial-section EM (e.g. the ATUMtome) allow large volumes to be reconstructed in three dimensions.

Applied / Clinical anatomy

- ▶ **Polarizing microscopy** is diagnostically important: Congo-red-stained **amyloid** shows pathognomonic apple-green birefringence; **monosodium urate crystals** (gout) show negative birefringence while calcium pyrophosphate crystals show weak positive birefringence, allowing crystal arthropathies to be distinguished on synovial-fluid microscopy; collagen and quartz are positively birefringent.
- ▶ **Frozen section (cryostat)** technique permits rapid intra-operative diagnosis (e.g. assessing a tumour margin) within minutes, and preserves lipids and labile enzymes lost during paraffin processing.
- ▶ **Electron microscopy** remains essential where light microscopy cannot resolve organelle-level pathology — e.g. renal biopsy evaluation of glomerular basement membrane and podocyte foot

processes, and assessment of ciliary ultrastructure (9+2 axoneme defects) in primary ciliary dyskinesia.

- ▶ **Immunohistochemistry** on routine sections is now the mainstay of tumour typing and diagnosis in surgical pathology.
- ▶ **Virtual/digital microscopy and telepathology** allow remote/expert second-opinion reporting and are increasingly used in PG teaching in place of physical slide collections.

High-yield exam pointers

- ▶ Resolving power (not magnification) is the true limiting factor of a microscope; light microscope resolution $\approx 0.2 \mu\text{m}$, oil-immersion NA up to 1.5.
- ▶ Formula: resolution (d) = $0.61\lambda/\text{NA}$ — shorter wavelength and higher NA both improve resolution.
- ▶ Types of light microscopy: bright-field, dark-field, phase-contrast, fluorescence, confocal, polarizing — know one principle and one use for each.
- ▶ TEM: transmitted electron beam, ultrathin (40–90 nm) resin sections, heavy-metal "staining" (osmium, lead, uranyl), resolution $\sim 3 \text{ nm}$.
- ▶ SEM: reflected/secondary electrons from a gold-coated surface, gives 3-D topographic image, no internal detail.
- ▶ Electron beam wavelength is far shorter than light, giving ~ 1000 -fold better resolution than LM.
- ▶ Polarizing microscopy: Congo red + amyloid = apple-green birefringence; urate crystals = negatively birefringent (gout).
- ▶ Cryostat frozen sections enable rapid intra-operative diagnosis and preserve lipids/enzymes lost in paraffin processing.

SOURCE & COVERAGE

Junqueira's Basic Histology — Text & Atlas 17e; Bancroft's Theory & Practice of Histological Techniques; Gray's Anatomy (Standring) 41e; Sontakke — Textbook of Human Histology 2e.



Microtome knives

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction — The microtome knife (or blade) is the cutting tool of a microtome that shears a thin, uniform ribbon of tissue from a wax- or resin-embedded block for microscopic study. It is made of high-quality carbon steel, tungsten carbide, glass or diamond, ground to a precise bevel so that its cutting edge is sharp enough to produce sections 2–10 μm thick without tearing, compressing or chattering the tissue. Selection of the correct knife type, material and angle setting is central to obtaining artefact-free sections and is a routine practical skill tested in histotechnology.

Classification based on shape (profile)

Profile	Shape	Features	Chief use
A — Plano-concave	One side plain, other side concave	Thin, very sharp blade	Originally for celloidin-embedded tissue; cuts soft tissue; now used infrequently
B — Bi-concave	Concave on both sides	Concavity hard to identify; less rigid, tends to vibrate during cutting	Used on the rocking microtome
C — Wedge	Plain on both sides	Most rigid and versatile; easy to sharpen	Most widely used knife for routine rotary/sliding microtomy; compatible with most microtome types
D — Tool-edge	Both faces plain but the edge is steep, resembling a woodworking chisel	Very stout; difficult to sharpen	Cutting hard tissue such as decalcified bone and resin-embedded blocks

□ Fig 5.3 — Different types of microtomy knives based on shape. Profile C is the most commonly used knife (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.67)

Classification based on material

- **Conventional (steel) knife** — high-quality carbon/steel, tempered from the cutting edge to about the inner one-third of the blade width; desirable hardness lies between **400 and 900 on the Vickers hardness scale**. Heavier steel knives measure ~16–18 cm for small (rotary) microtomes and up to 200–300 cm for base-sledge microtomes (e.g. the Polycut, for large blocks/whole-organ mounts). Needs periodic honing/stropping.
- **Tungsten-carbide knife** — used for **undecalcified bone** cryotomy and plastic (resin)-embedded tissue; unlike steel it must be professionally reconditioned rather than simply re-honed after repeated sharpenings.
- **Diamond knife** — costly; used to cut epoxy-resin blocks for **electron microscopy** (ultramicrotomy); needs special/specialised sharpening.
- **Glass knife** — also used in ultramicrotomy for very hard/resin-embedded tissue; its cutting edge lies parallel to one surface of a broken glass strip; disposable and cheaper than diamond.

Classification based on disposability

Type	Sub-type	Use	Clearance angle
Permanent (reusable) knife	Steel/tungsten-carbide	Routine and hard-tissue work; requires honing + stropping	Adjustable, 2°–5°
Disposable blade	Low-profile blade	Small biopsies, soft to moderately firm tissue	3°–5°
	High-profile blade	Firm to hard tissue (e.g. uterus, heart); slightly thicker/wider — chatters/vibrates less on dense tissue	5°–7°

- **Advantages of disposable blades:** replaced within seconds; no sharpening required; overall running cost is low as no knife sharpener/abrasive is needed; give a reliably sharp edge producing near-flawless 2–4 µm sections; some carry a PTFE coating that eases ribbon formation.
- **Disadvantages:** less rigid than a solid steel knife, so vibration artefact can occur; extremely hard tissue may still defeat a disposable blade; over-tightening in the clamp can itself produce thick-and-thin section artefact.

Angles of the microtome knife

Angle	Definition	Typical value	Significance
Rake angle	Angle between the knife's upper bevel and the perpendicular line dropped from the block surface	—	A higher rake angle eases cutting and gives smooth ribbon flow; a lower rake angle is used for hard tissue
Angle of clearance	Angle between the knife's lower bevel and the face of the block	~2°–5° (conventional); 3°–5° low-profile / 5°–7° high-profile disposable blades	Governs friction/compression between knife and block — too low → knife rubs/heats the block; too high → block chatters
Bevel (knife-edge) angle	Angle between the two bevelled planes of the knife	18°–30°	Smaller bevel angle → sharper knife, but less durable edge
Cutting edge	The line formed by intersection of the two bevel planes	—	The actual sectioning edge

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple side-on schematic (after the standard "angles of the knife" figure): the **paraffin block** with its **upper surface**; the **knife** shown in cross-section with its **upper bevel** and **lower bevel** meeting at the **cutting edge**; a **perpendicular line** dropped from the block surface; the **rake angle** marked between the upper bevel and this perpendicular; the **angle of clearance** marked between the lower bevel and the block surface; and the **cutting angle** (rake + clearance) marked at the point of contact.

Sharpening of the microtome knife

- ▶ **Honing** — abrasive grinding (iron oxide, aluminium oxide or silicon carbide) on a stone lubricated with light oil; the knife is held by its handle in one hand, its front tip in the other, and pushed forward diagonally across the stone several times on each face. Restores a keen edge on a blunted conventional knife.
- ▶ **Stropping** — done after honing; polishes the cutting edge and removes any "burr" (a fine metallic wire-edge) raised during honing. The strop is made of clean, grit-free leather.
- ▶ **Automatic knife sharpener** — the knife is laid horizontally on a rotating glass or copper plate coated with abrasive; saves time in laboratories that use conventional knives routinely; a multi-plate design can also recondition tungsten-carbide knives, though these need reconditioning (not simple honing) after repeated sharpenings.

Factors affecting section cutting

- ▶ **Temperature** — chilling the block (and sometimes the knife) facilitates cutting, especially of fatty/soft tissue.
- ▶ **Angle of rake** — a higher rake angle gives smoother ribbon flow; a lower rake angle is preferred for hard/dense tissue.
- ▶ **Consistency of the tissue** — soft tissue is cut at a slower wheel speed than firm/hard tissue.
- ▶ **Clamping** — the knife-clamping device must be clean, defect-free and evenly (not over-) tightened; the hand-wheel must be turned slowly and steadily during cutting.

Applied / Clinical anatomy

- ▶ **Knife selection by specimen:** routine soft-tissue paraffin sections → disposable low-profile blade; firm organs (uterus, heart, prostatectomy) → high-profile disposable blade or wedge (Profile C) steel knife; **undecalcified bone** → tool-edge (Profile D) or tungsten-carbide knife on a base-sledge/cryostat microtome; **electron-microscopy resin blocks** → glass knife (routine trimming) or diamond knife (final ultrathin sections, 40–100 nm).
- ▶ **Artefact recognition (viva-relevant):** alternating **thick-and-thin sections (chatter)** result from a loose knife/block clamp, a blunt knife, too small a clearance angle, or soft paraffin sticking to the knife (Pranab Dey) — or, for a disposable blade, from an **over-tightened** clamping device (Bancroft). **Tears/scratches** in the section point to a nicked or jagged cutting edge. Correcting the clamp tension, adjusting the clearance angle, or re-honing/replacing the knife resolves these — a standard bench-troubleshooting exercise in the viva.

- ▶ **Safety:** the exposed steel or disposable blade is a major sharps-injury hazard in the histopathology laboratory; a guarded knife holder, disposal of used blades into a sharps bin, and standard universal precautions are mandatory.
- ▶ **Correlation with microtome type:** the base-sledge microtome (specimen fixed, knife travels) uses long steel knives and suits large/hard blocks and whole-organ or bone/eye specimens; the rotary (Minot) microtome, the commonest bench instrument, is matched to disposable low/high-profile blades or a wedge steel knife; the rocking microtome uses the bi-concave (Profile B) knife; the ultramicrotome for electron microscopy uses glass or diamond knives.

High-yield exam pointers

- ▶ Four shape-based profiles: **A-Plano-concave**, **B-Biconcave** (rocking microtome), **C-Wedge** (commonest), **D-Tool-edge** (hard tissue/bone).
- ▶ Materials: conventional **steel** (400–900 Vickers hardness), **tungsten carbide** (undecalcified bone/resin), **glass** and **diamond** (ultramicrotomy/EM).
- ▶ Disposable blades: **low-profile** (soft tissue, clearance 3°–5°) vs **high-profile** (firm/hard tissue, clearance 5°–7°); advantages = no sharpening, quick change, low running cost; disadvantage = less rigid.
- ▶ Four angles to define: **rake angle**, **angle of clearance** (2°–5° conventional), **bevel/knife-edge angle** (18°–30°, smaller = sharper), and the **cutting edge**.
- ▶ Sharpening sequence: **honoring** (coarse, restores edge) → **stropping** (leather, removes burr, polishes) → automatic sharpener (routine labs).
- ▶ Diamond knife is reserved for epoxy-resin EM blocks; glass knife is its cheaper ultramicrotomy alternative.
- ▶ Remember the artefacts a wrong clearance/rake angle or blunt knife produces (chatter, thick-thin sections, tearing) — a favourite viva follow-up.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology, 2e (Ch. 5, Tissue Microtomy: Principle and Procedure); Bancroft's Theory & Practice of Histological Techniques (chapters on Microtomy for paraffin and frozen sections, and on techniques for analyzing bone).



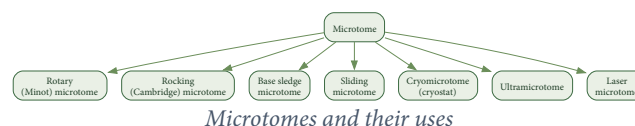
Microtomes and their uses

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction — A **microtome** (Greek *mikros* = small + *temnein* = to cut) is the principal instrument of the histology laboratory used to cut a paraffin-, resin- or frozen-embedded tissue block into uniformly thin sections (typically 2–10 μm for light microscopy, 40–100 nm for electron microscopy) suitable for mounting on a slide and microscopic study. Different clinical and research needs — routine diagnostic histopathology, rapid intra-operative reporting, undecalcified bone, whole-organ/brain blocks, or ultrastructural study — are met by different mechanical designs of microtome, each with its own knife, mechanism and characteristic uses. Correct choice of microtome, knife and cutting parameters is central to obtaining an artefact-free section and is a core practical skill examined in anatomy/histotechnology.

1. Classification of microtomes

Type	Mechanism	Typical section thickness
Rotary (Minot) microtome	Knife fixed; block moves vertically past it by rotation of a 360° hand-wheel	2–4 μm (routine 3–4 μm)
Rocking (Cambridge) microtome	Knife fixed; block moves in an arc-like rocking motion	Thin, slightly curved ribbons
Base sledge microtome	Block fixed in a carriage; knife slides to and fro over it	>10 μm (thick sections)
Sliding microtome	Knife fixed; block slides horizontally under it	Larger sections, for celloidin blocks
Cryomicrotome / cryostat	A rotary (or rotary-rocking) microtome housed in a refrigerated cabinet; tissue is frozen instead of wax-embedded	4–10 μm
Ultramicrotome	Precision advance; glass/diamond knife	40–100 nm (electron microscopy)
Laser microtome	Infra-red laser beam cuts unembedded, unprocessed fresh tissue	Variable; no thermal artefact



2. Traditional (paraffin-section) microtomes

- **Rotary microtome ("Minot")** — named after its inventor; a fine advance hand-wheel is rotated through 360°, moving the paraffin block vertically past a fixed knife and returning it to the starting position for the next stroke. It may be **manual** (fully hand-operated), **semi-automated** (one motor drives the fine or coarse hand-wheel) or **fully automated** (motors drive both fine and coarse advance); the block-advancement mechanism may be **retracting** or **non-retracting**.

- *Advantages* — cuts good-quality 2–3 μm thin sections; produces a continuous tissue ribbon; easily adapted to firm, fragile or small-biopsy tissue; automation reduces repetitive-motion occupational disorders in technologists.
- *Disadvantages* — expensive; unsuitable for very large blocks; the knife faces upward, a sharp hazard to staff.
- This is the **commonest bench instrument** in a routine histopathology/histology teaching laboratory.
- **Rocking microtome (Cambridge rocking microtome)** — one of the oldest microtome designs; the knife is static and the tissue block is moved in a rocking, arc-like motion. Produces thin ribboned, serial sections (useful for reconstruction studies), but the sections are **slightly curved**, not perfectly flat.
 - *Advantages* — simple, low-cost, reliable, easy to operate.
 - *Disadvantages* — sections are curved rather than flat; the light instrument may vibrate.
- **Base sledge microtome** — the specimen is held stationary in a steel carriage while the knife slides to and fro over its surface. Best suited to **large or hard tissue blocks**.
 - *Advantages* — can cut hard tissue and large samples; the microtome of choice for **ophthalmic** (whole eye) and **large neuropathological** (whole-brain) blocks.
 - *Disadvantages* — difficult to obtain thin ($<10\ \mu\text{m}$) sections; requires large glass slides.
- **Sliding microtome** — here the knife is static and the block itself slides horizontally beneath it.
 - *Advantages* — cuts large sections; simple design, easy maintenance; the traditional instrument for **celloidin-embedded** tissue and gives good **brain sections**.
 - *Disadvantages* — the knife may glide/jump on hard tissue; long knives are difficult to sharpen.

□ Fig 5.1 — Semi automated rotary microtome (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.65)

3. Specialised microtomes — cryostat and ultramicrotome

- **Cryomicrotome (cryostat)** — a refrigerated cabinet housing a modified rotary (often **rotary-rocking**) microtome, with all controls operated from outside the chamber; the retracting action on the rocking variant withdraws the block from the knife on the upstroke, giving a flat cut face. The cabinet temperature ranges from about 0°C to -35°C ; most tissue sections best between -15°C and -25°C , water-rich tissue at the warmer end and fat-rich tissue needing a colder setting, while pre-fixed tissue sections best at a warmer -7°C to -12°C . The block is mounted on a metal **chuck/specimen holder** using an **OCT (optimum-cutting-temperature) compound** — a water-soluble glycol-and-resin embedding medium — and cut with a low/high-profile disposable blade or a steel (C-profile) knife at a 5° – 7° **clearance angle**; an **anti-roll plate** (or a chilled sable brush) flattens the section as it is cut.
 - *Advantages* — rapid section for immediate intra-operative diagnosis; preserves labile enzymes and antigens destroyed by heat/fixation, permitting **enzyme histochemistry, immunofluorescence and demonstration of fat/carbohydrate**; useful for nerve biopsy.
 - *Disadvantages* — needs continuous supervision to maintain temperature; freezing artefacts (ice-crystal holes) are common; a very expensive instrument; fixed tissue is difficult to cut well.

- **Ultramicrotome** — used exclusively to cut **ultrathin sections for transmission electron microscopy (TEM)**, at **40–100 nm** thickness, with a **glass or diamond knife**. The resin-embedded tissue is first trimmed to a tiny $\sim 1 \text{ mm} \times 1 \text{ mm}$ block, sectioned under an optical microscope, and the ribbon of sections is floated on the water held in the knife's "boat" before being picked up on a metal grid. (Bancroft records comparable EM-section ranges: $\sim 40\text{--}90 \text{ nm}$ for routine TEM and $50\text{--}150 \text{ nm}$ for cryo-ultramicrotomy at temperatures down to about -180°C .)
- **Laser microtome** — an ultramodern instrument in which an **infra-red laser beam** of ultra-short pulse duration cuts the biological specimen **without any prior processing or embedding**; because almost no heat is generated, the tissue is cut without thermal artefact.

□ Fig 5.2 — Cryomicrotome used in frozen section (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.66)

4. Microtome knives and the sectioning procedure

- **Knife/blade selection** is matched to the microtome and tissue: the **wedge (Profile C)** knife, plain on both sides, is the most widely used all-purpose blade; the **bi-concave (Profile B)** knife is used on the rocking microtome; the **plano-concave (Profile A)** knife suits soft/cellogidin tissue; the **tool-edge (Profile D)** knife, resembling a chisel, is reserved for hard tissue such as decalcified bone. **Disposable low-profile blades** (clearance $3^\circ\text{--}5^\circ$) suit small/soft biopsies, **high-profile blades** (clearance $5^\circ\text{--}7^\circ$) suit firm organs; conventional steel knives (400–900 on the Vickers hardness scale) need periodic **honing** (abrasive stone) and **stropping** (leather, to remove the burr); **glass and diamond knives** are reserved for ultramicrotomy.
- **Steps in cutting a paraffin section**: (1) **trimming** the block till the tissue is fully exposed; (2) **cooling** the block on ice for 15–20 min; (3) **cutting** at the set thickness with a smooth, slow, even stroke to form a ribbon — Pranab Dey sets cellular tissue such as lymph node at $\sim 4 \mu\text{m}$ and routine sections at $\sim 6 \mu\text{m}$, while Bancroft recommends $3\text{--}4 \mu\text{m}$ for routine surgical material, so the exact micrometer setting is tissue- and laboratory-protocol dependent; (4) **floating** the ribbon on a water bath kept $6\text{--}10^\circ\text{C}$ below the paraffin's melting point; (5) **picking up** the flattened section on a glass slide; (6) **drying** the slide in a hot-air oven; (7) **storing** the block under fresh wax to prevent drying.
- **Common artefacts and causes** (viva-relevant): thick-and-thin sections ("chatter") from a loose clamp, blunt knife or too small a clearance angle; curved ribbons from non-parallel block edges; tissue splitting from a nicked knife edge; holes from air bubbles in an unevenly heated water bath.

5. Uses of microtomes

Microtome	Principal use
Rotary (Minot)	Routine diagnostic paraffin histopathology ; teaching histology slides; thin serial sections for most tissue types
Rocking (Cambridge)	Thin serial-ribbon sections for reconstruction studies (embryology/neuroanatomy)

Microtome	Principal use
Base sledge	Large or hard blocks — whole eyes (ophthalmic pathology), whole-brain/neuropathology sections, undecalcified bone
Sliding	Celloidin-embedded research tissue; good-quality brain sections
Cryomicrotome/cryostat	Rapid intra-operative diagnosis (including Mohs micrographic surgery margins and sentinel-lymph-node assessment), enzyme histochemistry, immunofluorescence, fat/carbohydrate demonstration
Ultramicrotome	Ultrathin sectioning for TEM/ultrastructural study
Laser microtome	Cutting fresh, unprocessed tissue with no thermal or embedding artefact

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple side-on schematic of the **rotary (Minot) microtome**: **fine-advance hand-wheel** (rotated 360° to move the block) → **specimen clamp/chuck** holding the paraffin block → **knife holder/clamp** with the fixed **knife** → **base/carriage** on which the block travels vertically past the knife → **coarse-feed lever** (for rough trimming) and **micrometer thickness-setting dial**. A second small inset may show the **cryostat cabinet** enclosing the same mechanism, with the **anti-roll plate** in front of the knife.

Applied / Clinical anatomy

- **Frozen sections (cryostat)** are the anatomical/pathological basis of rapid intra-operative decision-making — assessing a **tumour resection margin**, confirming **sentinel lymph node** metastasis, or guiding **Mohs micrographic surgery** for skin cancer — decisions that must be available to the surgeon within minutes.
- **Base sledge and sliding microtomes** remain indispensable for **whole-organ museum/teaching specimens** (whole eye, whole brain), directly supporting the anatomy department's dissection-hall and museum teaching material.
- **Ultramicrotomy** underlies the ultrastructural diagnosis of renal glomerular basement-membrane disease and ciliary axonemal (9+2) defects, where light microscopy is insufficient.
- **Knife choice tracks tissue hardness**: soft biopsy → low-profile disposable blade; firm organ → high-profile blade/wedge steel knife; **decalcified bone** → tool-edge or tungsten-carbide knife on a base-sledge/cryostat; **EM resin blocks** → glass (trimming) or diamond (final ultrathin) knife.
- **Correct cryostat cabinet temperature** (colder for fatty tissue, warmer for pre-fixed tissue) and correct **water-bath temperature** for paraffin ribbons are classic bench quality-control points tested in the practical/viva.

High-yield exam pointers

- ▶ Seven traditional/special types: **rotary (Minot)**, **rocking (Cambridge)**, **base sledge**, **sliding**, **cryomicrotome/cryostat**, **ultramicrotome**, **laser microtome**.
- ▶ Rotary microtome = knife fixed, block moves vertically via a 360° hand-wheel; cuts routine 2–4 µm sections; commonest bench instrument.
- ▶ Base sledge = knife moves over a fixed block; best for **large/hard tissue** (eye, brain, bone).
- ▶ Sliding microtome = block moves under a fixed knife; suited to **celloidin-embedded** and brain tissue.
- ▶ Cryostat = refrigerated cabinet housing a modified rotary microtome; cabinet range 0 to –35°C, most tissue cut at –15 to –25°C; block mounted with **OCT compound**; enables **rapid intra-operative diagnosis**, **enzyme histochemistry**, **immunofluorescence**.
- ▶ Ultramicrotome = ultrathin **40–100 nm** sections for TEM, glass/diamond knife.
- ▶ Laser microtome = infra-red laser, no processing/embedding, no heat artefact — the newest addition to the classification.
- ▶ Match the knife to the microtome: wedge (Profile C, most common), bi-concave (rocking), plano-concave (soft/celloidin), tool-edge/tungsten-carbide (hard tissue/bone), glass/diamond (ultramicrotomy).

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology, 2e (Ch. 5, Tissue Microtomy: Principle and Procedure; Ch. 6, Frozen Section: Principle and Procedure); Bancroft's Theory & Practice of Histological Techniques (chapter on Microtomy for paraffin and frozen sections).
Coverage note: this is a lab-technique/instrument topic outside the scope of Gray's Anatomy and the standard gross-anatomy texts; grounding is drawn entirely from the two dedicated histotechnique manuals in the specialty library above.



Principles of staining

Asked in: Paper III Nov 2018 — RGUHS MD/MS Anatomy

Definition / Introduction — A fixed tissue section is essentially colourless because the fixed protein has almost the same refractive index as glass; **staining** is the deliberate application of a coloured substance (**dye**) that binds selectively to different tissue components so that morphology becomes visible under the microscope. A dye is a **chromogen** (an aromatic, usually benzene-ring-based, compound) made coloured by a **chromophore** group and made tissue-binding by an **auxochrome** group. Staining is fundamentally a physicochemical/chemical reaction between dye and tissue, governed by the dye's electrical charge, its chemical class, the bonds it forms with tissue, and several tissue- and procedure-related factors — together these constitute the "principles of staining."

1. Chromogen, chromophore and auxochrome

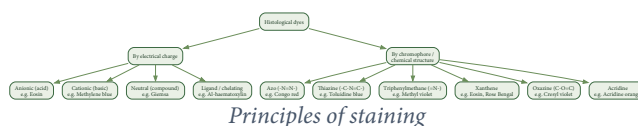
- ▶ **Chromogen**: benzene itself is colourless; replacing two H atoms of the ring with two O atoms yields **quinone**, a chromogen — the parent aromatic structure of a dye.
- ▶ **Chromophore group**: absorbs light in the visible range (400–800 nm) and imparts colour; it has free electrons that absorb specific wavelengths, and the dye emits the **complementary colour** of the light absorbed.
- ▶ **Auxochrome (ionising) group**: intensifies colour by donating additional electrons to the chromophore (allowing absorption of longer wavelengths) and is the part of the molecule that actually attaches the dye to the tissue.

2. Classification of dyes

(a) By electrical charge

Type of dye	Charge	Tissue bound (named "-philic")	Example
Anionic / acid dye	Negative (coloured anion); migrates to anode	Cytoplasmic proteins, collagen — acidophilic	Eosin
Cationic / basic dye	Positive (coloured cation); migrates to cathode	Nucleic acid, epithelial mucin — basophilic	Methyl green, ethyl green, Alcian blue
Neutral (compound) dye	Contains both acid + basic dye, combine by electron transfer	Both nucleus and cytoplasm	Giemsa (Romanowsky type — azure B = electron acceptor, eosin Y = electron donor)
Ligand / chelating dye	Weak acid, anionic, complexed with a metal ion ("metallochrome")	Nucleus and cytoplasm	Al ³⁺ –haematein (Harris', Mayer's haematoxylin); Fe ²⁺ –haematein (iron haematoxylin, for myelin/elastic fibres)

(b) By chemical structure / chromophore group



- ▶ **Azo dyes** ($-\text{N}=\text{N}-$ chromophore, mostly acidic): orange G, Congo red.
- ▶ **Thiazine dyes** ($-\text{C}-\text{N}=\text{C}-$ or $-\text{C}-\text{S}=\text{C}-$ group): toluidine blue, methylene blue.
- ▶ **Triphenylmethanes** ($=\text{N}-$ group): methyl violet, light green, malachite green.
- ▶ **Azin dyes** ($\text{C}-\text{O}=\text{C}$ and $\text{C}-\text{N}=\text{C}$ groups): celestine blue, Nile blue sulphate.
- ▶ **Diphenylmethanes** ($-\text{NH}$ group): auramine.
- ▶ **Xanthene dyes**: eosin, Rose Bengal, phloxine.
- ▶ **Oxazine dyes** ($\text{C}-\text{O}=\text{C}$ group): cresyl violet, celestine blue.
- ▶ **Acridine dyes**: acridine orange.
- ▶ **Anthraquinone dyes**: carminic acid.

3. Mechanisms (chemical bonds) of dye–tissue binding

Staining is the formation of a bond between dye and tissue strong enough to survive washing/dehydration. Recognised mechanisms:

- ▶ **Electrostatic (coulombic) bond**: the classical acid/basic dye–tissue attraction — colour ions of opposite charge to the tissue substrate attract (e.g., basic dyes bind phosphate/sulphate-rich nucleic acids and mucosubstances).
- ▶ **Van der Waals' attractions** (weak, non-coulombic): dipole–dipole interaction, dipole-induced-dipole interaction, and **dispersion (London) force** — important for large aromatic dyes/substrates, e.g. elastic-fibre staining by Miller's stain and Congo red.
- ▶ **Hydrogen bond**: a weak bond seen with, e.g., Best's carmine staining of glycogen.
- ▶ **Covalent bond**: two neutral atoms share electrons — basis of **Periodic Acid–Schiff (PAS)** staining of glycogen and the **Feulgen reaction**.
- ▶ **Hydrophobic bond/interaction**: not a true chemical bond (a form of van der Waals'/entropic effect); relevant to staining in aqueous solution and to **metachromatic** staining.
- ▶ **Dye aggregation**: dye molecules interact with each other (dye–dye interaction), favoured by high dye concentration, larger dye molecular size, and low temperature; the aggregate then penetrates the tissue.

4. Factors influencing staining intensity

Factor	Effect
Dye affinity to target tissue	Acid dyes (e.g. eosin) bind strongly to acidophilic (cytoplasmic protein) targets, minimally to basophilic substances; affinity is also modulated by pH and inorganic salt concentration of the solvent

Factor	Effect
Specimen geometry	Thick tissue → poor central penetration; paraffin sections (more even surface) stain better than cryostat sections; alcohol (coagulant fixative) disturbs microtopography and increases dye penetration; inner tissue geometry matters (e.g. bone-marrow canaliculi stain faster with Schmorl's thionine than adjacent connective tissue)
Target concentration	More target substance present → more intense staining
Rate of reaction	Short reaction time exposes fewer reactive groups (e.g. Feulgen reaction) → weaker staining
Rate of stain loss	Intentional loss = differentiation/de-staining ; excessive differentiation removes too much stain

5. Progressive and regressive staining; mordants and accentuators

- **Progressive staining:** dye is allowed to react only until the target structure is adequately stained; requires frequent microscopic checking and control of pH, tissue thickness and dye concentration to avoid over/under-staining.
- **Regressive staining:** tissue is deliberately **over-stained**, then selectively **differentiated** (excess dye removed) exploiting differing affinities of tissue components. Methods of differentiation: (1) acid in a basic dye or base in an acidic dye — e.g. haematoxylin removed from cytoplasm by 1% acid alcohol in Papanicolaou staining; (2) an **oxidising agent** (picric acid, potassium permanganate) that decolourises the dye; (3) a **mordant** that competes for and strips excess dye from tissue; (4) replacing the bound dye with another dye of lower affinity.
- **Mordant:** a multivalent metal ion (Al, Fe, Cu — divalent/trivalent) that bridges dye and tissue via covalent/coordinate ("chelation"/"lake") bonding, converting a weak dye (e.g. haematoxylin alone) into a strong one. Types: **pre-mordanting** (tissue treated with mordant first, e.g. Heidenhain's iron haematoxylin), **meta-mordanting** (mordant combined with dye, e.g. alum-haematoxylin solutions), **post-mordanting** (dye applied first, mordant after — not used in histopathology).
- **Accentuator vs mordant:**

	Mordant	Accentuator
Mechanism	Forms a bridge between dye and tissue	Only accelerates the dye–tissue reaction
Chemical role	Takes part in the reaction (forms a "lake")	Does not take part in any chemical reaction
Example	Alum + haematoxylin	Phenol in carbol fuchsin

6. Metachromasia

□ Fig 7.7 — Mechanism of dye aggregation in metachromasia. The cationic dye interacts with the polyanionic tissue and the bound water of the dye molecule is released; the aggregated dye-tissue complex changes the absorption of light (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.89)

"Meta" = altered, "chromasia" = colour — **metachromasia** is staining of a tissue in a **colour different from the dye's own colour**, without any change in the dye's chemical structure. Mechanism: the cationic dye is neutralised by tissue polyanions in aqueous solution, after which the non-polar aromatic rings of adjacent dye molecules bind each other by van der Waals' force, forming aggregates that absorb shorter wavelengths and shift the visible colour.

- ▶ **Metachromatic dyes:** mostly cationic — toluidine blue, methylene blue, azure A and B, methyl violet, brilliant cresyl blue; a few weakly metachromatic anionic dyes exist (Biebrich scarlet, bromophenol blue).
- ▶ **Types:** **alpha/orthochromatic** (monomeric dye → blue), **beta** (di-/trimeric form → purple), **gamma** (polymeric form → red); mixed orthochromatic-blue and polychromatic-red can give an intermediate purple.
- ▶ **Factors enhancing metachromasia:** higher dye concentration, low pH, decreased temperature, aqueous solvent.
- ▶ **Applications:** mast-cell granules, mucin, glycogen, amyloid, sulfatides.

7. Special nomenclature/categories

- ▶ **Colour Index (CI):** a unique code allotted by the Society of Dyers and Colourists to identify a dye unambiguously (e.g. CI 52000 = thionine), avoiding confusion from synonymous/duplicate dye names.
- ▶ **Total dye content (TDC):** the actual proportion of pure dye in a commercial dye powder (batch-variable; remainder is isomer/impurity) — must be corrected for when preparing a solution of known concentration.
- ▶ **Vital stain:** penetrates and stains living cells without killing them (methylene blue, Nile red); **supravital stain:** applied in vitro to living cells (e.g. brilliant cresyl blue for reticulocyte counting; also used in immunofluorescence). Vital DNA stains: Hoechst 33342, DAPI.
- ▶ **Argyrophil vs argentaffin:** **argyrophilic** substances bind silver salt and reduce it to metallic silver only with an **external reducing agent** (neuroendocrine granules — Grimelius silver stain); **argentaffin** substances reduce silver salt to metallic silver **without** an external reducing agent (melanin — Masson-Fontana stain).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled flow-schematic titled "Mechanisms of dye-tissue binding" with six boxes in sequence/branch: **Electrostatic (coulombic) bond** → **Van der Waals' forces (dipole-dipole / dipole-induced dipole / dispersion-London)** → **Hydrogen bond** → **Covalent bond** → **Hydrophobic interaction** → **Dye aggregation**; alongside, a small inset showing a **basic dye cation** binding a **negatively-charged (basophilic) nucleus** and an **acid dye anion** binding a **positively-charged (acidophilic) cytoplasmic protein**, mirroring the textbook's dye-tissue interaction schematic.

Applied / Clinical anatomy

- ▶ **Routine H&E:** hematoxylin (as an Al-mordanted "lake," a ligand dye) stains nuclei/basophilic structures blue-purple; eosin (an anionic xanthene dye) stains cytoplasm/collagen (acidophilic) pink — the everyday diagnostic application of acid/basic dye principles.
- ▶ **PAS reaction:** a covalent-bond-based stain used to demonstrate glycogen, basement membranes, mucin and fungal cell walls — diagnostically important in renal biopsy (BM thickening) and mycology.
- ▶ **Masson trichrome / connective tissue stains:** exploit differential dye affinity and differentiation to selectively colour collagen versus muscle, used to assess fibrosis.
- ▶ **Metachromatic stains** (toluidine blue) are used to identify mast cells and to screen for metachromatic leukodystrophy (sulfatide accumulation) on frozen sections/smears.
- ▶ **Reticulocyte counting** (supravital brilliant cresyl blue) and **argyrophil/argentaffin reactions** (Grimelius for neuroendocrine tumours; Masson-Fontana for melanoma) are direct clinical extensions of vital-staining and silver-impregnation principles.
- ▶ **Faulty differentiation** (under- or over-differentiation) is a common cause of poor-quality H&E and Papanicolaou smears in the histopathology/cytology laboratory, directly traceable to the regressive-staining principle.

High-yield exam pointers

- ▶ Dye = chromogen + chromophore (imparts colour) + auxochrome (imparts tissue affinity, ionising).
- ▶ Four dye classes by charge: **anionic/acidic** (acidophilic tissue), **cationic/basic** (basophilic tissue), **neutral/compound** (Giemsa), **ligand/chelating** (haematoxylin lakes).
- ▶ Six binding mechanisms: **electrostatic, van der Waals, hydrogen bond, covalent bond, hydrophobic interaction, dye aggregation**.
- ▶ **Progressive staining** = stain to endpoint and stop; **regressive staining** = overstain then **differentiate** (acid/base, oxidising agent, mordant, or dye-replacement).
- ▶ **Mordant** bridges dye-tissue via chelation ("lake") and takes part in the reaction; an **accentuator** merely speeds the reaction without reacting itself.
- ▶ **Metachromasia:** altered colour, same dye chemistry; alpha (blue, monomer) → beta (purple, dimer/trimer) → gamma (red, polymer).

- ▶ **Vital** stain colours living cells *in vivo*; **supravital** stain is applied *in vitro* to still-living cells.
- ▶ **Argyrophilic** = needs an external reducing agent to reduce silver; **argentaaffin** = reduces silver on its own (self-reducing).
- ▶ Colour Index (CI) gives each dye a unique numeric identity; Total Dye Content (TDC) corrects for batch impurity when preparing solutions.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (Ch. 7, "Staining Principle and General Procedure of Staining the Tissue"); Bancroft's *Theory & Practice of Histological Techniques* (Ch. "Theory of Histological Staining," Horobin). **Coverage note:** Gray's Anatomy (Standring, 41e) — the spine text for this bank — carries no histotechnique/staining content, as this is a laboratory-methods topic under Paper III (Microscopy); the answer is therefore grounded entirely in the specialty histotechnique texts above, per the topic spec.



Special stains

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction Routine haematoxylin and eosin (H&E) staining colours tissue on the broad basis of acidophilia/basophilia and cannot selectively demonstrate specific chemical constituents — carbohydrates, connective-tissue fibres, lipids, nucleic acids, pigments, amyloid or micro-organisms. **Special stains** are histochemical techniques that exploit a specific chemical or physical reaction between a dye (or metallic reagent) and a target tissue component to colour it selectively, distinct from and complementary to the surrounding tissue. They remain an essential, low-cost adjunct to immunohistochemistry in the routine histopathology laboratory for diagnosis, quantitation of fibrosis, and identification of organisms and abnormal deposits.

1. Principle of dye–tissue binding and classification

- ▶ A dye molecule has a **chromophore** group (imparts colour by absorbing visible light) and an **auxochrome** group (an ionising group that intensifies colour and anchors the dye to tissue).
- ▶ Dyes are classed by charge: **anionic/acid dyes** (e.g. eosin) bind basic, acidophilic tissue components (cytoplasm, collagen); **cationic/basic dyes** (e.g. methylene blue, Alcian blue) bind acidic, basophilic components (nucleic acid, acid mucin); **neutral dyes** (Giemsa) contain both; **ligand/chelating dyes** (haematoxylin with an Al or Fe mordant) bind via a metal bridge.
- ▶ **Metachromasia**: a tissue component stains in a colour different from the dye's own colour (e.g. mast-cell granules turn purple-red with toluidine blue) — due to polymerised dye stacking on closely-spaced polyanions (heparin, sulphated mucin).
- ▶ **Progressive staining** — the section is left in the dye only until the target achieves the desired intensity; **regressive staining** — the section is deliberately overstained and then selectively de-stained (differentiated) from unwanted sites by a mordant/acid, e.g. ferric chloride differentiation in Verhoeff's stain.

- **Classification of special stains** (by substance demonstrated — after Rosai): (1) carbohydrates/mucin, (2) connective-tissue fibres, (3) lipids, (4) nucleic acids, (5) pigments and minerals, (6) amyloid, (7) micro-organisms, (8) mast cells, (9) bone.



2. Stains for carbohydrates and glycoproteins (mucin)

Stain	Principle	Result
Periodic Acid–Schiff (PAS)	Periodic acid oxidises 1,2-glycol (–OH) groups of the carbohydrate to aldehyde (–CHO); the aldehyde reacts with Schiff's reagent (leuco-basic fuchsin) to form a magenta compound	Glycogen, neutral mucin, basement membrane, fungal capsule (Cryptococcus, Histoplasma) — magenta
Alcian blue	Copper-phthalocyanine dye with cationic isothiuronium groups binds anionic (carboxyl/sulphate) groups of acid mucin at pH 2.5	Acid mucin (sialomucin, sulphomucin), proteoglycans, hyaluronic acid — blue
Combined PAS–Alcian blue	Alcian blue first (acid mucin), then PAS (neutral mucin/glycogen)	Glycogen/neutral mucin magenta ; acid mucin blue
Mucicarmine (Southgate)	Aluminium-carmines chelate binds both neutral and acid mucin	Mucin, fungal (Cryptococcus) capsule — dark red
Colloidal iron	Positively charged colloidal ferric ion binds carboxyl/sulphate groups of acid mucopolysaccharide; potassium ferrocyanide then develops a Prussian-blue colour	Acid mucin blue ; collagen red, muscle yellow (Van Gieson counterstain)

Neutral mucin (stomach surface epithelium, Brunner's glands) is PAS-positive/Alcian-blue-negative; weakly acidic sialomucin is Alcian-blue-positive at pH ~2.5–2.8; strongly sulphated mucin needs a lower pH (~0.5–1.0) to stay Alcian-blue-positive while becoming PAS-negative — the basis of differentiating intestinal-type (sulphomucin-rich, malignancy-prone) from gastric-type metaplasia.

3. Stains for connective-tissue fibres

Stain	Collagen	Elastic fibre	Muscle	Nuclei	Chief use
Van Gieson (picro-fuchsin)	Red	—	Yellow	Black	Quantifying fibrosis; fibrosarcoma vs leiomyosarcoma

Stain	Collagen	Elastic fibre	Muscle	Nuclei	Chief use
Masson trichrome	Blue/green	—	Red	Black/blue	Liver fibrosis staging, cardiac/renal biopsy, soft-tissue tumours
Gordon & Sweet's silver impregnation	Reticulin (collagen III) → black	—	—	Red (counterstain)	Liver architecture/early cirrhosis, bone-marrow fibrosis, lymph-node architecture, tumour reticulin patterns
Verhoeff (– Van Gieson)	Red (counterstain)	Black	Yellow	Black	Elastic-tissue diseases, vessel wall
Weigert's resorcin-fuchsin	—	Purple	—	—	Elastic fibres (alternative)
Orcein	—	Brown	—	—	Elastic fibres; also HBsAg
Phosphotungstic-acid haematoxylin (PTAH)	Brown-pink	—	Cross-striations/fibrin blue	Blue	Fibrin thrombi, muscle cross-striation, glial fibres

Reticulin fibres are argyrophilic **type III collagen**; the Gordon-Sweet method oxidises their surface carbohydrate to aldehyde, which reduces ammoniacal silver to metallic black silver, toned with gold chloride. Diagnostic reticulin patterns: "weave-basket" in endometrial stromal sarcoma, "chicken-wire" in myxoid liposarcoma, "Zellballen" nesting in paraganglioma, abundant fibres around individual cells in glioma/sarcoma, and characteristic loss of reticulin in Ewing's sarcoma.

Fig 1.5 — A Masson trichrome stain of a renal biopsy taken for diagnostic histopathology. The arrangement of collagen (blue-green) and glomerular basement membrane can be assessed against tubular/vascular smooth muscle (red). (Gray's Anatomy — Standring — 41e, p.237)

□ Fig 1.5 — A Masson trichrome stain of a renal biopsy taken for diagnostic histopathology.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a low-power renal cortex field and label: **glomerular tuft** (capillary loops), **Bowman's capsule and space**, **mesangium**, **glomerular basement membrane** (blue-green), **proximal/distal tubules** (red-pink cytoplasm, blue-black nuclei), **interstitial collagen** (blue-green), **blood-vessel smooth muscle** (red). Colour key: collagen/basement membrane = blue-green, muscle/cytoplasm/fibrin = red, nuclei = black-blue.

4. Stains for lipids, nucleic acids and pigments

Target	Stain	Result	Note
Neutral lipid/triglyceride	Oil red O	Red	Needs fresh/frozen section — processed (alcohol/xylene) tissue dissolves the fat; useful in renal cell carcinoma, lipoblasts
Lipid (general)	Sudan black B	Black	More sensitive than Oil red O; frozen/air-dried section
Phospholipid	Ferric haematoxylin	Blue	Frozen section
DNA	Feulgen reaction	Reddish-purple	Acid hydrolysis exposes deoxyribose aldehyde group, which binds Schiff's reagent — used for DNA-ploidy studies
DNA + RNA	Methyl green–pyronin	DNA bluish-green, RNA red	RNA-rich cells (e.g. plasma cells) are strongly pyroninophilic
Haemosiderin	Perls' Prussian-blue (Pearl's reaction)	Blue (nuclei red)	Ferric iron + potassium ferrocyanide → insoluble ferric ferrocyanide
Bile pigment	Fouchet's stain	Green (collagen red, muscle yellow)	—
Melanin/argentaaffin granules	Masson-Fontana	Black (nuclei red)	Ammoniacal silver reduced without an external reducing agent (argentaaffin reaction)
Melanin/lipofuscin (reducing substances)	Schmorl's stain	Dark blue	Ferric ferricyanide reduction

Target	Stain	Result	Note
Calcium (as phosphate/carbonate)	Von Kossa	Black (nuclei red)	Silver nitrate + strong light — actually demonstrates phosphate/carbonate anions, not calcium ion itself, so a chemical (e.g. alizarin red) confirmation is preferred where doubt exists
Argyrophil cells	Grimelius	Black (background golden-yellow)	Neuroendocrine/ argyrophil cell granules

5. Stains for amyloid

Stain	Principle	Result
Alkaline Congo red (Bennhold/Puchtler)	Congo red intercalates between parallel β -pleated amyloid fibrils forming a non-polar hydrogen bond; the ordered dye array is birefringent	Bright-field: deep salmon-pink ; under polarised light : apple-green birefringence (gold-standard confirmatory finding)
Congo red (Highman)	Alcoholic Congo red, KOH-differentiated	Red
Thioflavin T	Binds amyloid β -sheet; fluoresces	Bright yellow-green fluorescence under UV — highly sensitive but not specific for amyloid

Amyloid is classified as **systemic** (primary AL — light-chain, associated with plasma-cell dyscrasia; secondary AA — serum amyloid A, associated with chronic inflammation/infection; haemodialysis-related A β 2m) or **localised** (senile cardiac/cerebral ATTR/A β , medullary thyroid carcinoma A-Cal).

Fig 15.4 — A Congo red-stained section of kidney demonstrating amyloid deposition (red). (Bancroft's Theory & Practice of Histological Techniques, p.255)

 Fig 15.4 — A Congo red-stained section of kidney demonstrating amyloid deposition (red).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Panel A (bright field): label **glomerular capillary loops**, **mesangium**, **amorphous eosinophilic/salmon-pink amyloid deposits** in the mesangium and capillary walls, **Bowman's capsule**. Panel B (same field under crossed polarisers): same structures now showing **apple-green birefringence** confined to the amyloid deposits, rest of the tissue dark. Caption: "Congo red — pink on bright field, apple-green birefringence under polarised light = diagnostic of amyloid."

6. Stains for micro-organisms

Organism group	Stain	Result
Acid-fast bacilli (M. tuberculosis)	Ziehl-Neelsen	Red bacilli on blue background

Organism group	Stain	Result
<i>M. leprae</i>	Fite / modified acid-fast	Red bacilli
Fungi (<i>Candida</i> , <i>Cryptococcus</i> , <i>Rhizomucor</i> , <i>Aspergillus</i>)	Gomori/Grocott methenamine-silver (GMS), PAS	Black (GMS) or magenta (PAS) fungal wall on green/pink background
<i>Spirochaetes</i> , <i>H. pylori</i>	Warthin-Starry (silver)	Black organisms
Bacteria (general)	Gram stain	Blue-violet (Gram-positive) / red (Gram-negative)

Fig 16.4 — *Rhizomucor* (a filamentous fungus) well demonstrated by PAS stain. (Bancroft's Theory & Practice of Histological Techniques, p.283) illustrates how PAS/GMS reveal the carbohydrate-rich fungal wall even after the organism has lost viability (and hence acid-fastness).

Applied / Clinical anatomy

- ▶ **Liver biopsy:** reticulin stain outlines the lobular architecture and is essential for grading early cirrhosis/regenerative nodules; Masson trichrome/Van Gieson quantify portal and bridging fibrosis in chronic liver disease.
- ▶ **Renal biopsy:** the standard triad — H&E, PAS, and silver (Jones methenamine)/trichrome — assesses glomerular basement membrane thickening, mesangial matrix and crescents; Congo red is mandatory when amyloid glomerulopathy is suspected.
- ▶ **Bone marrow trephine:** reticulin (Gordon-Sweet) grading is the histological criterion for diagnosing and staging myelofibrosis.
- ▶ **Amyloidosis work-up:** Congo red with polarised-light apple-green birefringence remains the gold-standard confirmatory test on any suspicious eosinophilic deposit (cardiac, renal, vascular, tongue biopsies).
- ▶ **Tumour diagnosis:** mucin stains (mucicarmine/Alcian-PAS) separate mucin-secreting adenocarcinoma from lymphoma; reticulin pattern helps distinguish Ewing's sarcoma (reticulin-poor) from other small round-cell tumours; PTAH highlights cross-striations in rhabdomyosarcoma.
- ▶ **Infective pathology:** ZN/Fite for mycobacteria, GMS/PAS for fungi, and Warthin-Starry for *H. pylori*/spirochaetes are routinely requested when H&E morphology is suspicious but organisms are not obviously seen.
- ▶ **Museum/teaching specimens:** special stains on archival paraffin blocks allow retrospective histochemical demonstration of fibres, pigments or organisms decades after the original H&E section was cut.

High-yield exam pointers

- ▶ H&E stains general morphology; special stains selectively demonstrate one chemical class — carbohydrate, fibre, lipid, nucleic acid, pigment, amyloid, or organism.
- ▶ PAS = magenta; positive for glycogen, neutral mucin, basement membrane, fungal capsule, Russell bodies.
- ▶ Alcian blue (pH 2.5) = blue; stains acid mucin, proteoglycans, hyaluronic acid.

- ▶ **Van Gieson**: collagen red, muscle/cytoplasm yellow, nuclei black. **Masson trichrome**: collagen blue/green, muscle red.
- ▶ **Reticulin (Gordon-Sweet)**: silver-impregnated collagen III, black fibres — key stain for liver, marrow and lymph-node architecture.
- ▶ **Verhoeff/Weigert/Orcein** = elastic fibre stains (black/purple/brown respectively).
- ▶ **Congo red + polarised light (apple-green birefringence)** = gold standard for amyloid; Thioflavin T is more sensitive but non-specific.
- ▶ **Perls'/Prussian blue** = haemosiderin blue; **Von Kossa** = calcium salts black (technically phosphate/carbonate); **Masson-Fontana** = melanin black.
- ▶ **ZN/Fite** = acid-fast bacilli red; **GMS/PAS** = fungal wall; **Warthin-Starry** = spirochaetes/H. pylori black.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (chapters on staining principle/general procedure, special stains for carbohydrate/protein/lipid/nucleic acid/pigments, connective-tissue stains, amyloid staining, and stains for microbial organisms); Bancroft's *Theory & Practice of Histological Techniques* (trichrome stains, connective/mesenchymal tissue stains, pigments/minerals, microorganism demonstration); Gray's Anatomy (Standring) 41e (reticular-fibre and trichrome-stain figures).



Special stains for connective tissue fibres

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction Connective tissue matrix contains three types of fibres — **collagen**, **reticular**, and **elastic** — which differ in chemical composition, physical properties and light-microscopic behaviour. Routine haematoxylin and eosin (H&E) stains collagen fibres pink/eosinophilic but fails to differentiate them clearly from elastic fibres, and does not selectively demonstrate reticular fibres or elastic laminae at all. **Special (selective) stains** exploit differences in dye-molecule size, ionic charge and tissue affinity to colour one fibre type distinctly from the others, and are essential in histology practicals and histopathological diagnosis (e.g. vascular elastic lamina integrity, hepatic fibrosis staging, amyloid–collagen distinction).

Why special stains are needed

- ▶ On H&E, **collagen** stains eosinophilic (pink) and **elastic fibres** are also eosinophilic — the two cannot be reliably distinguished from each other by colour alone.
- ▶ **Reticular fibres** (fine, type III collagen) are too delicate and too few to be seen on H&E; they require a technique exploiting their **argyrophilia** (affinity for silver salts).
- ▶ **Elastic fibres/laminae**, though present in arteries, lung, and ligaments, are often inconspicuous or blend with surrounding tissue on H&E and need contrast-enhancing dyes.

- **General principle of selective/trichrome staining:** dyes of low molecular weight penetrate all tissue components; the largest-molecular-weight dye is retained only by the least permeable, densest component (collagen), while intermediate components (muscle, cytoplasm) retain the intermediate dye — this differential permeability/molecular-sieve principle underlies Masson's trichrome and related methods.

Table: Special stains, by fibre type

Fibre type	Recommended special stain(s)	Colour result	Alternate name of fibre
Collagen fibres	Van Gieson's method	Collagen — red/pink; other tissue — yellow	White fibres
	Masson's trichrome	Collagen — blue/green (aniline blue/light green); muscle & cytoplasm — red	—
	Picrosirius red (+ polarised light)	Collagen — red on bright-field; intensely birefringent (yellow–orange, type I; greenish, type III) under polarising microscopy	—
Elastic fibres	Verhoeff's (Verhoeff–Van Gieson) method	Elastic fibres — black/blue-black; collagen counterstained red (Van Gieson); nuclei black	Yellow fibres
	Weigert's resorcin–fuchsin method	Elastic fibres — blue-black/purple	—
	Orcein stain	Elastic fibres — dark brown	—
	Aldehyde fuchsin (Gomori)	Elastic fibres — blue-black	—
Reticular fibres	Silver impregnation — Gordon & Sweet's method / Gomori's method	Reticular fibres — black (argyrophilic)	Argyrophilic fibres
	Periodic acid–Schiff (PAS)	Magenta (positive, due to carbohydrate-rich coat)	—

Van Gieson's method (for collagen)

- **Reagents:** saturated aqueous **picric acid** + 1% aqueous **acid fuchsin** (Van Gieson's solution), used after nuclear staining (celestine blue–haematoxylin, or Weigert's iron haematoxylin).

- **Steps:** deparaffinise → stain nuclei (iron haematoxylin) → differentiate in acid alcohol → apply Van Gieson's solution 1–5 minutes → dehydrate directly through ascending alcohols (no water wash, to avoid removing the dye) → clear and mount.
- **Principle:** picric acid, being of small molecular size, differentiates by dissolving out the acid fuchsin from loosely-packed tissue (muscle, cytoplasm — left yellow), while the densely packed collagen fibres retain the acid fuchsin and stain **red**.
- **Result:** collagen — red; nuclei — black/brown (from the haematoxylin step); muscle, cytoplasm, RBCs — yellow.
- **Uses:** demonstration of collagen/fibrous tissue in liver fibrosis, differentiating collagenous scar from smooth muscle, and (combined with Verhoeff's stain as **Verhoeff–Van Gieson, VVG**) simultaneous demonstration of elastic laminae (black) against a collagen (red) background — the standard method for vascular wall and lung sections.

Masson's trichrome stain (for collagen)

- **Principle:** sequential application of dyes of increasing molecular weight. A small-molecular dye (Weigert's iron haematoxylin) stains nuclei black; an intermediate dye (Biebrich scarlet–acid fuchsin) stains cytoplasm, muscle, keratin and RBCs red; a polyacid (phosphotungstic/phosphomolybdic acid) then differentiates by removing the red dye selectively from collagen; finally a large-molecular dye (aniline blue or light green) is retained only by collagen, which it renders **blue/green**.
- **Result:** nuclei — black; cytoplasm, muscle, keratin — red; collagen — blue/green.
- **Use:** standard method to assess fibrosis (e.g. hepatic, renal, cardiac) and to distinguish fibrous tissue from smooth muscle (e.g. leiomyosarcoma vs fibrosarcoma).

Verhoeff's method (for elastic fibres)

- **Reagents:** Verhoeff's iron haematoxylin (haematoxylin + ferric chloride + Lugol's iodine as oxidising agent) followed by differentiation in **2% aqueous ferric chloride** (a crucial, microscopically-monitored step) until elastic fibres appear black against a grey background; residual iodine is removed in 95% alcohol (sodium thiosulphate).
- **Result:** elastic fibres — **black**; nuclei — black; collagen (if counterstained with Van Gieson) — red; other tissue — yellow.
- **Use:** demonstration of internal/external elastic laminae of arteries, elastic cartilage, lung alveolar septa, and skin (loss of elastic fibres in solar/actinic elastosis).

Weigert's resorcin–fuchsin, orcein and aldehyde fuchsin (for elastic fibres)

- **Weigert's resorcin–fuchsin:** basic fuchsin + resorcinol + ferric chloride solution; stains elastic fibres **blue-black to purple**; a classical method for elastic laminae of arteries (e.g. aorta) and elastic cartilage.
- **Orcein:** a natural dye extracted from lichen, applied in acidified alcohol; stains elastic fibres **dark brown**; simple and reliable, widely used in histology practical classes (e.g. mesentery spreads, elastic cartilage of epiglottis/pinna).

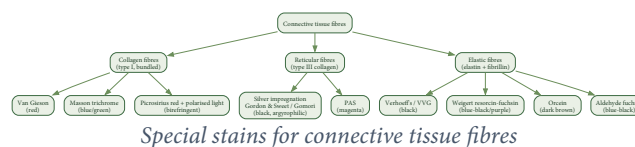
- ▶ **Aldehyde fuchsin (Gomori):** stains elastic fibres **blue-black**; also demonstrates other sulphated substances (e.g. mast-cell granules, pancreatic islet B-cell granules).

Silver impregnation for reticular fibres

- ▶ **Principle:** reticular fibres (type III collagen) have an abundant carbohydrate coat and are **argyrophilic** — they bind and reduce ammoniacal silver nitrate to visible **metallic black silver** without themselves being the reducing agent (unlike truly argentaffin tissues, e.g. neuroendocrine cells, which reduce silver directly).
- ▶ **Gordon & Sweet's method:** ammoniacal silver solution is prepared by adding concentrated ammonia dropwise to aqueous silver nitrate until the precipitate first formed redissolves, followed by sodium hydroxide and further ammonia titration; sections are impregnated, then reduced (formalin) to deposit black metallic silver on the fibres.
- ▶ **Gomori's silver impregnation method:** an alternative silver technique giving comparable results.
- ▶ **Result:** reticular fibres — **black**, forming a fine branching network; background counterstained (e.g. neutral red, van Gieson, or nuclear fast red).
- ▶ **Use:** classical demonstration of the reticular framework of liver, spleen, lymph node and bone marrow; assessment of hepatic architecture (reticulin framework collapse in cirrhosis) and of tumour reticulin patterns.
- ▶ **PAS positivity:** reticular fibres are also PAS-positive (magenta) because of their glycoprotein/carbohydrate content, though PAS is non-specific (also stains basement membranes, glycogen, mucin).

