Lecture 1: Histology, Cells and Basic Tissues.

Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs. This subject involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ. Histology, or microscopic anatomy, can be known as a visual, colorful science.

With the simplest light microscopes, examination of mammalian cells showed a nucleus and a cytoplasm, surrounded by cell membrane. Much initial information in histology was gained by examining tissue slides with a light microscope, its resolving power was too limited, but with the advent of transmission electron microscopy, superior resolution, and higher magnification of cells, examination of the contents of the cytoplasm became possible. Histologists are now able to describe the ultrastructure of the cell, its membrane, and the numerous organelles that are present in the cytoplasm of different cells.

Tissues have two interacting components: **cells and extracellular matrix (ECM)**. The ECM consists of many kinds of macromolecules, most of which form complex structures, such as collagen fibrils. The ECM supports the cells and contains the fluid transporting nutrients to the cells, and carrying away their wastes and secretory products. Cells produce the ECM locally and are in turn strongly influenced by matrix molecules. Many matrix components bind to specific cell surface receptors that span the cell membranes and connect to structural components inside the cells, forming a continuum in which cells and the ECM function together in a well coordinated manner. During development, cells and their associated matrix become functionally specialized and give rise to fundamental types of tissues with characteristic structural features. Organs are formed by an orderly combination of these tissues, and their precise arrangement allows the functioning of each organ and of the organism as a whole.

Cell Differentiation

The average adult human body consists of nearly 40 trillion cells. These cells exist as hundreds of histologically distinct cell types, all derived from the zygote. The first zygotic cellular divisions produce cells called blastomeres, and as part of the early

embryo's inner cell mass blastomeres give rise to all tissue types of the fetus. Explanted to tissue culture cells of the inner cell mass are called embryonic stem cells. Most cells of the fetus undergo a specialization process called differentiation in which they predominantly express sets of genes that mediate specific cytoplasmic activities, becoming efficiently organized in tissues with specialized functions and usually changing their shape accordingly. For example, muscle cell precursors elongate into long, fiber-like cells containing large arrays of actin and myosin. All animal cells contain actin filaments and myosins, but muscle cells are specialized for using these proteins to convert chemical energy into forceful contractions.

Cell components

The plasma membrane

- The plasma membrane (or plasmalemma) is the lipid bilayer with embedded proteins that surrounds a cell and is seen only with the TEM.
- The lipid bilayer forms from amphipathic phospholipids, stabilized by cholesterol, and contains many embedded (integral) proteins and many peripheral proteins on its cytoplasmic surface (figure 1).
- Membrane proteins move laterally within the lipid bilayer, with less movement in areas referred to as lipid rafts, which have higher concentrations of cholesterol and saturated fatty acids.
- Integral membrane proteins include receptors for external ligands, channels for passive or active movement of molecules across the membrane, and pumps for active membrane transport (figure 2).
- Endocytosis is cellular uptake of macromolecules or fluid by plasma membrane engulfment or invagination, followed by the "pinching off " of a filled membranous vesicle in the cytoplasm.
- Major types of endocytosis include phagocytosis (uptake of particulate material), pinocytosis (uptake of dissolved substances), and receptor-mediated endocytosis (uptake of specific molecules bound to integral membrane receptor proteins).
- Exocytosis is a type of cellular secretion in which cytoplasmic membrane vesicles fuse with the plasma membrane and release their contents to the extracellular space.

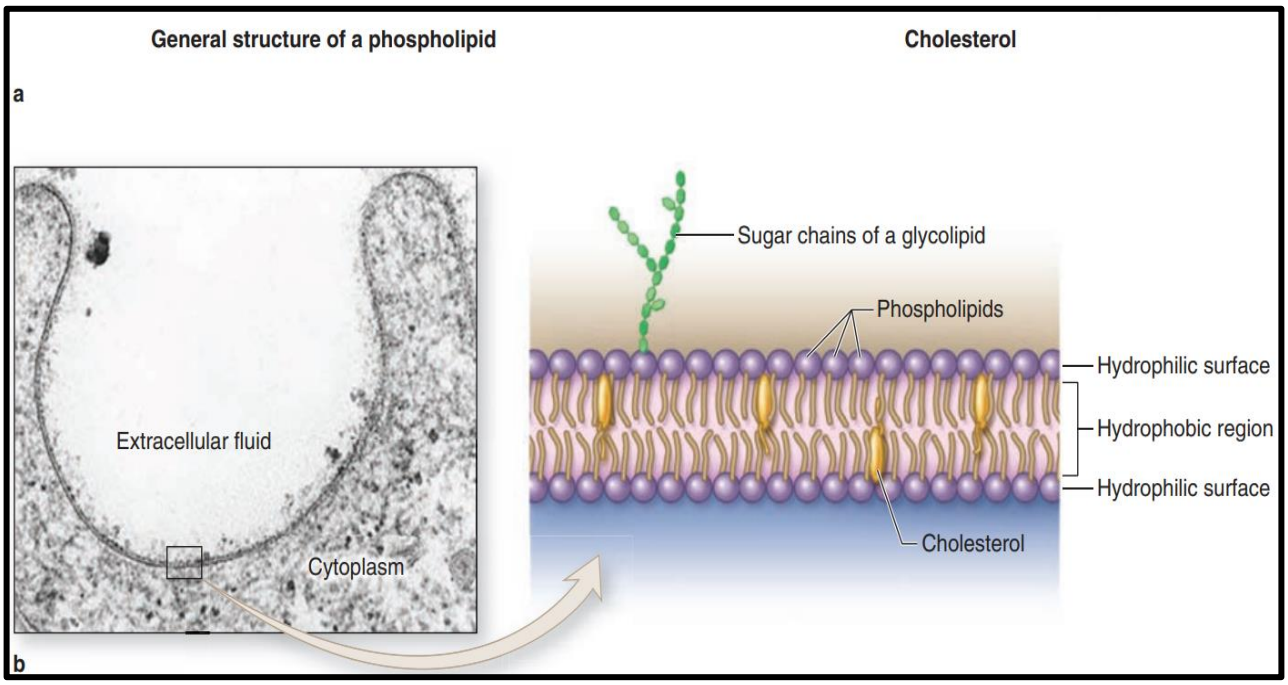

Figure 1: The plasma membrane.

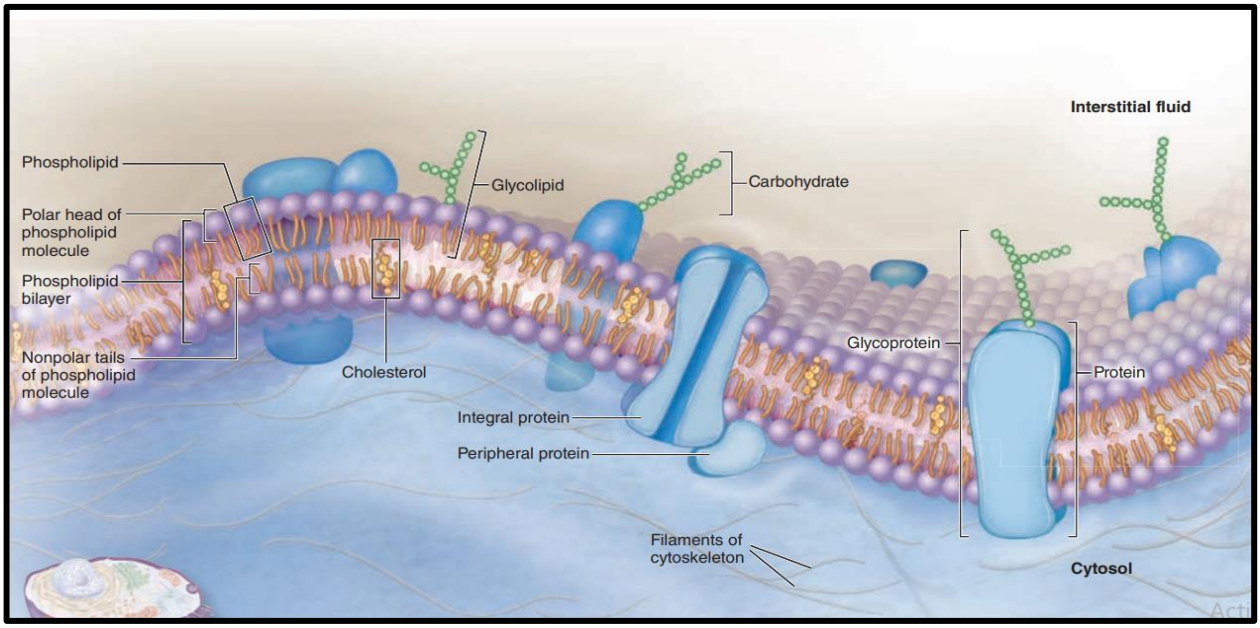

Figure 2: Proteins that make up the plasma membrane.

Cytoplasmic cell organelles

Inside the cell membrane the fluid cytoplasm (or cytosol) bathes metabolically active structures called organelles, which may be membranous (such as mitochondria) or nonmembranous protein complexes (such as ribosomes and proteasomes). Most organelles are positioned in the cytoplasm by movements along the polymers of the cytoskeleton, which also determines a cell's shape and motility. Cytosol also contains hundreds of enzymes, such as those of the glycolytic pathway. Oxygen, CO_2,

electrolytic ions, low-molecular-weight substrates, metabolites, and waste products all diffuse through cytoplasm, either freely or bound to carrier proteins.

➢ The Nucleus

The nucleus usually appears as a large rounded or oval structure, often near the cell's center (**Figure 3**). Typically the largest structure within a cell, it consists of a nuclear envelope containing chromatin, the mass of DNA and its associated proteins, with one or more specialized regions of chromatin called nucleoli. In specific tissues, the size and shape of nuclei normally tend to be uniform.

By Containing the code for all of a cell's enzymes and other proteins, the nucleus is the command center of the cell. The nucleus also contains the molecular machinery to replicate the DNA and to synthesize and process all types of RNA.

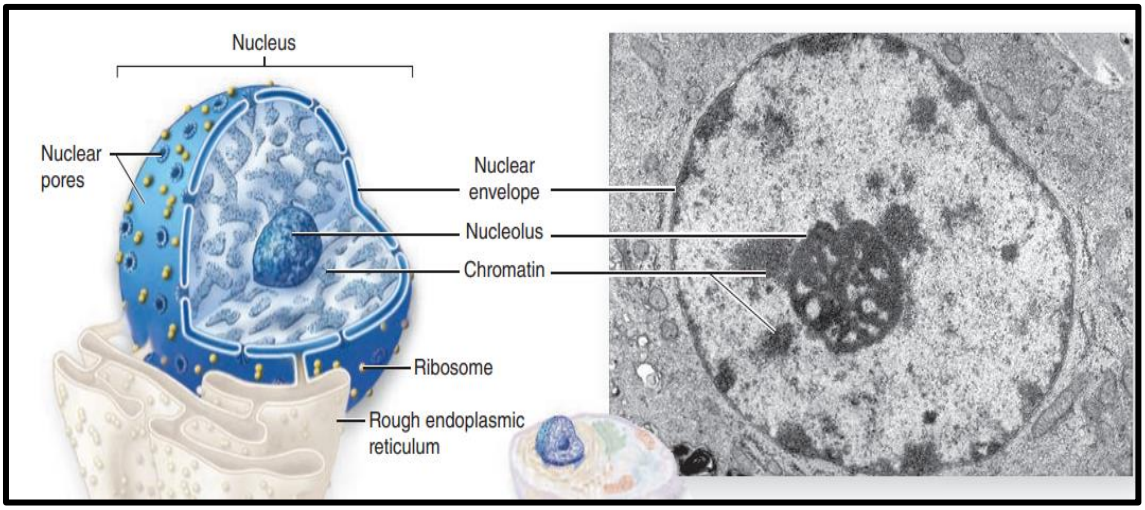

Figure 3: The nucleus.

➢ Ribosomes

- The two ribosomal subunits, each a complex of rRNA and many proteins, attach to mRNA and translate that message into protein.
- Multiple ribosomes on the same mRNA make up a polyribosome (polysome), and an abundance of these produces basophilic cytoplasm after H&E staining.

➢ Endoplasmic Reticulum

- The ER (figure 4) is a convoluted network of membrane enclosing continuous spaces called cisternae and extending from the nucleus to the plasma membrane.
- Rough ER has a granular, basophilic cytoplasmic surface due to the presence of polysomes making most membrane proteins, proteins in certain other organelles,

or for exocytosis; RER is always well developed in cells actively secreting proteins.

- Proteins to be processed through the RER contain initial signal peptides which bind receptors in the ER membrane, localizing them to that organelle.
- After translocation across the membrane into the cisterna, the proteins undergo posttranslational modification and folding in a process monitored by RER molecular chaperones and enzymes.
- Smooth ER (SER) lacks ribosomes, but includes enzymes for lipid and glycogen metabolism, for detoxification reactions, and for temporary Ca2+ sequestration.

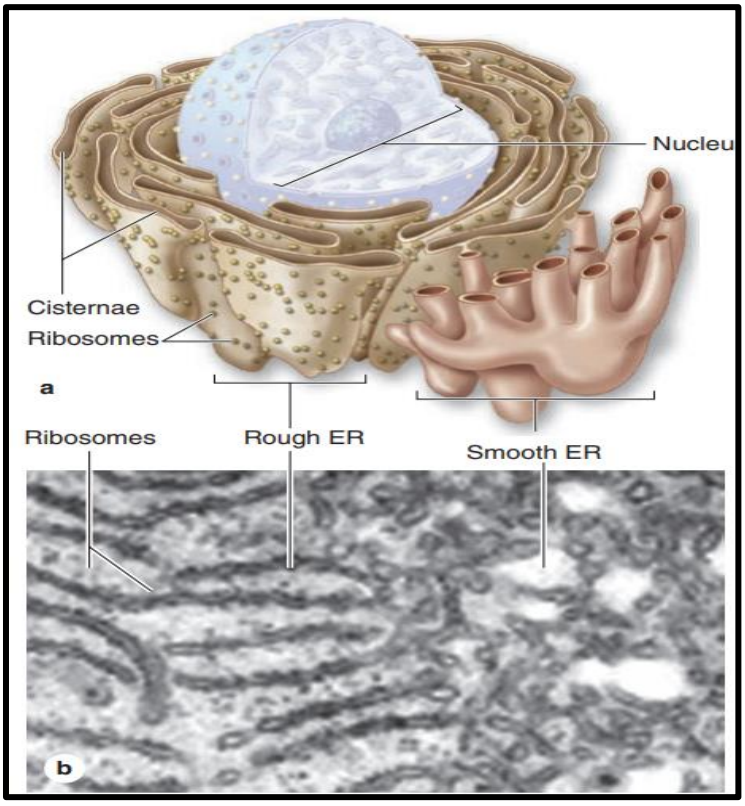

Figure 4: Endoplasmic Reticulum

➢ Golgi Apparatus

- The Golgi apparatus (figure 5) is a dynamic organelle consisting of stacked membranous cisternae in which proteins made in RER are processed further and packaged for secretion or other roles.
- Proteins in transport vesicles enter the Golgi apparatus, move through medical cisternae of the Golgi network for enzymatic modifications, and are released in other vesicles at the trans face.
- Vesicle movement through the Golgi apparatus is guided by specific coat proteins such as COPII and COPI.

- Important protein modifications in the Golgi apparatus include sulfation and many glycosylation reactions.
- Modified proteins leave the Golgi apparatus after packaging in vesicles with coat proteins that direct movement to lysosomes, the plasma membrane, or secretion by exocytosis.

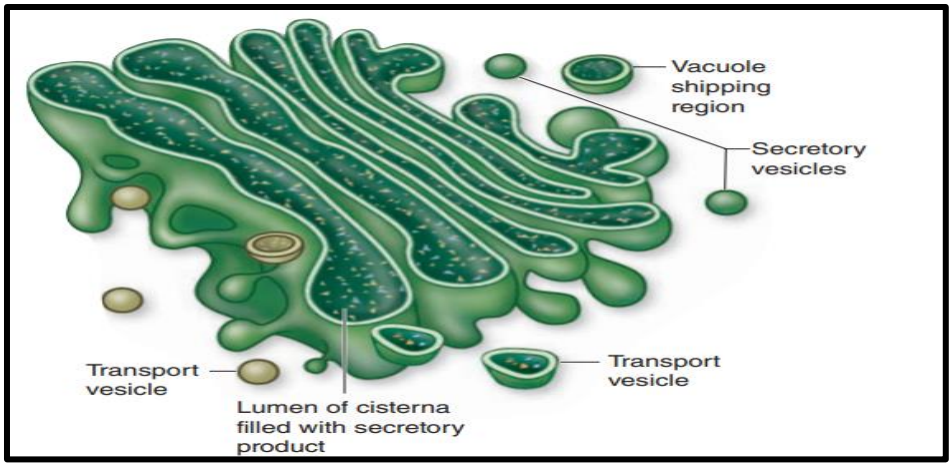

Figure 5: Golgi Apparatus

➢ Lysosomes

- Primary lysosomes (figure 6) emerge from the Golgi apparatus containing inactive acid hydrolases specific for degrading a wide variety of cellular macromolecules.
- Secondary lysosomes are more heterogeneous, having fused with vesicles produced by endocytosis that contain material to be digested by the hydrolytic enzymes.
- During autophagy, lysosomes digest unneeded or nonfunctional organelles after these are surrounded by membrane that then fuses with a lysosome.
- Products of digestion in secondary lysosomes are released to the cytoplasm for reuse; final condensed vesicles containing any indigestible molecules are called residual bodies.

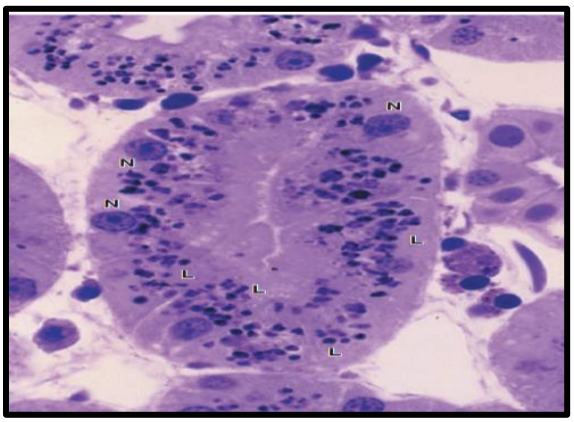

Figure 6: Lysosomes

➢ Proteasomes

Proteasomes are small cytoplasmic protein complexes which degrade improperly folded proteins after they are tagged with the polypeptide ubiquitin (figure 7).

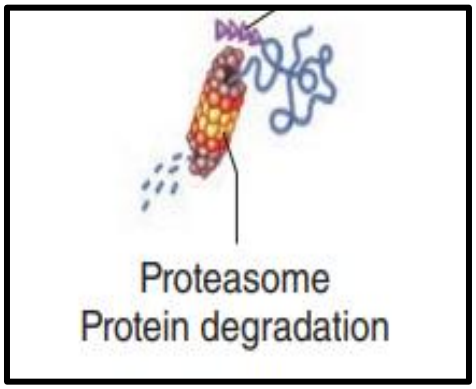

Figure 7: Proteasomes

➢ Mitochondria

- Mitochondria are the major sites of ATP synthesis and are abundant in cells or cytoplasmic regions where large amounts of energy are expended.
- Mitochondria are usually elongated organelles and form by fission of preexisting mitochondria (figure 8).
- Mitochondria have two membranes: a porous outer membrane encloses the intermembrane space and an inner membrane with many folds (cristae) enclosing a gel-like matrix.
- The mitochondrial matrix contains enzymes for β-oxidation of fatty acids and the citric acid (Krebs) cycle.
- The inner membrane includes enzyme assemblies of the electrontransport system and ATP synthase.

- Mitochondria of stressed cells may release cytochrome c from the inner membrane, triggering a regulated series of events culminating in cell death (apoptosis).

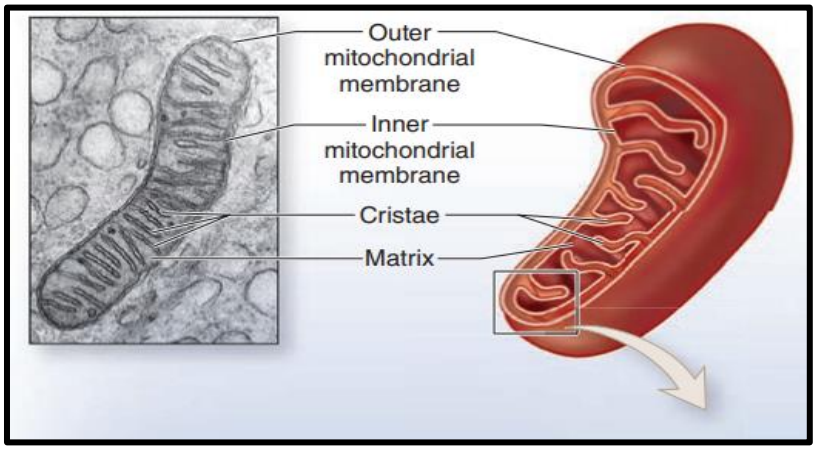

Figure 8: Mitochondria

➢ Peroxisomes

Peroxisomes are small spherical organelles containing enzymes for various metabolic reactions, notably for oxidation and detoxification, and catalase that breaks down the H_2O_2 resulting from those reactions (figure 9).

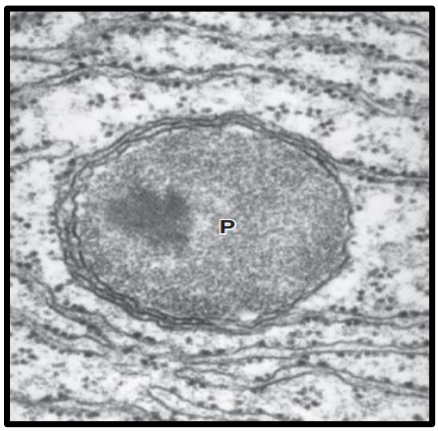

Figure 9: Peroxisomes

➢ Cytoskeleton

The cytoskeleton contains three types of polymers as shown in **Figure 10**:

(1) microtubules (25 nm in diameter); **(2) actin filaments or microfilaments** (5-7 nm); and **(3) intermediate filaments** (8-10 nm).

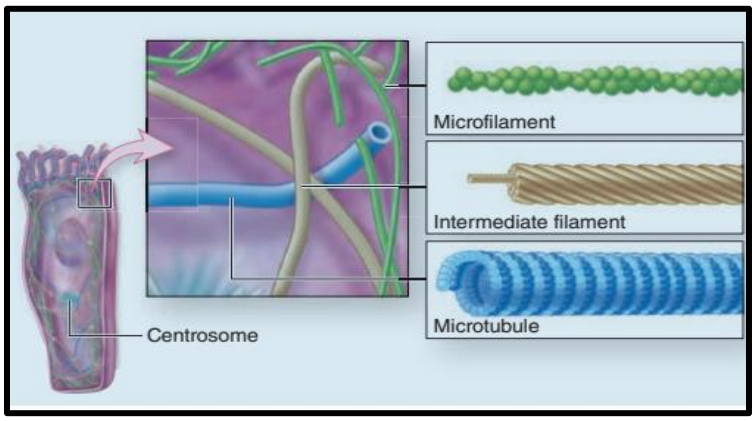

Figure 10:Polymers found in the cytoskeleton

- Microtubules are semirigid tubular structures with walls composed of polymerized tubulin heterodimers; their structure is often very dynamic, with steady addition and dissociation of tubulin.
- Microtubules are important in maintaining cell shape and as tracks for transport of vesicles and organelles by the motor proteins kinesin and dynein.
- Microfilaments are short, flexible, highly dynamic filaments of actin subunits, in which changes in length and interactions with binding proteins regulate cytoplasmic viscosity and movement.
- Myosins are motor proteins that bind and move along actin filaments, carrying vesicles or producing cytoplasmic movement.
- Movements of cytoplasm produced by actin filaments and myosins are important for endocytosis, cell cleavage after mitosis, and cell locomotion on substrates.

➢ Inclusions

Unlike organelles, inclusions are not metabolically active and are primarily storage sites, such as lipid droplets, glycogen granules, pigment granules, or residual bodies (also called lipofuscin).

Projections from the Cell Surface

Many cells show projections from the cell surface. The various types of projections are described below.

➢ Cilia

Cilia These can be seen, with the light microscope, as minute hair-like projections from the free surfaces of some epithelial cells (**Figure 11**).

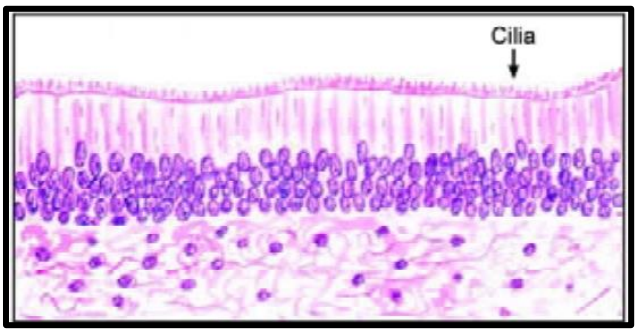

Figure 11: Cilia are projections from the cell surface.

➢ Flagella

These are somewhat larger processes having the same basic structure as cilia. In the human body the best example of a flagellum is the tail of the spermatozoon.

Microvilli & Basolateral folds

Microvilli are finger-like projections from the cell surface that can be seen by EM (**Figure 12**).

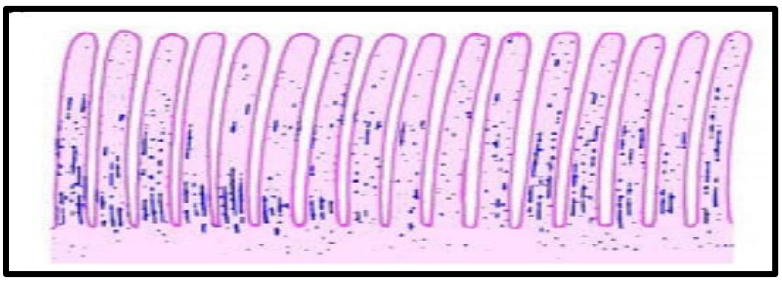

Figure 12: Microvilli as seen in longitudinal section. The regular arrangement of microvilli is characteristic of the striated border of intestinal absorptive cells.

Each microvillus consists of an outer covering of plasma membrane and a cytoplasmic core in which there are numerous microfilaments (actin filaments). The filaments are continuous with actin filaments of the cell cortex. Numerous enzymes, and glycoproteins, concerned with absorption have been located in microvilli. With the light microscope the free borders of epithelial cells lining the small intestine appear to be thickened: the thickening has striations perpendicular to the surface. This striated border of light microscopy (**Figure 13**) has been shown by EM to be made up of long microvilli arranged parallel to one another. In some cells the microvilli are not arranged so regularly.

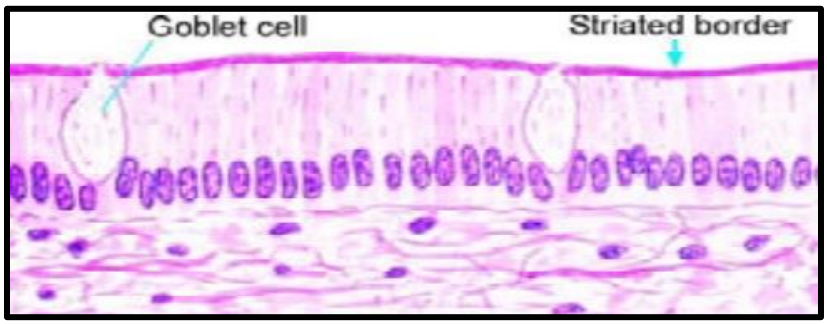

Figure 13: Light microscopic appearance of striated border formed by microvilli.

Microvilli greatly increase the surface area of the cell and are, therefore, seen most typically at sites of active absorption e.g., the intestine, and the proximal and distal convoluted tubules of the kidneys.

Applied Histology

Mitochondrial DNA can be abnormal. This interferes with mitochondrial and cell functions, resulting in disorders referred to as mitochondrial cytopathy syndromes. The features (which differ in intensity from patient to patient) include **muscle weakness**, **degenerative lesions in the brain**, and **high levels of lactic acid**. The condition can be diagnosed by EM examination of muscle biopsies. The mitochondria show characteristic paracrystalline inclusions. Genetic defects can lead to absence of specific **acid hydrolases** that are normally present in **lysosomes**. **As a result, some molecules cannot be degraded, and accumulate in lysosomes. Examples of such disorders are lysosomal glycogen storage disease in which there is abnormal accumulation of glycogen**, and **Tay-Sachs disease** in which lipids accumulate in lysosomes and lead to neuronal degeneration. Defects in **enzymes of peroxisomes** can result in **metabolic disorders** associated with storage of abnormal lipids in some cells (brain and adrenal).

Cilia can be abnormal in persons with genetic defects that interfere with synthesis of ciliary proteins. This leads to **the immotile cilia syndrome**. As secretions are not removed from respiratory passages, the patient has repeated and severe chest infections. **Women affected by the syndrome** may be sterile as movement of ova along the uterine tube is affected. **Ciliary proteins are present in the tails of spermatozoa**, and an affected male may be sterile because of interference with the motility of spermatozoa.

Basic Tissue Types

1. Epithelial tissues.
2. Supporting/connective tissues.
3. Nervous tissues.
4. Muscular tissue.

Epithelial tissues.

- An epithelium is a tissue in which cells are bound tightly together structurally and functionally to form a sheetlike or tubular structure with little extracellular material between the cells.
- Cells in epithelia each have an apical side facing the sheet's free surface and a basal side facing a basement membrane and underlying connective tissue.
- Epithelia are often specialized for absorption or transcytosis, pinocytosis of material at the apical side and exocytosis at the basolateral side (or vice versa).
- Cells of most epithelia exhibit continuous renewal, with the locations of stem cells and rates of cell turnover variable in various specialized epithelia.

Supporting/connective tissues.

- Connective tissue is specialized to physically support and connect other tissues and maintain the water required for metabolite diffusion to and from cells.
- Connective tissues all consist primarily of extracellular material rather than cells.
- Within most organs connective tissue proper forms the supportive stroma, which supports the organ's unique functional components or parenchyma.
- The ECM of connective tissue proper usually consists of both large protein fibers and nonfibrous areas of unstained ground substance rich in various GAGs and water.
- All adult connective tissues are derived from an embryonic form of connective tissue called mesenchyme, which contains uniformly undifferentiated cells scattered in a gel-like matrix.

Nervous tissues.

- Nervous tissue develops in the early embryo when the dorsal ectoderm neural plate folds lengthwise to form the neural tube, the precursor of the CNS, and releases neural crest cells, precursors for much of the PNS.

- The nervous system contains two types of cells, one is functional neurons and the other is supportive glial cells.
- There are many kinds of neurons, but all consist of a cell body (perikaryon) containing the nucleus, a long cytoplasmic extension called the axon, and one or more shorter processes called dendrites.
- Neurons use the common cell property of excitability to produce and move an action potential (nerve impulse) along the axon to excite another neuron or other effector cell.
- Such nerve communication is transmitted to another neuron or effector cell via a synapse, where neurotransmitter is released at the presynaptic membrane and binds receptors on the postsynaptic cell, initiating a new action potential there.
- Glial Cells (glia), required to support neurons in many ways, consist of six major types.

Muscular tissue

Muscular tissue is a specialized tissue in animals which applies forces to different parts of the body by contraction. It is made up of thin and elongated cells called muscle fibers. It controls the movement of an organism. The cytoplasm in the muscle fibers is called sarcoplasm. It contains a network of membrane called the sarcoplasmic reticulum. The membrane surrounding the muscle fibers is called sarcolemma.

Structure of Muscular Tissue

- The muscular tissues are bundled together and surrounded by a tough connective tissue similar to cartilage known as epimysium.
- The bundle of nerve cells that run in long fibers called fascicles are surrounded by the epimysium.
- The fascicles are surrounded by a protective layer known as perimysium. It allows the flow of nerves and blood to the individual fibers.
- Another protective layer, the endomysium surrounds the fibers.
- These layers and muscles help in the contraction of different parts of the muscles. The different bundles slide past one another as they contract.

- The epimysium connects to the tendons attached to the periosteum connective tissue that surrounds the bones. This helps in the movement of the skeleton when the muscles contract.

- The epimysium connects to other connective tissues to produce a force on the organs and control everything from circulation to food processing.

Types of Muscular Tissue

The muscular tissue is of three types:

- Skeletal Muscle Tissue
- Smooth Muscle Tissue
- Cardiac Muscle Tissue

Muscular Tissue Function

The muscular tissues are connected to the same nerve bundles. The nerve impulse from the brain tells the muscles to contract. Each muscle cell contains the proteins actin and myosin. These proteins slide past one another when the signal is received for contraction. A single cell contracts up to 70% in length. The entire muscle shortens during contraction. Muscular tissues help in the movement of bones, squeeze different organs, or compress chambers.

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Lecture 2: Epithelial Tissues

Despite its complexity, the organs of the human body are composed of only four basic tissue types: **epithelial**, **connective**, **muscular**, and **nervous** tissues. Each tissue is an assemblage of similarly specialized cells united in performing a specific function. The basic tissues, each containing extracellular matrix (ECM) as well as cells, associate with one another in the variable proportions and morphologies characteristic of each organ. The main features of the basic tissue types are summarized in **Table 1**. Connective tissue is characterized by cells producing very abundant ECM; muscle tissue is composed of elongated cells specialized for contraction and movement; and nervous tissue is composed of cells with long, fine processes specialized to receive, generate, and transmit nerve impulses. Most organs can be divided into the parenchyma, which is composed of the cells responsible for the organ's specialized functions, and the stroma, the cells of which have a supporting role in the organ. Except in the brain and spinal cord, the stroma is always connective tissue. Epithelial tissues are composed of closely aggregated polyhedral cells adhering strongly to one another and to a thin layer of ECM, forming cellular sheets that line the cavities of organs and cover the body surface. Epithelia (Gr. epi, upon + thele, nipple) line all external and internal surfaces of the body and all substances that enter or leave an organ must cross this type of tissue. The principal functions of epithelial tissues include the following:

- Covering, lining, and protecting surfaces (eg, epidermis).
- Absorption (eg, the intestinal lining).
- Secretion (eg, parenchymal cells of glands) Specific cells of certain epithelia may be contractile (myoepithelial cells) or specialized sensory cells, such as those of taste buds or the olfactory epithelium.

Table 1: Main characteristics of the four basic types of tissues.

Tissue	Cells	Extracellular Matrix	Main Functions
Epithelial	Aggregated polyhedral cells	Small amount	Lining of surface or body cavities; glandular secretion
Connective	Several types of fixed and wandering cells	Abundant amount	Support and protection of tissues/organs
Muscle	Elongated contractile cells	Moderate amount	Strong contraction; body movements
Nervous	Elongated cells with extremely fine processes	Very small amount	Transmission of nerve impulses

Characteristic Features of Epithelial Cells

The shapes and dimensions of epithelial cells are quite variable, ranging from tall columnar to cuboidal to low squamous cells. The cells' size and morphology are generally dictated by their function. Epithelial cell nuclei vary in shape and may be elliptic (oval), spherical, or flattened, with nuclear shape corresponding roughly to cell shape. Columnar cells generally have elongated nuclei, squamous cells have flattened nuclei, and cuboidal or pyramidal cells have more spherical nuclei (**Figure 1**). Because the lipid-rich membranes of epithelial cells are frequently indistinguishable by light microscopy, the number and shape of their stained nuclei are important indicators of cell shape and density. The nuclei also allow one to determine the number of cell layers in an epithelium, a primary morphologic criterion for classifying epithelia. Most epithelia are adjacent to connective tissue containing blood vessels from which the epithelial cells receive nutrients and O_2 . Even thick epithelia do not themselves normally contain blood vessels. The connective tissue that underlies the epithelia lining the organs of the digestive, respiratory, and urinary systems is called the lamina propria. The area of contact between the two tissues may be increased by small evaginations called papillae (L. papula, nipple) projecting from the connective tissue into the epithelium. Papillae occur most frequently in epithelial tissues subject to friction, such as the covering of the skin or tongue.