Structural note: why the stains differ

- ▶ **Collagen (type I):** thick banded fibrils forming bundles; strong tensile strength; abundant hydroxyproline/hydroxylysine cross-links → large, densely-packed protein network retains large-molecular acid dyes (van Gieson/trichrome).
- ▶ **Reticular fibres (type III collagen):** thin, non-bundled, richly glycosylated → do not stain well with ordinary collagen dyes but avidly reduce silver (argyrophilia).
- ▶ **Elastic fibres:** elastin core (rich in glycine, valine, desmosine/isodesmosine cross-links) + fibrillin-1 microfibrillar sheath; amorphous, non-banded, non-argyrophilic, poorly eosinophilic → require distinct dyes (orcin, resorcin-fuchsin, Verhoeff's) that bind the elastin core.



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Whole-mount/spread preparation of loose connective tissue (mesentery), as used in histology practicals, labelled to show:

- ▶ **Collagen bundles** — thick, wavy, branching bundles (stain red with van Gieson).
- ▶ **Elastic fibres** — thin, single, branching fibres forming a network, darker than collagen (stain black-brown with orcein/Verhoeff's).
- ▶ **Reticular fibres** — very fine argyrophilic fibres forming a delicate mesh (not visible without silver impregnation — show as a separate labelled inset, e.g. liver reticulin framework).
- ▶ Label the **fibroblast nucleus** amid the fibres and indicate the ground substance as the background.

□ Fig 13.5 — Photomicrograph. Elastic artery (Verhoeff-van Gieson stain, low magnification). (Sontakke — Textbook of Human Histology — 2e, p.170)

□ Fig 7.4 — Photomicrograph. Reticular fiber stained by Gomori's silver impregnation staining technique (Liver, high magnification). (Sontakke — Textbook of Human Histology — 2e, p.79)

Applied / Clinical anatomy

- ▶ **Verhoeff–Van Gieson (VVG)** is the standard stain to assess the **internal/external elastic lamina** in vascular pathology (e.g. arteritis, aneurysm, tumour vascular invasion) and to distinguish arteries from veins histologically.
- ▶ **Reticulin (silver) stains** are routinely used in liver biopsy reporting to assess **architectural collapse/fibrosis** in cirrhosis and hepatitis, and in distinguishing **hepatocellular carcinoma** (reticulin loss/disruption) from benign nodules.
- ▶ **Masson's trichrome/van Gieson** are used to quantify **fibrosis** in cardiac, renal and pulmonary biopsies, and to differentiate **fibrosarcoma (collagen-rich)** from **leiomyosarcoma (muscle-rich)**.
- ▶ **Picrosirius red with polarised microscopy** distinguishes thick, orange-red birefringent type I collagen from thin, greenish type III collagen — useful in wound-healing and fibrosis research.
- ▶ **Elastic-stain loss** is seen in **solar/actinic elastosis** of sun-damaged skin and in **cystic medial degeneration/aortic dissection** (fragmented elastic laminae), and elastin/fibrillin defects underlie **Marfan syndrome** (FBN1 mutation) and cutis laxa.
- ▶ **Amyloid** can mimic collagen on H&E and even shows green birefringence under polarised light after Congo red staining — an important differential when interpreting connective-tissue special stains in renal/vascular biopsies.

High-yield exam pointers

- ▶ Three fibre types: **collagen (white fibres)**, **elastic (yellow fibres)**, **reticular (argyrophilic fibres)** — remember by their traditional names.
- ▶ **Van Gieson** = picric acid + acid fuchsin → collagen red, background yellow.
- ▶ **Verhoeff–Van Gieson (VVG)** = combination stain → elastic fibres black + collagen red — the go-to stain for vessel wall elastic lamina.

- ▶ **Masson's trichrome** works on the **molecular-sieve/differential-permeability principle** — small dye stains everything, large dye retained only by densest tissue (collagen).
- ▶ **Reticular fibres = type III collagen**, thin, non-bundled, richly glycosylated, demonstrated by **silver impregnation (Gordon & Sweet's/Gomori's)** — black, "argyrophilic."
- ▶ Elastic-fibre special stains: **Verhoeff's (black)**, **Weigert's resorcin-fuchsin (blue-black/purple)**, **orcein (dark brown)**, **aldehyde fuchsin (blue-black)**.
- ▶ **PAS** stains reticular fibres and basement membranes **magenta** (carbohydrate-based reaction), but is non-specific.
- ▶ **Picrosirius red + polarised light**: collagen shows birefringence — type I orange-yellow, type III greenish — used to type collagen without immunostaining.
- ▶ Clinical correlate: **VVG** for vascular elastic lamina integrity; **reticulin** stain for liver architecture/fibrosis staging; **trichrome/van Gieson** for general fibrosis assessment.

SOURCE & COVERAGE

Sontakke — Textbook of Human Histology (2e); IB Singh — Textbook of Human Histology (9e); Junqueira's Basic Histology — Text & Atlas (17e); Ross Histology — A Text and Atlas (7e); Brijesh Kumar — Histology Text & Atlas (3e); Bancroft's Theory & Practice of Histological Techniques; Pranab Dey — Lab Techniques in Histopathology & Cytology (2e); Gray's Anatomy (Standring) 41e.



Special stains for nervous tissue

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction Nervous tissue is made up of **neurons** (perikaryon, dendrites, axon) and **neuroglia**, the axons of which are wrapped in a lipid-rich **myelin sheath**. Routine haematoxylin and eosin (H&E) stains nuclei and general cytoplasm but cannot selectively resolve Nissl substance, the fine neurofibrillary/dendritic architecture, glial processes, or myelin, because these components differ from the surrounding neuropil mainly in fibrillar organisation and lipid content rather than in simple acidophilia/basophilia. **Special stains** for nervous tissue are, therefore, a set of basic-dye, metal-impregnation (silver/gold) and lipid-affinity techniques — supplemented today by immunohistochemistry — devised specifically to demonstrate these components for neurohistology teaching and diagnostic neuropathology.

Why routine H&E is inadequate for neural elements

- ▶ **Nissl substance** (rough endoplasmic reticulum + free ribosomes) is basophilic but is only faintly and inconsistently resolved on H&E; a **basic aniline dye** is needed to bind its ribosomal RNA and render it deeply and reproducibly stained.
- ▶ **Neurofibrils, axons and dendritic processes** are extremely fine and have a refractive index close to that of the surrounding neuropil, so they are essentially invisible by light microscopy unless a **metal (silver/gold) impregnation** deposits an opaque precipitate along their length.

- **Neuroglial cell processes** (astrocyte foot-processes, fibrous gliosis) are unstained by H&E, which shows only small dark glial nuclei; differentiating astrocytes/oligodendrocytes from neurons needs metal impregnation or immunohistochemistry.
- **Myelin** is a compacted lipoprotein lamellar membrane; H&E does not bind this lipid selectively, so grey and white matter, and normal versus demyelinated tracts, cannot be told apart without a lipid-affinity stain.

Classification of special stains for nervous tissue

Structure demonstrated	Special stain(s)	Category / principle
Neuronal perikaryon — Nissl substance	Cresyl violet (Nissl stain), toluidine blue	Basic aniline dye binding ribosomal RNA
Neurofibrils / axons / whole-neuron outline	Bielschowsky's silver method, Bodian's protargol method	Silver impregnation
Entire neuron with dendritic tree	Golgi method (silver chromate precipitation)	Random/complete metallic impregnation
Neuroglia — astrocytes / glial fibres	Cajal's gold-sublimate method, Holzer's method, phosphotungstic acid haematoxylin (PTAH)	Metal impregnation / acid-haematin dye
Myelin sheath — CNS	Weigert's (iron-)haematoxylin method, Luxol fast blue (LFB)	Iron mordant / copper-phthalocyanine dye with lipoprotein affinity
Myelin + neurons together (CNS)	Kluver–Barrera method (LFB + cresyl violet counterstain)	Combined lipid dye + basic dye
Myelin sheath — PNS	Solochrome cyanin; osmium tetroxide (and Marchi's method for <i>degenerating</i> myelin)	Metal-mordant dye / osmication of unsaturated lipid
Connective-tissue framework of peripheral nerve	Masson's trichrome, van Gieson	Polychrome connective-tissue stain
Cell-specific antigens (modern adjunct)	GFAP (astrocytes), S-100 (Schwann cells/glia), synaptophysin/NeuN (neurons), neurofilament protein (axons)	Immunohistochemistry



Stains for neurons — Nissl substance and neurofibrils

- **Nissl stain (cresyl violet / toluidine blue)**: a basic (cationic) aniline dye is applied to a fixed section; it binds the phosphate groups of ribosomal RNA in the rough endoplasmic reticulum, so the **Nissl granules stain deep violet/blue** while the nucleus remains pale and the axon (which is devoid of Nissl substance) stays unstained. It demonstrates perikaryal size and shape, shows Nissl granules extending into dendrites but stopping abruptly at the axon hillock, and — clinically —

reveals **chromatolysis** (dispersal/loss of Nissl substance with peripheral migration of the nucleus) after axonal injury.

- ▶ **Bielschowsky's silver method / Bodian's protargol method:** sections are impregnated with an ammoniacal silver or protargol solution; silver ions adsorb onto neurofibrillary/axoplasmic proteins and are then chemically reduced to **black metallic silver**, outlining axons, dendrites and neurofibrils against a golden-brown background. The (modified) Bielschowsky technique is also used to demonstrate fine cutaneous nerve endings such as Meissner's corpuscles.
- ▶ **Golgi method (silver chromate impregnation):** a block of fixed nervous tissue is treated with potassium dichromate and then silver nitrate; by chance, only an occasional, randomly-selected neuron takes up a dense black silver-chromate precipitate, but in that neuron the **entire cell body, dendritic tree and axon** are outlined against an unstained background, and synapses appear as small oval "buttons" (boutons) on the receptor neuron's surface. This all-or-none impregnation is the classical technique with which Golgi and later Cajal elaborated the structure and connections of the neuron.

Stains for neuroglia and myelin

- ▶ **Cajal's gold-sublimate method, Holzer's method and phosphotungstic acid haematoxylin (PTAH)** selectively demonstrate the fibrillary processes of **astrocytes**, differentiating them from neurons and oligodendrocytes and highlighting reactive/fibrous **gliosis**.
- ▶ **Weigert's (iron-)haematoxylin method and Luxol fast blue (LFB):** an iron-mordanted haematoxylin, or a copper-phthalocyanine dye dissolved in alcohol, has chemical affinity for the myelin lipoprotein and stains intact CNS myelin sheaths **blue-black/blue-green**, leaving grey matter and demyelinated foci unstained; classical Weigert-stained photomicrographs are the standard way tract anatomy is illustrated in neuroanatomy atlases.
- ▶ **Kluver–Barrera method** combines LFB (myelin, blue) with a **cresyl-violet counterstain** (Nissl substance/neurons, violet), displaying both white- and grey-matter detail on one section — the routine combination stain for CNS sections.
- ▶ **Solochrome cyanin** stains peripheral (non-CNS) myelin, while **osmium tetroxide** blackens myelin generally by binding the unsaturated lipid of the sheath; **Marchi's method** (osmium + potassium dichromate) is more selective, blackening only the breakdown products of *degenerating* myelin, and is the classical technique used to trace degenerating fibre tracts after a nerve/CNS lesion (Wallerian degeneration).
- ▶ **Masson's trichrome/van Gieson** differentiate the collagenous **endoneurium, perineurium and epineurium** (green or red) from the Schwann cells and axoplasm, useful for assessing the connective-tissue framework in a peripheral-nerve biopsy.
- ▶ **Immunohistochemistry** (GFAP for astrocytes, S-100 for Schwann cells/glia, synaptophysin and NeuN for neurons, neurofilament protein for axons) is the modern adjunct that supplements and often supersedes these classical stains in diagnostic practice.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled diagram of a **multipolar neuron** stained to show **Nissl substance**, showing:

- ▶ **Cell body/perikaryon** with a large, central, vesicular **nucleus** and prominent **nucleolus**.
- ▶ **Nissl granules** — coarse violet/blue clumps filling the perikaryal cytoplasm.
- ▶ **Dendrites** — multiple, tapering processes, with **Nissl granules extending into them**.
- ▶ **Axon hillock** — the pale, Nissl-free conical region where the axon arises.
- ▶ **Axon** — single, uniform-diameter process, devoid of Nissl substance throughout its length.
- ▶ (Inset) the same neuron impregnated by a **silver method**, showing **neurofibrils** running through the perikaryon into both axon and dendrites.

□ Fig 9.2 — Neuron stained to show Nissl substance. Note that the Nissl substance extends into (IB Singh — Textbook of Human Histology — 9e, p.268)

Applied / Clinical anatomy

- ▶ **Alzheimer's disease:** intracellular neurofibrillary tangles (tau protein) and neuritic plaques are classically highlighted by silver-impregnation methods of the Bielschowsky type, using the same silver-neurofibril affinity that demonstrates normal neurofibrils.
- ▶ **Chromatolysis:** after axotomy, the neuronal cell body swells, the nucleus becomes eccentric, and Nissl substance is dispersed/diminished on Nissl staining — the histological hallmark used to identify and time nerve-cell injury and the onset of regeneration.
- ▶ **Demyelinating disease (e.g. multiple sclerosis):** LFB/Kluver–Barrera staining shows sharply demarcated pale (demyelinated) plaques against normally-stained white matter; because CNS myelin is made by oligodendrocytes, it degenerates in MS while Schwann-cell (PNS) myelin is spared.
- ▶ **Wallerian degeneration and tract-tracing:** Marchi's osmium-dichromate method selectively blackens degenerating myelin distal to an experimental or traumatic lesion, historically used to map fibre-tract connections.
- ▶ **Neuro-oncology:** immunohistochemistry differentiates glial tumours (GFAP-positive astrocytomas, the commonest primary brain tumour) from neuronal/embryonal tumours (synaptophysin/NeuN-positive medulloblastoma, arising from neural precursors) and from Schwann-cell tumours (S-100-positive schwannomas).
- ▶ **Peripheral-nerve biopsy:** osmium-stained teased-fibre/semithin preparations together with trichrome-stained paraffin sections distinguish demyelinating from axonal neuropathies and assess the endoneurial connective-tissue framework and ischaemic/pressure injury to nerves.

High-yield exam pointers

- ▶ **Nissl stain (cresyl violet/toluidine blue)** → basic dye → Nissl substance (RNA-rich RER) → violet; absent in axon/axon hillock; used to detect **chromatolysis**.
- ▶ **Silver impregnation** — Bielschowsky's and Bodian's methods → neurofibrils/axons black; **Golgi method** (silver chromate) → complete, random single-neuron impregnation (dendrites + axon + synaptic boutons) — the technique that established the neuron doctrine.

- ▶ **Weigert's method / Luxol fast blue (LFB)** → CNS myelin blue-black/blue-green; **Kluver–Barrera** = LFB + cresyl violet → myelin and Nissl substance together on one section.
- ▶ **Solochrome cyanin** → PNS myelin; **osmium tetroxide/Marchi's method** → myelin generally / selectively *degenerating* myelin (Wallerian degeneration tracing).
- ▶ **PTAH, Holzer's method, Cajal's gold-sublimate method** → astrocytes/glia fibres (gliosis).
- ▶ **Masson's trichrome/van Gieson** → connective-tissue framework of peripheral nerve (endo-/peri-/epineurium).
- ▶ **Immunohistochemical markers**: GFAP (astrocytes), S-100 (Schwann cells/glia), synaptophysin & NeuN (neurons), neurofilament protein (axons).
- ▶ **Clinical pearl**: Bielschowsky-type silver stains also demonstrate **Alzheimer neurofibrillary tangles** and fine sensory nerve endings (e.g. Meissner's corpuscles).

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Junqueira's Basic Histology — Text & Atlas 17e; Ross Histology — A Text and Atlas 7e; IB Singh — Textbook of Human Histology 9e; IB Singh — Textbook of Human Neuroanatomy; Sontakke — Textbook of Human Histology 2e; Snell — Clinical Neuroanatomy 7e; Bancroft's Theory & Practice of Histological Techniques. Coverage note: individual named silver/gold techniques (Bielschowsky, Bodian, Golgi, Cajal's gold-sublimate, Holzer's) appear in the corpus mainly as figure captions and applied-histology asides rather than as full step-by-step protocols; their principles are standard, universally-taught neurohistology consistent with these references.



Specific staining characteristics of Giemsa stain

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

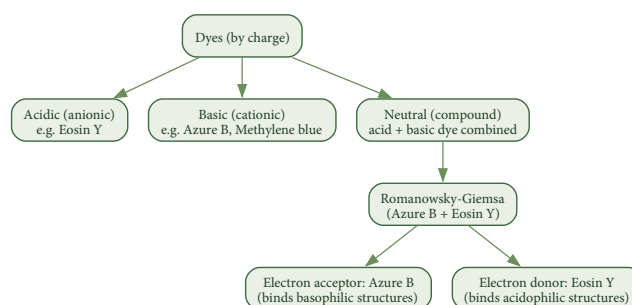
Definition / Introduction — Giemsa stain is a **Romanowsky-type, neutral (compound) dye** made by combining an acidic dye (**eosin Y**) with a basic thiazine dye (**azure B**, with some methylene blue) dissolved in methanol-glycerol. It is the routine stain for **peripheral blood and bone marrow smears**, **chromosome (G-)banding** in cytogenetics, and demonstration of **blood/tissue parasites** and mast-cell/basophil granules. Its defining feature is that different cellular components take up contrasting, reproducible colours from the *same* dye mixture, allowing precise morphological identification — the "specific staining characteristics" asked here.

1. Chemical composition and classification

Component	Chemical nature	Role
Azure B	Cationic (basic) thiazine dye	Electron acceptor; binds acidic/basophilic structures (nucleic acids) — stains them blue-purple

Component	Chemical nature	Role
Eosin Y	Anionic (acidic) xanthene dye	Electron donor ; binds basic/eosinophilic (acidophilic) structures (proteins, haemoglobin) — stains them pink-red
Methylene blue	Basic thiazine dye	Precursor component contributing further blue tone
Methanol	Solvent + fixative	Fixes air-dried smears before staining
Glycerol	Stabilising solvent	Keeps stock solution stable on storage

- ▶ Giemsa is classed as a **neutral (compound) dye**: in aqueous solution the acid and basic dye components exchange electrons and precipitate together on the tissue/cells — an **electron-transfer (charge-transfer) reaction**, unlike simple acidic dyes (e.g. eosin, orange G) or simple basic dyes (e.g. methyl green, Alcian blue) used alone.
- ▶ **Stock solution**: Giemsa powder 4 g + glycerol 250 ml + methanol 250 ml, dissolved at 60 °C with regular shaking.
- ▶ **Working solution**: stock diluted in acetate-buffered distilled water at **pH 6.8** (e.g. 4 ml stock in 96 ml buffer) — buffer pH is critical, since over-acidic or over-alkaline buffer distorts the pink/blue balance.
- ▶ Dye batches are standardised by **Total Dye Content (TDC)** and identified by **Colour Index (CI) number**, since azure B content varies between commercial lots and must be corrected for when preparing solutions.



Specific staining characteristics of Giemsa stain

2. Principle of Giemsa (Romanowsky) staining

- ▶ Air-dried smear is first **fixed in absolute methanol**, then flooded with diluted Giemsa working solution.
- ▶ The **azure B–eosin charge-transfer complex** produced during Romanowsky-Giemsa staining is responsible for the characteristic **purple colour of nuclear chromatin** — neither dye alone produces this colour.
- ▶ **Basophilic (acidic) structures** — nucleic acids of nucleus/chromatin, ribosome-rich cytoplasm (e.g. lymphocyte rim, immature erythroid precursors) — bind the basic azure dye → stain **blue/purple**.

- ▶ **Acidophilic (eosinophilic) structures** — hemoglobin, eosinophil specific granules, mature cytoplasm — bind eosin Y → stain **pink/red-orange**.
- ▶ **Metachromasia**: azurophilic granules (containing sulphated glycosaminoglycans, as in basophil and mast-cell granules) stain a **different colour (reddish-purple) from the dye's own colour (blue)** — the same phenomenon seen with toluidine blue on mast cells.
- ▶ Gentle, slow agitation of the slide in the staining bath prevents precipitate/staining-deposit artefacts and improves uniformity.

3. Method of staining (May-Grünwald-Giemsa, MGG technique)

Step	Duration
May-Grünwald solution	5 min
Running water rinse	1 min
Giemsa stain (working solution)	15 min
Running water rinse	1 min
Air drying, then clearing and mounting	—

- ▶ MGG is **metachromatic** and **not transparent** (unlike Papanicolaou stain); it gives **poor nuclear chromatin detail** and **cannot demonstrate keratin**, but is superior for showing **extracellular mucin/necrotic background** — this is why MGG and PAP are complementary, not interchangeable, stains in cytology practice.

4. Specific staining characteristics — colour reactions of individual structures

This is the core exam point: Giemsa gives each haematological/parasitic element a **specific, reproducible colour**, enabling differential identification on a single slide.

Structure	Giemsa staining reaction
Erythrocyte (RBC)	Pale pink to salmon-pink (eosinophilic; anucleate)
Nucleus/chromatin (all nucleated cells)	Purple-violet (azure B–eosin complex)
Nucleolus	Pale blue
Neutrophil	Nucleus purple, multilobed; specific granules fine, neutral, pale lilac-pink — do not obscure the nucleus
Eosinophil	Nucleus usually bilobed, purple; granules coarse, refractile, bright orange-red, fill the cytoplasm
Basophil	Granules coarse, deep purple/blue-black, metachromatic — often obscure the nucleus
Lymphocyte	Thin rim of sky-blue (basophilic) cytoplasm around a dense, deep-purple nucleus

Structure	Giemsa staining reaction
Monocyte	Grey-blue, "ground-glass" cytoplasm; nucleus indented/kidney- or horseshoe-shaped, lighter-staining chromatin
Platelet	Small clumps of purple granules on a pale-blue background
Erythroid maturation series (bone marrow) — proerythroblast → basophilic → polychromatophilic → orthochromatophilic erythroblast	Progressive decrease in cytoplasmic basophilia (deep blue → mixed/polychromatic → pink) as haemoglobin accumulates and the nucleus condenses and is finally extruded
Myeloid maturation series — myeloblast → promyelocyte → myelocyte → metamyelocyte → band → segmented neutrophil	Progressive nuclear condensation (round with nucleoli → kidney-shaped → lobed) with appearance of specific granules
Megakaryocyte / bone marrow smear	Best demonstrated with May-Grünwald-Giemsa (MGG)
Malaria parasite / Leishmania (LD body) / Toxoplasma	Nuclear chromatin stains red-purple ("hematoxyphilic"), cytoplasm blue — allows parasite identification against pink RBC background
Giardia (fresh duodenal aspirate)	Kite-shaped, binucleate trophozoite, 11–18 µm
Metaphase chromosomes (G-banding)	Alternating dark bands (AT-rich, heterochromatic, late-replicating) and light bands (GC-rich) after trypsin pre-treatment

□ Fig 5.10 — Bone Marrow: Smear. Stain: May-Grünwald-Giemsa. Oil immersion. (diFiore's Atlas of Histology — Eroschenko, p.84)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled peripheral blood smear (Giemsa stain), oil-immersion view — label each element with its specific colour:

- ▶ **RBCs** — pale pink, biconcave, no nucleus
- ▶ **Neutrophil** — multilobed purple nucleus + fine lilac cytoplasmic granules
- ▶ **Eosinophil** — bilobed purple nucleus + coarse bright orange-red granules
- ▶ **Basophil** — nucleus obscured by coarse dark purple/blue-black granules
- ▶ **Lymphocyte** — large, dense purple nucleus + thin sky-blue cytoplasmic rim
- ▶ **Monocyte** — kidney-shaped lighter-purple nucleus + grey-blue "ground-glass" cytoplasm
- ▶ **Platelets** — small purple granule-clumps scattered on pale-blue background

5. Applications of Giemsa stain

- ▶ **Haematology:** routine peripheral blood film for differential leukocyte count and RBC/WBC morphology (anaemia work-up, leukaemia blast morphology).
- ▶ **Bone marrow cytology:** May-Grünwald-Giemsa (MGG) staining of aspirate/trephine smears — megakaryocyte and erythroblast morphology, myelodysplasia and leukaemia diagnosis.

- ▶ **Clinical cytogenetics: Giemsa (G-) banding** of metaphase chromosome spreads — after trypsin pre-treatment, chromosomes show alternating dark/light bands used for karyotyping. Developed in the early 1970s, it was the **first widely used whole-genome analytical tool** and remains the **gold standard, first-line technique** for detecting numerical and structural chromosomal abnormalities (standard resolution 400–550 bands per haploid set; high-resolution banding detects deletions/duplications of ~5–10 Mb).
- ▶ **Parasitology:** thick/thin blood film for **malaria parasites**; splenic/bone-marrow/lymph-node aspirate for ***Leishmania donovani*** (kala-azar) and cutaneous *L. tropica* amastigotes; tissue sections/smears for ***Toxoplasma gondii***, ***Entamoeba histolytica***, and ***Giardia duodenalis***.
- ▶ **Exfoliative/aspiration cytology:** MGG as a complementary rapid stain alongside Papanicolaou (PAP) stain — MGG for cytoplasmic/background detail and metachromasia, PAP for superior nuclear chromatin and keratin detail.
- ▶ Historically also used to stain metaphase chromosome spreads before fluorescence in situ hybridisation (FISH) became available for finer resolution.

Applied / Clinical anatomy

- ▶ The **peripheral blood smear** remains the first-line, cheapest investigation in anaemia, leukaemia and infection work-up — its interpretation depends entirely on correct Giemsa/Romanowsky colour reactions described above.
- ▶ **Bone marrow aspiration/biopsy smears (MGG-stained)** are essential in diagnosing leukaemias, myelodysplastic syndromes and marrow infiltration; correlate with sternal or iliac crest marrow sampling sites.
- ▶ **G-banded karyotyping** underlies prenatal diagnosis of aneuploidies and postnatal/acquired diagnosis of constitutional and cancer-related chromosomal rearrangements — the standard first-tier cytogenetic test, now often complemented (not replaced) by FISH/microarray for sub-microscopic changes.
- ▶ **Splenomegaly work-up:** Giemsa-stained splenic/marrow aspirate smear is diagnostic in malaria and visceral leishmaniasis (kala-azar), both classical infective causes of splenomegaly.
- ▶ Decalcified bone sections may show **artefactually pink (over-eosinophilic) Giemsa staining** if decalcification exceeds 37 °C — a recognised technical pitfall in bone histopathology.

High-yield exam pointers

- ▶ Giemsa = Romanowsky-type, neutral/compound dye = Azure B (basic, electron acceptor) + Eosin Y (acidic, electron donor).
- ▶ Purple nuclear colour arises from an **azure-eosin charge-transfer complex**, not from either dye alone.
- ▶ Working solution buffered at **pH 6.8** in methanol/glycerol vehicle.
- ▶ **Metachromasia** of basophil/mast-cell granules — stain a colour different from the dye itself.
- ▶ MGG steps: May-Grünwald 5 min → water → Giemsa 15 min → water → air dry.
- ▶ Key colour reactions: RBC pink; nucleus purple; neutrophil granules fine pale-lilac; eosinophil granules coarse orange-red; basophil granules coarse purple/black; lymphocyte cytoplasm sky-blue.

- ▶ Three big applications: **haematology smears**, **G-banding karyotyping**, **blood/tissue parasite demonstration** (malaria, Leishmania, Toxoplasma, Giardia).
- ▶ MGG vs PAP: MGG is metachromatic/opaque with poor nuclear but good background detail; PAP is transparent with excellent nuclear/keratin detail.
- ▶ Dye standardisation uses **Total Dye Content (TDC)** and **Colour Index (CI) number** because azure B content varies by batch.

SOURCE & COVERAGE

Junqueira's Basic Histology (Text & Atlas) 17e; Ross Histology — A Text and Atlas 7e; Bancroft's Theory & Practice of Histological Techniques; Pranab Dey — Lab Techniques in Histopathology & Cytology 2e; Gray's Anatomy (Standring) 41e; Thompson & Thompson — Genetics in Medicine 8e; IB Singh — Textbook of Human Histology 9e; diFiore's Atlas of Histology (Eroschenko).



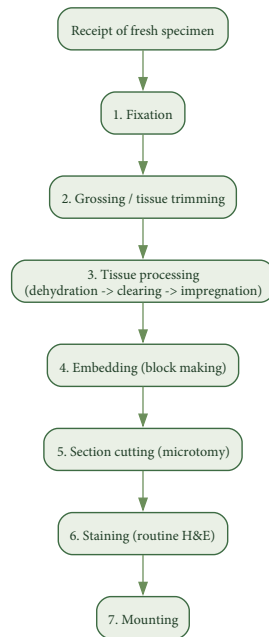
Stages of tissue preparation

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction

Tissue preparation (histological tissue processing, in the broad sense) is the sequence of laboratory steps that converts a fresh, soft, opaque piece of tissue into a thin, transparent, stained section mounted permanently on a glass slide for light-microscopic study. The aim throughout is to preserve cellular and tissue architecture as close to the living state as possible while giving the tissue enough rigidity to be cut into sections only a few micrometres thick. The classical pipeline for routine paraffin histology has seven linked stages: **fixation** → **grossing/trimming** → **tissue processing (dehydration–clearing–impregnation)** → **embedding** → **section cutting (microtomy)** → **staining** → **mounting**.

Overview of the pipeline



Stages of tissue preparation

Stage	Purpose	Key agent/instrument
1. Fixation	Arrest autolysis/putrefaction; stabilise proteins	10% neutral buffered formalin (NBF)
2. Grossing	Select and trim representative, cassette-sized pieces	Scalpel, cassette, tissue-marking ink
3. Processing	Remove water and replace it with a medium miscible with wax	Graded alcohols → xylene → molten paraffin
4. Embedding	Give the tissue a rigid block for cutting	Paraffin wax mould, embedding centre
5. Microtomy	Cut the block into thin ribbon sections	Rotary microtome, water bath
6. Staining	Colour cellular components for contrast	Haematoxylin and eosin (H&E)
7. Mounting	Preserve the stained section permanently	DPX / Canada balsam + coverslip

Stage 1 – Fixation

- **Aim:** preserve the tissue nearest its living state, prevent autolysis and bacterial putrefaction, prevent shape/volume change, harden the tissue for handling, and permit good, reproducible staining.
- **Fixative of choice:** 10% neutral buffered formalin (NBF) — 40% formaldehyde 100 ml + water 900 ml + sodium/disodium phosphate buffer. It penetrates tissue at ~1 mm/hour by the relation $D = k\sqrt{T}$ (depth of penetration proportional to the square root of time).

- ▶ **Practical rules:** tissue is sliced 3–5 mm thick and should be blood-free; fixative volume must be ≥ 20 times the tissue volume; small biopsies fix in ~6 hours, larger specimens need 12–24 hours (special organs such as brain need about a week in formol saline).
- ▶ **Mechanism:** formaldehyde reacts with protein side chains to form a hydroxymethyl group that subsequently cross-links to a stable methylene bridge, converting soluble protein to an insoluble, morphologically stable gel. (A separate detailed classification of fixatives — Bouin's, Zenker's, glutaraldehyde, osmium tetroxide, etc. — is covered under the dedicated "Fixatives" question; only the routine agent is repeated here.)

Stage 2 – Grossing / tissue trimming

- ▶ The fixed specimen is examined grossly, representative blocks are selected, and each piece is trimmed to the standard 3–4 mm thickness needed for adequate reagent penetration during processing.
- ▶ The cut surface/margin of interest is marked with **tissue-marking ink** (India ink is commonest; also silver nitrate or Rose Bengal) so orientation and resection margins remain identifiable after processing and staining.
- ▶ Correctly oriented, labelled tissue is loaded into a perforated **cassette** — the unit that travels through all subsequent processing baths.

Stage 3 – Tissue processing (dehydration → clearing → impregnation)

Processing gives the tissue enough rigidity for thin sectioning by replacing tissue water with a solid support medium (paraffin wax), which is not miscible with water — hence the three linked sub-steps:

- ▶ **Dehydration** — removes free/unbound water using a graded series of alcohols (50% → 70% → 90% → absolute), so that the sharp osmotic gradient of jumping straight to strong alcohol does not distort delicate tissue.
- ▶ **Clearing** — removes the dehydrating alcohol using an agent miscible with both alcohol and molten wax; **xylene** is the standard clearing agent (toluene and, historically, chloroform are alternatives). The tissue becomes translucent — the visual end-point of clearing.
- ▶ **Infiltration/impregnation** — the clearing agent is replaced by **molten paraffin wax** (melting point ~56–60 °C, kept 3–4 °C above its melting point) which fills the tissue spaces.

Bath	Typical schedule (automated processor)
50–70–90% alcohol	1 h each
Absolute alcohol (×3)	1 h each
Xylene/toluene (×3)	1 h each
Molten paraffin wax (×3)	1 h each

Factors influencing processing quality: size of tissue (smaller = better penetration), agitation, heat, viscosity of the medium, and vacuum (removes trapped air and speeds fluid exchange — used routinely for lung and spleen). Very hard tissue such as **bone or calcified cartilage** needs an additional step — **decalcification** (in an acid or EDTA solution) — inserted after fixation and before processing, so that the block can later be cut without shattering the microtome knife.

Stage 4 – Embedding and section cutting (microtomy)

Embedding: the processed tissue is oriented and surrounded by molten paraffin wax in a mould, which is then cooled on a cold plate to solidify into a **block**. Embedding (a) supports the tissue, (b) prevents distortion during cutting, and (c) allows archival storage. Orientation rules: tubular structures (vessel, fallopian tube) are placed to give a transverse section through all layers; epithelial-surfaced tissue is placed vertically at right angles to the surface; multiple small fragments (e.g. endometrial curettings) are centred together; long membranous tissue (amnion) is rolled ("Swiss roll"). Alternative embedding media exist for special purposes — **epoxy resin** (electron microscopy), **methacrylate**, **agar** (double-embedding of friable endoscopic/curettage fragments), and **gelatin**.

Microtomy ("mikros" = small + "temnein" = to cut) is performed on the **rotary microtome** for routine paraffin blocks (other types include the rocking, base-sledge and sliding microtomes; the **cryostat/cryomicrotome** for rapid frozen sections; and the **ultramicrotome**, using glass/diamond knives, for 40–100 nm ultrathin electron-microscopy sections). Practical steps: the block is trimmed and chilled (15–20 min) for even consistency; sections are cut at **4–6 µm** (cellular tissue such as lymph node at ~4 µm, routine tissue at ~6 µm) with a wedge-profile steel or disposable knife; the resulting ribbon is floated on a **water bath** kept 6–10 °C below the wax melting point (this flattens wrinkles); the section is picked up on a clean/adhesive-coated glass slide and dried in a hot oven slightly above the wax melting point.

Frozen section (cryostat, tissue frozen and cut without chemical fixation or wax processing) is the rapid alternative used for intra-operative diagnosis, enzyme histochemistry, and immunofluorescence, where speed or preservation of labile antigens/enzymes matters more than the highest morphological detail.

Stage 5 – Staining and mounting

Unstained tissue is colourless because fixed protein shares the refractive index of glass, so dyes with a selective chemical affinity for tissue components are used to generate contrast. The universal routine stain is **Haematoxylin and Eosin (H&E)**: haematoxylin (used with a mordant, e.g. alum) is a basic/cationic dye that binds nucleic acid and stains **nuclei blue-black** (basophilic structures); **eosin** is an acidic/anionic xanthene dye that binds cytoplasmic protein and collagen, staining them **pink** (acidophilic structures).

Standard H&E sequence: deparaffinise in xylene → rehydrate through descending grades of alcohol to water → stain nuclei in haematoxylin → differentiate excess stain in 1% acid alcohol → "**blue**" the nuclei in an alkaline solution (running tap water or Scott's tap water, raising pH to ~8.5) → counterstain the cytoplasm with 1% aqueous eosin → dehydrate through ascending alcohols → clear in xylene → **mount**.

Mounting is the final stage: a drop of mounting medium is placed on the section and a coverslip applied, giving a permanent seal that protects the tissue and renders it optically transparent. An ideal mounting medium has a refractive index close to glass (1.52–1.54), is colourless, dries quickly, and does not fade the stain. **DPX** (Distyrene–Plasticiser–Xylene) is the standard permanent synthetic-resin medium today; **Canada balsam** (natural resin) is now obsolete because it yellows with age; aqueous media (glycerine, polyvinyl alcohol) are used only for temporary mounts or stains (e.g. fat stains) destroyed by alcohol/xylene.

□ Fig 8.3 — Well stained tissue by haematoxylin and eosin stain (haematoxylin and eosin stain $\times 200$) (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.99)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A simple flow box diagram with these seven labelled boxes joined by downward arrows (reproduce the Graphviz sequence above by hand): **Fixation** → **Grossing/trimming** → **Processing** (Dehydration → Clearing → Impregnation) → **Embedding** → **Microtomy** (section cutting) → **Staining (H&E)** → **Mounting**.

Applied / Clinical anatomy

- ▶ **Frozen section** bypasses fixation/processing entirely for rapid intra-operative reporting (e.g. margin assessment during tumour surgery) — a direct clinical extension of the microtomy stage.
- ▶ **Decalcification**, an extra stage inserted before processing, is essential for bone/tooth/calcified specimens routinely used in anatomy museum preparations and osteology teaching sets.
- ▶ Faulty execution at any stage produces recognisable artefacts examiners test on: incomplete fixation → fuzzy nuclear detail; inadequate dehydration → tissue shrinkage/holes and poor wax infiltration; poor clearing/dewaxing → uneven or "milky" staining; wrong water-bath temperature during microtomy → air-bubble tears in the ribbon; too little bluing → reddish-brown (not blue) nuclei.
- ▶ **Immunohistochemistry and molecular pathology** place additional constraints on fixation time (over-fixation cross-links epitopes and degrades nucleic acids), making standardised processing schedules clinically important beyond routine morphology.
- ▶ **Electron microscopy** diverts the pipeline after fixation: glutaraldehyde/osmium fixation, resin (not paraffin) embedding, and ultramicrotomy to 40–100 nm sections — the same conceptual stages, different reagents.

High-yield exam pointers

- ▶ Seven linked stages: **Fixation** → **Grossing** → **Processing** (dehydration–clearing–impregnation) → **Embedding** → **Microtomy** → **Staining** → **Mounting**.
- ▶ Routine fixative = **10% neutral buffered formalin**; penetration ~ 1 mm/hour; tissue sliced 3–5 mm; fixative volume $\geq 20\times$ tissue volume.
- ▶ Processing = dehydration (graded alcohols) → clearing (xylene, tissue becomes translucent) → impregnation (molten paraffin, m.pt. ~ 56 – 60 °C).
- ▶ Embedding gives a paraffin **block**; routine section thickness at microtomy is **4–6 μm** , cut on a **rotary microtome**, floated on a water bath 6 – 10 °C below the wax melting point.
- ▶ Routine stain = **H&E**: haematoxylin (basic dye, needs a mordant) → nuclei blue; eosin (acidic dye) → cytoplasm/collagen pink.
- ▶ "Bluing" (alkaline wash, pH ~ 8.5) converts soluble red-brown hemalum to the stable insoluble blue form.
- ▶ Mounting medium of choice today is **DPX** (refractive index 1.523, matching glass); Canada balsam is obsolete.

- ▶ Decalcification is an extra stage for bone/calcified tissue, inserted between fixation and processing.
- ▶ Frozen section (cryostat) skips fixation/processing/embedding for a rapid intra-operative section.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (Chapters on Fixation, Processing, Embedding, Microtomy, Staining, and Haematoxylin & Eosin Staining); Bancroft's *Theory & Practice of Histological Techniques* (Chapters on Fixation of Tissues, Gross Room/Surgical Cut-up, Tissue Processing, Microtomy).



Stains for neuron and nerve fibres

Asked in: Paper III May 2009 — RGUHS MD/MS Anatomy

Definition / Introduction

A neuron and its processes are almost transparent in a routine haematoxylin & eosin section — the perikaryon, dendrites, axon and myelin sheath are all built of components (ribonucleoprotein, neurofilaments, lipoprotein membrane) that need targeted physical or chemical reactions to be brought out selectively. Three structurally distinct components are therefore stained by three fundamentally different principles: **Nissl substance** by basic (cationic) tinctorial dyes, **neurofibrils/axons (the whole neuron outline)** by metallic (silver/gold) impregnation, and the **myelin sheath** by lipid-reactive stains. A fourth, smaller group of special methods demonstrates degenerating fibres, living tissue, or specific proteins by immunohistochemistry.

Classification of stains for neuron and nerve fibres



1. Tinctorial (basic dye) stains — for Nissl substance

- ▶ **Principle:** Nissl substance is rough endoplasmic reticulum with attached and free ribosomes, concentrated in the perikaryon and extending into the dendrites; it is rich in RNA and hence strongly **basophilic**, taking up basic (cationic) dyes intensely.
- ▶ **Distribution:** present throughout the cell body and large dendrites but **absent from the axon hillock and axon** — a key differentiating feature used to identify the axon on a Nissl section.
- ▶ **Common stains:** **cresyl violet (cresyl fast violet)** — the standard, most widely used Nissl stain; **toluidine blue**; **thionine**; **methylene blue**.
- ▶ **Method (cresyl violet, typical):** deparaffinise → stain in filtered cresyl fast violet 10–20 min → differentiate in dilute acetic acid/alcohol → dehydrate, clear, mount.
- ▶ **Result:** Nissl granules stain deep violet/blue; nucleus pale with a prominent nucleolus. Motor neurones (e.g. anterior horn cells of the spinal cord) show characteristically coarse, "tigroid" Nissl substance and are the classic tissue used to demonstrate this stain.

- **Combination:** Nissl preparations are frequently combined with the luxol fast blue myelin stain in one section, so that Nissl substance (violet) and myelin (blue) are demonstrated together.

□ Fig 9.2 — Neuron stained to show Nissl substance. Note that the Nissl substance extends into the dendrites but not into the axon (IB Singh — Textbook of Human Histology — 9e, p.268)

2. Metallic impregnation (silver/gold) methods — for neurofibrils, axons and the whole neuron

- **Principle:** reduced metallic silver (occasionally gold) is deposited selectively on neurofilaments/axoplasm and on the neuronal membrane, blackening structures that basic dyes cannot show — in particular the fine dendritic and axonal processes.

Method	Typical fixative	Structure demonstrated
Golgi's method ("black reaction" / rapid Golgi)	Potassium dichromate, then silver nitrate	Impregnates only a small, random fraction (1–5%) of neurons but completely — soma, entire dendritic tree and axon in silhouette. Historic basis of the neuron doctrine (Golgi & Cajal, Nobel Prize 1906)
Cajal's reduced silver nitrate method	—	Neurofibrils
Bielschowsky's silver method	10% neutral buffered formalin (NBF)	Neurofibrils and axis cylinders (axons); also nerve endings (e.g. Meissner's corpuscle) and neurofibrillary tangles
Bodian's method (protargol/silver proteinate)	9 parts ethanol : 1 part formalin	Both myelinated and non-myelinated nerve fibres — useful on peripheral nerve biopsies
Nonidez's method	50% ethanol + chloral hydrate	Neurofibrils and axis cylinders
Rio-Hortega's silver carbonate method	10% NBF	Neurofibrils (a modified version is used for microglia)
Palmgren's method	—	A rapid, selective modern silver stain for nerve fibres and nerve endings in paraffin sections; together with cresyl violet and immunohistochemistry (neurofilament, MAP-2) it has largely superseded the older silver techniques

- **Result:** neurofibrils/axons black to dark brown against a pale yellow-brown background.
- **Use:** demonstrating axons and their terminals, dendritic branching (e.g. Purkinje cell dendritic arborisation), sympathetic ganglion architecture, and pathological neurofibrillary accumulation.

□ Fig 9.4 — Neuron stained to show neurofibrils. Note that the fibrils extend into both axons and dendrites (IB Singh — Textbook of Human Histology — 9e, p.269)

3. Lipid stains — for the myelin sheath

- **Principle:** the myelin sheath is a concentrically wrapped lipoprotein membrane (Schwann cell in the PNS, oligodendrocyte in the CNS), rich in phospholipid; it is demonstrated by reagents that react with, or dissolve in, this lipid.

Stain	Principle	Result
Osmium tetroxide (osmic acid)	Reacts with unsaturated lipid of myelin	Myelin stains black/dark brown; also the standard fixative for electron microscopy and for semithin, toluidine-blue-counterstained peripheral-nerve sections
Weigert's (iron hematoxylin) method	Lipid-mordant haematoxylin lake	Myelin blue-black
Luxol fast blue	Copper-phthalocyanine dye with affinity for myelin lipoprotein	Myelin blue; commonly combined with cresyl violet in one section
Solochrome cyanine	Simple, rapid dye technique	Myelin blue-black; works on both CNS and PNS material
Sudan black B / Sudan III / Oil red O	Lipid-soluble dyes, need frozen (unprocessed) sections	Myelin/lipid stains black (Sudan black) or red (Sudan III, Oil red O)

- **Note:** the axoplasm itself remains unstained/clear with these methods, so the myelin appears as a ring around a pale axonal core — the basis of morphometric assessment of myelinated fibres in peripheral nerve biopsy.

□ Fig 8.11 — Osmium-stained preparation of a peripheral nerve in (a) longitudinal and (b) transverse section (Brijesh Kumar — *Histology Text & Atlas* — 3e, p.122)

4. Special methods

- **Marchi's method** — stains **degenerating myelin selectively**, not normal myelin. Tissue is fixed in Orth's fluid and treated with an osmium tetroxide/potassium-dichromate-based mixture; intact, normal myelin resists reduction of the osmium and remains unstained, whereas the neutral-fat breakdown products of degenerating myelin reduce osmium tetroxide to black osmium dioxide. Used classically to trace a tract undergoing **Wallerian degeneration** after a lesion.
- **Vital/supravital methylene blue (Ehrlich's method)** — methylene blue applied to fresh, unfixed tissue selectively stains living nerve fibres and their terminals/motor end-plates; used for demonstrating peripheral nerve endings and neuromuscular junctions.
- **Immunohistochemistry** — antibodies to neurofilament protein and MAP-2 (axons/dendrites), synaptophysin (nerve terminals), S-100 and GFAP (glia) are the modern standard, now largely supplementing or replacing the classical silver methods in diagnostic neuropathology.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Schematic of a multipolar (motor) neuron showing where each stain acts — label:

- ▶ Perikaryon (cell body)
- ▶ Nucleus with prominent nucleolus
- ▶ Nissl granules — shown in cell body and dendrites (basic-dye target); mark them **absent** from the axon hillock and axon
- ▶ Dendrites (with Nissl substance extending into them)
- ▶ Axon hillock (Nissl-free zone)
- ▶ Axon with neurofibrils running its length (silver-impregnation target)
- ▶ Myelin sheath around the axon (lipid-stain target — osmium/Weigert/luxol fast blue)
- ▶ Node of Ranvier
- ▶ Axon terminal / synaptic bouton (site of vital methylene blue staining)

Applied / Clinical anatomy

- ▶ **Chromatolysis**: after axotomy, Nissl stains show dispersal ("chromatolysis") and loss of Nissl substance in the injured neuron's soma, with peripheral migration of the nucleus — used experimentally and diagnostically to time and localise nerve injury.
- ▶ **Neurofibrillary tangles** of Alzheimer's disease and senile plaques are classically demonstrated with Bielschowsky's/silver impregnation methods.
- ▶ **Demyelinating disease** (e.g. multiple sclerosis): plaques of demyelination show loss of luxol fast blue/Weigert staining in white matter, correlating with clinical deficits.
- ▶ **Peripheral nerve biopsy**: osmium-fixed, toluidine-blue-stained semithin sections are used to assess myelinated fibre density and distinguish axonal loss from primary demyelinating neuropathy.
- ▶ **Marchi's method** provided the historical basis for experimental tract-tracing — mapping degenerating fibre pathways after a surgical or traumatic lesion, before the era of modern axonal-transport tracers.
- ▶ **Golgi's and Cajal's methods** were historically decisive in establishing the **neuron doctrine** (individual, discrete neurons) over the older reticular theory; the Golgi (and Golgi-Cox) technique remains in use in neuroscience research to study dendritic spine morphology.

High-yield exam pointers

- ▶ Three principal targets of neuronal/nerve-fibre stains: **Nissl substance** (basic dyes), **neurofibrils/axon/whole neuron** (silver impregnation), **myelin sheath** (lipid stains).
- ▶ Nissl stains — cresyl violet, toluidine blue, thionine, methylene blue; Nissl substance is absent from the axon hillock and axon.
- ▶ Golgi's method impregnates only 1–5% of neurons but stains each one completely (soma + dendrites + axon) — basis of the neuron doctrine.
- ▶ Classical silver methods for neurofibrils/axons: Cajal's, Bielschowsky's, Bodian's (protargol), Nonidez's, Rio-Hortega's, Palmgren's.

- ▶ Myelin stains: osmium tetroxide, Weigert's haematoxylin, luxol fast blue, solochrome cyanine, Sudan black B/Oil red O (frozen sections).
- ▶ **Marchi's method is unique** — it stains only **degenerating** myelin, not normal myelin (osmium tetroxide + potassium dichromate after Orth's fixation).
- ▶ Ehrlich's vital methylene blue method stains living nerve fibres and nerve endings/motor end-plates in fresh tissue.
- ▶ Immunohistochemistry (neurofilament, MAP-2, synaptophysin, S-100, GFAP) is now the mainstay in diagnostic neuropathology, supplementing classical silver techniques.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; Bancroft's Theory & Practice of Histological Techniques; IB Singh — Textbook of Human Histology 9e; Junqueira's Basic Histology (Text & Atlas) 17e; Brijesh Kumar — Histology Text & Atlas 3e; Sontakke — Textbook of Human Histology 2e; Vishram Singh — Clinical Neuroanatomy 4e / General Anatomy 4e (Wallerian degeneration context).



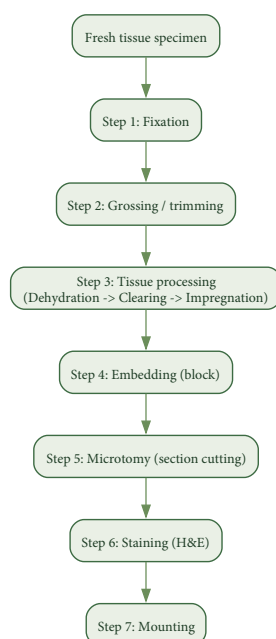
Steps in preparing a Tissue section

Asked in: Paper III May 2019 — RGUHS MD/MS Anatomy

Definition / Introduction

A tissue section is a thin, transparent, permanently stained slice of tissue mounted on a glass slide for light-microscopic examination. Preparing it from a fresh surgical/biopsy/autopsy specimen requires a fixed sequence of laboratory steps whose single purpose is to preserve cellular architecture as close to the living state as possible while giving the soft, opaque tissue enough rigidity to be cut into sections only a few micrometres thick and enough optical contrast to be interpreted. The routine paraffin-section pipeline has seven linked steps: **fixation** → **grossing/trimming** → **tissue processing** (dehydration → clearing → impregnation) → **embedding** → **section cutting (microtomy)** → **staining** → **mounting**.

Sequence of steps



Steps in preparing a Tissue section

Step	What is done	Key reagent/instrument
1. Fixation	Arrest autolysis/putrefaction, stabilise proteins	10% neutral buffered formalin (NBF)
2. Grossing/trimming	Select representative pieces, orient and ink margins	Scalpel, cassette, marking ink
3. Processing	Remove tissue water, replace with a wax-miscible medium	Graded alcohols → xylene → molten paraffin
4. Embedding	Cast the processed tissue into a rigid block	Paraffin wax, embedding mould/centre
5. Microtomy	Cut the block into a ribbon of thin sections	Rotary microtome, water bath
6. Staining	Impart selective colour/contrast to tissue components	Haematoxylin and eosin (H&E)
7. Mounting	Seal the stained section permanently under glass	DPX + coverslip

Step 1 — Fixation

- **Purpose:** prevents autolysis (self-digestion by tissue enzymes) and bacterial putrefaction, hardens the tissue for handling, prevents shrinkage/swelling, and renders the tissue amenable to subsequent processing and staining.
- **Fixative of choice:** **10% neutral buffered formalin** — 100 ml of 40% formaldehyde made up to 1000 ml with water and buffered with phosphate salts to keep pH neutral.
- **Rules of good fixation:** tissue is cut into slices about 3–5 mm thick; the volume of fixative should be **at least 15–20 times** the volume of the tissue; small biopsies fix within a few hours, larger specimens need 12–24 hours.

- ▶ **Mechanism:** formaldehyde cross-links protein amino groups through methylene bridges, converting soluble cytoplasmic protein into an insoluble stable gel that resists subsequent processing reagents.
- ▶ Hard, calcified specimens (bone, calcified cartilage) need an extra step — **decalcification** in an acid or EDTA solution — inserted immediately after fixation so the tissue can later be sectioned without damaging the microtome knife.

Step 2 — Grossing (tissue trimming)

- ▶ The fixed specimen is examined grossly; the pathologist/technician selects representative areas and trims each block down to a standard **2–4 mm thickness**, which allows adequate penetration of all subsequent reagents.
- ▶ Resection margins or the surface of interest are marked with **tissue-marking ink** (India ink is the commonest) so orientation is retained through processing and staining.
- ▶ The oriented, labelled piece is placed in a small perforated **cassette**, the carrier that travels through every remaining bath.

Step 3 — Tissue processing (dehydration → clearing → impregnation)

Processing is the step that actually gives the tissue enough rigidity for thin cutting, by exchanging tissue water for a solid support medium that is otherwise immiscible with water. It has three linked sub-stages, traditionally carried out on an automated tissue processor:

- ▶ **Dehydration** — removes free tissue water through a graded series of alcohols (50% → 70% → 90% → absolute); grading avoids the tissue distortion that a sudden strong-alcohol bath would cause.
- ▶ **Clearing** — the dehydrating alcohol (immiscible with wax) is removed by a "clearing agent" miscible with both alcohol and molten paraffin; **xylene** is standard (toluene is an alternative). The tissue turns translucent — the visible end-point of clearing.
- ▶ **Impregnation (infiltration)** — the clearing agent is replaced by **molten paraffin wax** (melting point ~56–60 °C), which fills every tissue space, cell and vessel lumen.

Bath	Approx. schedule
Alcohols 50→70→90%	~1 hour each
Absolute alcohol (×2–3 changes)	~1 hour each
Xylene (×2–3 changes)	~1 hour each
Molten paraffin (×2–3 changes, under vacuum)	~1 hour each

Factors that speed and improve processing: smaller tissue pieces, gentle heat, agitation of the fluid, and vacuum (removes trapped air, speeds fluid exchange — routinely used for spongy organs such as lung).

Step 4 — Embedding

- ▶ The fully impregnated tissue is oriented in a mould, surrounded by fresh molten paraffin, and cooled rapidly on a cold plate to solidify into a firm **paraffin block**.
- ▶ Embedding (a) supports the delicate tissue during cutting, (b) prevents distortion/tearing, and (c) allows the block to be archived indefinitely for future re-cutting.

- ▶ **Orientation rule:** tubular organs (vessel, fallopian tube, appendix) are embedded to give a transverse cut through all wall layers; epithelium-bearing tissue is embedded on edge, at right angles to the cutting plane, so the full epithelial-to-stromal thickness is captured.
- ▶ Other embedding media exist for special purposes: **epoxy/araldite resin** (electron microscopy, requires much thinner, harder support), **agar** (for friable curettings/biopsy fragments, double-embedded), and **gelatin/OCT** (for frozen sections).

Step 5 — Microtomy (section cutting)

- ▶ Performed on a **rotary microtome**: the block is clamped, chilled for 15–20 minutes to firm the wax, and advanced against a steel or disposable knife by a calibrated hand-wheel that cuts a section of a set thickness with every turn.
- ▶ **Routine section thickness** is 4–6 μm (thin, cellular tissue such as lymph node may be cut nearer 3–4 μm).
- ▶ The ribbon of cut sections is floated on a **water bath** kept a few degrees below the wax melting point — this flattens knife-induced wrinkles — then picked up on a clean, adhesive-coated glass slide and dried in a warm oven.
- ▶ Other microtome types: the **base-sledge/sliding microtome** for large or hard blocks, the **cryostat** for rapid frozen sections (5–10 μm , no prior fixation/processing/embedding — used for intra-operative diagnosis), and the **ultramicrotome** with a glass/diamond knife for 40–100 nm ultrathin electron-microscopy sections.

Step 6 — Staining

- ▶ Fixed protein has a refractive index close to glass, so an unstained section shows almost no contrast; a dye with selective chemical affinity for a tissue component is therefore required.
- ▶ The universal routine stain is **Haematoxylin and Eosin (H&E)**. Haematoxylin, used together with a mordant (commonly alum), is a basic/cationic dye that binds acidic nucleic acid and stains **nuclei blue-black** (basophilic structures). **Eosin**, an acidic/anionic xanthene dye, binds basic cytoplasmic protein and collagen and stains them shades of **pink** (acidophilic/eosinophilic structures).
- ▶ **Sequence:** deparaffinise the slide in xylene → rehydrate through descending alcohols to water → stain nuclei in haematoxylin → differentiate excess dye in 1% acid alcohol → "**blue**" the nuclei in an alkaline solution (running tap water/Scott's tap water, pH ~8) → counterstain in aqueous eosin → dehydrate through ascending alcohols → clear in xylene.

Step 7 — Mounting

- ▶ A drop of mounting medium is placed on the stained, cleared section and a glass **coverslip** applied, sealing it permanently and rendering it optically clear for microscopy.
- ▶ An ideal mounting medium is colourless, has a refractive index close to glass (~1.52–1.54), dries without shrinkage, and does not fade the stain over time.
- ▶ **DPX** (Distyrene–Plasticiser–Xylene) is the standard synthetic-resin permanent mountant used today; **Canada balsam** (natural resin) is now largely obsolete because it yellows with age; aqueous mountants (glycerine jelly) are reserved for special stains destroyed by alcohol/xylene.

□ Fig 8.3 — Well stained tissue by haematoxylin and eosin stain (haematoxylin and eosin stain $\times 200$) (Pranab Dey — *Lab Techniques in Histopathology & Cytology* — 2e, p.99)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A simple downward flow-box diagram with these seven labelled boxes joined by arrows (reproduce the Graphviz sequence above by hand): **Fixation** → **Grossing/trimming** → **Processing** (Dehydration → Clearing → Impregnation) → **Embedding (block)** → **Microtomy (section cutting)** → **Staining (H&E)** → **Mounting**.

Applied / Clinical anatomy

- ▶ **Frozen section** on a cryostat bypasses fixation, processing and paraffin embedding entirely, giving a section within minutes for intra-operative reporting (e.g. margin/lymph-node assessment during tumour surgery) — a direct diagnostic use of the microtomy step alone.
- ▶ **Decalcification**, an added step between fixation and processing, is essential for sectioning bone, tooth, and calcified museum/osteology specimens.
- ▶ Errors at any single step generate recognisable artefacts that examiners like to test: incomplete/delayed fixation → autolytic, blurred nuclear detail; inadequate dehydration → tissue shrinkage and processing "holes"; poor clearing → uneven, milky-looking sections; wrong water-bath temperature at microtomy → wrinkles or knife-line tears in the ribbon; inadequate bluing after haematoxylin → reddish-brown rather than blue nuclei.
- ▶ **Immunohistochemistry/molecular pathology** impose strict fixation-time limits (over-fixation over-cross-links epitopes and degrades nucleic acid), making the standardised sequence of steps clinically important well beyond routine morphology.
- ▶ **Electron microscopy** diverts the same conceptual pipeline after fixation — glutaraldehyde/osmium fixation, resin (not paraffin) embedding, ultramicrotomy — to obtain sections thin enough for the electron beam.

High-yield exam pointers

- ▶ Seven sequential steps: **Fixation** → **Grossing/trimming** → **Processing** (dehydration–clearing–impregnation) → **Embedding** → **Microtomy** → **Staining** → **Mounting**.
- ▶ Routine fixative = **10% neutral buffered formalin**; tissue sliced 3–5 mm; fixative volume $\geq 15\text{--}20\times$ tissue volume.
- ▶ Processing = dehydration (graded alcohols) → clearing (xylene; tissue turns translucent) → impregnation (molten paraffin wax, m.pt. $\sim 56\text{--}60^\circ\text{C}$).
- ▶ Embedding gives a solid paraffin **block**; routine microtomy section thickness is **4–6 μm** , cut on a **rotary microtome**, floated on a water bath below the wax melting point to remove wrinkles.
- ▶ Routine stain = **H&E** — haematoxylin (basic dye + mordant) stains nuclei blue; eosin (acidic dye) stains cytoplasm/collagen pink.
- ▶ "Bluing" after haematoxylin (alkaline wash) converts the soluble red-brown dye-tissue complex into its stable insoluble blue form.
- ▶ Mounting medium of choice = **DPX** (refractive index close to glass); Canada balsam is obsolete.

- Decalcification is an added step for bone/calcified tissue, inserted between fixation and processing.
- Frozen section (cryostat) skips fixation, processing and embedding for a rapid intra-operative section.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (chapters on Fixation, Tissue Processing, Embedding, Microtomy, and Haematoxylin & Eosin Staining); Bancroft's *Theory & Practice of Histological Techniques* (chapters on Fixation of Tissues, Gross Room/Surgical Cut-up, Tissue Processing, and Microtomy).



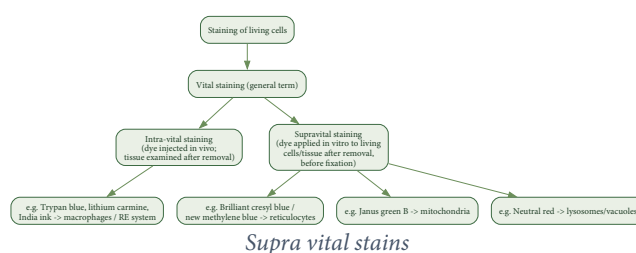
Supra vital stains

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction A **vital stain** is a dye that penetrates a living cell and colours a specific cell component without immediately killing the cell. A **supravital stain** is the special case in which this dye is applied **in vitro** to living cells or a small piece of tissue after it has been removed from the body, but before fixation — i.e., the cells are still alive at the moment of staining, even though they are no longer inside the organism. This is distinguished from **intra-vital staining**, in which the dye is introduced into a living animal/organism and the tissue is examined only after subsequent removal. Supravital staining is a key applied-cytology technique in Anatomy and Haematology, best known for demonstrating **reticulocytes** and for the classical microscopic demonstration of living cell organelles such as **mitochondria** and **lysosomes**, structures that are lost or altered by routine fixation and paraffin processing.

1. Terminology — vital, supravital and intra-vital staining

Term	What is stained	When dye is applied	Classic example
Vital stain (broad term)	Living cells, without killing them	Dye taken up by cells that remain alive	Methylene blue; Nile red
Intra-vital staining	Cells/tissue still within the living organism	Dye injected in vivo ; tissue harvested and examined later	Trypan blue, lithium carmine, India ink → demonstrate macrophages of the reticuloendothelial (mononuclear phagocyte) system
Supravital staining	Cells/tissue removed from the body but still alive	Dye applied in vitro , after removal, before fixation	Brilliant cresyl blue / new methylene blue → reticulocytes; Janus green B → mitochondria; neutral red → lysosomes



2. Principle

- ▶ Supravital dyes are **weak, non-toxic basic dyes of low concentration** that diffuse into living cells without immediately disturbing their metabolism, so the cell can still take up, concentrate and react with the dye.
- ▶ Uptake exploits a **living, functioning cell membrane and organelle system**: e.g. Janus green B is reduced (decolourised) by non-respiring cytoplasm but remains oxidised (coloured blue-green) specifically within actively respiring mitochondria, because mitochondrial cytochrome oxidase keeps it in the oxidised state; neutral red is a weak base that becomes trapped and concentrated in the acidic interior of lysosomes/vacuoles.
- ▶ Because the cell is alive, the dye reveals a **dynamic, near-physiological picture** of the organelle or inclusion (shape, number, movement) rather than the fixed/shrunken appearance seen after chemical fixation.
- ▶ Staining must be carried out promptly on fresh material (blood, thin tissue slices, tissue-culture cells) since the "vital" property is lost once the cells die.

3. Common supravital/vital stains and what they demonstrate

Stain	Structure/inclusion demonstrated	Typical use
Brilliant cresyl blue	Residual ribosomal RNA of young erythrocytes, precipitated into a visible reticular (basophilic) network	Reticulocyte count
New methylene blue	Same RNA-precipitation principle	Alternative reticulocyte stain (reference/standard method)
Janus green B	Mitochondria — stays oxidised (blue-green) only in respiring mitochondria	Classical cytology demonstration of mitochondria in living cells
Neutral red	Lysosomes / vacuoles — weak base trapped in the acidic vacuolar lumen	Classical demonstration of lysosomes/Golgi-associated vacuoles
Methylene blue	General vital stain of living cells	Cytoplasmic/nuclear demonstration
Nile red	Intracellular lipid droplets (lipophilic fluorochrome)	Vital stain for lipid inclusions

Stain	Structure/inclusion demonstrated	Typical use
Trypan blue / lithium carmine / India ink	Phagocytic macrophages (reticuloendothelial system)	Intra-vital dye, injected into the living animal; also used as a dye-exclusion test of cell viability (dead cells with damaged membranes take up the dye, live cells exclude it)
Hoechst 33342, DAPI	Nuclear DNA	Vital DNA-binding fluorochromes used to quantitate DNA/for cell-cycle and immunofluorescence work

Note: not every Romanowsky-type blood stain is vital — **May-Grünwald-Giemsa** is applied to an already air-dried, methanol-fixed smear and is therefore **not** a vital/supravital stain, a common examiner distractor.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled sketch of a **supravitally-stained reticulocyte preparation (wet mount)**, showing:

- ▶ A field of **mature erythrocytes** — plain pink/orange biconcave discs, no internal network.
- ▶ **Reticulocytes** — same size as mature RBCs, but with a **blue-stained reticular (net-like/granulofilamentous) meshwork** of precipitated RNA scattered through the cytoplasm.
- ▶ Label the **percentage reticulocyte count** convention: number of reticulocytes per 1000 (or 500) erythrocytes counted, expressed as a percentage.
- ▶ A small inset showing **Janus green B-stained mitochondria** (blue-green rod/granular structures) in a living cell, for the organelle-demonstration application.

4. Technique — supravital staining for the reticulocyte count

- ▶ **Sample:** a drop of fresh, anticoagulated venous or capillary blood (must be examined promptly, as the effect is lost once cells die).
- ▶ **Method (equal-volume mixing):**
- ▶ Mix equal volumes of fresh blood and the supravital stain (brilliant cresyl blue or new methylene blue, prepared in normal saline/absolute alcohol) in a small test tube.
- ▶ Incubate at room temperature for 15–20 minutes to allow dye uptake by living reticulocytes.
- ▶ Prepare a thin smear (or wet coverslip preparation) from the stained blood-dye mixture.
- ▶ Examine under oil-immersion; count reticulocytes (cells showing a blue reticular network) per 1000 erythrocytes; express as a **percentage** (normal $\approx$ 0.5–1.5% of total RBCs; higher in neonates).
- ▶ Because the RNA-precipitation reaction requires living cells, this counting technique **cannot be done on a routinely fixed and Romanowsky-stained smear** — it is the defining reason reticulocyte counting is a supravital procedure.

5. Advantages and limitations

- ▶ **Advantages:** demonstrates structures (mitochondria, lysosomes, reticular RNA) that are destroyed, shrunk or rendered invisible by chemical fixation; shows the cell/organelle in a near-physiological, dynamic state; technically simple and rapid (no fixation, processing or embedding required).
- ▶ **Limitations:** the dye must be non-toxic and used at low concentration, or it kills the cell before the desired reaction is seen; the stained preparation is not permanent (cannot usually be archived like a fixed, mounted slide) and must be examined promptly; only a limited number of structures are amenable (RNA-rich reticular material, mitochondria, lysosomes, DNA with vital fluorochromes) — most tissue morphology still requires conventional fixation and staining.

6. Applications

- ▶ **Reticulocyte count** — the principal clinical application; brilliant cresyl blue or new methylene blue precipitates residual ribosomal RNA into a stainable reticular network in young erythrocytes.
- ▶ **Immunofluorescence technique** — supravital stains/fluorochromes are used to label live cells for immunofluorescence-based analysis.
- ▶ **DNA quantitation** — Hoechst 33342 and DAPI are vital DNA-binding fluorochromes used to quantitate DNA content (e.g., in cell-cycle/flow-cytometric studies).
- ▶ **Classical cytology demonstrations** — Janus green B for mitochondria and neutral red for lysosomes/vacuoles, historically important in teaching the morphology of living cell organelles before electron microscopy became available.
- ▶ **Cell-viability testing** — the related dye-exclusion principle (trypan blue) distinguishes live cells (dye excluded by an intact membrane) from dead/damaged cells (dye taken up), used in tissue-culture and embryology laboratories.

Applied / Clinical anatomy

- ▶ **Reticulocyte count as a marker of erythropoietic activity:** a raised reticulocyte count indicates increased marrow erythropoiesis (e.g., haemolytic anaemia, acute blood loss, response to haematinic/erythropoietin therapy); a low count in the presence of anaemia suggests marrow hypoplasia/aplastic process.
- ▶ **Neonatal physiology:** reticulocyte percentage is physiologically higher in the newborn, reflecting the brisk erythropoiesis of intrauterine/early neonatal life — relevant to the anatomy/embryology of haemopoiesis.
- ▶ **Cell-viability (dye-exclusion) testing:** used to assess sperm and oocyte/embryo viability in reproductive biology/embryology laboratories, and routinely in tissue-culture quality control before an experiment.
- ▶ **Historical link to cell biology teaching:** supravital demonstration of mitochondria (Janus green B) and lysosomes/vacuolar system (neutral red) underpins the classical descriptions of these organelles in general anatomy/cytology, later confirmed and refined by electron microscopy.
- ▶ **Reticuloendothelial (mononuclear phagocyte) system demonstration:** intra-vital injection of trypan blue, lithium carmine or India ink into a living animal, followed by tissue harvest, is the

classical method of identifying macrophages by their phagocytosed dye particles — foundational to the anatomy of the mononuclear phagocyte system.

High-yield exam pointers

- ▶ **Vital stain** = dye stains living cells without killing them (e.g., methylene blue, Nile red).
- ▶ **Supravital stain** = dye applied **in vitro**, to cells/tissue **already removed from the body but still alive**, before fixation.
- ▶ **Intra-vital stain** = dye injected **in vivo**; tissue examined only after subsequent removal (trypan blue, lithium carmine, India ink → macrophages).
- ▶ **Reticulocyte count** is the classic supravital application — **brilliant cresyl blue / new methylene blue** precipitate residual RNA into a reticular network; normal range $\approx 0.5\text{--}1.5\%$.
- ▶ **Janus green B** → vitally/supravitaly demonstrates **mitochondria** (stays oxidised only in respiring mitochondria).
- ▶ **Neutral red** → demonstrates **lysosomes/vacuoles** (weak base trapped in acidic compartment).
- ▶ **Hoechst 33342 and DAPI** are vital DNA-binding fluorochromes, used to quantitate DNA.
- ▶ Supravital stain is also used in the **immunofluorescence** technique.
- ▶ **May-Grünwald-Giemsa is NOT a vital stain** — it is applied to a fixed, air-dried smear (common distractor).
- ▶ Trypan blue's **dye-exclusion principle** (live cells exclude the dye; dead cells take it up) is the basis of cell-viability testing.

SOURCE & COVERAGE

Pranab Dey — *Lab Techniques in Histopathology & Cytology*, 2e (Ch. 7, "Staining Principle and General Procedure of Staining the Tissue"); Ross — *Histology: A Text and Atlas*, 7e (mitochondria/vital staining, reticulocyte morphology); Bancroft's *Theory & Practice of Histological Techniques*; IB Singh — *Textbook of Human Histology*, 9e (macrophage demonstration by vital dyes). **Coverage note:** Gray's Anatomy (Standring, 41e) — the spine text for this bank — carries no histotechnique/staining content, as this is a laboratory-methods topic under Paper III (Microscopy); the answer is therefore grounded in the specialty histotechnique/histology texts above, per the topic spec.



Types of clearing agents

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction Clearing (also called **dealcoholization** or **clarification**) is the third step of routine tissue processing, performed after fixation and dehydration and before infiltration and embedding. It is the process by which the dehydrating agent (usually alcohol) is removed from the tissue and replaced by a fluid that is miscible with both the dehydrant and the embedding medium (molten paraffin wax). A clearing agent has a refractive index close to that of the tissue proteins, so a properly cleared tissue becomes translucent/transparent — this transparency is the visual end-point of the step. Clearing agents are essential to routine histopathology processing, to museum/preservation histotechnique, and to preparing gross specimens (bone, dense fibrous/fatty tissue) for paraffin-section mounting.

1. Position and aim of clearing in tissue processing

- **Sequence:** Fixation → Dehydration → **Clearing** → Infiltration → Embedding → Sectioning.



Types of clearing agents

- **Aims of clearing:**
- Removal of the dehydrating agent (alcohol) from tissue.
- Rendering the tissue clear/transparent so that its refractive index approximates that of the tissue proteins, permitting good microscopic examination.
- Preparing the tissue for complete infiltration by molten paraffin wax (a clearing agent must be miscible with both the preceding dehydrant and the succeeding wax).
- **End-point detection:** the tissue becomes translucent/transparent; residual opacity signals incomplete dehydration (and hence unsatisfactory clearing).
- **Volume and duration:** approximately 40 times the volume of the specimen is used; a small biopsy needs about 1 hour, while larger tissue requires three changes of xylene/toluene, 60 minutes each.

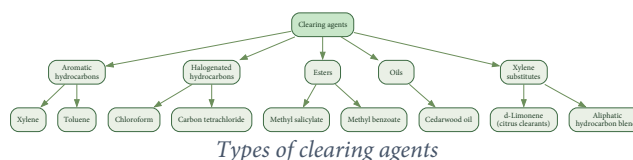
- **Hazard of over-clearing:** prolonged exposure to the clearing agent makes the tissue brittle and friable, so the optimal clearing time must not be exceeded.

2. Ideal properties of a clearing agent

- Low viscosity and high penetration rate.
- Low melting point (so it is easily and quickly displaced by the molten wax during infiltration).
- Miscible with both the dehydrating alcohol and the embedding (paraffin) wax.
- Good lipid solvent (so residual lipid does not impede wax penetration).
- Causes minimal tissue damage — no significant shrinkage or hardening/brittleness.
- Non-toxic and non-inflammable (low health hazard).
- Inexpensive.

3. Classification of clearing agents

Category	Examples
Aromatic hydrocarbons	Xylene, toluene
Halogenated hydrocarbons	Chloroform, carbon tetrachloride
Esters	Methyl salicylate, methyl benzoate
Oils	Cedarwood oil
Terpene (citrus-based) "xylene substitutes"	d-Limonene and proprietary citrus clearants
Aliphatic (straight-chain) hydrocarbon "xylene substitutes"	Proprietary aliphatic hydrocarbon blends



4. Individual clearing agents

- **Xylene:** the most commonly used clearing agent in the laboratory; a clear, inflammable liquid; clears small pieces of tissue rapidly (30–60 minutes). It is an excellent lipid solvent and, being practically insoluble in water, effectively removes residual bound water from tissue, but this same property causes a drying action; prolonged exposure makes tissue hard and brittle. Health hazards of xylene exposure (neurological and mucosal irritant effects) are well documented.
- **Toluene:** properties almost similar to xylene, but its action is slightly slower and, importantly, it does **not** harden the tissue even on prolonged exposure. It is also flammable and toxic.
- **Chloroform:** a highly volatile, non-inflammable but expensive and toxic (carcinogenic) agent. Its penetrating power/clearing action is slower than xylene, but it does not cause tissue shrinkage, which historically made it the agent of choice for dense tissues such as the uterus and muscle. It is now rarely used — it was also excluded from many earlier closed automatic tissue processors because it dissolved their plastic components.

- ▶ **Esters (methyl salicylate, methyl benzoate):** less toxic than the hydrocarbons and esters may be used in manual tissue processing; they do not cause tissue hardening even with prolonged exposure.
- ▶ **Cedarwood oil:** an expensive but rapid clearing agent, mainly used for clearing dense tissue.
- ▶ **d-Limonene:** a clear liquid that does not cause tissue hardening; however, removal of limonene from the tissue by the infiltrating paraffin wax is difficult.
- ▶ **Newer "xylene substitutes":** developed as safer alternatives to xylene and chloroform. They fall into two proprietary groups — **citrus-based clearants** (made from d-limonene; characteristic citrus odour and oily consistency; some are marketed with the FDA "Generally Regarded As Safe" (GRAS) food-additive designation, though this label is not truly appropriate for a laboratory clearant, and d-limonene remains a skin sensitizer/irritant) and **aliphatic (straight-chain) hydrocarbon blends** (practically odourless, non-sensitizing, non-irritating; do not remove bound water and therefore do not dry the tissue; their oiliness/flammability depends on carbon-chain length).

5. Comparison of the principal clearing agents

Property	Xylene	Toluene	Chloroform	Esters
Tissue shrinkage	Yes	Yes	Minimal	No
Tissue hardening	Yes	No	No	No
Inflammable	Yes	Yes	No	Yes
Harmful effect	Irritant, but less harmful	Irritant	Toxic gas liberated on heating; carcinogenic	Relatively safe
Cost	Cheap	Cheap	Very expensive	High cost

6. Selection of an appropriate clearing agent

Choice depends on:

- ▶ **Type of tissue** — larger specimens need longer clearing time than smaller ones; dense/fibrous tissue (uterus, muscle) traditionally cleared better with chloroform or cedarwood oil.
- ▶ **Type of processor** — manual versus automatic/closed processing systems (some agents, e.g. chloroform, damage plastic components of certain processors).
- ▶ **Processing conditions** — temperature, use of vacuum.
- ▶ **Ease of removal by the embedding medium** — a low-melting-point, low-viscosity clearant is displaced by molten wax more readily than a high-melting-point/viscous one.
- ▶ **Safety factors** — flammability and toxicity of the reagent.
- ▶ **Cost.**

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- ▶ **Diagnostic accuracy:** incomplete clearing leaves the tissue opaque, impairs wax infiltration, and produces poor sectioning with staining artefacts on the finished slide; over-clearing (prolonged exposure) renders the tissue brittle and friable and equally compromises section quality — both

are recognised causes of processing artefact in routine histopathology and museum-specimen sectioning.

- ▶ **Occupational/laboratory safety:** the well-documented neurotoxic and irritant effects of xylene exposure, and the carcinogenicity of chloroform, have driven modern histopathology and anatomy-museum laboratories toward citrus-based (d-limonene) and aliphatic-hydrocarbon "xylene substitutes," which are less hazardous to processing-room personnel even though costlier.
- ▶ **Choice by specimen type:** dense, fibrous or fatty specimens (e.g. uterus, skeletal muscle, fat-laden tissue) require an agent with strong lipid-solvent action and slower, gentler penetration (chloroform, cedarwood oil) rather than the more rapid but hardening xylene, which is preferable for small, delicate biopsies.
- ▶ **Relevance to anatomical preservation:** the same principle of matching solvent miscibility (dehydrant ↔ clearant ↔ mounting/embedding medium) underlies degreasing and clearing steps used while preparing osteological and wet/plastic-mounted museum specimens.

High-yield exam pointers

- ▶ Clearing is the **third step** of tissue processing — between dehydration and infiltration — and its visual end-point is tissue **transparency**.
- ▶ **Xylene** is the most commonly used clearing agent but hardens/shrinks tissue and has documented health hazards; **toluene** is a similar but non-hardening alternative.
- ▶ **Chloroform** is non-inflammable, causes minimal shrinkage, and was classically used for dense tissue (uterus, muscle), but is expensive, toxic/carcinogenic, and now rarely used.
- ▶ **Esters** (methyl salicylate, methyl benzoate) and **cedarwood oil** clear without hardening the tissue but are costly.
- ▶ **d-Limonene** does not harden tissue but is difficult to remove with paraffin wax; it is the basis of modern citrus-based "xylene substitutes."
- ▶ Ideal clearing agent: low viscosity/high penetration, low melting point, miscible with dehydrant + wax, minimal tissue damage, non-toxic, non-inflammable, cheap.
- ▶ Volume of clearing agent used ≈ **40 times** the tissue volume.
- ▶ Modern laboratories increasingly use aliphatic-hydrocarbon and citrus-based xylene substitutes for occupational safety.

SOURCE & COVERAGE

Pranab Dey — Lab Techniques in Histopathology & Cytology, 2e (Ch. 2, "Processing of Tissue in the Histopathology Laboratory"); Bancroft's Theory & Practice of Histological Techniques (Ch. 7, "Tissue processing"). Coverage note: this topic is a laboratory histotechnique subject not addressed by Gray's Anatomy (Standring) or by this bank's assigned Preservation/Museum-technique texts (Halдар; Handbook of Museum Technique, Govt. Museum Chennai) — grounding was therefore drawn from the corpus's dedicated microscopy/histotechnique manuals, which directly cover tissue-processing clearing agents.



Museum, embalming & organ donation



Embalming fluids

Asked in: Paper III 02-Aug-2021 · Paper III Nov 2018 · Paper III May 2018 (3×) — RGUHS MD/MS Anatomy

Definition / Introduction — Embalming is the chemical treatment of a dead human body to achieve an antiseptic condition, a pre-mortem appearance, and preservation, by injecting an embalming fluid into the vascular system and body cavities. An embalming fluid is therefore a formulated mixture of a preservative, a germicide, and several adjuvant chemicals (buffer, wetting agent, anticoagulant, dye, vehicle, perfume) that together coagulate tissue protein, fix organs, and arrest putrefaction. Depending on the route and purpose of injection, embalming fluids are classed as arterial, cavity, or pre-injection fluids; a separate low-formaldehyde "soft" fluid (Thiel's method) is used when tissue pliability must be preserved for cadaveric-surgery workshops. In India, embalming fluid is what makes body-donation programmes and the dissection hall possible for undergraduate and postgraduate anatomy teaching.

Composition of an ideal embalming fluid

The standard (ideal) arterial embalming fluid, formulated for a 70 kg body requiring 10 litres of fluid (of which ~10% is lost through drains and purging), is built as follows:

Ingredient	Role	Quantity
Formalin	Preservative	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 mL
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 mL
Soluble oil of wintergreen	Perfume	90 mL
Water	Vehicle	made up to 10 L

Sodium borate and sodium citrate are first dissolved in hot water and allowed to cool; the remaining ingredients are added and diluted to volume; the mixture is left to stand for a few hours and then filtered before use.

Constituents of embalming fluid and their functions

Every embalming fluid, whichever route it is given by, is built from the same nine functional classes of chemical:

Category	Representative agents	Function
Preservative	Formaldehyde (as 37% formalin), methanol, phenol	Coagulate/fix tissue protein; arrest putrefaction

Category	Representative agents	Function
Germicide/disinfectant	Phenolic derivatives, zephiran chloride, glutaraldehyde	Surface disinfection; kill microbes (not necessarily spores)
Buffer	Sodium borate, sodium bicarbonate, sodium/magnesium carbonate	Stabilise pH — chemical stability of the fluid depends on pH
Wetting agent	Glycerine, glycol, sorbitol, sodium lauryl sulphate	Lower the surface tension of water; aid penetration and even distribution
Anticoagulant	Sodium citrate, sodium oxalate	Chelate calcium to keep blood fluid, permitting its removal and even distribution of arterial fluid
Dye (cosmetic)	Eosin (tetrabromofluorescein), Ponceau S, erythrosine, amaranth, acid fuchsin, toluidine red, rhodamine	Simulate natural tissue colouring
Vehicle	Water, alcohol, glycerine, sorbitol	Diluent/solvent that keeps ingredients in stable, uniform solution during transport through the vascular tree
Perfuming/masking agent	Methyl salicylate (oil of wintergreen), clove oil, cinnamon oil, menthol, lavender	Mask the odour of preservatives
Muscle relaxant	Magnesium chloride	Relaxes arterial smooth muscle, assisting fluid flow through the vascular system

Of the preservatives: **formaldehyde** (discovered 1856 by A.W. von Hofmann) is a colourless, pungent gas, sold commercially as formalin (37% formaldehyde + ~7% methanol + water); **methanol** is volatile, inflammable and poisonous (causes blindness/death if ingested), precipitates protein, stabilises formalin, and aids penetration; **phenol** (carbolic acid) is a poisonous, colourless crystalline coal-tar derivative that darkens on exposure to light, is insoluble in water but soluble in ether/ethanol/chloroform/glycerine, is a powerful germicide and fungicide, and causes greying of tissue.

Types of embalming fluid



Arterial fluid — injected into the vascular system; dilution is adjusted for dehydrated, obese, or oedematous bodies, and for special situations (refrigerated, burnt, infant bodies):

Constituent	Role	Obese subject	Thin/emaciated subject
Formalin + Methanol	Preservative	10% + 55%	10% + 55%
Sodium borate	Buffer	15 g	15 g

Constituent	Role	Obese subject	Thin/emaciated subject
Sodium citrate	Anticoagulant	15 g	15 g
Glycerine	Wetting agent	15%	20%
Phenol	Germicide	5%	5%
Water	Vehicle	15%	10%
Thymol	Fungicide	few crystals	few crystals
Eosin (1%)	Dye	5 mL	5 mL
Oil of wintergreen	Perfume	10 mL	10 mL

Note the reciprocal shift — glycerine (wetting agent) is raised and water reduced in the emaciated body, to compensate for the smaller vascular/interstitial fluid volume and aid penetration.

Cavity fluid — injected via trocar into the thoracic, abdominal and pelvic cavities (after aspiration of hollow-viscus contents), to preserve and disinfect the walls and parenchyma of organs, the contents of hollow viscera, and the space between visceral organs. Composition: Formalin 60%, Methanol 25%, Phenol 10%, Sodium lauryl sulphate 1%, Mercuric chloride 1%, Eucalyptus oil 1%. It is used concentrated (undiluted by residual blood/lymph, which would otherwise dilute it and permit decomposition); roughly 1 litre is injected into each of the thoracic and abdominal cavities of an average (70 kg) body.

Pre-injection fluid — injected before the arterial fluid proper; contains anticoagulants and buffers. At death, contraction of the arterial system redistributes blood (~5% in arteries, 10% in veins, 85% in capillaries); 4–5 litres of pre-injection fluid are given, with a wait of about 30 minutes before the arterial fluid is injected, to loosen clots and improve subsequent drainage.

Fluids used in the dissection laboratory — paint mixture (keeps the exposed cadaver surface moist between sessions); tank/immersion fluid (used for immersing stored cadavers); cloth fluid (soaked cloths that prevent drying of the area under active dissection and of isolated dissected parts).

Newer / soft-embalming fluid — Thiel's method

Conventional formaldehyde-based fluids leave the body stiff and fragile. Thiel's soft-fix method uses a much lower formaldehyde concentration (kept to a strict minimum, ~0.8% in the embalming solution; final formaldehyde concentration works out to <0.5%) combined with:

- ▶ **4-Chloro-3-methylphenol** — the principal fixative/preservative agent.
- ▶ **Boric acid** — disinfectant.
- ▶ **(Mono-)ethylene glycol** — maintains tissue plasticity (humectant).
- ▶ **Ammonium nitrate, potassium nitrate, sodium sulphite** — buffering/reducing salts; sodium sulphite and formalin are added only just before perfusion.

Two stem solutions (A and B) are prepared and combined (with a small amount of formalin and sodium sulphite) to form the embalming solution, which is perfused through the great saphenous vein (femoral or carotid artery if the saphenous vein cannot be used); about 14 litres are perfused, after which the

body is immersed in a separate, larger-volume immersion solution and allowed to mature for 4–6 weeks. The resulting cadaver retains a flexible skin and musculature — limbs remain mobile and organs remain identifiable and respond to the scalpel as if alive — making the technique ideal for cadaveric surgical-skills workshops (flap raising, endoscopic sinus surgery, laparoscopic and arthroscopic training). Bodies begin to deteriorate after about one month unless the formalin concentration is raised to 4%, which extends usable preservation to about six months (at the cost of the tissue's natural pliability).

Mechanism of action

- ▶ Embalming chemicals combine with tissue proteins to form a lattice of **inert, firm, cross-linked material** that resists breakdown by bacterial or autolytic enzymes.
- ▶ Native body proteins carry numerous reactive centres with a strong affinity for water; the preservatives chemically saturate/destroy these reactive centres, so the resulting protein-aldehyde complex loses much of its water-holding capacity — tissue becomes firmer, more stable, and longer-lasting.
- ▶ Germicides and preservatives simultaneously inactivate endogenous (autolytic) tissue enzymes and destroy both pathogenic and non-pathogenic bacteria — the tissue is sanitised as well as fixed.
- ▶ The net effect is a chemical stiffening resembling rigor mortis, but — unlike true rigor mortis, which resolves spontaneously — the rigidity produced by embalming is **permanent**.
- ▶ For the best result, embalming should be carried out within about **6 hours of death**; if delayed substantially, bacterial decomposition and putrefaction will already be under way and the fluid can no longer arrest or reverse these changes.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Outline of the body with the six standard sites for arterial-fluid injection and venous drainage, labelled:

- ▶ **Right and left common carotid artery** (with accompanying internal jugular vein) — perfuses head and neck.
- ▶ **Right and left axillary/brachial artery** (with vein) — perfuses the upper limb.
- ▶ **Right and left femoral artery** (with vein) — perfuses the lower limb.

Show arrows for direction of fluid injection (peripherally) and blood/fluid drainage (centrally, toward the heart), and mark each vessel as ligated once its segment is complete.

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- ▶ **Six-point injection** is used for autopsied bodies and advanced decomposition, since a single injection site leaves patches of tissue unfixed; each vessel is raised, injected, and ligated in turn (legs, then arms, then trunk via the right common carotid, then left and right sides of the head).
- ▶ **Factors governing fluid distribution:** capillary resistance, chemical composition/concentration of the fluid, injection pressure, osmosis, diffusion and gravity; choice of artery is guided by its calibre, depth, branches, and proximity to the arch of the aorta, and choice of vein by calibre, depth, and proximity to the right atrium.

- ▶ **HIV-infected, badly decomposed, or autopsied bodies** require a higher concentration of both arterial and cavity fluid; in autopsied bodies the viscera (whose circulatory continuity has been disrupted) are treated separately with cavity fluid or an embalming powder in a viscera bag ("shake and bake").
- ▶ **Occupational/biosafety anatomy:** embalmers are exposed to bloodborne and airborne pathogens (HIV, hepatitis B/C, multidrug-resistant TB, *C. difficile*, MRSA, CJD prions) and to formaldehyde vapour, a recognised chemical hazard — personal protective equipment and safe permissible formaldehyde exposure levels are mandatory in the embalming room and dissection hall.
- ▶ **Medico-legal relevance:** an unautopsied body requires appropriate legal clearance before embalming; correct fluid choice and timing (within ~6 hours) are essential parts of the chronology followed in voluntary body-donation programmes that supply cadavers to the anatomy dissection hall.

High-yield exam pointers

- ▶ Ideal arterial embalming fluid (70 kg body, 10 L): formalin 1.5 L + sodium borate 600 g + sodium citrate 900 g + glycerine 600 mL + NaCl 800 g + eosin 30 mL + oil of wintergreen 90 mL, made up with water.
- ▶ Three types by route/purpose: **arterial** (vascular system), **cavity** (thoracic/abdominal/pelvic cavities via trocar, ~1 L/cavity), **pre-injection** (anticoagulant + buffer flush before arterial fluid).
- ▶ Nine functional constituent classes: preservative, germicide, buffer, wetting agent, anticoagulant, dye, vehicle, perfume, muscle relaxant.
- ▶ Formaldehyde (as 37% formalin) is the principal preservative; phenol is the principal germicide/fungicide (also greys tissue); glycerine/glycol are the standard wetting agents.
- ▶ Mechanism = protein cross-linking/coagulation (loss of water-binding reactive centres) + germicidal sanitisation → a permanent chemical rigidity, unlike self-resolving true rigor mortis.
- ▶ Six-point injection (bilateral common carotid, axillary, femoral) is the standard whole-body technique, especially for autopsied bodies.
- ▶ Thiel's soft-embalming fluid keeps formaldehyde to <0.5–0.8% (using boric acid, ethylene glycol, and 4-chloro-3-methylphenol) to preserve tissue pliability for cadaveric surgical workshops.
- ▶ Embalming is ideally performed within 6 hours of death, before bacterial putrefaction sets in.

SOURCE & COVERAGE

Halдар, *Notes on Embalming and Museum Techniques* — sole textbook in this corpus covering embalming-fluid composition, constituent chemistry, and mechanism of action; this content lies outside the scope of Gray's Anatomy (Standring) and the standard gross-anatomy texts, which do not describe embalming-fluid formulation. *Coverage note:* standard gross/embryology/histology spine texts do not carry this material; the answer is grounded entirely in the dedicated embalming/mortuary-science reference specified for Paper III (Organ donation and preservation of the human body).



Plastination

Asked in: Paper III 02-Aug-2021 · Paper III May 2010 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — Plastination is a modern museum/preservation technique in which the water and lipids of a fixed biological specimen are replaced, under vacuum, by a curable polymer (silicone rubber, epoxy or polyester resin), which is then hardened in situ. The result is a dry, coloured, non-toxic, durable, odourless and natural-looking specimen that retains gross and even microscopic structural detail indefinitely. It is one of the artificial methods of preservation of the human body (alongside embalming, formalin preservation, paraffin impregnation, freezing and taxidermy) and was devised to overcome the well-known drawbacks of conventional wet, formalin-fixed museum specimens.

Why plastination — drawbacks of conventional wet (formalin-fixed) preservation

- ▶ **Irritating odour** of formalin vapour, hazardous for prolonged student/staff exposure.
- ▶ **Bleached, colourless** specimens that lose all natural tissue colour.
- ▶ Specimens **do not give a naturalistic impression** of the living organ.
- ▶ **Difficulty in maintaining** wet sections over long-term storage (leakage, fading, fungal growth).
- ▶ The **luminal architecture, calibre and branching pattern** of hollow/tubular structures is almost impossible to appreciate on a routine dissection or a wet-fixed specimen.

Principle

- ▶ Complete **dehydration and defatting** of a previously fixed and dissected specimen, followed by **forced impregnation** of a liquid, curable polymer under vacuum in place of a volatile intermediary solvent (acetone).
- ▶ The polymer is finally **cured/hardened**, so it sets within every cell and interstitial space, faithfully preserving the exact three-dimensional morphology of the tissue while rendering it dry, rigid, and permanent.

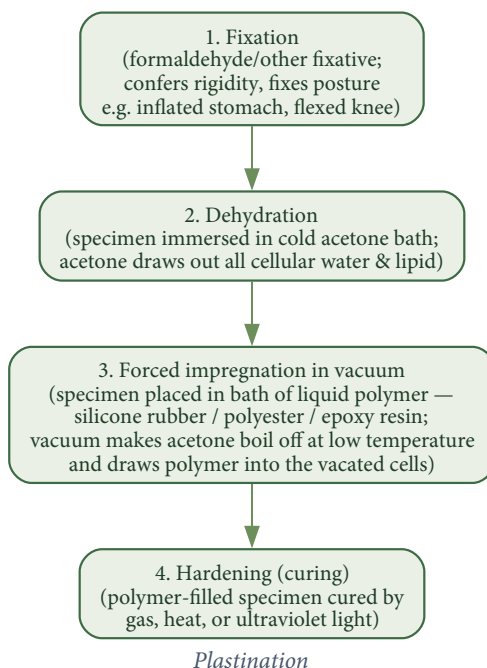
Types of plastination

Type	Method (essence)	Chief use
Whole organ plastination	Entire dissected organ/specimen is fixed, dehydrated in acetone, then impregnated with resin and mounted on a base	Demonstrates the total structure and natural relationships of an organ
Sheet plastination	Deep-frozen body/organ is cut into thin slices (~1 cm) with a band saw; each slice is cast between glass plates in resin	Thin transparent or thick opaque, portable body sections; best shows internal relationships
Luminal cast plastination	Lumen of a hollow/tubular structure is filled with a resin mixture and the surrounding tissue is then digested away	Studies dimensions, branching pattern and variations of tubular/cavitary structures

Materials used

- ▶ Jars for storage and processing.
- ▶ **Acetone** — dehydrating agent and intermediary solvent.
- ▶ **Silicone rubber**; general-purpose **resin** (polyester or epoxy) with **catalyst** and **accelerator** (or hardener) — the impregnating/curing medium.
- ▶ Colour paints — to restore a natural, life-like appearance.
- ▶ Dissection instruments, syringes and needles.

Steps of whole-body / whole-organ plastination



- ▶ **Fixation** — formaldehyde or other preserving solutions prevent tissue decomposition and confer a degree of rigidity; this stage is also used to fix the specimen's shape or posture (e.g. an inflated stomach, or a leg bent at the knee) before further processing.
- ▶ **Dehydration** — after all necessary dissection is completed, the specimen is placed in an acetone bath; under freezing conditions the acetone extracts all the water from the tissue cells.
- ▶ **Forced impregnation in a vacuum** — the dehydrated specimen is transferred to a bath of liquid polymer (silicone rubber, polyester or epoxy resin); a vacuum is created so that the acetone within the tissue boils off at a low temperature, and as it vaporises and leaves the cells, it draws the liquid polymer in behind it, filling the cell with liquid plastic.
- ▶ **Hardening** — the polymer-impregnated specimen is finally cured using gas, heat, or ultraviolet light, which hardens the plastic within the tissue and yields a rigid, durable, permanent specimen.

Method — illustrative technical detail for each type

- ▶ **Whole organ plastination:** specimen washed under tap water → immersed in a preservative mixture (95% alcohol 280 mL, formalin 1320 mL, glycerine 80 mL, phenol 120 mL, distilled water 800 mL) for 2–3 days for complete fixation → washed free of formalin under running tap

water → transferred to an acetone jar → transferred to a resin jar for **1–2 weeks** → finally immersed in a mixture of general-purpose resin, **5% catalyst** and **0.1% accelerator**; after a few hours the specimen becomes non-sticky and is mounted on a suitable base in proper anatomical orientation.

- ▶ **Sheet plastination:** after deep-freezing for **24 hours**, thin (~1 cm) sections are cut with a band saw → each section is cast as a sheet between two glass plates (4 mm thick) separated by rubber tubing (6–8 mm diameter) held together by clips to form a leak-proof chamber → the chamber is filled with the resin + catalyst + accelerator mixture → after **24 hours** the clips are removed and the glass sheets separated from the set resin → the sheet's edges are trimmed, polished and the specimen labelled.
- ▶ **Luminal cast plastination:** the lumen is cleaned under tap water → the specimen is kept in **KOH solution for 1–2 hours** → cleaned again under running tap water and dried → the lumen is filled with a mixture of resin, catalyst and hardener → after **1 day** the cast is obtained by digesting/removing the surrounding tissue. This method is used for tracheobronchial casts of the lung, cerebral ventricular casts, bony labyrinth casts, and vascular casts of kidney, liver, lung, spleen and coronary vessels, including their variations.

Advantages of plastination as a museum technique

- ▶ Produces **dry, non-toxic, odourless, durable and natural-coloured** specimens that can be freely handled by students without fixative exposure.
- ▶ Eliminates the irritating fumes and biohazard risk associated with wet formalin-fixed specimens.
- ▶ Provides a **unique specimen at low cost**, comparable in quality to internationally produced plastinated specimens.
- ▶ Has a comparatively **simple protocol**.
- ▶ Best suited for **teaching purposes, designing and practising newer surgical procedures, skill training, and research**.

Applied / Clinical anatomy

- ▶ Plastinated whole-organ and part specimens form the backbone of modern gross-anatomy museums, replacing fragile, odorous, fading wet specimens with permanent teaching aids.
- ▶ **Sheet plastination** slices correspond directly to CT/MRI cross-sectional planes, so they are widely used to teach cross-sectional and radiological anatomy and to correlate imaging with true anatomical relationships.
- ▶ **Luminal cast plastination** specimens (bronchial tree, cerebral ventricles, bony labyrinth, renal/hepatic/pulmonary vascular trees, coronary vessels) demonstrate three-dimensional branching patterns and anatomical variations that cannot be appreciated by dissection alone — useful for teaching vascular/ductal variations relevant to surgery and interventional radiology.
- ▶ Because plastinated specimens are dry, non-toxic and durable, they remove the formaldehyde-exposure hazard to students and museum staff, an important occupational-safety consideration in the dissection hall/museum.

- ▶ Plastinated cadaveric material and models are used for **rehearsing and designing new surgical approaches** and for skills training, since the specimens retain realistic tissue planes without the handling constraints of wet cadavers.
- ▶ (Widely known applied context, not from this corpus:) the technique was devised by **Gunther von Hagens** at the University of Heidelberg and popularised through public anatomical exhibitions of whole-body plastinated cadavers.

High-yield exam pointers

- ▶ Plastination = technique producing **dry, coloured, non-toxic, durable, odourless, natural-looking** specimens.
- ▶ **3 types**: Whole organ, Sheet, and Luminal cast plastination.
- ▶ **4 steps** of whole-body plastination: **Fixation** → **Dehydration (acetone)** → **Forced impregnation in vacuum (polymer)** → **Hardening (curing by gas/heat/UV)**.
- ▶ Key materials: acetone (dehydration/intermediary solvent); silicone rubber/polyester/epoxy resin + catalyst + accelerator (impregnation and curing medium).
- ▶ Sheet plastination: ~1 cm slices cast between glass plates in resin — correlates with CT/MRI cross-sections.
- ▶ Luminal cast plastination: lumen filled with resin, surrounding tissue digested (KOH) to leave a cast of the branching pattern.
- ▶ Overcomes drawbacks of wet formalin-fixed specimens: odour, bleaching, unnatural appearance, poor demonstration of luminal/branching anatomy.
- ▶ Advantages: low cost, simple protocol, safe (non-toxic, odourless), durable — used for teaching, surgical skill practice and research.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (specialty text; sole substantive source for this topic in the corpus).

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

Coverage note: Gray's Anatomy (Standring) 41e does not cover museum/preservation techniques, so this answer is grounded entirely in the specialty preservation text (Haldar). The historical attribution to Gunther von Hagens/Heidelberg (1977) is standard, universally accepted background not found verbatim in the local corpus and has been kept brief and clearly separated from the source-grounded technical content.



Preservation and maintenance of museum specimens

Asked in: Paper III Nov 2017 · Paper III Apr 2006 (2×) — RGUHS MD/MS Anatomy

Definition / Introduction — An anatomy museum specimen is a gross (or macerated bone) specimen obtained at dissection/autopsy that is fixed, mounted and displayed so that its natural size, shape and colour are retained indefinitely for undergraduate and postgraduate teaching. Preservation requires (i) careful selection and fixation of the specimen, (ii) restoration/maintenance of its natural colour, (iii) correct mounting in a jar or Perspex box, and (iv) systematic storage, labelling and cataloguing. Modern museums increasingly supplement or replace fluid-mounted "wet" specimens with dry, odourless plastinated specimens.

1. Selection and preparation of the specimen

- ▶ Good museum specimens are obtained by **care and planning at the time of autopsy/dissection**; careless removal of a section can easily ruin an otherwise good specimen.
- ▶ The cut surface must be **smooth and even** — a single continuous stroke of a long-bladed sharp knife (not sawing); tissue taken for histology is removed from the **back** of the specimen, or from the front with a scalpel, so the display surface is not disfigured.
- ▶ The specimen is placed in a **fixative almost immediately** — containers of 10% formal-saline are kept ready in the dissection hall/operation theatre — and specimens with much blood must **never be washed in water** (leaches colour and pigment).
- ▶ Fresh specimens are laid on cotton wool covered with lint; unopened cystic cavities are inflated and opened cavities packed with fixative-soaked cotton wool, so the natural shape is maintained. The specimen must be fixed **with its attached structures** intact (vessels, ducts, nerves) to preserve anatomical relations; bile-stained specimens are fixed and stored separately.
- ▶ A museum specimen is correlated with its **relevant photograph, radiograph, catalogue label and clinical history** — considered as important as the physical preservation itself.

2. Fixation and colour restoration of the specimen

- ▶ **Primary fixation** is in 10% formal-saline; the specimen is then transferred to a special fixative for colour maintenance.
- ▶ The most widely used method is a modification of **Kaiserling's technique (1900)**, which classically uses three solutions — a fixing fluid, a fluid to restore original colour, and a mounting fluid.
- ▶ **Pulvertaft's modification (1936)** restores colour by adding a reducing agent — **sodium hydrosulphite** — to the mounting medium itself; specimens mounted by this method show remarkably little fading even after 35 years.

Solution	Composition	Purpose / notes
Kaiserling's fluid I	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + water to 2000 ml	Fixing fluid — specimen transferred to this either after formal-saline fixation or fixed directly in it

Solution	Composition	Purpose / notes
Kaiserling's fluid II	Ethyl alcohol 80%	Restores colour (30 min–4 h); continued immersion causes permanent bleaching, so timing must be controlled; not needed if sodium hydrosulphite mounting fluid is used
Pulvertaft–Kaiserling mounting fluid III	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + water to 1000 ml	Final mounting fluid; 0.4% sodium hydrosulphite added immediately before sealing the jar to restore/maintain natural colour

- ▶ **Israel & Young (1978)** used pure liquid paraffin as the final mountant after alcohol colour-restoration, reducing discoloration of the mounting fluid by pigments from the specimen.
- ▶ **Wentworth's method (1938–1957)** dispensed with the alcohol step altogether, using sodium hydrosulphite alone (fixation ≥ 1 month); the final mounting solution is chosen by the pH of the fixed specimen (solution III if pH > 6.5 , solution II if pH < 6.5).
- ▶ **Drawbacks of routine formalin-fixed "wet" specimens:** irritating odour; bleached/colourless parts; unnatural appearance; sections hard to maintain; luminal architecture and branching patterns of hollow organs almost impossible to appreciate.

3. Mounting and display of the specimen

- ▶ Before mounting, surface irregularities are re-cut, natural cavities are re-filled with **arsenious acid gelatin**, and coloured Perspex arrows/rods or black horse-hair are used to indicate delicate structures.
- ▶ The specimen is laid on a flat, water-proofed (formica) bench in as **anatomically correct a position as possible**, and measured allowing 1 cm clearance at top/sides and 2 cm at the bottom (space for the label).
- ▶ **Perspex boxes** are now used almost universally in place of glass jars — light, unbreakable, and fabricated (or bought) to fit each specimen exactly.
- ▶ The specimen is stitched to a **centre plate** that fits into the box with ~5 mm clearance, using nylon thread (2 holes) or linen thread with a glass-bead retainer (1 hole) knotted with a reef knot, avoiding pathological lesions; alternatively it is pressed onto cemented Perspex spikes.
- ▶ **Filling and sealing:** museum fluid (with 0.4% sodium hydrosulphite) is run in to within 1 cm of the top, air bubbles removed, and topped up through a small drilled hole that is then plugged and cemented shut.
- ▶ **Glass-jar mounting** follows the same principle, except the lid is sealed onto the ground-glass rim with heated asphaltum–rubber (Picein) compound, later trimmed and edge-painted with black enamel/asphaltum varnish.
- ▶ **Embedding in solid plastic blocks** is the ideal presentation method, but is limited because no technique yet protects the colour of soft-tissue specimens embedded this way.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a mounted museum specimen (Perspex box, sectional view):

- ▶ **Outer Perspex box** (transparent, custom-fitted)
- ▶ **Specimen**, anatomically oriented, with attached structures intact
- ▶ **Centre plate** (Perspex) to which the specimen is sutured
- ▶ **Nylon/linen sutures with glass-bead retainer**, tied with a reef knot, avoiding the lesion
- ▶ **Museum mounting fluid** (Kaiserling III / Pulvertaft's fluid + 0.4% sodium hydrosulphite) filled to 1 cm from the top
- ▶ **Sealed lid** (Perspex-cemented plug / asphaltum–Picein seal for a glass jar)
- ▶ **Catalogue label** tied to the specimen with linen thread, showing specimen number, diagnosis and clinical details

4. Storage, labelling and cataloguing

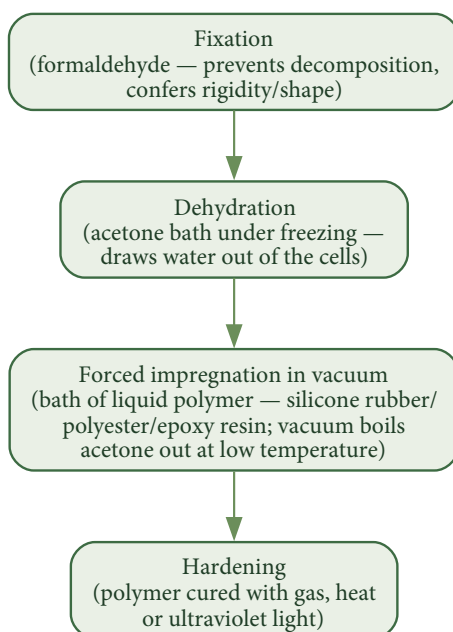
- ▶ Storage must permit **easy and unique identification** of every specimen by a serial catalogue number, recorded in a **reference/catalogue register** along with clinical history, diagnosis, source and date.
- ▶ Specimens are stored in **separate containers** to prevent damage from contact with other specimens.
- ▶ Every container is **labelled on the outside**, and the label is additionally **tied to the specimen itself with linen thread**, so identity is never lost even if the outer label is separated.

5. Preservation of hard-tissue (bone) specimens — maceration

- ▶ **Maceration** is used to demonstrate bony lesions such as osteogenic sarcoma, osteoma, chronic osteomyelitis and tuberculosis of bone.
- ▶ The technique chosen depends on the degree and type of lesion:
 - For preserving the **finest bone spicules** — the **putrefaction method** is used.
 - As a **routine method**, the specimen is boiled in tap water or very dilute (N/100) sodium hydroxide, with softened tissue removed at intervals.
 - A **gross method for compact bone** is autoclaving in N/10 sodium hydroxide for 5 minutes — faster, but it also removes fine bony spicules along with soft tissue.
- ▶ **Degreasing and bleaching**: fat is removed by immersing the bone in chloroform for 3–4 hours; the specimen is then dried in an incubator and bleached in hydrogen peroxide.
- ▶ **Mounting**: macerated bones are mounted dry, either on the centre plate or on individually designed Perspex supports, tied with nylon thread; a drop of Perspex cement over the knot gives added support.
- ▶ **Calculi**: older methods mounted them dry in boxes with reversible glass lids, or in gelatin with added formalin (drawbacks: specimens become dirty, or the gelatin slowly dissolves). The **recent method** uses both halves of a bisected calculus — one half is polished and mounted for display, the other half is labelled with Indian ink and kept for students to handle and study more closely.

6. Plastination — the modern museum technique

Plastination produces a **dry, coloured, non-toxic, durable and odourless** specimen that looks natural, overcoming the drawbacks of fluid-fixed specimens.



Preservation and maintenance of museum specimens

Type	Principle / method	Typical use
Whole organ plastination	Fix in alcohol–formalin–glycerine–phenol mixture (2–3 days) → wash → acetone jar → resin jar (1–2 weeks) → immerse in resin + catalyst (5%) + accelerator (0.1%) → mount on a base	Better understanding of total organ structure and relationships
Sheet plastination	Specimen deep-frozen 24 h, cut into ~1 cm slices with a band saw, cast between two glass sheets (resin + catalyst + accelerator) for 24 h, then trimmed, polished and labelled	Thin, transparent or thick opaque body-section slices; totally portable, best shows internal relationships
Luminal cast plastination	Lumen cleaned, soaked in KOH (1–2 h), cleaned again, filled with resin + catalyst + hardener; surrounding tissue removed after 1 day	Studies dimensions/architecture of tubular structures — tracheobronchial tree, cerebral ventricles, bony labyrinth, vascular pattern of kidney/liver/lung/spleen, coronary vessels

- **Materials used:** jars for storage/processing, acetone, silicone rubber, resin with catalyst and accelerator, colour paints, dissection instruments, syringes and needles.
- **Advantages:** provides a unique specimen of international quality at low cost; simple protocol; best suited for teaching, designing new surgical procedures, practising skills, and research.

Applied / Clinical anatomy

- ▶ A well-run museum lets a candidate correlate a **gross specimen with its paired radiograph/CT image** — a core RGUHS viva/practical skill — and macerated bone specimens of osteosarcoma, chronic osteomyelitis and skeletal TB allow direct correlation with radiological features.
- ▶ **Calculi museums** (one polished, one rough Indian-ink-labelled half) permit tactile study of internal laminae otherwise invisible on the surface.
- ▶ Formalin-preserved wet specimens carry an **occupational hazard** (irritant, carcinogenic vapour) for students and staff; odourless plastinated specimens are safer for long-term handling in teaching collections.
- ▶ Specimens obtained via the body-donation/dissection programme must be **ethically sourced, catalogued and labelled**, to the same documentation standard (register, unique number, clinical history) required for embalmed cadavers.
- ▶ Plastinated whole-organ and luminal-cast specimens are now also used in **cadaveric surgical skill workshops** (endoscopic/laparoscopic training), extending the museum's teaching role beyond undergraduate anatomy.

High-yield exam pointers

- ▶ Sequence of Kaiserling technique: fix in **Kaiserling I** (formalin + KNO_3 + K-acetate) → restore colour in **Kaiserling II** (80% ethyl alcohol, 30 min–4 h) → mount in **Kaiserling III / Pulvertaft's fluid** (glycerin + sodium acetate + formalin) with **0.4% sodium hydrosulphite** added just before sealing.
- ▶ **Pulvertaft's modification (1936)**: sodium hydrosulphite as reducing agent gives remarkably stable colour retention (>35 years).
- ▶ **Wentworth's method (1938–1957)**: omits the alcohol step, uses sodium hydrosulphite alone, mounting solution chosen by the pH of the fixed specimen.
- ▶ **Maceration of bone**: routine method = boiling in dilute (N/100) NaOH; fast/gross method = autoclaving in N/10 NaOH for 5 min (loses fine spicules); putrefaction method best preserves the finest bony spicules.
- ▶ **Plastination — 4 steps**: Fixation → Dehydration (acetone) → Forced impregnation (vacuum, polymer) → Hardening (gas/heat/UV).
- ▶ **Plastination — 3 types**: Whole organ, Sheet, Luminal cast.
- ▶ Calculi: modern method mounts one polished half for display and keeps the other (Indian-ink labelled) half for tactile student study.
- ▶ **Perspex has replaced glass** as the standard museum container material — light, unbreakable, and custom-fabricated to the specimen.
- ▶ An ideal museum specimen preserves natural size, shape and colour, and is always paired with a labelled catalogue card and, where relevant, a photograph/radiograph.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (primary source for fixation/colour-restoration methods, mounting, storage, maceration and plastination). **Coverage note:** the Handbook of Museum Technique (Govt Museum, Chennai) was also checked but is a natural-history/taxidermy manual (zoological specimen preservation) rather than an anatomical wet-specimen text, so it was not used to source facts in this answer; standard anatomy-museum practice is otherwise not covered in Gray's Anatomy (Standring) or the gross-anatomy texts.