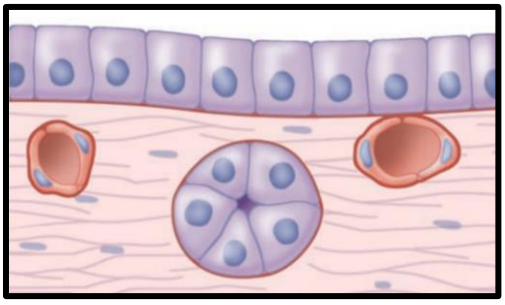


Figure1: Epithelia and adjacent connective tissue.

Epithelial cells generally show polarity, with organelles and membrane proteins distributed unevenly within the cell. The region of the cell contacting the ECM and connective tissue is called **the basal pole** and the opposite end, usually facing a space, is **the apical pole**, with the two poles differing significantly in both structure and function. Regions of cuboidal or columnar cells that adjoin neighboring cells comprise the cells' lateral surfaces; cell membranes here often have numerous folds which increase the area and functional capacity of that surface.

Basement Membranes

The basal surface of all epithelia rests on a thin extracellular, felt-like sheet of macromolecules referred to as the basement membrane (**Figure 1**), a semipermeable filter for substances reaching epithelial cells from below. Glycoproteins and other

components in this structure can often be stained and visualized with the light microscope (**Figure 2**). With the transmission electron microscope (TEM) two parts of the basement membrane may be resolved. Nearest the epithelial cells is the basal lamina, a thin, electron-dense, sheet-like layer of fine fibrils, and beneath this layer is a more diffuse and fibrous reticular lamina (**Figure 3a**).

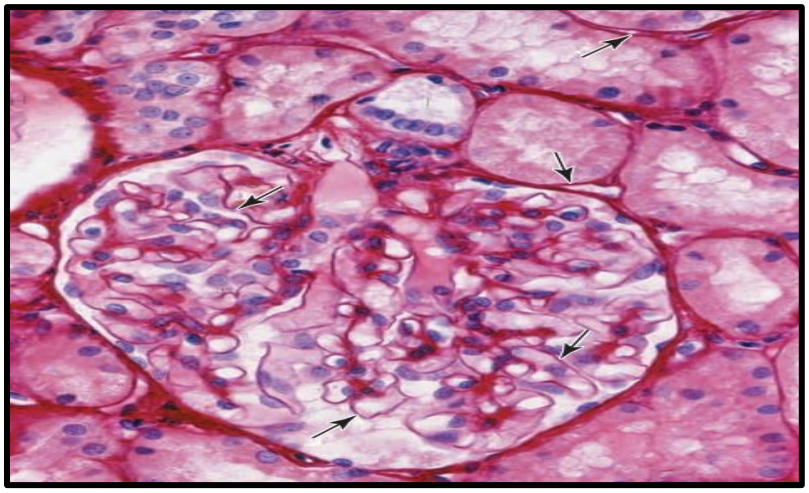


Figure 2: Basement membranes.

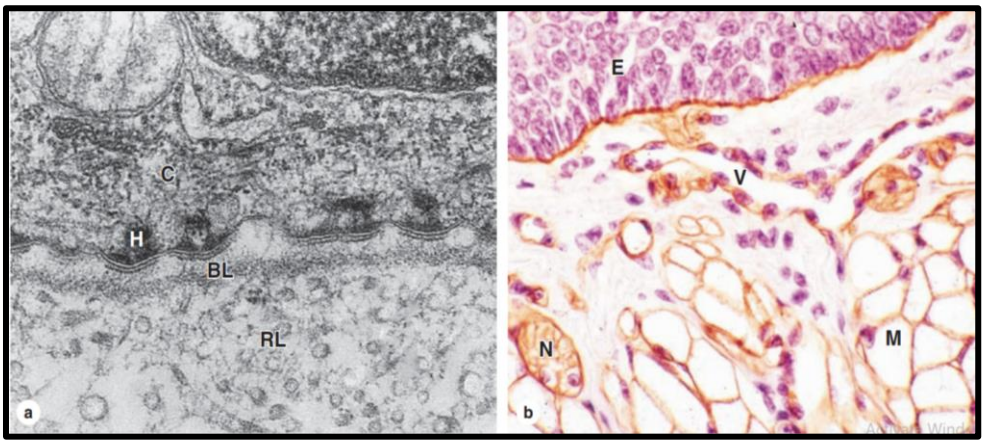


Figure 3: Basal and reticular laminae of basement membranes.

The terms "**basement membrane**" and "**basal lamina**" are sometimes used interchangeably, but "basal lamina" usually denotes the fine extracellular layer seen ultrastructurally and "basement membrane" the entire structure beneath the epithelial cells visible with the light microscope. The macromolecules of the basal lamina are secreted from the basal sides of the epithelial cells and form a sheet-like array. the basal lamina characteristically include the following:

■ **Type IV collagen**: Monomers of type IV collagen selfassemble into a two-dimensional network of evenly spaced subunits resembling the mesh of a window screen.

■ **Laminin**: These are large glycoproteins that attach to transmembrane integrin proteins in the basal cell membrane and project through the mesh formed by the type IV collagen.

■ **Nidogen and perlecan**: Respectively a short, rod-like protein and a proteoglycan, both of these cross-link laminins to the type IV collagen network, helping to provide the basal lamina's three-dimensional structure, to bind the epithelium to that structure, and to determine its porosity and the size of molecules able to filter through it.

The function of Basal laminae

Basal laminae often called external laminae but with similar composition also exist as thin sleeves surrounding muscle cells, nerves (**Figure 3b**), and fat-storing cells, where they serve as semipermeable barriers regulating macromolecular exchange between the enclosed cells and connective tissue. The more diffuse meshwork of the reticular lamina contains type III collagen and is bound to the basal lamina by anchoring fibrils of type VII collagen, both of which are produced by cells of the connective tissue (**Figure 3**). Besides acting as filters, functions of basement membranes include helping to provide structural support for epithelial cells and attach epithelia to underlying connective tissue. Basal lamina components help organize integrins and other proteins in the plasma membrane of epithelial cells, maintaining cell polarity and helping to localize endocytosis, signal transduction, and other activities. Basement membrane proteins also mediate many cell-to-cell interactions involving epithelia and mark routes for certain cell migrations along epithelia. Finally, the basement membrane also serves as a scaffold that allows rapid epithelial repair and regeneration.

Apical Structures of Epithelial Cells

■ Microvilli are small membrane projections with cores of actin filaments that generally function to increase epithelial cells' apical surface area for absorption.

■ Stereocilia are long microvilli with specialized mechanosensory function in cells of the inner ear and for absorption in tissues of the male reproductive tract.

■ Cilia are larger projecting structures with a well-organized core of microtubules (in a 9 + 2 arrangement called the axoneme) in which restricted, dynein-based sliding of microtubules causes ciliary movement that propel material along an epithelial surface.

Intercellular Adhesion & Other Junctions

Several membrane-associated structures provide adhesion and communication between cells. Some are present in other tissues but all are particularly numerous and prominent in epithelia. Epithelial cells adhere strongly to neighboring cells and basal laminae, particularly in epithelia subject to friction or other mechanical forces. As shown in **Figure 4**, lateral surfaces of epithelial cells have complexes of several specialized intercellular junctions with different functions:

■ **Tight or occluding junctions** are formed by interacting transmembrane proteins such as claudin and occludin; linear arrangements of these linked proteins surround the apical ends of the cells and prevent paracellular passage of substances (between the cells.)

■ **Adherent or anchoring junctions**, formed by interacting proteins of the cadherin family, are points of strong attachment holding together cells of the epithelium.

■ **Adherent junctions** may form **zonula adherens** that encircle epithelial cells just below their tight junctions or scattered, spot-like attachment sites called desmosomes or maculae adherens, both of which are attached to cytoplasmic keratins.

■ **Hemidesmosomes** composed of transmembrane integrins attach cells to proteins of the basal lamina.

■ **Gap** or **communicating junctions** are points of cell contact where both plasma membranes have numerous hexameric complexes of transmembrane connexons, each forming a channel allowing passage of small molecules from one cell to the other.

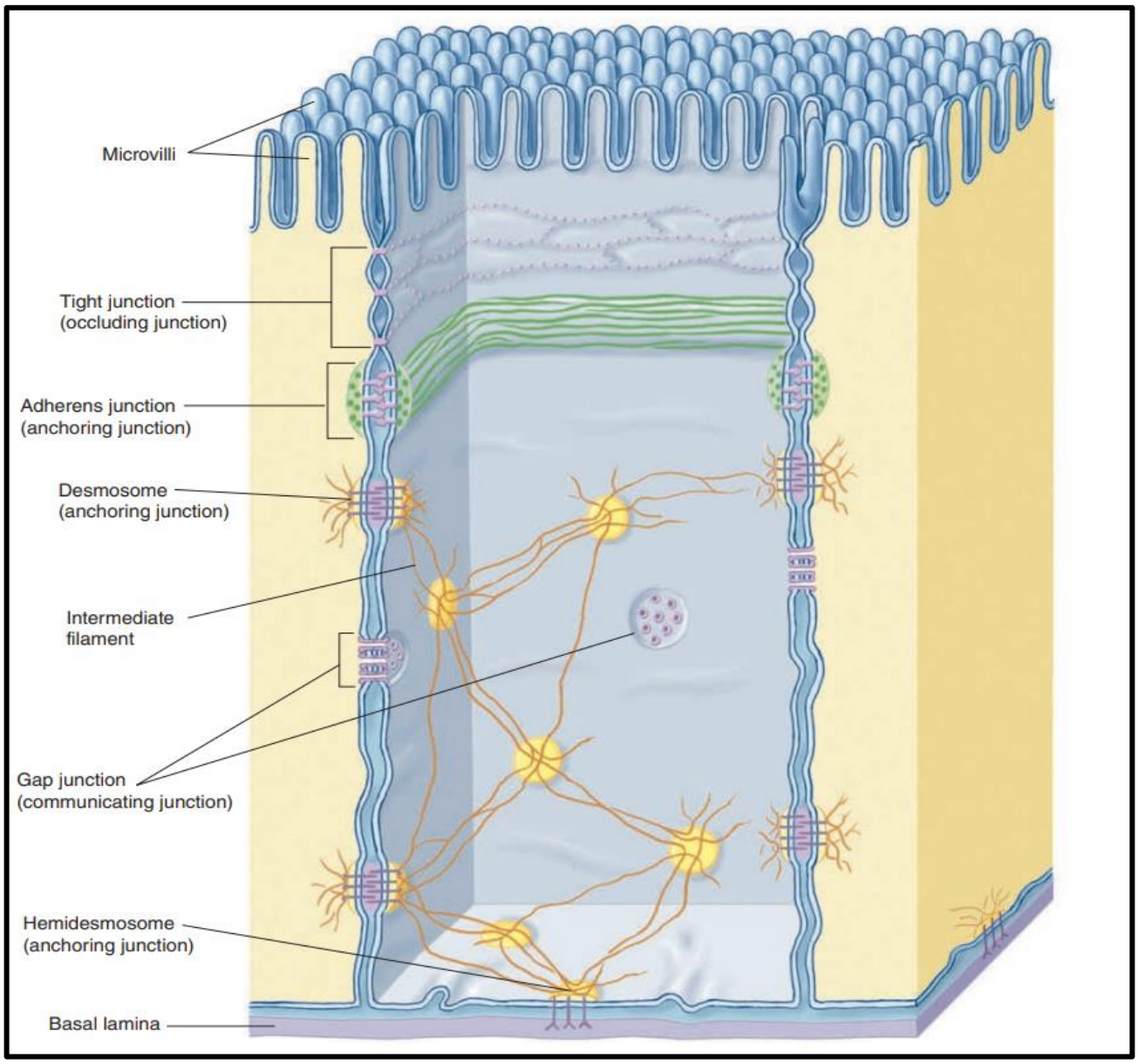


Figure 4: Junctional Complexes Of Epithelial Cells.

Types of Epithelia

Epithelia can be divided into **two** main groups**: covering (or lining) epithelia and secretory (glandular) epithelia**. This is an arbitrary functional division for there are lining epithelia in which all the cells also secrete (eg, the lining of the stomach) or in which glandular cells are distributed among the lining cells (eg, mucous cells in the small intestine or trachea). Covering or Lining Epithelia Cells of covering epithelia are organized into one or more layers that cover the surface or line the cavities of an organ. As summarized in **Table 2**, such epithelia are classified according to:

- The number of cell layers.
  Simple epithelia contain **one** cell layer and **stratified epithelia** contain **two or more layers.**

- The cell morphology in the outer layer.

simple epithelia are further classified as **squamous** (thin cells), **cuboidal** (cell width and thickness roughly similar), or **columnar** (cells taller than they are wide). Most **stratified epithelia** (**Figure 8**) are classified according to the cell shape of the superficial outer layer(s): squamous, cuboidal, or columnar.

Table 2: Common types of covering epithelia.

Major Feature	Cell Form	Examples of Distribution	Main Function
Simple (one layer of cells)	Squamous	Lining of vessels (endothelium); Serous lining of cavities: pericardium, pleura, peritoneum (mesothelium)	Facilitates the movement of the viscera (mesothelium), active transport by pinocytosis (mesothelium and endothelium), secretion of biologically active molecules (mesothelium)
	Cuboidal	Covering the ovary, thyroid	Covering, secretion
	Columnar	Lining of intestine, gallbladder	Protection, lubrication, absorption, secretion
Stratified (two or more layers of cells)	Squamous keratinized (dry)	Epidermis	Protection; prevents water loss
	Squamous nonkeratinized (moist)	Mouth, esophagus, larynx, vagina, anal canal	Protection, secretion; prevents water loss
	Cuboidal	Sweat glands, developing ovarian follicles	Protection, secretion
	Transitional	Bladder, ureters, renal calyces	Protection, distensibility
	Columnar	Conjunctiva	Protection
Pseudostratified (layers of cells with nuclei at different levels; not all cells reach surface but all adhere to basal lamina)		Lining of trachea, bronchi, nasal cavity	Protection, secretion; cilia-mediated transport of particles trapped in mucus out of the air passages

Examples of these epithelial types are shown in **Figures 5** through **Figures 8**.

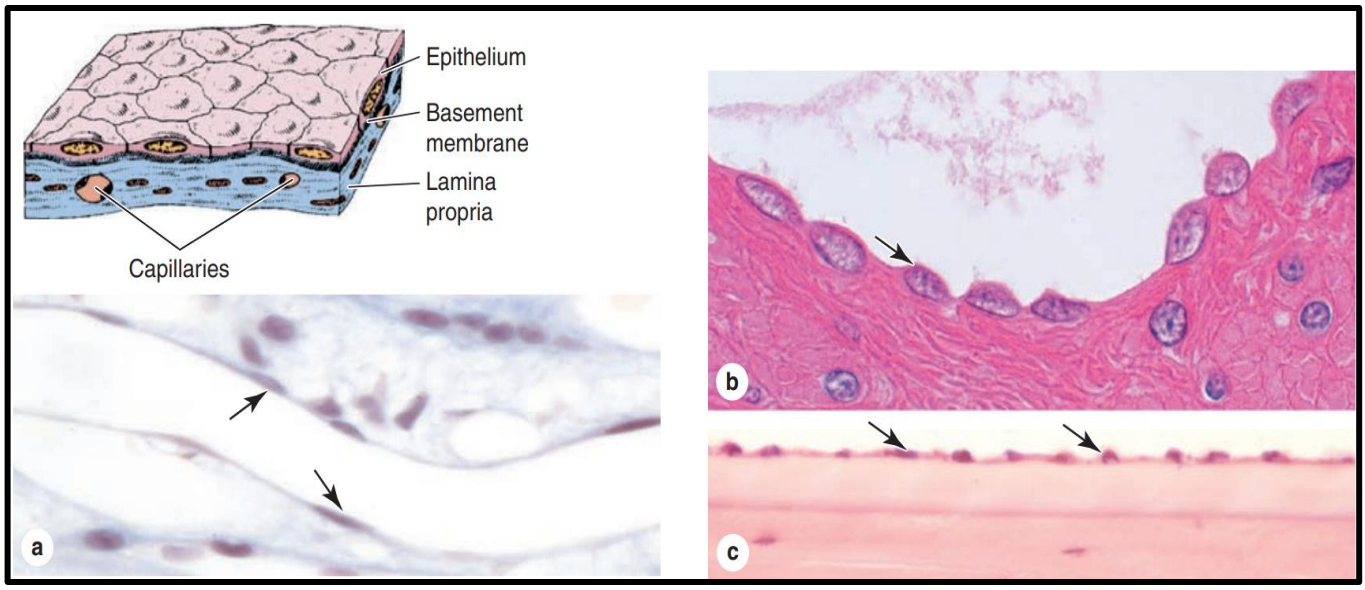


Figure 5: Simple squamous epithelium. This is a single layer of thin cells, in which the cell nuclei are the thickest and most visible structures. Examples shown here are those lining the thin renal loops of Henle (a), covering the outer wall of the intestine (b), and lining the inner surface of the cornea (c).

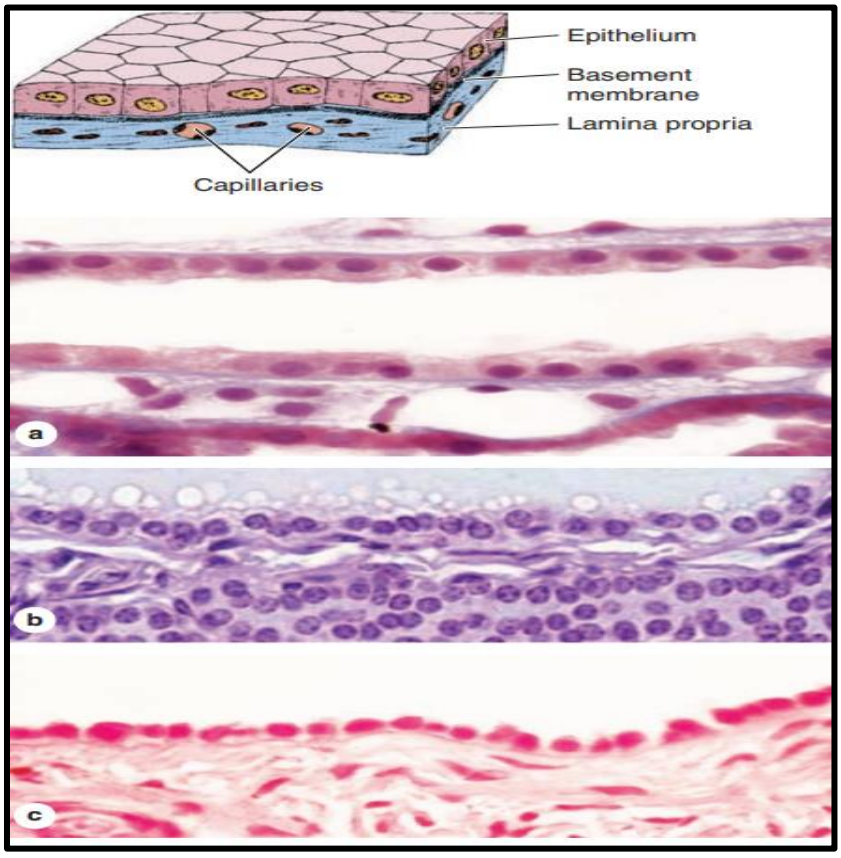


Figure 6: Simple cuboidal epithelium. Cells here are roughly as tall as they are wide. Their greater thickness allows cytoplasm to be rich in mitochondria and other organelles for a high level of active

transport across the epithelium and other functions. Examples shown here are from a renal collecting tubule (a), a large thyroid follicle (b), and the thick mesothelium covering an ovary (c).

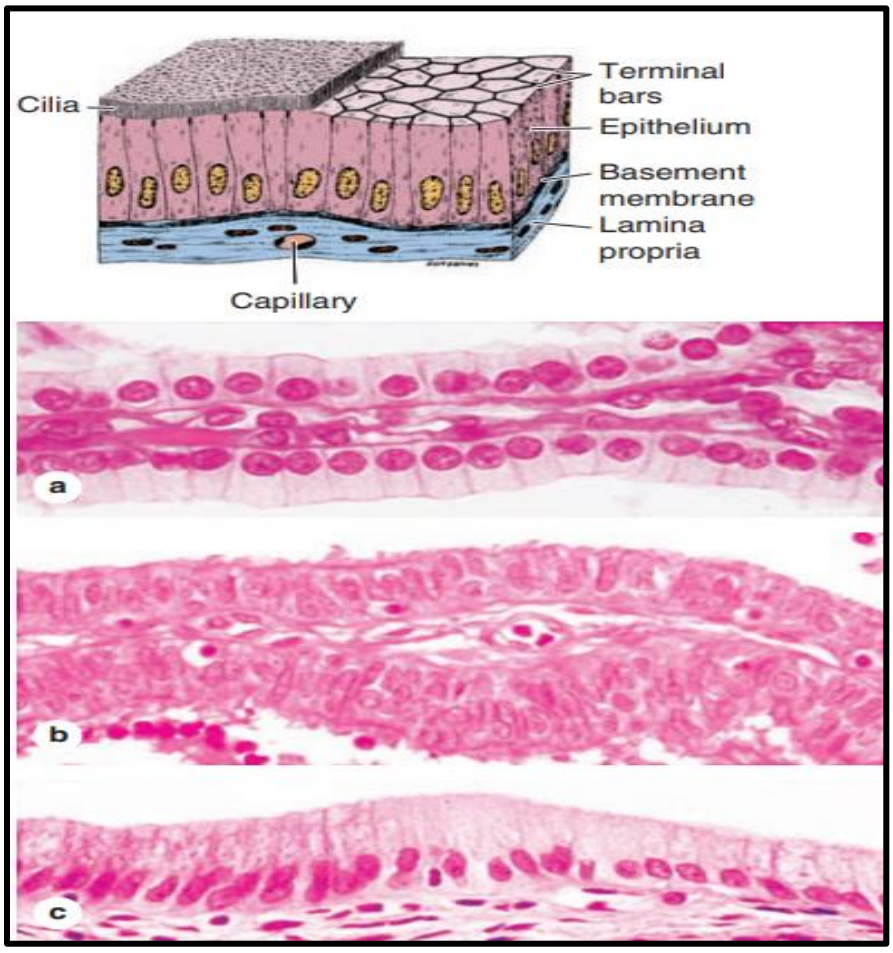


Figure 7: Simple columnar epithelium. Cells here are always taller than they are wide. The examples shown here are from a renal collecting duct (a), the oviduct lining, with both secretory and ciliated cells (b), and the lining of the gallbladder (c).

The very thin surface cells of stratified squamous epithelia can be "keratinized" (packed with keratin filaments) or "nonkeratinized" (with relatively sparse keratin). **Stratified squamous keratinized epithelium** is found mainly in the epidermis of skin, where it helps prevent dehydration from the tissue (**Figure 8 a**). Its cells form many layers, with the less differentiated cuboidal cells near the basement membrane. These cells have many desmosomes and become more irregular in shape and then flatten as they accumulate keratin in the process of **keratinization** and are moved progressively toward the skin surface, where they become thin, metabolically inactive packets (squames) of keratin lacking nuclei. As discussed with skin, this surface layer of cells helps protect against water loss across this epithelium. **Stratified squamous nonkeratinized epithelium** (**Figures 8 b and c**) lines moist internal cavities (eg,

mouth, esophagus, and vagina) where water loss is not a problem. Here the flattened cells of the surface layer retain their nuclei and most metabolic functions.

Stratified cuboidal and **stratified columnar epithelia** are both relatively rare. Stratified cuboidal epithelium occurs in the excretory ducts of salivary and sweat glands (**Figure 8 d**). Stratified columnar epithelium is seen in the conjunctiva lining the eyelids, where it is both protective and mucus secreting.

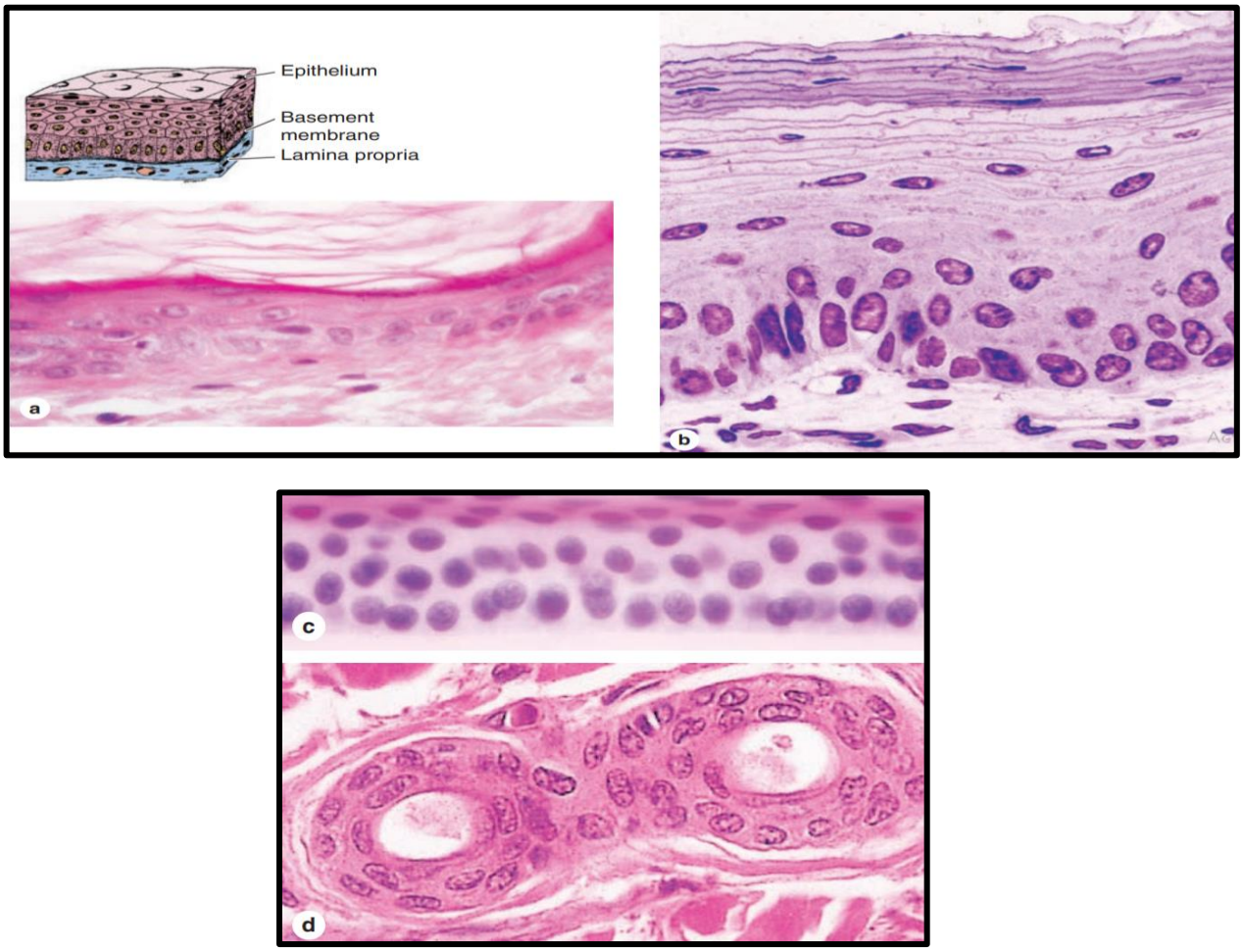


Figure 8: Stratified epithelium.

Unique **transitional epithelium** or **urothelium** lines much of the urinary tract, extending from the kidneys to the proximal part of the urethra, and is characterized by a superficial layer of large, dome-like cells sometimes called umbrella cells (**Figure 9**). As discussed further with the urinary system, these cells are specialized to protect underlying tissues from the hypertonic and potentially cytotoxic effects of urine. Importantly, unique morphological features of the cells allow distension of transitional epithelium as the urinary bladder fills.

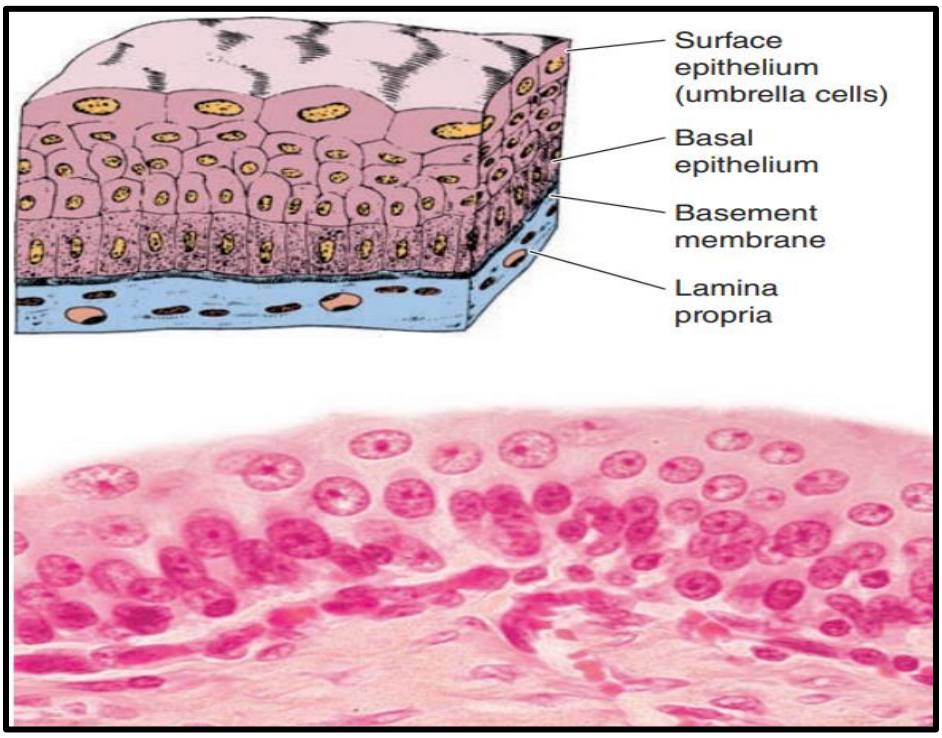


Figure 9: Transitional epithelium or urothelium. The superficial cells are rounded or dome-shaped, and have specialized membrane features enabling them to withstand the hypertonic effects of urine and protect underlying cells from this toxic solution. Cells of this epithelium are also able to adjust their relationships with one another and undergo a transition in their appearance as the urinary bladder fills and the wall is distended.

›››MEDICAL APPLICATION

In individuals with chronic vitamin A deficiency, epithelial tissues of the type found in the bronchi and urinary bladder may gradually be replaced by stratified squamous epithelium.

A final morphological type of epithelium is called **pseudostratified columnar epithelium** (**Figure 10**). Here tall, irregular cells all are attached to the basement membrane but their nuclei are at different levels and not all cells extend to the free surface, giving a stratified appearance. A good example of pseudostratified columnar epithelium is that lining the upper respiratory tract, where the cells are also heavily ciliated.

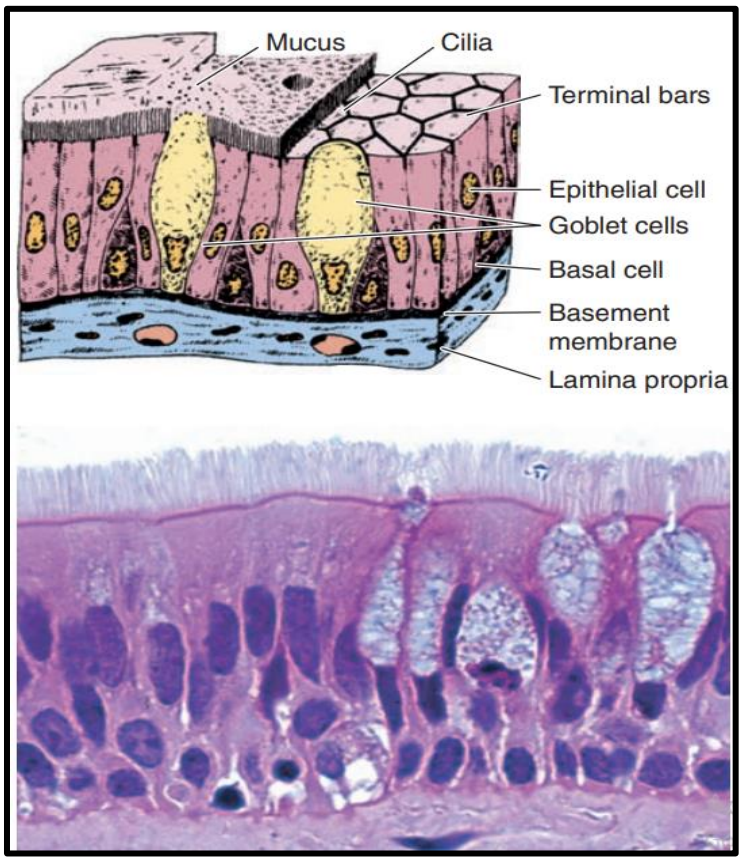


Figure 10: Pseudostratified epithelium. Cells of pseudostratified epithelia appear to be in several layers, but their basal ends all rest on the basement membrane. The pseudostratified columnar epithelium of the upper respiratory tract shown here contains many ciliated cells, as well as other cells with their nuclei at different levels.

›››MEDICAL APPLICATION

In chronic bronchitis, common among habitual smokers, the number of goblet cells in the lining of airways in the lungs often increases greatly. This leads to excessive mucus production in areas where there are too few ciliated cells for its rapid removal and contributes to obstruction of the airways. The ciliated pseudostratified epithelium lining the bronchi of smokers can also be transformed into stratified squamous epithelium by metaplasia.

Secretory Epithelia & Glands

Epithelial cells that function mainly to produce and secrete various macromolecules may occur in epithelia with other major functions or comprise specialized organs called **glands**. Secretory cells may synthesize, store, and release proteins (eg, in the pancreas), lipids (eg, adrenal, sebaceous glands), or complexes of carbohydrates and proteins (eg, salivary glands). Epithelia of mammary glands secrete all three substances. The cells of some glands (eg, sweat glands) have little synthetic activity and secrete mostly water and electrolytes (ions) transferred from the blood.

Scattered secretory cells, sometimes called unicellular glands, are common in simple cuboidal, simple columnar, and pseudostratified epithelia. An important, easily seen example is **the goblet cell** abundant in the lining of the small intestine (**Figure 11**) and respiratory tract (**Figure 10**), which secretes lubricating mucus that aids the function of these organs.