Body donation

Asked in: Paper III May 2010 — RGUHS MD/MS Anatomy

Definition / Introduction **Body donation** is the voluntary act by which a living person pledges (or, after death, the next of kin consents) to gift the person's own dead body to a medical/teaching institution for **anatomical examination, dissection, skill training and research**, free of cost and without commercial consideration. In India the supply and use of dead bodies for this purpose is regulated by the **Anatomy Act (1949)**, which covers both **unclaimed bodies** and **willed (voluntarily donated) bodies**. The cadaver is often called the "**first teacher**" of a medical student — no plastic model, 3-D reconstruction or virtual-reality simulation can replace the tactile, three-dimensional learning of a real dissection, which makes an adequate, ethically-sourced supply of donated bodies central to anatomy education.

1. Need for body donation

- ▶ **Irreplaceable teaching tool** — cadaveric dissection remains the only method that teaches true tissue-plane relationships, variations and 3-D spatial orientation; models/CT-reconstructions/VR are adjuncts, not substitutes.
- ▶ **Chronic shortage of cadavers** in most medical colleges (rising student intake under CBME vs static cadaver supply) makes voluntary body donation programmes essential to supplement the traditional **unclaimed-body route**.
- ▶ **Multiple downstream uses** of one donated body: undergraduate/postgraduate dissection, museum-specimen preparation, hands-on **cadaveric skill workshops** (endoscopic, laparoscopic, arthroscopic, intraoral, flap-raising surgery), research, and forensic/radiological correlation.
- ▶ Several medical schools administer a formal "**oath**" before the first dissection, to uphold the dignity of the donor's mortal remains and sensitise students to the sanctity of the body being studied.

2. Legal framework governing body donation in India

- ▶ **Anatomy Act (1949)** — an Act to provide for the supply of **unclaimed bodies** of deceased persons to hospitals and medical/teaching institutions for the purpose of anatomical examination and dissection.
 - Originated as the **Bombay Anatomy Act**; subsequently adopted, with local amendments, by most Indian States (including Karnataka) as their own State Anatomy Act.

- Permits collection of a dead body for teaching only if death occurs in a **State/government hospital or a public place** within the prescribed jurisdiction of the medical institution, and the police have certified after a **48-hour** waiting period that **no claimant** has come forward.
- **Willed/voluntary body donation** — a separate, ethically preferred route in which a person (or, after death, the family/next of kin) directly hands over the body to the Anatomy Department of a medical college, bypassing the "unclaimed" route; increasingly promoted because it carries **informed, documented consent**.
- **Distinguish from organ donation** — the **Transplantation of Human Organs Act (1994)**, amended as **THOTA (2011)**, governs the removal and therapeutic transplantation of organs/tissues (kidney, liver, cornea, etc.) from living or brain-dead donors; it is a **separate statute** from the Anatomy Act and serves a **therapeutic**, not **educational**, purpose — an important distinction to draw in the exam.

3. Sources of cadavers: unclaimed body vs willed (donated) body

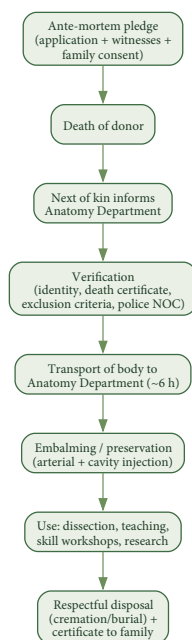
Feature	Unclaimed body (Anatomy Act)	Willed/voluntary donated body
Consent	Presumed by law after 48-h no-claimant period; no personal antemortem consent	Antemortem pledge by the donor (or postmortem consent by next of kin)
Route	Police/hospital → Anatomy Department	Donor/family → Anatomy Department directly
Identity/history	Usually unknown; social/medical history unavailable	Known identity; medical history often available
Ethical standing	Historically controversial (no personal consent)	Ethically preferred — true "gift"
Trend	Traditional, still common source	Actively encouraged; growing source in India

4. Procedure of body donation

- **Eligibility (standard practice)**: any adult of sound mind may pledge; application is usually countersigned by two witnesses and the near relatives/next of kin (who must reaffirm consent at the time of death).
- **Common exclusion criteria**: death from a notifiable/highly infectious disease (HIV, viral hepatitis B/C, active tuberculosis, gangrene/septicaemia), an unautopsied **medico-legal case**, gross decomposition/putrefaction, major mutilating trauma, or extremes of body build — since these compromise safe/adequate embalming and dissection.
- **Timing** is critical — for a good embalming result the body should reach the department and be embalmed **within about 6 hours of death**; delay allows bacterial decomposition/putrefaction to set in.
- **Legal aspects to be completed before embalming**: identification of the body by near relatives, **written consent** for the procedure, a **No-Objection Certificate from the police**, the **death certificate**, and — for a foreign national — **Embassy clearance**; a medico-legal body may be

embalmed only **after** autopsy. On completion, the embalmer/competent authority issues a certificate (multiple copies for onward transport).

- **Disposal:** after use, the dissected/used remains are **cremated or buried with full dignity** according to the family's rites, and a certificate/acknowledgement is issued to the family.



Body donation

5. Preservation of the donated body — embalming

- **Purpose:** arrest putrefaction/autolysis, produce a stable, safe, dissectible specimen; embalming causes a **chemical stiffening resembling rigor mortis**, but this rigidity, unlike true rigor, is **permanent**.
- **Composition of an ideal arterial embalming fluid** (per 10 L, for a ~70 kg body — of which ~10% is lost through drainage/purging during injection):

Ingredient	Role	Quantity
Formalin	Preservative (fixative)	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 mL
Sodium chloride	Controls pH	800 g
Eosin (1%)	Cosmetic tint	30 mL
Water	Vehicle	up to 10 L

- **Cavity embalming fluid** (for viscera): Formalin 60%, Methanol 25%, Phenol 10%, Sodium lauryl sulphate 1%, Mercuric chloride 1%, Eucalyptus oil 1%.
- **Hard vs soft embalming** — two techniques of practical, exam-relevant significance:

- **Hard (conventional/high-formalin) embalming** — formalin concentration raised to ~4% preserves the cadaver for up to **6 months**, but the tissue becomes stiff/rigid, limiting its use to gross dissection.
- **Soft embalming (Thiel's method)** — uses boric acid (fixation), ethylene glycol (preserves tissue plasticity) and salts, keeping **formalin to a strict minimum (~0.8%)**; the body retains a natural look with **flexible skin, muscle and joints**, ideal for **cadaveric surgical skill workshops** (endoscopic sinus surgery, laparoscopic and arthroscopic training, flap-raising) — such prepared cadavers, vacuum-sealed and mortuary-stored, remain usable for about **3 weeks** of intensive workshop use before deterioration begins.
- ▶ **Occupational precautions for the embalmer:** PPE against blood/airborne pathogens (HIV, Hepatitis B/C, MRSA, TB), and against chemical (formaldehyde) exposure.

6. Ethical aspects of body donation

- ▶ The cadaver dissected is a **person's final, selfless gift** to medical education — "**the dead teach the living**", and students pledge to use this knowledge for the upliftment of humankind.
- ▶ **Respect and dignity** must be maintained throughout — many institutions administer an **oath before dissection**; body parts are handled, stored and eventually disposed of with the same reverence as during life.
- ▶ **Confidentiality** of the donor's identity is preserved; the family is treated with empathy and gratitude at every step.
- ▶ Every dissected part is finally **disposed or cremated with full dignity**, honouring the donor and family — this remains the **prime responsibility of the health professional**.

Applied / Clinical anatomy

- ▶ **Surgical/endoscopic skill training** — soft-embalmed (Thiel-type) cadavers are increasingly used for hands-on workshops in laparoscopic, arthroscopic, endoscopic sinus, skull-base and flap-raising surgery, because near-normal tissue pliability is preserved.
- ▶ **Museum specimen preparation** — organs harvested during dissection/autopsy of donated bodies are fixed (formal-saline) and preserved (e.g., Kaiserling-type colour-restoration techniques) for teaching museums.
- ▶ **Radiological/surface anatomy correlation** — donated cadavers allow CT/MRI-cadaver correlation studies used in modern anatomy teaching.
- ▶ **Forensic and medico-legal training** — unautopsied vs autopsied embalming protocols differ, and understanding legal formalities (death certificate, police NOC, medico-legal clearance) is directly examinable applied knowledge.
- ▶ **Limitations to remember clinically/medico-legally:** embalming before autopsy can create artefacts, obscure wound interpretation, destroy toxicological evidence (alcohol, opiates, CO, cyanide) and preclude bacteriological/blood-grouping studies — hence embalming of a medico-legal case must **follow**, never precede, autopsy.

High-yield exam pointers

- ▶ Two legal routes of cadaver procurement: **unclaimed body** (Anatomy Act, 1949 — 48-h no-claimant rule) vs **willed/voluntary body donation** (antemortem pledge/consent).
- ▶ Anatomy Act (1949) originated as the **Bombay Anatomy Act**, later adopted by most States; distinct from the **Transplantation of Human Organs Act (1994/THOTA 2011)**, which governs organ donation for transplantation, not dissection.
- ▶ Embalming must be completed **within ~6 hours** of death for a good result; medico-legal bodies are embalmed **only after autopsy**.
- ▶ Legal formalities before embalming: identification, written consent, police NOC, death certificate, Embassy clearance (foreigner).
- ▶ Ideal arterial embalming fluid: **Formalin 1.5 L** + buffer/anticoagulant/wetting agent/salt in **10 L** water (per ~70 kg body).
- ▶ **Hard embalming** (~4% formalin) → preserves ~6 months, rigid tissue; **soft/Thiel embalming** (~0.8% formalin + ethylene glycol) → flexible tissue, ideal for surgical skill workshops (~3 weeks usable).
- ▶ Ethical core: cadaver = "**first teacher**"; oath before dissection; respectful disposal/cremation after use; donor confidentiality and family gratitude.

SOURCE & COVERAGE

Haldar — *Notes on Embalming & Museum Techniques*; BD Chaurasia — *Human Anatomy* Vol 3–4, 8e (section: "Ethical Aspects of Cadaveric Dissection").

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

The library corpus for this bank does not carry a dedicated chapter on Indian willed-body-donation-programme administration (application forms, State-specific eligibility clauses); the procedural/eligibility content above follows the Anatomy Act framework and universally-accepted, standard Indian body-donation-programme practice rather than a single cited passage.



Brief note on preservation of specimens in the museum

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction

A museum ("wet") specimen is a dissected/prosected body part preserved permanently in a fixative-cum-mounting medium so that its natural size, shape, colour and anatomical relations remain available for teaching, research and medico-legal reference. Preservation begins at autopsy/dissection itself — careless removal and delayed fixation are the commonest causes of a poor specimen. Two broad approaches are used: (i) the classical **wet technique** — fixation, colour restoration and mounting in a fluid-filled jar or Perspex box (the Kaiserling family of methods), and (ii) **plastination**, a modern dry,

odourless, durable alternative. Special methods (maceration, degreasing) are reserved for bony and calculous specimens.

Aims / requisites of a good museum specimen

- ▶ Natural size, shape and colour retained as closely as possible to the fresh state.
- ▶ Durable and non-fading — should withstand decades of storage and repeated handling.
- ▶ Odourless, non-toxic and safe for students/staff to examine.
- ▶ Anatomical relations preserved; important structures (vessels, ducts, nerves) clearly identifiable, if necessary with coloured markers.
- ▶ Properly labelled with a unique identification number and catalogued in a reference register.
- ▶ Ideally accompanied by correlated clinical history, radiograph or photograph to enhance teaching value.

Preparation of the specimen

- ▶ Good museum specimens are obtained by care and planning at the time of autopsy, not as an afterthought — careless removal of the section can easily ruin a specimen.
- ▶ The cut surface should be smooth and even, made with a single continuous stroke of a long-bladed sharp knife (a sawing action roughens the surface).
- ▶ Tissue required for histology is taken from the back of the piece intended for museum preservation, or with a scalpel from the front, so the display surface is not disturbed.
- ▶ The specimen must be placed in fixative almost immediately after removal; containers of 10% formal-saline should always be kept ready to hand in the operating theatre/mortuary.
- ▶ Blood-stained specimens are **never washed in water**, before or after fixation, as this favours haemolysis and discolouration; a fresh specimen is laid on a thick layer of cotton wool covered with lint.
- ▶ Hollow viscera and cystic cavities: if unopened, they are gently inflated with fixative; if opened, they are packed with fixative-soaked cotton wool to maintain their natural shape.
- ▶ Bile-stained or bile-containing specimens are fixed and stored separately, as bile pigment discolours neighbouring specimens.

Fixation and colour restoration — the Kaiserling technique and its modifications

The most widely used method of colour maintenance is a modification of **Kaiserling's technique (1900)**, which originally employed three solutions — a fixing fluid, a colour-restoring fluid, and a mounting fluid.

Step	Solution / composition	Purpose
Primary fixation	10% formal-saline	Hardens tissue, arrests autolysis immediately after removal
Kaiserling's fluid I	Formalin 400 mL + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 mL	Fixation — specimen transferred here after formal-saline, or fixed directly in it

Step	Solution / composition	Purpose
Kaiserling's fluid II	Ethyl alcohol 80%	Restores colour in an emergency (e.g., for colour photography); time carefully controlled (30 min – 4 h) — prolonged immersion causes permanent bleaching
Pulvertaft–Kaiserling fluid III (mounting)	Glycerin 300 mL + sodium acetate 100 g + formalin 5 mL + water to 1000 mL, with 0.4% sodium hydrosulphite added immediately before sealing	Final mounting fluid that maintains the restored colour
Wentworth's modification (1957)	Three graded formaldehyde–sodium-acetate solutions ($\pm$ sodium phosphate, $\pm$ glycerine), chosen according to the pH of the specimen after fixation	Dispenses with the alcohol step; colour restored by sodium hydrosulphite alone
Israel & Young (1978)	Pure liquid paraffin as final mountant, after colour restoration with alcohol	Reduces discolouration of the mounting fluid by pigments leaching from the specimen

- **Pulvertaft's modification (1936)** restores tissue colour by adding the reducing agent sodium hydrosulphite to the mounting medium; specimens mounted by this technique show remarkably little fading even after 35 years.
- Additional rules for fixation: specimens are injected with fixative where necessary to ensure adequate penetration (e.g., the brain); specimens with much blood are never washed in water at any stage.

Storage, mounting and sealing

- Each specimen is given a unique identification number and entered in a reference (catalogue) register recording relevant clinical and pathological details.
- Specimens are stored in separate, individually labelled containers to prevent damage from contact with other specimens; the outer label is also tied to the specimen with linen thread.
- Before mounting: slight surface irregularities are re-cut; membranes/skin/intestine that were pinned on a corkboard have their edges trimmed; after removal of the cotton-wool packing, natural cavities are filled with arsenious-acid gelatin; friable fragments (e.g., a blood clot) are held in position with a thin layer of the same gelatin.
- Two classical mounting methods:
 - **Centre-plate (Perspex box) mounting** — the specimen is anatomically positioned and stitched (nylon thread through two 1 mm holes, or linen thread with a retaining glass bead through one hole) onto a Perspex centre plate, or fixed using cemented Perspex spikes; the plate is fitted into a close-fitting Perspex box, filled with mounting fluid containing 0.4% sodium hydrosulphite, trapped air bubbles are removed, and the box is sealed with Perspex/ethylene-dichloride cement under a lead weight.
 - **Glass-jar mounting** — the specimen is fixed to a glass plate by drilled holes (using camphor-in-turpentine as a lubricant while drilling); the jar is filled with mounting fluid to within 1

cm of the top and sealed with an asphaltum–rubber compound (picein), melted with gentle flaming, with the edges finished in black enamel/asphaltum varnish.

- Specimens may also be embedded in solid plastic blocks, though there is as yet no reliable method to preserve soft-tissue colour by this technique.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled sketch of a **mounted museum jar specimen (centre-plate technique)** — label:

- Perspex/glass jar with sealed lid
- Perspex centre plate
- Specimen anatomically oriented and stitched/pinned to the centre plate
- Mounting fluid (Kaiserling/Pulvertaft fluid III) filling the jar
- Coloured Perspex marker rods identifying key structures (vessel/duct/nerve)
- Outer label bearing the unique specimen ID number

Special museum techniques

- **Maceration** — used to demonstrate bony lesions such as osteogenic sarcoma, osteoma, and chronic osteomyelitis/tuberculosis. The routine method is boiling the specimen in tap water or very dilute (N/100) sodium hydroxide, removing softened tissue at intervals; a gross method for compact bone is autoclaving in N/10 sodium hydroxide for 5 minutes, though this also removes fine bony spicules. The putrefaction method is preferred when the finest spicules of bone must be preserved.
- **Degreasing and bleaching** — fat is removed by immersing the bone in chloroform for 3–4 hours, followed by drying in an incubator and bleaching in hydrogen peroxide. Macerated bones are mounted dry, on a centre plate or Perspex supports, using nylon thread reinforced with a drop of Perspex cement at the knot.
- **Calculi** — older methods dry-mount them in boxes with reversible glass lids, or mount them in formalin-containing gelatin (both are disadvantageous — the former becomes dirty, the latter's gelatin slowly dissolves); the modern method polishes and mounts one half of the calculus while the other half is labelled with Indian ink and kept for students to handle and study closely.

Plastination

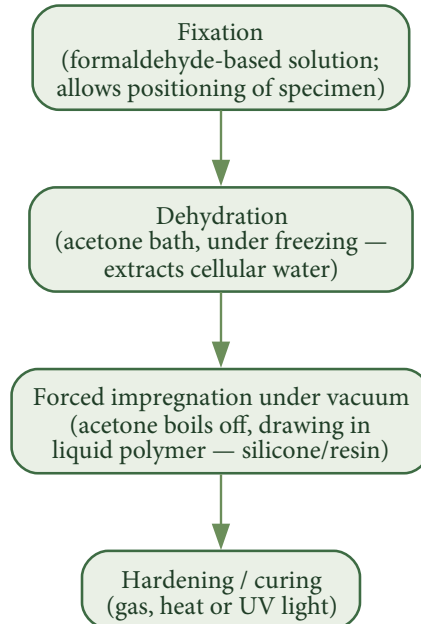
Plastination is a technique for preparing **dry, coloured, non-toxic, durable, odourless, natural-looking specimens** — the principal modern alternative to wet (fluid) preservation, developed to overcome the irritating odour, bleached/colourless appearance, and difficulty in demonstrating luminal architecture inherent to routine formalin-fixed wet specimens.

Types

- Whole-organ plastination — best appreciates total organ structure and relationships.
- Sheet plastination — thin transparent or thick opaque body slices; the whole body can be cut into portable, dry-stored sheets that best show internal relationships.
- Luminal-cast plastination — fills the lumen of a hollow structure and digests away the surrounding tissue to yield a cast of its architecture (e.g., tracheobronchial tree of the lung,

cerebral ventricles, bony labyrinth, and the vascular pattern of kidney, liver, spleen and coronary vessels).

Materials used: jars for storage/processing, acetone, silicone rubber, resin with catalyst and accelerator, colour paints, dissection instruments, syringes and needles.



Brief note on preservation of specimens in the museum

- ▶ **Whole-organ method** in brief: wash in tap water → immerse in a preservative (95% alcohol 280 mL + formalin 1320 mL + glycerine 80 mL + phenol 120 mL + distilled water 800 mL) for 2–3 days → wash off formalin in running water → transfer to acetone → transfer to resin for 1–2 weeks → final immersion in resin (general-purpose) + 5% catalyst + 0.1% accelerator until non-sticky → mount on a base in the desired orientation.
- ▶ Advantages over wet fixation: no irritating odour, natural colour and dry handling, non-toxic, durable over years, and far better demonstration of luminal dimensions, branching pattern and three-dimensional architecture than a dissected wet specimen.

Applied / Clinical anatomy

- ▶ Museum specimens are the backbone of undergraduate/postgraduate teaching in gross anatomy and pathology, permanently recording morphology, congenital anomalies and disease progression not always re-demonstrable on a fresh cadaver — "the common diseases of today may well be the rarities of tomorrow" (Silverstone).
- ▶ Procurement of unclaimed human material rests on the same legal basis as cadaver procurement — the **Anatomy Act (1949)**, which permits use of an unclaimed body for anatomical examination/dissection only after police certify, following the prescribed waiting period, that there are no claimants.
- ▶ Medico-legal specimens need the same safeguards as embalming — identification, written consent/NOC, and reference to the death certificate/PM report — before retention for the museum.

- ▶ Correlated clinical photographs, radiographs and case summaries displayed alongside the jar substantially enhance its diagnostic and teaching value.
- ▶ Plastinated specimens, being dry and durable, are increasingly used for hands-on surgical-skills training and research, tolerating handling a fragile wet specimen cannot.
- ▶ A specimen washed in water while blood-stained, or fixed late, permanently loses colour/diagnostic detail — a caution directly relevant to museum curation.

High-yield exam pointers

- ▶ Two broad preservation routes: **wet mounting** (fixation → colour restoration → mounting fluid, Kaiserling-type) and **plastination** (dry).
- ▶ Kaiserling's original technique used three solutions — I fixing, II colour-restoring (alcohol), III mounting; **Pulvertaft's modification** adds sodium hydrosulphite to the mounting fluid for long-lasting colour (little fading even at 35 years).
- ▶ Golden rules: place the specimen in fixative immediately after removal; never wash a blood-stained specimen in water.
- ▶ **Centre-plate (Perspex box)** and **glass-jar** are the two classical wet-mounting methods, sealed respectively with Perspex/ethylene-dichloride cement or an asphaltum-rubber (picein) compound.
- ▶ **Maceration** = for bone lesions (dilute NaOH boiling, or autoclaving in N/10 NaOH); **degreasing** with chloroform; **bleaching** with hydrogen peroxide.
- ▶ Plastination steps (must-recall sequence): **Fixation** → **Dehydration (acetone)** → **Forced impregnation under vacuum** → **Hardening (gas/heat/UV)**.
- ▶ Three types of plastination: **whole-organ, sheet, and luminal-cast**.
- ▶ Every museum specimen requires a unique ID number and a catalogue/reference-register entry for storage and retrieval.
- ▶ Legal basis for procurement of human material: the Anatomy Act (1949).

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques.

Coverage note: Museum/embalming technique lies outside Gray's Anatomy's scope (confirmed absent on grep of the spine text), so this answer is grounded entirely in the dedicated preservation-specialty text. The other specialty resource, the Handbook of Museum Technique (Govt Museum Chennai), covers zoological and anthropological (non-anatomical) exhibit preservation and was therefore not drawn upon for this human-anatomy museum-specimen answer.



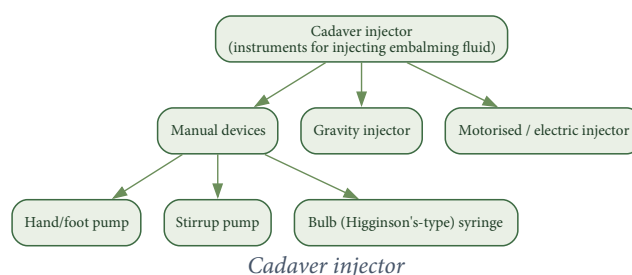
Cadaver injector

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction A cadaver injector is the mechanical apparatus used to deliver embalming fluid into the vascular system of a dead body — the core instrument of **arterial embalming**, the standard artificial method of preserving cadavers for anatomical dissection, demonstration and cadaveric-surgery training. It works by generating and sustaining a fluid-pressure gradient (by gravity or by a pump) that drives preservative solution from a reservoir, through delivery tubing and a cannula, into a raised artery, while venous drainage simultaneously evacuates blood so the fixative can perfuse every vascular territory. The type of injector used, the site(s) chosen, and the pattern of injection together decide how complete and even the fixation is — a matter of direct practical importance in every dissection hall.

Types of cadaver injector (instruments used for injecting embalming fluid)

Type	Mechanism	Key features
Hand/foot pump	Manually operated reciprocating pump	Simplest manual device; limited, operator-dependent pressure
Stirrup pump	Foot-stirrup-braced manual pump	Frees both hands for cannula control during injection
Bulb syringe	Rubber bulb + rubber tubing at both ends, similar to a Higginson's syringe	Valves permit suction on one side and ejection on the other; low-pressure manual device
Gravity injector	10-litre graduated glass bottle/percolator suspended 4–6 feet above the embalming table; outlet corked, fitted with a nozzle, transparent rubber tube and a screw clamp to regulate flow, connected to the injecting needle/cannula	Simplest, safest, slowest method; a rise of 1 metre gives ~ 0.6 kg/cm ² and 2 metres ~ 1 kg/cm ² fluid pressure; distribution is comparatively uneven and time-consuming
Motorised (electric) injector	Fluid forced from the injection tank into the vascular system by compressed air from a compression tank; a regulating device controls pressure and flow rate	Delivers 8–10 litres of arterial solution in 30–45 minutes at an injection pressure of about 2 kg/cm ² ; provides steady, high, controllable pressure — the standard instrument in institutional embalming rooms today



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a **gravity cadaver injector set-up** (safest, most-tested-in-exam version):

- ▶ Graduated glass bottle/percolator (10 L capacity) filled with arterial embalming fluid
- ▶ Support stand holding the bottle 4–6 feet above the embalming table
- ▶ Corked outlet with nozzle
- ▶ Transparent rubber delivery tube
- ▶ Screw clamp (flow-rate regulator)
- ▶ Injecting needle/cannula
- ▶ Raised artery (e.g., right common carotid artery) with cannula tied in
- ▶ Embalming table with the cadaver, head end elevated
- ▶ Accompanying vein (e.g., internal jugular vein) with drain tube for venous outflow

Principle & factors governing fluid flow

- ▶ **Sequence of action:** Injection → Distribution → Diffusion → Drainage.
- ▶ **Factors determining flow of fluid into tissues:** capillary resistance, chemical composition of the fluid, injection pressure, and osmosis/diffusion/gravity.
- ▶ **Criteria for selecting the artery:** size/diameter, practicality of drainage from the accompanying vein, depth/location of the vessel, its branches, and proximity to the arch of the aorta.
- ▶ **Criteria for selecting the vein:** size/diameter, proximity to the right atrium, depth, and ease of raising the vessel.
- ▶ **Drainage:** brought about by displacement — the injected arterial solution forces blood out; drainage consists of blood/blood clots, interstitial and lymphatic fluid, and embalming fluid. Common drainage sites are the internal jugular vein, femoral vein, right atrium and inferior vena cava; drainage may be alternate, concurrent or intermittent. Its purposes are to make room for further arterial fluid, ensure even distribution, avoid discoloration/odour/gas formation, and reduce microbial activity.

Injection sites & techniques

Technique	Site(s)	Notes
One-point injection	One location for both injection and drainage — commonest: right common carotid artery + internal jugular vein; 2nd commonest: femoral artery/vein; least common: axillary/brachial	Simple, but a single site can leave a patch of tissue unfixed
Split injection/drainage	Injection from one site, drainage from another (e.g., right femoral artery for injection, right internal jugular vein for drainage)	Reduces short-circuiting of fluid
Multipoint injection	Two or more arteries injected, drainage from one or more sites	Solves the problem of poor/uneven distribution

Technique	Site(s)	Notes
Six-point injection (sectional embalming)	Right & left common carotid (head/neck), right & left axillary/brachial (upper limb), right & left femoral (lower limb) arteries; drainage usually via right internal jugular vein	Always used for autopsied bodies ; also used as the primary technique in advanced decomposition. Standard injection order: right leg → left leg → right arm → left arm → trunk (via right common carotid) → left side of head → right side of head
Restricted cervical injection	Head embalmed separately via right and left common carotid arteries with stopcocks; trunk injected separately, drainage via internal jugular vein	Indicated in general oedema, bodies likely to purge, poor fluid distribution, facial/head trauma, eye enucleation, and delayed embalming

Each artery, once injected, is **ligated before injecting the next artery**, and every drainage point is ligated on completion to prevent leakage of embalming fluid. Each side of the face is injected separately to avoid facial distortion from over-injection.

Patterns of injection

Pattern	Technique	Result
Continuous injection & drainage	Vein tube kept open throughout	Embalming time reduced, but venous drainage and tissue saturation are poor — least satisfactory
Continuous injection with disrupted drainage	Vein tube closed during injection, builds resistance for better diffusion, then intermittently opened to discharge thick blood	Better than plain continuous injection/drainage
Alternate injection & drainage	Injected with drain tube closed until superficial veins swell → drain opened until flow stops → injection resumed; cycle repeated	Balances distribution and drainage
Discontinuous injection & drainage	Repeated small-volume arterial injections at ~2-hour intervals, 3–4 times, with the drain tube opened briefly before and after each dose; total fluid volume exceeds a single ordinary injection	Considered the best method

Embalming fluid delivered by the injector

- **Ideal arterial embalming fluid** (for a 70 kg body needing 10 L, of which ~10% is lost through drains/purging): Formalin (preservative) 1.5 L, sodium borate (buffer) 600 g, sodium citrate (anticoagulant) 900 g, glycerine (wetting agent) 600 mL, sodium chloride (pH control) 800 g, 1% eosin (dye/cosmetic) 30 mL, soluble wintergreen oil (perfume) 90 mL, water made up to 10 L.

Sodium borate and sodium citrate are dissolved in hot water and cooled first, the remaining ingredients added, diluted to volume, allowed to stand, then filtered.

- ▶ **Fluid categories used with the injector:** arterial fluids (injected through the vascular system via the injector), cavity fluids (injected via trocar into thoracic/abdominal/pelvic cavities, about 2 L per average body), and pre-injection fluids (4–5 L, injected first — allowed to stand 30 minutes — to loosen clots, drain blood from the vascular system, and improve subsequent arterial fluid distribution; these contain anticoagulants and buffers).
- ▶ **Modes of embalming delivered through/alongside the injector:** arterial embalming, cavity embalming, hypodermic embalming (subcutaneous injection for isolated limbs or parts, e.g. after blast/air-crash/rail injuries, using an 8–19 gauge hypodermic syringe), surface embalming (chemical-soaked packs applied to burns/bed sores/surface lesions), embalming of the autopsied body, and embalming of the HIV/AIDS-positive body (higher fluid concentration with added handling precautions).

Applied / Clinical anatomy

- ▶ Embalming should ideally begin **within 6 hours of death** for the best fixation; delayed embalming already shows bacterial decomposition and typically needs a restricted-cervical or higher-concentration protocol.
- ▶ **Autopsied bodies mandatorily require six-point injection**, because the post-mortem examination has disrupted normal circulatory continuity; the viscera are treated separately with cavity fluid or embalming powder in a viscera bag ("shake and bake").
- ▶ A **motorised injector's controlled, steady, high pressure and short delivery time** (8–10 L in 30–45 minutes) make it the preferred instrument for cadaveric-surgery workshop bodies used in skull-base/endoscopic sinus, laparoscopic, intraoral and arthroscopic training, where the specimen must remain usable for weeks under vacuum-sealed cold storage.
- ▶ For **HIV/AIDS or other high-risk cadavers**, the concentration of both arterial and cavity fluid is increased, and universal precautions must be followed by the embalmer during injection and drainage, given the occupational risk of bloodborne/airborne pathogens (HIV, Hepatitis B/C, MRSA, *C. difficile*, TB-MDR, CJD).
- ▶ Poor injector technique — single-point injection with uneven pressure — leaves a "patch" of tissue unfixed by embalming fluid, which is why anatomy departments preparing teaching cadavers routinely favour multipoint or six-point injection protocols.

High-yield exam pointers

- ▶ Cadaver injector = the apparatus that delivers embalming fluid into the arterial system; **5 types** — hand/foot pump, stirrup pump, bulb (Higginson's-type) syringe, gravity injector, motorised/electric injector.
- ▶ **Gravity injector:** 10 L bottle, 4–6 feet above the table; simplest, safest, slowest.
- ▶ **Motorised/electric injector:** compressed-air driven, 8–10 L in 30–45 min, $\sim 2 \text{ kg/cm}^2$ pressure; standard in institutions today.
- ▶ Four sequential steps of embalming action: **injection** → **distribution** → **diffusion** → **drainage**.
- ▶ Injection techniques: **one-point, split, multipoint, six-point (sectional), restricted cervical**.

- ▶ Six-point injection sites: right/left common carotid, right/left axillary or brachial, right/left femoral arteries.
- ▶ **Autopsied body = mandatory six-point injection** plus separate viscera treatment.
- ▶ Ideal arterial fluid (10 L): formalin 1.5 L + sodium borate 600 g + sodium citrate 900 g + glycerine 600 mL + NaCl 800 g + eosin 30 mL + wintergreen 90 mL + water to 10 L.
- ▶ Embalming is best started within 6 hours of death.

SOURCE & COVERAGE

Notes on Embalming & Museum Techniques (Arpan Haldar).

Coverage note: Gray's Anatomy (Standring) and the standard gross-anatomy specialty texts carry no embalming/mortuary-science content; this being a Paper III "Museum, embalming & organ donation" topic, the answer is grounded entirely in the dedicated preservation-specialty text on the syllabus.



Cadaveric laboratory

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction — A **cadaveric laboratory** (dissection hall) is the designated facility of an Anatomy department where human cadavers are lawfully procured, legally documented, embalmed/preserved, stored and dissected for undergraduate and postgraduate teaching, research and surgical-skill workshops. Running it requires a legal framework for procurement, a physical layout suited to preservation and dissection, a chemical/technical protocol for embalming, and an occupational-safety and record-keeping system. The cadaver is traditionally regarded as the "**first teacher**" of medicine, and its dignified handling is an ethical obligation of the department.

1. Lawful procurement of cadavers

- ▶ **Historical background** — indiscriminate demand for teaching cadavers once drove body-snatching; the notorious Burke and Hare murders (Edinburgh, 1828) forced the enactment of the **Anatomy Act, 1832** in Britain, which legalised the requisitioning of unclaimed bodies from workhouses and hospital mortuaries for anatomical study.
- ▶ **Present-day sources of cadavers:**
 - **Body/whole-body donation ("willed body") programme** — a person pledges their body before death, or next of kin consent after death, purely for medical education/research.
 - **Unclaimed bodies** — governed in India by the **Anatomy Act, 1949** (e.g. the Bombay Anatomy Act, the model adopted by several states), which provides for supply of unclaimed bodies of the deceased to hospitals and medical/teaching institutions for anatomical examination and dissection. A body qualifies only if death occurred in a state hospital or public place within the prescribed zone of the institute, **and the police have certified after a lapse of 48 hours that no claimant has come forward.**

- **Legal procedures before embalming/receipt** — verification of the death certificate and police no-claimant/NOC certificate (for unclaimed bodies) or the signed donation-consent form (for donated bodies); entry in the departmental cadaver-receipt register with donor/next-of-kin details, cause of death and date of receipt; and screening (where feasible) for transmissible infection (HIV, HBsAg, HCV) so that special embalming precautions can be applied.

2. Layout and infrastructure of the dissection hall

- **Location & environment** — spacious, well-lit, cross-ventilated hall with a dedicated mechanical exhaust system to clear formalin vapour; non-slip, non-porous, acid-resistant flooring sloped to floor drains for wash-down.
- **Dissection tables** — stainless-steel, height-adjustable tables with a perforated top and under-tray/gutter for drainage, spaced to allow free movement of a small dissecting group around each cadaver; a running-water point and sink near each table row.
- **Adjoining rooms** — an embalming/injection room (with the embalming pump, injector and fluid reservoir) directly connected to a **cold-storage/mortuary tank room** where embalmed cadavers are immersed and stored; a separate museum for prepared specimens; an instrument and consumables store; staff and demonstrator rooms; and student locker/changing rooms.
- **Safety fittings** — eye-wash station and first-aid box, fire extinguishers, adequate general and task lighting, and signage for hazard/biosafety instructions.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple floor-plan sketch of the dissection hall with: (1) rows of dissection tables with spacing and orientation to natural light; (2) exhaust/ventilation points above the tables; (3) embalming/injection room; (4) cold-storage tank room; (5) museum; (6) instrument store; (7) student lockers/changing area; (8) sink/running-water points and floor drains; (9) eye-wash and first-aid station; (10) entry/exit with hand-wash point.

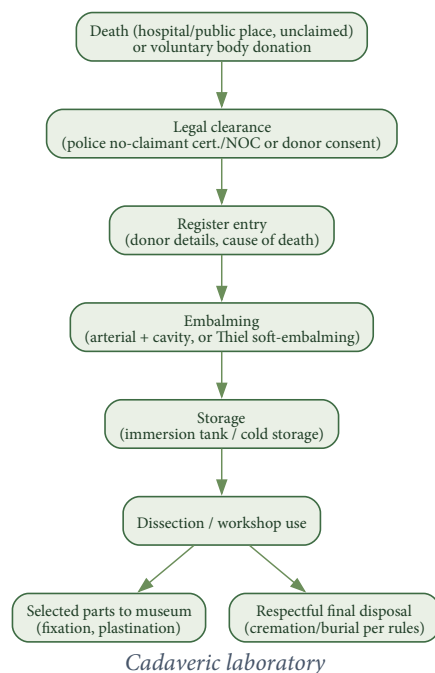
3. Embalming and preservation of the cadaver

- **Definition** — the chemical treatment of a dead body to delay decomposition, sanitise it and preserve it in a life-like state for anatomical study and research.
- **Purposes** — temporary preservation for teaching/research, sanitisation (destruction of pathogenic and non-pathogenic organisms), and restoration of a natural body image.
- **Standard arterial embalming fluid (composition):**

Component	Agent	Concentration
Preservative	Formalin + Methanol	10% + 55%
Buffer	Sodium borate	15 g
Anticoagulant	Sodium citrate	15 g
Wetting agent	Glycerine	15–20%
Germicide	Phenol	5%

Component	Agent	Concentration
Vehicle	Water	10–15%
Fungicide	Thymol	Few crystals
Dye	1% Eosin	5 mL
Perfume	Wintergreen oil	10 mL

- **Types of embalming:** arterial, cavity, hypodermic (subcutaneous injection for isolated limbs/parts, e.g. after trauma), surface (topical application for local lesions such as burns/bedsores), embalming of an already-autopsied body (six-point injection through both femoral/iliac, both subclavian/axillary and both common carotid vessels, viscera treated separately with cavity fluid/embalming powder), and embalming of an HIV/high-risk body (raised fluid concentration with added precautions in handling).
- **Vessel choice** — the vessel nearest the heart gives the best perfusion and drainage; the **common carotid artery with internal jugular vein** is the commonest single site, the femoral vessels the second choice, and the axillary/brachial vessels the least used.
- **Injection technique** — one-point injection (single site for both injection and drainage); split injection/drainage (injection and drainage from different sites, reducing short-circuiting of fluid); multipoint/six-point (sectional) injection for autopsied or badly damaged bodies.
- **Cavity embalming** — after arterial embalming has hardened the viscera, a trocar (~30 cm) is passed through a small midline incision above the umbilicus to aspirate the stomach, heart, pleural sacs and hollow viscera, and about 1–2 litres of cavity fluid is instilled to preserve and disinfect the body cavities.
- **Fluids used routinely in the dissection laboratory** — a **paint mixture** to keep the cadaver surface moist between sessions, a **tank (immersion) fluid** for long-term storage of the whole body, and a **cloth fluid** soaked into wrapping cloth to prevent drying of the area under active dissection and of isolated dissected parts.
- **Post-embalming storage** — the embalmed cadaver is immersed in a storage tank (tank fluid) in the mortuary/storage room until required for dissection.
- **Thiel's soft-embalming method** — a modern alternative that keeps the final formaldehyde concentration below 0.5%, using stem solutions containing boric acid, ammonium/potassium nitrate and ethylene glycol together with 4-chloro-3-methylphenol as the germicide; about 14 litres of the embalming solution is perfused (commonly via the great saphenous vein, or the femoral/carotid vessels if the saphenous vein cannot be used) and the cadaver is then matured in an immersion tank for 4–6 weeks. It retains natural tissue colour, softness and joint mobility (unlike conventional formalin-fixed cadavers, which are stiff and fragile), and vacuum-sealed, cold-stored Thiel cadavers can be reused for about 3 weeks of surgical-skill workshop training.



4. Occupational health, safety and biosafety

- ▶ **Biological hazard** — bloodborne and airborne pathogens including HIV, hepatitis B and C, multidrug-resistant tuberculosis, MRSA/VRE and other resistant organisms, and prion disease (CJD); embalming and dissection staff must follow standard precautions and use of proper personal protective equipment (gloves, mask, apron, goggles).
- ▶ **Chemical hazard** — formalin vapour is an irritant and a recognised occupational hazard; the hall must be maintained within safe permissible formaldehyde exposure levels through adequate exhaust ventilation, and staff/students must wear PPE and maintain good personal hygiene.
- ▶ **Special precaution** — female staff who are pregnant require additional caution against TORCH agents, human parvovirus B19 and syphilis in addition to routine chemical-hazard precautions.
- ▶ **Anatomical (biomedical) waste** — dissected tissue, viscera and other anatomical remains generated in the hall are segregated as anatomical waste and disposed of through the institution's biomedical-waste-management protocol (incineration/deep burial as applicable), distinct from routine general waste.

5. Administration, record-keeping and ethical conduct

- ▶ **Records maintained** — cadaver receipt/donation register, embalming and storage log, dissection allotment register (batch/table-wise), and disposal record.
- ▶ **Ethical conduct** — respect for the cadaver as the "first teacher"; many institutions administer a formal oath of respect and confidentiality before the batch begins dissection; unauthorised photography or removal of tissue from the hall is prohibited.
- ▶ **Final disposal** — after teaching use is complete, the remains are disposed of respectfully by cremation or burial as per institutional/state rules; select structures may instead be retained, fixed and prepared as permanent museum specimens (including by plastination for durable, dry, odourless long-term teaching specimens).

Applied / Clinical anatomy

- ▶ **Surgical-skill training** — Thiel-embalmed, life-like cadavers are increasingly preferred over stiff formalin-fixed cadavers for hands-on workshops in raising skin/muscle/bone flaps, skull-base and endoscopic sinus surgery, laparoscopic procedures, intra-oral procedures, and knee/shoulder arthroscopy training.
- ▶ **Museum correlation** — well-prepared, correctly labelled dissection-hall specimens (fresh or plastinated) feed directly into the departmental museum and support both undergraduate teaching and postgraduate viva preparation.
- ▶ **Medicolegal relevance** — documented consent/no-claimant clearance under the Anatomy Act protects the institution and staff from legal liability over the use of a cadaver.
- ▶ **Digital adjuncts** — virtual/digital cadaver resources (e.g. the Visible Human Project) supplement, but do not replace, hands-on cadaveric dissection.

High-yield exam pointers

- ▶ Two lawful sources of a teaching cadaver: voluntary body donation and unclaimed bodies under the Anatomy Act, 1949 (48-hour police no-claimant rule).
- ▶ Anatomy Act, 1832 (Britain) followed the Burke-and-Hare body-snatching scandal — historical trigger for legalising cadaver supply.
- ▶ Standard arterial embalming fluid: formalin 10% + methanol 55%, with sodium borate (buffer), sodium citrate (anticoagulant), glycerine (wetting agent), phenol (germicide), thymol (fungicide), eosin (dye).
- ▶ Commonest embalming vessel: common carotid artery with internal jugular vein (single-site injection).
- ▶ Cavity embalming uses a trocar to aspirate and instil fluid into thoracic, abdominal and pelvic viscera.
- ▶ Three special dissection-hall fluids: paint mixture (surface moisture), tank/immersion fluid (storage), cloth fluid (prevents drying of dissected areas).
- ▶ Thiel's soft embalming keeps formaldehyde below ~0.5%, preserves flexibility/colour, and is preferred for surgical-skill cadaver workshops.
- ▶ Occupational hazards: bloodborne/airborne pathogens (HIV, HBV/HCV, TB, prions) and chemical exposure to formalin vapour — mitigated by PPE and ventilation.
- ▶ Ethical practice: respect for the cadaver, consent documentation, and respectful final disposal/cremation after teaching use.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques; Gray's Anatomy (Standing) 41e; Vishram Singh — General Anatomy 4e; BD Chaurasia — Human Anatomy Vol 3-4 8e.

Coverage note: Dissection-hall design, day-to-day management and biosafety protocol are administrative/practical topics not addressed by Gray's Anatomy proper; content here is drawn from Haldar's dedicated embalming/museum-technique text (procurement law, embalming formulae, occupational hazards) and cross-checked against the historical and ethical material in Vishram Singh's General Anatomy and BD Chaurasia, supplemented by universally-taught standard practice for hall layout where the corpus itself is silent.



Corrosion cast

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction — A **corrosion cast** is a museum/research technique that reproduces, in permanent three-dimensional relief, the exact calibre, course and branching pattern of a hollow, tubular or vascular system that is otherwise very difficult to appreciate by ordinary dissection. Its core principle — filling the lumen of the structure with a setting material and then dissolving away the surrounding soft tissue — is shared by a whole family of injection-and-maceration museum preparations, of which the modern resin-based **luminal cast plastination** is a direct descendant. The technique is used chiefly to demonstrate the vascular pattern of solid organs (kidney, liver, lung, spleen, heart) and the branching of hollow viscera (tracheobronchial tree, cerebral ventricles, bony labyrinth), and is of special value in research on anatomical variations of arteries and ducts relevant to surgery and interventional radiology.

1. Principle

- ▶ The lumen of the structure to be studied (artery, vein, duct, bronchus, ventricle, etc.) is completely filled with a material that can (a) flow into fine branches while still liquid and (b) set into a firm, chemically resistant solid.
- ▶ The specimen is then subjected to a **corroding/macerating agent** that digests and dissolves every soft tissue around the set cast — parenchyma, fat, connective tissue — but does not attack the hardened injection material.
- ▶ What remains after thorough washing is a rigid, life-size replica of the internal lumen alone; the name "corrosion" cast reflects the fact that it is literally obtained by corroding away the surrounding tissue. As stated in the specialty text on museum techniques, the principle of luminal cast preparation is simply "**filling up of the lumen with material and dissolving the surrounding tissue.**"
- ▶ The technique thus converts an otherwise invisible internal cavity or branching system into a solid, dry, permanently displayable museum specimen.

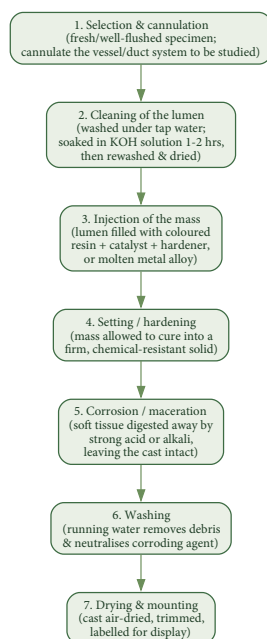
2. Injection mass (materials used)

Material	Nature / example	Remarks
Resin mixture	General-purpose resin + catalyst + hardener	The modern injection medium; sets to a firm, colour-stable, chemically resistant solid; can be tinted for teaching (conventionally red = artery, blue = vein, yellow = duct/ureter)
Low-melting metal alloy	e.g. Wood's metal	Poured molten and allowed to set on cooling; the classical injection medium before synthetic resins became available
Coloured wax or gelatin mass	Wax/gelatin loaded with pigment or a radio-opaque salt	Used in older, softer casts and in combined radiographic-corrosion studies

3. Corroding (macerating) agents

- ▶ **KOH (potassium hydroxide) solution** — used to clean and soften the lumen surface of the specimen before the final resin injection (a soak of about 1–2 hours in the confirmed museum-technique protocol).
- ▶ **Concentrated strong acid (e.g. HCl) or strong alkali (NaOH/KOH)**, applied over a longer period once the cast has fully hardened, is the classical corroding agent that gives the technique its name — it digests the bulk soft tissue en masse, leaving only the set cast.
- ▶ **Dilute sodium hydroxide** (used by boiling, or a gross method by autoclaving) is the analogous tissue-removal agent used in this same museum-technique tradition for **macerating bone specimens** to expose fine bony lesions — confirming that alkaline maceration is the standard tissue-digestion principle running through museum preparation methods generally.
- ▶ After corrosion/maceration, the specimen is thoroughly **washed under running water** to remove digested tissue debris and neutralise residual acid/alkali before the cast is dried.

4. Procedure



Corrosion cast

- ▶ **Selection & cannulation** — a fresh or adequately flushed organ is chosen and the vessel/duct system of interest is cannulated (e.g. renal artery and ureter for the kidney; hepatic artery, portal vein and bile duct for the liver; bronchus and pulmonary vessels for the lung).
- ▶ **Cleaning of the lumen** — the lumen is first cleaned under running tap water, then kept in KOH solution for about 1–2 hours, and cleaned again under running water and dried, so that the injection mass adheres well to the luminal surface.
- ▶ **Injection** — the coloured resin (or molten alloy) mixture is injected under gentle, controlled pressure so that it fills the lumen down to its finer branches without rupturing the walls.
- ▶ **Setting/hardening** — the mass is allowed to cure; a resin cast obtained by this route is typically ready within about a day.
- ▶ **Corrosion (maceration)** — the specimen is then exposed to the corroding agent, which dissolves all the surrounding soft tissue while leaving the set cast unaffected; the cast "is obtained by removing the surrounding tissue."
- ▶ **Washing, drying and mounting** — the corroded/macerated debris is washed away, the cast is dried, trimmed of extraneous material, and mounted/labelled as a permanent museum exhibit.

□ Fig 57.44 — A variation of the anulus of Vieussens (*). This corrosion cast specim (Gray's Anatomy — Standring — 41e, p.1414)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Draw a kidney corrosion cast and label:

- ▶ Renal artery and its segmental branches — coloured red
- ▶ Renal vein and its tributaries — coloured blue
- ▶ Pelvicalyceal system (major/minor calyces, renal pelvis) and ureter — coloured yellow
- ▶ The cortical and medullary branching pattern, showing progressive diminution of calibre from the main renal artery to interlobar/arcuate/interlobular branches
- ▶ A label/legend box stating the colour code: red = artery, blue = vein, yellow = collecting/ductal system

5. Structures/organs commonly demonstrated

Organ / system	Structure demonstrated
Kidney	Segmental branching of the renal artery, renal vein tributaries, pelvicalyceal system and ureter
Liver	Hepatic arterial, portal venous and biliary ductal pattern
Lung	Tracheobronchial tree; pulmonary arterial and venous branching
Heart	Coronary arterial pattern and anastomotic variations
Brain	Cerebral ventricular system
Inner ear	Bony labyrinth
Suprarenal (adrenal) gland	Arterial supply pattern and its variations

6. Advantages

- ▶ Produces a **three-dimensional, permanent, dry** specimen that needs no fluid preservation and can be freely handled for teaching.
- ▶ Faithfully demonstrates the **fine branching pattern, calibre and variations** of vessels and ducts that dissection or a wet-fixed specimen cannot show.
- ▶ A valuable **research tool** for documenting anatomical variations of direct surgical/radiological relevance.
- ▶ **Colour-coding** of arteries, veins and ducts aids comprehension and teaching.
- ▶ Once completed, the cast is **non-toxic and odourless**, unlike a conventional formalin-fixed wet specimen.

7. Disadvantages / limitations

- ▶ All surrounding soft tissue and organ relations are **destroyed** in the corrosion step — only the luminal/branching pattern survives, so the technique cannot demonstrate topographical relations.

- ▶ **Laborious and time-consuming**; setting of the injection mass and subsequent corrosion/maceration together may take several days.
- ▶ Requires an **intact, clot-free vascular bed** — post-mortem clotting, autolysis or vessel damage limits the quality of filling, so a fresh or well-flushed specimen is essential.
- ▶ The corroding agents (strong acid/alkali) are **hazardous** and require fume-hood extraction and personal protective precautions during preparation.
- ▶ Very fine, capillary-level branches below the resin's flow limit are **not filled** and therefore not represented in the final cast.
- ▶ The finished cast, especially its finer branches, is **brittle and fragile**, and may fracture during washing, handling or transport.

Applied / Clinical anatomy

- ▶ Corrosion casts document anatomical variations of surgical/radiological importance — e.g. a study of the **renal apical segmental artery** by the corrosion cast method (relevant to nephron-sparing/partial nephrectomy), cited in BD Chaurasia's kidney references, and a study of the **arterial supply to the suprarenal gland** by vascular corrosion casts (relevant to adrenal surgery), cited in both Gray's Anatomy and BD Chaurasia.
- ▶ A corrosion cast of the coronary arterial tree can reveal small anastomotic channels — such as a variant **anulus of Vieussens** — that are important in understanding collateral coronary flow, relevant to coronary angioplasty and bypass surgery planning.
- ▶ Vascular corrosion casts of the kidney, liver and lung correlate directly with **CT/MR angiography and cross-sectional imaging**, helping trainees relate a three-dimensional branching pattern to two-dimensional imaging slices.
- ▶ Because it destroys all soft-tissue relations, a corrosion cast is purely an **educational/research adjunct**; it can never substitute for a dissected specimen, a wet-fixed museum piece, or in-vivo cross-sectional imaging for actual surgical planning.

High-yield exam pointers

- ▶ Corrosion cast = **injecting** the lumen of a hollow/tubular/vascular structure with a setting material, then **dissolving/corroding** away the surrounding soft tissue, leaving a solid 3-D replica of the lumen.
- ▶ Core principle: "**filling up of the lumen with material and dissolving the surrounding tissue.**"
- ▶ Injection mass: modern **resin + catalyst + hardener** (colour-coded red = artery, blue = vein, yellow = duct); classically a low-melting **metal alloy** or coloured wax/gelatin.
- ▶ Corroding/macerating agent: **KOH/NaOH solution or strong acid**; dilute NaOH is used analogously for bone maceration in museum technique.
- ▶ Steps: clean lumen → soak in KOH → inject coloured resin mass → allow to set → corrode/digest surrounding tissue → wash → dry & mount.
- ▶ Demonstrates: renal/hepatic/pulmonary/coronary vascular pattern; tracheobronchial tree; cerebral ventricles; bony labyrinth.
- ▶ The modern refinement of the same principle is **luminal cast plastination**, one of the three types of plastination.

- Advantage: permanent, dry, 3-D specimen showing fine branching/variation; Disadvantage: destroys all soft-tissue relations, laborious, hazardous chemicals, fragile finished cast.
- Research application: documents anatomical variations (renal apical segmental artery, suprarenal arterial supply, coronary anastomoses) relevant to surgery/interventional radiology.

SOURCE & COVERAGE

Halder — Notes on Embalming & Museum Techniques (specialty text; source of the confirmed "luminal cast" principle, KOH/resin/catalyst/hardener protocol, and the alkaline bone-maceration analogy); Gray's Anatomy (Standring) 41e (confirms genuine research use of vascular corrosion casts for the suprarenal-gland variation study and supplies the embedded corrosion-cast specimen figure of the coronary anulus of Vieussens); BD Chaurasia Human Anatomy Vol 2 8e (confirms the renal apical segmental artery corrosion-cast variation study and the suprarenal-gland variation study).

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

Coverage note: neither of this topic's dedicated specialty texts carries a section explicitly titled "Corrosion cast." The Handbook of Museum Technique (Govt Museum Chennai) uses the word "corrosion" only for the conservation of ancient metal/coin objects, and its cast/mould content concerns taxidermy (wax/plaster fish and frog moulds), not human vascular casting, so it was not used. The technique described above is grounded in Halder's confirmed "**Luminal Cast Plastination**" method (identical core principle and procedure) and in Halder's separate bone-maceration protocol (alkaline tissue removal), with classical/historical details — metal-alloy injection media, prolonged strong-acid/alkali corrosion, and colour-coding conventions — added as standard, universally accepted museum-technique teaching. Reference lists in Gray's Anatomy and BD Chaurasia (not their main narrative text) independently confirm two real corrosion-cast variation studies (suprarenal gland; renal apical segmental artery) and supply the one genuine corrosion-cast photograph available in the figure library (coronary anulus of Vieussens, Gray's Fig 57.44).



Dawson's technique for bone in fetuses

Asked in: Paper III Oct 2006 — RGUHS MD/MS Anatomy

Definition / Introduction Dawson's technique is a "clear-and-double-stain" museum/laboratory method that converts a whole fetal (or embryonic) specimen into an optically transparent preparation while selectively colouring its skeleton — mineralized (ossified) bone red with **Alizarin red S** and unossified cartilage blue with **Alcian blue** — so that the pattern, timing and extent of skeletal ossification can be studied in situ without dissection. It is named after A. B. Dawson (1926), who introduced Alizarin red S as a stain for the bony skeleton of cleared specimens; the method was later combined with Alcian blue cartilage staining to give a true double stain that demonstrates both the cartilaginous and bony components of the developing skeleton together. It is a core Paper III (museum, embalming & preservation) practical technique in anatomy, used both for teaching prenatal osteogenesis and for skeletal-anomaly/teratology screening.

Principle

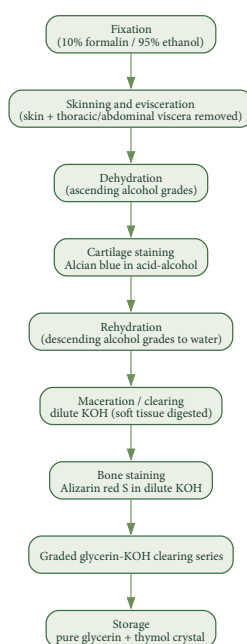
- ▶ The method exploits two independent tissue chemistries so that cartilage and bone can be stained differentially in the **same** intact specimen:
 - **Alcian blue** is a large copper-phthalocyanine basic dye that, at low (acid) pH, binds electrostatically to the sulfated and carboxylated glycosaminoglycans (acid mucopolysaccharides) of cartilage matrix, giving a stable blue colour.
 - **Alizarin red S** is an anthraquinone dye that chelates calcium ions; wherever matrix has been mineralized (i.e. ossified bone), it forms an insoluble orange-red calcium–dye lake. This is the same calcium–chelation chemistry used for Dahl's alizarin red S method for calcium on histological sections, adapted here to a whole, cleared specimen.
- ▶ Soft, non-skeletal tissue (skin, muscle, viscera) must be made optically transparent so the coloured skeleton shows through:
 - Dilute **potassium hydroxide (KOH)** macerates/digests non-collagenous soft tissue proteins, leaving the more resistant skeletal and ligamentous framework intact.
 - **Glycerin**, used in a graded series with KOH and finally as a pure clearing/storage medium, gradually replaces water in the tissue and matches its refractive index, rendering the specimen glass-like and transparent.
- ▶ Net effect: a transparent, uncut "glass" fetus in which the skeleton stands out clearly — red where ossified, blue where still cartilaginous — permitting the observer to map every ossification centre present at that gestational age.