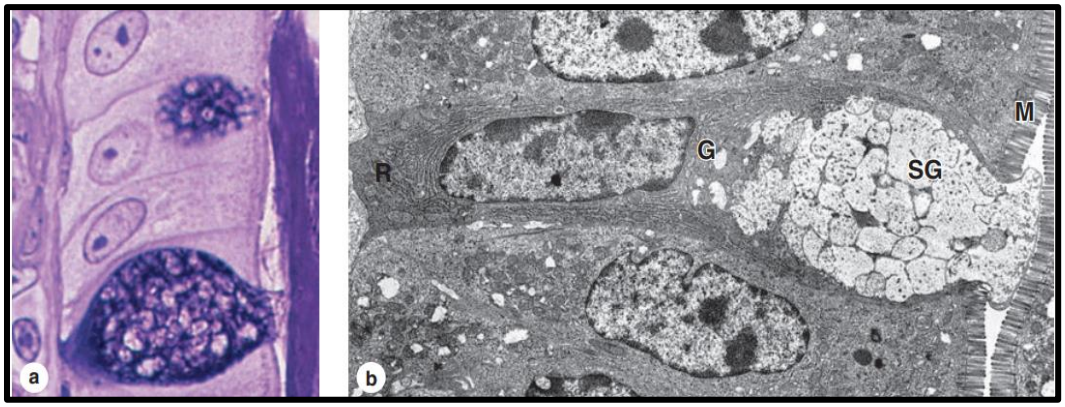


Figure 11: Goblet cells: unicellular glands. The simple columnar epithelium lining the small intestine shows many isolated goblet cells secreting mucus into the lumen. (a) With a stain for the oligosaccharide components of mucin glycoproteins, the cytoplasmic secretory granules of two goblet cells and secreted mucus are stained purple. (b) As shown ultrastructurally, goblet cells always have basal nuclei surrounded by RER (R), a large Golgi complex (G), and abundant apical cytoplasm filled with large secretory granules (SG). After exocytosis mucin components are hydrated and become mucus. A brush border of microvilli (M) is seen on neighboring columnar cells.

Glands develop from covering epithelia in the fetus by cell proliferation and growth into the underlying connective tissue, followed by further differentiation (**Figure 12**). Exocrine glands remain connected with the surface epithelium, the connection forming the tubular ducts lined with epithelium that deliver the secreted material where it is used. Endocrine glands lose the connection to their original epithelium and therefore lack ducts. Thin-walled blood vessels (capillaries) adjacent to endocrine cells absorb their secreted hormone products for transport in blood to target cells throughout the body.

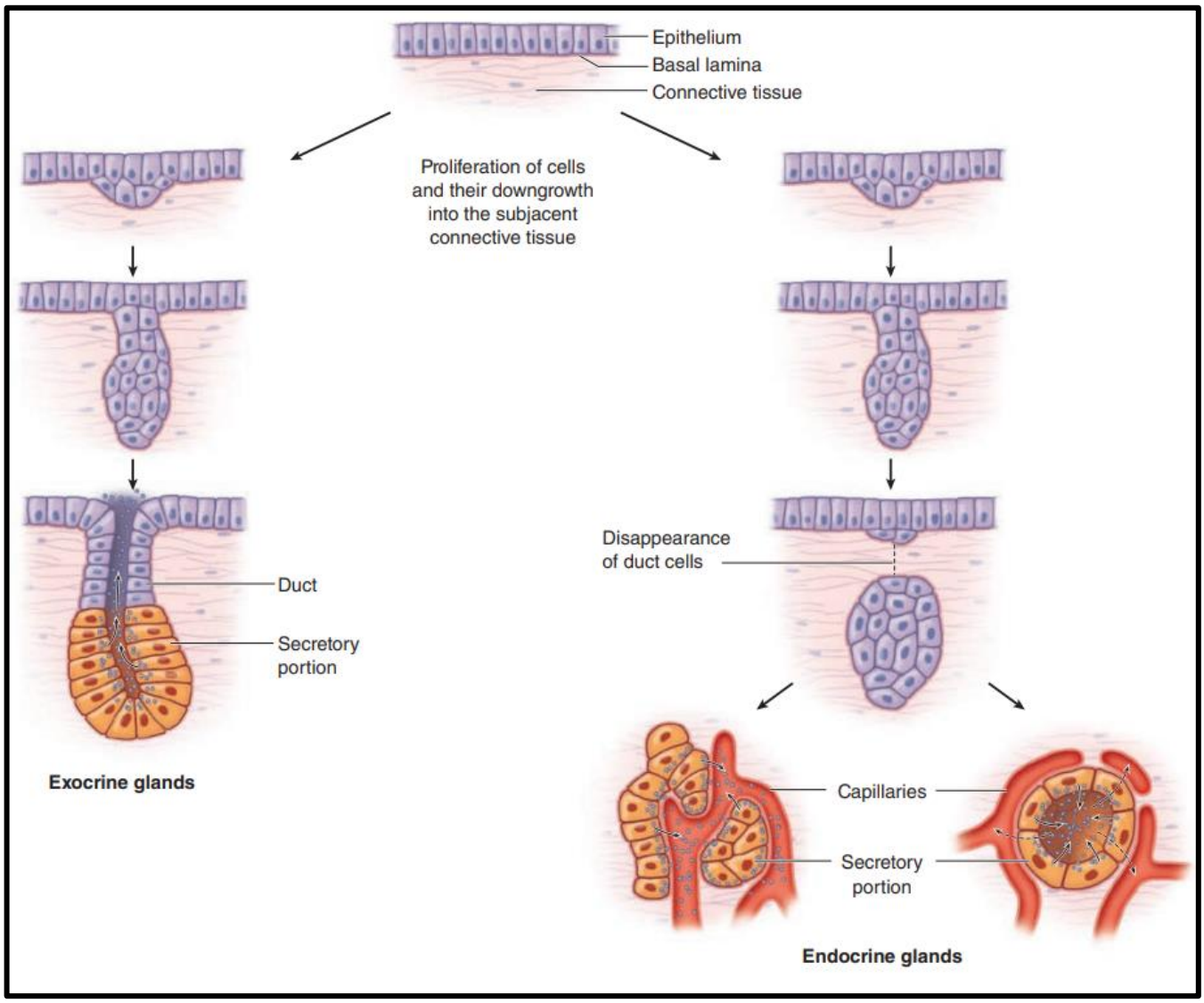


Figure 12: Formation of glands from covering epithelia.

As shown in **Figure 13**, epithelia of exocrine glands are organized as a continuous system of many small **secretory portions** and **ducts** that transport the secretion out of the gland. In both exocrine and endocrine glands, the secretory units are supported by a stroma of connective tissue. In larger glands, layers of connective tissue also surround the larger ducts, form partitions or septa separating the gland into lobules, each containing secretory units connected to a small part of the duct system, and enclose the entire gland as its capsule (**Figure 13**).

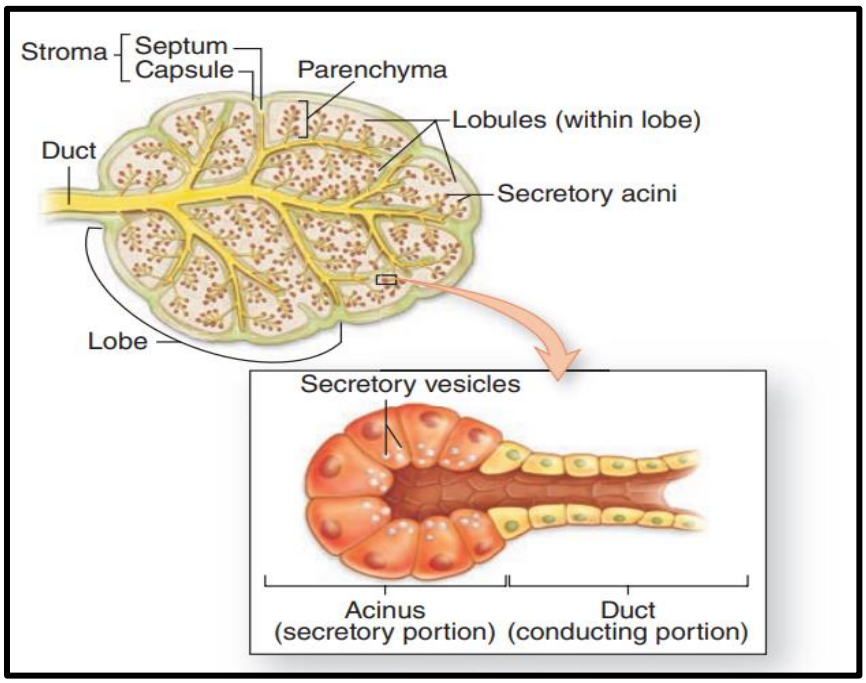


Figure 13: General structure of exocrine glands. Exocrine glands by definition have ducts that lead to another organ or the body surface. Inside the gland the duct runs through the connective tissue of septa and branches repeatedly, until its smallest branches end in the secretory portions of the gland.

The structures of their secretory portions and ducts allow exocrine glands to be classified as shown schematically in **Table 3**. Although the three-dimensional morphology is often not prominent in histologic sections, the key points are summarized as follows:

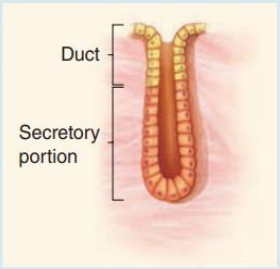
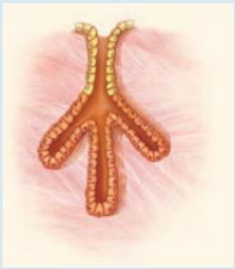
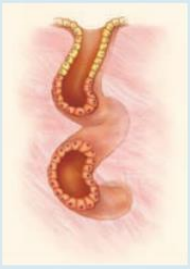
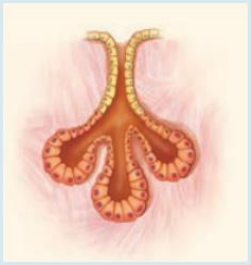
■ **Glands** can be simple (ducts not branched) or compound (ducts with two or more branches).

■ **Secretory portions** can be tubular (either short or long and coiled) or acinar (rounded and saclike); either type of secretory unit may be branched, even if the duct is not branched.

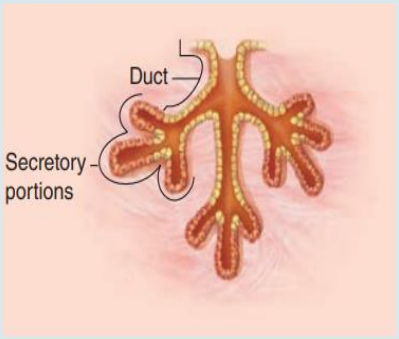
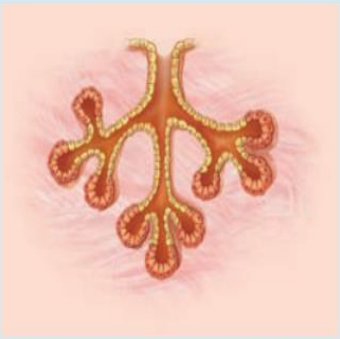
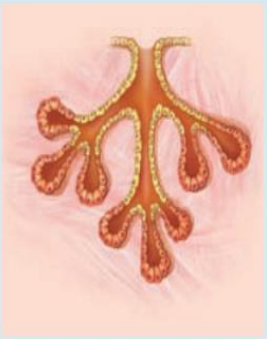
■ **Compound glands** can have branching ducts and can have multiple tubular, acinar, or tubuloacinar secretory portions.

Table 3: Structural classes of exocrine glands, features of each class, and examples.

SIMPLE Glands **(Ducts Do Not Branch)**

Class	Simple Tubular	Branched Tubular	Coiled Tubular	Acinar (or Alveolar)	Branched Acinar
					
Features	Elongated secretory portion; duct usually short or absent	Several long secretory parts joining to drain into 1 duct	Secretory portion is very long and coiled	Rounded, saclike secretory portion	Multiple saclike secretory parts entering the same duct
Examples	Mucous glands of colon; intestinal glands or crypts (of Lieberkühn)	Glands in the uterus and stomach	Sweat glands	Small mucous glands along the urethra	Sebaceous glands of the skin

COMPOUND Glands **(Ducts from Several Secretory Units Converge into Larger Ducts)**

Class	Tubular	Acinar (Alveolar)	Tubuloacinar
			
Features	Several *elongated* coiled secretory units and their ducts converge to form larger ducts	Several *saclike* secretory units with small ducts converge at a larger duct	Ducts of both tubular and acinar secretory units converge at larger ducts
Examples	Submucosal mucous glands (of Brunner) in the duodenum	Exocrine pancreas	Salivary glands

Three basic mechanisms for releasing the product are commonly used by cells specialized for secretion (**Figure 14**).

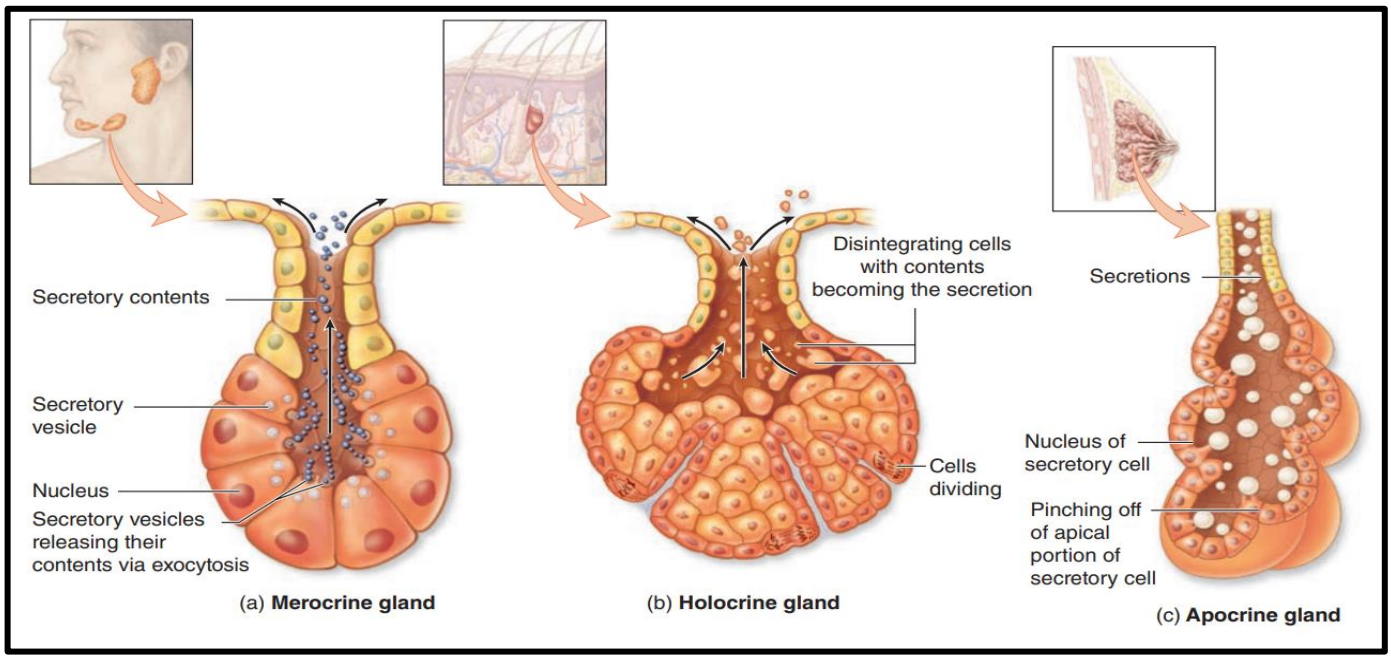


Figure 14: Mechanisms of exocrine gland secretion.

The cells engaged in each type of secretion can be distinguished histologically:

1. Merocrine secretion: This is the most common method of protein or glycoprotein secretion and involves typical exocytosis from membrane-bound vesicles or secretory granules.

2. Holocrine secretion: Here cells accumulate product continuously as they enlarge and undergo terminal differentiation, culminating in complete cell disruption that releases the product and cell debris into the gland's lumen. This is best seen in the sebaceous glands producing lipidrich material in skin (**Figure 15**).

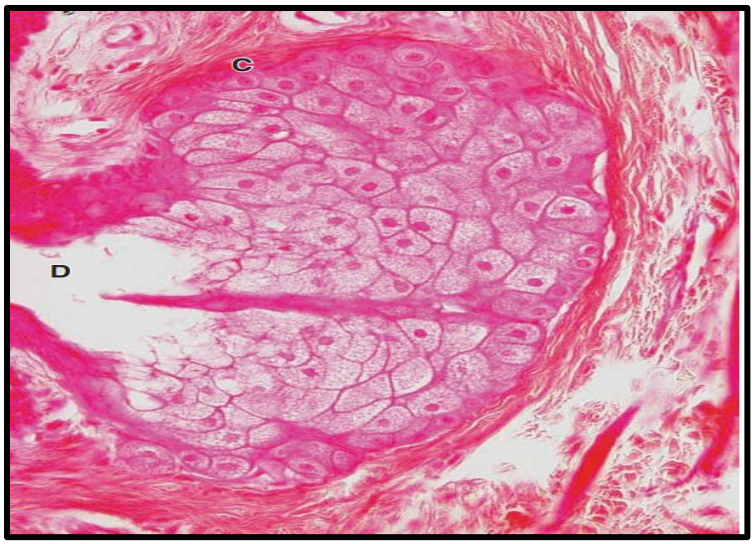


Figure 15: Holocrine secretion in a sebaceous gland.

3. Apocrine secretion: Here product accumulates at the cells' apical ends, portions of which are then extruded to release the product together with small amounts of cytoplasm and cell membrane. Lipid droplets are secreted in the mammary gland in this manner (**Figure 16**).

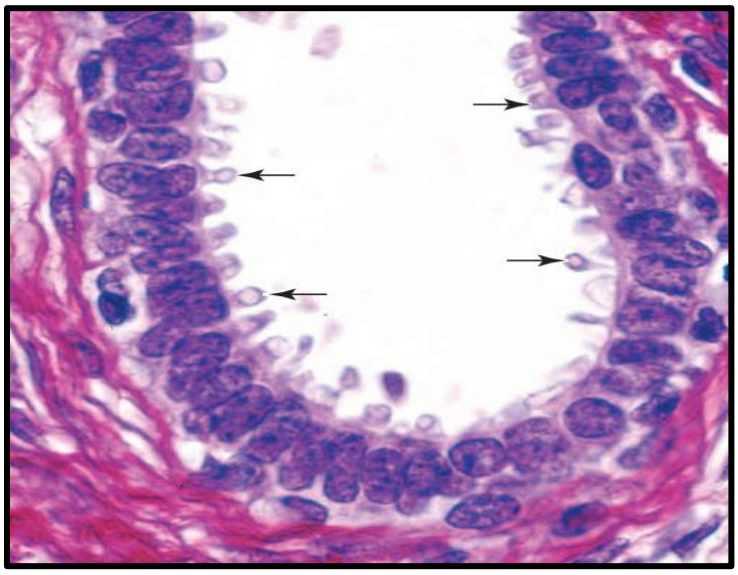


Figure 16: Apocrine secretion in the mammary gland.

Exocrine glands with merocrine secretion can be further categorized as either **serous** or **mucous** according to the nature of their secretory products, which give distinct staining properties to the cells. Serous cells synthesize proteins that are mostly not glycosylated, such as digestive enzymes. The cells have well-developed RER and Golgi complexes and are filled apically with secretory granules in different stages of maturation (**Figure 17**). Serous cells therefore stain intensely with basophilic or acidophilic stains. Acini of the pancreas and parotid salivary glands are composed of serous cells.

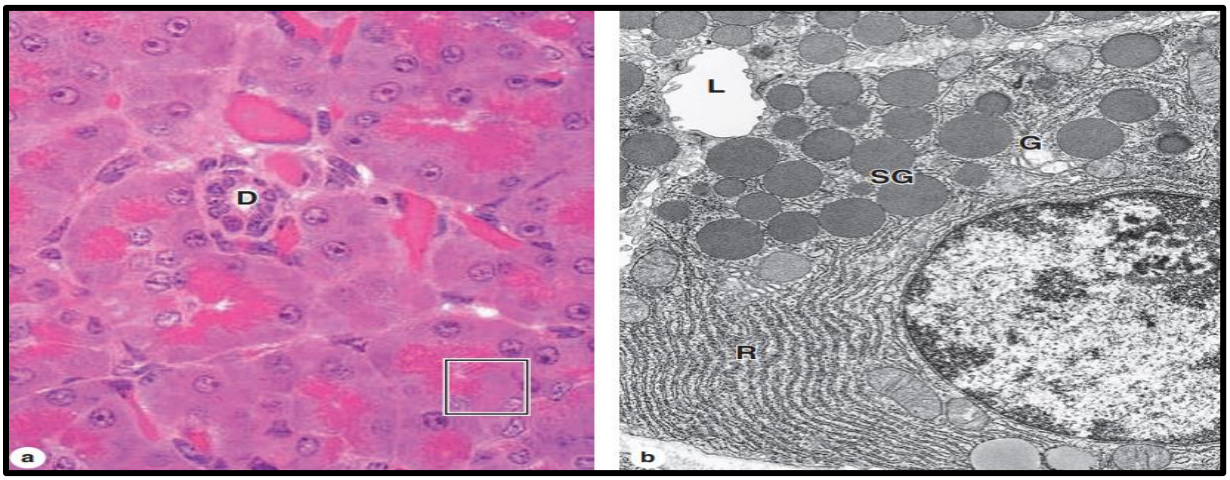


Figure 17: Serous cells. The small serous acini of the exocrine pancreas each have 5-10 cells facing a very small central lumen. Each acinar cell is roughly pyramidal, with its apex at the lumen. (a) As seen by light

microscopy, the apical ends are very eosinophilic due to the abundant secretory granules present there. The cells' basal ends contain the nuclei and an abundance of RER, making this area basophilic. A small duct (D) is seen, but lumens of acini are too small to be readily visible. The enclosed area is comparable to that shown in part b. (b) A portion of one acinar cell is shown ultrastructurally, indicating the abundant RER (R), a Golgi complex (G), apical secretory granules (SG), and the small acinar lumen (L).

Mucous cells, such as goblet cells, also have RER and Golgi complexes and are filled apically with secretory granules, but these contain heavily glycosylated proteins called mucins. When mucins are released from the cell, they become hydrated and form a layer of mucus. The hydrophilic mucins are usually washed from cells during routine histological preparations, causing the secretory granules to stain poorly with eosin (**Figure 18**). Sufficient oligosaccharides remain in developing mucinogen granules, however, to allow mucous cells to be stained by the PAS method (**Figure 11a**).

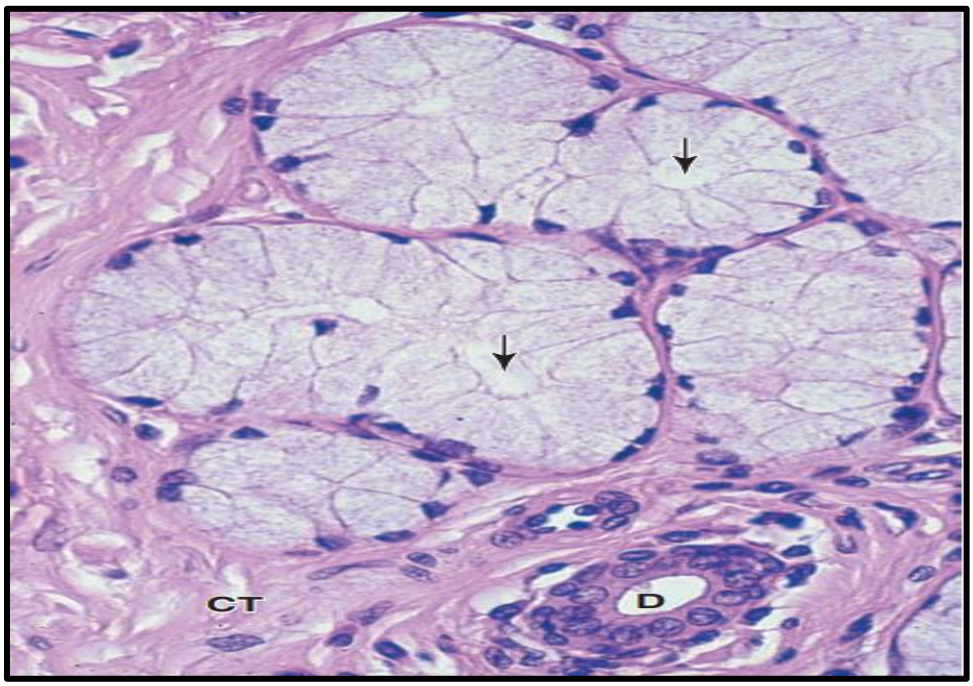


Figure 18: Mucous cells. Mucous cells of salivary glands are typically larger than serous cells, with flattened basal nuclei. Most of the cytoplasm is filled with secretory granules containing mucinogen like that of goblet cells. The RER and Golgi complexes of mucous cells produce heavily glycosylated glycoproteins with water-binding properties. The lumens (arrows) of mucous tubules are larger than those of serous acini. Much connective tissue surrounds the mucous tubules and ducts (D).

Some salivary glands are mixed **seromucous glands**, having both serous acini and mucous tubules with clustered serous cells. The product of such glands is a mixture of digestive enzymes and watery mucus.

In addition to secretory cells, epithelia of many exocrine glands (eg, sweat, lachrymal, salivary, and mammary glands) contain contractile **myoepithelial cells** at the basal ends of the secretory cells (**Figure 19**). Long processes of these cells embrace an

acinus as an octopus might embrace a rounded boulder. Bound to the basal lamina by hemidesmosomes and connected to the other epithelial cells by both gap junctions and desmosomes, myoepithelial cells are rich in actin filaments and myosins. Strong contractions in these cells serve to help propel secretory products from acini into the duct system.

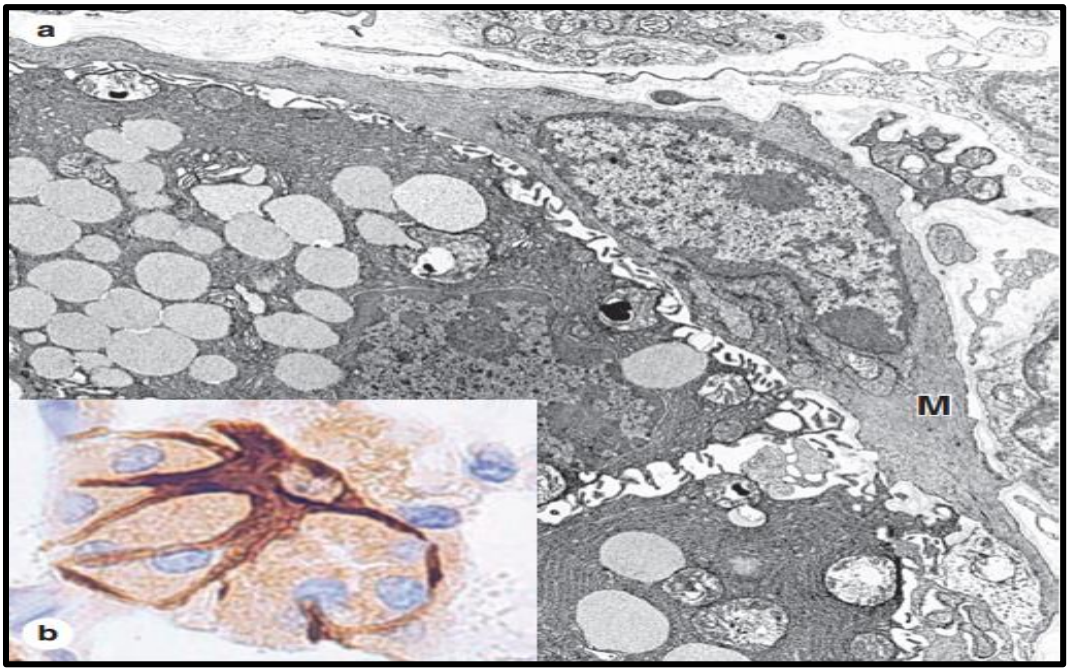


Figure 19: myoepithelial cells. (a) The TEM shows two salivary gland cells containing secretory granules, with an associated myoepithelial cell (M). (b) A myoepithelial cell immunostained brown with antibodies against actin shows its association with cells of an acinus stained by H&E. Contraction of the myoepithelial cell compresses the acinus and aids in the expulsion of secretory products into the duct.

Endocrine glands lack myoepithelial cells and are specialized either for protein or steroid **hormone** synthesis, with cytoplasmic staining characteristic of RER or SER, respectively. The proteins are released by exocytosis and the lipophilic steroids by diffusion through the cell membrane for uptake by binding proteins outside the cell. As mentioned previously, endocrine signaling involves hormone transport in the blood to target cells throughout the body, often within other endocrine glands. The receptors may also be on cells very close to the hormone-secreting cell or on the secreting cell itself, signaling which is termed paracrine or autocrine, respectively.

Important but inconspicuous endocrine or paracrine cells also occur singly or in small groups in epithelia of the digestive, respiratory, and other organ systems. Hormones are also secreted from some cells specialized for other functions, such as certain cardiac muscle cells or fat cells. The pancreas contains both endocrine and exocrine cells. Liver cells exert both functions in the same cells, secreting bile components into a duct system and releasing other products to the bloodstream.

Transport Across Epithelia

Many cells have the ability to actively transport certain ions against concentration and electrical potential gradients. An important example is the extrusion of Na+ from cells by the transmembrane protein Na+/K+-ATPase, also called the Na+/K+ pump, which allows cells to maintain the required low intracellular sodium concentration (5-15 mmol/L vs ~140 mmol/L in extracellular fluid). Some epithelial cells specialize in the transfer of ions (by ion pumps) and water (via the membrane channels called aquaporins) in either direction across the epithelium, the process is known as transcellular transport (**Figure 20**).

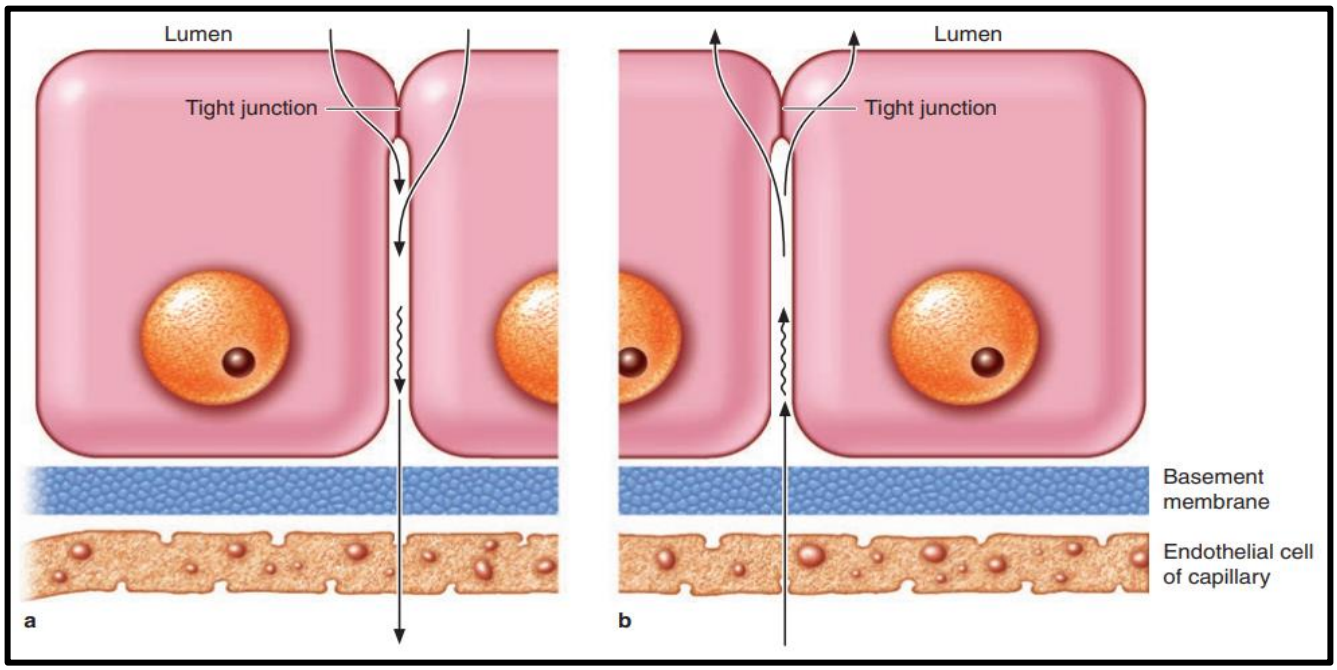


Figure 20: Ion and water absorption and secretion. Ion and water transport across epithelia can occur in either direction, depending on the organ involved. (a) Absorption is the process of transport from an organ or duct's lumen to capillaries near the epithelial basement membrane and involves movement from the apical to the basolateral cell membrane domains. Absorption occurs for example in the epithelium of the gallbladder and intestine where it serves to concentrate bile or obtain water and ions from digested material. (b) Secretion involves transport in the other direction from the capillaries into a lumen, as in many glands and the choroid plexus. Secretion by epithelial cells removes water from the neighboring interstitial fluid or plasma and releases it as part of the specialized aqueous fluids in such organs.

Apical tight junctions prevent paracellular diffusion or backflow between the cells. Epithelia of kidney tubules are key sites for ion and water transport, maintaining the body's overall balance of salts and water. Cells of the proximal renal tubules are specialized structurally for transcellular transport. The apical surface at the tubule lumen is freely permeable to Na+, and the basolateral cell membranes have sodium pumps for the active extrusion of Na+ into the interstitial fluid outside the tubules. Osmotic and electrical balance is maintained by the passive transfer of chloride ions (Cl−) and water into the cell. The basal membrane of these cells is elaborately folded, with mitochondria

located between the folds to supply ATP for Na+/K+ pumps (**Figure 21**). Lateral membrane folds interdigitating between the cells further increase the surface area for transport. Regulated transfer of ions and water by various epithelial cells along the renal tubules maintains the ionic balance within the body and allows excretion of excess water and salts in the urine.

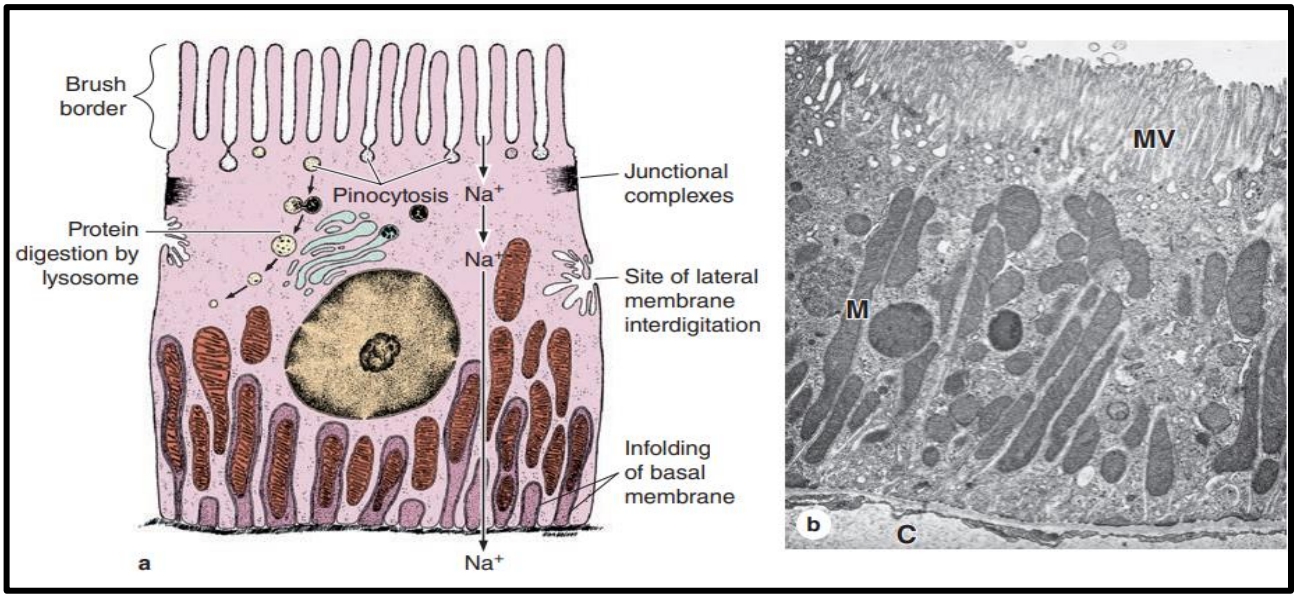


Figure 21: Features of absorptive cells.

All cells can also internalize extracellular molecules and fluid using endocytosis and formation of cytoplasmic, membrane-bound vesicles. This activity is clearly observed in the simple squamous epithelial cells lining blood and lymphatic capillaries (endothelia) or body cavities (mesothelia). These thin cells have few organelles other than the abundant pinocytotic vesicles, which cross the thin cells in both directions and release their contents on the opposite side by exocytosis. This process of **transcytosis** also occurs between the apical and basolateral membranes domains in cells of simple cuboidal and columnar epithelia and is important in many physiologic processes.

Renewal Of Epithelial Cells

Epithelial tissues are relatively labile structures whose cells are renewed continuously by mitotic activity and stem cell populations. The rate of renewal varies widely; it can be fast in tissues such as the intestinal epithelium, which is replaced every week, or slow, as in the large glands. In stratified epithelial tissues, stem cells and mitosis occur only within the basal layer in contact with the basal lamina. In some functionally complex epithelia, stem cells are located only in restricted niches some distance from the transit amplifying cells and differentiating cells. For example, the epithelium lining the small intestine is derived completely from stem cells found in the

simple glands between the intestinal villi. In the epidermis, many stem cells are located at a characteristic position along the wall of hair follicles.

›››MEDICAL APPLICATION

Both benign and malignant tumors can arise from most types of epithelial cells. Malignant tumors of epithelial origin are called carcinomas (Gr. karkinos, cancer + oma, tumor). Malignant tumors derived from glandular epithelial tissue are called adenocarcinomas (Gr. adenos, gland + karkinos). Adenocarcinomas are by far the most common tumors in adults after age 45.

Epithelia are normally capable of rapid repair and replacement of apoptotic or damaged cells. In some large glands, most notably the liver, mitotic activity is normally rare but is actively renewed following major damage to the organ. When a portion of liver tissue is removed surgically or lost by the acute effects of toxic substances, cells of undamaged regions quickly begin active proliferation and a mass of liver tissue with normal function is regenerated.

Some epithelial cells are prone to abnormal growth or dysplasia, which can progress to precancerous growth called neoplasia. Early neoplastic growth is often reversible and does not always result in cancer. Under certain abnormal conditions, one type of epithelial tissue may undergo transformation into another type in another reversible process called **metaplasia**. In heavy cigarette smokers, the ciliated pseudostratified epithelium lining the bronchi can be transformed into stratified squamous epithelium.

Lecture 3: Connective Tissues

Connective tissue provides a matrix that supports and physically connects other tissues and cells together to form the organs of the body. The interstitial fluid of connective tissue gives metabolic support to cells as the medium for diffusion of nutrients and waste products.

Unlike the other tissue types (epithelium, muscle, and nerve), which consist mainly of cells, the major constituent of connective tissue is **the extracellular matrix (ECM).** Extracellular matrices consist of different combinations of **protein fibers** (collagen and elastic fibers) and **ground substance**. Ground substance is a complex of anionic, hydrophilic proteoglycans, glycosaminoglycans (GAGs), and multiadhesive glycoproteins (laminin, fibronectin, and others). As described briefly with the basal lamina, such glycoproteins help stabilize the ECM by binding to other matrix components and to integrins in cell membranes. Water within this ground substance allows the exchange of nutrients and metabolic wastes between cells and the blood supply.

The variety of connective tissue types in the body reflects differences in composition and amount of the cells, fibers, and ground substance which together are responsible for the remarkable structural, functional, and pathologic diversity of connective tissue.

All connective tissues originate from embryonic **mesenchyme**, a tissue developing mainly from the middle layer of the embryo, the mesoderm. Mesenchyme consists largely of viscous ground substance with few collagen fibers (Figure 1). **Mesenchymal cells** are undifferentiated and have large nuclei, with prominent nucleoli and fine chromatin. They are often said to be “spindle-shaped,” with their scant cytoplasm extended as two or more thin cytoplasmic processes. Mesodermal cells migrate from their site of origin in the embryo, surrounding and penetrating developing organs. In addition to producing all types of connective tissue proper and the specialized connective tissues bone and cartilage, the embryonic mesenchyme includes stem cells for other tissues such as blood, the vascular endothelium, and muscle.

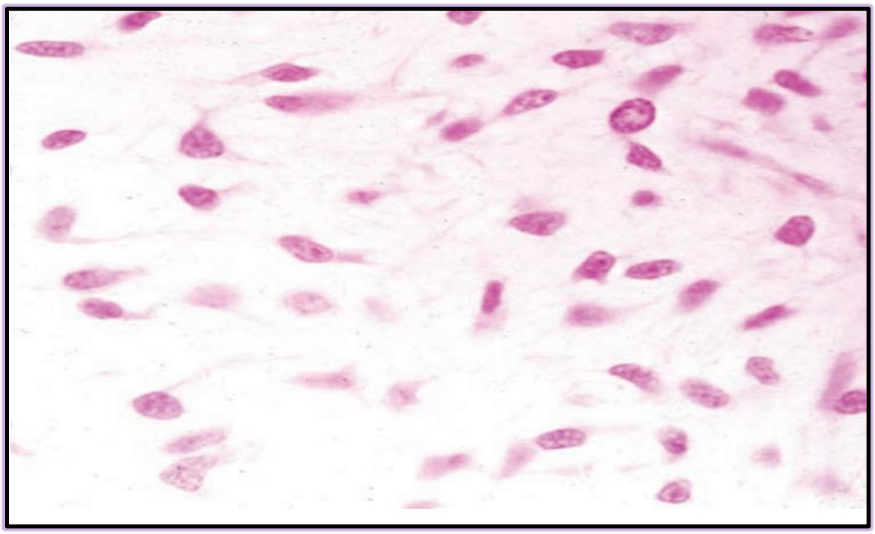


Figure 1: Embryonic mesenchyme.

Cells of Connective Tissue

Fibroblasts are the key cells in connective tissue proper (Figure 2 and Table 1). Fibroblasts originate locally from mesenchymal cells and are permanent residents of connective tissue. Other cells found here, such as **macrophages**, **plasma cells**, and **mast cells**, originate from hematopoietic stem cells in bone marrow, circulate in the blood, and then move into connective tissue where they function. These and other white blood cells (leukocytes) are transient cells of most connective tissues, where they perform various functions for a short period as needed and then die by apoptosis.

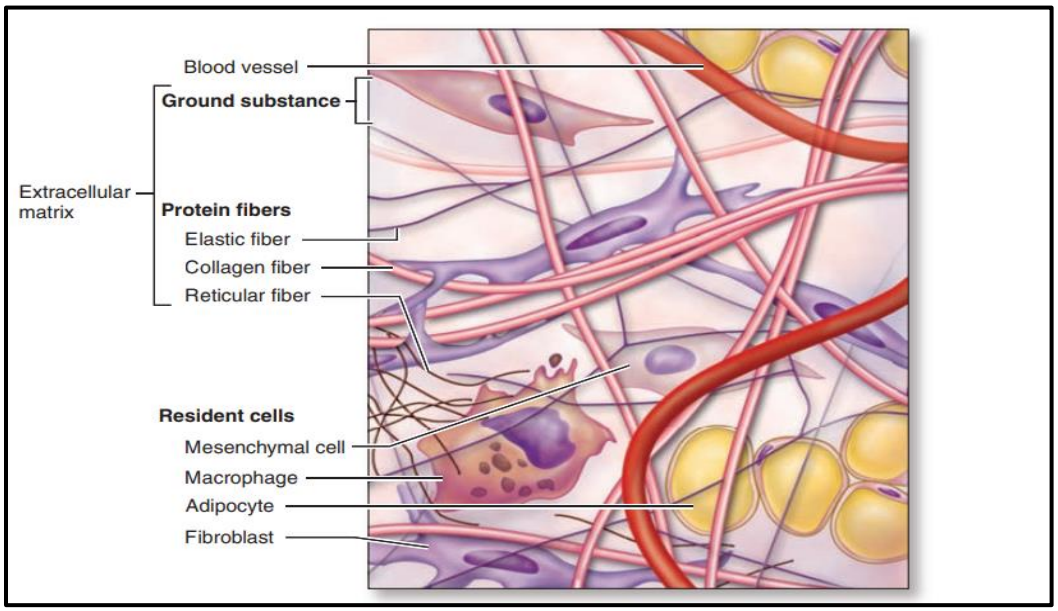


Figure 2: Cellular and extracellular components of connective tissue.

Table 1: Functions of cells in connective tissue proper.

Cell Type	Major Product or Activity
Fibroblasts (fibrocytes)	Extracellular fibers and ground substance
Plasma cells	Antibodies
Lymphocytes (several types)	Various immune/defense functions
Eosinophilic leukocytes	Modulate allergic/vasoactive reactions and defense against parasites
Neutrophilic leukocytes	Phagocytosis of bacteria
Macrophages	Phagocytosis of ECM components and debris; antigen processing and presentation to immune cells; secretion of growth factors, cytokines, and other agents
Mast cells and basophilic leukocytes	Pharmacologically active molecules (eg, histamine)
Adipocytes	Storage of neutral fats

➢ Fibroblasts

Fibroblasts (Figure 3), the most common cells in connective tissue proper, produce and maintain most of the tissue's extracellular components. Fibroblasts synthesize and secrete collagen (the most abundant protein of the body) and elastin, which both form large fibers, as well as the GAGs, proteoglycans, and multiadhesive glycoproteins that comprise the ground substance. As described later, most of the secreted ECM components undergo further modification outside the cell before assembling as a matrix.

Distinct levels of fibroblast activity can be observed histologically (Figure 3b). Cells with intense synthetic activity are morphologically different from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. Some histologists reserve the term "fibroblast" to denote the active cell and "fibrocyte" to denote the quiescent cell. The active fibroblast has more abundant and irregularly branched cytoplasm, containing much rough endoplasmic reticulum (RER) and a welldeveloped Golgi apparatus, with a large, ovoid, euchromatic nucleus and a prominent nucleolus. The quiescent cell is smaller than the active fibroblast, is usually

spindle-shaped with fewer processes, much less RER, and a darker, more heterochromatic nucleus.

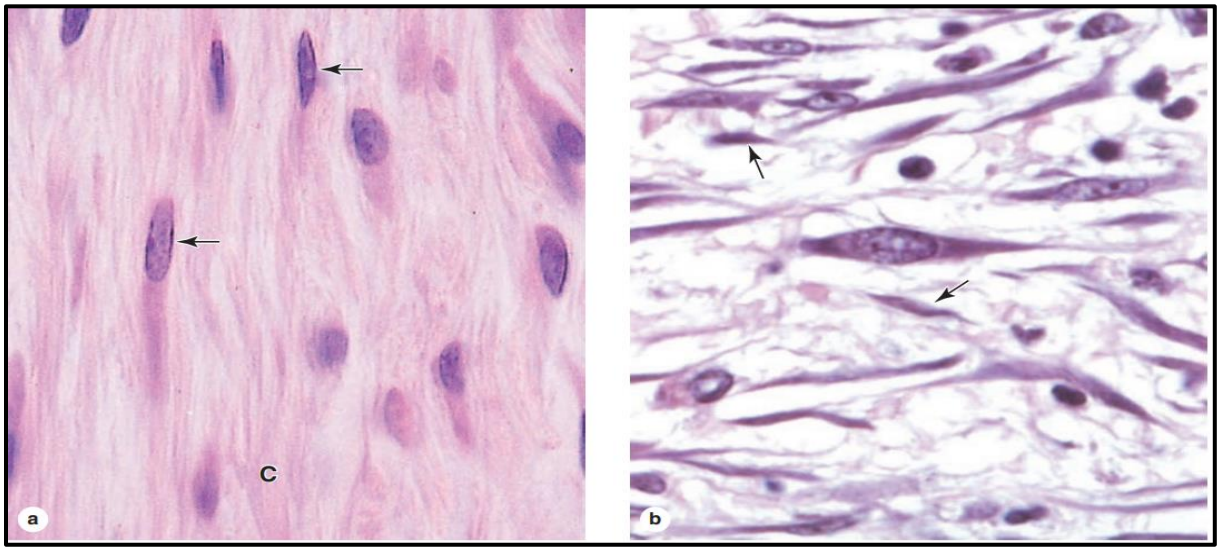


Figure 3: Fibroblasts. (a) Fibroblasts typically have large active nuclei and eosinophilic cytoplasm that tapers off in both directions along the axis of the nucleus, a morphology often referred to as "spindle-shaped." Nuclei (arrows) are clearly seen, but the eosinophilic cytoplasmic processes resemble the collagen bundles (C) that fill the ECM and are difficult to distinguish in H&E-stained sections. (b) Both active and quiescent fibroblasts may sometimes be distinguished, as in this section of dermis. Active fibroblasts have large, euchromatic nuclei and basophilic cytoplasm, while inactive fibroblasts (or fibrocytes) are smaller with more heterochromatic nuclei (arrows). The round, very basophilic round cells are in leukocytes. (Both X400; H&E).

Fibroblasts are targets of many families of proteins called **growth factors** that influence cell growth and differentiation. In adults, connective tissue fibroblasts rarely undergo division. However, stimulated by locally released growth factors, cell cycling and mitotic activity resume when the tissue requires additional fibroblasts, for example, to repair a damaged organ. Fibroblasts involved in wound healing, sometimes called **myofibroblasts**, have a well-developed contractile function and are enriched with a form of actin also found in smooth muscle cells.

➢ Adipocytes

Adipocytes (L. adeps, fat + Gr. kytos, cell), or fat cells, are found in the connective tissue of many organs. These large, mesenchymally derived cells are specialized for cytoplasmic storage of lipid as neutral fats, or less commonly for the production of heat. Tissue with a large population of adipocytes, called adipose connective tissue, serves to cushion and insulate the skin and other organs.

➢ Macrophages & the Mononuclear Phagocyte System

Macrophages have highly developed phagocytic ability and specialize in turnover of protein fibers and removal of apoptotic cells, tissue debris, or other particulate material, being especially abundant at sites of inflammation. Size and shape

vary considerably, corresponding to their state of functional activity. A typical macrophage measures between 10 and 30 μm in diameter and has an eccentrically located, oval or kidney-shaped nucleus. Macrophages are present in the connective tissue of most organs and are sometimes referred to by pathologists as "histiocytes.

In the TEM, macrophages are shown to have a characteristic irregular surface with pleats, protrusions, and indentations, features related to their active pinocytotic and phagocytic activities (Figure 4). They generally have well-developed Golgi complexes and many lysosomes.

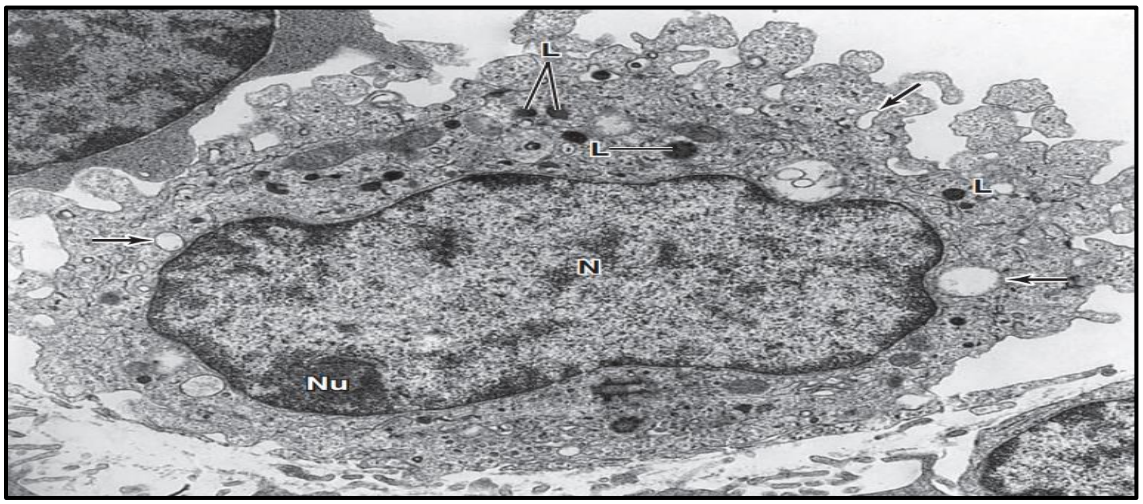


Figure 4: Macrophage ultrastructure. Characteristic features of macrophages seen in this TEM of one such cell are the prominent nucleus (N) and the nucleolus (Nu) and the numerous secondary lysosomes (L). The arrows indicate phagocytic vacuoles near the protrusions and indentations of the cell surface.

Macrophages derive from precursor cells called **monocytes** circulating in the blood. Monocytes cross the epithelial wall of small venules to enter connective tissue, where they differentiate, mature, and acquire the morphologic features of macrophages. Monocytes formed in the yolk sac during early embryonic development circulate and become resident in developing organs throughout the body, comprising a group of related cells called the **mononuclear phagocyte system**. Many of these macrophage-like cells with prominent functions in various organs have specialized names (Table 2). All are long-living cells, surviving with relative inactivity in tissues for months or years. During inflammation and tissue repair which follow organ damage, macrophages become activated and play a very important role. Under such conditions these cells increase in number, mainly in the connective tissue stroma, both by proliferation and by recruiting additional monocytes formed in the bone marrow. The transformation from monocytes to macrophages in connective tissue involves increases in cell size, increased protein synthesis, and increases in the number of Golgi complexes and lysosomes. In addition to debris removal, macrophages secrete growth factors important for tissue repair and also function in the uptake, processing, and presentation of antigens for lymphocyte activation, a role discussed later with the immune system.

Table 2: Distribution and main functions of the cells of the mononuclear phagocyte system.

Cell Type	Major Location	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow, pleural and peritoneal cavities	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing, and presentation
Kupffer cell	Liver (perisinusoidal)	Same as macrophages
Microglial cell	Central nervous system	Same as macrophages
Langerhans cell	Epidermis of skin	Antigen processing and presentation
Dendritic cell	Lymph nodes, spleen	Antigen processing and presentation
Osteoclast (from fusion of several macrophages)	Bone	Localized digestion of bone matrix
Multinuclear giant cell (several fused macrophages)	In connective tissue under various pathological conditions	Segregation and digestion of foreign bodies

➢ Mast Cells

Mast cells are oval or irregularly shaped cells of connective tissue, between 7 and 20 μm in diameter, filled with basophilic secretory granules that often obscure the central nucleus (Figure 5). These granules are electron dense and of variable size, ranging from 0.3 to 2.0 μm in diameter. Because of the high content of acidic radicals in their sulfated GAGs, mast cell granules display **metachromasia**, which means that they can change the color of some basic dyes (eg, toluidine blue) from blue to purple or red. The granules are poorly preserved by common fixatives, so mast cells may be difficult to identify in routinely prepared slides.

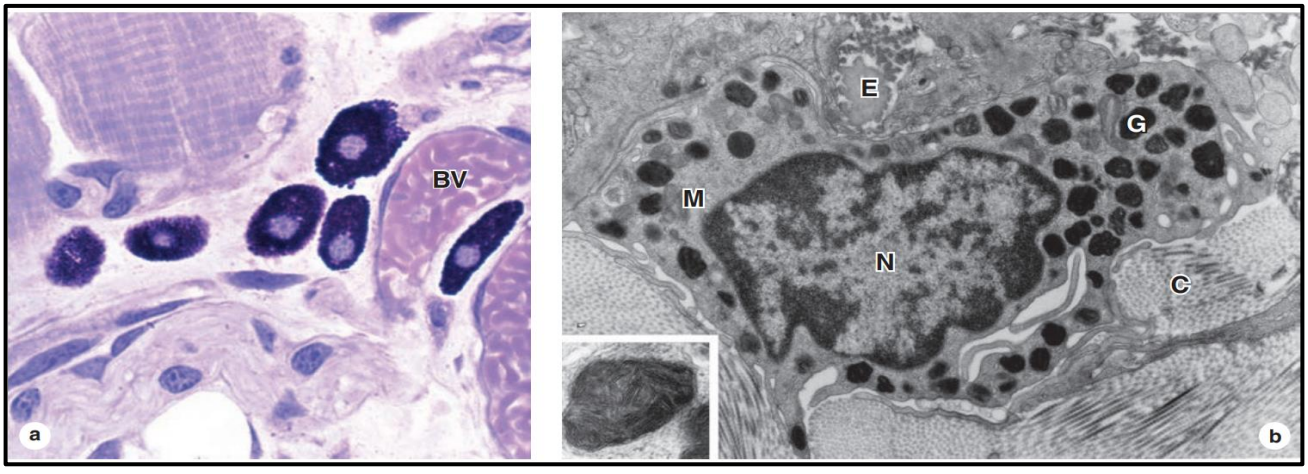


Figure 5: Mast cells are components of loose connective tissues, often located near small blood vessels (BV). (a) They are typically oval shaped, with cytoplasm filled with strongly basophilic granules. (X400;

PT) .(b) Ultrastructurally mast cells show little else around the nucleus (N) besides these cytoplasmic granules (G), except for occasional mitochondria (M). The granule staining in the TEM is heterogeneous and variable in mast cells from different tissues; at higher magnifications some granules may show a characteristic scroll-like substructure (inset) that contains preformed mediators such as histamine and proteoglycans. The ECM near this mast cell includes elastic fibers (E) and bundles of collagen fibers (C).

Mast cells function in the localized release of many bioactive substances important in the local inflammatory response, innate immunity, and tissue repair. A partial list of molecules released from these cells' secretory granules includes the following:

■ **Heparin**, a sulfated GAG that acts locally as an anticoagulant.

■ **Histamine**, which promotes increased vascular permeability and smooth muscle contraction.

■ **Serine proteases**, which activate various mediators of inflammation.

■ **Eosinophil** and **neutrophil chemotactic factors**, which attract those leukocytes.

■ **Cytokines**, polypeptides directing activities of leukocytes and other cells of the immune system

■ **Phospholipid** precursors, which are converted to prostaglandins, leukotrienes, and other important lipid mediators of the inflammatory response.

Occurring in connective tissue of many organs, mast cells are especially numerous near small blood vessels in skin and mesenteries (perivascular mast cells) and in the tissue that lines digestive and respiratory tracts (mucosal mast cells); the granule content of the two populations differs somewhat. These major locations suggest that mast cells place themselves strategically to function as sentinels detecting invasion by microorganisms.

Release of certain chemical mediators stored in mast cells promotes the allergic reactions known as **immediate hypersensitivity reactions** because they occur within a few minutes after the appearance of an antigen in an individual previously sensitized to that antigen.

Like macrophages, mast cells originate from progenitor cells in the bone marrow, which circulate in the blood, cross the wall of small vessels called venules, and enter connective tissues, where they differentiate. Although mast cells are in many respects similar to basophilic leukocytes, they appear to have a different lineage at least in humans.

➢ Plasma Cells

Plasma cells are lymphocyte-derived, antibody-producing cells. These relatively large, ovoid cells have basophilic cytoplasm rich in RER and a large

Golgi apparatus near the nucleus that may appear pale in routine histologic preparations (Figure 6).

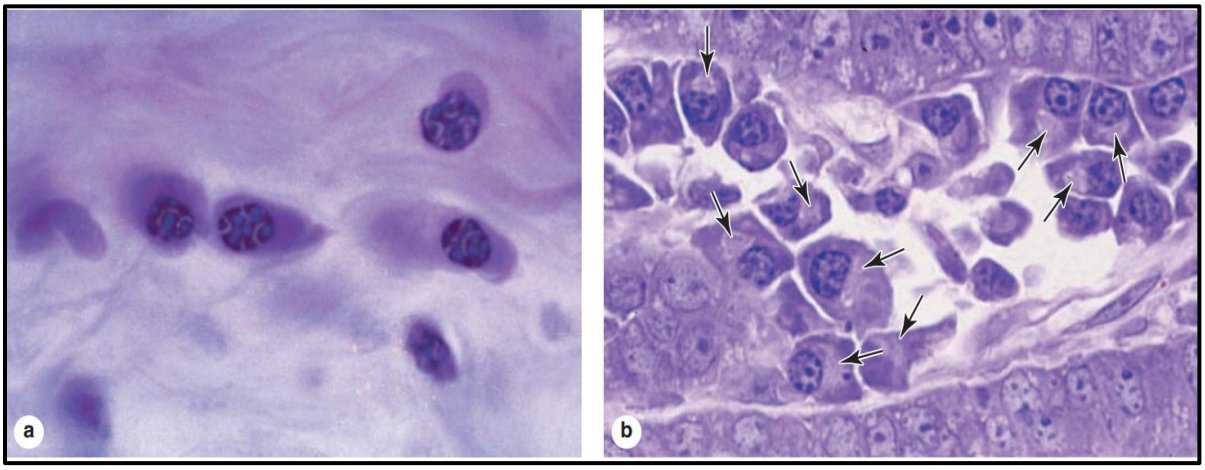


Figure 6: Plasma cells. (a) Plasma cells are large, ovoid cells, with basophilic cytoplasm. The round nuclei frequently show peripheral clumps of heterochromatin, giving the structure a "clock-face" appearance. (X640; H&E) (b) Plasma are often more abundant in infected tissues, as in the inflamed lamina propria shown here. A large pale Golgi apparatus (arrows) at a juxtanuclear site in each cell is actively involved in the terminal glycosylation of the antibodies (glycoproteins).

The nucleus of the plasma cell is generally spherical but eccentrically placed. Many of these nuclei contain compact, peripheral regions of heterochromatin alternating with lighter areas of euchromatin. At least a few plasma cells are present in most connective tissues. Their average life span is only 10-20 days.

➢ Leukocytes

Other white blood cells, or **leukocytes**, besides macrophages and plasma cells normally comprise a population of wandering cells in connective tissue. Derived from circulating blood cells, they leave blood by migrating between the endothelial cells of venules to enter connective tissue. This process increases greatly during inflammation, which is a vascular and cellular defensive response to injury or foreign substances, including pathogenic bacteria or irritating chemical substances.

Inflammation begins with the local release of chemical mediators from various cells, the ECM and blood plasma proteins. These substances act on local blood vessels, mast cells, macrophages, and other cells to induce events characteristic of inflammation, for example, increased blood flow and vascular permeability, entry and migration of leukocytes, and activation of macrophages for phagocytosis. Most leukocytes function in connective tissue only for a few hours or days and then undergo apoptosis.

Fibers Of Connective Tissue

The fibrous components of connective tissue are elongated structures formed from proteins that polymerize after secretion from fibroblasts (Figure 2). The three main types of fibers include **collagen**, **reticular**, and **elastic fibers**. Collagen and reticular

fibers are both formed by proteins of the collagen family, and elastic fibers are composed mainly of the protein **elastin**. These fibers are distributed unequally among the different types of connective tissue, with the predominant fiber type conferring most specific tissue properties.

➢ Collagen

The **collagens** constitute a family of proteins selected during evolution for their ability to form various extracellular fibers, sheets, and networks, all of which extremely strong and resistant to normal shearing and tearing forces. Collagen is a key element of all connective tissues, as well as epithelial basement membranes and the external laminae of muscle and nerve cells.

Collagen is the most abundant protein in the human body, representing 30% of its dry weight. A major product of fibroblasts, collagens are also secreted by several other cell types and are distinguishable by their molecular compositions, morphologic characteristics, distribution, functions, and pathologies. A family of 28 collagens exists in vertebrates, numbered in the order they were identified, they can be categorized according to the structures formed by their interacting α-chains subunits:

■ **Fibrillar collagens**, notably **collagen types I, II, and III**, have polypeptide subunits that aggregate to form large fibrils clearly visible in the electron or light microscope (Figure 7). Collagen type I, the most abundant and widely distributed collagen, forms large, eosinophilic bundles usually called **collagen fibers**. These often densely fill the connective tissue, forming structures such as tendons, organ capsules, and dermis.

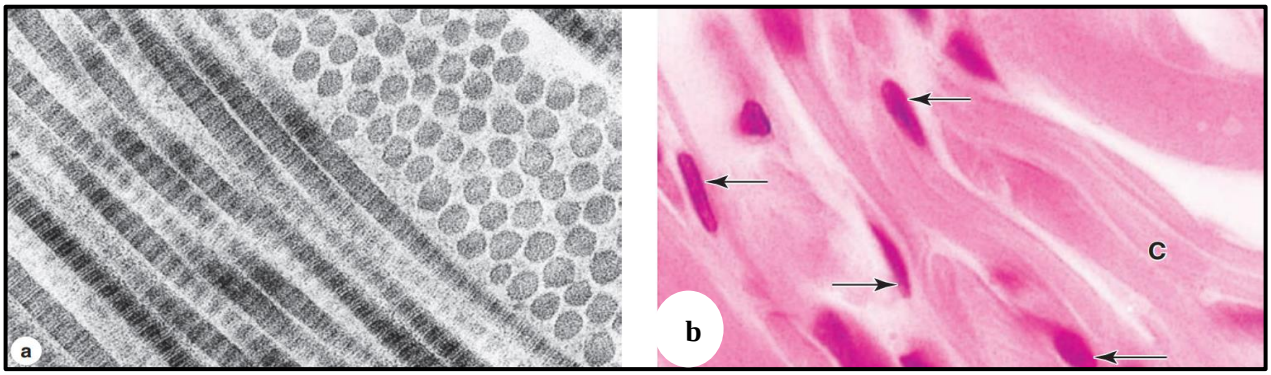


Figure 7: Type I collagen. (a) TEM shows fibrils cut longitudinally and transversely. In longitudinal sections fibrils display alternating dark and light bands; in cross section the cut ends of individual collagen molecules appear as dots. Ground substance completely surrounds the fibrils. (X100,000) (b) The large bundles of type I collagen fibrils (C) appear as acidophilic collagen fibers in connective tissues, where they may fill the extracellular space.

■ **Network or sheet-forming collagens** such as **type IV collagen** have subunits produced by epithelial cells and are major structural proteins of external laminae and all epithelial basal laminae.

■ **Linking/anchoring collagens** are short collagens that link fibrillar collagens to one another (forming larger fibers) and to other components of the ECM. **Type VII collagen** binds type IV collagen and anchors the basal lamina to the underlying reticular lamina in basement membranes.

Collagen synthesis occurs in many cell types but is a specialty of fibroblasts. The initial **procollagen α chains** are polypeptides made in the RER. Several different α chains of variable lengths and sequences can be synthesized from the related collagen genes. In the ER three α chains are selected, aligned, and stabilized by disulfide bonds at their carboxyl terminals, and folded as a **triple helix**, another defining feature of collagens. The triple helix undergoes exocytosis and is cleaved to a rodlike **procollagen molecule** (Figure 8) that is the basic subunit from which the fibers or sheets are assembled. These subunits may be homotrimeric, with all three chains identical, or heterotrimeric, with two or all three chains having different sequences. Different combinations of procollagen α chains produce the various types of collagen with different structures and functional properties.

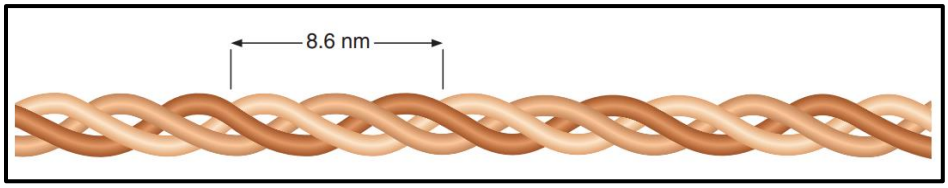


Figure 8: The collagen subunit.

An unusually large number of posttranslational processing steps are required to prepare collagen for its final assembly in the ECM. These steps have been studied most thoroughly for type I collagen, which accounts for 90% of all the body's collagen. The most important parts of this process are summarized in Figure 9.

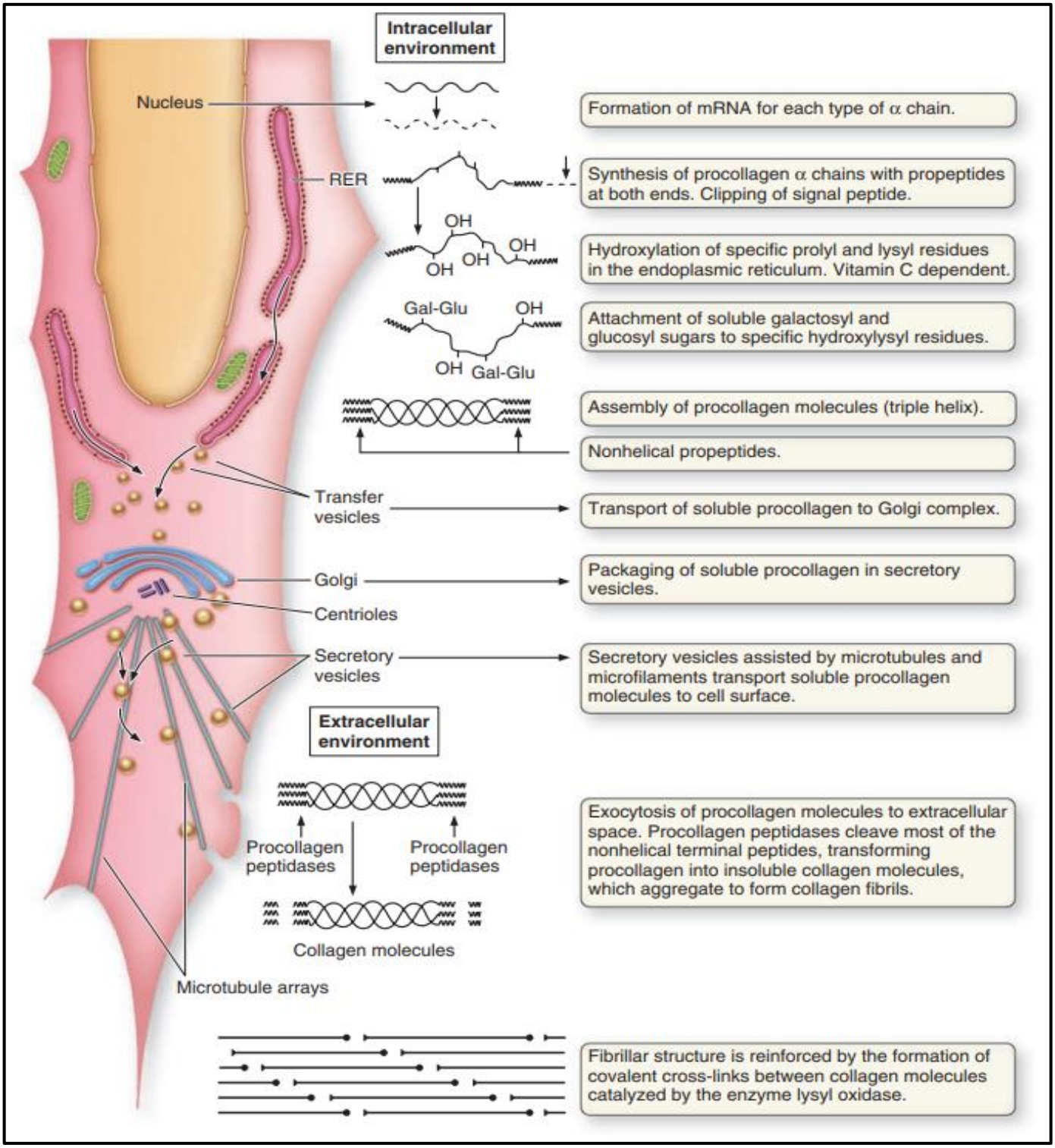


Figure 9: Collagen synthesis.

Collagen turnover and renewal in normal connective tissue is generally a very slow but ongoing process. In some organs, such as tendons and ligaments, the collagen is very stable, whereas in others, as in the periodontal ligament surrounding teeth, the collagen turnover rate is high. To be renewed, the collagen must first be degraded.