Requirements / Reagents

Reagent/material	Role in the technique
10% formalin or 95% ethyl alcohol	Fixation of the fetal specimen
Alcian blue (in acid-alcohol — ethanol + glacial acetic acid)	Stains cartilage matrix blue
Graded ethanol series	Dehydration before, and rehydration after, alcian blue staining

Reagent/material	Role in the technique
Dilute potassium hydroxide (KOH), ~1–2% aqueous	Macerates and clears soft tissue
Alizarin red S (dissolved in dilute KOH)	Stains ossified bone red
Graded glycerin–KOH series, then pure glycerin	Final clearing, de-staining of background and mounting/storage medium
Thymol crystal (or a trace of phenol)	Antifungal preservative in the stored glycerin
Wide-mouthed glass museum jar with airtight lid	Permanent transparent storage/display

Procedure (stepwise)



Dawson's technique for bone in fetuses

- **Fixation** — the fetus is fixed whole in 10% formalin (or 95% ethanol), the duration depending on specimen size.
- **Skinning and evisceration** — skin is carefully reflected and the thoracic/abdominal viscera removed through a small ventral incision without disturbing the skeleton, to prevent putrefaction and permit reagent penetration.
- **Dehydration** — through ascending grades of alcohol, since Alcian blue is applied in an acid-alcohol vehicle.
- **Cartilage staining** — the specimen is immersed in Alcian blue (acid-alcohol solution) for one to several days depending on size; cartilage takes up a stable blue colour.
- **Rehydration** — the specimen is brought back down through descending alcohol grades to water.
- **Maceration/clearing** — immersion in dilute KOH digests skin, muscle and residual soft tissue, which becomes translucent and gelatinous; the KOH is changed periodically and the process monitored closely.
- **Bone staining** — the macerated, semi-cleared specimen is transferred to Alizarin red S dissolved in dilute KOH; ossified bone stains red/orange-red while cartilage, already blue, is unaffected.

- ▶ **Final clearing** — the specimen is passed through a graded series of glycerin–KOH mixtures (progressively increasing glycerin, decreasing KOH) to remove excess background stain and render the whole specimen transparent.
- ▶ **Storage/mounting** — the finished specimen is stored in pure glycerin with a thymol crystal as preservative, in a sealed, labelled glass museum jar.

Precautions

- ▶ Maceration in KOH must be monitored closely and stopped as soon as the soft tissue is adequately cleared — over-maceration digests ligaments and periosteum, and the disarticulated skeleton falls apart or is lost.
- ▶ Only a well-fixed, non-putrefied specimen should be used; autolysed tissue macerates unevenly and stains poorly.
- ▶ Handle gently at every stage — bones in a cleared, decalcified-looking specimen are fragile, especially unossified cartilaginous epiphyses.
- ▶ Avoid prolonged exposure to strong light during storage/display, as the alizarin red S/alcian blue colours can fade over time.
- ▶ Label the jar with the estimated gestational age/crown–rump length so the ossification pattern seen can be correlated with the expected developmental stage.

Results/Interpretation

- ▶ **Ossified (mineralized) bone** — stains orange-red with Alizarin red S.
- ▶ **Unossified cartilage** (epiphyses, costal cartilages, cranial vault margins/fontanelles, etc.) — stains blue with Alcian blue.
- ▶ **Soft tissue** (skin, muscle, connective tissue) — rendered transparent/colourless.
- ▶ The finished preparation lets the observer read off, without dissection, exactly which centres of ossification (primary and secondary) have appeared by a given gestational age, and the still-cartilaginous portions of the same bones — a direct visual demonstration of endochondral ossification proceeding from a primary centre outward, with epiphyseal ends remaining cartilaginous until their own secondary centres appear.

Applied / Clinical anatomy

- ▶ **Teratology/developmental toxicology screening** — the clear-and-double-stain technique is the standard method used to examine fetal skeletons for malformations (missing, extra, fused, malformed or delayed ossification centres) induced by teratogenic drugs or chemicals in reproductive/developmental toxicity studies, since the whole skeleton can be inspected without disturbing anatomical relations.
- ▶ **Forensic and radiological correlation** — the sequence in which ossification centres appear is used clinically to estimate gestational/fetal age from radiographs or ultrasound (e.g. a primary diaphyseal centre for long bones such as the femur/tibia typically appears around the seventh intrauterine week, with epiphyseal secondary centres appearing later, into infancy). A Dawson-stained specimen provides a direct, dissection-free correlate of this radiological staging.

- ▶ **Museum and teaching specimens** — produces permanent, transparent "glass" mounts that demonstrate prenatal osteogenesis, the cartilage–bone relationship, and normal skeletal variation for undergraduate and postgraduate teaching, without needing repeated dissection of fresh material.
- ▶ **Comparative and developmental anatomy** — allows side-by-side comparison of ossification patterns across different gestational ages or species.

High-yield exam pointers

- ▶ Also called the "**clear-and-stain**" / **double-staining technique** for demonstrating the fetal skeleton.
- ▶ Named for **A. B. Dawson (1926)**, who introduced Alizarin red S staining of the bony skeleton in cleared specimens; later combined with Alcian blue to also demonstrate cartilage.
- ▶ Two dyes, two chemistries: **Alizarin red S** chelates calcium → **red/orange-red bone**; **Alcian blue** binds acid mucopolysaccharides at low pH → **blue cartilage**.
- ▶ **KOH** macerates and clears soft tissue; **glycerin** (graded, then pure) gives the final optical clearing and is the storage medium.
- ▶ Alcian blue staining is done **before** KOH maceration (acid-alcohol vehicle, intact matrix); Alizarin red S staining is done **after** maceration (dissolved in the same dilute KOH).
- ▶ A thymol crystal in the stored glycerin acts as antifungal preservative.
- ▶ Demonstrates both primary and secondary ossification centres together, in their true anatomical relationship.
- ▶ Chief uses: teaching prenatal osteogenesis, museum specimens, and skeletal teratology/malformation screening.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A labelled sketch of a Dawson clear-and-stained fetal specimen mounted in a glass jar, showing:

- ▶ Skull — red-stained ossified vault bones with blue-stained unossified sutures/fontanelles.
- ▶ Vertebral column — red vertebral centra with blue cartilaginous neural arches/costal cartilages.
- ▶ Limb long bones — red diaphysis (primary centre) with blue cartilaginous epiphyses (still unossified).
- ▶ Ribs — red bony shaft continuing into blue costal cartilage.
- ▶ Translucent, colourless soft-tissue outline of the whole fetus.
- ▶ Wide-mouthed glass jar containing clear glycerin with a thymol crystal, labelled with the estimated gestational age.

SOURCE & COVERAGE

Gray's Anatomy (Standing) 41e (ossification centre biology, primary/secondary centre timing, endochondral ossification); Bancroft's Theory & Practice of Histological Techniques (Alizarin red S–calcium chelation chemistry [Dahl's method]; Alcian blue–acid mucopolysaccharide staining chemistry).

Coverage note: The RGUHS Paper III preservation specialty texts available in this library — Haldar's *Notes on Embalming & Museum Techniques* and the *Handbook of Museum Technique* (Govt Museum, Chennai) — do not carry a dedicated chapter on this specific human fetal clear-and-stain protocol (the Haldar excerpt on disk is limited to its introductory author-profile pages, and the Museum Handbook chapters on disk cover natural-history — zoological/geological — specimen preservation rather than human anatomical material). The stepwise protocol above therefore follows the standard, universally taught Dawson (1926) double-staining/clear-and-stain method, with its dye chemistry cross-verified against Bancroft's Histological Techniques and its ossification-centre biology verified against Gray's Anatomy 41e.



Describe briefly the techniques for preparation of Museum specimens

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction A museum specimen is a gross anatomical or pathological preparation selected, fixed, colour-restored, mounted and stored so that its natural shape, colour and structural relations are preserved indefinitely for teaching, research and medico-legal record. Good specimens begin with careful handling at autopsy/dissection and end as a properly labelled, catalogued preparation. Preparations are broadly of two types: **wet (fluid) preparations**, mounted in a colour-preserving fluid (the classical Kaiserling technique and its modifications), and **dry preparations** — macerated bone, calculi and the modern plastinated specimen. Relevant photographs, radiographs and accession records are maintained alongside every specimen.

1. Selection and handling of the specimen at autopsy

- ▶ A good museum specimen is obtained and preserved by careful planning at the time of autopsy; careless removal of a section can easily ruin an otherwise good specimen.
- ▶ Cut surfaces are made smooth and even with a single continuous stroke of a long-bladed sharp knife; tissue is sectioned either from the back of the piece intended for preservation or from the front with a scalpel, so that the exhibited surface is not marred.
- ▶ The specimen is placed in fixative almost immediately after removal; containers of formal-saline are kept readily available to the operating/dissection staff for this purpose.

2. Fixation

- ▶ **Primary fixation** is with 10% formal-saline; the specimen is later transferred to a special colour-restoring fixative.

► Additional rules followed while fixing museum specimens:

- Specimens should be injected with fixative wherever possible to ensure adequate penetration and fixation throughout the tissue (e.g., the brain).
- Specimens containing much blood must never be washed in water, either before or after fixation, as this leaches and discolours the tissue.
- A fresh specimen is rested on a thick layer of cotton wool covered with lint while fixing, so that its shape is not distorted.
- The specimen must be fixed together with its attached structures intact.
- Cystic cavities, if unopened, are inflated before fixation; if already opened, they are packed with fixative-soaked cotton wool to maintain their natural shape.
- Bile-stained or bile-containing specimens are fixed and stored separately from other specimens.

3. Colour restoration and mounting-fluid techniques (wet preparations)

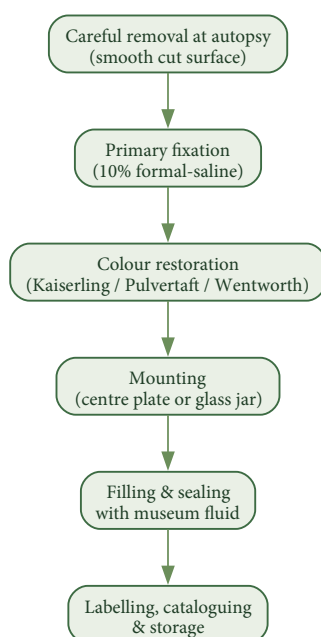
The most widely used method is a modification of **Kaiserling's technique (1900)**, which employs three solutions in sequence — a fixing fluid, a fluid to restore the original colour of the specimen, and a final mounting fluid.

Method	Modification	Key detail
Kaiserling original (1900)	3-solution sequence	Sol. I fixes; Sol. II restores colour; Sol. III mounts
Pulvertaft's modification (1936)	Reducing agent in mountant	Sodium hydrosulphite added to the mounting fluid — original Pulvertaft-mounted specimens show remarkably little fading even after 35 years
Pulvertaft–Kaiserling method	Standard 3 solutions	Sol. I (fixing): formalin 400 mL + KNO ₃ 30 g + K-acetate 60 g + water to 2000 mL. Sol. II (colour restoration): 80% ethyl alcohol, 30 min–4 h — emergency/photography use only, as prolonged immersion bleaches permanently. Sol. III (mounting): glycerin 300 mL + Na-acetate 100 g + formalin 5 mL + water to 1000 mL, with 0.4% Na hydrosulphite added just before sealing (filter through paper-pulp if turbid, add camphor-in-alcohol)
Israel & Young (1978)	Alternative mountant	Pure liquid paraffin as the final mountant after alcohol colour restoration — reduces mounting-fluid discolouration by leached pigment

Method	Modification	Key detail
Wentworth method (1938–1957)	No alcohol step	Only sodium hydrosulphite restores colour; glycerol omitted from the final mountant; fixation in Sol. I needs ≥ 1 month; mounting solution is chosen by specimen pH — pH > 6.5 goes directly to Sol. III, pH < 6.5 needs Sol. II first, and final mounting uses fresh Sol. III with Na hydrosulphite (3 g/1000 g) added just before sealing

4. Mounting techniques

- ▶ **Museum jars and boxes:** Perspex boxes are used almost universally today, bought commercially or made in the laboratory to fit each specimen exactly.
- ▶ **Pre-mounting care:** specimens taken out of storage are inspected first — irregular surfaces are re-cut; pinned membranes/skin/intestine are trimmed at the edges; natural cavities (after removing packing cotton wool) are filled with arsenious acid gelatin; delicate structures are marked with coloured Perspex rods or black horse hair; friable fragments (e.g., a blood clot) are held with a thin layer of arsenious acid gelatin; and bile-stained specimens are soaked in saturated calcium chloride for 24 hours.
- ▶ **Centre-plate mounting:** the specimen is stitched, in an anatomically correct position, to a Perspex centre plate; the number of stitches depends on the tissue's weight and consistency, and none passes through a pathological lesion. Nylon thread uses two 1 mm holes; linen thread uses a single hole retained by a glass bead larger than the hole. The threads emerge about 1 cm apart to form a "V" of tissue, secured with a non-slipping reef knot; alternatively, cemented Perspex spikes can hold the specimen.
- ▶ **Sizing:** the specimen is laid on a flat, formica-covered bench in an anatomical position and measured for its box — 1 cm clearance at the top and sides, 2 cm at the bottom for the label, and depth plus about 5 mm.
- ▶ **Filling and sealing:** museum fluid with 0.4% sodium hydrosulphite is run in to within 1 cm of the top, air bubbles are released, and the lid is cemented (Perspex cement/ethylene dichloride) under a lead weight for 1–3 hours; after 24–48 hours the box is topped up through a 3 mm fill-hole and the plug finally sealed.
- ▶ **Glass-jar mounting** follows the same principle — holes drilled with an engraver's tool using camphor-in-turpentine as lubricant, the jar sealed with a heated asphaltum-rubber compound (picein), and the edge finished with black enamel or asphaltum varnish.
- ▶ **Embedding in solid plastic blocks** is the ideal presentation method, but no technique yet protects the natural colour of a soft-tissue specimen mounted this way.



Describe briefly the techniques for preparation of Museum specimens

5. Special techniques for hard and calcified specimens

- ▶ **Maceration** demonstrates bony lesions such as osteogenic sarcoma, osteoma, chronic osteomyelitis and skeletal tuberculosis. The putrefaction method best preserves the finest bone spicules; the routine method boils the specimen in tap water or very dilute (N/100) NaOH, removing softened tissue at intervals; a quicker gross method for compact bone is autoclaving in N/10 NaOH for 5 minutes, though this also strips away fine spicules.
- ▶ **Degreasing and bleaching:** fat is removed by immersing the bone in chloroform for 3–4 hours; the specimen is then dried in an incubator and bleached in hydrogen peroxide.
- ▶ **Mounting of macerated bone:** done dry, on a centre plate or on custom Perspex supports, with nylon thread tied and secured with a drop of Perspex cement.
- ▶ **Calculi:** older methods present them as a dry mount in a reversible-glass-lid box, or mounted in formalin-added gelatin (the former gets dirty, the latter's gelatin slowly dissolves). The modern method bisects the calculus, polishes and mounts one half, and labels the other half with Indian ink for students to handle and study.

6. Plastination — the modern museum technique

Plastination produces a dry, coloured, non-toxic, durable, odourless and natural-looking specimen, overcoming the drawbacks of routine formalin-fixed wet preparations — irritating odour, bleached and colourless parts, an unnatural appearance, difficulty maintaining thin sections, and an inability to demonstrate luminal architecture, dimensions or branching patterns.

- ▶ **Types:** (i) whole-organ plastination, (ii) sheet plastination, (iii) luminal cast plastination.
- ▶ **Materials used:** storage/processing jars, acetone, silicone rubber, resin with catalyst and accelerator, colour paints, dissection instruments, and syringes/needles.
- ▶ **Four steps of plastination:**

- ▶ **Fixation** — a formaldehyde-based (or other preserving) solution prevents tissue decomposition and confers rigidity, which helps maintain a specimen's shape or arrangement (e.g., an inflated stomach, or a leg bent at the knee).
- ▶ **Dehydration** — after all necessary dissection, the specimen is placed in an acetone bath under freezing conditions, which draws all the water out of the cells.
- ▶ **Forced impregnation in a vacuum** — the specimen is transferred to a bath of liquid polymer (silicone rubber, polyester or epoxy resin); a vacuum is created so that the acetone boils at a low temperature and vaporises out of the tissue, drawing the liquid polymer in behind it.
- ▶ **Hardening** — the polymer-filled specimen is cured with gas, heat or ultraviolet light to harden the plastic.
- ▶ **Whole-organ plastination:** washed in tap water, immersed 2–3 days in a preservative mixture (95% alcohol 280 mL, formalin 1320 mL, glycerine 80 mL, phenol 120 mL, distilled water 800 mL), washed free of formalin, transferred to acetone then to resin for 1–2 weeks, and finally immersed in general-purpose resin with 5% catalyst and 0.1% accelerator; it becomes non-sticky within hours and is mounted on a base in the desired orientation.
- ▶ **Sheet plastination:** gives thin transparent or thick opaque body slices — portable, storable dry, and best for showing internal relationships. After 24-hour deep-freezing, ~1 cm slices are cut with a band saw and cast between two 4 mm glass sheets (separated by 6–8 mm rubber tubing) filled with resin, catalyst and accelerator; after 24 hours the sheet is separated, trimmed, polished and labelled.
- ▶ **Luminal cast plastination** fills an organ's lumen with casting material and then dissolves away the surrounding tissue, to study the dimensions, architecture and branching of tubular structures — used for tracheobronchial casts of the lungs, cerebral ventricles, the bony labyrinth, and the vascular pattern of kidney, liver, lung, spleen and coronary vessels. The lumen is cleaned, soaked in KOH for 1–2 hours, dried, and filled with resin + catalyst + hardener; the cast is obtained after 1 day by removing the surrounding tissue.
- ▶ **Advantages of plastination:** a unique specimen at low cost, comparable to international-quality plastinated specimens, with a simple protocol; best suited for teaching, designing new surgical procedures, skills practice, and research.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled wet-mounted museum jar (centre-plate technique) — label the following:

- ▶ Perspex or glass museum jar/box
- ▶ Museum mounting fluid (e.g., Kaiserling/Pulvertaft solution III, with sodium hydrosulphite)
- ▶ Perspex centre plate
- ▶ Specimen sutured to the centre plate (nylon thread or linen thread with a glass-bead retainer)
- ▶ Coloured Perspex marker rod/black horse hair identifying a delicate structure
- ▶ Fill-hole with Perspex plug
- ▶ Sealed lid (Perspex cement / asphaltum-rubber compound)
- ▶ Outer label bearing the unique accession/identification number

Applied / Clinical anatomy

- ▶ Museum specimens are a core teaching resource for gross, systemic and surgical pathology, correlated with photographs/radiographs taken at autopsy.
- ▶ Medico-legal specimens retained at post-mortem must be labelled with the case/PM number and a maintained chain of custody, since they may later serve as evidence.
- ▶ Macerated bone specimens of osteogenic sarcoma, osteoma, chronic osteomyelitis and tuberculosis directly correlate with the pathology curriculum.
- ▶ Luminal cast plastinated specimens (tracheobronchial tree, cerebral ventricles, bony labyrinth, renal/hepatic/pulmonary/coronary vasculature) teach branching patterns and variations difficult to appreciate on routine dissection.
- ▶ The bisect-and-label technique for calculi lets students palpate an unpreserved half without damaging the polished, permanently mounted half.
- ▶ Being dry, odourless and durable, plastinated specimens are increasingly used for surgical skill training and planning new operative approaches, besides routine teaching.
- ▶ Formalin fumes generated during fixation and mounting are an occupational hazard for museum/dissection-hall staff, requiring adequate ventilation.

High-yield exam pointers

- ▶ Kaiserling's technique (1900) is the classical 3-solution method: fixation → colour restoration (alcohol) → mounting fluid.
- ▶ Pulvertaft's modification (1936) adds sodium hydrosulphite (a reducing agent) to the mounting fluid, giving markedly better colour retention (little fading even after 35 years).
- ▶ The Wentworth method (1938–1957) omits the alcohol colour-restoration step and glycerol, and selects the mounting solution based on the specimen's pH.
- ▶ Museum boxes are almost universally made of Perspex; specimens are sutured to a Perspex centre plate with nylon or linen-and-glass-bead thread, secured with a reef knot.

- ▶ Maceration (boiling in dilute NaOH, or autoclaving in N/10 NaOH) is the technique of choice for demonstrating bony lesions such as osteosarcoma, osteoma, osteomyelitis and skeletal tuberculosis.
- ▶ Plastination has four steps — Fixation → Dehydration (acetone) → Forced vacuum impregnation (polymer) → Hardening (gas/heat/UV) — and three types: whole-organ, sheet, and luminal cast plastination.
- ▶ The modern method for calculi is to bisect, polish and mount one half while labelling the other half with Indian ink for student handling.
- ▶ Every museum specimen must carry a unique identification/accession number, correlated with a maintained reference/record book, and be stored in its own separate container.

SOURCE & COVERAGE

Haldar — *Notes on Embalming & Museum Techniques* (chapter on Museum Techniques — preparation, fixation, colour-restoration and mounting-fluid methods, mounting procedures, maceration, calculi, and plastination). Handbook of Museum Technique (Govt Museum Chennai) — general principles of fluid (formalin/spirit) preservation, corroborating standard museum practice. **Coverage note:** this is a laboratory/preservation-technique topic outside the scope of Gray's Anatomy (Standring), which does not describe museum specimen preparation; grounding is therefore drawn entirely from the specialty preservation texts above.



Describe the various stages involved in museum techniques in terms of specimen preparation, mounting, painting and display

Asked in: Paper III Nov 2016 — RGUHS MD/MS Anatomy

Definition / Introduction A museum technique is the systematic art and science of selecting, fixing, preserving, mounting and displaying gross anatomical/pathological specimens obtained at autopsy or dissection, so their natural size, shape, colour and relations are permanently retained for teaching, examination (spotters) and record purposes. Beyond teaching, a museum records the natural history of disease — "the common disease of today may well be the rarity of tomorrow." The classical sequence — preparation, fixation, colour maintenance ("painting"), mounting, labelling and display — is best described using the Kaiserling technique and its later modifications, the standard method in Indian anatomy/pathology museums.

1. Selection and Preparation of the Specimen

- ▶ Good museum specimens are obtained and preserved by care and planning **at the time of autopsy/dissection itself**; careless removal easily ruins a specimen.
- ▶ The cut surface must be smooth and even, made with a **single continuous stroke of a long-bladed, sharp knife**.
- ▶ Tissue is sectioned from the **back** of the specimen intended for preservation wherever possible, or from the front with a scalpel, so the display surface is not disturbed.

- ▶ The specimen is put into a **fixative almost immediately** after removal; containers of formal-saline should always be kept ready and available to the operating/dissection theatre staff to avoid autolytic change.

2. Fixation of the Specimen

- ▶ **Primary fixation** is with 10% formal-saline; the specimen is later transferred to a special fixative for colour maintenance.
- ▶ Additional rules for museum specimens:
 - Specimens should be **injected with fixative directly** wherever possible to ensure adequate penetration (e.g., the brain).
 - Blood-rich specimens must **never be washed in water**, either before or after fixation — washing removes blood pigment and dulls the natural colour.
 - A fresh specimen is rested on a thick layer of cotton wool covered with lint before fixation.
 - The specimen, together with its **attached structures**, is fixed in continuity.
 - **Unopened cystic cavities** are inflated; if already opened, they are packed with fixative-soaked cotton wool to maintain their natural shape.
 - **Bile-stained/bile-containing specimens** are fixed and stored separately, as bile pigment contaminates other specimens and the mounting fluid.

3. Painting and Colour Restoration ("Museum Fluids")

The word "painting" in museum technique refers to **restoring and maintaining the natural colour** of the fixed specimen, classically by the **Kaiserling method (1900)** and its modifications, followed by literal painting of the sealed jar rim.

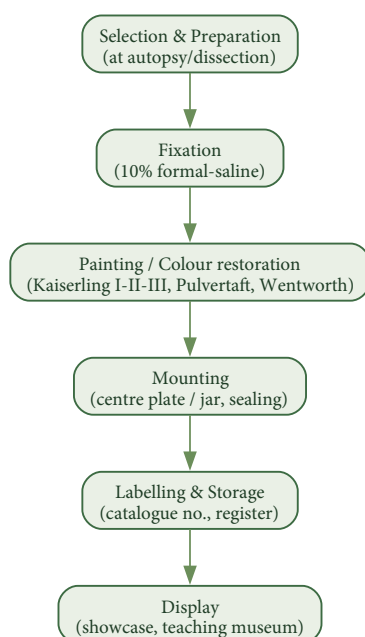
Solution	Composition	Purpose
Kaiserling I — fixing fluid	Formalin 400 ml, Potassium nitrate 30 g, Potassium acetate 60 g, Tap water to 2000 ml	Primary/transfer fixation before colour restoration
Kaiserling II — colour-restoring fluid	Ethyl alcohol 80%	Restores natural colour over 30 min–4 hr (time must be carefully controlled); continued immersion causes permanent bleaching , not reversed by later mounting fluid
Kaiserling III — mounting fluid	Glycerin 300 ml, Sodium acetate 100 g, Formalin 5 ml, Tap water to 1000 ml + 0.4% sodium hydrosulphite added just before sealing	Final mounting fluid; maintains restored colour long-term

- ▶ **Pulvertaft's modification (1936)**: colour is restored simply by adding a **reducing agent (sodium hydrosulphite)** directly to the mounting medium, dispensing with a separate alcohol-restoration step; specimens mounted by this method show remarkably little fading even after 35 years.

- ▶ **Israel & Young (1978)**: used pure liquid paraffin as the final mountant after alcohol colour-restoration — this reduces discoloration of the mounting fluid caused by pigments leaching from the specimen.
- ▶ **Wentworth's method (1938, 1939, 1942, 1957)**: further modified Pulvertaft's technique, dispensing with the alcohol step and glycerol, using only sodium hydrosulphite. Mounting is **pH-guided**: fixation for at least one month in solution I, then if pH > 6.5 the specimen is placed directly into solution III, and if pH < 6.5 it is first placed in solution II; sodium hydrosulphite (3 g/1000 g) is added to fresh solution III immediately before final sealing.
- ▶ **Literal "painting" step**: once a glass jar is sealed with asphaltum-rubber (Picein) compound, the excess cement is trimmed after setting and the ground rim/edges are **painted with black enamel or asphaltum varnish** to give a neat, airtight, presentable finish.

4. Mounting of the Specimen

- ▶ **Pre-mounting care**: irregular surfaces are re-cut; pinned edges of membrane/skin/intestine specimens are trimmed; natural cavities (cotton wool removed) are filled with **arsenious-acid gelatin**; coloured Perspex arrows/rods and black horsehair mark delicate structures; friable specimens (e.g., a blood clot) are held with a thin gelatin coat; bile-stained specimens are soaked in saturated calcium chloride for 24 hours.
- ▶ **Procedure**: the specimen is laid on a flat, waterproofed (formica) bench in an anatomically correct position; measured, allowing **1 cm clearance at top/sides and 2 cm at the bottom** (for the label); depth + ~5 mm is added for the centre plate; a suitably sized box is selected, ordered or made.
- ▶ **Museum jars and boxes**: Perspex boxes are used almost universally today — commercial or lab-made to fit each specimen exactly, built by cementing four sides plus a top/bottom, or by bending a single Perspex strip.
- ▶ **Centre plates**: most specimens are simply stitched to a centre plate fitting the box (~5 mm clearance); coloured opaque Perspex plates enhance colour or allow double-sided viewing.
- ▶ **Attaching the specimen**: marked on the plate with a scribe, 1–2 mm holes drilled (avoiding pathological lesions); **nylon thread** (two 1 mm holes) or **linen thread with a glass bead** (one hole, bead as retainer) is used; a **reef knot** gives a tight, non-slipping tie. Alternatively, Perspex spikes are cemented on and the specimen pressed onto them.
- ▶ **Filling and sealing**: museum fluid with 0.4% sodium hydrosulphite is run to within 1 cm of the top; air bubbles released with a broad spatula; a 3 mm topping-up hole is drilled in the lid; the lid is cemented, weighted for ≥1 hour, and after 24–48 hours the plug is removed, the box topped up, and resealed.
- ▶ **Glass-jar mounting** follows the same principle: holes drilled with an engraver's tool lubricated with camphor-in-turpentine; the jar is sealed with asphaltum-rubber (Picein) flamed onto the ground rim.
- ▶ **Embedding in solid plastic blocks** is an ideal presentation method, but no technique yet protects the natural colour of soft-tissue specimens so embedded.



Describe the various stages involved in museum techniques in terms of specimen preparation, mounting, painting and display

5. Display, Labelling and Storage

- ▶ The purpose of a museum is educational and archival — it "entertains, informs, tells stories, and translates the unfamiliar into the accessible"; even a small departmental museum records the history of disease over time.
- ▶ To fulfil this purpose, the **original shape and colour of the specimen must be retained**, and relevant **photographs, radiographs, presentation notes, labelling and cataloguing** are of equal importance to the specimen itself.
- ▶ **Storage**: each specimen is given a **unique identification number**, and a reference/catalogue book is maintained recording relevant clinical and pathological details; specimens are stored in **separate containers** to prevent damage from contact with other specimens; the outer container is adequately labelled, and the label is additionally **tied to the specimen with linen thread**.
- ▶ Final **display** is in glass-fronted showcases in the teaching museum, specimens arranged systematically (by organ system/disease), adequately lit, each jar bearing its catalogue number, diagnosis and brief clinical history so it can serve as a "spotter" in practical examinations.

6. Allied and Special Preservation Techniques

- ▶ **Maceration** — for bony lesions (osteogenic sarcoma, osteoma, chronic osteomyelitis, TB): specimen boiled in tap water or dilute (N/100) NaOH, softened tissue periodically removed (**putrefaction method** best preserves fine spicules; autoclaving in N/10 NaOH is a quicker gross method but loses spicules). Fat removed by chloroform (3–4 hr), then dried and bleached in hydrogen peroxide; mounted dry on a centre plate.
- ▶ **Calculi**: mounted dry in reversible-glass-lid boxes or formalin-gelatin (older methods); modern method polishes and mounts one half, labelling the other in Indian ink for student handling.
- ▶ **Plastination** produces dry, coloured, non-toxic, durable, odourless, natural-looking specimens — a superior modern technique. Types: **whole-organ, sheet, and luminal-cast plastination**. Steps: (1) **fixation** (formaldehyde solution — prevents decomposition, confers rigidity); (2)

dehydration in acetone (under freezing, draws water out of cells); (3) **forced impregnation** in a liquid polymer bath (silicone/polyester/epoxy resin) under vacuum, so acetone boils out and polymer is drawn in; (4) **hardening/curing** with gas, heat or UV light. Valued for teaching, surgical-skills training and low-cost, durable demonstration.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Labelled diagram of a mounted museum specimen (Perspex/glass jar, Kaiserling technique):

- ▶ Sealed lid/top (Perspex or glass) with painted black-enamel/asphaltum-varnished rim
- ▶ Museum (Kaiserling III) mounting fluid filled to 1 cm below the rim
- ▶ Specimen stitched with nylon/linen thread + glass bead onto the **centre plate**
- ▶ Coloured Perspex marker rods/arrows and black horsehair indicating key structures
- ▶ Label plate at the base bearing the catalogue number, diagnosis and brief history
- ▶ 1 cm clearance at top/sides, 2 cm clearance at the bottom for the label

Applied / Clinical anatomy

- ▶ Museum specimens form the permanent teaching/record archive of an anatomy/pathology department and serve as "spotters" in practical/viva examinations.
- ▶ Medico-legal and autopsy-derived specimens need separate labelling, cataloguing and storage with complete documentation — linking museum technique to the curriculum on organ retention, the Anatomy Act, and body/organ preservation.
- ▶ Specimens washed in water, or left too long in colour-restoring alcohol, permanently lose diagnostic colour — hence correct handling **at autopsy itself** is stressed as the first stage.
- ▶ Plastinated specimens (dry, odourless, durable, repeatedly handleable) are increasingly used for surgical-skills training, extending classical wet-museum technique into modern practice.

High-yield exam pointers

- ▶ Kaiserling's technique (1900) uses **three solutions**: I — fixing fluid; II — colour-restoring fluid (80% ethyl alcohol); III — mounting fluid (glycerin + sodium acetate + formalin + sodium hydrosulphite).
- ▶ **Pulvertaft's modification** (1936) adds the reducing agent sodium hydrosulphite directly to the mounting fluid — specimens show minimal fading even at 35 years.
- ▶ **Wentworth's method** dispenses with the alcohol step and uses pH-guided mounting into solution II or III.
- ▶ Blood-rich specimens must **never** be washed in water before/after fixation; bile-stained specimens are fixed and stored **separately**.
- ▶ Perspex boxes with a **centre plate** (nylon/linen-thread stitching, reef knot) are the modern universal mounting method; glass-jar mounting uses Picein/asphaltum sealing, finished with painted black enamel/varnish.
- ▶ **Maceration** demonstrates bone lesions (osteogenic sarcoma, osteomyelitis, TB); **plastination** (whole-organ/sheet/luminal-cast) gives dry, durable, natural-coloured specimens via fixation → dehydration (acetone) → forced polymer impregnation (vacuum) → curing.

- Every mounted specimen must carry a **unique catalogue number** cross-referenced in a museum register with clinical/pathological details.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques. **Coverage note:** Museum technique/museology is not addressed in Gray's Anatomy (Standring) or the standard gross-anatomy specialty texts; this answer is grounded entirely in the dedicated PRESERVATION-specialty monograph (Haldar) mapped to this topic in the curriculum.



Digital museum display

Asked in: Paper III Nov 2016 — RGUHS MD/MS Anatomy

Definition / Introduction

A **digital museum display** is the computer-based digitisation, cataloguing and presentation of anatomical/pathological museum material — gross wet and dry specimens, radiographs, and histology slides — as high-resolution photographs, three-dimensional scans or whole-slide scans, stored in a searchable digital repository and shown to students on monitors, touch-screen kiosks, or through a networked/online "virtual museum". It supplements (rather than replaces) the traditional Perspex-box/glass-jar museum by converting the age-old preservation → mounting → labelling → cataloguing sequence into an equivalent digital imaging → database → on-screen display workflow. It is increasingly adopted by departments of anatomy and pathology to overcome the space, fading, breakage and formalin-hazard problems of the conventional wet museum while retaining its teaching value.

Traditional museum technique — the baseline being digitised

- **Preparation & fixation:** specimen fixed immediately in 10% formal-saline, then transferred to a colour-restoring fixative — the **Kaiserling** technique (1900), modified by **Pulvertaft** (1936) using sodium hydrosulphite as the reducing agent, and further modified by **Wentworth** (1938–57).
- **Mounting:** the fixed specimen is stitched to a centre-plate or suspended in a glass jar/Perspex box filled with museum (mounting) fluid; **plastination** (whole-organ, sheet, or luminal-cast) is used for dry, odourless, durable display specimens.
- **Storage & identification:** "the method must permit easy & have unique identification no. for each specimen" and "a reference book should be maintained to record necessary details about the specimens" — i.e. a manual catalogue was already integral to classical museum technique.
- **Presentation:** "relevant photographs, radiographs, presentation, labelling, cataloguing are of equal importance" to the specimen itself — photographic documentation is the direct forerunner of today's digital image.
- **Classical display principles** (general museum practice): restrict exhibits to the essential minimum; display at eye level with adequate elbow-room; use brief, neutral, non-obtrusive labels; and provide controlled lighting (natural vs artificial) so the exhibit — not the fixture — draws attention. A digital screen/kiosk must still respect these same principles of framing, legible on-screen labelling and adequate brightness.

Concept and components of a digital museum

Layer	Function
Image-capture	High-resolution digital/macro-photography of wet, dry and plastinated specimens; 2-D/3-D surface or laser scanning of bones/models; digitisation of the specimen's radiograph or CT image; whole-slide imaging (WSI) scanners for histology slides
Cataloguing/database	Each digitised image is tagged with a unique specimen ID/barcode (the digital equivalent of the old "reference book"), linked to metadata — organ, region, diagnosis, clinical history
Display/output	Networked touch-screen kiosk or LCD monitor placed beside (or instead of) the physical jar; QR-code/barcode linking a smartphone to further data; an intranet/LAN or cloud-hosted "virtual museum" web page accessible off-campus
Optional add-ons	3-D-printed tactile replicas generated from the scan data; augmented-reality label overlay on a live camera view of the real specimen

Whole-slide imaging — the closest textbook-grounded model for digitisation

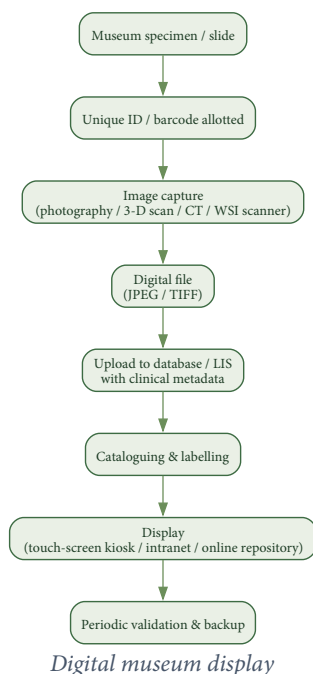
The histopathology literature's **digital pathology (DP)/whole-slide imaging (WSI)** system is the best-documented example of exactly this transformation and is directly transferable to museum-specimen digitisation:

- **Workflow:** barcoding of specimen/slide → imaging of the gross specimen into the laboratory information system (LIS) → whole-slide scanning (digitises the entire section) → upload of the image file with clinical data → interpretation on a workstation → validation and archiving.
- **Hardware:** slide loader, microscope with 20×/40× objective, robotics, and a CCD/LED digital camera; common file formats are JPEG/TIFF (average 600–800 MB per slide; 2–3 GB for a Z-stack); commercial scanners include Hamamatsu Nanozoomer, Leica Aperio AT2, 3D Histech Panoramic, Philips UltraFast and Ventana iScan HT.
- **Software:** image-viewing/annotation tools (pan, zoom, mark areas of interest) and optional artificial-neural-network modules for quantitative/pattern analysis.

Feature	Conventional glass slide / wet jar	Digital slide / image
Resolution	Always possible	Possible with adequate scan quality
Viewing	One specimen/limited multi-headed viewers at a time	Multiple viewers/terminals simultaneously
Storage	Physical shelf/cupboard space; fragile	Virtual server/cloud space

Feature	Conventional glass slide / wet jar	Digital slide / image
Fading/damage	Stain/colour fades with time; jar can break	No fading, no physical damage
Retrieval	Slow, manual search of shelf/register	Rapid, searchable database
Transport/sharing	Specimen must be physically carried	Transferred online in seconds (tele-education)
Access	Needs museum staff to issue/re-shelve	Can be accessed without museum staff, at any time
Cost	Comparatively low running cost	High initial equipment cost

Digitisation workflow (structural diagram)



Advantages of digital display over the conventional wet/dry museum

- ▶ **Simultaneous, multi-user access** — several students/terminals view the same specimen at once, unlike a single physical jar.
- ▶ **No deterioration** — no colour fading, no jar breakage, and repeated re-sectioning does not exhaust a rare specimen.
- ▶ **Rapid retrieval and tele-education** — images are searched and shared in seconds; distant colleges and self-learners can access the "virtual" specimen without museum staff supervision.
- ▶ **Space-saving and disaster-safe** — a large specimen collection occupies only server/cloud storage, and a digital backup protects rare, one-of-a-kind teaching specimens against permanent loss.
- ▶ **Easy updating/annotation** — labels, clinical correlation and radiology can be added or corrected without re-mounting the specimen; standardises OSPE/viva stations across batches.

Limitations / disadvantages

- ▶ **High initial cost** of scanners, kiosks, servers and networking, and the need for continued IT maintenance.
- ▶ **Large storage-capacity requirement** for high-resolution image/scan files.
- ▶ **Loss of tactile, three-dimensional hands-on experience** of handling a real wet specimen — only partly offset by 3-D-printed replicas.
- ▶ **Dependence on electricity/network**, and the need to validate the digitised image against the original specimen before it can substitute for it in assessment.
- ▶ **Unfamiliarity/training need** for faculty and students accustomed to the conventional format.

Applied / Clinical anatomy

- ▶ Fits the **competency-based medical education (CBME)** emphasis on self-directed and e-learning resources; a digital-museum kiosk supports pre- and post-dissection orientation and formative assessment stations.
- ▶ Enables direct **correlation of the digitised gross specimen with its radiograph/CT/MRI image**, reinforcing radiological and cross-sectional anatomy alongside the museum specimen.
- ▶ Permits **tele-education/sharing of rare teaching specimens** (congenital anomalies, tumours, variant vasculature) across affiliated colleges without risking damage to the original.
- ▶ Maintains continuity of **self-learning when physical access to the wet museum/laboratory is restricted** (e.g. during an infectious outbreak).
- ▶ The digital catalogue doubles as an **accession/audit record** for the specimen register expected at departmental inspection — the electronic counterpart of the traditional museum reference book.

High-yield exam pointers

- ▶ Define digital museum display as digitisation + cataloguing + on-screen presentation of museum specimens, supplementing the wet/dry museum.
- ▶ Recall that photographic documentation and a unique-ID reference-book catalogue were already part of classical museum technique (Halдар) — digitisation formalises this.
- ▶ Name the digitisation modes: high-resolution photography, 3-D/laser scanning, CT digitisation, and whole-slide imaging (for histology).
- ▶ Quote the WSI vs glass-slide comparison — multi-user viewing, no fading, rapid retrieval, virtual storage, high initial cost — as the model comparison table.
- ▶ List classical display principles (minimum exhibits, eye level, neutral background, unobtrusive label, controlled lighting) that a digital screen must still respect.
- ▶ Give at least 4 advantages (multi-access, no deterioration, tele-education, disaster-safe backup) and 3 limitations (cost, storage, loss of tactile 3-D experience).
- ▶ Mention CBME/self-directed learning and radiology correlation as the key applied points.
- ▶ Sketch the digitisation workflow: specimen → barcode → image capture → database → display → backup.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques; Handbook of Museum Technique (Govt Museum Chennai); Pranab Dey — Lab Techniques in Histopathology & Cytology 2e (Ch 18, Digital Pathology/Whole-Slide Imaging).

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

"Digital museum display" as a named topic is not addressed verbatim by any single textbook in this corpus (correctly flagged as a coverage gap in the topic spec, since Gray's Anatomy and the standard gross-anatomy texts do not deal with museum technique). This answer combines the classical museum-preservation/display principles above with the whole-slide-imaging/digital-pathology principles — the closest textbook-grounded digital analogue — and standard, universally-accepted medical-education technology practice.



Embalming

Asked in: Paper III Oct 2009 — RGUHS MD/MS Anatomy

Definition / Introduction

Embalming is the chemical treatment of a dead human body — by arterial injection and cavity treatment with preservative-cum-disinfectant fluids — to inhibit putrefaction, achieve an antiseptic state, and restore an acceptable pre-mortem appearance for a defined period. In the anatomy department it is the standard method of long-term cadaver preservation for dissection and cadaveric-surgery workshops; it is equally practised for funeral, religious, and legal-transport purposes. It should ideally be carried out within 6 hours of death; delayed embalming leads to bacterial decomposition and disintegration of the body within months.

1. Definition, Goals & Principle of Action

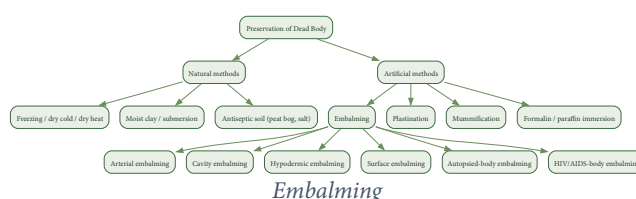
- ▶ **Definition (American Board of Funeral Service Education):** "the chemical treatment of the dead human body to reduce the presence and growth of micro-organisms, to temporarily inhibit organic decomposition, and to restore the dead human body to an acceptable physical appearance."
- ▶ Also defined as preservation of the dead body by treatment with antiseptics and preservatives to prevent putrefaction.
- ▶ **Effects produced:** proteins are coagulated; tissues are fixed; organs are bleached and hardened; blood is coagulated and transformed into a pinkish-brown mass.
- ▶ Embalming produces a chemical stiffening resembling rigor mortis, but unlike true rigor mortis (which is temporary), the **rigidity of embalming is permanent**.
- ▶ **Goals:** sanitisation of the cadaver; presentation and restoration of a life-like appearance after disease, trauma, or post-mortem change; preservation for anatomical teaching and research; safe

transport of the deceased to a distant location; allows time for families to organise funeral rites and to accept the reality of death.

- **Mechanism of action:** embalming chemicals occupy the water-retaining reactive centres of body proteins, forming a stable, inert, firm latticework resistant to bacterial/autolytic enzymes; germicides simultaneously inactivate enzymes and kill pathogenic and non-pathogenic bacteria (sanitisation). Net effect: **desiccation rather than putrefaction.**

2. Methods of Body Preservation & Types of Embalming

Category	Examples
Natural preservation	Extreme cold/dry heat, moist clay grave or water submersion, soil/water with natural antiseptics (arsenic, lime), peat-bog burial
Artificial preservation	Freezing, embalming, taxidermy, mummification, formalin/paraffin preservation, plastination, powders (sawdust + zinc sulphate), evisceration & immersion/drying, arterial or cavity injection, artificial cold (refrigeration)



Type of embalming	Method / indication
Arterial embalming	Fluid injected into the vascular system — the principal, definitive method
Cavity embalming	Fluid instilled into thoracic/abdominal/pelvic cavities via trocar, after arterial embalming
Hypodermic embalming	Arterial/cavity fluid injected subcutaneously — for isolated limbs / mutilated parts (blast, air-crash, rail injuries)
Surface embalming	Chemical-soaked packs on external skin — for burns, bed sores, surface lesions
Autopsied-body embalming	Six-point injection + separate viscera treatment
HIV/AIDS-body embalming	Increased fluid concentration + added precautions

3. Composition of Embalming Fluid

Ideal arterial embalming fluid (for one average 70 kg cadaver; total 10 L, of which ~10% is lost through drainage/purging):

Ingredient	Role	Quantity
Formalin	Preservative	1.5 L

Ingredient	Role	Quantity
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 ml
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 ml
Wintergreen oil	Perfume	90 ml
Water	Vehicle	up to 10 L

Sodium borate and sodium citrate are dissolved in hot water and cooled; remaining ingredients are added and made up to volume, allowed to stand, then filtered before use.

Cavity fluid (concentrated, used for organ/viscera treatment): Formalin 60%, Methanol 25%, Phenol 10%, Sodium lauryl sulphate 1%, Mercuric chloride 1%, Eucalyptus oil 1%.

Functional categories: Preservatives (formaldehyde, methanol, phenol) · Germicides (phenolics, zephiran chloride, glutaraldehyde) · Buffers (sodium borate/bicarbonate) · Wetting agents (glycerine, glycol, sodium lauryl sulphate) · Anticoagulants (sodium citrate/oxalate — precipitate calcium, keep blood liquid for displacement) · Dyes (eosin, Ponceau S, erythrosine — simulate natural colour) · Vehicles (alcohol, glycerine, water) · Perfumes (wintergreen, clove, lavender) · Muscle relaxant (magnesium chloride — relaxes arterial smooth muscle).

Formaldehyde (discovered 1856, A. W. von Hofmann): commercial "formalin" = 37% formaldehyde + 7% methanol + water. **Methanol** precipitates protein and stabilises formalin. **Phenol** (carbolic acid) is a powerful germicide/fungicide but darkens tissue on exposure to light.

4. Technique of Arterial Embalming

Preliminary steps: mouth/eyelids closed; sunken cheeks packed with fluid-soaked cotton, sunken eyeball corrected by orbital injection; head elevated 8–10 cm, feet raised for drainage; anal orifice and vagina plugged.

Choice of vessel — *artery*: size, depth, branches, drainage-vein practicality, proximity to aortic arch; *vein*: size, proximity to right atrium, depth, ease of raising. Principle: the nearer the vessel to the heart, the better the drainage.

Injection techniques:

Technique	Description
One-point injection	Single site for injection + drainage — commonest: right common carotid artery + internal jugular vein; then femoral; least common axillary/brachial
Split injection/drainage	Injection one site, drainage another (e.g., right femoral artery in, right internal jugular vein out) — reduces short-circuiting

Technique	Description
Multipoint injection	2+ arteries injected — corrects poor distribution
Six-point injection	For autopsied bodies/advanced decomposition
Restricted cervical injection	Head embalmed separately from the trunk

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Six-point injection sites for arterial embalming — label: right & left common carotid artery (head/neck, drained via right internal jugular vein); right & left axillary/brachial artery (upper limb); right & left femoral artery (lower limb). Order of injection: legs → arms → trunk (via right common carotid) → left then right side of head.

Restricted cervical injection is indicated in: generalised oedema, likely purging, poor distribution, facial/head trauma, eye enucleation, delayed embalming.

Instruments: hand/foot pump; stirrup pump; bulb (Higginson's-type) syringe; **gravity injector** (10 L bottle raised above table — simplest, safest, slowest; ~ 1 m rise ≈ 0.6 kg/cm²); **motorised injector** (10 L in ~ 30 min at ~ 2 kg/cm², using compressed air).

Methods of injection: continuous injection with continuous drainage (least satisfactory); continuous injection with disrupted drainage (better diffusion); alternate injection and drainage; **discontinuous injection and drainage at 2-hour intervals — the best method.**

Sequence of steps: injection → distribution → diffusion → drainage.

Drainage: blood/clots + interstitial/lymphatic fluid + embalming fluid, displaced to make room for arterial fluid, ensure even distribution, and avoid discolouration/odour/gas. Sites: internal jugular vein, femoral vein, right atrium, IVC. Patterns: alternate, concurrent, or intermittent.

5. Cavity Embalming

- ▶ Fluid is instilled directly into the thoracic, abdominal and pelvic cavities using a **trocár**; it is not a visually apparent process and comprises two steps — **aspiration followed by injection**; performed after arterial embalming (immediately, or several hours later).
- ▶ **Organs treated:** pancreas, spleen, kidney, liver, lungs, heart, stomach, intestines, brain and brainstem.
- ▶ **Instruments:** scalpel and blade, electric aspirator, hydro-aspirator, pointed trocar, rubber tube, hand pump, nasal-tube aspirator, autopsy aspirator, needle.
- ▶ **Trocár entry point:** 2 inches to the left and 2 inches superior to the umbilicus.
- ▶ **Thoracic aspiration:** trocar along a line from left ASIS to right ear lobule aspirates the heart, then anterior pleural cavities/lungs; directed either side of the vertebral column to reach the lung hilum and aspirate the bronchial tree/trachea.
- ▶ **Abdominal aspiration:** trocar toward the 5th intercostal space/left mid-axillary line pierces/aspirates the stomach; withdrawn and "fanned" to aspirate intestines and liver; then

directed to the pubic symphysis (midline) to pierce/aspirate the bladder, sigmoid colon and rectum (caecum reached $\frac{3}{4}$ of the way from pubic symphysis to right ASIS).

- ▶ **Fluid quantity:** ~1 litre of concentrated cavity fluid per thoracic cavity and per abdominal cavity (~2 L total for a 70 kg body), injected via a 100 ml syringe or a lumbar-puncture needle. Openings are closed with two nylon sutures on completion.

6. Special Embalming Situations

- ▶ **Embalming of an autopsied body:** the vascular circuit is disrupted by organ removal, so a **six-point injection** is made via the iliac/femoral, subclavian/axillary and common carotid vessels; viscera are treated separately with cavity fluid or embalming powder in a bag ("shake and bake"), then packed back with fluid-soaked cotton and the cavity sutured.
- ▶ **HIV/AIDS-infected body:** concentration of both arterial and cavity embalming fluid is increased, with recommended additional handling precautions.
- ▶ **Thiel's method of soft embalming** (for cadaveric surgical-skill workshops): retains natural flexibility of skin and muscle — limbs can be moved and tissue responds to the scalpel "as if alive," unlike stiff, fragile formaldehyde-fixed bodies. Uses 4-chloro-3-methylphenol with salts for fixation, boric acid for disinfection, and ethylene glycol for tissue plasticity, with formalin kept to a strict minimum (final concentration <0.5%); perfused (~14 L) through the great saphenous vein (femoral/carotid if difficult), then matured 4–6 weeks in an immersion tank before use.

Applied / Clinical anatomy

- ▶ **Legal aspects:** identification by near relatives; written consent; police NOC; death certificate referenced (+ Embassy clearance for foreigners); in MLC cases embalming follows autopsy; embalmer issues a certificate (3 copies local, 5 international transport); notifiable-disease deaths (cholera, rabies, plague, tetanus, Hep B, AIDS, TB) reported to authority.
- ▶ **Medico-legal aspects (why embalming must never precede autopsy):** induces artefacts; chemical stiffening mimics rigor mortis, confounding time-since-death estimation; alters appearance, hampering injury interpretation; destroys cyanide/alcohol/opiates/CO (toxicology unreliable) and bacteria (bacteriology useless); prevents blood grouping and thrombus/embolism detection; trocar may modify or create wounds. Heavy metals must be avoided in an MLC embalming mixture.
- ▶ **Anatomy Act (1949)** (enacted as the Bombay Anatomy Act): governs supply of **unclaimed bodies** of deceased persons to hospitals and medical/teaching institutions for anatomical examination and dissection — applicable only if death occurred in a state hospital or public place within the prescribed zone of the institute, and the police, after a lapse of 48 hours, declare there are no claimants.
- ▶ **Occupational risks:** blood-borne/airborne pathogens (*C. difficile*, MRSA, VRE/VRSA, *Pseudomonas*, CJD, HIV, Hepatitis B/C, MDR-TB) plus chemical hazard from formalin; mitigated by hygiene, PPE, safe (OSHA) formaldehyde limits.
- ▶ **Religious note:** most Christian groups permit embalming; Islam and Zoroastrianism do not. Notable embalmed bodies: Lenin, Lincoln, Stalin, Lord Nelson.

High-yield exam pointers

- ▶ Embalming = chemical treatment of the dead body for antiseptis, temporary arrest of decomposition, and restoration of life-like appearance; ideally within **6 hours** of death.
- ▶ Produces stiffening resembling rigor mortis, but unlike true rigor this rigidity is **permanent**.
- ▶ Ideal arterial fluid for a 70 kg body: **10 litres** (formalin, sodium borate, sodium citrate, glycerine, sodium chloride, eosin, wintergreen oil, water).
- ▶ Cavity fluid is far more concentrated than arterial fluid (Formalin 60%); ~1 L instilled per thoracic and abdominal cavity via trocar.
- ▶ **Six-point injection** (bilateral common carotid, axillary/brachial, femoral) is standard for autopsied or badly decomposed bodies.
- ▶ Embalming must **never precede autopsy** in MLC cases — destroys toxicological/bacteriological evidence and mimics rigor mortis.
- ▶ **Thiel's soft-embalming** keeps tissue pliable (minimal formalin, <0.5%) for cadaveric surgical-skill workshops.
- ▶ The **Anatomy Act (1949)** governs legal procurement of unclaimed cadavers for dissection in India.

SOURCE & COVERAGE

Haldar — *Notes on Embalming and Museum Techniques* (specialty text on preservation & body-donation techniques for RGUHS Paper III).

Coverage note: Embalming is a mortuary-science/preservation-technique topic and is not covered in Gray's Anatomy (Standring) 41e, the spine text for this bank; the answer is therefore grounded entirely in the dedicated specialty preservation text, as flagged in the topic note.



Embalming a body where death was due to metastasis in lungs

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction — Embalming is defined (American Board of Funeral Service Education) as the chemical treatment of the dead human body to reduce the presence and growth of micro-organisms, temporarily inhibit organic decomposition, and restore an acceptable physical appearance; it coagulates protein, fixes tissue, and produces a chemical stiffening resembling rigor mortis, but permanent. This body's antemortem disease was disseminated (metastatic) malignancy in the lung — a **natural, non-medicolegal death**, but one that predictably leaves cachexia, malignant pleural effusion, mediastinal nodal disease and sometimes venous obstruction, each of which forces specific modifications to otherwise standard technique. The answer below gives the routine embalming procedure and flags, at each step, the change required by this special case.

1. Legal and preliminary (medico-legal) requirements

Requirement	Detail
Identification	Body identified by near relatives before the procedure
Consent	Written consent for embalming obtained
Police NOC	No-objection certificate from police obtained
Death certificate	Referred to and must accompany the body before embalming begins
Notifiable disease	If the body suffered from a notifiable disease (cholera, rabies, plague, tetanus, Hepatitis B, AIDS, TB, etc.) the concerned health authority is informed — a co-existing infection (e.g., TB) in a lung-cancer patient falls under this rule
Foreign national	Embassy clearance obtained in addition
Medicolegal case (MLC)	Embalming is never done before autopsy in an MLC — it produces artefacts, destroys toxicology (cyanide, alcohol, opiates, CO), abolishes bacteriologic/blood-grouping value, and masks thrombus/embolism and wound dating
This case	Death from metastatic malignancy is a natural (non-MLC) death — a treating-physician death certificate stating the cause suffices; no forensic autopsy is mandatory, so routine (unautopsied-body) chronology is followed unless the body is destined for teaching dissection under the Anatomy Act (1949), which additionally requires the body to be unclaimed after 48 hours' police notice before use
Certification	On completion, the embalmer/competent authority issues a certificate (3 copies for local, 5 for international transport)

2. Pre-embalming clinical assessment specific to this case

- ▶ Review case notes: primary site, extent/sites of metastasis, chemotherapy/radiotherapy history (radiation skin change; chemotherapy-induced anaemia/thrombocytopenia raises bleeding risk at trocar/vessel-raising sites), any intercostal drainage tube, central line, colostomy or other indwelling device — these must be removed, the tract disinfected and sutured (as for any hospital death).
- ▶ Anticipated findings on external examination: **cachexia/emaciation**, pallor, occasional jaundice (hepatic metastases), dependent oedema or ascites (hypoalbuminaemia of malignancy), digital clubbing, and cervical/supraclavicular lymphadenopathy.
- ▶ A bulky mediastinal nodal mass from lung metastasis can compress the **superior vena cava**, producing facial, neck and upper-limb oedema and distended neck veins in life — this "general oedema" picture is an explicit indication in embalming manuals for using **restricted cervical injection** rather than a single right-carotid point, because oedematous tissue disperses fluid poorly and purges easily.