Degradation is initiated by specific enzymes called **collagenases**, which are members of an enzyme class called **matrix metalloproteinases (MMPs)**, which clip collagen fibrils or sheets in such a way that they are then susceptible to further degradation by nonspecific proteases. Various MMPs are secreted by macrophages and play an important role in remodeling the ECM during tissue repair.

➢ Reticular Fibers

Found in delicate connective tissue of many organs, notably in the immune system, **reticular fibers** consist mainly of collagen type III, which forms an extensive network (reticulum) of thin (diameter 0.5-2 μm) fibers for the support of many different cells. Reticular fibers are seldom visible in hematoxylin and eosin (H&E) preparations but are characteristically stained black after impregnation with silver salts (Figure 10) and are thus termed **argyrophilic** (Gr. argyros, silver). Reticular fibers are also periodic acid–Schiff (PAS) positive, which, like argyrophilia, is due to the high content of sugar chains bound to type III collagen α chains.

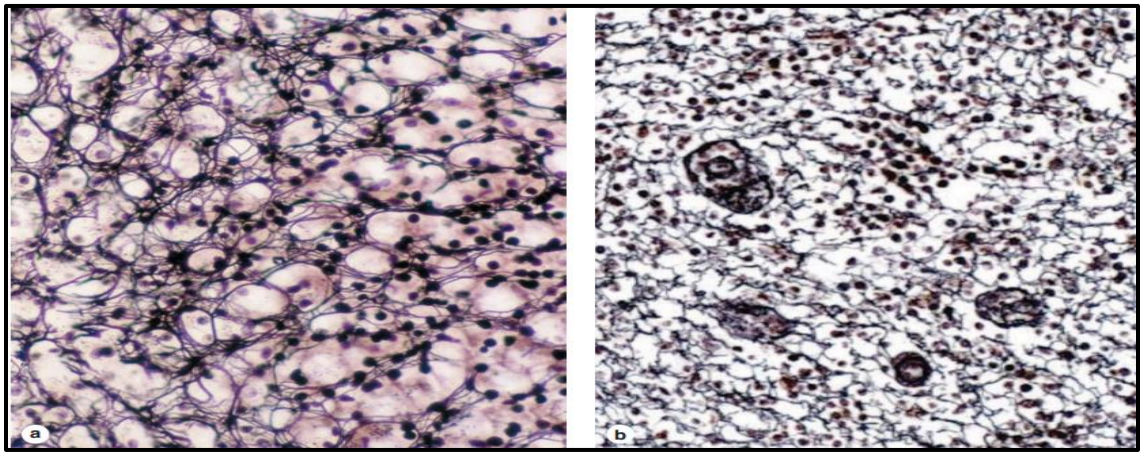


Figure 10: Reticular Fibers. In these silver-stained sections of adrenal cortex (a) and lymph node (b), networks of delicate, black reticular fibers are prominent. These fibers serve as a supportive stroma in most lymphoid and hematopoietic organs and many endocrine glands. The fibers consist of type III collagen that is heavily glycosylated, producing the black argyrophilia. Cell nuclei are also dark, but cytoplasm is unstained.

Reticular fibers contain up to 10% carbohydrate as opposed to 1% in most other collagen fibers. Reticular fibers produced by fibroblasts occur in the reticular lamina of basement membranes and typically also surround adipocytes, smooth muscle and nerve fibers, and small blood vessels. Delicate reticular networks serve as the supportive stroma for the parenchymal secretory cells and rich microvasculature of the liver and endocrine glands. Abundant reticular fibers also characterize the stroma of hemopoietic tissue (bone marrow), the spleen, and lymph nodes where they support rapidly changing populations of proliferating cells and phagocytic cells.

➢ Elastic Fibers

Elastic fibers are also thinner than the type I collagen fibers and form sparse networks interspersed with collagen bundles in many organs, particularly those subject to regular stretching or bending. As the name implies, elastic fibers have rubberlike properties that allow tissue containing these fibers, such as the stroma of the lungs, to be stretched or distended and return to their original shape. In the wall of large blood vessels, especially arteries, elastin also occurs as fenestrated sheets called **elastic lamellae**. Elastic fibers and lamellae are not strongly acidophilic and stain poorly with H&E; they are stained more darkly than collagen with other stains such as orcein and aldehyde fuchsin (Figure 11).

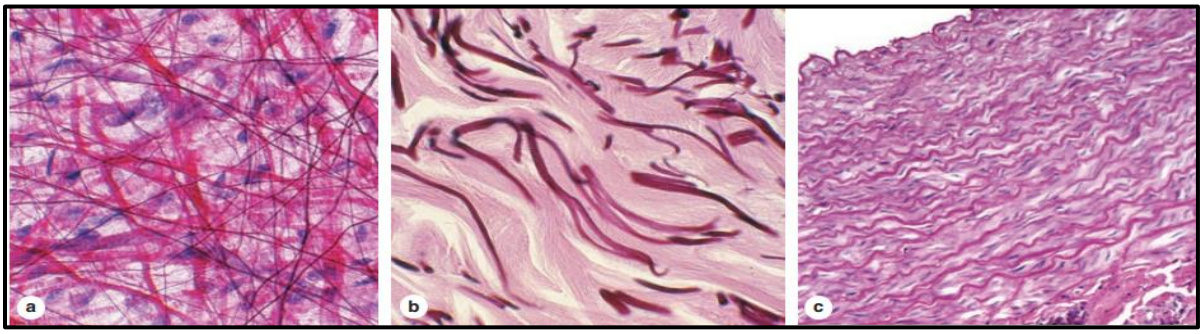


Figure 11: Elastic fibers. (a) The length, diameter, distribution, and density of dark elastic fibers are easily seen in this spread preparation of nonstretched connective tissue in a mesentery. (X200; Hematoxylin and orcein) (b) In sectioned tissue at higher magnification, elastic fibers can be seen among the acidophilic collagen bundles of dermis. (X400; Aldehyde fuchsin) (c) Elastic lamellae in the wall of the aorta are more darkly stained, incomplete sheets of elastin between the layers of eosinophilic smooth muscle. (X80; H&E).

Elastic fibers (and lamellae) are a composite of **fibrillin** (350 kDa), which forms a network of **microfibrils**, embedded in a larger mass of cross-linked **elastin** (60 kDa). Both proteins are secreted from fibroblasts (and smooth muscle cells in vascular walls) and give rise to elastic fibers in a stepwise manner are shown in Figure 12.

Stages in the formation of elastic fibers can be seen by TEM.

(a) Initially, a developing fiber consists of many 10-nm-diameter microfibrils composed of fibrillin subunits secreted by fibroblasts and smooth muscle cells.

(b) Elastin is deposited on the scaffold of microfibrils, forming growing, amorphous composite structures. The elastin molecules are also secreted by the fibroblasts and quickly become covalently cross-linked into larger assemblies.

(c) Elastin accumulates and ultimately occupies most of the electron-dense center of the single elastic fiber shown here. Fibrillin microfibrils typically remain visible at the fiber surface. Collagen fibrils, seen in cross section, are also present surrounding the elastic fiber.

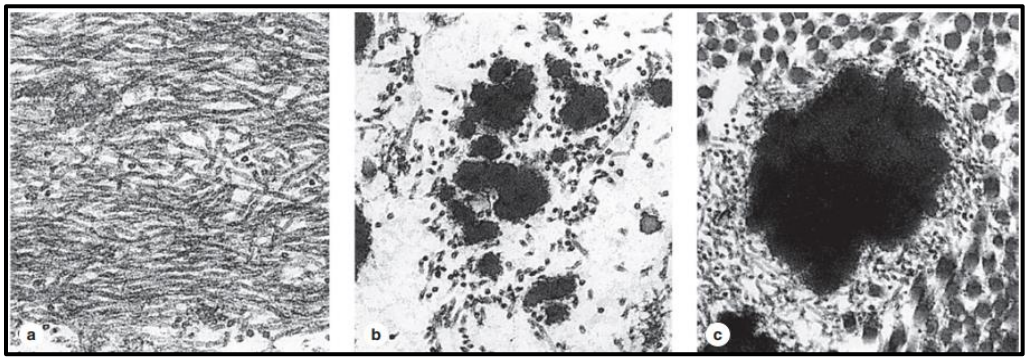


Figure 12: Formation of elastic fibers.

The elastic properties of these fibers and lamellae result from the structure of the elastin subunits and the unique crosslinks holding them together. Elastin molecules have many lysine-rich regions interspersed with hydrophobic domains rich in lysine and proline that are thought to form extensible, random-coil conformations (like natural rubber). Elastin resists digestion by most proteases, but it is hydrolyzed by pancreatic **elastase**.

Ground Substance

The ground substance of the ECM is a highly hydrated (with much bound water), transparent, complex mixture of three major kinds of macromolecules: **glycosaminoglycans** (GAGs), **proteoglycans**, and **multiadhesive** glycoproteins. Filling the space between cells and fibers in connective tissue, ground substance allows diffusion of small molecules and, because it is viscous, acts as both a lubricant and a barrier to the penetration of invaders. Physical properties of ground substance also profoundly influence various cellular activities. When adequately fixed for histologic analysis, its components aggregate as fine, poorly resolved material that appears in TEM preparations as electron-dense filaments or granules (Figure 13).

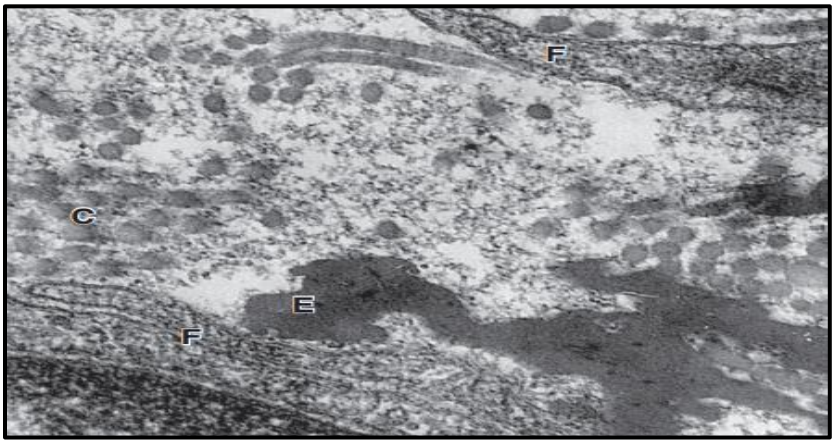


Figure 13: Ground substance of the ECM. (a) TEM of connective tissue ECM reveals ground substance as areas containing only fine granular material among the collagen (C) fibers, elastic (E) fibers and fibroblast processes (F). X100,000.

Water in the ground substance of connective tissue is referred to as interstitial fluid and has an ion composition similar to that of blood plasma. Interstitial fluid also contains plasma proteins of low molecular weight that pass through the thin walls of the smallest blood vessels, the capillaries. * Capillaries in connective tissue also bring the various nutrients required by cells and carry away their metabolic waste products to the detoxifying and excretory organs, the liver and kidneys. Interstitial fluid is the solvent for these substances.

Types of Connective Tissue

Different combinations and densities of the cells, fibers, and other ECM components produce graded variations in histological structure within connective tissue. Descriptive names or classifications used for the various types of connective tissue typically denote either a structural characteristic or a major component. Table 3 gives a classification commonly used for the main types of connective tissue.

Table 3: Classification of connective or supporting tissues.

	General Organization	Major Functions	Examples
Connective Tissue Proper			
Loose (areolar) connective tissue	Much ground substance; many cells and little collagen, randomly distributed	Supports microvasculature, nerves, and immune defense cells	Lamina propria beneath epithelial lining of digestive tract
Dense irregular connective tissue	Little ground substance; few cells (mostly fibroblasts); much collagen in randomly arranged fibers	Protects and supports organs; resists tearing	Dermis of skin, organ capsules, submucosa layer of digestive tract
Dense regular connective tissue	Almost completely filled with parallel bundles of collagen; few fibroblasts, aligned with collagen	Provide strong connections within musculoskeletal system; strong resistance to force	Ligaments, tendons, aponeuroses, corneal stroma
Embryonic Connective Tissues			
Mesenchyme	Sparse, undifferentiated cells, uniformly distributed in matrix with sparse collagen fibers	Contains stem/progenitor cells for all adult connective tissue cells	Mesodermal layer of early embryo
Mucoid (mucous) connective tissue	Random fibroblasts and collagen fibers in viscous matrix	Supports and cushions large blood vessels	Matrix of the fetal umbilical cord
Specialized Connective Tissues			
Reticular connective tissue	Delicate network of reticulin/ collagen III with attached fibroblasts (reticular cells)	Supports blood-forming cells, many secretory cells, and lymphocytes in most lymphoid organs	Bone marrow, liver, pancreas, adrenal glands, all lymphoid organs except the thymus

➢ Loose and Dense Connective tissues

Connective Tissue Proper Connective tissue proper is broadly classified as “loose” or “dense,” terms that refer to the amount of collagen present (Figure 14). **Loose connective tissue** is common, forming a layer beneath the epithelial lining of many organs and filling the spaces between fibers of muscle and nerve (Figure 14).

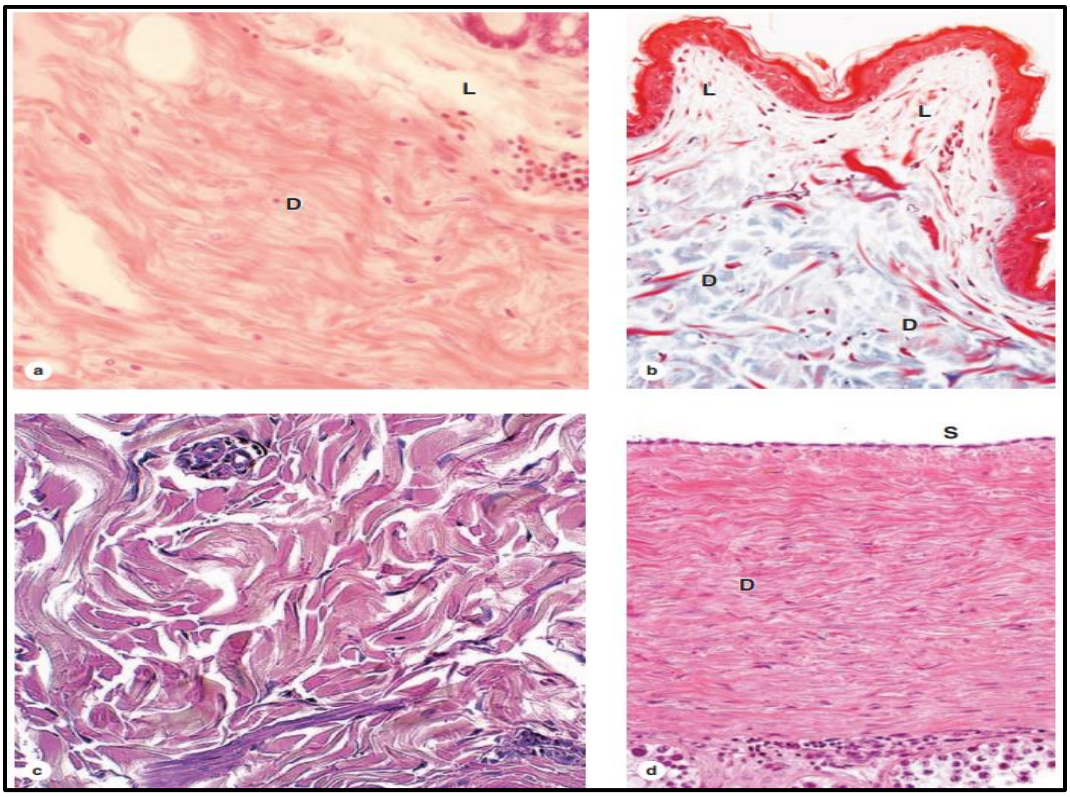


Figure 14: Loose connective tissue and dense irregular connective tissue. (a) Loose connective tissue (L) of a gland contains faintly stained ground substance with fine fibers of collagen and frequently forms a thin layer near epithelia, while dense irregular connective tissue (D) forms a thicker layer and is invariably much richer in larger bundles of collagen. (X100; H&E) (b) Trichrome staining of a section from skin demonstrates the blue staining of collagen with this method and its relative density in loose (L) and dense irregular (D) connective tissue. (X100; Mallory trichrome) (c) Another example of dense irregular connective tissue, showing the randomly arranged large collagen bundles.(X150; H&E) (d) Dense irregular connective tissue (D) forms a thick, protective capsule around many organs such as the testis shown here. Here the capsule is covered by a simple squamous epithelium of serous mesothelial cells (S),(X150; H&E).

Also called **areolar tissue**, the loose connective tissue typically contains cells, fibers, and ground substance in roughly equal parts. The most numerous cells are fibroblasts, but the other types of connective tissue cells are also normally found, along with nerves and small blood vessels. Collagen fibers predominate, but elastic and reticular fibers are also present. With at least a moderate amount of ground substance, loose connective tissue has a delicate consistency; it is flexible and not very resistant to stress.

Dense connective tissue has similar components as loose connective tissue, but with fewer cells, mostly fibroblasts, and a clear predominance of bundled type I

collagen fibers over ground substance. The abundance of collagen here protects organs and strengthens them structurally. In **dense irregular connective tissue**, bundles of collagen fibers appear randomly interwoven, with no definite orientation. The tough three-dimensional collagen network provides resistance to stress from all directions. Examples of dense irregular connective tissue include the deep dermis layer of skin and capsules surrounding most organs. Dense irregular and loose connective tissues are often closely associated, with the two types grading into each other and making distinctions between them somewhat arbitrary (Figure 14).

Dense regular connective tissue consists mostly of type I collagen bundles and fibroblasts aligned in parallel for great resistance to prolonged or repeated stresses from the same direction (Figure 15).

The best examples of dense regular connective tissue are the very strong and flexible **tendons** (Figure 15), cords connecting muscles to bones; **aponeuroses**, which are sheetlike tendons; and **ligaments**, bands or sheets that hold together components of the skeletal system. Consisting almost entirely of densely packed parallel collagen fibers separated by very little ground substance and having very few blood vessels, these inextensible structures are white in the fresh state. Fibrocytes with elongated nuclei lie parallel to the collagen fibers of dense regular connective tissue, with cytoplasmic folds enveloping portions of the collagen bundles (Figure 15b). Cytoplasm in these "tendinocytes" is difficult to distinguish in H&E-stained preparations because it is very sparse and has acidophilia like that of the collagen.

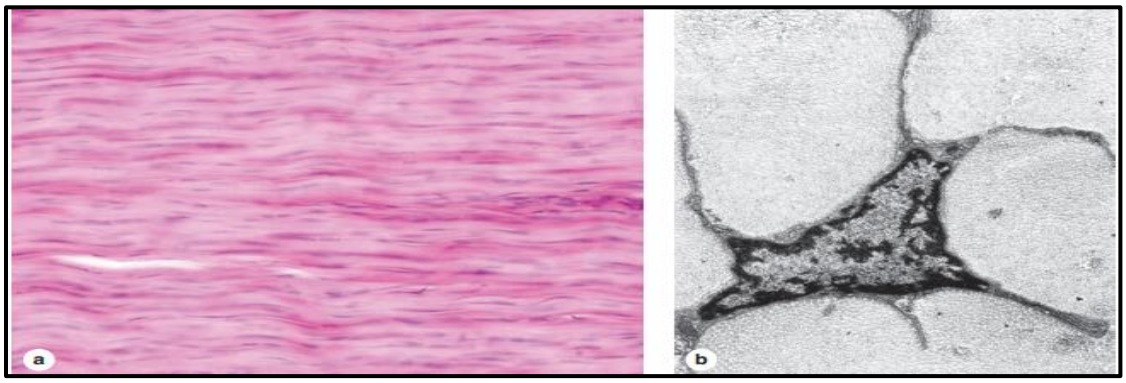


Figure 15: Dense regular connective tissue. (a) Micrograph shows a longitudinal section of dense regular connective tissue in a tendon. Long, parallel bundles of collagen fibers fill the spaces between the elongated nuclei of fibrocytes. (X100; H&E stain) (b) The electron micrograph shows one fibrocyte in a cross section of tendon, revealing that the sparse cytoplasm of the fibrocytes is divided into numerous thin cytoplasmic processes extending among adjacent collagen fibers. (X25,000).

➢ Reticular Tissue

Reticular tissue is characterized by abundant fibers of type III collagen forming a delicate network that supports various types of cells. This collagen is also known as reticulin and is produced by modified fibroblasts often called **reticular cells** that remain

associated with and partially cover the fibers (Figure 16). The loose disposition of glycosylated reticular fibers provides a framework with specialized microenvironments for cells in hemopoietic tissue and some lymphoid organs (bone marrow, lymph nodes, and spleen). The resulting cell-lined system creates a meshwork for the passage of leukocytes and lymph. Macrophages and dendritic cells (also in the mononuclear phagocyte family) are also dispersed within these reticular tissues to monitor cells formed there or passing through and to remove debris.

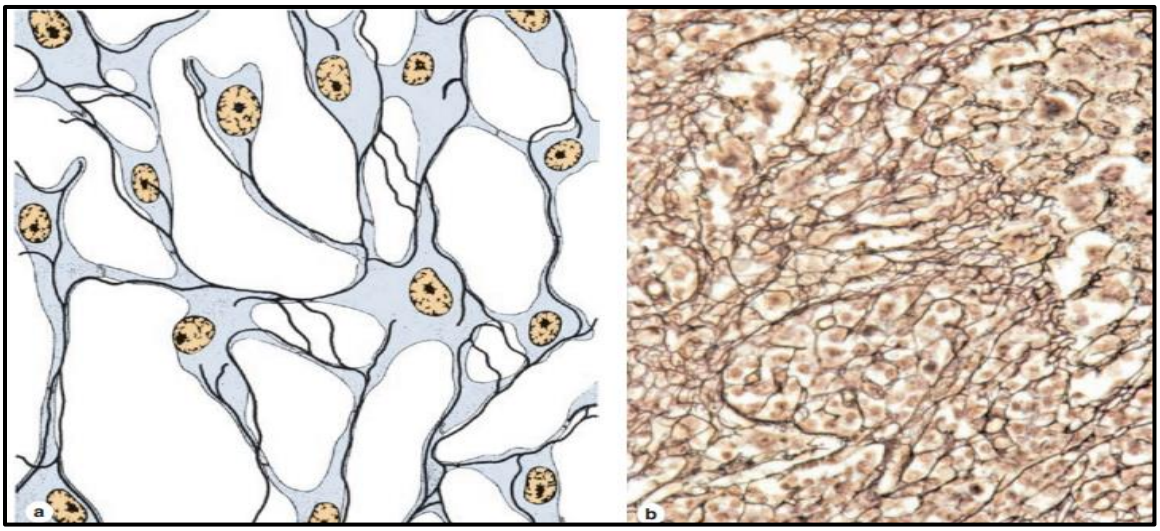


Figure 16: Reticular Tissue. (a) The diagram shows only the fibers and attached reticular cells (free, transient cells are not represented). Reticular fibers of type III collagen are produced and enveloped by the reticular cells, forming an elaborate network through which interstitial fluid or lymph and wandering cells from blood pass continuously. (b) The micrograph shows a silver-stained section of lymph node in which reticular fibers are seen as irregular black lines. Reticular cells are also heavily stained and dark. Most of the smaller, more lightly stained cells are lymphocytes passing through the lymph node. (X200; Silver).

➢ Mucoid Tissue

Mucoid (or mucous) connective tissue is the principal component of the fetal umbilical cord, where it is referred to as **Wharton's jelly**. With abundant ground substance composed chiefly of hyaluronan, mucoid tissue is gelatinous, with sparse collagen fibers and scattered fibroblasts (Figure 17). Included among the fibroblastic cells are many mesenchymal stem cells, which are being studied for their potential in regenerative medicine. Mucoid connective tissue is similar to the tissue found in the vitreous chambers of eyes and pulp cavities of young teeth.

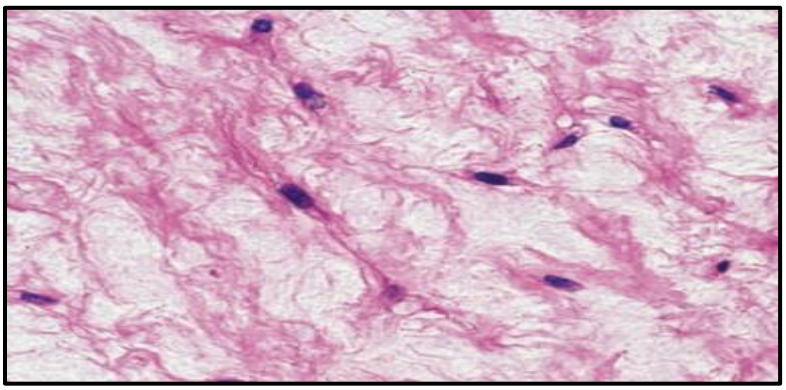


Figure 17: Mucoid tissue. A section of umbilical cord shows large fibroblasts surrounded by a large amount of very loose ECM containing mainly ground substances very rich in hyaluronan, with wisps of collagen. Histologically mucoid (or mucous) connective tissue resembles embryonic mesenchyme in many respects and is rarely found in adult organs. (X200; H&E).

➢ Adipose Tissue

The defining cells of **adipose tissue** (fat), **adipocytes**, are very large cells derived from **mesenchyme** and specialized for energy storage in lipid droplet(s) with triglycerides. Adipocytes store lipids from **three sources**: from dietary fats packaged as chylomicrons in the intestine; from triglycerides produced in the liver and circulating as VLDLs; and from fatty acids synthesized locally. Lipids are mobilized from adipocytes by **hormone-sensitive lipase** activated by **norepinephrine** released from the adrenal gland and various peptide hormones. Cells of adipose tissue are supported by reticular fibers, with connective tissue septa dividing the tissue into lobules of various sizes.

There are two types of adipose tissue: white fat and brown fat.

- **White Adipose Tissue**

White adipose tissue is found in many organs throughout the body, typically forming about 20% of the body weight in adults. Adipocytes of white fat (Figure 18) are typically very large cells, ranging in diameter from 50 to 150 μm. These cells each contain primarily one large lipid droplet (they are **unilocular**), causing the nucleus and remaining cytoplasm to be pushed against the **plasmalemma**. Fatty acids are released from white adipocytes by lipase activity when nutrients are needed and carried throughout the body on plasma proteins such as albumin. **Leptin** is a polypeptide hormone with target cells in the hypothalamus that is released from white adipocytes and helps regulate eating behavior.

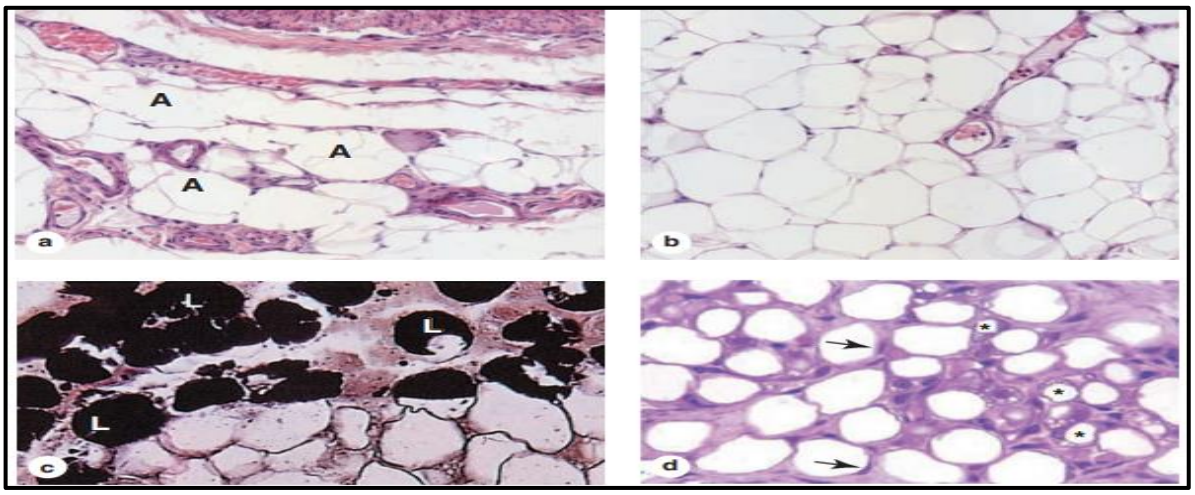


Figure 18: (a) Large white adipocytes (A) are seen in the connective tissue associated with small blood vessels. The fat cells are empty because lipid was dissolved away in slide preparation. Nuclei at the cell membranes are visible in some of the fat cells. (X100; H&E) (b) Large (empty) adipocytes predominate in this typical white adipose tissue, which shows only a small portion of microvasculature. In a single histologic section, nuclei of most very large adipocytes are not included. (X100; H&E) (c) Tissue was fixed here with osmium tetroxide, which preserves lipid (L) and stains it black. Many adipocytes in this slide retain at least part of their large lipid droplets. (X440; Osmium tetroxide) (d) In this specimen from a young mammal the smaller adipocytes marked with asterisks are not unilocular, having many lipid droplets of various sizes. Such cells in white fat represent those in which differentiation is incomplete as well as a small subpopulation of beige cells with brown fat-forming potential. The eccentric nuclei of the unilocular cells are indicated by arrowheads. (X200; PT).

- **Brown Adipose Tissue**

Brown fat comprises up to 5% of the newborn body weight but smaller amounts in adults. **Adipocytes** of this tissue are typically smaller than those of white fat and contain primarily many small lipid droplets (they are **multilocular**) in cytoplasm containing many mitochondria and a central nucleus (Figure 19). Fatty acids released in adipocytes of brown fat are metabolized in mitochondria of these cells for thermogenesis rather than ATP synthesis, using uncoupling protein-1.

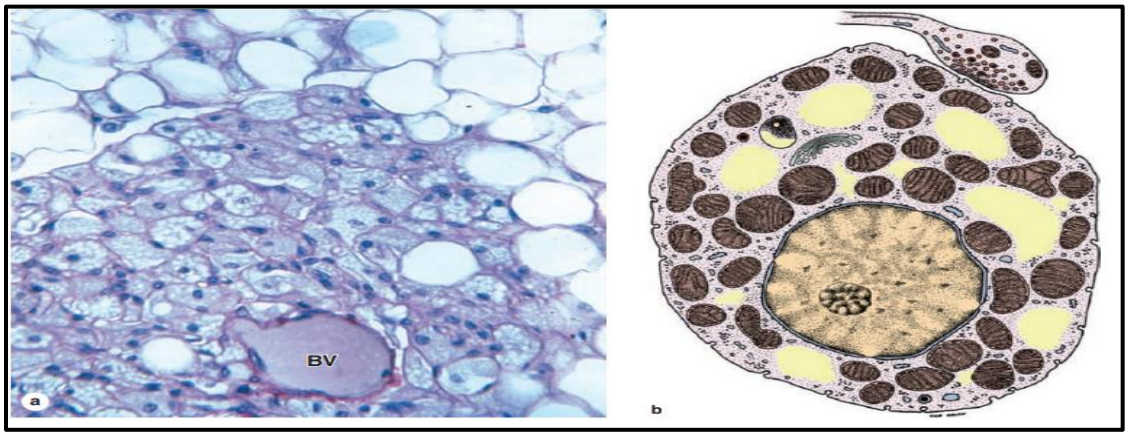


Figure 19: Brown adipose tissue. a) Brown adipose tissue is shown around a small blood vessel (BV) and adjacent white adipose tissue at the top of the photo. Brown adipocytes are slightly smaller and contain

many small lipid droplets and central spherical nuclei. If the lipid has been dissolved from the cells, as shown here, the many mitochondria among the lipid spaces are retained and can be easily discerned. (X200; PT). (b) A diagram of a single multilocular adipocyte showing the central nucleus, numerous small lipid droplets (yellow), and many mitochondria.

➢ Cartilage

Cartilage is a tough, resilient type of connective tissue that structurally supports certain soft tissues, notably in the respiratory tract, and provides cushioned, low-friction surfaces in joints. Cells of cartilage, **chondrocytes**, make up a small percentage of the tissue's mass, which is mainly a flexible mass of extracellular matrix (ECM). **Chondrocytes** are embedded within lacunae surrounded by the ECM. Cartilage ECM typically includes **collagen** as well as **abundant proteoglycans**, notably **aggrecan**, which bind a large amount of water. Cartilage always lacks blood vessels, **lymphatics**, and **nerves**, but it is usually surrounded by a **dense connective tissue perichondrium** that is vascularized. The components of cartilage are shown in figure 20.

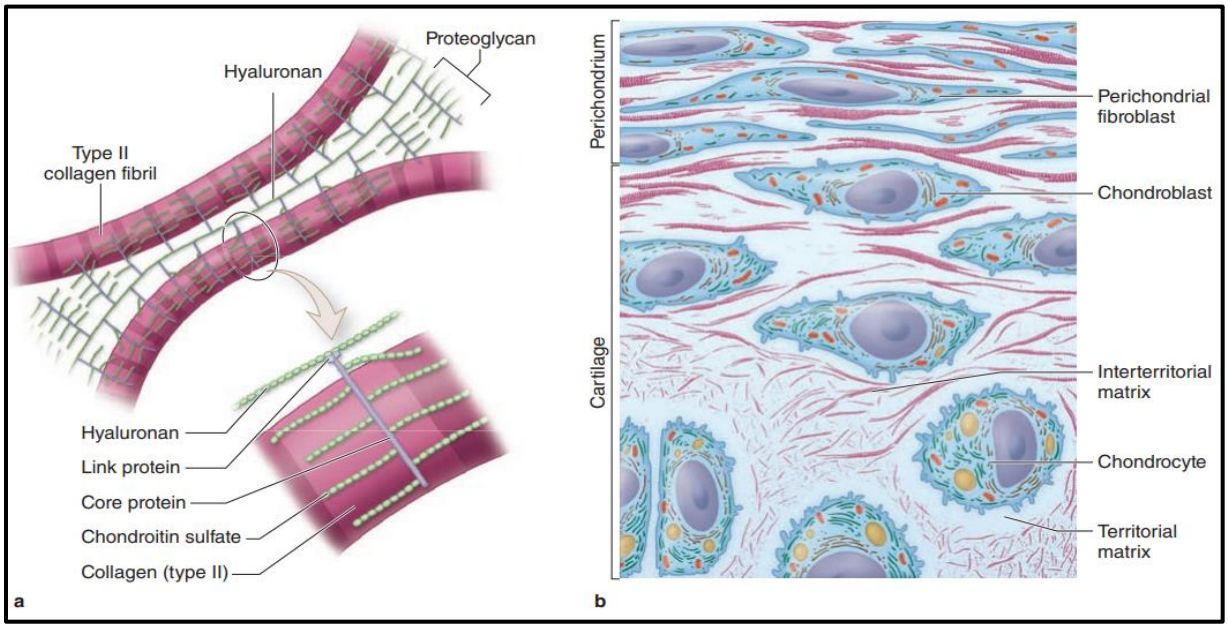


Figure 20: The structure of cartilage matrix and cells.

There are three major forms of cartilage: (1) hyaline cartilage, (2) elastic cartilage, and (3) fibrocartilage.

❖ Hyaline Cartilage

The ECM of hyaline cartilage is homogenous and glassy, rich in fibrils of **type II collagen** and **aggrecan** complexes with **bound water**. The ECM has less collagen and more proteoglycan immediately around the lacunae, producing slight staining differences in this **territorial matrix**. **Chondrocytes** occur singly or in small,

mitotically derived **isogenous groups**. **Perichondrium** is usually present, but not at the hyaline cartilage of articular surfaces or the epiphyses of growing long bones. The general appearance of the hyaline cartilage is shown in figure 21.

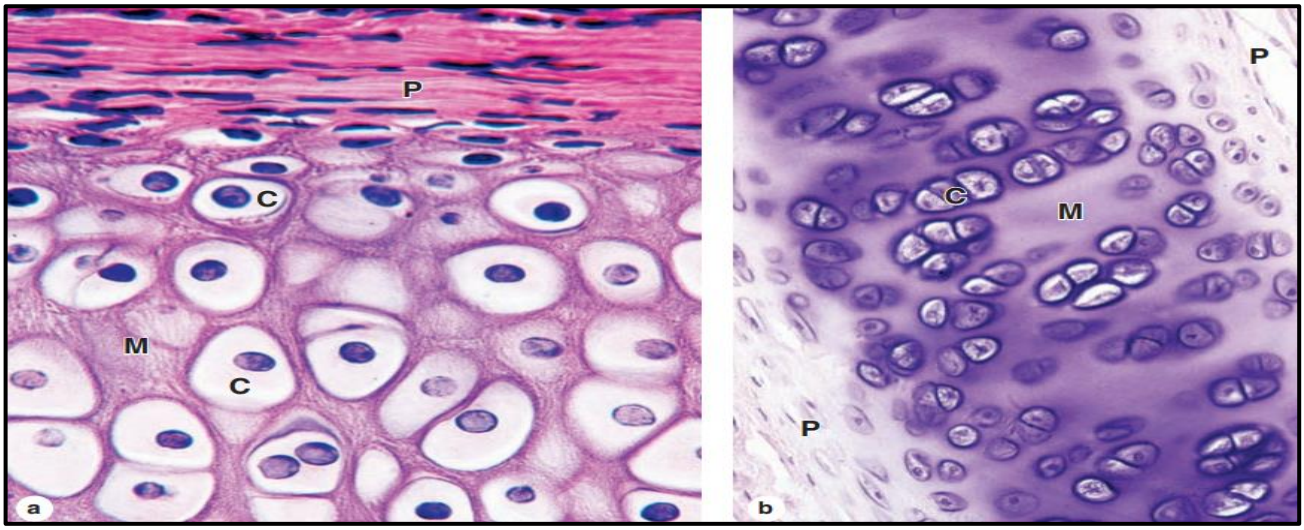


Figure 21: Hyaline Cartilage. (a) The upper part of the photo shows the perichondrium (P). Among the fibroblastic cells of the perichondrium are indistinguishable mesenchymal stem cells. There is a gradual transition and differentiation of cells from the perichondrium to the cartilage, with some elongated fibroblast-like cells becoming larger and more rounded as chondroblasts and chondrocytes (C). These are located within lacunae surrounded by the matrix (M) which these cells secreted. (X200; H&E) (b) The thin region of hyaline cartilage shown here has perichondrium (P) on both sides and shows larger lacunae containing isogenous groups of chondrocytes (C) within the matrix (M). Such groups of two, four, or more cells are produced by mitosis; the cells will separate into individual lacunae as they begin to secrete matrix. Territorial matrix immediately around the chondrocytes is more basophilic than interterritorial matrix farther from the cells. (X160; H&E).

❖ Elastic Cartilage

Elastic cartilage (Figure 22) generally resembles **hyaline cartilage** in its chondrocytes and major ECM components, but its matrix includes abundant **elastic fibers**, visible with special stains, which increase the tissue's flexibility. Elastic cartilage provides flexible support for the external ear as well as certain structures of the middle ear and larynx; it is always surrounded by **perichondrium**.

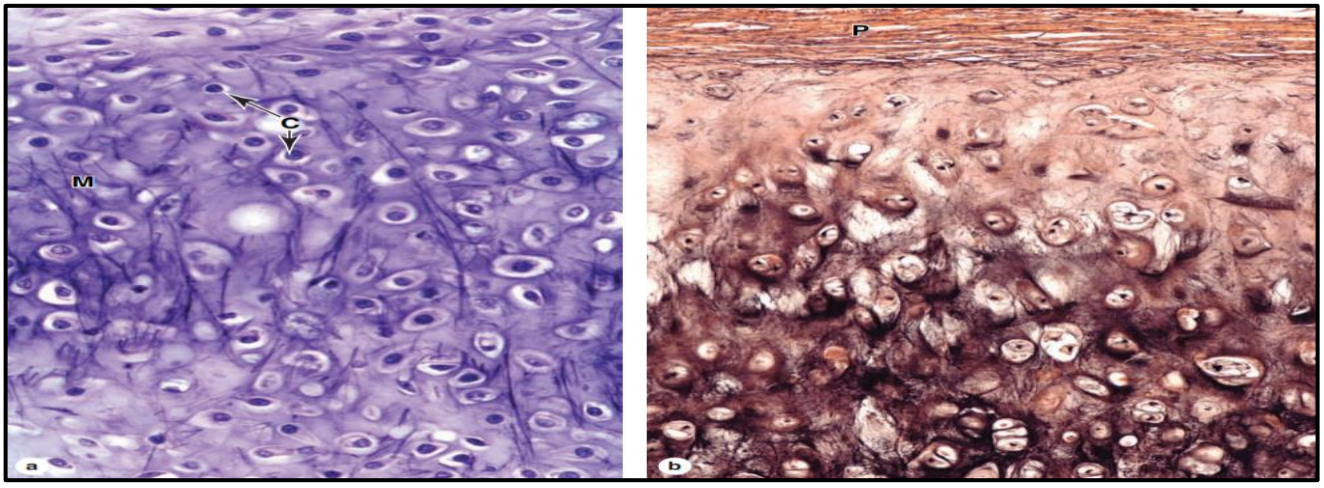


Figure 22: Elastic Cartilage. The chondrocytes (C) and overall organization of elastic cartilage are similar to those of hyaline cartilage, but the matrix (M) also contains elastic fibers that can be seen as darker

components with proper staining. The abundant elastic fibers provide greater flexibility to this type of cartilage. The section in part b includes perichondrium (P) that is also similar to that of hyaline cartilage. (a) X160; Hematoxylin and orcein. (b) X180; Weigert resorcin and van Gieson.

❖ Fibrocartilage

Fibrocartilage contains varying combinations of **hyaline cartilage** in small amounts of dense connective tissue (figure 23). Histologically it consists of small **chondrocytes** in a hyaline matrix, usually layered with larger areas of bundled type I collagen with **scattered fibroblasts**. Fibrocartilage provides very tough, strong support at tendon insertions and in **intervertebral discs** and certain other joints.

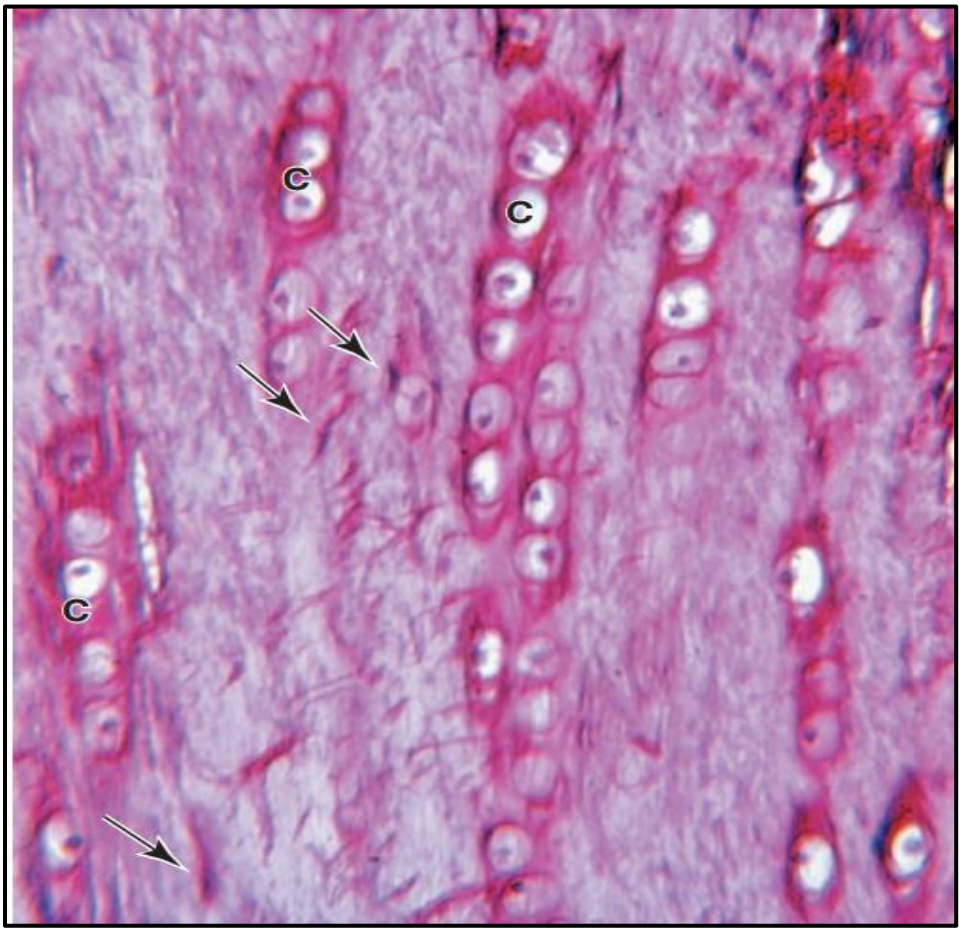


Figure 23: Fibrocartilage. In a small region of intervertebral disc, the axially arranged aggregates of chondrocytes (C) are seen to be surrounded by small amounts of matrix and separated by larger regions with dense collagen and scattered fibroblasts with elongated nuclei (arrows). (X250; Picrosirius-hematoxylin).

Cartilage Formation, Growth, & Repair

All forms of cartilage form from **embryonic mesenchyme**. Cartilaginous structures grow by mitosis of existing **chondroblasts** in lacunae (interstitial growth) or formation of new chondroblasts peripherally from **progenitor cells** in the perichondrium (appositional growth). Repair or replacement of injured cartilage is very slow and ineffective, due in part to the tissue's avascularity and low metabolic rate.

➢ Bone

Bone is a type of connective tissue with a **calcified extracellular matrix (ECM)**, specialized to support the body, protect many internal organs, and act as the body's Ca^{2+} reservoir.

Major Cells & Matrix Components of Bone

Osteoblasts differentiate from (stem) **osteoprogenitor** cells and secrete components of the initial matrix, called **osteoid**, that allow matrix mineralization to occur. Important components of osteoid include **type I collagen**, the protein osteocalcin, which binds **Ca2+** and matrix vesicles with enzymes generating **$PO4^-$**. High concentrations of Ca2+ and $PO4^-$ ions cause formation of **hydroxyapatite crystals**, whose growth gradually calcifies the entire matrix. **Osteocytes** differentiate further from **osteoblasts** when they become enclosed within matrix lacunae and act to maintain the matrix and detect mechanical stresses on bone. **Osteocytes** maintain communication with adjacent cells via a network of long dendritic processes that extend through the matrix via narrow **canaliculi** radiating from each **lacuna**. **Osteoclasts** are very large cells, formed by fusion of several blood **monocytes**, which locally erode bone matrix during **osteogenesis** and bone remodeling (figure 24).

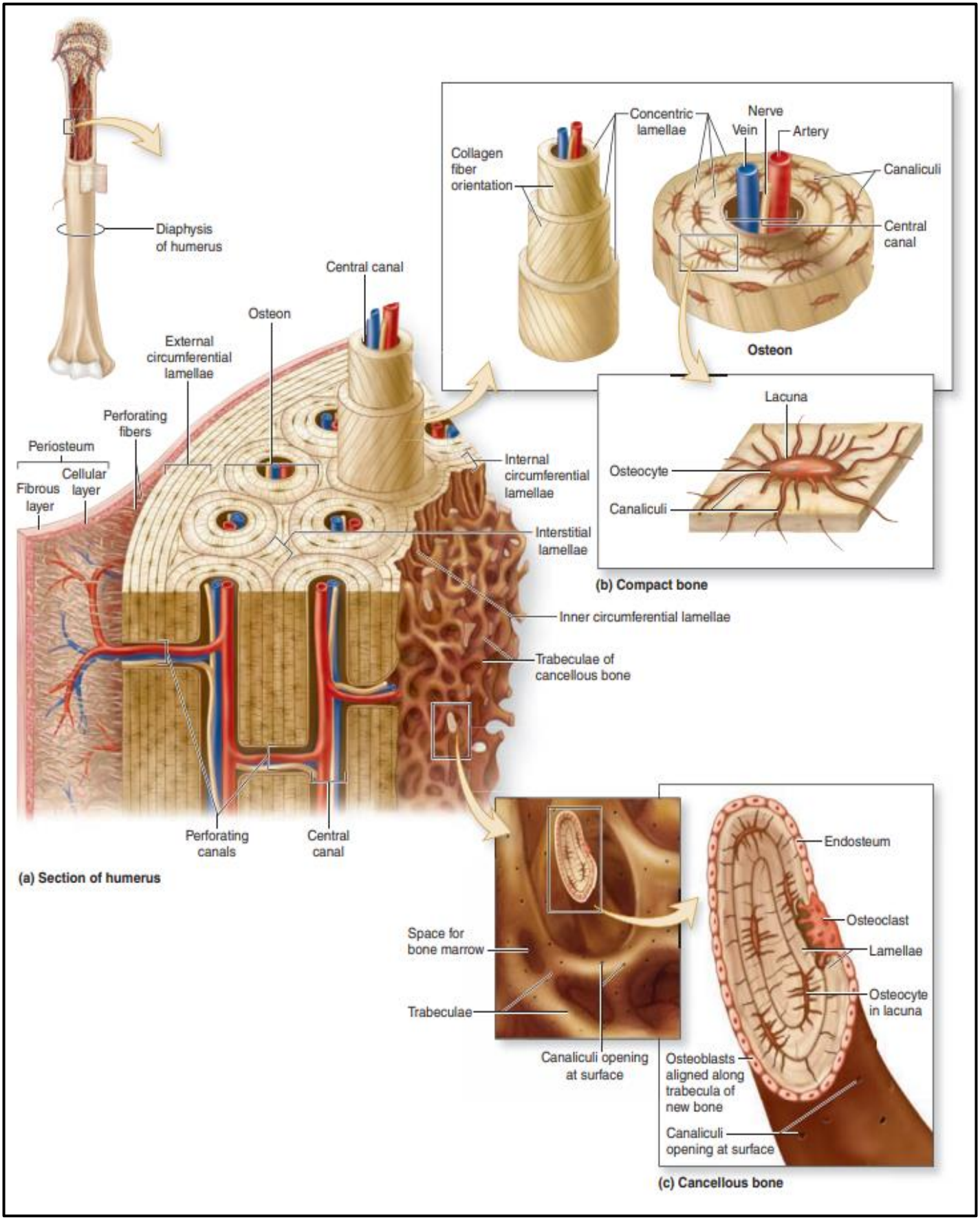


Figure 24: A schematic overview of the basic features of bone.

As for bone cells, Figure 25 shows this.

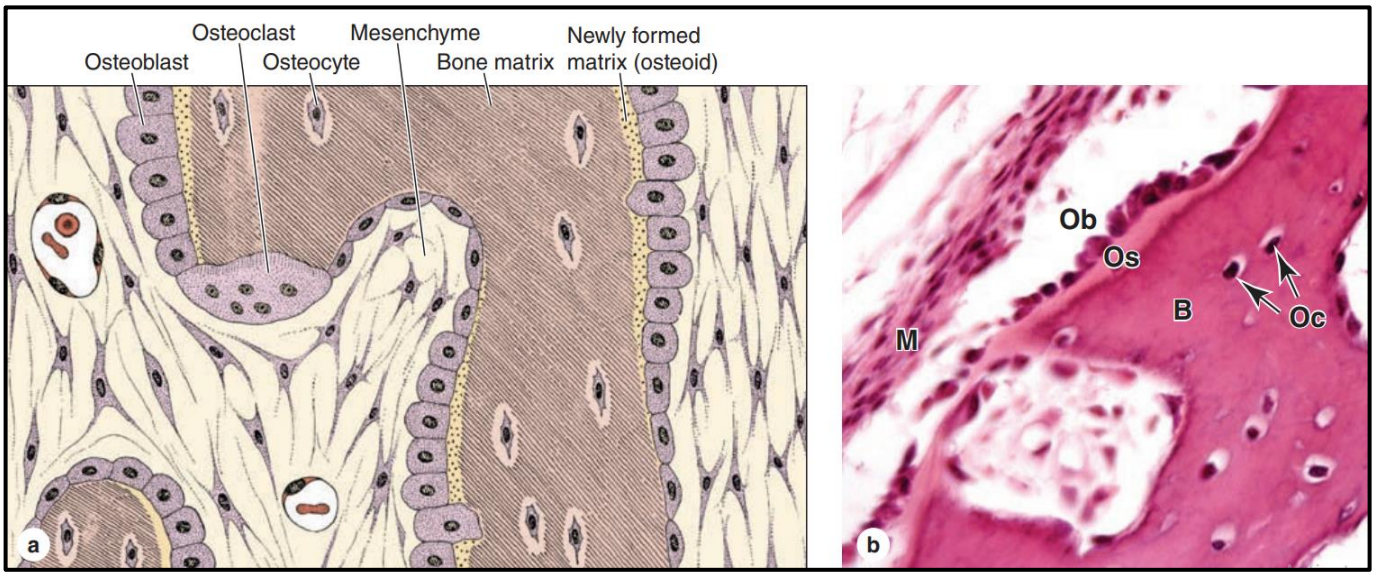


Figure 25: Osteoblasts, osteocytes, and osteoclasts. (a) Diagram showing the relationship of osteoblasts to the newly formed matrix called "osteoid," bone matrix, and osteocytes. Osteoblasts and most of the larger osteoclasts are part of the endosteum covering the bony trabeculae. (b) The photomicrograph of developing bone shows the location and morphologic differences between active osteoblasts (Ob) and osteocytes (Oc). Rounded osteoblasts, derived from progenitor cells in the adjacent mesenchyme (M), cover a thin layer of lightly stained osteoid (Os) on the surface of the more heavily stained bony matrix (B). Most osteoblasts that are no longer actively secreting osteoid will undergo apoptosis; others differentiate either as flattened bone lining cells on the trabeculae of bony matrix or as osteocytes located within lacunae surrounded by bony matrix. (X300; H&E).

Periosteum & Endosteum

Periosteum is a layer of dense connective tissue on the outer surface of bone, bound to bone matrix by bundles of **type I collagen** called **perforating** (or **Sharpey**) fibers. Regions of periosteum adjacent to bone are rich in **osteoprogenitor cells** and **osteoblasts** that mediate much bone growth and remodeling. The **endosteum** is a thin layer of active and inactive osteoblasts, which lines all the internal surfaces within bone; **osteoblasts** here are also required for bone growth.

Types & Organization of Bone (Table 4)

■ **Dense bone** immediately beneath the **periosteum** is called **compact bone**; deep to the compact bone are small bony **trabeculae** or **spicules of cancellous** (or **spongy bone**) (figure 26).

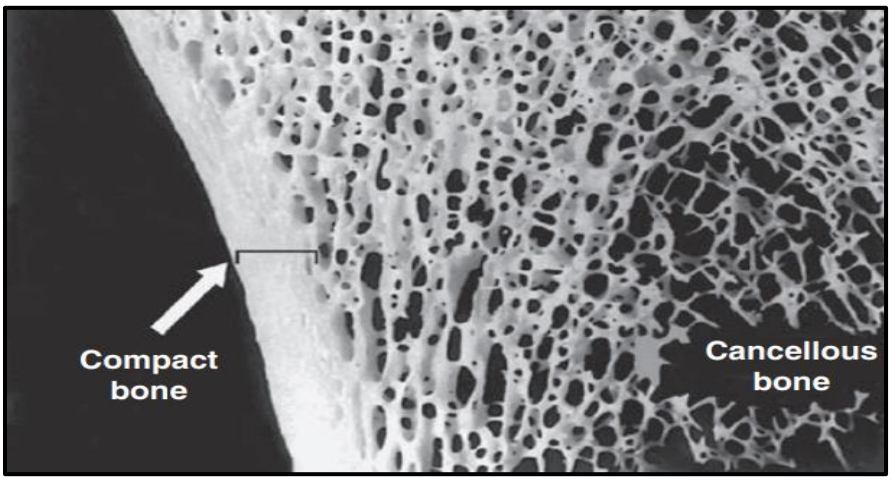


Figure 26: Compact and cancellous bone.

■ **In long bones of the limbs**, these two types of mature bone tissue occur in both the knobby, bulbous ends, called **epiphyses**, and in the intervening shaft or **diaphysis**.

■ **Immature bone**, called **woven bone**, is formed during **osteogenesis** or repair and has a calcified matrix with randomly arranged collagen fibers.

■ By the action of **osteoclasts** and **osteoblasts**, **woven bone** undergoes rapid turnover and is remodeled into **lamellar bone** with new matrix deposited in distinct layers with parallel collagen bundles; both **compact** and **cancellous** bone is **lamellar** bone (figure 27).

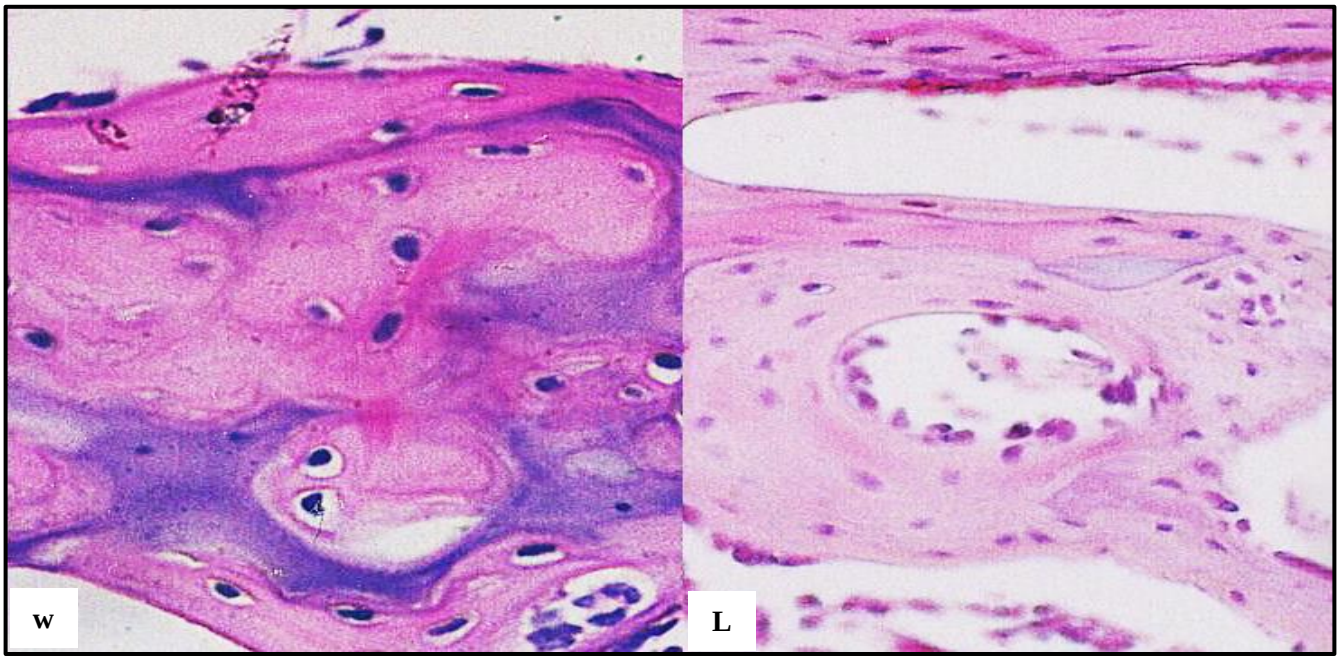


Figure 27: woven bone and lamellar bone.

■ Most **lamellar bone** consists of **lamellae** organized concentrically around small central canals containing **blood vessels** and **nerves**; this organization is called an **osteon** or **Haversian system**.

■ Within each **osteon**, **osteocytic lacunae** occur between the **lamellae**, with **canaliculi** radiating through the **lamellae**, which allow all cells to communicate with the central canal.

Table 4: Summary of bone types and their organization.

Type of Bone	Histological Features	Major Locations	Synonyms
Woven bone, newly calcified	Irregular and random arrangement of cells and collagen; lightly calcified	Developing and growing bones; hard callus of bone fractures	Immature bone; primary bone; bundle bone
Lamellar bone, remodeled from woven bone	Parallel bundles of collagen in thin layers (lamellae), with regularly spaced cells between; heavily calcified	All normal regions of adult bone	Mature bone; secondary bone
Compact bone, ~80% of all lamellar bone	Parallel lamellae or densely packed osteons, with interstitial lamellae	Thick, outer region (beneath periosteum) of bones	Cortical bone
Cancellous bone, ~20% of all lamellar bone	Interconnected thin spicules or trabeculae covered by endosteum	Inner region of bones, adjacent to marrow cavities	Spongy bone; trabecular bone; medullary bone

Bone Growth, Remodeling, & Repair

Growth of bones occurs throughout life, with cells and matrix turning over continuously through activities of **osteoblasts** and **osteoclasts**. **Lamellae** and **osteons** are temporary structures and are replaced and rebuilt continuously in a process of bone remodeling by which bones change size and shape according to changes in mechanical stress. Bone repair after fracture or other injury involves the activation of **periosteal fibroblasts** to produce an initial soft callus of fibrocartilage-like tissue. The **soft callus** is gradually replaced by a **hard callus** of woven bone that is soon remodeled to produce stronger lamellar bone.

Metabolic Role of Bone

Ca2+, a key ion for all cells, is stored in bone when dietary calcium is adequate and mobilized from bone when dietary calcium is deficient. Maintenance of proper **blood calcium levels** involves activity of all three major bone cells and is largely regulated by subtle paracrine interaction among these and other cells. Hormones affecting calcium deposition and removal from bone include **PTH**, which indirectly stimulates osteoclasts to elevate levels of **calcium** in blood, and **calcitonin**, which can inhibit **osteoclast** activity, lowering blood calcium level.

Lecture 4-5: Hemopoiesis

Mature blood cells have a relatively short life span and must be continuously replaced with new cells from precursors developing during **hemopoiesis** (Gr. haima, blood + poiesis, a making). In the early embryo these blood cells arise in the **yolk sac** mesoderm. In the second trimester, hemopoiesis (also called **hematopoiesis**) occurs primarily in the developing **liver**, with the spleen playing a minor role. Skeletal elements begin to ossify and **bone marrow** develops in their medullary cavities, so in the third-trimester marrow of specific bones becomes the major hemopoietic organ.

Throughout childhood and adult life, erythrocytes, granulocytes, monocytes, and platelets continue to form from stem cells located in bone marrow. The origin and maturation of these cells are termed, respectively, **erythropoiesis** (Gr. erythros, red + poiesis), **granulopoiesis**, **monocytopoiesis**, and **thrombocytopoiesis**. **Lymphopoiesis** or lymphocyte development occurs in the marrow and in the lymphoid organs to which precursor cells migrate from marrow.

› Stem Cells & Differentiation

stem cells are **pluripotent** cells capable of asymmetric division and self-renewal. Some of their daughter cells form specific, irreversibly committed progenitor cells, and other daughter cells remain as a small pool of slowly dividing stem cells.

Hemopoietic Stem Cells

All blood cells arise from a single type of pluripotent **hemopoietic stem cell** in the bone marrow that can give rise to all the blood cell types (Figure 1). These pluripotent stem cells are rare, proliferate slowly, and give rise to two major lineages of progenitor cells with restricted potentials (committed to produce specific blood cells): one for **lymphoid cells** (lymphocytes) and another for **myeloid cells** (Gr. myelos, marrow), which develop in bone marrow. Myeloid cells include granulocytes, monocytes, erythrocytes, and megakaryocytes. The lymphoid progenitor cells migrate from the bone marrow to the thymus or the lymph nodes, spleen, and other lymphoid structures, where they proliferate and differentiate.

Progenitor & Precursor Cells

The progenitor cells for blood cells are often called colony **forming units (CFUs)**, because they give rise to colonies of only one cell type when cultured in vitro or injected into a spleen. As shown in Figure 1, there are four major types of progenitor cells/CFUs:

- **Erythroid lineage of erythrocytes.**
- **Thrombocytic lineage of megakaryocytes for platelet formation.**
- **Granulocyte-monocyte lineage of all three granulocytes and monocytes.**

■ **Lymphoid lineage of B lymphocytes, T lymphocytes, and natural killer cells.**

Each progenitor cell lineage produces precursor cells (or blasts) that gradually assume the morphologic characteristics of the mature, functional cell types they will become (Figure 1). In contrast, stem and progenitor cells cannot be morphologically distinguished and simply resemble large lymphocytes. While stem cells divide at a rate only sufficient to maintain their relatively small population, progenitor and precursor cells divide more rapidly, producing large numbers of differentiated, mature cells (3 × 109 erythrocytes and 0.85 × 109 granulocytes/kg/d in human bone marrow). The changing potential and activities of cells during hemopoiesis are shown graphically in Figure 2.

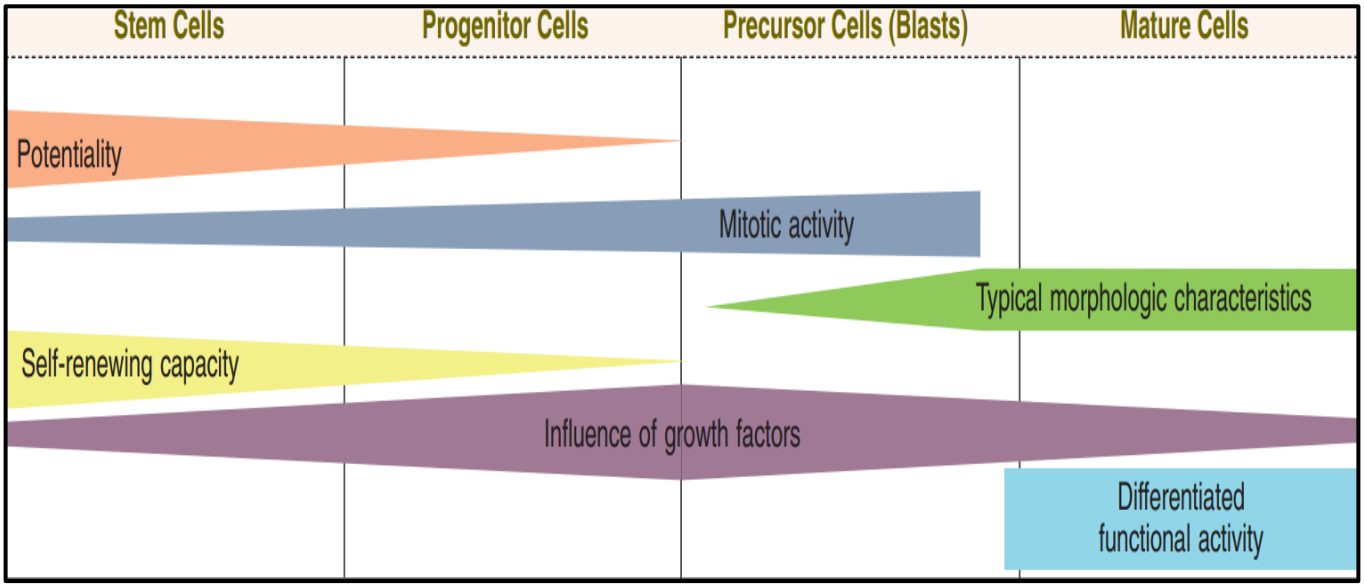


Figure 2: Major changes in developing hemopoietic cells.

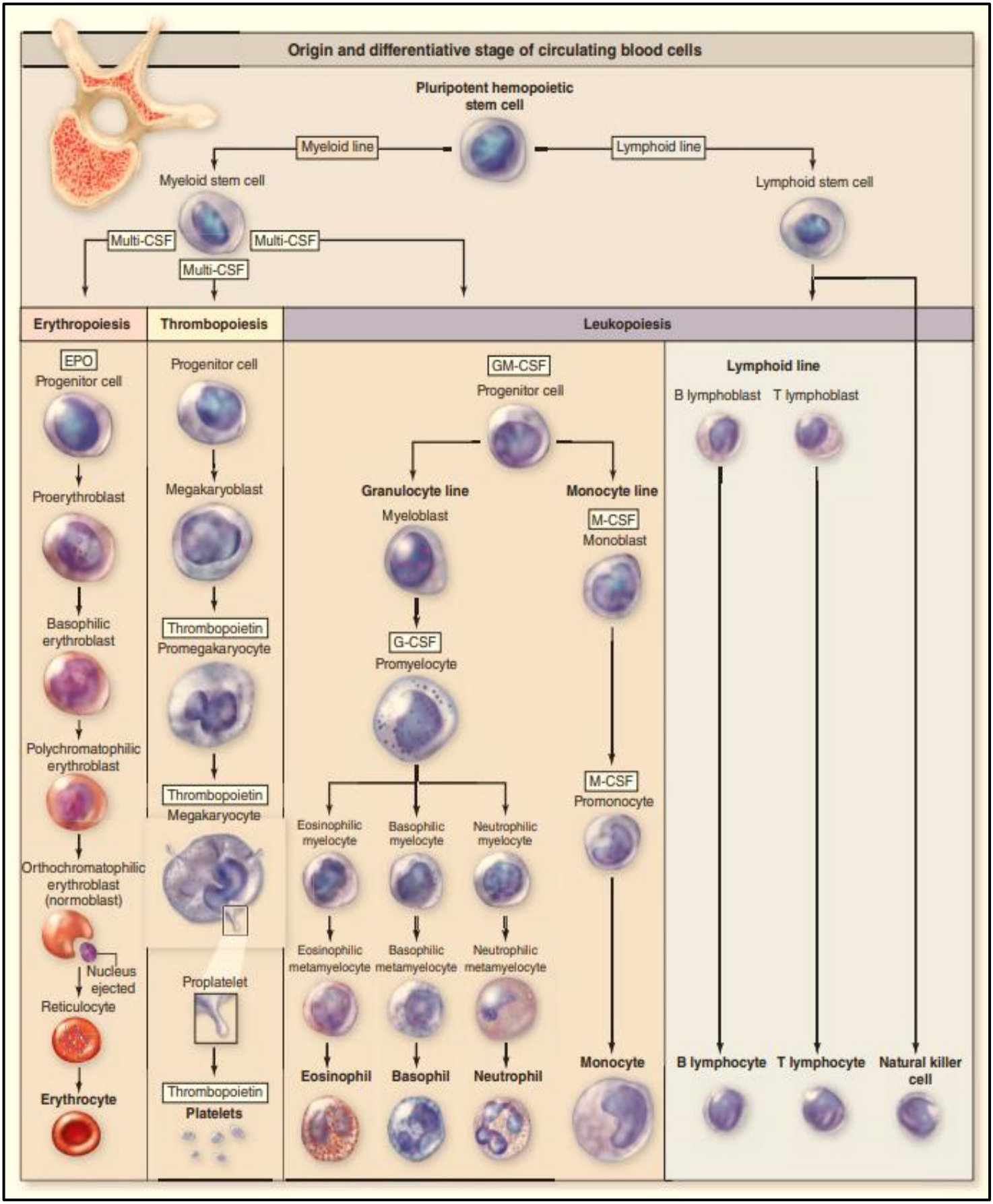


Figure 1: Origin and differentiative stages of blood cells.

Hemopoiesis depends on a microenvironment, or niche, with specific endocrine, paracrine, and juxtacrine factors. These requirements are provided largely by the local cells and extracellular matrix (ECM) of the hemopoietic organs, which together create the niches in which stem cells are maintained and progenitor cells develop. Hemopoietic growth factors, often called **colonystimulating factors (CSF)** or cytokines, are glycoproteins that stimulate proliferation of progenitor and precursor cells and promote cell differentiation and maturation within specific lineages (Table 1).