3. Embalming fluid — composition and adjustment for the cachectic body

The standard ideal arterial fluid (per 10 L, sufficient for a 70 kg body, allowing ~10% loss through drains/purging) is built from a preservative, buffer, anticoagulant, wetting agent, pH-control salt, cosmetic dye and perfume:

Ingredient	Role	Quantity (per 10 L)
Formalin	Preservative	1.5 litres
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 ml
Sodium chloride	pH control	800 g
Eosin 1%	Cosmetic dye	30 ml
Winter-green oil	Perfume	90 ml
Water	Vehicle	to 10 litres

Dilution must be varied for body build — the manual specifies distinct arterial-fluid formulae for **obese** and **thin** subjects (formalin 10%, methanol 55%, sodium borate 15 g, sodium citrate 15 g, phenol 5%, thymol crystals, eosin 5 ml, wintergreen 10 ml common to both):

Component	Obese subject	Thin/cachectic subject
Glycerine (wetting agent)	15%	20%
Water (vehicle)	15%	10%

A cancer-cachexia body — the expected build here — is treated as the **thin-subject formula**: a more concentrated solution (more wetting agent, less water) is used because reduced subcutaneous fat and

tissue bulk mean a standard dilution under-penetrates and risks poor fixation and post-embalming skin wrinkling. Cavity fluid (for aspirated body cavities) is a separate, stronger concentrate: Formalin 60%, Methanol 25%, Phenol 10%, Sodium lauryl sulphate 1%, Mercuric chloride 1%, Eucalyptus oil 1%.

4. Arterial embalming — technique and vessel-choice modification

- ▶ Position: body supine, head elevated 8–10 cm on a headrest, feet raised to aid drainage; features "set" — mouth and eyelids closed (eye caps if globe sunken, arterial solution injected into the orbit), cheeks packed with soaked cotton if hollowed by cachexia; anal and vaginal orifices plugged.
- ▶ **Choice of vessels** — the vessel nearer the heart gives the best result, especially for drainage; a **single-point injection (right common carotid artery + internal jugular vein)** is standard but leaves patches unfixed. **Multi-point/six-point injection** — right and left common carotid arteries (head/neck), right and left axillary or brachial arteries (upper limb), right and left femoral arteries (lower limb), each ligated on completion — is indicated for traumatic death, autopsied bodies, and **wherever venous/arterial distribution is expected to be poor**, which applies here if mediastinal nodal disease has compromised great-vein return, or if peripheral vessels are friable from cachexia.
- ▶ **Restricted cervical injection** (head embalmed separately via both carotids, trunk injected and drained via the internal jugular) is specifically indicated for a **body with general oedema**, bodies likely to purge, poor distribution, or delayed embalming — directly relevant if SVC-obstruction-related facial/neck oedema is present.
- ▶ Drainage (by displacement of blood, interstitial/lymphatic fluid and embalming fluid) may be alternate, concurrent or intermittent; its purpose is to make room for fluid, ensure even distribution, and prevent discoloration, gas formation and decomposition.

5. Cavity embalming — thoracic modification for pulmonary metastasis

Cavity embalming (aspiration followed by injection) is done after arterial embalming and treats the pancreas, spleen, kidney, liver, **lungs**, heart, stomach, intestines and brain/brain-stem, using a trocar, hand or electric/hydro-aspirator.

- ▶ **Thoracic aspiration:** trocar inserted along the line from the left anterior superior iliac spine to the right ear lobule to aspirate the heart; then directed along either side of the vertebral column to reach the **hilum of the lung**, aspirating the root of the bronchial tree toward the trachea.
- ▶ **Case-specific modification:** pulmonary metastases very commonly coexist with a **malignant pleural effusion**; the thoracic cavity therefore often yields a larger-than-average aspirate, and loculated pockets (from pleural adhesions/tumour deposits) may need repeated trocar passes to empty completely — residual blood/lymph/effusion fluid dilutes the concentrated cavity fluid and can itself cause decomposition, so thorough aspiration must precede injection. Firm, solid tumour-replaced lung parenchyma is less porous than normally aerated lung, so a deliberate fanning motion of the trocar is used to break up nodularity and ensure the cavity fluid reaches all lobes; the volume injected per thoracic cavity (average 1 L for a 70 kg body) may need to be increased if the lung is bulky with confluent metastatic deposits.

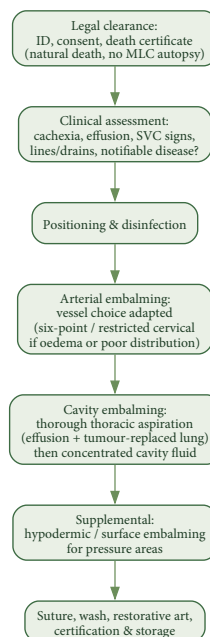
- **Abdominal aspiration:** trocar moved between the 5th intercostal space and mid-axillary line to pierce and aspirate the stomach, then withdrawn to pierce intestines and liver by a fanning movement, then directed to the pelvis to aspirate bladder, sigmoid colon and rectum (screening for occult hepatic secondaries at the same time).
- After aspiration, concentrated cavity fluid is injected into all cavities and over the viscera (about 1 L per thoracic and per abdominal cavity for a 70 kg body), and the puncture openings closed with two sutures (commonly nylon).

6. Supplemental methods as required

- **Hypodermic embalming** (arterial or cavity fluid via an 8–19 gauge hypodermic syringe) for any focally emaciated, discoloured or bed-sore-affected area over bony prominences — common in a terminally cachectic, bed-bound cancer patient.
- **Surface embalming** — packing exposed/damaged skin areas with cotton soaked in embalming fluid — for any pressure ulcer or radiotherapy-related skin change.

7. Completion, restorative art and documentation

Arterial tubes and drains are removed, incisions dried and sutured, the body re-washed, dried, all orifices packed with cotton, and restorative art performed to correct the wasted, cachectic facial contour before dressing and storage; the embalming/competent-authority certificate is issued as above.



Embalming a body where death was due to metastasis in lungs

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Outline of a supine body labelled with the **six-point injection scheme** and thoracic/abdominal **trocars guide-lines**:

- ▶ Right and left **common carotid artery** + right **internal jugular vein** (head/neck; drain tube toward heart)
- ▶ Right and left **axillary/brachial artery** (upper limb)
- ▶ Right and left **femoral artery** (lower limb)
- ▶ Thoracic trocar line: **left anterior superior iliac spine** → **right ear lobule** (aspirates heart), then paravertebral to **hilum of lung**
- ▶ Abdominal trocar points: **5th intercostal space/mid-axillary line** (stomach), umbilical region (liver/intestines by fanning), **suprapubic** (urinary bladder)

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- ▶ The **hilum of the lung** (root — bronchus, pulmonary artery/veins, bronchial vessels, lymph nodes) is the anatomical target the embalmer's trocar must reach to aspirate the bronchial tree — the same route by which metastatic and lymphatic spread reaches mediastinal nodes.
- ▶ Mediastinal nodal enlargement from pulmonary metastasis can compress the **superior vena cava**, producing SVC-obstruction facies (facial/neck oedema, distended neck veins) — the direct clinical correlate that dictates restricted cervical or multi-point injection.
- ▶ Malignancy is a hypercoagulable state (Trousseau's phenomenon); intravascular clot may impede free perfusion of arterial fluid and necessitate multi-point injection with adequate drainage.
- ▶ Malignant pleural effusion is the radiological/clinical counterpart of the "larger than average thoracic aspirate" the embalmer should expect; a pre-embalming chest X-ray/CT report, where available, usefully forewarns the embalmer of effusion, nodularity and nodal bulk.
- ▶ Embalming must never precede autopsy in a medicolegal case, but a certified natural death from malignancy carries no such restriction.

High-yield exam pointers

- ▶ Embalming = chemical treatment for antisepsis, arrest of decomposition and cosmetic restoration (ABFSE definition); produces protein coagulation and permanent chemical rigidity.
- ▶ This is a **natural, non-MLC death** — routine unautopsied-body legal steps (identification, consent, police NOC, death certificate) suffice; MLC-style embalming-after-autopsy-only does not apply here.
- ▶ Cachexia of malignancy → treat as a **thin-subject arterial fluid** (glycerine 20%, water 10%) for better penetration.
- ▶ **General oedema / poor distribution** (e.g., from SVC compression by mediastinal nodal metastasis) → indication for restricted cervical or six-point/multi-point injection instead of single right-carotid injection.
- ▶ Thoracic trocar route to aspirate lungs: left ASIS → right ear-lobule line, then paravertebral to the **hilum of lung** and bronchial root.

- ▶ Pulmonary metastasis commonly coexists with **malignant pleural effusion** — complete aspiration before injecting concentrated cavity fluid (residual fluid dilutes fixative and risks decomposition).
- ▶ Organs treated in cavity embalming: pancreas, spleen, kidney, liver, lungs, heart, stomach, intestines, brain/brain-stem.
- ▶ Notifiable-disease reporting rule applies if a co-existing infective disease is present alongside the malignancy.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (primary source for definitions, legal/medicolegal aspects, fluid composition, vessel choice, six-point/restricted-cervical injection, and thoracic/abdominal cavity-aspiration technique). Handbook of Museum Technique (Govt Museum Chennai) consulted for cross-check (specimen-preparation focus; no disease-specific embalming section). **Coverage note:** neither embalming text contains a dedicated sub-section on "embalming a body with pulmonary metastasis"; the case-specific modifications above (cachexia → thin-subject fluid; malignant effusion → thorough thoracic aspiration; nodal/SVC compromise → multi-point or restricted-cervical injection) are standard technique choices from the source text applied by reasoning from the well-established systemic effects of disseminated malignancy (cachexia, malignant pleural effusion, mediastinal lymphadenopathy, hypercoagulability), not fabricated values.



Embalming an AIDS body

Asked in: Paper III Oct 2010 — RGUHS MD/MS Anatomy

Definition / Introduction Embalming is the chemical treatment of the dead human body — by injecting antiseptic/preservative fluids into the vascular system and body cavities — to reduce microbial growth, temporarily inhibit putrefaction, and restore an acceptable pre-mortem appearance. An AIDS body (a body known or suspected to be HIV-positive) is embalmed under the same general principles as any specialist/high-risk case, but the procedure is modified in two respects that examiners expect: (1) the **concentration of both the arterial and cavity embalming fluids is increased**, to ensure more complete disinfection and fixation of tissues that may carry a high bloodborne viral load, and (2) **enhanced infection-control (universal) precautions** are mandatory for the embalmer, since HIV — together with hepatitis B and C — is one of the recognised high-risk bloodborne pathogens transmissible through mortuary practice.

1. Legal and administrative aspects before embalming

Step	Requirement
Identification	Body identified by near relatives before the procedure
Consent	Written consent for embalming obtained

Step	Requirement
Police clearance	No-objection certificate from police obtained
Death certificate	Death certificate / post-mortem report referred to and kept with the body
Foreigner	Additional embassy clearance required
Medicolegal case	Embalming is never done before autopsy — it induces artefacts (chemical stiffening mimicking rigor mortis, destruction of cyanide/alcohol/opiates/CO so toxicology fails, bacteriologic evaluation becomes useless, blood grouping and thrombus/embolism detection are lost) and alters wound interpretation
Notifiable disease	If the deceased suffered from a disease of public-health concern — cholera, rabies, plague, tetanus, hepatitis B, AIDS, tuberculosis, etc. — the concerned (notifying) authority must be informed before embalming
Certification	On completion, the embalmer/competent authority issues a certificate (3 copies for local transport, 5 for international transport)

- For an AIDS body, this notification step is the trigger for the mortuary/embalming team to escalate to **enhanced universal precautions** described below.

2. Occupational hazards to the embalmer from an HIV/AIDS body

- **Bloodborne pathogens:** HIV, hepatitis B, hepatitis C — the principal risk group; transmitted through needle-stick/trocar injury, mucocutaneous splash of blood or body fluid, or contact with non-intact skin.
- **Airborne/other pathogens** also carried in the broader occupational-risk profile of embalming: multidrug-resistant TB (TB-MDR), *Clostridioides difficile* (mutated toxin up to 20 times more potent), MRSA, VRE/VRSA/VISA, multi-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and prion disease (CJD).
- **Special-group risk:** a pregnant female embalmer carries added risk from TORCH agents, human parvovirus B19, and syphilis.
- **Chemical hazard:** concurrent exposure to formalin vapour (embalming fluid), which must be kept within safe OSHA formaldehyde exposure limits.
- Good personal hygiene and proper personal protective equipment (PPE) are the two blanket safeguards recommended against this entire hazard profile.

3. Universal (standard) precautions for handling an infectious body

The mortuary/embalming team must treat **every** body — and an identified AIDS body in particular — as a potential source of bloodborne infection, because the HIV/HBV/HCV status of any given case may be unknown or in the window period.

- **High-risk pathogens** requiring universal precautions: hepatitis B, hepatitis C, HIV.

- ▶ **Body fluids needing universal precautions:** blood, pleural and peritoneal fluid, CSF, semen, vaginal secretions, synovial fluid and faecal material — all of which the embalmer contacts during cavity aspiration and injection.
- ▶ **Barrier measures / PPE:** hand washing (alcohol-based hand rub preferred), gloves (latex/nitrile, not re-used), mask, goggles/face shield, water-impervious gown or apron, and footwear that fully covers the foot.
- ▶ **Sharps safety** (the trocar, cannula and injection needles are the chief hazard):
 - never recap a used needle; use a needle cutter;
 - discard all sharps into a leak-proof, puncture-proof container;
 - ligate each injection/drainage site immediately after use to prevent leakage;
 - minimise the number of separate punctures (a single well-chosen six-point injection is preferred to repeated re-puncturing).
- ▶ **Environmental measure:** clean the embalming table and work area before and after the procedure with 10% sodium hypochlorite solution (freshly prepared, corrosive — not stored in a metal container).
- ▶ **Post-exposure protocol:** any needle-stick/sharps injury must be reported immediately, followed by post-exposure prophylaxis (PEP) and serological follow-up; risk of transmission after a single percutaneous exposure is approximately HIV 0.3%, hepatitis C 1.8%, hepatitis B 6–30% (unvaccinated), which is why HBV vaccination and prompt PEP are emphasised for embalming/mortuary staff.
- ▶ A staff member with a weeping dermatitis or open hand wound should be excluded from handling the body until healed.

4. Modified embalming technique for the HIV/AIDS body

- ▶ **Core modification:** the concentration of the arterial embalming fluid and the cavity embalming fluid is increased above the standard strength, and the recommended handling precautions above are strictly enforced throughout.
- ▶ **Vessel selection/injection technique:** a **six-point injection** (bilateral common carotid arteries for the head and neck, bilateral axillary arteries for the upper limb, bilateral femoral arteries for the lower limb) is preferred over a single-point injection, since it ensures complete distribution of the higher-strength fluid to all regions while allowing each vessel to be ligated as soon as it is used — reducing the time any single puncture site is left open and thereby reducing exposure risk.
- ▶ **Sequence** (unchanged in principle, intensified in practice):
- ▶ Disinfect the body surface and all orifices with a germicidal solution before any instrumentation.
- ▶ **Arterial embalming** — raise and cannulate the chosen artery/vein pair, inject the concentrated arterial fluid (continuous, disrupted, or alternate injection-and-drainage method), ligate the vessel on completion.
- ▶ **Cavity embalming** — aspirate the thoracic, abdominal and pelvic cavities with a trocar and inject the concentrated cavity fluid (this fluid additionally disinfects the walls and parenchyma of organs and the contents of hollow viscera).

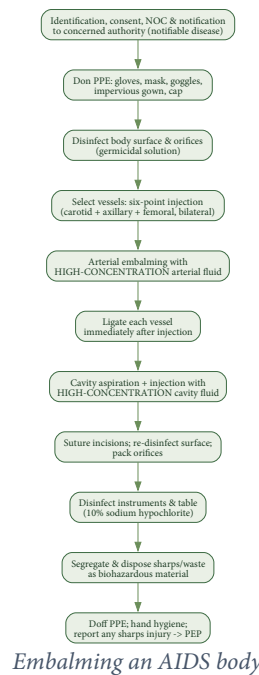
- ▶ Supplementary **hypodermic or surface embalming** if a local area (wound, bed sore) needs additional fixation.
- ▶ Dry and suture all incisions/punctures securely; disinfect the body surface again; pack orifices with cotton.
- ▶ Disinfect all instruments and the embalming table; segregate and dispose of contaminated waste as biohazardous material.
- ▶ **Rationale:** formalin and phenol — the preservative and germicide components of embalming fluid — inactivate both pathogenic and non-pathogenic organisms in the tissue; raising their concentration in an AIDS body is intended to ensure more thorough microbial/viral inactivation given the higher expected pathogen load.

5. Embalming fluid composition (baseline, before the AIDS-body concentration increase)

Component	Agent	Typical strength
Preservative	Formalin + Methanol	~10% + ~55%
Buffer	Sodium borate	15 g
Anticoagulant	Sodium citrate	15 g
Wetting agent	Glycerine	15–20%
Germicide	Phenol	5%
Vehicle	Water	10–15%
Fungicide	Thymol	few crystals
Dye	1% Eosin	5 mL
Perfume	Wintergreen oil	10 mL

- ▶ **Cavity fluid:** ~2 litres per average body; disinfects walls/parenchyma of organs and contents of hollow viscera. Three graded strengths are described — (i) glycerine 75%, alcohol 10%, phenol 5%, water 10%; (ii) formalin 15%, glycerine 20%, phenol 5%, water 60%; (iii) formalin 5%, glycerine 50%, phenol 5%, water 40%.
- ▶ **Pre-injection fluid:** injected first (4–5 L, wait ~30 min) to loosen clots and improve drainage before the arterial fluid is given — formalin 60%, methanol 25%, glycerine 2.5%, phenol 10%, mercuric chloride 1%, lavender 1%; also contains anticoagulants and buffers.
- ▶ For an AIDS body, the **same fluid classes are used but at a higher concentration** — the corpus does not give a specific numeric percentage for this increase, so no fixed figure should be quoted in the exam beyond "increased concentration."

6. Protocol flow (steps for embalming an HIV/AIDS body)



7. Disinfection and biomedical waste disposal after the procedure

- ▶ Instruments are cleaned and, where possible, autoclaved or soaked in a fresh chemical disinfectant (10% sodium hypochlorite is the standard broad-spectrum agent for laboratory/mortuary surfaces; iodophors, quaternary ammonium compounds and phenolics are alternative disinfectants).
- ▶ Blood-soaked cotton, tissue remnants, and sharps generated during embalming are **biohazardous waste** and must be segregated at source, stored in leak-proof, puncture-proof, labelled containers, and transported/treated separately from general waste — never stored in an open or metallic container for sharps, and never mixed with incompatible chemical waste.

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- ▶ **Cadaver donation for teaching:** bodies received under the Anatomy Act for dissection are, ideally, HIV-screened; even so, embalming staff apply universal precautions to every unclaimed/donated body because a negative test does not exclude the serological window period.
- ▶ **Medicolegal AIDS deaths:** embalming must follow, never precede, autopsy; the pathologist/mortuary team is informed of the HIV status so that heightened precautions and increased-concentration fluids are used from the outset of the post-mortem embalming.
- ▶ **Occupational health surveillance:** embalmers and mortuary technicians are advised hepatitis B vaccination, ready access to post-exposure prophylaxis, and a written post-exposure protocol, given the measurable seroconversion risk after a percutaneous sharps injury.
- ▶ **Museum/teaching specimens** derived from an HIV-positive body are handled with the same enhanced fixation logic — a higher formalin/phenol concentration ensures the specimen is rendered non-infective before long-term storage and handling by students.

High-yield exam pointers

- ▶ Two defining modifications for an AIDS body: (1) ↑ concentration of arterial and cavity fluid, (2) strict recommended precautions during handling.
- ▶ Notifiable diseases requiring authority intimation before embalming: cholera, rabies, plague, tetanus, hepatitis B, AIDS, TB.
- ▶ High-risk bloodborne pathogens under universal precautions: HIV, HBV, HCV.
- ▶ Six-point injection sites: bilateral common carotid, axillary, femoral vessels.
- ▶ Standard mortuary disinfectant: 10% sodium hypochlorite (freshly prepared, not stored in metal containers).
- ▶ Needle-stick seroconversion risk: HIV ~0.3%, HCV ~1.8%, HBV 6–30% (unvaccinated) — basis for PEP protocols.
- ▶ Embalming must never precede autopsy in a medicolegal case (destroys toxicology, bacteriology, blood grouping and injury-interpretation evidence).
- ▶ PPE checklist: gloves, mask, goggles, impervious gown/apron, cap, sharps discipline, hand hygiene.
- ▶ Biomedical waste from an infectious embalming is handled as biohazardous waste — segregated, leak-proof, labelled, separately transported.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (definition, goals, legal/notifiable-disease requirement, occupational risks of embalming, types of embalming, six-point injection, arterial/cavity/pre-injection fluid compositions, the "Embalming of HIV/AIDS body" section). Pranab Dey — Lab Techniques in Histopathology & Cytology 2e, Ch. 30 "Laboratory Safety and Laboratory Waste Disposal" (universal precautions, high-risk pathogens, PPE, sharps/needle-stick management, seroconversion risk figures, chemical disinfectants, biomedical waste categories). Coverage note: the corpus's direct treatment of "embalming an HIV/AIDS body" (Haldar) is brief — two core bullets (increased fluid concentration; recommended handling precautions). This answer expands those two points using the corpus's own general embalming-technique and universal-precautions/biosafety material rather than inventing an HIV-specific numeric fluid concentration, which is not given in the source.



Embalming methods

Asked in: Paper III 11-May-2022 — RGUHS MD/MS Anatomy

Definition / Introduction — Embalming is the chemical treatment of the dead human body, by injecting an antiseptic/preservative fluid into the vascular system and body cavities, to reduce microbial growth, temporarily inhibit organic decomposition, and restore an acceptable physical appearance (American Board of Funeral Service Education). In anatomy departments it is done to preserve cadavers for dissection, demonstration and cadaveric-surgery training, and to permit transport of a body. To achieve the desired antiseptic, non-putrefactive result, embalming must ideally be started within 6 hours of death — delayed embalming allows bacterial decomposition to set in and gives poor, uneven fixation.

1. Principles, goals and the ideal embalming fluid

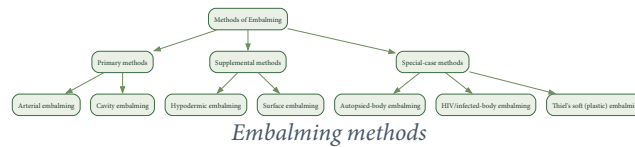
- ▶ **Biochemical basis** — the embalming chemicals cross-link body proteins into an inert, firm latticework that resists breakdown by bacterial and autolytic enzymes; this destroys the reactive, water-binding centres of proteins so the tissue no longer imbibes/retains water (stable, long-lasting fixation). Germicides and preservatives simultaneously inactivate tissue enzymes and kill pathogenic and non-pathogenic bacteria (sanitisation).
- ▶ **Effects produced** — (1) proteins are coagulated, (2) tissues are fixed, (3) organs are bleached and hardened, (4) blood is coagulated into a pinkish-brown mass, (5) a chemical stiffening resembling rigor mortis is produced — but, unlike true rigor, this rigidity is **permanent**.
- ▶ **Factors governing flow of fluid into tissue** — capillary resistance, chemical composition of the fluid, injection pressure, and osmosis/diffusion/gravity.
- ▶ **Composition of an ideal embalming (arterial) fluid** — a 70 kg body requires about 10 L of fluid ($\approx 10\%$ is lost through drainage/purging):

Ingredient	Role	Quantity (per 10 L)
Formalin	Preservative	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 mL
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 mL
Methyl salicylate (wintergreen)	Perfume	90 mL
Water	Vehicle	up to 10 L

- ▶ **Component classes** — preservatives (formaldehyde, methanol, phenol), germicides (phenolic derivatives, zephiran chloride, glutaraldehyde), buffers (sodium borate/bicarbonate), wetting agents (glycerine, glycol, sodium lauryl sulphate), anticoagulants (sodium citrate, sodium oxalate), dyes (eosin, ponceau S), vehicles (water, alcohol, glycerine), perfumes (wintergreen, clove oil) and muscle relaxants (magnesium chloride, aids fluid flow through the arterial wall).
- ▶ Formalin = 37% formaldehyde + 7% methyl alcohol in water (formaldehyde discovered 1856 by A.W. von Hofmann).

2. Classification of embalming methods

Embalming fluids are of three kinds — **arterial fluid** (injected into the vascular system), **cavity fluid** (injected into the body cavities with a trocar), and **pre-injection fluid** (a dilute anticoagulant/buffer fluid injected first to loosen clots and improve later distribution). Based on route, the methods of embalming are classified as follows.



3. Arterial embalming — vessels, instruments and injection technique

Arterial embalming (the primary method) fills the vascular tree with arterial fluid and displaces blood out through a vein.

- ▶ **Criteria for choosing the artery** — size/diameter, practicality of drainage from the accompanying vein, depth/location, its branches, and proximity to the arch of the aorta.
- ▶ **Criteria for choosing the vein** — size/diameter, proximity to the right atrium, depth, and ease of raising the vessel.
- ▶ **Injection techniques:**
 - *One-point injection* — one site used for both injection and drainage; right common carotid artery + internal jugular vein is commonest, femoral artery/vein next, axillary/brachial vessels least common.
 - *Split injection/drainage* — injection from one site, drainage from another (commonly right femoral artery for injection, right internal jugular vein for drainage) — reduces short-circuiting of fluid.
 - *Multipoint injection* — two or more arteries injected to overcome poor distribution.
 - *Six-point (sectional) injection* — used routinely for **autopsied bodies** or advanced decomposition. The right/left common carotid, right/left axillary (or brachial), and right/left femoral arteries are raised and cannulated (with the right internal jugular vein as the drain); injection order is right leg → left leg → right arm → left arm → trunk (via right common carotid) → left side of head → right side of head.
 - *Restricted cervical injection* — head embalmed separately from the trunk via the carotids; indicated in general oedema, bodies prone to purging, poor distribution, facial/head trauma, eye enucleation, and delayed embalming.
- ▶ **Instruments for injection** — hand/foot pump; bulb (Higginson's-type) syringe with valved rubber tubing; **gravity injector** (simplest/safest/slowest — a 10 L bottle raised 4–6 feet above the table, a 1-metre rise giving $\approx 0.6 \text{ kg/cm}^2$ fluid pressure); and **motorised/electric injectors**, which deliver 8–10 L of fluid within 30–45 minutes at a controlled pressure of about 2 kg/cm^2 .
- ▶ **Method of injection (rate/drainage pattern)** — continuous injection with continuous drainage (least satisfactory — poor saturation); continuous injection with disrupted (intermittently closed) drainage (better diffusion); alternate injection and drainage (injection halted when superficial veins swell, then drained); and discontinuous injection and drainage — repeated small-volume injections at ~ 2 -hour intervals, 3–4 times, considered the best method.

- **Drainage** — occurs by fluid displacing blood, interstitial and lymphatic fluid from the internal jugular vein, femoral vein, right atrium or inferior vena cava; it makes room for further fluid, ensures even distribution, and prevents discoloration, gas formation and decomposition.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Outline of the trunk, head and limbs with the **six embalming injection sites** marked and labelled:

- Right and left common carotid artery (head & neck; trunk injected through the right)
- Right and left axillary/brachial artery (upper limbs)
- Right and left femoral artery (lower limbs)
- Right internal jugular vein (drainage site)

Arrows showing the injection order: right leg → left leg → right arm → left arm → trunk → left head → right head.

4. Cavity, hypodermic, surface and special-case embalming

- **Cavity embalming** — performed after arterial embalming ($\frac{1}{2}$ –1 hour later, to let the viscera harden); a trocar (with electric/hydro-aspirator) is passed through a small stab incision about 5–6 cm above the umbilicus in the midline. Aspiration sequence: stomach (trocar directed upward, backward and left) → right side of the heart → pleural sacs (via the diaphragm) → small intestine, caecum and colon (multiple punctures) → urinary bladder, sigmoid colon and rectum. About 1 L of concentrated cavity fluid is then instilled into each of the thoracic and abdominal cavities ($\approx$ 2 L total for a 70 kg body); a representative cavity-fluid composition is formalin 60%, methanol 25%, phenol 10%, sodium lauryl sulphate 1%, mercuric chloride 1%, eucalyptus oil 1%. Instruments include scalpel/blade, pointed trocar, rubber tubing, hand pump, and electric/hydro/nasal-tube/autopsy aspirators. Incisions are finally closed with two sutures (nylon).
- **Pre-injection fluid** — a dilute anticoagulant-and-buffer fluid (4–5 L) injected before the arterial fluid; it loosens clots and improves later drainage/distribution — useful because after death nearly 85% of blood pools in the capillary bed, 10% in veins and only 5% remains in arteries.
- **Hypodermic embalming** (supplemental) — fluid (arterial or cavity type) injected subcutaneously with an 8–19-gauge hypodermic syringe/needle to preserve localised areas — isolated limbs or parts from blast, air-crash or rail-accident injuries.
- **Surface embalming** (supplemental) — cotton or gauze packs soaked in embalming fluid are applied directly to the skin surface (or wrapped around the whole body); used for burns, bedsores and surface lesions.
- **Embalming of the autopsied body** — six-point arterial injection (both iliac/femoral, subclavian/axillary, and common carotid vessels) since the circulatory circuit is disrupted; the eviscerated organs are treated separately with cavity fluid or embalming powder in a viscera bag ("shake and bake") before replacement and closure.
- **Embalming an HIV/AIDS body** — concentration of both arterial and cavity fluids is increased, with strict barrier precautions for the embalmer.

5. Thiel's method of soft (plastination-free) embalming

A modern technique used mainly for cadaveric surgical-skills workshops, preferred because conventional formalin-fixation leaves tissue stiff and fragile.

- ▶ Retains the body's natural colour and texture; skin and muscle stay pliable, joints remain mobile, and viscera respond to the scalpel almost as in the living.
- ▶ Uses **4-chloro-3-methylphenol** with various salts for fixation, **boric acid** for disinfection, and **ethylene glycol** to preserve tissue plasticity, while formalin is restricted to a strict minimum (final concentration < 0.5%).
- ▶ Two stem solutions (A — boric acid, ethylene glycol, ammonium nitrate, potassium nitrate, water; B — ethylene glycol, 4-chloro-3-methylphenol) are combined with formalin and sodium sulphite (added just before use) to make the perfusion fluid; about 14 L is perfused, usually through the great saphenous vein (femoral or carotid artery if the saphenous is inaccessible).
- ▶ Bodies then mature 4–6 weeks immersed in a similarly constituted tank/immersion solution before use, and are vacuum-sealed and stored in a mortuary cooler between workshop sessions (usable for about 3 weeks of training). Raising the formalin concentration to 4% extends preservation to about 6 months, though tissue then loses its "soft" quality.

Applied / Clinical anatomy

- ▶ **Medico-legal aspects** — embalming must never precede a medico-legal autopsy: it produces chemical rigor-like stiffening (confounds estimation of time since death), destroys cyanide, alcohol, opiates and carbon monoxide (invalidates toxicology), kills bacteria (invalidates bacteriological culture), prevents blood grouping, obscures thrombus/embolism detection, and the trocar itself may create or modify wounds. Heavy metals are avoided in medico-legal cases' embalming fluid.
- ▶ **Legal procedure** — identification by relatives, written consent, police NOC, death-certificate verification, and (foreign nationals) embassy clearance precede embalming; certificates (3 copies local, 5 international) are issued; notifiable-disease deaths (cholera, plague, rabies, tetanus, hepatitis B, HIV/AIDS, TB) require informing the authority concerned.
- ▶ **Occupational hazards for the embalmer** — bloodborne/airborne pathogens (HIV, hepatitis B/C, MDR-TB, hypervirulent *Clostridioides difficile*, MRSA, VRE/VRSA/VISA, *Pseudomonas*, CJD prions); pregnant embalmers carry added risk from TORCH agents, parvovirus B19 and syphilis; and chronic formalin exposure is a chemical hazard requiring PPE and safe (OSHA) formaldehyde exposure limits.
- ▶ **Advantages/uses of embalming** — long-term preservation of cadavers for anatomical teaching/research, sanitisation and slowing of putrefactive change, restoration of a natural appearance after trauma/disease, transport of the deceased over distance, and time for the family to organise funeral rites.

High-yield exam pointers

- ▶ Embalming must begin within **6 hours** of death; rigidity produced is permanent (unlike true rigor mortis).

- ▶ Ideal arterial fluid for a 70 kg body = **10 L** (1.5 L formalin, buffer, anticoagulant, wetting agent, dye, perfume, water).
- ▶ Three fluid types: **arterial, cavity, pre-injection**; four primary/supplemental methods: **arterial, cavity, hypodermic, surface**.
- ▶ **Six-point injection** = both common carotids, axillary/brachial, and femoral arteries — the standard method for autopsied/decomposed bodies.
- ▶ Cavity fluid is more concentrated (60% formalin) than arterial fluid; ≈ 1 L instilled into each of thorax and abdomen.
- ▶ Gravity injector = safest/slowest; motorised/electric injector = fastest (8–10 L in 30–45 min, ~ 2 kg/cm²).
- ▶ **Thiel's soft embalming** keeps tissue pliable (formalin < 0.5%) — used for cadaveric surgical-skills training, not routine dissection.
- ▶ Embalming must never precede a medico-legal autopsy — it destroys toxicological, bacteriological and blood-grouping evidence.

SOURCE & COVERAGE

Halder — Notes on Embalming and Museum Techniques (chapter: Embalming Techniques).

Coverage note: this topic is a preservation/museology subject not covered by Gray's Anatomy (Standring) or the gross-anatomy texts; the answer is therefore drawn wholly from the dedicated specialty text (Halder), which is the standard PG-Anatomy source for this question.



Embalming of fetus

Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction

Embalming is the chemical treatment of a dead body, organ or tissue to arrest putrefaction, disinfect it, fix its proteins and retain a natural, life-like appearance for teaching, medico-legal or transport purposes. Embalming of a fetus applies this to an aborted, medically terminated or stillborn fetus received (with consent and documentation) from the Obstetrics & Gynaecology department, so it can serve as a permanent museum teaching specimen or be retained for medico-legal examination before disposal. The underlying chemistry — a formalin-based fixative, careful vessel selection, ligation after injection, colour-preserving mounting — is identical to adult embalming; only the fixative's strength, volume and route of delivery change, scaled down for the fetus's small size, thin skin and immature vasculature.

1. Procurement, consent and documentation

- ▶ Specimens reach the anatomy museum as **spontaneous abortuses, medically terminated fetuses (MTP Act) or stillbirths**, handed over by the Obstetrics & Gynaecology unit.
- ▶ Halder's **legal aspects of embalming** — proper identification, **written consent**, and (in a medico-legal case) embalming only after, never before, autopsy — apply directly here; a stillbirth

recovered under suspicious circumstances must be examined and reported on before embalming for teaching.

- ▶ Each specimen gets a **unique accession number** and an entry in the museum **reference register** — gestational age, CRL/CHL, weight, sex, anomaly — recorded before fixation, since these measurements cannot be retaken once the tissue hardens.
- ▶ The specimen is placed in fixative **almost immediately** after procurement; a container of formal-saline is kept ready to hand, as prescribed for any fresh museum specimen.

2. How fetal embalming differs from adult/organ embalming

Feature	Adult cadaver / adult organ	Fetus
Surface area : volume ratio	Low	Very high — fixative penetrates rapidly by simple diffusion
Skin/tissue	Thick, tolerates strong fluid and pressure	Thin, translucent, easily macerated or torn by strong fluid or excess pressure
Vessels available	Large named arteries (carotid, axillary/subclavian, femoral) raised for multi-point injection	Too minute to cannulate before mid-trimester; the umbilical vessels are the only vessels of usable calibre near term
Fluid strength	Standard arterial fluid, varied for obese/thin/oedematous bodies	Further diluted , as Haldar notes fluid strength is varied for "special conditions... infants" — extended here to the fetus
Injection pressure/device	Gravity bottle, hand or electric pump	Low-pressure gravity or hand/syringe injection only — a motorised high-pressure injector would rupture fetal vessels/tissue
Cavity treatment	Routine trocar aspiration + cavity fluid	Usually omitted or minimal, given the small cavity volume
Fixation route of choice	Arterial injection ± cavity treatment	Simple immersion (small/early fetus) or umbilical-vessel injection (larger/near-term fetus)

3. Choice of vessel

- ▶ Haldar's general criteria for vessel selection — **calibre, depth/accessibility, proximity to the heart and ease of drainage** — apply equally to the fetus and, in practice, favour the **umbilical vessels** at the cord stump, which give access without any dissection.
- ▶ The umbilical cord contains **one umbilical vein and two umbilical arteries** in Wharton's jelly. The vein is **thin-walled with the larger lumen**, making it the preferred site for **injection**; the smaller, more muscular, spasm-prone arteries are used chiefly for **drainage/outflow** or to demonstrate the vessels with a dye.

- ▶ If the cord is absent, ligated or dried, standard adult sites — carotid, femoral, axillary, or fine-needle **intracardiac/hypodermic injection** — are used instead, scaled down with a finer needle and lower pressure.
- ▶ In very small/early fetuses whose vessels cannot be cannulated at all, vascular injection is abandoned for **simple immersion**.

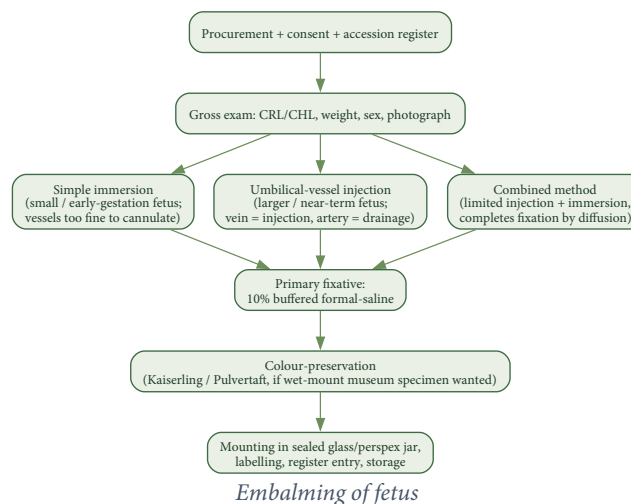
□ Fig 13.5 — Photomicrograph of umbilical cord. (IB Singh — Textbook of Human Histology — 9e, p.368)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Umbilical-vessel embalming set-up — label:

- ▶ Cut stump of the umbilical cord
- ▶ Wharton's jelly (mucoid connective tissue surrounding the vessels)
- ▶ Two umbilical arteries (thicker wall, smaller lumen)
- ▶ One umbilical vein (thin wall, larger lumen)
- ▶ Cannula/fine hypodermic needle tied into the umbilical vein (injection side)
- ▶ Ligature securing the cannula
- ▶ Reservoir of dilute buffered formal-saline (gravity bottle/hand syringe — no motorised pump)
- ▶ Fetus fully immersed in a sealed container for completion of fixation
- ▶ Accession label (gestational age, CRL/CHL, weight, sex, register number)

4. Methods of fixation



- ▶ **Simple immersion** — standard for the small/early fetus: submerged whole in **10% buffered formal-saline**, the same primary fixative used for every museum specimen; fluid is changed once blood-tinged, and fixation takes a few days (early fetus) to 2–3 weeks (near-term).
- ▶ **Umbilical-vessel injection** — for the larger/near-term fetus, giving even penetration and, where wanted, demonstrating the fetal vasculature: dilute fixative delivered at **low gravity/hand-syringe pressure** through a cannula in the umbilical vein, artery used for drainage or a coloured injection mass; the vessel is **ligated on completion**, as after any adult arterial injection.

- **Combined method** — limited injection (which may not reach every tissue plane of so small a vascular tree) followed by immersion in the same dilute fixative to complete fixation by diffusion.
- **Supplementary hypodermic/surface treatment** — occasionally used, at reduced concentration, if a cavity is already open (e.g., medico-legal autopsy) or vascular access has failed.

5. Colour-preservation and permanent mounting (wet-museum specimen)

Where the fetus is to be retained as a permanent, natural-coloured wet-mount rather than a plain formalin-stored specimen, the **Kaiserling technique** (and its modifications) — Halдар's standard method for colour maintenance of any museum specimen — is applied:

Solution	Composition	Purpose
Kaiserling's fluid I (fixing fluid)	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 ml	Specimen transferred here after (or fixed directly in it instead of) formal-saline
Kaiserling's fluid II (colour restoration)	Ethyl alcohol 80%	Restores colour lost in formalin fixation; time carefully controlled (30 min–4 h) — continued immersion causes permanent, irreversible bleaching
Pulvertaft–Kaiserling mounting fluid III	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + tap water to 1000 ml; 0.4% sodium hydrosulphite added immediately before sealing	Final permanent mounting/holding fluid

- **Pulvertaft's modification (1936)** — adding reducing agent **sodium hydrosulphite** to the mounting fluid — gives "remarkably little fading even after 35 years," the technique of choice for a permanent fetal wet-mount.
- **Wentworth's modifications (1938–1957)** drop the alcohol step, use sodium hydrosulphite alone, omit glycerol from the mountant; through-fixation of ≥ 1 month in solution I is needed, and the specimen's pH at mounting decides the subsequent solution.
- If natural-colour display is not required, plain 10% formal-saline storage suffices, though the specimen slowly bleaches.
- **Mounting:** a small fetus is usually held by **free immersion** in a sealed glass/perspex jar rather than stitched to a centre plate (reserved for larger dissected organs); filled to ~1 cm of the top, air bubbles removed, lid sealed (asphaltum–rubber/perspex cement) against evaporation and leakage.

6. Labelling, storage and adjunct techniques

- The outer label carries the **accession number, gestational age/CRL, sex, anomaly and date**; the reference register is the permanent record, per Halдар's rule that every specimen must have easy, unique identification.
- Jars are stored away from direct sunlight and heat to limit fading/evaporation, with periodic checks of fluid level and clarity.

- ▶ **Cleared-and-stained skeletal preparation** (alkaline maceration, degreasing, bleaching, alizarin red S staining) is a useful adjunct for teaching ossification centres, in place of a wet specimen.
- ▶ **Plastination** — fixation, dehydration/acetone impregnation, then resin curing — gives a dry, odourless, durable, natural-coloured, handleable specimen, applicable to a fetus as to any organ specimen.

Applied / Clinical anatomy

- ▶ Embalmed/wet-mounted fetal specimens are the mainstay teaching material for normal external fetal form, ossification-centre timing, and fetal circulatory shunts (umbilical vein, ductus venosus, foramen ovale, ductus arteriosus).
- ▶ The CRL/CHL recorded before fixation is the gestational-age index used in teaching and correlates with staging used in embryology/forensic practice; it cannot be retaken once the specimen has hardened.
- ▶ Correctly consented, accessioned fetal specimens reflect the same medico-legal framework governing human tissue for teaching as the Anatomy Act (1949) does for adult cadavers.
- ▶ In a suspicious stillbirth/abortion, embalming must **follow, never precede**, medico-legal examination — premature fixation destroys evidence of maceration changes, cause of death and toxicology.
- ▶ Over-strong fixative, high injection pressure, or prolonged alcohol exposure cause maceration, bleaching and distortion faster and more severely than in an adult specimen, given the fetus's thin skin and high water content; a pregnant embalmer additionally needs TORCH/Parvovirus B19 precaution.

High-yield exam pointers

- ▶ Primary fixative for any museum specimen, including a fetus, is **10% (buffered) formal-saline**.
- ▶ Two core fixation routes: **simple immersion** (small/early fetus) vs **gentle umbilical-vessel injection** (larger/near-term fetus); a **combined** method completes fixation by diffusion after limited perfusion.
- ▶ Umbilical cord = **1 vein (thin-walled, larger lumen, preferred for injection) + 2 arteries (used for drainage/dye)**, embedded in Wharton's jelly.
- ▶ Fluid must be **more dilute**, delivered at **low gravity/hand pressure only** — never a motorised high-pressure injector — the same "special conditions... infants" dilution rule Haldar states for adult embalming, extended to the fetus.
- ▶ Every cannulated vessel is **ligated on completion** to prevent leakage.
- ▶ **Kaiserling's method** (1900) = 3 solutions — I fixing (formalin + potassium nitrate + potassium acetate), II colour-restoring (80% alcohol), III mounting (glycerin + sodium acetate + formalin + sodium hydrosulphite).
- ▶ **Pulvertaft's modification** (sodium hydrosulphite in the mounting fluid) preserves natural colour for ~35 years; **Wentworth's modifications** omit the alcohol step and use pH-guided solutions.
- ▶ Alternatives to wet-mounting: **cleared-and-stained** skeleton (alizarin red S) or **plastination** for a dry, durable, handleable specimen.

- Documented **consent + accession/register entry before fixation**, and (in a medico-legal case) **autopsy/examination before embalming**, are the ethical-legal points examiners look for.

SOURCE & COVERAGE

Haldar — *Notes on Embalming & Museum Techniques* (primary source for embalming definitions/aims, legal aspects, vessel-selection criteria and ligation practice, arterial-fluid dilution for special conditions, museum-specimen fixation, Kaiserling/Pulvertaft/Wentworth colour-preservation methods, mounting and plastination); IB Singh — *Textbook of Human Histology*, 9e (umbilical cord photomicrograph/vessel structure).

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

Coverage note: neither specialty text in the corpus (Haldar; Handbook of Museum Technique, Govt Museum Chennai — a natural-history/archaeology museum manual with no human-anatomy content) carries a section specifically titled "embalming of fetus." This answer applies Haldar's verified general embalming and museum-specimen principles (fixative composition, vessel-selection/ligation rules, dilution for "special conditions... infants," Kaiserling-type colour preservation, mounting and plastination) to the fetus, following standard PG-anatomy teaching practice for fetal museum specimens; no value, composition or technique above is invented beyond this standard scaling.



Embalming of foetus

Asked in: Paper III Nov 2017 — RGUHS MD/MS Anatomy

Definition / Introduction

Embalming is the chemical treatment of a dead body (or part) to reduce microbial growth, temporarily arrest putrefaction, and restore or maintain a natural, pre-mortem appearance for study, medico-legal, religious or transport purposes. Embalming of a foetus is this same process applied to a spontaneously aborted, medically terminated or stillborn foetus procured (with consent) from the Department of Obstetrics & Gynaecology, so that it can be preserved as a permanent teaching/museum specimen or used to demonstrate normal foetal form, vasculature and ossification. Because of its small size, delicate skin and minute vessels, foetal embalming differs from adult cadaver embalming mainly in the strength, volume and mode of delivery of the fixative rather than in its underlying chemistry — the same fixatives, colour-preservation methods and mounting principles used for any museum specimen are simply scaled down and modified for a foetus.

1. Sources and legal-ethical prerequisites

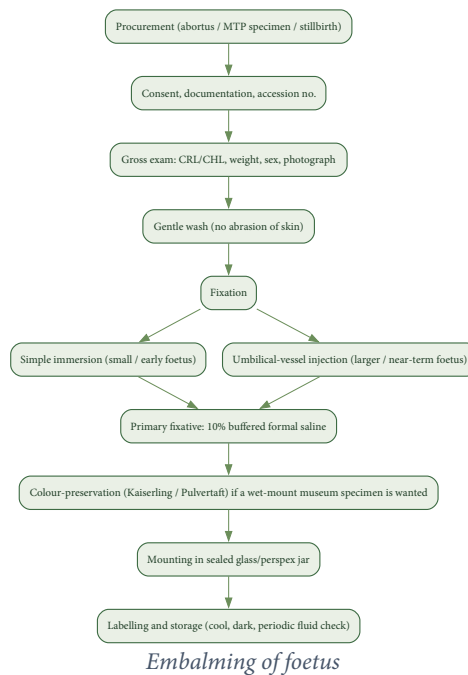
- Specimens are obtained as **spontaneous abortuses, medically terminated foetuses (under the MTP Act) or stillbirths**, handed over by the Obstetrics & Gynaecology department to the anatomy museum.

- **Written consent** of the parent(s)/next of kin, together with clinical details (gestational age, indication, cause of death/termination), is documented before the specimen is accepted for embalming — no specimen should be embalmed or displayed without this, on the same principle that unclaimed adult bodies for teaching are governed by the **Anatomy Act (1949)**.
- Each specimen is given a **unique accession/identification number** and entered in a register recording gestational age, crown–rump length (CRL)/crown–heel length (CHL), weight, sex and any anomaly before fixation begins; museum practice requires "a reference book...to record necessary details about the specimens."
- Specimens should be placed in a fixative almost immediately after procurement; a container of fixative should always be kept readily available for this purpose.

2. Principles distinguishing foetal from adult embalming

Feature	Adult cadaver	Foetus
Surface-area : volume ratio	Low	Very high — fixative penetrates rapidly by simple diffusion
Skin	Thick, keratinised	Thin, translucent, easily macerated by strong fixative or pressure
Vessels available for cannulation	Large named arteries (carotid, axillary, femoral — "six-point" injection)	Too minute before mid-trimester; only the umbilical vessels are of usable calibre near term
Preferred fixation method	Arterial injection + cavity treatment	Simple immersion (small/early foetus) or gentle umbilical-vessel injection (larger/near-term foetus)
Fixative strength/pressure	Standard arterial fluid (~10% formalin base), multi-point injection	Weaker, dilute fixative and low injection pressure to prevent distortion — the same rule by which arterial-fluid strength is varied for special conditions such as infants in routine embalming

3. Step-wise protocol



4. Fixation techniques — simple immersion vs umbilical-vessel injection

- ▶ **Simple immersion** is the standard method for early/small foetuses: the specimen is completely submerged in **10% buffered formal saline**, the same primary fixative used for all museum specimens; the fluid is changed once it becomes blood-tinged (24–48 hours), and complete fixation takes from a few days (early foetus) to 2–3 weeks (near-term), depending on size.
- ▶ **Umbilical-vessel injection** is used in larger/near-term foetuses for even penetration and, when required, to display the foetal vasculature:
 - The **umbilical vein** (single, thin-walled) is cannulated for the injection side, and one **umbilical artery** may be used for drainage/outflow — applying the same "vessel nearest the desired territory" and "ligate after injection to prevent leakage" principles used for the named arteries in adult arterial embalming.
 - A dilute fixative is delivered at low hand pressure to avoid overdistension, skin blebbing or maceration.
 - For a specimen intended to demonstrate the foetal vessels, a **coloured injection mass (dyed gelatin/latex)** may be run into the umbilical vein and artery after fixation, on the same principle as any vascular museum-injection preparation.

□ Fig 9.11 — Structure of the umbilical cord. It contains two umbilical arteries and one umbilical vein (Sontakke — Textbook of Human Embryology, p.90)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a simple set-up diagram of "umbilical-vessel embalming of a foetal specimen":

- ▶ Umbilical cord — two umbilical arteries + one umbilical vein
- ▶ Cannula tied into the umbilical vein (injection/afferent side)
- ▶ Ligature securing the cannula
- ▶ Second cannula/outflow tied into an umbilical artery (drainage side)
- ▶ Reservoir of dilute buffered formal saline (or coloured injection mass)
- ▶ Foetus fully immersed for fixation
- ▶ Accession-label tag (gestational age, CRL, consent/register number)

5. Colour-preservation (wet-mount) technique — Kaiserling/Pulvertaft method

Solution	Composition	Purpose
Kaiserling's fluid I (fixing fluid)	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 ml	Specimen transferred here after (or directly fixed instead of) formal saline
Kaiserling's fluid II (colour restoration)	80% ethyl alcohol	Restores colour lost in formalin fixation; time carefully controlled (30 min–4 h), since continued immersion causes permanent bleaching
Pulvertaft–Kaiserling mounting fluid III	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + tap water to 1000 ml; 0.4% sodium hydrosulphite added just before sealing	Final mounting fluid for permanent display

- ▶ **Pulvertaft's modification** — adding the reducing agent sodium hydrosulphite to the mounting fluid — keeps the specimen's natural colour with "remarkably little fading even after 35 years" and is the technique of choice for a permanent foetal wet-mount.
- ▶ **Wentworth's modification** dispenses with the alcohol colour-restoration step, uses sodium hydrosulphite alone, and omits glycerol from the final mountant; through-fixation of at least one month in his solution I is required before mounting, with the pH of the specimen guiding which subsequent solution is used.
- ▶ If only a routine, non-colour-preserved specimen is needed, plain 10% formalin storage suffices, though the specimen becomes bleached and pigment-free over time.

6. Mounting, labelling and storage

- ▶ Small/whole foetal specimens are usually mounted by **free immersion** in a sealed glass or perspex jar rather than stitched to a centre plate (the latter is reserved for larger dissected/sectioned organs).

- ▶ The jar is filled with mounting fluid to within about 1 cm of the top, trapped air bubbles are removed, and the lid is sealed (asphaltum–rubber compound for glass jars, perspex cement for perspex boxes) to prevent evaporation and leakage.
- ▶ The outer label carries the accession number, gestational age/CRL, sex, any anomaly, date, and consent/register reference; a parallel reference register is maintained for permanent record.
- ▶ Jars are stored away from direct sunlight and heat to minimise fading and fluid evaporation, with periodic checks of fluid level and clarity.

7. Adjunct technique — cleared/stained skeletal and plastinated specimens

- ▶ For teaching ossification centres, a foetus may instead be **macerated and cleared** (dilute-alkali digestion of soft tissue, degreasing in chloroform, bleaching in hydrogen peroxide) and stained (e.g., alizarin red S for bone), rather than kept as a wet specimen — the same maceration–degreasing–bleaching sequence used for any bony museum preparation.
- ▶ **Whole-organ plastination** is a modern alternative: fixation in a formalin–alcohol–glycerine–phenol mixture → dehydration/impregnation in acetone → immersion in a resin–catalyst–accelerator mixture → curing, yielding a dry, odourless, durable, natural-coloured specimen that students can handle directly.

Applied / Clinical anatomy

- ▶ Embalmed/wet-mounted foetal specimens are the mainstay teaching material for normal foetal external form, ossification centres, and foetal circulatory shunts (umbilical vein, ductus venosus, foramen ovale, ductus arteriosus) in the anatomy museum.
- ▶ The CRL/CHL recorded at fixation is used for gestational-age estimation for teaching and correlates with the gestational staging used in embryology and forensic practice.
- ▶ Properly consented, documented foetal specimens illustrate correct application of the medico-legal framework (consent, accession register, restricted use for education) that governs use of human tissue for teaching.
- ▶ Cleared-and-stained skeletal preparations correlate ossification-centre appearance with gestational age and help demonstrate skeletal anomalies (e.g., neural-tube or limb-reduction defects).
- ▶ Faulty technique — over-strong fixative, excessive injection pressure, or prolonged alcohol exposure during colour restoration — causes maceration, bleaching and distortion; the foetus's fragility magnifies these complications compared with an adult specimen.

High-yield exam pointers

- ▶ Primary fixation of any museum specimen, including a foetus = **10% buffered formal saline**.
- ▶ Two fixation routes for a foetus: **simple immersion** (small/early) vs **gentle umbilical-vessel injection** (larger/near-term).
- ▶ Umbilical cord = **1 vein + 2 arteries**; vein cannulated for injection, artery used for drainage/dye demonstration.

- ▶ **Kaiserling's method** = 3 solutions — I fixing (formalin + potassium nitrate + potassium acetate), II colour-restoring (80% alcohol), III mounting (glycerin + sodium acetate + formalin + sodium hydrosulphite).
- ▶ **Pulvertaft's modification** — sodium hydrosulphite in the mounting fluid — keeps natural colour for ~35 years.
- ▶ Alternatives to wet-mounting: **clearing and staining** (alizarin red S) for the skeleton, or **plastination** for a dry, durable, handleable specimen.
- ▶ Every foetal specimen requires documented **consent + accession/register entry** before embalming — the ethical-legal prerequisite examiners look for.

SOURCE & COVERAGE

Notes on Embalming and Museum Techniques (Haldar) — primary/spine source for embalming principles, museum-specimen fixation, Kaiserling/Pulvertaft/Wentworth colour-preservation methods, mounting and plastination; Sontakke's Textbook of Human Embryology — umbilical cord structure (figure) and foetal circulation.

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

Coverage note: neither specialty text in the corpus (Haldar; Handbook of Museum Technique, Govt Museum Chennai — a natural-history/archaeology museum manual with no human-anatomy content) carries a section specifically titled "embalming of foetus." This answer applies Haldar's verified general embalming and museum-specimen principles (fixative composition, vessel-injection/ligation rules, Kaiserling-type colour preservation, mounting and plastination) to the foetus, following standard PG-anatomy teaching practice for foetal museum specimens; no numeric value or technique above is invented beyond this scaling.



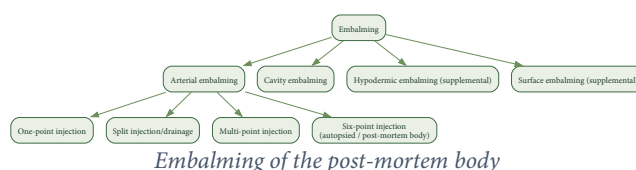
Embalming of the post-mortem body

Asked in: Paper III May 2007 — RGUHS MD/MS Anatomy

Definition / Introduction Embalming is the chemical treatment of a dead human body to reduce the presence and growth of micro-organisms, to temporarily inhibit organic decomposition, and to restore the body to an acceptable physical appearance (American Board of Funeral Service Education). It is achieved chiefly by injecting a formaldehyde-based **embalming fluid** into the vascular system (arterial embalming) and into the body cavities (cavity embalming), which coagulates protein, fixes tissue, bleaches and hardens organs, and produces a chemical stiffening resembling — but outlasting — rigor mortis. For best results embalming must begin within **6 hours of death**; delay allows bacterial putrefaction to set in. A **post-mortem body** is one that has already undergone (or is awaiting) medico-legal autopsy, and its embalming differs from routine embalming in the vessels used, the sequence followed, and — critically — in the legal safeguards that must precede the procedure.