Table 1: Major hemopoietic growth factors or colony-stimulating factors.

Cytokine	Major Activities and Target Cells[a]	Important Sources
Stem cell factor (SCF)	Mitogen for all hemopoietic progenitor cells	Stromal cells of bone marrow
Erythropoietin (EPO)	Mitogen for all erythroid progenitor and precursor cells, also promoting their differentiation	Peritubular endothelial cells of the kidney; hepatocytes
Thrombopoietin (TPO)	Mitogen for megakaryoblasts and their progenitor cells	Kidney and liver
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Mitogen for all myeloid progenitor cells	Endothelial cells of bone marrow and T lymphocytes
Granulocyte colony-stimulating factor (G-CSF or filgrastim)	Mitogen for neutrophil precursor cells	Endothelial cells of bone marrow and macrophages
Monocyte colony-stimulating factor (M-CSF)	Mitogen for monocyte precursor cells	Endothelial cells of marrow and macrophages

› Bone Marrow

Under normal conditions, the production of blood cells by the bone marrow is adjusted to the body's needs, increasing its activity several-fold in a very short time. Bone marrow is found in the medullary canals of long bones and in the small cavities of cancellous bone, with two types based on their appearance at gross examination: blood-forming **red bone marrow**, whose color is produced by an abundance of blood and hemopoietic cells, and **yellow bone marrow**, which is filled with adipocytes that exclude most hemopoietic cells. In the newborn all bone marrow is red and active in blood cell production, but as the child grows, most of the marrow changes gradually to the yellow variety. Under certain conditions, such as severe bleeding or hypoxia, yellow marrow reverts to red.

Red bone marrow (Figure 3) contains a reticular connective tissue **stroma** (Gr. stroma, bed), **hemopoietic cords** or **islands** of cells, and **sinusoidal capillaries**. The stroma is a meshwork of specialized fibroblastic cells called **stromal cells** (also called **reticular or adventitial cells**) and a delicate web of reticular fibers supporting the hemopoietic cells and macrophages. The matrix of bone marrow also contains collagen type I, proteoglycans, fibronectin, and laminin, the latter glycoproteins interacting with integrins to bind cells to the matrix. Red marrow is also a site where older, defective erythrocytes undergo phagocytosis by macrophages, which then reprocess heme-bound iron for delivery to the differentiating erythrocytes.

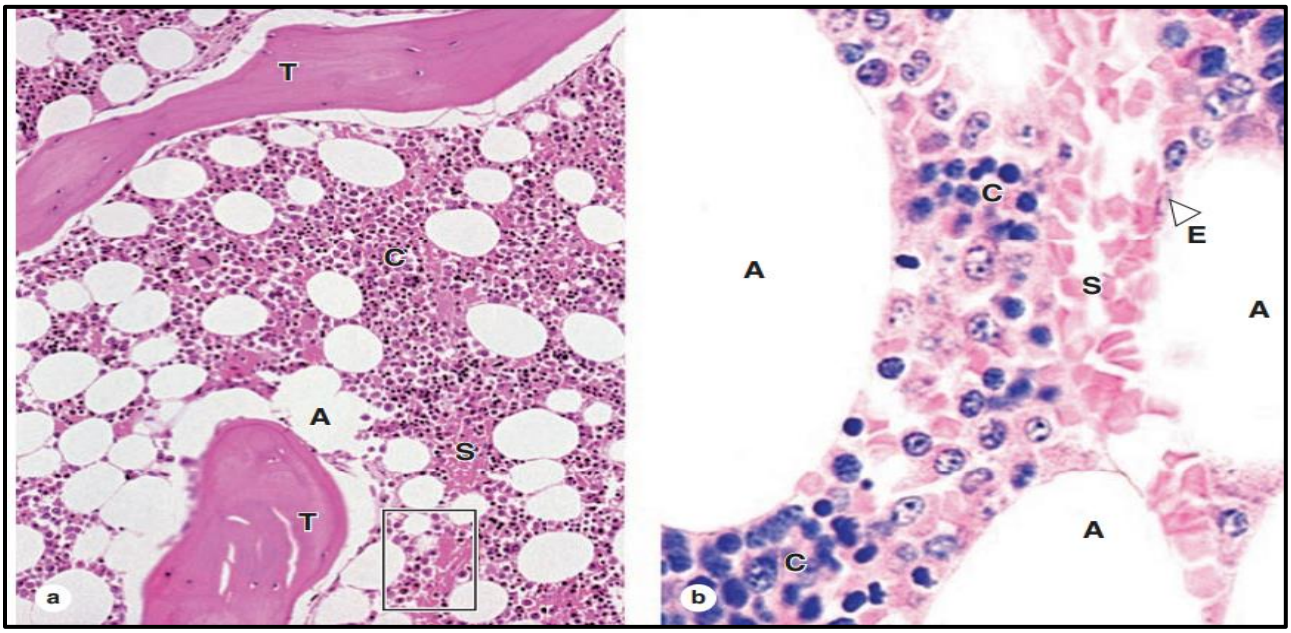


Figure 3: Red bone marrow .(a) Sections of red bone marrow include trabeculae (T) of cancellous bone, adipocytes (A), and blood-filled sinusoids (S) between hemopoietic cords (C) (X140; H&E). (b) At higher magnification the flattened nuclei of sinusoidal endothelial cells (E) can be distinguished, also packed hemopoietic cells in the cords (C) between the sinusoids (S) and adipocytes (A) (X400; H&E).

The hematopoietic niche in marrow includes the stroma, osteoblasts, and megakaryocytes. Between the hematopoietic cords run the sinusoids, which have discontinuous endothelium, through which newly differentiated blood cells and platelets enter the circulation (Figure 4).

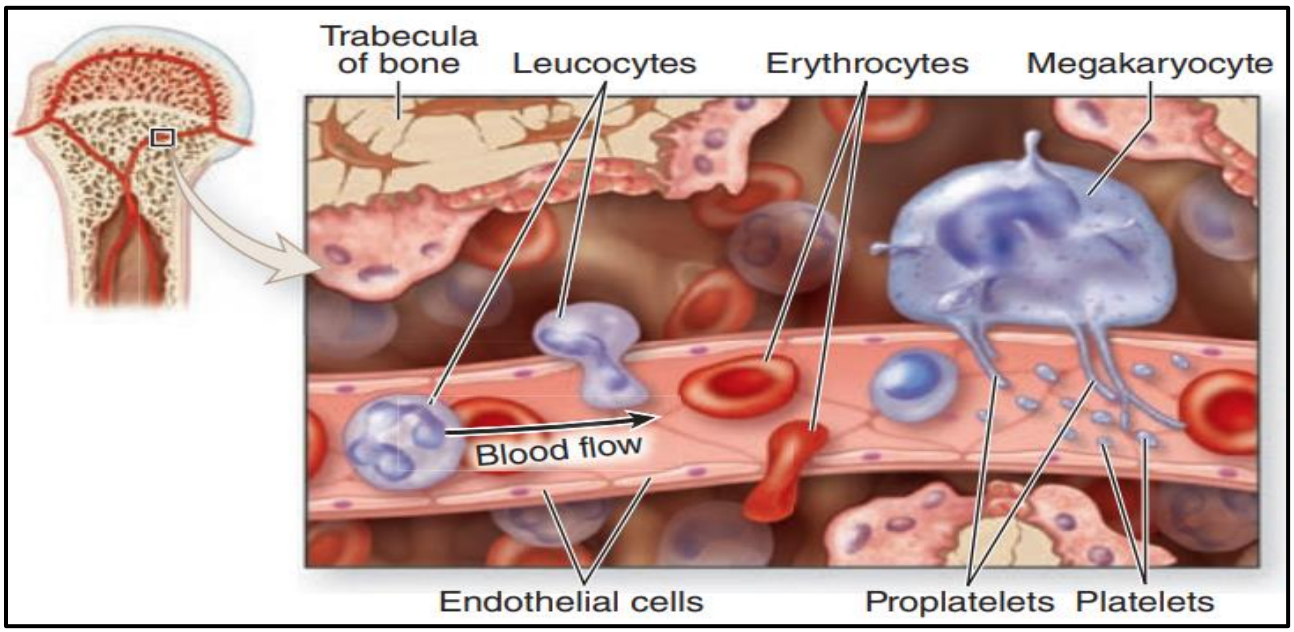


Figure 4: Sinusoidal endothelium in active marrow. The diagram shows that mature, newly formed erythrocytes, leukocytes, and platelets in marrow enter the circulation by passing through the discontinuous sinusoidal endothelium. Leukocytes cross the wall of the sinusoid by their own activity, but the non-motile erythrocytes cannot migrate through the wall actively and enter the circulation pushed by a pressure gradient across the wall. Megakaryocytes form thin processes that also pass through such apertures and liberate platelets at their tips.

› Maturation of Erythrocytes

A mature cell is one that has differentiated to the stage at which it can carry out its specific functions. Erythrocyte maturation is an example of terminal cell differentiation involving hemoglobin synthesis and formation of a small, enucleated, biconcave corpuscle. Several major changes take place during **erythropoiesis** (Figures 5 and 6). Cell and nuclear volumes decrease, while the nucleoli diminish in size and disappear. Chromatin density increases until the nucleus presents a pyknotic appearance and is finally extruded from the cell. There is a gradual decrease in the number of polyribosomes (basophilia), with a simultaneous increase in the amount of hemoglobin (a highly eosinophilic protein). Mitochondria and other organelles gradually disappear.

Erythropoiesis requires approximately a week and involves three to five cell divisions between the progenitor cell stage and the release of functional cells into the circulation. The glycoprotein **erythropoietin**, a growth factor produced by cells in the kidneys, stimulates production of mRNA for the protein components of hemoglobin and is essential for erythrocyte production.

The distinct erythroid progenitor cell (Figure 5) is the **proerythroblast**, a large cell with loose, lacy chromatin, nucleoli, and basophilic cytoplasm. The next stage is represented by the early **basophilic erythroblast**, slightly smaller with cytoplasmic basophilia and a more condensed nucleus. The basophilia is caused by the large number of free polysomes synthesizing hemoglobin. During the next stage cell volume is

reduced, polysomes decrease, and some cytoplasmic areas begin to be filled with hemoglobin, producing regions of both basophilia and acidophilia in the cell and the name **polychromatophilic** erythroblast. Cell and nuclear volumes continue to condense and basophilia is gradually lost, producing cells with uniformly acidophilic cytoplasm—the **orthochromatophilic** erythroblasts (also called normoblasts). Late in this stage the cell nucleus is ejected and undergoes phagocytosis by macrophages. The cell still retains a few polyribosomes which, when treated with the dye brilliant cresyl blue, form a faintly stained network and the cells are termed **reticulocytes** (Figure 6b). These cells enter the circulation (where they may constitute 1% of the red blood cells), quickly lose all polyribosomes, and mature as erythrocytes.

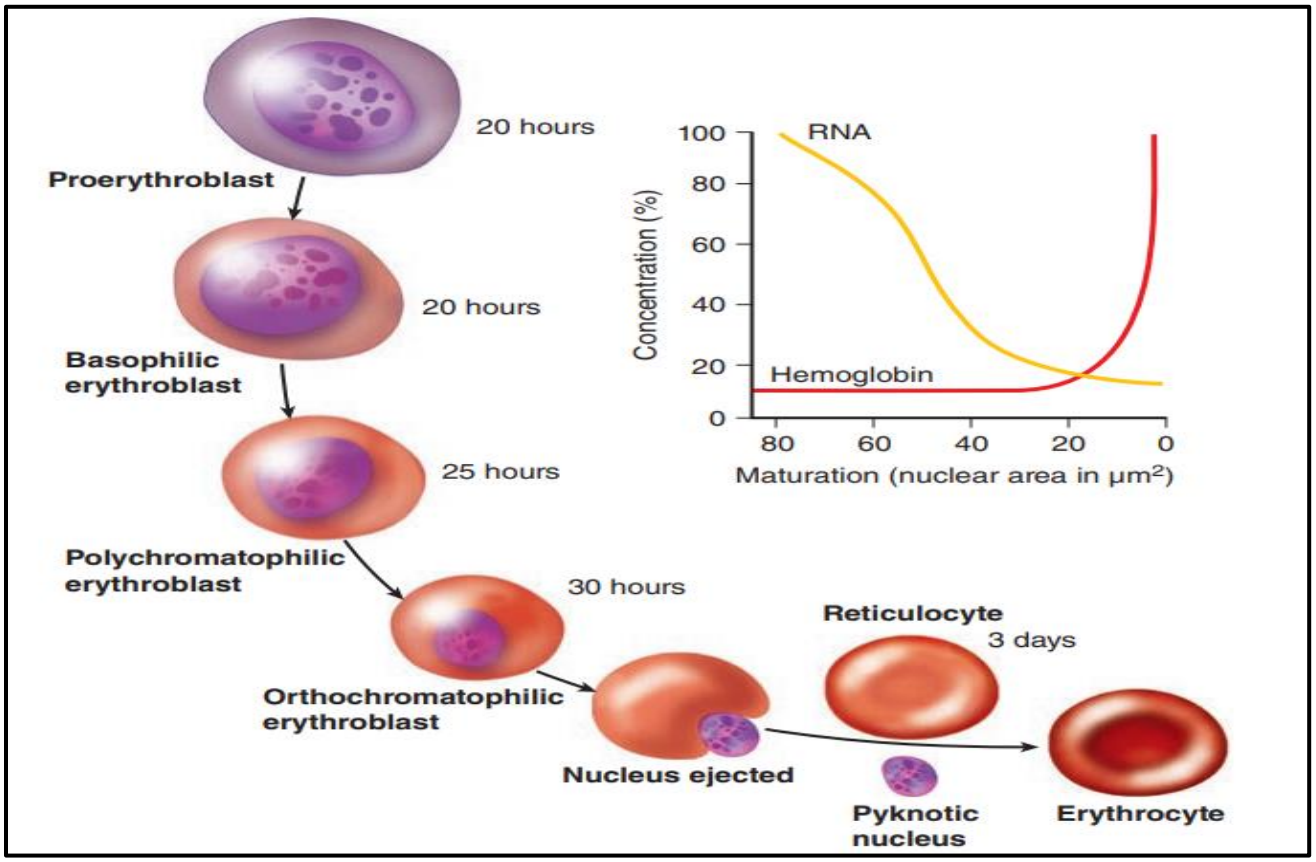


Figure 5: Summary of erythrocyte maturation. The color change in the cytoplasm shows the continuous decrease in basophilia and the increase in hemoglobin concentration.

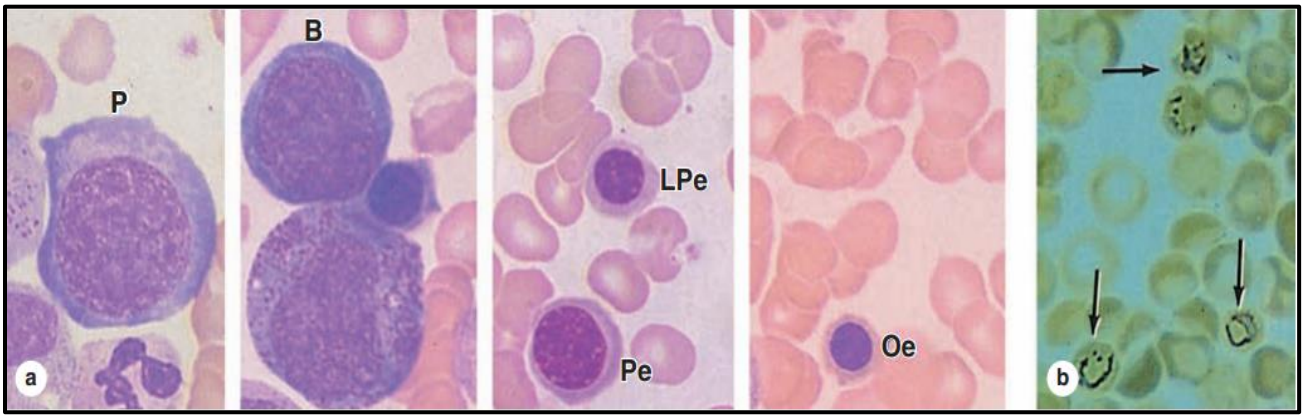


Figure 6: Major erythrocyte precursors. (a) Micrographs showing a very large proerythroblast (P), a smaller basophilic erythroblast (B), typical and late polychromatophilic erythroblasts (Pe and LPe), and a small orthochromatophilic erythroblast (Oe) (All X1400; Wright). (b) Micrograph containing reticulocytes (arrows), which are cells that have lost their nuclei but have not yet completely lost the polyribosomes (X1400; Brilliant cresyl blue).

› Maturation Of Granulocytes

Granulopoiesis involves cytoplasmic changes dominated by synthesis of proteins for the **azurophilic granules** and **specific granules**. These proteins are produced in the rough ER and in the prominent Golgi apparatus in two successive stages (Figure 7).

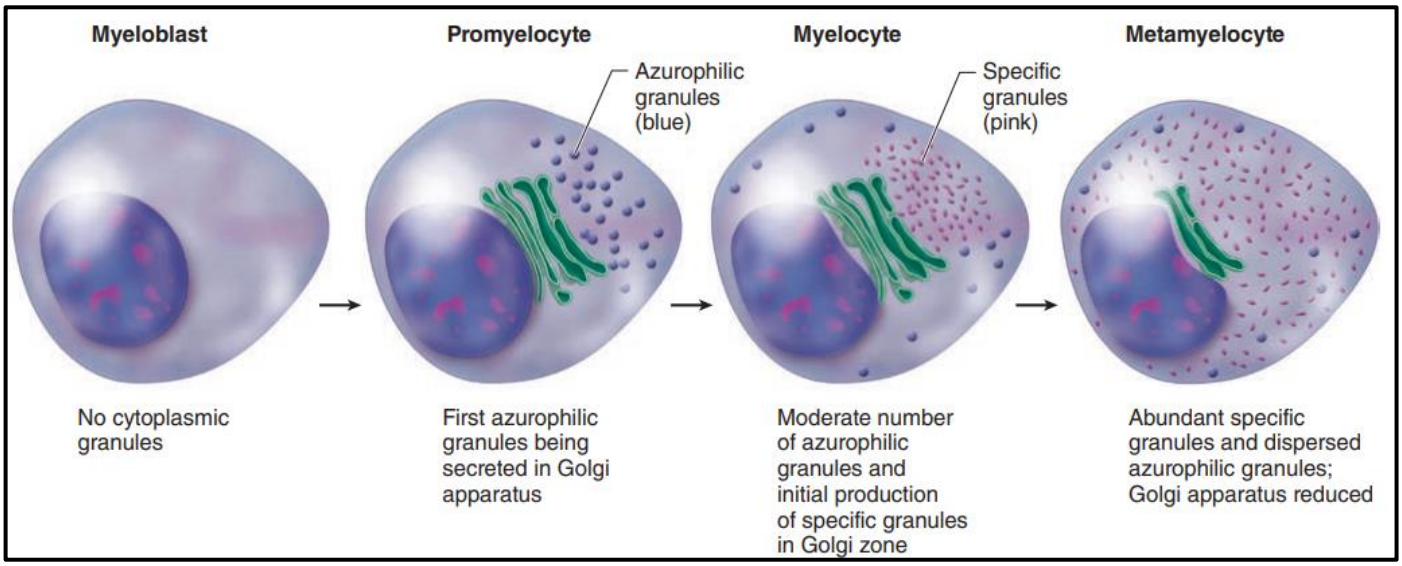


Figure 7: Granulopoiesis. Illustrated is the sequence of cytoplasmic events in the maturation of granulocytes from myeloblasts. Modified lysosomes or azurophilic granules form first at the promyelocyte stage and are shown in blue; the specific granules of the particular cell type form at the myelocyte stage and are shown in pink. All granules are fully dispersed at the metamyelocyte stage, when indentation of the nucleus begins.

Formed first are the azurophilic granules, which contain lysosomal hydrolases, stain with basic dyes, and are generally similar in all three types of granulocytes. Golgi activity then changes to package proteins for the specific granules, whose contents differ in each of the three types of granulocytes and endow each type with certain different properties. In sections of bone marrow cords of granulopoietic cells can be distinguished from erythropoietic cords by their granule-filled cytoplasm (Figure 8).

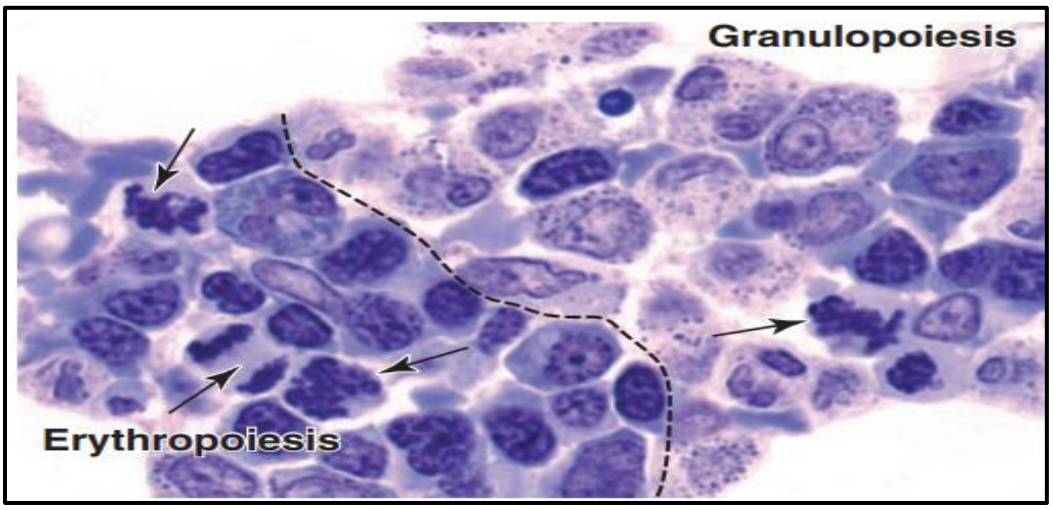


Figure 8: Developing erythrocytes and granulocytes in marrow. Precursor cells of different hemopoietic lineages develop side by side. This plastic section of red bone marrow shows mitotic figures (arrows) and fairly distinct regions of erythropoiesis and granulopoiesis. Most immature granulocytes are in the myelocyte stage: their cytoplasm contains large, dark-stained azurophilic granules and small, less darkly stained specific granules. (X400; Giemsa).

The **myeloblast** is the most immature recognizable cell in the myeloid series (Figures 1). Typically these have finely dispersed chromatin, and faint nucleoli. In the next stage, the **promyelocyte** is characterized by basophilic cytoplasm and azurophilic granules containing lysosomal enzymes and myeloperoxidase. Different promyelocytes activate different sets of genes, resulting in lineages for the three types of granulocytes (Figure 1). The first visible sign of this differentiation appears in the **myelocyte** stage (Figure 9), in which specific granules gradually increase in number and eventually occupy most of the cytoplasm at the **metamyelocyte** stage. These neutrophilic, basophilic, and eosinophilic metamyelocytes mature with further condensation of their nuclei. Before its complete maturation the neutrophilic granulocyte passes through an intermediate stage, **the band cell**, in which the nucleus is elongated but not yet polymorphic.

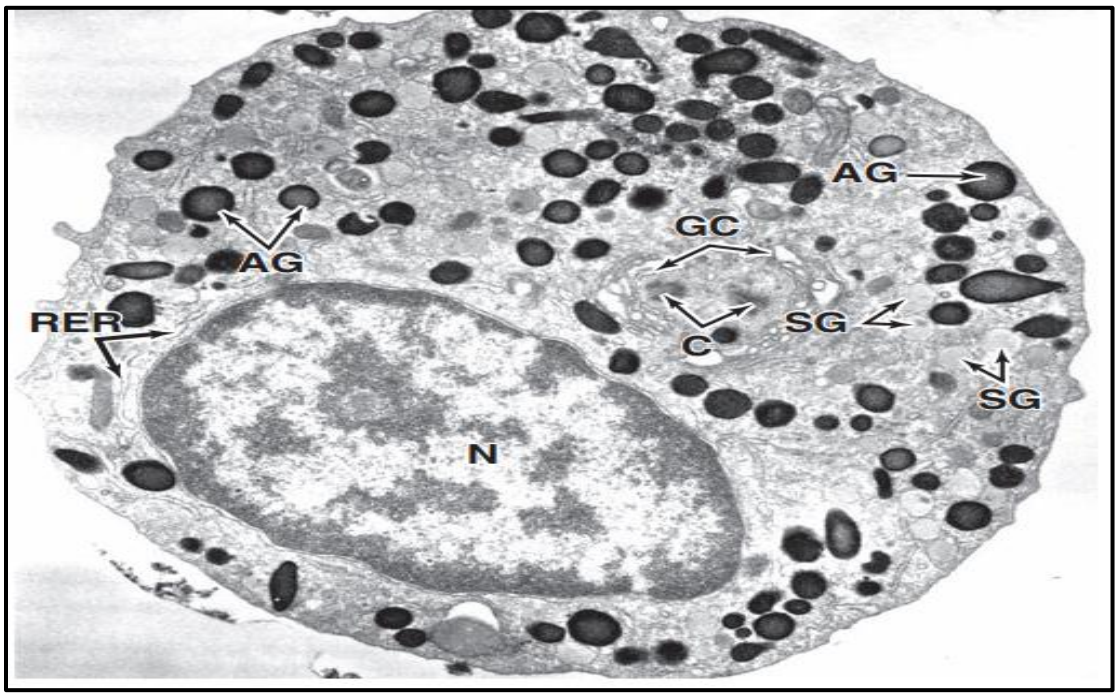


Figure 9: Neutrophilic myelocyte. This micrograph shows ultrastructurally a peroxidase stained section of a neutrophilic myelocyte with cytoplasm containing both large, peroxidase-positive azurophilic granules (AG) and smaller specific granules (SG), which do not stain for peroxidase. The peroxidase reaction product is present only in mature azurophilic granules and is not seen in the rough ER (RER) or Golgi cisternae (GC), which are located around the centriole (C) near the nucleus (N). (X15,000).

The vast majority of granulocytes are neutrophils and the total time required for a myeloblast to produce mature, circulating neutrophils ranges from 10 to 14 days. Five mitotic divisions normally occur during the myeloblast, promyelocyte, and neutrophilic myelocyte stages. As diagrammed below, developing and mature neutrophils exist in four functionally and anatomically defined compartments: (1) the granulopoietic compartment in active marrow; (2) storage as mature cells in marrow until release; (3) the circulating population; and (4) a population undergoing margination, a process in which neutrophils adhere loosely and accumulate transiently along the endothelial surface in venules and small veins. Margination of neutrophils in some organs can persist for several hours and is not always followed by the cells' emigration from the microvasculature.

At sites of injury or infection, neutrophils and other granulocytes enter the connective tissues by migrating through intercellular junctions between endothelial cells of postcapillary venules in diapedesis. Inflamed connective tissues thus form a fifth terminal compartment for neutrophils, where the cells reside for a few days and then die by apoptosis, regardless of whether they have performed their major function of bacterial phagocytosis.

› Maturation of Agranulocytes

The precursor cells of monocytes and lymphocytes do not show specific cytoplasmic granules or nuclear lobulation, both of which facilitate the distinction of cells in the granulopoietic series. We will explain below the maturation process of these cells.

➢ Monocytes

The **monoblast** is a committed progenitor cell. Further differentiation leads to the **promonocyte**, a large cell (up to 18 μm in diameter) with basophilic cytoplasm and a large nucleus (Figures 1). The chromatin is lacy and nucleoli are evident. Promonocytes divide twice as they develop into **monocytes**. Differentiating monocytes contain extensive RER and large Golgi complexes forming lysosomes, which are observed as fine azurophilic granules at maturity.

➢ Lymphocytes

The first identifiable progenitor of lymphoid cells is the **lymphoblast**, a large cell capable of dividing two or three times to form **lymphocytes** (Figures 1). As lymphocytes develop their nuclei become smaller, nucleoli disappear, and cell size decreases. In the bone marrow and in the thymus, these cells synthesize the specific cell surface proteins that characterize B or T lymphocytes, respectively.

Mature and functionally active B and T cells are generally larger than newly formed lymphocytes. Subsets of lymphocytes acquire distinctive cell surface and other proteins during differentiation.

› Origin of Platelets

The small, membrane-enclosed formed elements called **platelets** or thrombocytes originate by fragmentation from mature megakaryocytes (Gr. megas, big + karyon, nucleus, + kytos), which in turn differentiate from **megakaryoblasts** in a process driven by **thrombopoietin**. The megakaryoblast contain a basophilic cytoplasm and a large ovoid or kidney-shaped nucleus (Figure 10), often with several nucleoli. Before differentiating, these cells undergo endomitosis, with repeated rounds of DNA replication not separated by cell divisions, resulting in a nucleus that is highly polyploid (from 8N to 64N).

Megakaryocytes are giant cells, up to 150 μm in diameter, and the polyploid nuclei are large and irregularly lobulated with coarse chromatin. Their cytoplasm contains numerous mitochondria, a well-developed RER, and an extensive Golgi apparatus. Best seen in bone marrow, megakaryocytes also occur in the interstitial tissue of the spleen, lungs and vascular sinusoids or capillaries.

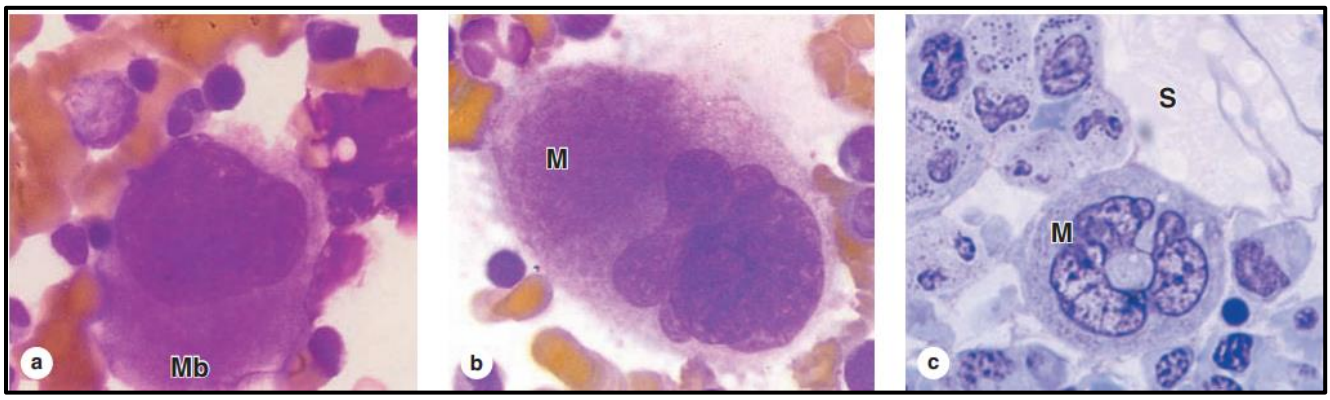


Figure 10: Megakaryoblast and megakaryocytes. (a) Megakaryoblasts (Mb) with very basophilic cytoplasm. (X1400; Wright) (b) Megakaryoblasts undergo endomitosis and differentiate into megakaryocytes (M) (X1400; Wright) (c) Micrograph of sectioned bone marrow in which a megakaryocyte (M) is shown near sinusoids (S). (X400; Giemsa).

For platelet formation, megakaryocytes rapidly extend several long branching processes called **proplatelets**, which penetrate adjacent microvascular endothelium and are exposed in the circulating blood (Figure 4). Internally proplatelets have a framework of actin filaments and loosely bundled, mixed microtubules along which membrane vesicles and specific granules are transported. A loop of microtubules forms a teardrop shaped enlargement at the distal end of the proplatelet, and cytoplasm within these loops is pinched off to form platelets with their characteristic marginal bundles of microtubules and actin filaments surrounding cytoplasmic granules and vesicles of the **open canalicular system**.

During proplatelet growth microtubules polymerize in both directions. Proplatelet elongation depends on both this polymerization. Mature megakaryocytes also have numerous invaginations of plasma membrane ramifying throughout the cytoplasm, called **demarcation membranes** (Figure 11), which were formerly considered "**fracture lines**" or "**perforations**" for the release of platelets but are now thought to represent a membrane reservoir facilitating continuous proplatelet elongation. From the proplatelets each megakaryocyte's cytoplasm is apportioned into a few thousand platelets, after which the remainder of the cell undergoes apoptosis and is removed by macrophages.

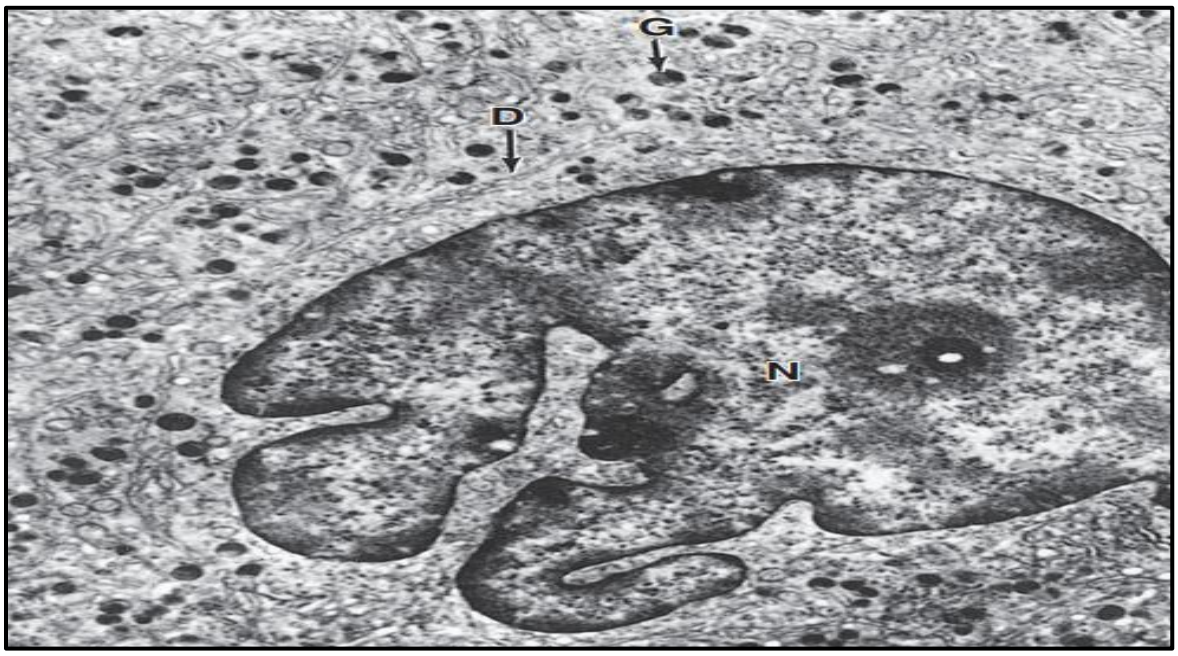


Figure 11: Megakaryocyte ultrastructure. This TEM of a megakaryocyte shows the lobulated nucleus (N), numerous cytoplasmic granules (G), and an extensive system of demarcation membranes (D) through the cytoplasm (X10,000).

➢ MEDICAL APPLICATION

Hematopoiesis is an important step in the medical treatment of people with bone marrow disease. Stem cell and bone marrow transplant recipients rely on hematopoiesis to make new healthy blood cells to treat conditions like leukemia and other blood cancers, hereditary blood conditions, and certain immune disorders.