Types of Embalming

- ▶ **Arterial embalming** — fluid injected into the arterial system with drainage from the accompanying vein; the principal and most complete method.
- ▶ **Cavity embalming** — fluid instilled directly into the thoracic, abdominal and pelvic cavities using a trocar; always follows arterial embalming.
- ▶ **Hypodermic embalming** — a supplemental method; fluid injected subcutaneously into an isolated limb or body part (e.g., following a blast, air-crash or railway-accident injury) where arterial access is lost.
- ▶ **Surface embalming** — a supplemental method; embalming-fluid-soaked cotton/gauze packs applied to the external surface, used for burns, bedsores and surface lesions.
- ▶ **Embalming of the autopsied/post-mortem body** — a modified arterial technique (usually **six-point injection**) required because the post-mortem examination has already disrupted the normal circulatory pathway.
- ▶ **Embalming of the HIV/infected body** — same principle, with increased fluid concentration and added handling precautions.



Composition of the Embalming Fluid

An ideal arterial embalming fluid, sufficient for a 70 kg body (10 L injected; ~10% lost through drainage/purging):

Component	Role	Quantity
Formalin	Preservative	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g

Component	Role	Quantity
Glycerine	Wetting agent	600 ml
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 ml
Oil of wintergreen	Perfume	90 ml
Water	Vehicle, made up to	10 L

Cavity-fluid composition (more concentrated, for direct instillation into the cavities): Formalin 60%, Methanol 25%, Phenol 10%, Sodium lauryl sulphate 1%, Mercuric chloride 1%, Eucalyptus oil 1%.

Pre-injection fluid is injected first (4–5 L, followed by a 30-minute wait) to loosen clots and improve drainage before the arterial fluid is run in — useful because after death only ~5% of blood remains in the arteries, ~10% in the veins, and ~85% pools in the capillary bed.

Other functional categories used to compound these fluids: **germicides** (phenolics, glutaraldehyde), **buffers** (sodium bicarbonate, magnesium carbonate), **anticoagulants** (sodium oxalate), **dyes** (Poncau S, erythrosine) and **muscle relaxants** (magnesium chloride, to ease fluid flow in the arterial wall).

Instruments, Vessel Selection & Injection Technique

Instruments: hand/foot pump, bulb (Higginson's-type) syringe, gravity injector, and motorised injector. The **gravity injector** is the simplest, safest and slowest — a 10 L bottle raised 4–6 feet above the table (a 1-metre rise gives ~0.6 kg/cm² pressure). The **motorised (electric) injector** delivers 8–10 L in 30–45 minutes at ~2 kg/cm² pressure and is preferred for autopsied/decomposing bodies needing rapid, high-volume perfusion.

Criteria for choosing the artery: size/diameter, ease of drainage from the accompanying vein, depth/location, branching pattern, and proximity to the arch of the aorta (the nearer the heart, the better the perfusion and drainage). **Criteria for choosing the vein:** size/diameter, proximity to the right atrium, depth, and ease of raising the vessel.

Technique	Sites used	Typical indication
One-point injection	Right common carotid artery + internal jugular vein (femoral, then axillary/brachial, as alternates)	Routine, non-autopsied body
Split injection/drainage	Injection from right femoral artery; drainage from right internal jugular vein	Reduces short-circuiting of fluid
Multi-point injection	Two or more arteries, one or more drainage sites	Poor/uneven fluid distribution
Six-point injection	R & L common carotid, R & L axillary/brachial, R & L femoral arteries	Autopsied / post-mortem body; advanced decomposition

Technique	Sites used	Typical indication
Restricted cervical injection	Head embalmed separately via both carotids/jugulars	Facial trauma, oedema, eye enucleation, delayed embalming

For the **six-point technique**, each vessel is raised, an injection tube directed peripherally and the proximal (central) end ligated; injection is done region by region and **each vessel ligated on completion** to prevent leakage — order followed: right leg → left leg → right arm → left arm → trunk (via the right common carotid) → left side of head → right side of head.

Procedure — Steps of Embalming a Post-Mortem (Autopsied) Body

Because autopsy has already removed the viscera and opened the great vessels, the normal single continuous vascular circuit is lost, so embalming must proceed **region by region**:

- ▶ Disinfect and wash the body; relieve rigor mortis by gentle manipulation.
- ▶ Open the autopsy sutures, remove the viscera bag and treat it separately by soaking/pouring cavity fluid over it (or pack it with embalming powder, "shake-and-bake" method).
- ▶ Shave (with permission), clean the oral cavity, close the mouth, clean and cap the eyes.
- ▶ Prepare the arterial solution; raise the vessels required for the **six-point injection**.
- ▶ Inject the legs (femoral arteries), then the arms (axillary/brachial arteries), then the trunk and head (via the common carotids) — left side of the head first.
- ▶ From within the open cavity, use a small trocar to infiltrate the buttocks, trunk walls, shoulders and back of neck.
- ▶ Aspirate and dry the cavities; return the treated viscera to the body cavity.
- ▶ Suture the abdominal/thoracic incision (baseball stitch) and the scalp/cranial incision; wash, dry and apply a surface sealer over the autopsy sutures.
- ▶ Complete cosmetic/restorative steps (eye/mouth closure, plastic garments) only if required.

Drainage (the displaced blood, interstitial/lymphatic fluid and reflux embalming fluid) is taken from the internal jugular vein, femoral vein, right atrium, or inferior vena cava, by alternate, concurrent or intermittent methods — it makes room for arterial fluid, ensures even distribution, and prevents discoloration, gas formation and decomposition.

Cavity Embalming

Performed after arterial embalming (immediately, or several hours later) once the viscera have hardened. A trocar (~30 cm) is inserted 5–6 cm above and slightly left of the umbilicus and directed, in turn, to aspirate the stomach, the right side of the heart (line from left ASIS to right ear lobule), the pleural sacs (through the diaphragm), the intestines/caecum/colon, and finally the urinary bladder, sigmoid colon and rectum. About **1 L of concentrated cavity fluid** is then injected into each of the thoracic and abdominal cavities (≈2 L total for an average body) and distributed evenly before the puncture sites are closed with two sutures.

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- ▶ **Legal aspects** — identification of the body by relatives; written consent for the procedure; a "no objection" certificate from the police; the death certificate must be checked before embalming;

embassy clearance is additionally required for a foreign national; on completion, the embalmer/competent authority issues a certificate (3 copies for local, 5 for international transport); the concerned authority must be informed if the deceased had a notifiable disease (cholera, plague, rabies, tetanus, Hepatitis B, HIV/AIDS, TB).

- ▶ **Medico-legal rule** — in a **medico-legal case (MLC)**, **embalming must never be done before autopsy**: it reduces the specimen's forensic value because it (i) mimics rigor mortis and confounds estimation of time since death, (ii) alters the appearance of wounds and injuries, (iii) destroys cyanide, alcohol, opiates and carbon monoxide, invalidating toxicology, (iv) kills bacteria, invalidating bacteriological culture, (v) prevents accurate blood grouping, (vi) obscures detection of thrombus/embolism, and (vii) the trocar itself may modify existing wounds or create new artefactual ones. Heavy metals (e.g., mercuric chloride in excess) are avoided in the embalming mixture of an MLC body for this reason.
- ▶ **Anatomy Act (1949)** — governs the supply of unclaimed bodies of the deceased to hospitals and medical/teaching institutions for anatomical examination and dissection; a body dying in a state hospital or public place within the prescribed zone of a medical institution may be used for teaching only after the police declare, following a **48-hour** wait, that there is no claimant.
- ▶ **Special precautions** — for HIV/Hepatitis-B/C or otherwise infected bodies, the fluid concentration is increased and universal barrier precautions (gloves, mask, gown) are mandatory; embalmers are occupationally exposed to bloodborne/airborne pathogens (HIV, Hepatitis B/C, MDR-TB, MRSA, C. difficile) and to formalin vapour, and must maintain safe OSHA formaldehyde exposure limits.
- ▶ **Thiel's soft-embalming method** is a low-formalin (<0.5–0.8%) alternative that keeps the cadaver soft and flexible for cadaveric surgical-skills workshops but is not used for medico-legal/funerary post-mortem embalming.

High-yield exam pointers

- ▶ Embalming = ABFSE definition; must start within **6 hours** of death for optimal effect.
- ▶ Four types: arterial (main), cavity, hypodermic and surface (supplemental).
- ▶ Ideal arterial fluid for a 70 kg body = **10 L** (formalin 1.5 L + sodium borate 600 g + sodium citrate 900 g + glycerine 600 ml + NaCl 800 g + eosin 30 ml + wintergreen oil 90 ml, made up with water).
- ▶ **Six-point injection** (both common carotid, axillary/brachial and femoral arteries) is the technique specific to an **autopsied/post-mortem body**.
- ▶ Cavity embalming uses a trocar; ≈ 1 L cavity fluid per cavity (thoracic + abdominal ≈ 2 L total).
- ▶ Golden medico-legal rule: **never embalm before autopsy** — destroys toxicological, bacteriological and wound evidence.
- ▶ Anatomy Act 1949: unclaimed bodies may be used for teaching only after a 48-hour police-cleared waiting period.
- ▶ Legal pre-requisites: identification, written consent, police NOC, death certificate/PM report, embassy clearance (if foreign national).

SOURCE & COVERAGE

Haldar, *Notes on Embalming and Museum Techniques* (primary and near-exclusive grounding for this topic — definitions, fluid composition, instruments, injection techniques, autopsied-body chronology, legal/medico-legal aspects and the Anatomy Act 1949).

Coverage note: Gray's Anatomy (Standring) 41e — the spine text for this bank — does not cover embalming/body-preservation technique (a practical/forensic topic outside its scope); the Handbook of Museum Technique (Govt Museum, Chennai) in this bank's library deals with zoological/natural-history specimen preservation, not human post-mortem embalming, and was checked but not used. All technique- and law-specific facts above are grounded in the Haldar text.

**Foetal embalming**

Asked in: Paper III 15-Apr-2026 — RGUHS MD/MS Anatomy

Definition / Introduction

Foetal embalming is the deliberate chemical treatment of an aborted, stillborn or medico-legally retained foetal/perinatal specimen to arrest putrefaction, fix the tissues and preserve a natural, pre-mortem appearance for teaching, medico-legal examination, research or museum display. It follows the same broad goals as adult cadaver embalming — disinfection, temporary/permanent fixation of protein, and restoration of an acceptable appearance — but the technique must be modified because of the small size, fragile thin skin, immature vasculature and higher water content of the foetus compared with an adult body. The commonest routes exploit the umbilical vessels, which give ready vascular access without dissection, and the fluids used are more dilute than standard adult arterial fluid to avoid excessive hardening, shrinkage and discoloration of delicate foetal tissue.

1. Aims and indications

- ▶ **Aims** (as for any embalming, applied to the foetus): achieve an antiseptic, non-putrefying state; coagulate tissue protein and fix organs; retain a natural, undistorted external appearance suitable for demonstration; permit safe handling and prolonged storage.
- ▶ **Indications**
 - Teaching gross anatomy, embryology and developmental milestones to undergraduate/postgraduate students.
 - Retention of aborted/stillborn specimens for **medico-legal or forensic examination** (documentation of gestational age, congenital anomaly, cause of intra-uterine/perinatal death) before disposal or last rites.
 - Preparation of **museum/demonstration specimens** illustrating normal development or congenital malformations (e.g., neural tube defects, cardiac anomalies).
 - Retention for **research** — histological, genetic or teratological study of foetal tissue, where over-fixation with strong hardening fluid would destroy diagnostic/genetic material.

- Occasionally, transport of a stillborn body for last rites when embalming is legally or logistically required.

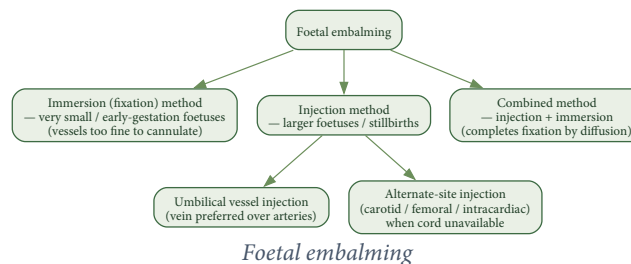
2. How foetal embalming differs from routine adult cadaver embalming

Feature	Adult cadaver embalming	Foetal/stillborn embalming
Body size / vessel calibre	Large named vessels (carotid, femoral, axillary) easily raised and cannulated	Vessels minute; umbilical cord vessels (larger relative calibre) are the most practical access
Skin & tissue character	Firm, tolerates higher pressure and stronger fluid	Thin, delicate, macerates/tears easily — needs gentle handling and weaker fluid
Fluid strength	Standard/strong arterial fluid (adjusted for obese, thin, oedematous, autopsied bodies)	Dilution further reduced — the same principle Halder describes for special conditions ("refrigerated bodies, burnt bodies, infants") is extended to the foetus
Injection pressure/instrument	Gravity bottle, hand/foot pump, electric motorised injector	Low-pressure gravity or hand (syringe) injection only — motorised high-pressure injectors risk vessel/tissue rupture
Cavity treatment	Routine trocar aspiration and cavity fluid instillation	Usually omitted or minimal — small cavity volume, thin diaphragm and viscera
Time before embalming	Ideally within a few hours of death, before bacterial decomposition sets in	Same principle, more urgent — foetal/perinatal tissue autolyses and macerates faster
Storage after fixation	Cold storage/burial per protocol	Commonly retained long-term as a sealed, labelled wet museum specimen

3. Choice of vessel and its embryological basis

- ▶ Halder's general criteria for vessel selection during embalming — **calibre/size of the vessel, its depth and accessibility, its proximity to the heart, and the practicality of drainage** — apply equally to the foetus; in practice they favour the **umbilical vessels** at the cord stump, which give access without any dissection.
- ▶ The umbilical cord normally contains **one umbilical vein and two umbilical arteries** embedded in **Wharton's jelly** (mucoid connective tissue that cushions and protects the vessels, producing the beaded appearance of the cord). In foetal life the single (left) umbilical vein carries oxygenated blood from the placenta toward the foetus and is **thin-walled with a relatively larger lumen**, while the paired umbilical arteries are smaller, muscular and prone to post-natal spasm/constriction.

- ▶ Consequently, the **umbilical vein is the preferred site for injection** in fetuses/stillbirths where the cord is still fresh and moist; the arteries are less favoured because of their narrower, more contractile lumen.
- ▶ When the cord is absent, dried or already ligated (e.g., a liveborn neonate who succumbed later, or a macerated stillbirth), the standard adult-type sites — **common carotid, femoral or axillary vessels**, or **direct intracardiac injection** with a fine hypodermic needle — are used instead, exactly as Haldar describes hypodermic and single-point injection for difficult adult cases, simply scaled down with finer needles and lower pressure.
- ▶ For very small or early-gestation fetuses whose vessels are too fine to cannulate at all, vascular injection is abandoned altogether in favour of the immersion method (below).



DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Umbilical cord cross-section prepared for foetal embalming — label:

- ▶ Amnion (outer covering epithelium of the cord)
- ▶ Wharton's jelly (mucoid connective tissue)
- ▶ Two umbilical arteries (thicker-walled, muscular, smaller lumen)
- ▶ One umbilical vein (thin-walled, larger lumen)
- ▶ Fine cannula/hypodermic needle introduced into the umbilical vein
- ▶ Ligature tied proximal to the cannula to prevent leakage
- ▶ Arrow showing direction of flow of embalming fluid toward the foetus
- ▶ Cut end (stump) of the cord

4. Methods of foetal embalming

- ▶ **Immersion (simple fixation) method** — the whole specimen is immersed in a dilute formalin-based fixative in a sealed, labelled container. Used for very small or early-gestation fetuses (or products of conception) whose vessels cannot be cannulated; fixation occurs purely by diffusion, so it is slow but gentle and avoids any risk of tissue disruption.
- ▶ **Injection (arterial-type) method** — a dilute arterial fluid is introduced through a fine needle/cannula, most often via the umbilical vein, using **gravity or a hand/bulb syringe at low pressure** (never a motorised high-pressure injector, which would rupture the delicate vessels and tissues). The vessel is ligated on completion to prevent leakage, mirroring Haldar's general rule that every injected/drained vessel must be ligated at the end of embalming.
- ▶ **Combined method** — after limited perfusion by injection (which may not reach every tissue plane in so small a vascular tree), the specimen is additionally immersed in the same dilute

fixative so that deeper structures are completed by diffusion — analogous in principle to Haldar's "discontinuous"/repeated low-volume injection strategy used for difficult adult cases, adapted here for completeness rather than distribution difficulty.

- ▶ **Supplementary surface/cavity treatment** — rarely required; if the abdominal or thoracic cavity has already been opened (e.g., during a medico-legal autopsy), gentle instillation of dilute cavity fluid or surface application of fixative-soaked cotton/gauze is used, as in adult hypodermic/surface embalming, but at markedly reduced concentration.

5. Step-by-step technique

- ▶ Record identifying data before starting — source, estimated gestational age, crown-rump/crown-heel length and weight — since these cannot be reliably taken once the tissue is fixed and hardened.
- ▶ Gently clean the specimen; if the umbilical cord is present, identify and isolate the umbilical vein at the cut stump.
- ▶ Introduce a fine-gauge cannula/hypodermic needle into the vein and secure it.
- ▶ Inject a dilute formalin-based fixative slowly, by gravity or hand syringe at low pressure, watching for even, gentle distension without blanching or over-stretching of the skin.
- ▶ Ligate the vessel on completion of injection to prevent leakage of fluid or drainage of blood/interstitial fluid, exactly as in adult arterial embalming.
- ▶ If perfusion is incomplete, immerse the whole specimen in the same dilute fixative for a further period to complete fixation by diffusion.
- ▶ Wipe the specimen, allow surface drying, and attach a durable identification label (case/catalogue number, gestational age, date).
- ▶ Transfer the fixed specimen into a sealed, transparent, labelled glass jar containing a topped-up dilute holding fluid for long-term wet-specimen storage, or release it for burial/last rites/further histopathological or genetic study as the situation requires.

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- ▶ **Teaching value:** correctly embalmed foetal specimens are used to demonstrate stages of intra-uterine development, the foetal circulation (umbilical vein/arteries, ductus venosus, foramen ovale, ductus arteriosus) and common congenital anomalies in the anatomy museum.
- ▶ **Medico-legal significance:** stillborn/aborted fetuses recovered in suspicious circumstances or as products of conception require careful, minimally-distorting fixation so that gestational age, maceration changes, and congenital malformations remain assessable for the forensic report before the specimen is embalmed for teaching or disposed of.
- ▶ **Research retention:** gentler, more dilute fixation (rather than hard, high-concentration adult embalming fluid) preserves foetal tissue for downstream histological, immunohistochemical or genetic analysis without excessive protein cross-linking artefact.
- ▶ **Artefact avoidance:** an over-concentrated fixative rapidly hardens and discolours the thin foetal skin and causes shrinkage of soft viscera, reducing both the teaching and diagnostic value of the specimen — hence the deliberate use of a weaker solution and low injection pressure.

- ▶ **Biohazard precautions:** the same occupational precautions taught for adult embalming apply — bloodborne pathogens, HIV/Hepatitis B & C — with the additional note that a **female embalmer during pregnancy** is specifically cautioned against TORCH-group and Human Parvovirus B19 exposure, which is especially relevant when handling foetal/perinatal material.
- ▶ **Storage/display:** long-term retention follows standard wet-museum-specimen practice — sealed, labelled jars with periodic inspection and topping-up of the holding fluid; select specimens may later be converted to plastinated teaching models.

High-yield exam pointers

- ▶ Foetal embalming = same aims as adult embalming (antisepsis, fixation, natural appearance) but scaled down for a small, fragile, water-rich specimen.
- ▶ Two core methods: **immersion** (very small/early foetuses, vessels too fine) and **injection** (larger foetuses/stillbirths); a **combined** approach completes fixation by diffusion after limited perfusion.
- ▶ **Umbilical vein preferred over the umbilical arteries** for injection — larger lumen, thinner wall, less prone to spasm; alternate sites (carotid/femoral/intracardiac) used only when the cord is unavailable.
- ▶ Fluid must be **more dilute** than standard adult arterial fluid — the same "special condition" dilution principle Haldar applies to infants extends to the foetus.
- ▶ **Low-pressure gravity/hand injection only** — never a motorised high-pressure injector.
- ▶ Ligate every cannulated vessel on completion, as in adult arterial embalming.
- ▶ Three practical indications to remember: **teaching, medico-legal retention, museum/research.**
- ▶ Long-term storage = sealed, labelled wet-specimen jar with periodically topped-up dilute holding fluid.
- ▶ Extra biohazard note: TORCH/Parvovirus B19 precaution for a pregnant female embalmer.

SOURCE & COVERAGE

Haldar — *Notes on Embalming & Museum Techniques* (definitions and goals of embalming; composition and dilution principles of arterial/cavity fluids; criteria for choice of vessel; injection techniques, ligation and drainage; occupational-risk precautions); Sontakke — *Textbook of Human Embryology* and BD Chaurasia — *Human Anatomy Vol 1, 8e* (umbilical vein/artery number, calibre and Wharton's jelly; foetal circulation).

Coverage note: the local specialty corpus (Haldar; Handbook of Museum Technique, Govt Museum Chennai) does not contain a dedicated fetus-specific embalming protocol or a foetal fluid-strength table — the Chennai Handbook covers zoological specimens only. The technique above therefore applies Haldar's general embalming principles (vessel-selection criteria, ligation practice, and his explicit note that fluid dilution is varied for "special conditions" including infants) to the foetal context, combined with standard, universally-taught anatomy-museum/mortuary-science practice (immersion for very small specimens; gentle low-pressure umbilical-vessel injection with a weaker fixative for larger stillbirths).

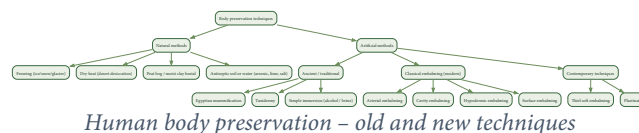


Human body preservation – old and new techniques

Asked in: Paper III 12-Jul-2023 — RGUHS MD/MS Anatomy

Definition / Introduction Body preservation is the arrest of post-mortem decomposition by physical or chemical means so that a cadaver remains in a near-life-like, dissectible state for anatomical teaching, forensic/funerary transport, or museum display. **Embalming** — defined by the American Board of Funeral Service Education as "the chemical treatment of the dead human body to reduce the presence and growth of micro-organisms, to temporarily inhibit organic decomposition, and to restore the dead human body to an acceptable physical appearance" — is the commonest artificial method used in medical colleges. Techniques have evolved from ancient natural and mummification methods through classical arterial embalming to contemporary soft (Thiel) embalming and plastination.

1. Classification of preservation techniques



2. Historical evolution of body preservation

- ▶ **Chinchorro culture** (Atacama Desert, Chile/Peru) practised the earliest known artificial mummification, 5000–6000 BC, predating Egypt.
- ▶ **1st period (3200 BC – 650 AD)** — originated in **Egypt** with specialised priest-embalmers; religious motive (belief in resurrection); also practised by Ethiopians, Guanches, Peruvians, Jivaro Indians, Aztecs, Toltecs, Mayans and Tibetan/Nigerian tribes.
- ▶ **2nd period (650 AD – 1861)** — knowledge spread through Europe, termed the "**Anatomists' period**"; Middle Age and Renaissance anatomists refined techniques to advance dissection.

- ▶ **3rd/modern period (1861–present)** — spread from Europe to America; American Civil War casualties drove demand for transportable embalmed dead; **Dr Richard Burr (1865)** is credited as the first cadaver embalmer, licensed under the 1865 Final Military Order; **Sir Jean Nicolas Gannal (1791–1852)**, a chemist, pioneered arsenic-based arterial fluids.
- ▶ **Notable embalmed bodies** — Lord Nelson, Lincoln, Stalin, and most famously **Lenin**, displayed in his Moscow mausoleum since 1924.
- ▶ Rising numbers of Indian medical institutes have increased demand for embalmed teaching cadavers.

3. Natural methods of preservation

- ▶ **Freezing** — ice, snow or glacier entombment (dry-cold preservation), e.g. the naturally preserved body of Bishop Peder Winstrup (1605–1679).
- ▶ **Dry heat** — desiccation in dry, warm desert air, exemplified by Egyptian mummies.
- ▶ **Nature of soil** — long-term burial in peat bogs, or soil impregnated with salt, aluminium or copper.
- ▶ **Antiseptic soil/water** — shallow moist clay-soiled graves, or submersion in water/soil containing arsenic, lime or similar antiseptics.

4. Artificial methods — traditional (old) techniques

Technique	Key feature
Ancient Egyptian mummification	Evisceration and immersion; earliest organised state method
Guanche method	Evisceration and drying
European method	Evisceration, local incision and immersion
Simple immersion	Alcohol, brine or other liquid preservative
Arterial injection & evisceration	Introduced by the Hunter brothers
Cavity injection & immersion	Gabriel Clauderus' method
Arterial injection alone	Gannal and Sucquet's method
Arterial injection + cavity treatment	Present-day standard method
Simple heat	Slow oven-drying with slaked lime
Powders	Sawdust with zinc sulphate or other preservative
Artificial cold	Refrigeration, inhibits bacterial activity
Taxidermy / paraffin impregnation	Used for display specimens

5. Modern embalming — mechanism, ideal fluid and types

- ▶ **Mechanism** — embalming chemicals bind the reactive centres of body proteins into an inert, water-resistant latticework resistant to bacterial/autolytic enzymes; germicides inactivate enzymes and destroy bacteria (sanitisation); blood coagulates into a pinkish-brown mass; the

resulting chemical stiffening resembles rigor mortis but, unlike true rigor, is permanent. Ideal window is within 6 hours of death — beyond this, bacterial decomposition sets in.

- **Composition of an ideal (arterial) embalming fluid** (per 10 L batch, for a ~70 kg body; ~10% is lost via drainage/purging):

Ingredient	Role	Quantity
Formalin	Preservative	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 mL
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 mL
Oil of wintergreen	Perfume	90 mL
Water	Vehicle	up to 10 L

- **Chemical categories** — preservatives (formaldehyde, methanol, phenol), germicides (phenolics, zephiran chloride, glutaraldehyde), buffers (sodium borate/bicarbonate), wetting agents (glycerine, sorbitol), anticoagulants (sodium citrate/oxalate), dyes (eosin), vehicles (alcohol, water) and perfumes (methyl salicylate).
- **Types of embalming** — arterial, cavity, hypodermic (isolated limbs/traumatic parts), surface (burns/bedsores), autopsied-body, and HIV-body embalming (higher fluid concentration, extra precautions).
- **Vessel selection** — the vessel nearest the heart gives best distribution/drainage; **six-point injection** (R & L common carotid for head/neck, R & L axillary/brachial for upper limb, R & L femoral for lower limb) is used for autopsied, traumatic or badly decomposed bodies; each vessel is ligated after use.
- **Injection techniques** — one-point (right common carotid + internal jugular vein, commonest), split injection/drainage, multipoint, six-point (sectional), and restricted cervical injection (head embalmed separately — for facial trauma, oedema, or eye enucleation).
- **Instruments** — gravity injector (simplest, slowest), bulb/stirrup/hand pump, and electric/motorised injector (delivers 8–10 L in 30–45 min at ~2 kg/cm²).
- **Cavity embalming** — performed 30–60 min after arterial embalming (allows visceral hardening); a trocar aspirates stomach, right heart, pleural sacs, intestines, bladder and rectum, then ~1 L concentrated cavity fluid is injected per cavity (thoracic, abdominal) for a 70 kg body; openings closed with sutures.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a schematic of the **six-point arterial embalming vessels**: right and left common carotid arteries (with accompanying internal jugular veins) for the head and neck; right and left axillary/brachial arteries (with veins) for the upper limbs; right and left femoral arteries (with veins) for the lower limbs — mark the direction of injection (toward the periphery) and the site of drainage (toward the heart) at each point.

6. Contemporary (new) techniques

- ▶ **Thiel's soft-fix embalming** — retains a life-like, flexible body: skin/muscles stay pliable (limbs mobile) and viscera respond to the scalpel like fresh tissue, unlike rigid conventional formalin fixation; used chiefly for **cadaveric surgical workshops**. Two stem solutions (A: boric acid, ethylene glycol, ammonium/potassium nitrate; B: ethylene glycol, 4-chloro-3-methylphenol) are combined with formalin and sodium sulphite just before perfusion; final formaldehyde concentration is kept **below 0.5%**. Perfusion is via the great saphenous vein (or femoral/carotid artery), ~14 L infused, then immersion in a maturation tank for **4–6 weeks**; bodies are vacuum-sealed and stored in a mortuary cooler between sessions; raising formalin to 4% extends usable preservation to ~6 months.
- ▶ **Plastination** — produces dry, coloured, odourless, non-toxic, durable, natural-looking specimens. Types: **whole-organ**, **sheet** (thin transparent or thick opaque body slices, for internal relationships) and **luminal-cast plastination** (fills hollow viscera/vessels and dissolves surrounding tissue to display ductal/vascular architecture — bronchial trees, cerebral ventricles, bony labyrinth, vascular casts). Steps: (1) **Fixation** in formalin — arrests decomposition, maintains shape; (2) **Dehydration** in cold acetone (draws out cellular water); (3) **Forced impregnation under vacuum** with liquid polymer (silicone rubber/polyester/epoxy resin) — vacuum lowers acetone's boiling point so it vaporises out, drawing polymer in; (4) **Hardening/curing** with gas, heat or UV light. Advantages: low cost, simple protocol; ideal for teaching, novel surgical-technique design, skills practice and research.

7. Museum preservation of anatomical specimens

- ▶ Good specimens are secured **at the time of autopsy**: smooth even cut surfaces (single continuous stroke of a long sharp knife), immediate transfer into fixative (10% formal-saline); hollow viscera inflated or packed with fixative-soaked cotton to retain shape.
- ▶ **Kaiserling's method (1900)** — classical technique for colour-retaining wet museum specimens, using three solutions: **Fluid I** (formalin, potassium nitrate, potassium acetate) fixes/hardens; **Fluid II** (80% ethyl alcohol) restores natural colour (over-exposure permanently bleaches); **Fluid III** (glycerin, sodium acetate, formalin) is the mounting/storage fluid.
- ▶ **Pulvertaft's modification (1936)** adds reducing agent **sodium hydrosulphite** directly to the mounting fluid — little fading even after 35 years.
- ▶ **Wentworth's modification (1938–57)** dispenses with the alcohol colour-restoration step, using sodium hydrosulphite alone.

- ▶ **Maceration** — displays bony lesions (osteoma, osteosarcoma, chronic osteomyelitis, TB) by boiling in water/dilute NaOH, then degreasing in chloroform and bleaching in hydrogen peroxide.
- ▶ **Mounting** — specimens stitched to a Perspex centre-plate (nylon/linen thread), sealed in Perspex boxes or glass jars with mounting fluid; containers individually labelled and catalogued.

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- ▶ **Medico-legal rule** — embalming must **never precede** a medico-legal autopsy: it destroys toxicological evidence (masks cyanide, alcohol, opiates, carbon monoxide), sterilises the body (bacteriological culture invalid), prevents blood grouping and detection of thrombus/embolism, and can add wound-like trocar marks, complicating injury interpretation and time-since-death estimation (embalming rigidity, unlike rigor mortis, is permanent).
- ▶ **Legal procedure** — needs relative identification, written consent, police NOC, and the death certificate; foreign nationals additionally need embassy clearance; an embalming certificate must accompany the body (3 copies local, 5 international transport).
- ▶ **Religious perspectives** — generally permitted in Christianity, LDS and Iglesia ni Cristo (which nonetheless forbids autopsy/cremation); some Orthodox communities ban it unless legally mandated; **not practised in Islam** (washed, wrapped in a plain shroud "kafan", buried without coffin), Zoroastrianism (Tower of Silence sky burial) or many Neopagan traditions.
- ▶ **Anatomy Act (1949)** (Bombay Anatomy Act) — governs legal supply of unclaimed cadavers to medical institutions for dissection; applies when death occurs in a government hospital/public place within the institute's prescribed zone and the body stays unclaimed 48 hours post police declaration.
- ▶ **Occupational hazards** — bloodborne/airborne pathogens (HIV, hepatitis B/C, MDR-TB, a hypervirulent *Clostridium difficile* strain, MRSA, CJD); TORCH agents/parvovirus B19 risk pregnant embalmers; formaldehyde exposure mandates PPE and OSHA-compliant limits.
- ▶ **Cadaveric skills training** — pliable soft/Thiel-embalmed cadavers, with realistic tissue planes, are preferred over hard-fixed cadavers for laparoscopic, endoscopic and flap-raising surgical training.

High-yield exam pointers

- ▶ Embalming produces **chemical stiffening resembling rigor mortis but is permanent**; ideal window is within **6 hours** of death.
- ▶ Ideal arterial embalming fluid: **formalin (preservative) + sodium borate (buffer) + sodium citrate (anticoagulant) + glycerine (wetting agent) + NaCl + eosin (dye) + wintergreen oil (perfume) + water to 10 L**.
- ▶ **Six-point injection** = bilateral common carotid + axillary/brachial + femoral vessels; used for autopsied or badly decomposed bodies.
- ▶ **Cavity embalming** follows arterial embalming (after 30–60 min); ~1 L concentrated cavity fluid per cavity via trocar.
- ▶ **Thiel's soft embalming** keeps tissue pliable for cadaveric workshops; final formaldehyde concentration kept **<0.5%**; maturation in tank for 4–6 weeks.

- ▶ **Plastination steps:** fixation → dehydration (cold acetone) → forced impregnation (vacuum + polymer) → curing; three types — whole-organ, sheet, luminal-cast.
- ▶ **Kaiserling's technique (1900)** uses three solutions (fix / restore colour / mount) for wet museum specimens; **Pulvertaft (1936)** modification adds sodium hydrosulphite to the mounting fluid for long-term colour retention.
- ▶ Embalming must **never precede** a medico-legal autopsy — it destroys toxicological and bacteriological evidence and can permanently mask time-since-death and wound findings.
- ▶ **Anatomy Act (1949)** governs the legal supply of unclaimed bodies to medical colleges for dissection.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (2019).

Coverage note: the corpus's other listed specialty text, the Handbook of Museum Technique (Govt Museum, Chennai), covers zoological/natural-history specimens (fish, echinoderms, taxidermy), not human cadaveric techniques, and was not drawn upon. Gray's Anatomy 41e has no dedicated embalming chapter — this niche PRESERVATION topic is covered almost entirely by the dedicated embalming manual above.



Luminal cast plastination

Asked in: Paper III 29-Sep-2025 — RGUHS MD/MS Anatomy

Definition / Introduction — Plastination is a museum-preservation technique that produces dry, coloured, non-toxic, durable, odourless and natural-looking specimens by replacing tissue water and lipid with a curable polymer. It has three forms — whole-organ, sheet, and **luminal cast plastination**. Luminal cast plastination is the variant used specifically to study the three-dimensional dimensions, architecture and branching pattern of hollow cavities and tubular structures (arteries, veins, ducts and their variations) by filling the lumen with a resin mixture and then digesting away the surrounding soft tissue, leaving behind a rigid positive replica ("cast") of the lumen. It is the modern, resin-based descendant of the classical corrosion-cast method and is far superior to conventional wet (formalin) preservation for demonstrating internal luminal geometry.

Why luminal cast plastination is needed — limitations of conventional preservation

Routine formalin-fixed wet specimens and dissection have well-recognised drawbacks that luminal cast plastination is designed to overcome:

- ▶ **Irritating odour** of formalin, unpleasant for prolonged handling.
- ▶ **Bleached, colourless** tissue that loses its natural appearance.
- ▶ **Non-naturalistic** look — specimens do not resemble the living structure.
- ▶ **Difficulty in maintaining sections** — wet slices distort, macerate or discolour with storage.

- Most importantly, **the luminal architecture, dimensions and branching pattern of a hollow viscus or vessel are almost impossible to appreciate on ordinary dissection** — the surrounding tissue obscures the cavity, and cutting into it destroys the very geometry being studied.

Principle

- The **lumen** of the organ or vessel is **filled with a casting material** (a resin-catalyst-hardener mixture) that subsequently hardens.
- The **surrounding soft tissue is then dissolved/removed**, leaving a rigid, permanent, positive replica that faithfully reproduces the internal dimensions, course, branching and calibre of the cavity or tube.
- This is conceptually the resin-plastination analogue of the older **corrosion-cast technique**, in which a vessel/duct system was injected with a setting material (classically Wood's metal, celluloid or vinyl acetate) and the surrounding tissue was then corroded away with concentrated acid — luminal cast plastination replaces acid corrosion with a gentler alkaline (KOH) digestion and uses a modern synthetic resin instead of a metal alloy, giving a lighter, safer, more durable and better-coloured cast.

Classical corrosion cast vs luminal cast plastination

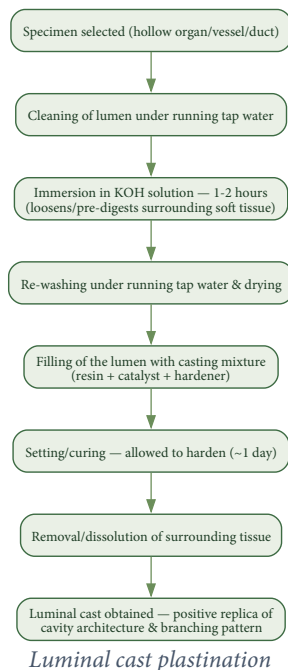
Feature	Classical corrosion cast	Luminal cast plastination
Casting material	Wood's metal, celluloid or vinyl acetate injected into lumen	Resin + catalyst + hardener mixture
Tissue removal	Concentrated acid corrosion of surrounding tissue	KOH pre-treatment + dissection/digestion of surrounding tissue
Handling safety	Hazardous acid fumes; heavy metal casts	Safer chemicals; light, non-toxic resin cast
Durability/colour	Durable but often dull grey/metallic	Durable, can be coloured, natural-looking
Cost & equipment	Needs metal-casting/acid-corrosion set-up	Simple protocol, low-cost, no vacuum equipment needed
Modern status	Largely historical	Current museum-preservation method of choice for luminal studies

Materials used

Category	Material
Storage/processing	Jars for storage & processing
Dehydrating agent	Acetone
Polymer	Silicone rubber / general-purpose resin
Curing agents	Catalyst, accelerator/hardener

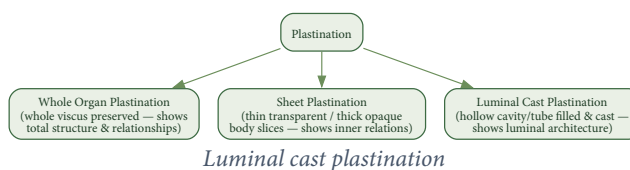
Category	Material
Colour	Colour paints
Instruments	Dissection instruments, syringes, needles
Tissue-digestion agent	KOH (potassium hydroxide) solution

Technique / steps



- **Step 1 — Cleaning:** the lumen of the fresh/preserved specimen is cleaned under tap water to remove blood, mucus or debris.
- **Step 2 — KOH treatment:** the specimen is kept in KOH solution for about 1–2 hours; this helps loosen and begin digestion of the peri-luminal soft tissue.
- **Step 3 — Re-washing:** the specimen is again cleaned under running tap water and dried.
- **Step 4 — Filling:** the cleaned, dried lumen is filled with a mixture of resin, catalyst and hardener, taking care to fill the entire cavity/branching system without air bubbles.
- **Step 5 — Setting:** the filled specimen is left to cure; after about 1 day the resin has fully hardened into a rigid replica.
- **Step 6 — Corrosion/removal of tissue:** the surrounding soft tissue is removed (dissected away/digested), and the hardened cast is obtained as a free-standing, permanent, positive replica of the lumen.

Types of plastination — luminal cast in context



- ▶ **Whole organ plastination** — the entire organ is fixed, dehydrated in acetone, impregnated with resin/catalyst/accelerator and mounted; it demonstrates overall structure and relationships.
- ▶ **Sheet plastination** — deep-frozen tissue is band-sawn into thin (or up to 1 cm) slices, cast between glass plates with resin, and yields portable, dry, labelled body-section sheets that best show internal relationships.
- ▶ **Luminal cast plastination** — as described above, specifically targets **hollow cavities and tubular systems** rather than the solid organ or a body slice.

Structures/organs demonstrated by luminal cast plastination

- ▶ **Tracheobronchial cast** of the lungs (bronchial tree, showing divisions and branching pattern).
- ▶ **Cerebral ventricular cast** (shape, size and communications of the ventricular system).
- ▶ **Bony labyrinth cast** (cochlea, vestibule, semicircular canals — internal ear architecture).
- ▶ **Vascular casts** of the kidney, liver, lung and spleen (arterial/venous branching pattern and organ vasculature).
- ▶ **Coronary vessel casts** (coronary arterial pattern and its variations).

Advantages of plastination (applicable to the luminal cast type)

- ▶ Produces **dry, coloured, non-toxic, durable, odourless, natural-looking** specimens — none of the drawbacks of wet formalin preservation.
- ▶ Gives a **unique, permanent record of luminal geometry/branching** that cannot otherwise be reproduced by dissection or wet fixation.
- ▶ **Low-cost**, yet the resulting specimen is of a quality equivalent to expensive international plastination specimens.
- ▶ **Simple protocol** — does not require sophisticated vacuum-impregnation equipment (unlike whole-body plastination).
- ▶ Specimens are **best suited for**: undergraduate/postgraduate **teaching**, **designing newer surgical procedures**, **practice of dissection/surgical skills**, and **research** into normal branching patterns and their variations.

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- ▶ Luminal cast plastination is the direct, museum-safe evolution of the classical **corrosion-cast technique** long used to study vascular and ductal patterns (e.g., renal arterial and pelvicalyceal casts, coronary arterial casts) — where the older method used acid corrosion of tissue around an injected Wood's-metal/vinyl mass, the resin-KOH method achieves the same demonstration with better safety, colour retention and durability.
- ▶ **Bronchial-tree casts** are used to teach segmental bronchopulmonary anatomy relevant to bronchoscopy and lobectomy/segmentectomy planning.
- ▶ **Coronary vessel casts** demonstrate the pattern of coronary dominance and branching used in cardiac surgical and interventional cardiology training.
- ▶ **Renal/hepatic/splenic vascular casts** illustrate segmental vascular anatomy relevant to partial nephrectomy, hepatic resection and splenic-artery embolisation.

- ▶ **Cerebral ventricular and bony-labyrinth casts** correlate directly with CT/MRI cross-sectional and inner-ear imaging, aiding radiological anatomy teaching.
- ▶ Modern cross-sectional imaging (CT/MR angiography with 3-D volume reconstruction) has largely supplemented, but not replaced, the teaching value of a permanent, handleable luminal cast specimen in the anatomy museum.

High-yield exam pointers

- ▶ Luminal cast plastination is **one of three types of plastination** — whole organ, sheet, and luminal cast.
- ▶ **Principle:** fill the lumen with a resin-catalyst-hardener mixture, then remove the surrounding tissue to obtain a positive cast of the cavity.
- ▶ Method sequence: **clean lumen → KOH (1–2 h) → wash & dry → fill with resin+catalyst+hardener → set (~1 day) → remove surrounding tissue → cast obtained.**
- ▶ Used for: **tracheobronchial tree, cerebral ventricles, bony labyrinth, vascular pattern of kidney/liver/lung/spleen, coronary vessels.**
- ▶ It is the **modern equivalent of the classical corrosion-cast** technique, replacing acid corrosion with KOH digestion and metal/vinyl injection with resin.
- ▶ Advantages of plastination generally: **dry, coloured, non-toxic, durable, odourless, natural-looking;** low-cost yet international-quality; simple protocol.
- ▶ Best suited for **teaching, surgical-procedure design, practice of skills, and research.**
- ▶ Overcomes the key limitation of conventional dissection/wet fixation — the **luminal architecture is otherwise almost impossible to appreciate.**

SOURCE & COVERAGE

Halder — Notes on Embalming & Museum Techniques (primary and near-sole source for this technique). Coverage note: Luminal cast plastination is a museum/preservation-technique topic that is not covered in Gray's Anatomy (Standring) or standard gross-anatomy texts (BD Chaurasia, AK Datta, Moore); it is grounded here entirely in Halder's dedicated embalming/museum-technique reference, the specialty text for this Paper III (Preservation) topic. The general Handbook of Museum Technique (Govt Museum, Chennai) was checked but covers zoological/taxidermy casting, not this human-anatomical technique, and contributed no additional verbatim-critical facts.



Methods of preservation of cadavers

Asked in: Paper III Oct 2008 — RGUHS MD/MS Anatomy

Definition / Introduction — Preservation of a cadaver is the arrest (or marked slowing) of post-mortem putrefaction so that the body remains fit for dissection, demonstration or cadaveric-surgery training. Methods are broadly **natural** (physical conditions that retard decay without added chemicals) and **artificial** (deliberate chemical/physical treatment, of which **embalming** — injection of an antiseptic-preservative fluid into the vascular system and body cavities — is the standard method in a medical college). Embalming is defined by the American Board of Funeral Service Education as "the chemical treatment of the dead human body to reduce the presence and growth of micro-organisms, to temporarily inhibit organic decomposition, and to restore the dead human body to an acceptable physical appearance." To be effective it should ideally begin within **6 hours of death**; beyond this, bacterial decomposition sets in and fixation becomes uneven.

1. Classification of methods of preservation



2. Natural methods

- ▶ **Freezing** — entombment in ice, snow or a glacier (dry-cold preservation).
- ▶ **Dry heat** — desiccation in hot, dry desert air/sand, as in naturally mummified pre-dynastic Egyptian burials.
- ▶ **Dry cold** — preservation by cold, dry ambient air.
- ▶ **Nature of the soil/water at interment** — long-term burial in peat bogs; soil impregnated with salt, aluminium or copper; a shallow moist clay-soiled grave; or submersion in water/soil containing an antiseptic such as arsenic or lime.

3. Artificial methods — historical/traditional techniques

Before modern arterial-cavity embalming became standard (present-day method), several older techniques were used:

Technique	Key feature
Ancient Egyptian mummification	Evisceration and immersion (earliest organised state method, from 3200 BC)
Guanche method	Evisceration and drying
European method	Evisceration, local incision and immersion
Simple immersion	Alcohol, brine or other liquid preservative
Arterial injection & evisceration	Hunter brothers' method
Cavity injection & immersion	Gabriel Clauderus' method
Arterial injection alone	Gannal and Sucquet's method

Technique	Key feature
Simple heat	Slow oven-drying with a mixture of slaked lime
Powders	Sawdust with zinc sulphate or other preservative
Artificial cold (refrigeration)	Inhibits bacterial activity
Taxidermy / paraffin impregnation	Chiefly for display specimens

The earliest known artificial mummification is that of the **Chinchorro culture** (Atacama Desert, 5000–6000 BC), predating Egypt; the modern period began in 1865, when **Dr Richard Burr** became the first licensed cadaver embalmer during the American Civil War.

4. Modern embalming — mechanism, ideal fluid and types

- **Mechanism** — embalming chemicals cross-link the reactive, water-binding centres of body proteins into an inert, firm latticework resistant to bacterial/autolytic enzymes; germicides simultaneously inactivate tissue enzymes and destroy bacteria (sanitisation). Effects: proteins coagulate, tissues fix, organs bleach and harden, and blood clots into a pinkish-brown mass; the resulting chemical stiffening resembles rigor mortis but is **permanent**.
- **Composition of an ideal (arterial) embalming fluid** — a 70 kg body needs about 10 L ($\approx 10\%$ is lost through drainage/purging):

Ingredient	Role	Quantity (per 10 L)
Formalin	Preservative	1.5 L
Sodium borate	Buffer	600 g
Sodium citrate	Anticoagulant	900 g
Glycerine	Wetting agent	600 mL
Sodium chloride	pH control	800 g
Eosin (1%)	Cosmetic dye	30 mL
Oil of wintergreen	Perfume	90 mL
Water	Vehicle	up to 10 L

- **Component classes** — preservatives (formaldehyde, methanol, phenol), germicides (phenolic derivatives, zephiran chloride, glutaraldehyde), buffers (sodium borate/bicarbonate), wetting agents (glycerine, sorbitol, sodium lauryl sulphate), anticoagulants (sodium citrate/oxalate), dyes (eosin), vehicles (water, alcohol, glycerine), perfumes (methyl salicylate) and muscle relaxants (magnesium chloride). Formalin = 37% formaldehyde + 7% methyl alcohol in water (formaldehyde discovered 1856, A.W. von Hofmann).
- **Types of embalming** — **arterial** (into the vascular tree), **cavity** (into thoracic/abdominal/pelvic cavities via trocar), **hypodermic** (subcutaneous, for isolated limbs/traumatic parts), **surface** (topical, for burns/bedsores/surface lesions), and special-case embalming of the **autopsied body** and the **HIV/AIDS body** (fluid concentration increased, extra barrier precautions).

5. Arterial embalming — vessel selection, injection technique and instruments

- ▶ **Choosing the artery** — size/diameter, practicality of drainage from the accompanying vein, depth/location, its branches, and proximity to the aortic arch. **Choosing the vein** — size/diameter, proximity to the right atrium, depth, and ease of raising the vessel.
- ▶ **Injection techniques:**
 - *One-point injection* — one site for both injection and drainage; right common carotid + internal jugular vein commonest, femoral vessels next, axillary/brachial least common.
 - *Split injection/drainage* — injection from one site (right femoral artery), drainage from another (right internal jugular vein) — reduces short-circuiting.
 - *Multipoint injection* — two or more arteries injected to overcome poor distribution.
 - *Six-point (sectional) injection* — routine method for **autopsied/badly decomposed bodies**: right/left common carotid, axillary/brachial and femoral arteries are raised, cannulated and ligated after use; order — right leg → left leg → right arm → left arm → trunk (via right carotid) → left head → right head.
 - *Restricted cervical injection* — head embalmed separately via the carotids; for general oedema, bodies likely to purge, facial/head trauma, eye enucleation, or delayed embalming.
- ▶ **Instruments** — hand/foot pump; bulb (Higginson's-type) syringe; **gravity injector** (simplest, safest, slowest — a 10 L bottle raised 4–6 ft above the table; a 1 m rise gives $\approx 0.6 \text{ kg/cm}^2$); **motorised/electric injectors** deliver 8–10 L in 30–45 min at $\approx 2 \text{ kg/cm}^2$.
- ▶ **Method of injection** — continuous injection/continuous drainage (least satisfactory); continuous injection with disrupted drainage (better diffusion); alternate injection and drainage (halted when veins swell); and **discontinuous injection and drainage** (repeated small injections at ~2-hour intervals, 3–4 times) — the **best method**.

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Outline of the trunk, head and limbs with the **six-point embalming injection sites** labelled:

- ▶ Right and left common carotid arteries (head & neck; trunk perfused via the right)
- ▶ Right and left axillary/brachial arteries (upper limbs)
- ▶ Right and left femoral arteries (lower limbs)
- ▶ Right internal jugular vein (drainage site)

Mark the injection order with arrows: right leg → left leg → right arm → left arm → trunk → left head → right head.

6. Cavity embalming and contemporary techniques

- ▶ **Cavity embalming** — performed ½–1 hour after arterial embalming (allows visceral hardening); a trocar (with electric/hydro-aspirator) enters through a stab incision 5–6 cm above the umbilicus. Aspiration sequence: stomach → right side of heart → pleural sacs (via diaphragm) → small intestine, caecum, colon → bladder, sigmoid colon, rectum. About 1 L of concentrated cavity fluid (formalin 60%, methanol 25%, phenol 10%, sodium lauryl sulphate 1%, mercuric

chloride 1%, eucalyptus oil 1%) is then instilled into each of the thoracic and abdominal cavities (~2 L total, 70 kg body); incisions are closed with nylon sutures.

- ▶ **Thiel's soft embalming** — for cadaveric surgical-skills workshops: skin/muscle stay pliable and viscera respond to the scalpel almost as in life, unlike stiff conventional formalin-fixed tissue. Stem solutions A (boric acid, ethylene glycol, ammonium/potassium nitrate, water) and B (ethylene glycol, 4-chloro-3-methylphenol) are combined with formalin and sodium sulphite just before perfusion; formalin is kept to a strict minimum, the final formaldehyde concentration in the perfusate being <0.5%. About 14 L is perfused (great saphenous vein, or femoral/carotid artery), after which the body matures **4–6 weeks** in an immersion tank; between workshop sessions it is vacuum-sealed and stored in a mortuary cooler (usable ~3 weeks). Raising formalin to 4% extends preservation to ~6 months but loses the "soft" quality.
- ▶ **Plastination** — produces dry, coloured, odourless, durable, natural-looking whole-body/organ specimens. Steps: **Fixation** (formalin, arrests decomposition, fixes posture) → **Dehydration** in cold acetone (draws water from cells) → **Forced impregnation under vacuum** with liquid polymer (silicone rubber/polyester/epoxy resin — the vacuum lowers acetone's boiling point so it vaporises out, drawing polymer in) → **Hardening/curing** with gas, heat or UV light. Low-cost and simple; useful for teaching, new surgical-technique design, skills practice and research.

Applied / Clinical anatomy

- ▶ **Medico-legal rule** — embalming must **never precede** a medico-legal autopsy: it masks toxicological findings (cyanide, alcohol, opiates, carbon monoxide), invalidates bacteriological culture, prevents blood grouping and detection of thrombus/embolism, and the trocar may itself create or modify wounds — the permanent rigidity also confounds time-since-death estimation. Heavy metals (e.g. mercuric chloride) are avoided in a medico-legal case's fluid.
- ▶ **Legal procedure before embalming** — relative identification, written consent, police NOC, and death-certificate verification (embassy clearance for foreign nationals); a certificate is issued after (3 copies local, 5 international transport), and notifiable-disease deaths (cholera, plague, rabies, tetanus, hepatitis B, HIV/AIDS, TB) require informing the authority.
- ▶ **Anatomy Act (1949)** (Bombay Anatomy Act) — governs legal supply of unclaimed cadavers to medical institutions for dissection, when death occurs in a government hospital/public place within the institute's zone and the body stays unclaimed 48 hours after police declaration.
- ▶ **Occupational hazards** — bloodborne/airborne pathogens (HIV, hepatitis B/C, MDR-TB, hypervirulent *Clostridioides difficile*, MRSA, VRE/VRSA/VISA, *Pseudomonas aeruginosa*, CJD); pregnant embalmers carry added risk from TORCH agents, parvovirus B19, syphilis; chronic formalin exposure needs PPE and OSHA-compliant limits.
- ▶ **Advantages** — long-term preservation for teaching/research; sanitisation, slowed putrefaction; restores natural appearance after trauma/disease; permits transport of the deceased; gives time to organise funeral rites. Soft (Thiel)- and plastinated cadavers are additionally preferred for laparoscopic/endoscopic surgical-skills training (realistic tissue planes).

High-yield exam pointers

- ▶ Two broad groups: **natural** (freezing, dry heat/cold, antiseptic soil/water) and **artificial** (embalming, plastination, and older methods — mummification, taxidermy, simple immersion, refrigeration).
- ▶ Embalming must begin within **6 hours** of death; it produces a chemical stiffening resembling rigor mortis but, unlike true rigor, is **permanent**.
- ▶ Ideal arterial fluid for a 70 kg body = **10 L** (1.5 L formalin + buffer + anticoagulant + wetting agent + NaCl + dye + perfume + water).
- ▶ **Six-point injection** (both common carotids, axillary/brachial, femoral arteries) is the standard method for autopsied or badly decomposed bodies.
- ▶ Cavity fluid is more concentrated (60% formalin) than arterial fluid; ≈ 1 L is instilled into each of the thoracic and abdominal cavities.
- ▶ Gravity injector = safest/slowest (≈ 0.6 kg/cm² per metre of rise); motorised injector = fastest (8–10 L in 30–45 min, ≈ 2 kg/cm²).
- ▶ **Thiel's soft embalming** keeps tissue pliable (final formaldehyde $< 0.5\%$) for cadaveric surgical-skills training, not routine dissection.
- ▶ **Plastination** = fixation $\rightarrow$ dehydration (cold acetone) $\rightarrow$ forced vacuum impregnation (polymer) $\rightarrow$ curing; gives a dry, odourless, durable specimen.
- ▶ Embalming must **never precede** a medico-legal autopsy — it destroys toxicological, bacteriological and blood-grouping evidence.

SOURCE & COVERAGE

Haldar — Notes on Embalming and Museum Techniques (chapter: Embalming Techniques).

Coverage note: this preservation/museology topic is not covered by Gray's Anatomy (Standring) or the gross-anatomy texts; the answer is drawn from the dedicated specialty text (Haldar), the standard PG-Anatomy source for this question. The second specialty text (Handbook of Museum Technique, Govt Museum Chennai) covers zoological/natural-history museum specimens, not human cadaveric preservation, and was not drawn upon.



Mounting of cross section of Brain

Asked in: Paper III 16-Nov-2019 — RGUHS MD/MS Anatomy

Definition / Introduction — Mounting of a cross section of brain is the museum technique by which a cut slice of the (previously hardened) brain is fixed, colour-restored and permanently sealed in a fluid-filled container — or, by the modern alternative, plastinated — so that its natural size, shape, colour and internal spatial relations are preserved indefinitely for teaching and study. Because fresh brain is too soft to cut cleanly or to hold its shape once sectioned, the procedure has two distinct phases: (1) hardening the **whole, intact** brain by controlled fixation before any cut is made, and (2) applying standard museum wet-mounting (or plastination) technique to the cut slice itself. The wet-mounting technique used is the same Kaiserling–Pulvertaft–Wentworth method used for any museum specimen, with brain-specific modifications for hardening and orientation.

1. Why the brain needs special preparation before mounting

- ▶ Fresh brain has a soft, gelatinous consistency and no rigid stroma; an unfixed or under-fixed brain **cannot be cut with a clean, single stroke** — it distorts, tears and loses its natural gyral/sulcal pattern and internal landmarks (ventricles, basal nuclei, internal capsule) the moment a blade is drawn through it.
- ▶ Unlike a solid viscus, the brain is fixed **before** sectioning, as a whole intact specimen, and only cut once it has hardened enough to hold a smooth cut surface.
- ▶ The source material's general rule for museum fixation — "specimens should always be injected with fixative if possible, to ensure adequate fixation" — specifically names the **brain** as an example, because simple immersion fixes only the surface for a long time while the core stays soft; where feasible, fixative is introduced directly (e.g., via the internal carotid/vertebral arteries or by an intraventricular injection) to speed and even out hardening.
- ▶ Blood-laden tissue (as the brain often is) must **never be washed in water** at any stage, before or after fixation, since this leaches its natural colour.

2. Step 1 — Selection and hardening (fixation) of the whole brain

- ▶ The brain is removed intact at autopsy/dissection with **its attached structures preserved** (cranial nerve stumps, vessels, dura where relevant, pituitary stalk) — careless removal at this stage, per the general museum rule, can easily ruin an otherwise good specimen.
- ▶ **Primary fixation is in 10% formal-saline.** The whole brain is submerged in a **large volume** of fixative (so that the fluid-to-tissue ratio is generous and fixative is not exhausted), resting on or supported by a bed of cotton wool/lint, or suspended (e.g., by a thread passed beneath the basilar artery/through the falx) so that its own weight does not flatten the base or distort the natural convolutional pattern while it is still soft.
- ▶ **Hardening is allowed to proceed for roughly 3–4 weeks**, with the fixative changed once or twice, until the brain is firm enough to be cut with a single continuous stroke without tearing or the cut surface caving in.
- ▶ Cystic/ventricular cavities are handled exactly as for any museum specimen — natural cavities are kept expanded (rather than collapsed) during fixation so the true shape of the ventricular system is not lost.

3. Step 2 — Selecting the plane and cutting the section

- Once adequately hardened, the brain is placed on a flat, water-proofed (formica-covered) bench and **oriented in the anatomically correct position** before any cut is made.
- The section is made with a **long-bladed, sharp brain knife using a single continuous stroke** — not a sawing action — exactly as the general museum-specimen rule requires ("cut surfaces should be smooth and even, by using a continuous stroke with the long-bladed sharp knife"); a hesitant or multi-stroke cut leaves a ridged, uneven face that will not display well once mounted.
- The **plane of section is chosen to display the structures the specimen is meant to teach** — most commonly a coronal (transverse) slice through the cerebral hemispheres at the level of the basal nuclei/thalamus/internal capsule, or through the midbrain/pons, and/or a mid-sagittal slice through the brainstem, corpus callosum and ventricular system; a full teaching set typically carries several such slices at different levels, exactly as illustrated for gross brain sectioning.
- The cut slice, and not the whole brain, is what is then carried through colour restoration and mounting; the remaining brain may be further sliced to yield additional sections for the same or other specimens.

□ Fig 1.22 — Sections of brain in different planes: (A to C—coronal sections; D—horizontal section, E & F—sagittal sections, G—oblique section) (BD Chaurasia — Human Anatomy Vol 3-4 — 8e, p.434)

4. Step 3 — Colour restoration and mounting-fluid technique

The cut slice is now processed exactly like any wet museum specimen, by a modification of **Kaiserling's technique (1900)**, which uses three solutions in sequence — a fixing fluid, a colour-restoring fluid, and a final mounting fluid.

Solution	Composition	Purpose
Kaiserling's fluid I (fixing)	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 ml	The cut slice is transferred into this fluid (after the whole-brain formal-saline fixation, or fixed directly in it)
Kaiserling's fluid II (colour restoration)	Ethyl alcohol 80%	Restores colour over 30 min–4 h; timing must be controlled, as prolonged immersion causes permanent bleaching that the mounting fluid cannot reverse; not needed if sodium hydrosulphite is used in the mounting fluid
Pulvertaft–Kaiserling mounting fluid III	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + water to 1000 ml	Final mounting fluid; 0.4% sodium hydrosulphite is added immediately before sealing — Pulvertaft's (1936) modification, shown to give remarkably little fading even after 35 years

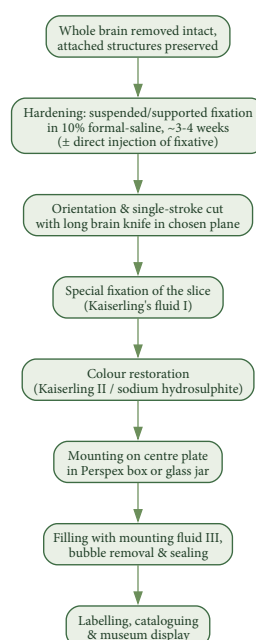
- If the mounting fluid is not crystal clear it is filtered through a paper-pulp filter, and 30 ml of a saturated solution of camphor in alcohol may be added per litre.
- **Wentworth's modification (1938–1957)** omits the alcohol step altogether and relies on sodium hydrosulphite alone; fixation in solution I is continued for at least a month, and the mounting

solution is then chosen by the **pH of the specimen** — solution III directly if pH > 6.5, solution II first if pH < 6.5.

- **Israel & Young (1978)** used pure liquid paraffin as the final mountant after alcohol colour-restoration, which reduces discoloration of the mounting fluid by pigment leaching from the specimen.

5. Step 4 — Mounting and sealing the cut section

- The colour-restored slice is laid on the formica bench in correct anatomical orientation and measured, allowing **1 cm clearance at the top and sides and 2 cm at the bottom** (for the label), with the container depth about 5 mm more than the specimen's own depth.
- **Perspex boxes are used almost universally today** in preference to glass, being light, unbreakable and either bought commercially or made to fit the individual slice exactly.
- The slice is attached to a **Perspex centre plate**: holes are drilled through the tissue at marked points and it is tied with nylon thread (two 1 mm holes) or linen thread retained by a glass bead (one hole), tied with a non-slip **reef knot**, keeping stitches away from any structure meant to be displayed; alternatively the slice is pressed onto Perspex spikes cemented to the plate. Coloured Perspex rods/arrows or black horse-hair may be used to indicate delicate structures such as small vessels or nerve stumps.
- The mounted plate is fitted into the box, which is then **filled with mounting fluid (0.4% sodium hydrosulphite added just before sealing) to within 1 cm of the top**; trapped air bubbles are worked out with a broad spatula, and the remaining fluid is topped up through a small drilled hole that is finally plugged with a Perspex rod/nylon screw and cemented shut, or — for a glass jar — the lid is sealed onto the ground-glass rim with a heated asphaltum-rubber (picein) compound and the edge finished with black enamel/asphaltum varnish.
- The finished specimen is given a **unique identification/accession number**, recorded in a reference/catalogue register, labelled on the outside of the container, and the label additionally tied to the specimen itself with linen thread so identity is never lost.



Mounting of cross section of Brain

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a **mounted coronal-section brain specimen** (Perspex box, museum display):

- ▶ Outer **Perspex box**, transparent, custom-fitted to the slice
- ▶ The **coronal slice of brain**, anatomically oriented, showing cerebral cortex, basal nuclei, internal capsule, thalamus and the lateral ventricle
- ▶ **Perspex centre plate** to which the slice is sutured
- ▶ **Nylon/linen sutures with glass-bead retainer**, tied with a reef knot, kept clear of the displayed structures
- ▶ **Coloured Perspex marker rod** identifying a specific structure (e.g., a vessel stump)
- ▶ **Museum mounting fluid** (Kaiserling III/Pulvertaft's fluid + 0.4% sodium hydrosulphite), filled to 1 cm from the top
- ▶ **Sealed fill-hole plug** and cemented lid
- ▶ **Catalogue label**, tied to the specimen, bearing the unique accession number

6. Newer/alternative mounting techniques for a brain section

- ▶ **Sheet plastination** is the modern method of choice for a slice specimen: after deep-freezing (~24 h) a ~1 cm slice is cast between two 4 mm glass sheets separated by a 6–8 mm rubber-tubing spacer, filled with resin + catalyst + accelerator, cured for ~24 h, then unmoulded, trimmed, polished and labelled — giving a dry, odourless, unbreakable, totally portable sheet that shows internal relations without the drawbacks of a fluid-fixed wet mount (irritating odour, bleached colour, unnatural appearance, and difficulty appreciating luminal architecture).
- ▶ **Luminal cast plastination** is specifically useful for the brain's **cerebral ventricular system**: the ventricles are cleaned, soaked in KOH solution for 1–2 hours, cleaned again, and filled with a resin + catalyst + hardener mixture; after about a day the surrounding brain tissue is removed to leave a cast reproducing the exact dimensions and branching architecture of the ventricles.
- ▶ **Whole-organ plastination** (fixation in an alcohol–formalin–glycerine–phenol mixture, acetone dehydration, resin impregnation, curing) is used where the intact, unsectioned brain itself is to be preserved dry rather than a cut slice.

Applied / Clinical anatomy

- ▶ A mounted coronal/sagittal brain section is the standard museum-viva specimen for identifying the **lateral ventricles, basal nuclei (caudate, lentiform, claustrum), internal capsule, thalamus and corpus callosum** in their true spatial relationship — the same relations a resident must read off an axial/coronal CT or MRI.
- ▶ Correlating the mounted gross slice with its matching CT/MRI cut is core practical training for radiology postings and for planning image-guided neurosurgical approaches.
- ▶ A **luminal cast of the cerebral ventricles** teaches ventricular size, shape and communications (interventricular foramen, cerebral aqueduct) far more vividly than a flat slice — directly relevant to understanding hydrocephalus and CSF-flow obstruction at a given level.

- ▶ Because formalin fumes generated during fixation, colour-restoration and mounting are an irritant/occupational hazard, plastinated brain sections are increasingly preferred over classical wet mounts in teaching museums.
- ▶ Poor hardening before cutting, or a hesitant multi-stroke cut, distorts the true gyral and deep-nuclear anatomy and can mislead a candidate correlating the specimen against imaging — hence the emphasis on adequate, even fixation and a single clean stroke.

High-yield exam pointers

- ▶ Two-phase process: (1) **harden the whole brain first** (suspended/supported fixation in 10% formal-saline, ~3–4 weeks, ± direct injection of fixative) — **then** (2) cut and mount the slice.
- ▶ The brain is one of the organs the source specifically names as needing **injection with fixative** for adequate, even fixation.
- ▶ Cutting rule: **single continuous stroke** of a long, sharp brain knife — never a sawing action.
- ▶ Kaiserling's technique (1900): fixation (Solution I) → colour restoration with 80% ethyl alcohol (Solution II, 30 min–4 h) → mounting fluid (Solution III, glycerin + sodium acetate + formalin) with **0.4% sodium hydrosulphite** added just before sealing.
- ▶ **Pulvertaft's modification (1936)**: sodium hydrosulphite in the mountant gives colour retention with little fading even after 35 years.
- ▶ **Wentworth's method**: omits the alcohol step; mounting solution chosen by the specimen's pH (>6.5 → Solution III directly; <6.5 → Solution II first).
- ▶ Mounting: slice sutured to a **Perspex centre plate** (nylon/linen-and-bead thread, reef knot) inside a Perspex box, or sealed in a glass jar with asphaltum-rubber (picein) compound; box filled to 1 cm from the top, bubbles removed, fill-hole plugged and cemented.
- ▶ Modern alternative: **sheet plastination** of the slice, or **luminal cast plastination** specifically for the cerebral ventricles.
- ▶ Every mounted specimen carries a unique accession number in a reference register and a label tied directly to the specimen.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (fixation of museum specimens including the specific rule on injecting the brain with fixative; Kaiserling–Pulvertaft–Wentworth colour-restoration and mounting-fluid technique; centre-plate/Perspex-box and glass-jar mounting and sealing procedure; sheet, whole-organ and luminal-cast plastination). BD Chaurasia — Human Anatomy Vol 3-4, 8e (real plate of brain sections in different planes). Vishram Singh — Clinical Neuroanatomy, 4e and IB Singh — Textbook of Human Neuroanatomy (coronal-section structures — thalamus, corpus callosum, basal nuclei — used to specify what a mounted brain slice displays). **Coverage note:** whole-brain hardening/suspension technique and standard slice planes are added as universally accepted gross-anatomy/dissection-hall practice; the corpus's dedicated museum-technique text (Haldar) covers general specimen fixation, colour restoration and mounting in depth but does not describe brain-specific hardening in a separate named section — the Handbook of Museum Technique (Govt Museum, Chennai) was checked but is a zoological/taxidermy manual and was not used to source facts here.



Plastination technique

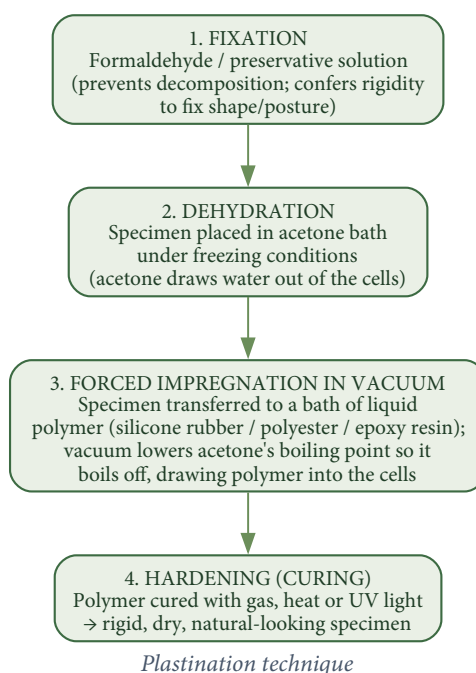
Asked in: Paper III May 2011 — RGUHS MD/MS Anatomy

Definition / Introduction Plastination is a modern museum/preservation technique in which the water and lipid of a fixed tissue are replaced, molecule for molecule, by a curable polymer (silicone rubber, epoxy or polyester resin) under forced vacuum impregnation, and the polymer is then hardened in situ. The end product is a **dry, non-toxic, odourless, durable, naturalistic (life-like) specimen** that retains gross structural detail indefinitely without the fumes, colour bleaching and handling problems of conventional formalin-wet museum jars. The method was devised by Gunther von Hagens (Heidelberg, Germany) in the late 1970s and has since become a standard adjunct to embalming in anatomy museum and dissection-hall preparation.

Principle

- ▶ Cellular and tissue water/lipid is an **exchange medium** — it is removed by dehydration and replaced by a liquid, curable polymer.
- ▶ Impregnation is driven under **vacuum**: lowering ambient pressure lowers the boiling point of the dehydrating agent (acetone), so it boils out of the tissue at a low temperature, and as it leaves the cells it draws the liquid polymer in behind it ("forced impregnation").
- ▶ The polymer is finally **cured/hardened** (by catalyst-accelerator reaction, heat, or UV/gas curing depending on the polymer used), fixing it permanently within the tissue architecture.

Steps of (whole body/organ) plastination



- ▶ **Fixation** — formaldehyde or another preservative solution halts autolysis/putrefaction and may confer enough rigidity to fix a desired shape or posture (e.g. an inflated stomach, a knee bent for a joint demonstration).
- ▶ **Dehydration** — after any necessary dissection, the specimen is immersed in an **acetone bath under freezing conditions**; acetone progressively draws all cellular water out of the tissue.
- ▶ **Forced impregnation in vacuum** — the dehydrated specimen is placed in a bath of liquid polymer (silicone rubber, polyester or epoxy resin); a vacuum is applied so the residual acetone boils at a low temperature and is drawn out, pulling the liquid polymer into the tissue spaces vacated by the acetone.
- ▶ **Hardening/curing** — the impregnated polymer is cured (gas-, heat- or UV-cured, according to the polymer system used) to harden it permanently, yielding the final dry specimen.

Types of plastination

Type	Method (brief)	Chief use
Whole organ plastination	Wash → fixation (95% alcohol 280 ml + formalin 1320 ml + glycerine 80 ml + phenol 120 ml + distilled water 800 ml; 2–3 days) → wash → acetone (dehydration) → immersion in resin bath 1–2 weeks (impregnation) → final immersion in general-purpose resin + catalyst (5%) + accelerator (0.1%) until non-sticky → mount on base	Demonstrates the total structure and spatial relationships of a whole organ

Type	Method (brief)	Chief use
Sheet plastination	Deep-freeze 24 h → cut with a band saw into ~1 cm (or thinner, transparent) slices → cast between two 4 mm glass sheets separated by 6–8 mm rubber tubing/clips, immersed in resin + catalyst + accelerator → cure 24 h → trim, polish, label	Thin transparent or thick opaque whole-body sections ; totally portable, storable dry; best demonstrates internal regional relationships
Luminal cast plastination	Clean lumen under tap water → KOH solution 1–2 h → wash and dry → fill lumen with resin + catalyst + hardener → after 1 day remove surrounding tissue by digestion, leaving the cast	Studies dimensions, architecture and branching of cavities/tubular structures — e.g. tracheobronchial tree, cerebral ventricles, bony labyrinth, vascular pattern of kidney/liver/lung/spleen, coronary vessels

□ Fig 66.49 — A transilluminated 2.5 mm thick coronal section of a male pelvis processed for epoxy resin E12 sheet plastination, showing the entire length of the anal canal and its muscular layers (Gray's Anatomy — Standing — 41e, p.1600)

Materials used

- ▶ Jars for storage and processing.
- ▶ **Acetone** (dehydrating/intermediary agent).
- ▶ **Silicone rubber**, polyester or epoxy resin (impregnating polymer).
- ▶ Catalyst and accelerator (to initiate and speed polymer curing).
- ▶ Colour paints (to restore/enhance natural colour).
- ▶ Dissection instruments; syringes and needles (for luminal filling).

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A vertical flow-box diagram of the four steps of plastination — label in sequence: **Fixation** (formaldehyde/preservative — arrests decomposition, fixes posture) → **Dehydration** (acetone bath, freezing conditions — extracts cellular water) → **Forced impregnation in vacuum** (liquid polymer bath — silicone/polyester/epoxy resin drawn into tissue as acetone boils off under vacuum) → **Hardening/curing** (gas, heat or UV) → final dry, life-like specimen. Alongside, sketch the three type-icons — a whole solid organ (whole-organ plastination), a thin glass-cast body slice (sheet plastination), and a branching tubular cast with the surrounding organ removed (luminal cast plastination).

Advantages of plastination over conventional (formalin-wet) museum technique

- ▶ Produces **dry, coloured, non-toxic, durable, odourless, naturalistic** specimens — no irritant formalin fumes on handling.
- ▶ Overcomes the classical drawbacks of routine formalin-fixed specimens: irritating odour, bleached/colourless tissue, non-naturalistic appearance, difficulty maintaining sections, and

near-impossibility of appreciating true **luminal architecture, dimensions and branching patterns** on wet dissection.

- ▶ Specimens can be freely handled by students without gloves, without deterioration or leakage.
- ▶ Provides internationally comparable specimen quality at **low cost** and with a **simple protocol**.
- ▶ Long shelf-life with minimal maintenance/storage requirements (no fluid top-up, no jar leakage).

Feature	Conventional formalin-wet specimen	Plastinated specimen
Physical state	Wet, immersed in fluid-filled jar	Dry, freely handled
Odour	Irritating (formalin fumes)	Odourless
Colour	Bleached, colourless with time	Natural/painted, retained long-term
Luminal/branching detail	Difficult to appreciate on dissection	Well shown (sheet/luminal-cast types)
Durability	Deteriorates, jar leakage, needs fluid top-up	Very durable, minimal maintenance
Handling by students	Needs gloves; fluid spillage risk	Direct handling, no biohazard
Cost/protocol	Simple but recurring (fluid, jars)	Simple protocol; low recurring cost once processed

Limitations of plastination itself: the process is comparatively **time-consuming** (impregnation may take days to weeks) and requires a **vacuum chamber, curing polymers and trained technical personnel**, so initial set-up cost is higher than a simple formalin jar even though long-term maintenance cost is lower.

Applied / Clinical anatomy

- ▶ **Teaching:** plastinated organs, sheet sections and luminal casts give trainees an appreciation of three-dimensional relationships and branching patterns that are difficult to visualise on conventional wet dissection — the mainstay use in anatomy museums and dissection halls.
- ▶ **Designing new surgical procedures and surgical skills practice:** durable, life-like plastinated specimens (and whole-body plastinates) are used to plan and rehearse operative approaches without the biohazard and short shelf-life of wet cadavers.
- ▶ **Correlative/research anatomy:** sheet plastination sections are used to correlate gross topography with cross-sectional imaging — e.g. epoxy-resin (E12) sheet-plastinated pelvic sections have been used to correlate the anatomy of the anal sphincter complex with endoanal ultrasonography (illustrated above).
- ▶ **Occupational safety:** because plastination specimens are dry and odourless, they eliminate the formaldehyde-fume exposure risk associated with routine wet museum jars and embalmed cadavers.
- ▶ **Public/medical education:** whole-body plastination underlies popular anatomical exhibitions, extending anatomical teaching beyond the dissection hall.
- ▶ **Luminal casts** are particularly valuable for demonstrating vascular/ductal anomalies (renal, coronary, bronchial) that are otherwise hard to teach from a fixed cadaver.

High-yield exam pointers

- ▶ Plastination = replacement of tissue water/lipid by a curable polymer (silicone/epoxy/polyester) under forced vacuum impregnation, followed by curing.
- ▶ Four steps: **Fixation** → **Dehydration (acetone)** → **Forced impregnation in vacuum** → **Hardening (curing)**.
- ▶ Three types: **Whole organ**, **Sheet**, **Luminal cast** plastination — know one clinical/teaching use for each.
- ▶ Materials: acetone (dehydration), silicone rubber/resin + catalyst + accelerator (impregnation/curing), colour paints, syringes/needles.
- ▶ Chief advantage: dry, odourless, non-toxic, durable, naturalistic specimen — overcomes formalin's fume, colour-bleaching and luminal-visualisation limitations.
- ▶ Best suited for teaching, surgical-procedure design/skills practice, and research; low cost, simple protocol.
- ▶ Luminal cast plastination specifically demonstrates cavity/duct dimensions and branching (bronchial tree, ventricles, bony labyrinth, vasculature).

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (primary source for principle, types, materials, steps and advantages of plastination); Gray's Anatomy (Standring) 41e (source of the embedded sheet-plastination figure and its applied correlative-anatomy use).



Preparation for Sectional Anatomy Museum specimens

Asked in: Paper III 18-Jul-2020 — RGUHS MD/MS Anatomy

Definition / Introduction A sectional (cross-sectional) anatomy museum specimen is a permanently preserved slice — thin and transparent, or thick and opaque — cut through a fixed or frozen cadaver, limb, or organ in a standard anatomical plane, so that the internal spatial relations of structures are displayed exactly as they would appear on a matching CT, MRI or ultrasound cut. Because they bridge gross anatomy and cross-sectional imaging, such specimens occupy a distinct place in the museum alongside wet-dissected specimens, prosecutions and osteology sets. Two broad approaches make the cut slice permanent: the classical **wet (fluid-mounted) method** — fixation, colour restoration and sealed-fluid mounting — and the modern **plastination method**, of which **sheet plastination** is the technique specifically designed for sectional slices.

1. Rationale and uses of sectional museum specimens

- ▶ Correlate normal gross topography with the axial/coronal/sagittal CT, MRI and USG images students meet in radiology postings.
- ▶ Demonstrate luminal and spatial relations of vessels, ducts and nerve bundles that layer-by-layer dissection cannot show as clearly.

- ▶ Provide durable, colour-retained, odourless teaching material for repeated handling by successive batches — unlike a routinely formalin-fixed wet specimen, which the source material notes has an irritating odour, bleached colourless parts and an unnatural look, and does not let the viewer imagine true luminal architecture, dimensions or branching patterns.
- ▶ Support museum-viva/OSPE stations and self-study of regional anatomy.
- ▶ Aid planning of image-guided and minimally invasive surgical approaches, and forensic reconstruction.

□ Fig 20.1 — ■ Cross section of the thorax showing the (Vishram Singh — Textbook of Anatomy Vol 1 — 4e, p.790)

2. Selection, fixation and orientation of the specimen

- ▶ The specimen is an unclaimed cadaver received under the applicable state Anatomy Act (e.g. the Bombay Anatomy Act, 1949) or a donated body/organ; either formalin-fixed or fresh (unembalmed, deep-frozen) tissue may be used — fresh-frozen tissue is often preferred for slices meant to correlate with CT/MRI, since fixation slightly shrinks and hardens soft tissue.
- ▶ Where fixation is used, **primary fixation is in 10% formal saline**; specimens should be injected with fixative where possible (e.g., the brain) to ensure uniform penetration, and blood-laden specimens must never be washed in water either before or after fixation.
- ▶ The plane of section (transverse/axial, coronal, or sagittal) is chosen to match the imaging plane being taught, and the specimen is oriented and marked for right/left and correct anatomical position before cutting.
- ▶ Cut surfaces are made smooth and even with a single continuous stroke of a long, sharp-bladed knife — careless removal or an unsteady cut easily ruins a specimen.

3. Freezing and slicing technique

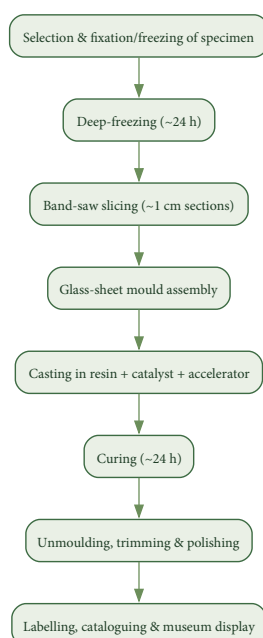
- ▶ The specimen is **deep-frozen for about 24 hours** to give it uniform rigidity suitable for cutting.
- ▶ Frozen parts are then cut with a **band saw**, conventionally into **slices about 1 cm thick**; thinner cuts yield transparent sheet sections, thicker cuts yield opaque block sections.
- ▶ Each slice is numbered sequentially in cranio-caudal or anteroposterior order so the series can be reassembled or displayed correctly — the same "freeze-slice-preserve" logic underlies large digital cross-sectional atlases built by serial cryosectioning of whole cadavers (see Applied anatomy below).

4. Methods of permanently preserving the cut section

Method	Principle	Key steps	Result
Wet (Kaiserling–Pulvertaft) mounting	Fixation + colour restoration + sealed fluid mounting	Fix in Kaiserling's fluid I → restore colour in fluid II (80% ethyl alcohol, 30 min–4 h) or by sodium hydrosulphite in the mounting fluid → mount in a fluid-filled perspex box/glass jar	Natural colour well retained (Pulvertaft's own specimens showed little fading even after 35 years); needs periodic fluid maintenance

Method	Principle	Key steps	Result
Sheet plastination	Deep-frozen slice impregnated with a curable resin between glass plates	Freeze 24 h → band-saw slice (~1 cm) → cast in a glass-sheet mould with resin + catalyst + accelerator → cure ~24 h → trim & polish	Thin/thick, dry, odourless, unbreakable, totally portable sheet — the standard technique for sectional anatomy specimens
Whole-organ / solid-block plastination	Water and lipids in fixed tissue are replaced by a curable polymer under vacuum	Fixation → acetone dehydration → forced impregnation with silicone rubber/polyester/epoxy resin under vacuum → curing by gas, heat or UV light	Dry, coloured, non-toxic, natural-looking whole-organ specimen retaining 3-D relations
Luminal cast plastination	Lumen is filled with resin and the surrounding tissue is digested away	Clean lumen → KOH solution 1–2 h → fill lumen with resin + catalyst + hardener → recover cast after ~1 day	Shows dimensions/branching architecture of ducts and vessels — tracheobronchial tree, cerebral ventricles, bony labyrinth, renal/hepatic/coronary vascular pattern

5. Step-by-step protocol — sheet plastination (the method of choice for sectional specimens)



Preparation for Sectional Anatomy Museum specimens

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

Label a sketch of the **sheet-plastination mould assembly**: (1) two glass sheets, 4 mm thick; (2) a rubber-tubing spacer, 6–8 mm in diameter, running round the margin between the plates; (3) clips holding the two glass sheets together to form a leak-proof chamber; (4) the ~1 cm frozen tissue slice placed centrally in the chamber; (5) the resin + catalyst + accelerator mixture filling the chamber around the slice; (6) the finished cured sheet after the clips are removed and the glass plates separated, with edges trimmed, polished and labelled.

6. Mounting, labelling and storage (wet-mounted specimens)

- ▶ After fixation and colour restoration, the slice is laid on a flat, formica-covered bench in correct anatomical position; it is measured allowing 1 cm clearance at the top and sides and 2 cm at the bottom (for the label), and a perspex box or glass jar of matching size and depth (+5 mm) is prepared or ordered.
- ▶ The slice is attached to a centre plate through drilled holes with nylon thread (two 1 mm holes) or linen thread with a retaining glass bead (one hole), tied with a reef knot, or cemented onto perspex spikes; the plate is then fitted into the box.
- ▶ The box/jar is filled to within 1 cm of the top with mounting fluid to which 0.4% sodium hydrosulphite has been added immediately before sealing (this reducing agent restores/retains natural colour); trapped air bubbles are expressed, and the container is sealed — perspex boxes with perspex cement, glass jars with a heated asphaltum-rubber (picein) compound, with the sealed edge later painted with black enamel/asphaltum varnish.
- ▶ Each specimen is given a unique identification number recorded in a reference/catalogue book and stored in a separate container to avoid damage from contact with adjacent specimens.

Applied / Clinical anatomy

- ▶ Sectional museum specimens are the anatomical reference against which trainees learn to interpret axial CT/MRI/USG cuts — essential for radiology postings and image-guided, minimally invasive surgery.
- ▶ Large digital cross-sectional atlases (e.g. the Visible Human Project of the US National Library of Medicine) apply the same freeze–slice–photograph principle at whole-body scale, milling serial cryosections of a cadaver and photographing each cut face to build a 3-D reference dataset.
- ▶ Luminal cast specimens (bronchial tree, renal/hepatic/coronary vascular pattern, cerebral ventricular cast, bony labyrinth) supplement flat sectional slices by showing the three-dimensional ductal/vascular architecture a single 2-D slice cannot.
- ▶ Plastinated sections are increasingly favoured over wet-mounted sections because they are odourless, non-toxic, unbreakable and need no fluid top-up — relevant where formalin exposure and disposal are regulated.
- ▶ Poor freezing or an unsteady cut distorts true anatomical relations and can mislead radiology correlation, so meticulous freezing and a single continuous cutting stroke are stressed at every stage.

High-yield exam pointers

- ▶ Sectional museum specimen = a preserved slice of fixed/frozen tissue cut in a standard plane (axial/coronal/sagittal) to show spatial relations correlating with cross-sectional imaging.
- ▶ Two families of technique: (1) wet fluid-mounted (Kaiserling–Pulvertaft/Wentworth) and (2) plastination (sheet / whole-organ / luminal cast).
- ▶ Sheet plastination steps: deep-freeze ~24 h → band-saw slice ~1 cm → mould between two 4 mm glass sheets with a 6–8 mm rubber-tubing spacer → cast in resin + catalyst + accelerator → cure ~24 h → trim, polish, label.
- ▶ Kaiserling's fluid I (fixing): formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + water to 2000 ml.
- ▶ Pulvertaft's mounting fluid III (glycerin, sodium acetate, formalin, water) with 0.4% sodium hydrosulphite added just before sealing restores/retains natural colour; Wentworth's modification omits the alcohol colour-restoration step.
- ▶ Luminal cast plastination: fill lumen with resin, digest surrounding tissue with KOH — used for bronchial tree, cerebral ventricles, bony labyrinth, vascular casts.
- ▶ Drawback of a plain formalin-fixed wet section: irritating odour, bleached colour, unnatural appearance, difficult to maintain, and luminal architecture almost impossible to appreciate — the reason plastination is preferred for sectional work.
- ▶ Real-world large-scale example: the Visible Human Project (serial cryosectioning and photography of whole cadavers).

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (Museum Techniques chapter: preparation and fixation of museum specimens, Kaiserling–Pulvertaft and Wentworth wet-mounting methods, mounting/sealing in perspex boxes and glass jars, and plastination — whole-organ, sheet and luminal-cast — including whole-body plastination steps). Vishram Singh — Textbook of Anatomy Vol 1, 4e (cross-sectional figure). Coverage note: the Visible Human Project and CT/MRI cross-sectional correlation are added as universally accepted, standard curriculum knowledge supplementing the corpus.



Preparation of museum specimen from dissected femoral triangle

Asked in: Paper III Sep 2007 — RGUHS MD/MS Anatomy

Definition / Introduction — A museum specimen of the femoral triangle is a permanently preserved, gross-dissected preparation of the antero-medial upper thigh that displays, in their natural anatomical position, the boundaries, roof, floor and contents (femoral sheath, femoral artery, vein, nerve, and lymph nodes) of this clinically important region. Its preparation has two inseparable components: (1) a **meticulous dissection** that lays open every boundary and content of the triangle without disturbing their mutual relations, and (2) a standard **wet-museum preservation technique** — fixation, colour restoration and mounting in a sealed, fluid-filled container — so that the dissected relations remain permanently visible for undergraduate and postgraduate teaching. A good specimen retains the original size, shape and colour of the tissue, and is accompanied by correlating labelling, cataloguing and (where possible) a photograph, since a museum functions as much as a permanent teaching record as a display.

Anatomy to be demonstrated: boundaries, roof, floor and contents of the femoral triangle

Feature	Detail
Site	A triangular, depressed intermuscular space on the antero-medial aspect of the upper one-third of the thigh, immediately distal to the inguinal ligament
Base	Inguinal ligament
Lateral boundary	Medial border of sartorius
Medial boundary	Medial border of adductor longus
Apex	Directed downwards, where sartorius crosses (overlaps) adductor longus; continuous below with the adductor canal
Roof	Skin; superficial fascia (containing superficial inguinal lymph nodes, femoral branch of the genitofemoral nerve, branches of the ilioinguinal nerve, superficial vessels and the upper great saphenous vein); deep fascia (fascia lata), including the cribriform fascia over the saphenous opening
Floor	Medially — pectineus and adductor longus; laterally — iliacus and psoas major
Principal contents	Femoral artery and its branches; femoral vein and tributaries; femoral sheath (with the femoral canal); femoral nerve and its branches; femoral branch of genitofemoral nerve; lateral cutaneous nerve of thigh (crossing the lateral angle); fat and lymph nodes

- The **femoral sheath** encloses the upper ~4 cm of the femoral vessels and is divided by septa into three compartments: lateral (femoral artery), intermediate (femoral vein), and medial (the

femoral canal, containing the lymph node of Cloquet/Rosenmüller, lymphatics and areolar tissue).

- ▶ The **femoral artery** enters at the mid-inguinal point (base) and leaves at the apex, giving six branches in the triangle — three superficial (superficial epigastric, superficial circumflex iliac, superficial external pudendal) and three deep (profunda femoris, deep external pudendal, muscular branches).
- ▶ The **femoral nerve** lies lateral to the artery, outside the femoral sheath, in the groove between iliacus and psoas major, and breaks up into its terminal branches on entering the triangle.

□ Fig 3.11c — Dissection of main contents of femoral triangle (BD Chaurasia — Human Anatomy Vol 2 — 8e, p.81)

DIAGRAMS TO DRAW — IMAGE SUGGESTIONS

A right femoral triangle dissection, labelled with:

- ▶ **Inguinal ligament** — base (superior boundary)
- ▶ **Sartorius** — lateral boundary (medial border)
- ▶ **Adductor longus** — medial boundary (medial border); apex where it crosses sartorius
- ▶ **Fascia lata / cribriform fascia** — roof, with the saphenous opening
- ▶ **Iliacus + psoas major** (lateral floor) and **pectineus + adductor longus** (medial floor)
- ▶ **Femoral nerve** (lateral, outside sheath) → its branches (nerve to pectineus, femoral branch of genitofemoral nerve, lateral cutaneous nerve of thigh)
- ▶ **Femoral sheath** with three compartments: **femoral artery** (lateral), **femoral vein** (intermediate), **femoral canal** with lymph node of Cloquet (medial)
- ▶ **Great saphenous vein** draining into the femoral vein through the saphenous opening
- ▶ Superficial and deep branches of the femoral artery, and superficial inguinal lymph nodes

Selection and dissection of the specimen

- ▶ Good museum specimens are obtained by **care and planning at the time of dissection** — careless removal or cutting can easily ruin an otherwise good specimen.
- ▶ The region is dissected in the standard sequence: skin and superficial fascia are reflected to expose the saphenous opening and superficial vessels/nodes, the deep fascia (fascia lata) is opened to reveal the boundaries, and the femoral sheath is carefully opened to display its three compartments without severing the femoral nerve, artery or vein.
- ▶ Cut surfaces should be smooth and even, produced by a **continuous stroke of a long-bladed sharp knife**; where extra tissue must be sampled (e.g., for histology), it is taken from the back of the piece to be preserved, or with a scalpel from the front, so the display face is undisturbed.
- ▶ To make the femoral artery and its branches stand out for teaching, the vessels are often **injected with a coloured mass** (red for arteries, blue for veins) before or during fixation; delicate structures such as the femoral nerve and small branches are later marked with coloured Perspex rods/arrows or black horse hair once mounted.
- ▶ The dissected block is placed into a **fixative almost immediately** after removal — a container of formal-saline should be kept ready at hand in the dissection room for this purpose.

Fixation of the specimen

- **Primary fixation is in 10% formal saline.** The specimen is subsequently transferred to a special fixative for long-term preservation and colour restoration.
- Additional rules of fixation apply to a vascular specimen such as this: the vessels should be **injected with fixative** if possible to ensure adequate penetration; a specimen containing much blood must **never be washed in water**, either before or after fixation; the fresh specimen is laid on a thick layer of **cotton wool covered with lint**; and the specimen is fixed **together with its attached structures** (nerve, vessels, fascia) so that natural relations are not lost.

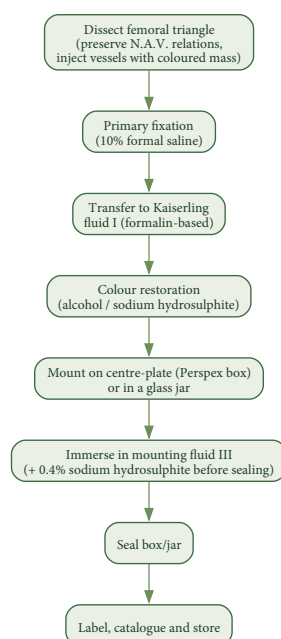
Colour restoration and mounting fluid

Formalin fixation alone bleaches the muscle and vessels to a grey-brown colour, so a dedicated **colour-restoring/mounting technique** is used — the classic method of **Kaiserling (1900)**, modified by **Pulvertaft (1936)** and later by **Wentworth (1938–1957)**.

Method	Solution I — fixing fluid	Solution II — colour restoration	Solution III — mounting fluid
Kaiserling (1900)	Original 3-solution technique: formalin-based fixing fluid	Restores original colour	Final mounting medium
Pulvertaft–Kaiserling	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 ml (specimen fixed directly in it, or transferred after formal-saline fixation)	Ethyl alcohol 80%, time carefully controlled (30 min–4 h); optional if a sodium-hydrosulphite mounting fluid is used, since continued alcohol immersion permanently bleaches tissue	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + tap water to 1000 ml; 0.4% sodium hydrosulphite (reducing agent) added immediately before sealing the jar
Wentworth (1957 method) — omits the alcohol step and the glycerol from the final mountant	Formaldehyde 40% 100 ml + sodium acetate 40 g + water to 1000 ml (fixation for ≥1 month)	Used if specimen pH < 6.5: formaldehyde 40% 10 ml + sodium acetate 40 g + Na ₂ HPO ₄ 1 g + water to 1000 ml	Used if pH ≥ 6.5: formaldehyde 40% 10 ml + sodium acetate 100 g + Na ₂ HPO ₄ 1 g + glycerine 200 ml + water to 1000 ml (sodium hydrosulphite 3 g/1000 g added fresh before sealing if the solution's own pH is ≥ 7.5)

- If the mounting solution is not crystal clear, it is filtered through a paper-pulp filter, and 30 ml of a saturated solution of camphor in alcohol is added per litre.
- Specimens mounted by Pulvertaft's sodium-hydrosulphite technique show remarkably little fading even after 35 years; **Israel & Young (1978)** used pure liquid paraffin as the final mountant after alcohol colour-restoration to reduce discolouration of the mounting fluid by leached pigments.

Mounting the specimen



Preparation of museum specimen from dissected femoral triangle

- **Centre-plate technique in a Perspex box:** the dissected triangle is laid on a flat, waterproofed bench in as close to an **anatomically correct position** as possible (as seen in situ), then measured, allowing 1 cm clearance at the top/sides and 2 cm at the bottom for a label. Crosses are scribed on the centre plate; stitches are placed to avoid the femoral vessels and nerve, using nylon thread (two 1 mm holes) or linen thread with a glass-bead retainer, tied with a reef knot. Museum fluid with 0.4% sodium hydrosulphite is run in to within 1 cm of the top, air bubbles are released with a broad spatula, and the lid is cemented, weighted for 1–3 hours, and left 24–48 hours before final topping-up and sealing.
- **Glass-jar method:** an alternative housing, drilled with an engraver's tool (camphor-in-turpentine lubricant) and sealed with an asphaltum-rubber (picein) compound flamed onto the ground edge; excess picein is trimmed and the join finished with black enamel/asphaltum varnish.
- Delicate structures of the specimen — the femoral nerve, its branches, and small vessels — are identified for the viewer using **coloured Perspex arrows/rods** or **black horse hair**, since these are easily lost to the eye once fixed and mounted.

Storage, labelling and cataloguing

- Every specimen is given a **unique identification number**, entered in a reference/catalogue book recording relevant details.
- Specimens are stored in **separate containers** to avoid damage from contact with other specimens; containers are labelled on the outside, with the label also tied to the specimen with linen thread.
- Good cataloguing — a correlating photograph, radiograph and descriptive label — is considered as important as the specimen itself, since the museum is also a permanent teaching and research record.

Applied / Clinical anatomy

- ▶ **Femoral hernia:** the femoral canal (medial compartment of the femoral sheath) is a site of potential weakness through which abdominal contents may herniate; it is more common in females owing to a wider femoral canal and pelvis. The **lacunar ligament**, forming the medial boundary of the femoral ring, must be incised with care during surgical enlargement because an **abnormal obturator artery** may occasionally run along its free margin.
- ▶ **Femoral pulse and compression:** the femoral artery is palpable at the mid-inguinal point (against the head of femur and psoas major tendon) and can be compressed here to control distal haemorrhage; a feeble or absent femoral pulse is a classic sign of **coarctation of the aorta**.
- ▶ **Vascular access:** the femoral triangle is the standard site for femoral artery/vein catheterisation (angiography, cardiac catheterisation, femoral line insertion) because the vessels are superficial and easily palpable/compressible.
- ▶ **Femoral nerve block and injury:** the femoral nerve, lying lateral to the sheath, is the target of femoral nerve blocks for hip/knee/thigh surgery; injury produces sensory loss over the anteromedial thigh and leg and weakness of knee extension (loss of patellar reflex).
- ▶ **Meralgia paraesthetica:** entrapment of the lateral cutaneous nerve of the thigh as it crosses the inguinal ligament near the lateral angle of the triangle causes pain/paraesthesia over the anterolateral thigh.
- ▶ **Saphenofemoral junction surgery:** the great saphenous vein's termination in the femoral vein at the saphenous opening (roof of the triangle) is the site of high ligation in varicose-vein surgery.
- ▶ **Legal aspects of specimen sourcing:** cadavers used for dissection and museum specimens are governed by the **Anatomy Act (1949)**, which permits use of an unclaimed body only when death occurs in a state hospital or public place within the prescribed zone, after police certify (following a 48-hour wait) that there are no claimants.

High-yield exam pointers

- ▶ Femoral triangle: base = inguinal ligament; lateral boundary = sartorius (medial border); medial boundary = adductor longus (medial border); apex = where the two cross, continuous with the adductor canal.
- ▶ Roof = fascia lata (+ skin, superficial fascia, cribriform fascia over saphenous opening); floor = iliacus + psoas major (lateral), pectineus + adductor longus (medial).
- ▶ Femoral sheath — 3 compartments: artery (lateral), vein (intermediate), femoral canal with lymph node of Cloquet/Rosenmüller (medial).
- ▶ Museum-technique sequence: dissect → fix in 10% formal saline → transfer to Kaiserling/Pulvertaft fluid → colour-restore (alcohol/sodium hydrosulphite) → mount on centre-plate or in a jar → seal → label/store.
- ▶ **Kaiserling (1900)** = original 3-solution technique; **Pulvertaft (1936)** adds sodium hydrosulphite to the mounting fluid; **Wentworth (1938–1957)** omits the alcohol step and selects solutions by specimen pH.
- ▶ Coloured vessel injection (arterial red, venous blue) and coloured Perspex rods/horse hair are used to make the femoral vessels/nerve stand out in the mounted specimen.

- Clinical triad to recall: femoral hernia (femoral canal), femoral pulse/coarctation, and femoral vessel catheterisation — all hinge on the triangle's contents and femoral sheath.

SOURCE & COVERAGE

Gray's Anatomy (Standring) 41e; BD Chaurasia — Human Anatomy Vol 2, 8e (femoral triangle anatomy, boundaries, contents and clinical anatomy); Haldar — Notes on Embalming & Museum Techniques (museum-specimen preparation, fixation, Kaiserling/Pulvertaft/Wentworth colour-restoration and mounting techniques, storage and legal aspects).



Wet anatomical museum preparation

Asked in: Paper III May 2018 — RGUHS MD/MS Anatomy

Definition / Introduction — Wet museum preparation is the technique of preserving a gross anatomical or pathological specimen, permanently immersed in a fixative-cum-mounting fluid inside a sealed transparent container (Perspex box or glass jar), so that its natural size, shape, colour and surface detail are retained for demonstration and teaching. It contrasts with dry preparation (maceration, corrosion casts) and with plastination, and remains the commonest method of building a departmental anatomy/pathology museum. A good wet specimen serves teaching, research, and as a permanent record of the natural history of disease, so accompanying photographs, radiographs, labelling and cataloguing are considered as important as the specimen itself.

Aims and requirements of an ideal museum specimen

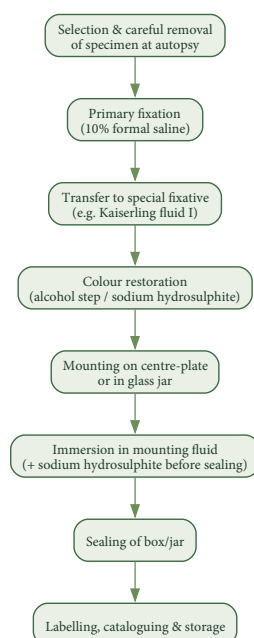
- Retains the **original shape, size and colour** of the tissue as closely as possible.
- Cut surface is **smooth, even and anatomically correct** in its mounted position.
- Is **durable** — resists fading, distortion and microbial spoilage over years of display.
- Is **adequately identified** — unique accession number, label, and an entry in a reference/catalogue book.
- Has genuine **teaching and research value**, ideally supplemented by a correlating photograph or radiograph.
- Is prepared and stored **safely and legally**, in line with the Anatomy Act, 1949, and hospital consent procedures.

Selection, preliminary care and fixation

- Good museum specimens are obtained by **care and planning at the time of autopsy/dissection**; careless removal or sectioning can easily ruin an otherwise good specimen.
- Cutting is done with a **long-bladed sharp knife using a single continuous stroke** so that the cut surface is smooth and even; any tissue needed separately (e.g., for histology) is sampled from the back of the piece to be preserved, or taken from the front with a scalpel, so the face to be displayed is undisturbed.
- The specimen must be placed into a **fixative almost immediately** after removal — containers of formal-saline are kept ready at hand in the operation theatre/dissection room for this purpose.

- ▶ **Primary fixation is in 10% formal saline.** The specimen is subsequently transferred to a special fixative for long-term preservation and colour restoration (see below).
- ▶ Additional rules of fixation:
 - Solid viscera should be **injected with fixative** wherever possible to ensure adequate penetration (e.g., the brain).
 - Specimens containing much blood must **never be washed in water**, either before or after fixation.
 - The fresh specimen is laid on a thick layer of **cotton wool covered with lint**.
 - The specimen is fixed **together with its attached structures**.
 - **Cystic cavities**, if unopened, are inflated; if opened, they are packed with cotton wool soaked in fixative, so as to maintain their natural shape.
 - **Bile-stained specimens** must be fixed and stored separately from other specimens.

Sequence of wet museum preparation



Wet anatomical museum preparation

Methods of colour maintenance

Formalin fixation alone bleaches tissue and turns it grey-brown, so a **colour-restoring/mounting technique** is needed for a display-quality wet specimen. The classic method is Kaiserling's (1900), later modified by Pulvertaft (1936) and by Wentworth (1938–1957).

Method	Solution I — fixing fluid	Solution II — colour restoration	Solution III — mounting fluid
Kaiserling (1900) — original 3-solution technique	Formalin-based fixing fluid	Restores original colour	Final mounting medium

Method	Solution I — fixing fluid	Solution II — colour restoration	Solution III — mounting fluid
Pulvertaft–Kaiserling	Formalin 400 ml + potassium nitrate 30 g + potassium acetate 60 g + tap water to 2000 ml (specimen fixed directly in this, or transferred to it after formal-saline fixation)	Ethyl alcohol 80%, time carefully controlled (30 min–4 h); optional if a sodium-hydrosulphite mounting fluid is used, since continued alcohol immersion permanently bleaches the tissue	Glycerin 300 ml + sodium acetate 100 g + formalin 5 ml + tap water to 1000 ml; 0.4% sodium hydrosulphite added immediately before sealing the jar as the reducing agent that restores colour
Wentworth (1957 method) — dispensed with the alcohol step; glycerol omitted from the final mountant	Formaldehyde 40% 100 ml + sodium acetate 40 g + water to 1000 ml (fixation for at least 1 month)	Formaldehyde 40% 10 ml + sodium acetate 40 g + Na ₂ HPO ₄ 1 g + water to 1000 ml (used if pH of the specimen is < 6.5)	Formaldehyde 40% 10 ml + sodium acetate 100 g + Na ₂ HPO ₄ 1 g + glycerine 200 ml + water to 1000 ml (used if pH ≥ 6.5, or if pH of solution III ≥ 7.5, sodium hydrosulphite 3 g/1000 g is added fresh just before sealing)

- ▶ If the mounting solution is not crystal clear, it is filtered through a paper-pulp filter, and 30 ml of a saturated solution of camphor in alcohol is added per litre to prevent fungal growth.
- ▶ **Israel & Young (1978)** used pure liquid paraffin as the final mountant after alcohol colour-restoration, which reduces discolouration of the mounting fluid by pigments leaching from the specimen.
- ▶ Specimens mounted by Pulvertaft's sodium-hydrosulphite technique show remarkably little fading even after 35 years.

Mounting techniques

Two principal methods of housing the fixed, colour-restored specimen are used.

A. Centre-plate technique in a Perspex box

- ▶ Perspex boxes are used almost universally today, either bought commercially or made in the laboratory (lab-made boxes can be tailored exactly to each specimen); they are assembled either by cementing four sides plus a top and bottom together with Perspex cement, or by bending a single Perspex strip to form the top and sides.
- ▶ A coloured, opaque Perspex **centre plate** is used to enhance the colour of the specimen, or to allow specimens to be attached on both faces; most specimens are simply stitched to the centre plate, which fits into the box with about 5 mm clearance at the top.
- ▶ The specimen is laid on a flat, waterproofed (formica-covered) bench in as close to an **anatomically correct position** as possible, then measured allowing 1 cm clearance at the top and sides and 2 cm at the bottom (space for a label), with about 5 mm added to the measured depth for the box.
- ▶ **Attaching the specimen:** crosses are scribed on the centre plate at the chosen points; the number of stitches depends on the weight and consistency of the tissue, and stitches are never placed

through a pathological lesion. Holes of 1–2 mm diameter are drilled — two 1 mm holes for nylon thread, or a single hole with a linen thread and a glass bead (the bead is slightly larger than the hole and acts as a retainer) for linen thread. Threads emerge from the specimen about 1 cm apart to form a "V" of tissue, and are tied with a reef knot, which holds tightly without slipping. An alternative is to cement Perspex spikes to the plate and press the specimen onto them.

- ▶ **Filling and sealing:** museum fluid containing 0.4% sodium hydrosulphite is run in to within 1 cm of the top, and trapped air bubbles are released with a broad-bladed spatula. A 3 mm hole is drilled in a corner of the lid for topping up; the lid is cemented with Perspex/ethylene-dichloride cement, weighted down for at least 1 hour (preferably 2–3 hours), and left for 24–48 hours before the fill is topped up through the hole and the plug cemented shut.

B. Glass-jar method

- ▶ Specimens are mounted in the jar in the same way as on a centre plate, except that the holes are drilled with an engraver's tool, using camphor dissolved in turpentine as a lubricant, with the glass sheet resting on an absolutely flat surface while drilling.
- ▶ The jar is sealed with an **asphaltum-rubber compound (picein)**, flamed with a Bunsen burner until it runs completely over the ground edge; the specimen is placed in the jar, which is filled with mounting fluid to within 1 cm of the top, and the heated lid is pressed firmly onto the jar using a cloth to hold it.
- ▶ After the seal has set, excess picein is trimmed away and the join is painted over with a black enamel/asphaltum varnish for a finished appearance.

Handling of special specimens, storage and cataloguing

- ▶ **Cystic/hollow organs:** inflated if unopened, or packed with fixative-soaked cotton wool if opened, to preserve their natural contour.
- ▶ **Bile-stained specimens:** fixed and stored separately, and soaked in a saturated solution of calcium chloride for 24 hours before final mounting.
- ▶ **Friable specimens:** covered with a thin layer of arsenious-acid gelatin for support; the same gelatin is used locally to hold loose fragments (e.g., a blood clot) in position, and to fill natural cavities once the packing cotton wool has been removed.
- ▶ **Delicate structures** (small vessels, nerves, ducts) are identified for the viewer using coloured Perspex arrows or rods, or black horse hair.
- ▶ **Storage** follows fixed principles: each specimen carries a unique identification number; a reference book records the relevant details of every specimen; specimens are stored in separate containers to prevent damage from contact with one another; and containers are labelled on the outside, with the label additionally tied to the specimen with linen thread.
- ▶ Good cataloguing — accompanying photographs, radiographs, descriptive labelling — is considered as important as the specimen, since a museum also functions as a permanent record of the natural history of disease.

Applied / Clinical anatomy

- ▶ **Legal aspects:** specimens sourced from unclaimed bodies for teaching/museum purposes are governed by the **Anatomy Act (1949)**, which permits collection of a body for anatomical

examination and dissection only when death occurs in a state hospital or a public place within the prescribed zone of the institute, and only after the police have certified, following a 48-hour wait, that there are no claimants. Proper identification, written consent, and (in medicolegal cases) completion of autopsy must precede any fixation/embalming, since embalming destroys toxicological evidence, prevents bacteriological/blood-group evaluation, and alters the interpretation of wounds.

- **Teaching/diagnostic value:** because natural size, shape and colour are retained, a well-mounted wet specimen remains unmatched for demonstrating gross and pathological anatomy to UG/PG students, and is best displayed alongside a correlating photograph or radiograph of the same lesion.
- **Limitations:** the routine formalin-fixed wet technique has an irritating odour, bleaches soft tissue to a colourless appearance if colour restoration is skipped, gives a non-naturalistic look to the specimen, and makes it difficult to maintain thin sections or to visualise the luminal architecture and branching pattern of tubular/ductal structures — this is why **plastination** (a dry, odourless, durable technique) has increasingly supplemented, though not replaced, wet museum preparation in modern departments.

High-yield exam pointers

- Primary fixation is always **10% formal saline**; the specimen is then transferred to a special fixative for permanent preservation and colour restoration.
- **Kaiserling (1900)** — original technique uses three solutions: fixing fluid, colour-restoring fluid, mounting fluid.
- **Pulvertaft's modification (1936)** — adds the reducing agent **sodium hydrosulphite** to the mounting fluid to restore colour; specimens show little fading even after 35 years.
- **Wentworth's modification (1938–1957)** — omits the alcohol colour-restoration step and the glycerol from the final mountant; choice of solution is guided by the specimen's pH after ≥ 1 month of fixation.
- Two standard mounting methods: **Perspex box with centre-plate** (specimen stitched with nylon or linen-and-glass-bead thread, reef knot) and **sealed glass jar** (asphaltum-rubber/picein seal).
- Special handling: cystic cavities inflated/packed with fixative-soaked cotton; bile-stained specimens soaked in saturated CaCl_2 ; friable areas supported with arsenious-acid gelatin; delicate structures marked with coloured Perspex rods/arrows or horse hair.
- Every specimen requires a unique ID number, an entry in a reference/catalogue book, individual storage, and adequate external labelling.
- The **Anatomy Act (1949)** is the legal basis for sourcing unclaimed bodies for anatomical/museum specimens.

SOURCE & COVERAGE

Haldar — Notes on Embalming & Museum Techniques (primary source for the wet-preparation technique, the Kaiserling/Pulvertaft/Wentworth fixation-mounting methods, centre-plate and glass-jar mounting, storage and legal aspects).

NOTE — FOR REFERENCE, NOT TO BE WRITTEN IN THE EXAM

This is a museum-preservation/technique topic that is not covered in Gray's Anatomy (Standring), the spine text for this bank; the answer is grounded entirely in the dedicated preservation reference text (Haldar) per the topic spec's grounding hint.