Red bone marrow also contains stem cells that can produce other tissues in addition to blood cells. These pluripotent cells may make it possible to generate specialized cells that are not rejected by the body because they are produced from stem cells from the marrow of the same patient. The procedure is to collect bone marrow stem cells, cultivate them in appropriate medium for their differentiation to the cell type needed for transplant, and then use the resulting cells to replace defective cells. These studies in regenerative medicine are at early stages, but results with animal models are promising.

Lec 6,7: The Circulatory System

The circulatory system pumps and directs blood cells and substances carried in blood to all tissues of the body. It includes both the blood and lymphatic vascular systems, and in an adult the total length of its vessels is estimated at between 100,000 and 150,000 km.

Blood

The liquid portion of circulating blood is **plasma**, while the cells and platelets comprise the **formed elements**; upon clotting, some proteins are removed from plasma and others are released from platelets, forming a new liquid termed **serum**. Important protein components of plasma include **albumin**, diverse α- and β-globulins, proteins of the complement system, and fibrinogen, all of which are secreted within the liver, as well as the **immunoglobulins**.

The element of The Blood

■ **RBCs or erythrocytes**, which make up the hematocrit portion (~45%) of a blood sample, are enucleated, biconcave discs 7.5 μm in diameter, filled with hemoglobin for the uptake, transport, and release of O2 , and with a normal life span of about 120 days.

■ **WBCs or leukocytes** are broadly grouped as **granulocytes** (**neutrophils, eosinophils, basophils**) or **agranulocytes** (**lymphocytes, monocytes**). All leukocytes become active outside the circulation, specifically leaving the microvasculature in a process involving cytokines, selective adhesion, changes in the endothelium, and **transendothelial migration** or **diapedesis**.

■ **Neutrophils**, the most abundant type of leukocyte, have polymorphic, multilobed nuclei, and faint pink cytoplasmic granules that contain many factors for highly efficient phagolysosomal killing and removal of bacteria.

■ **Eosinophils** have bilobed nuclei and eosinophilic-specific granules containing factors for destruction of helminthic parasites and for modulating inflammation.

■ **Basophils**, the rarest type of circulating leukocyte, have irregular bilobed nuclei and resemble mast cells with strongly basophilic specific granules containing factors important in allergies and chronic inflammatory conditions, including histamine, heparin, chemokines, and various hydrolases.

■ **Lymphocytes**, agranulocytes with many functions as T- and B-cell subtypes in the immune system, range widely in size, depending on their activation state, and have roughly spherical nuclei with little cytoplasm and few organelles.

■ **Monocytes** are larger agranulocytes with distinctly indented or C-shaped nuclei, which circulate as precursors of macrophages and other cells of the mononuclear phagocyte system.

■ **Platelets** are small (2-4 μm) cell fragments derived from megakaryocytes in bone marrow, with a marginal bundle of actin filaments, alpha granules and delta granules.

The blood vascular system,

The blood vascular system, or cardiovascular system (Figure 1), consists of the following structures:

■ **The heart** propels blood through the system.

■ **Arteries**, a series of vessels efferent from the heart that become smaller as they branch into the various organs, carry blood to the tissues.

■ **Capillaries**, the smallest vessels, are the sites of **O2 , CO2** , nutrient, and waste product exchange between blood and tissues. Together with the smallest arterial and venous branches carrying blood to and from them, capillaries in almost every organ form a complex network of thin tubules called the **microvasculature** or **microvascular bed.**

■ **Veins** result from the convergence of **venules** into a system of larger channels which continue enlarging as they approach the heart, toward which they carry the blood to be pumped again.

As shown in Figure 1, two major divisions of **arteries** and **veins** make up the **pulmonary circulation**, where blood is oxygenated in the lungs, and the **systemic circulation**, where blood brings nutrients and removes wastes in tissues throughout the body.

The internal surface of all components of the blood and lymphatic systems is lined by a simple squamous epithelium called **endothelium**. Cardiovascular endothelial cells have crucial physiologic and medical importance:

1. maintain a selectively permeable. **2.** antithrombogenic (inhibitory to clot formation) barrier. **3.** they also determine when and where white blood cells leave the circulation for the interstitial space of tissues. **4.** secrete a variety of paracrine factors for vessel dilation, constriction, and growth of adjacent cells.

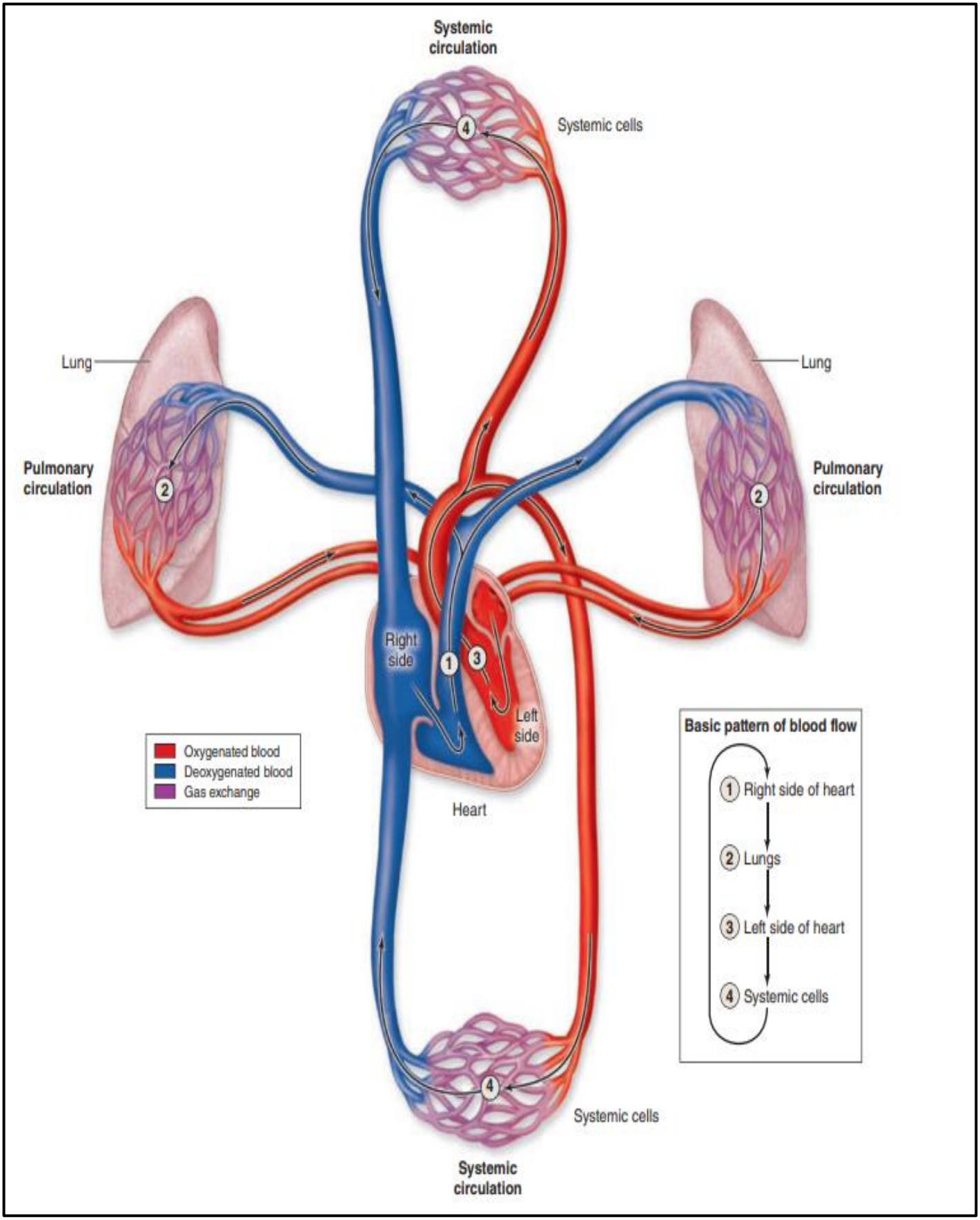


Figure1: Diagram of the cardiovascular system.

➢ Heart

Cardiac muscle in the four chambers of the heart wall contracts rhythmically, pumping the blood through the circulatory system (Figure 2). The right and left ventricles propel blood to the pulmonary and systemic circulations, respectively; right and left atria receive blood from the body and the pulmonary veins, respectively. The walls of all four heart chambers consist of three major layers: **the internal endocardium**, **the middle myocardium**, and **the external epicardium**.

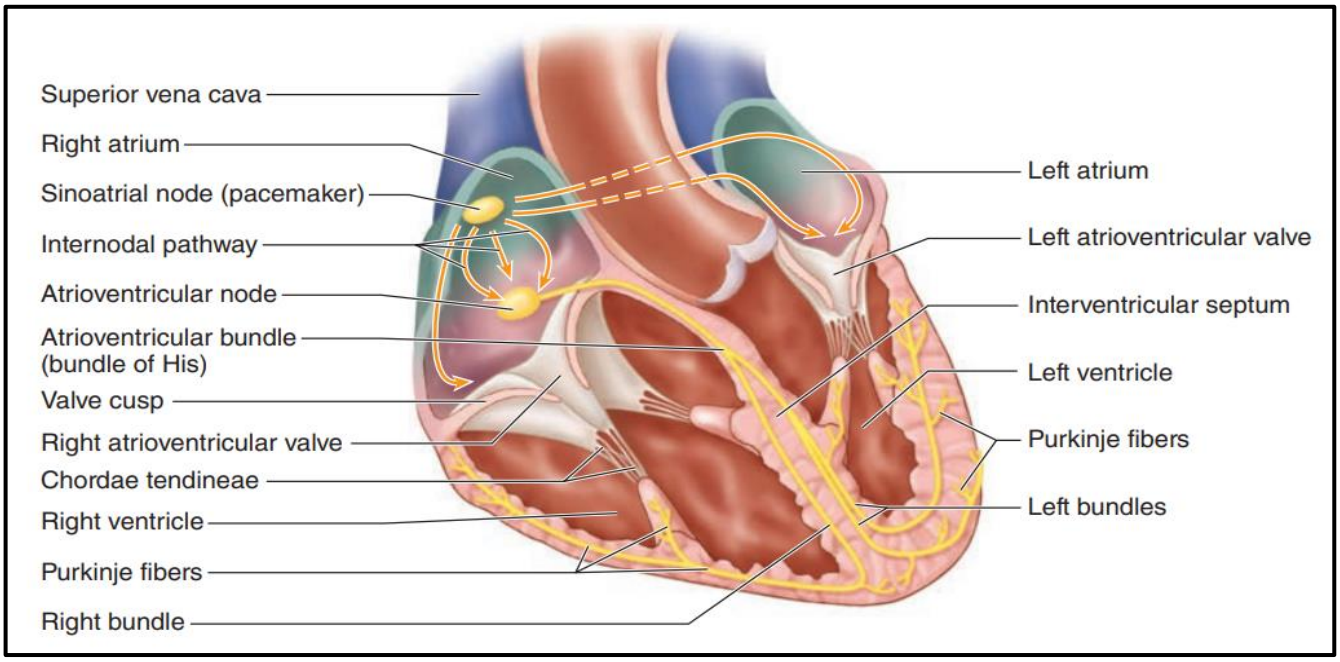


Figure 2: Overview of the heart.

■ **The endocardium** consists of the lining endothelium, its supporting layer of fibroelastic connective tissue with scattered fibers of smooth muscle, and a deeper layer of connective tissue (continuous with that of the myocardium called the subendocardial layer) surrounding variable numbers of modified cardiac muscle fibers which comprise the heart's impulse conducting system (Figure 3).

■ **The myocardium** consists mainly of typically contractile cardiac muscle fibers arranged spirally around each heart chamber. Because strong force is required to pump blood through the systemic and pulmonary circulations, the myocardium is much thicker in the walls of the ventricles, particularly the left, than in the atrial walls (Figure 3).

■ **The epicardium** is a simple squamous mesothelium supported by a layer of loose connective tissue containing blood vessels and nerves (Figure 4). The epicardium corresponds to the visceral layer of the pericardium, the membrane surrounding the heart. Where the large vessels enter and leave the heart, the epicardium is reflected back as the parietal layer lining the pericardium. During heart movements, underlying structures are cushioned by deposits of adipose tissue in the epicardium and friction

within the pericardium is prevented by lubricant fluid produced by both layers of serous mesothelial cells.

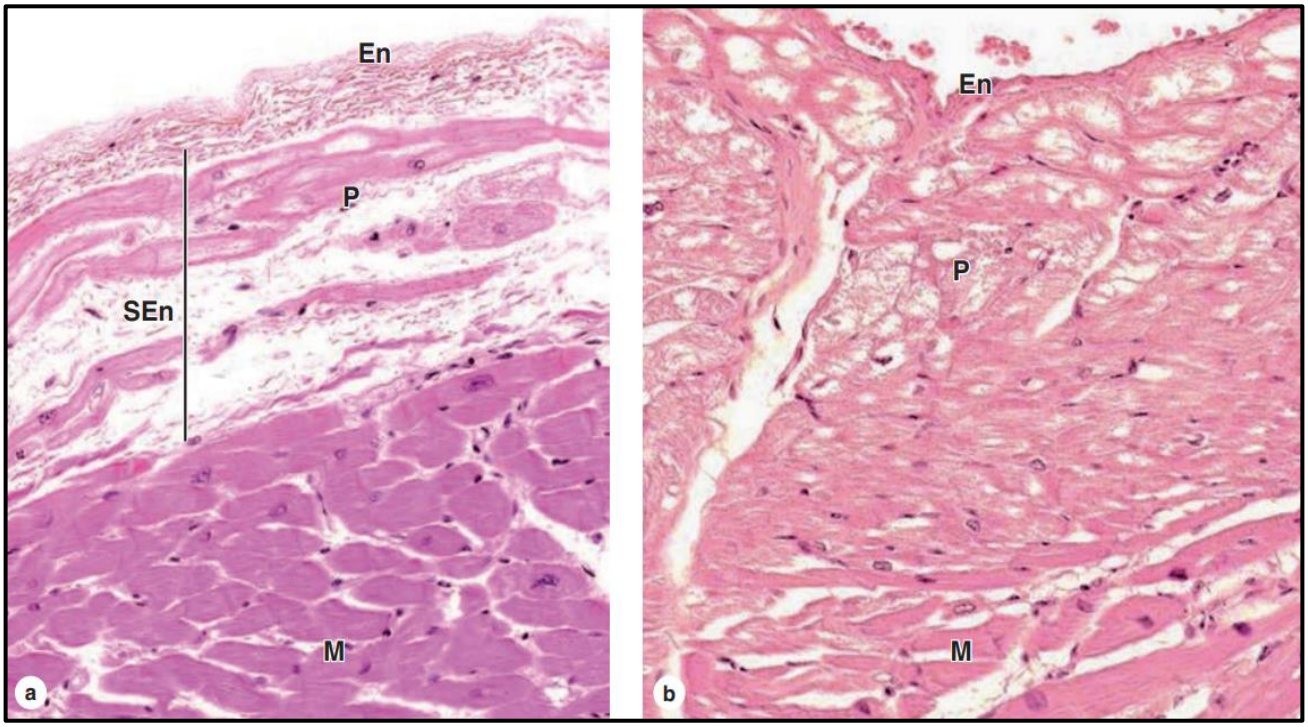


Figure 3: Endocardium, myocardium, and fibers of the subendocardial conducting network. (a) The subendocardial layer of connective tissue (SEn) in the ventricles surrounds Purkinje fibers (P) of the heart's impulse conducting network. Purkinje fibers typically are paler staining than contractile muscle fibers (M). (b) In the atrial walls conducting Purkinje-like fibers (P) often occupy most of the subendocardial layer, lying close to the endothelium (En), and merging with the contractile fibers of the myocardium (M) (Both X200; H&E).

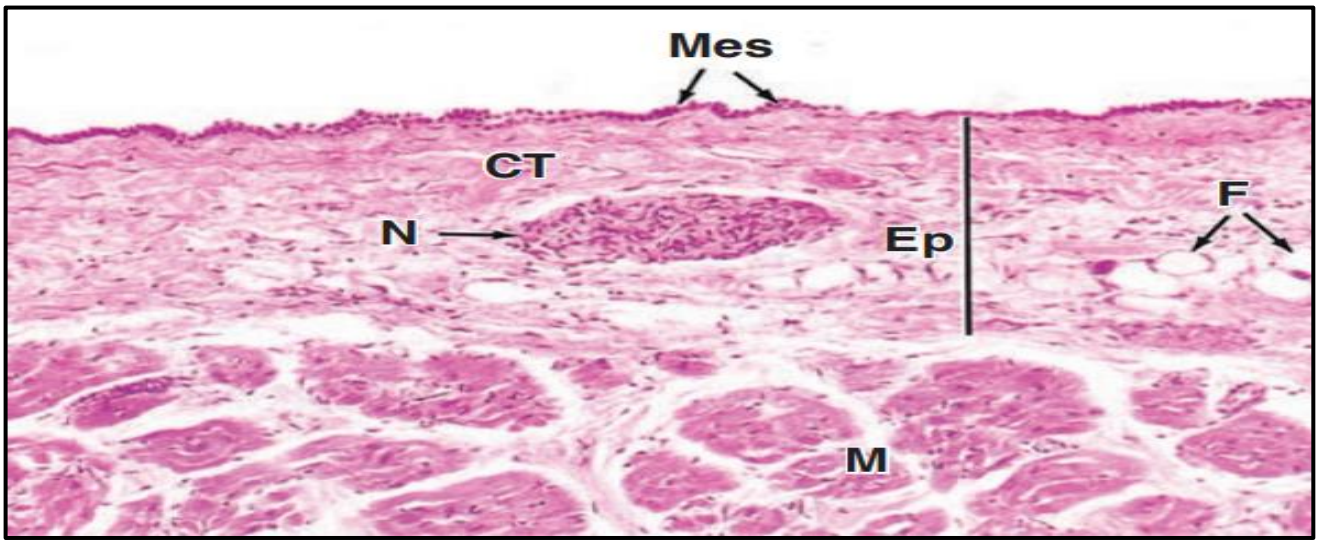


Figure 4: Epicardium or visceral pericardium. This section of atrium shows part of the myocardium (M) and epicardium (Ep). The epicardium consists of loose connective tissue (CT) containing autonomic nerves (N) and variable amounts of fat (F). The epicardium is the visceral layer of the pericardium and is covered by the simple mesothelium (Mes) which also lines the pericardial space (X100; H&E).

Within these major layers the heart contains other structures important for its overall function of moving blood. Prominent dense irregular connective tissue comprising the fibrous cardiac skeleton (Figures 2). Various regions of the cardiac skeleton have the following functions:

- **Surrounding, anchoring, and supporting all heart valves.**
- **providing firm points of insertion for cardiac muscle in the atria and ventricles.**
- **helping coordinate the heartbeat by acting as electrical insulation between atria and ventricles.**

In both the **subendocardial layer** and **the adjacent myocardium**, **modified cardiac muscle cells** make up the impulse conducting system of the heart, specialized to generate and conduct waves of depolarization which stimulate rhythmic contractions in adjacent myocardial fibers. This system (Figure 2) consists of two nodes of specialized myocardial tissue in the right atrial wall: **the sinoatrial (SA) node (or pacemaker) and the atrioventricular (AV) node, from which the AV bundle (bundle of His) emerges.**

At the apex of the heart, the bundles subdivide further into a subendocardial conducting network of cardiac muscle fibers, usually called **Purkinje fibers**. These are pale-staining fibers, larger than the adjacent contractile fibers, with sparse, peripheral myofibrils, and much glycogen (Figure 3). Purkinje fibers mingle distally with contractile muscle fibers of each ventricle and trigger waves of contraction through both ventricles simultaneously.

Both **parasympathetic** and **sympathetic** neural components innervate the heart. Ganglionic nerve cells and nerve fibers are present in the regions close to the **SA and AV nodes**, where they affect heart rate and rhythm, such as during physical exercise and emotional stress. Stimulation of the **parasympathetic division** (**vagus nerve**) slows the heartbeat, whereas stimulation of the **sympathetic nerve** accelerates activity of the pacemaker.

Tissues of The Vascular Wall

Walls of all blood vessels except capillaries contain **smooth muscle and connective tissue in addition to the endothelial lining**. The amount and arrangement of these tissues in vessels are influenced by mechanical factors, primarily blood pressure, and metabolic factors reflecting the local needs of tissues.

The endothelium is a specialized epithelium that acts as a semipermeable barrier between two major internal compartments: the blood and the interstitial tissue fluid. Vascular endothelial cells are squamous, polygonal, and elongated with the long axis in the direction of blood flow. Endothelium with its basal lamina is highly differentiated to mediate and actively monitor the bidirectional exchange of molecules by simple and active diffusion, receptor-mediated endocytosis, transcytosis.

Smooth muscle fibers occur in the walls of all vessels larger than capillaries and are arranged helically in layers. In arterioles and small arteries, the smooth muscle cells are connected by many more gap junctions and permit vasoconstriction and vasodilation that are of key importance in regulating the overall blood pressure.

Macroscopically visible blood vessels have three major layers or tunics: **(1) The intima** includes the endothelium, connective tissue, and an internal elastic lamina in larger vessels; **(2) the media** contains alternating layers of smooth muscle and collagen or elastic lamellae; and **(3) the adventitia** (or externa) contains connective tissue, small vessels (vasa vasorum), and nerves.

Vasculature

Large blood vessels and those of the microvasculature branch frequently and undergo gradual transitions into structures with different histologic features and functions. For didactic purposes vessels can be classified arbitrarily as the types discussed here:

➢ Elastic Arteries

Elastic arteries are the aorta, the pulmonary artery, and their largest branches; these large vessels are also called conducting arteries because their major role is to carry blood to smaller arteries. As shown in Figure 4a, the most prominent feature of elastic arteries is the thick tunica media in which elastic lamellae alternate with layers of smooth muscle fibers. The adult aorta has about 50 elastic lamellae (more if the individual is hypertensive).

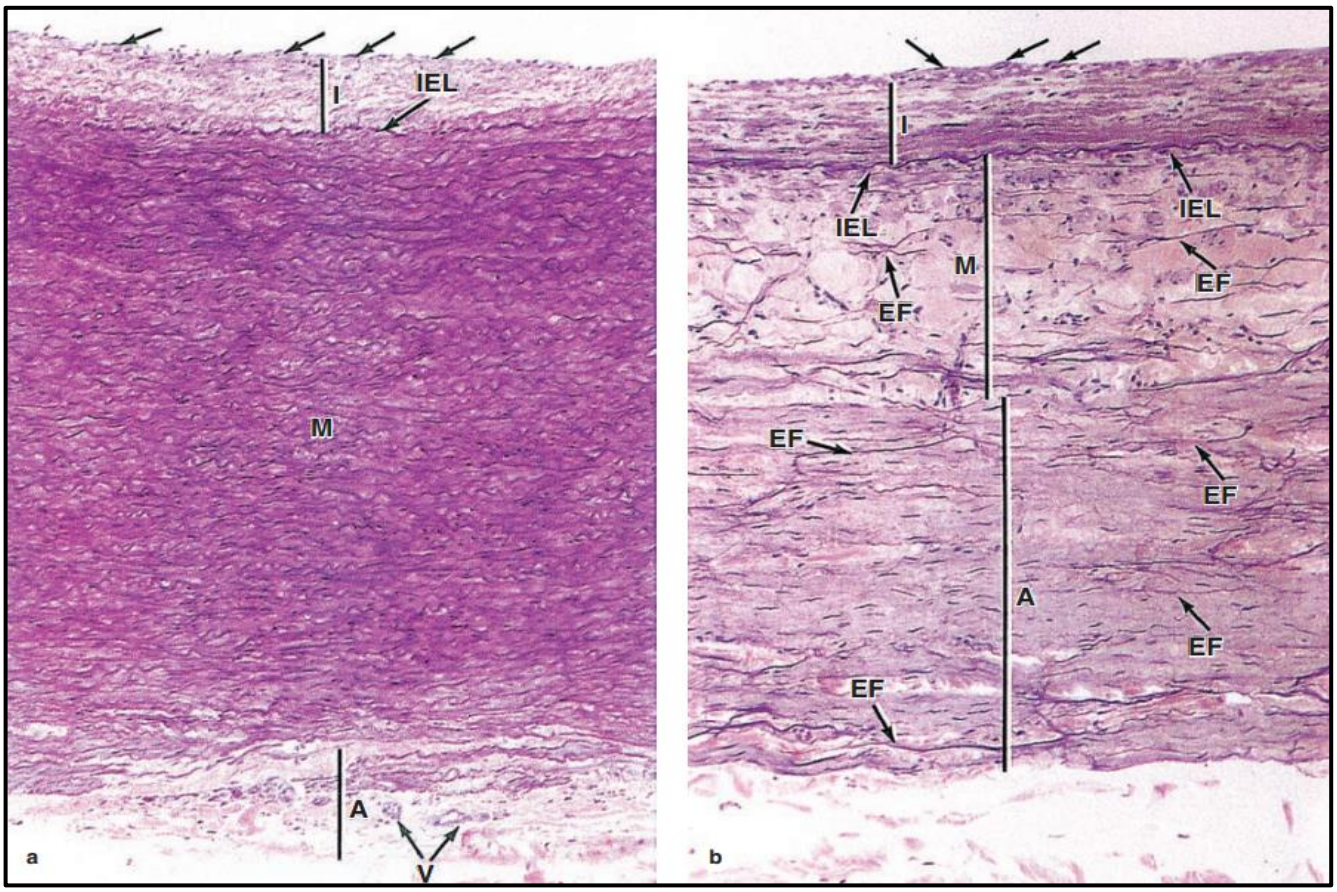


Figure 4: Tunics of the vascular wall. Comparison of the three major layers or tunics in the largest artery and vein. (a) Aorta, (b) vena cava. Simple squamous endothelial cells (arrows) line the intima (I) that also has subendothelial connective tissue and in arteries is separated from the media by an internal elastic lamina (IEL). The media (M) contains many elastic lamellae and elastic fibers (EF) alternating with layers of smooth muscle. The media is much thicker in large arteries than veins, with relatively more elastin. Elastic fibers are also present in the outer tunica adventitia (A), which is relatively thicker in large veins. Vasa vasorum (V) are seen in the adventitia of the aorta. (Both X122; Elastic stain).

Intima: connective tissue with smooth muscle. **Media**: Many elastic lamellae alternating with smooth muscle. **Adventitia**: Connective tissue, thinner than media, with vasa vasorum. **Roles in Circulatory System**: Conduct blood from heart and with elastic recoil help move blood forward under steady pressure.

Arterial Sensory Structures

Carotid sinuses are slight dilations of the bilateral internal carotid arteries where they branch from the (elastic) common carotid arteries; they act as important baroreceptors monitoring arterial blood pressure. At these sinuses the tunica media is thinner, allowing greater distension when blood pressure rises, and the adventitia contains many sensory nerve endings from cranial nerve IX, the glossopharyngeal nerve. The brain's vasomotor centers process these afferent impulses and adjust vasoconstriction, maintaining normal blood pressure. Functionally similar baroreceptors

present in the aortic arch transmit signals pertaining to blood pressure via cranial nerve X, the vagus nerve.

➢ Muscular Arteries

The muscular arteries, also called distributing arteries, distribute blood to the organs and help regulate blood pressure.

Intima: Endothelium; connective tissue with smooth muscle, internal elastic lamina prominent. **Media**: Many smooth muscle layers, with much less elastic material. **Adventitia**: Connective tissue, thinner than media; vasa vasorum maybe present. **Roles in Circulatory System**: Distribute blood to all organs and maintain steady blood pressure and flow with vasodilation and constriction (figure 5).

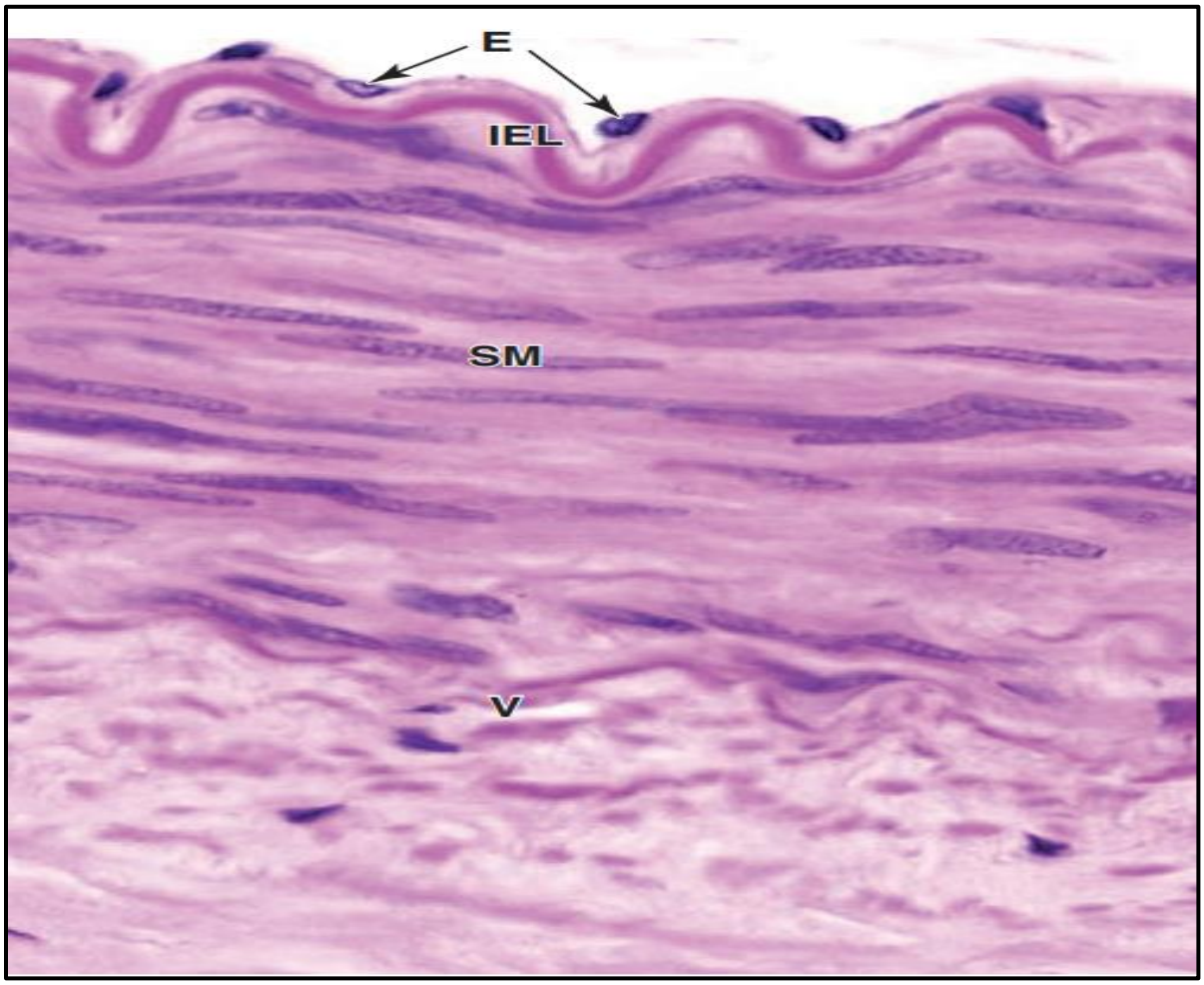


Figure 5: Muscular artery. A transverse section through a muscular artery shows a slightly folded intima with only sparse connective tissue between the endothelial cells (E) and internal elastic lamina (IEL). Multiple layers of smooth muscle (SM) in the media are thicker than the elastic lamellae and fibers with which they intersperse. Vasa vasorum (V) are seen in the adventitia (X100; H&E).

➢ Arterioles

Muscular arteries branch repeatedly into smaller and smaller arteries, until reaching a size with three or four layers of medial smooth muscle. The smallest arteries branch as arterioles, which have only one or two smooth muscle layers; these indicate the beginning of an organ's microvasculature (Figures 6) where exchanges between blood and tissue fluid occur. Arterioles are generally less than 0.1 mm in diameter, with lumens approximately as wide as the wall is thick.

Intima: Endothelium; no connective tissue or smooth muscle. **Media**: 1-3 layers of smooth muscle. **Adventitia**: Very thin connective tissue layer. **Roles in Circulatory System**: Resist and control blood flow to capillaries; major determinant of systemic blood pressure.

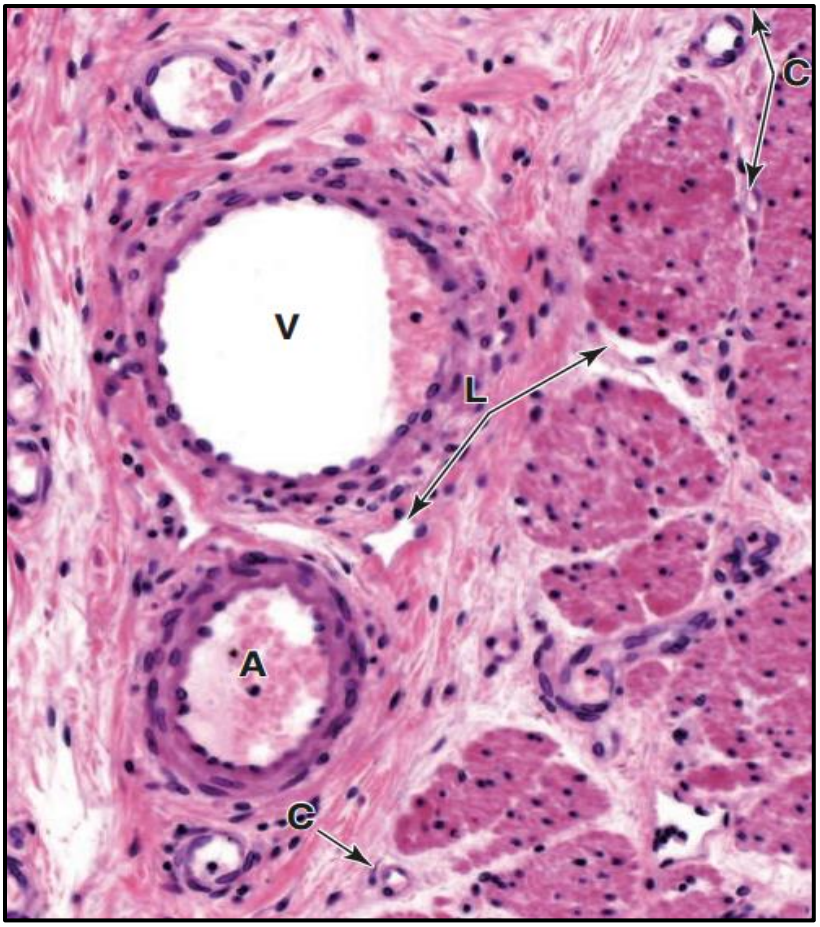


Figure 6: Microvasculature. Arterioles (A), capillaries (C), and venules (V) comprise the microvasculature in almost every organ. (L) denote to irregularly shaped lymphatic vessels (200X; H&E).

In certain tissues and organs, arterioles deviate from this simple path to accommodate various specialized functions (Figure 7). For example, thermoregulation by the skin involves arterioles that can bypass capillary networks and connect directly to venules. The media and adventitia are thicker in these arteriovenous shunts and richly innervated by sympathetic and parasympathetic nerve fibers. The autonomic fibers

control the degree of vasoconstriction at the shunts, regulating blood flow through the capillary beds. High capillary blood flow in the skin allows more heat dissipation from the body, while reduced capillary blood flow conserves heat—important functions when the environmental temperature is hot or cold, respectively.

Another important alternative microvascular pathway is a venous portal system (Figure 7), in which blood flows through two successive capillary beds separated by a portal vein. This arrangement allows for hormones or nutrients picked up by the blood in the first capillary network to be delivered most efficiently to cells around the second capillary bed before the blood is returned to the heart for general distribution. The best examples are in the liver and the hypothalamic.

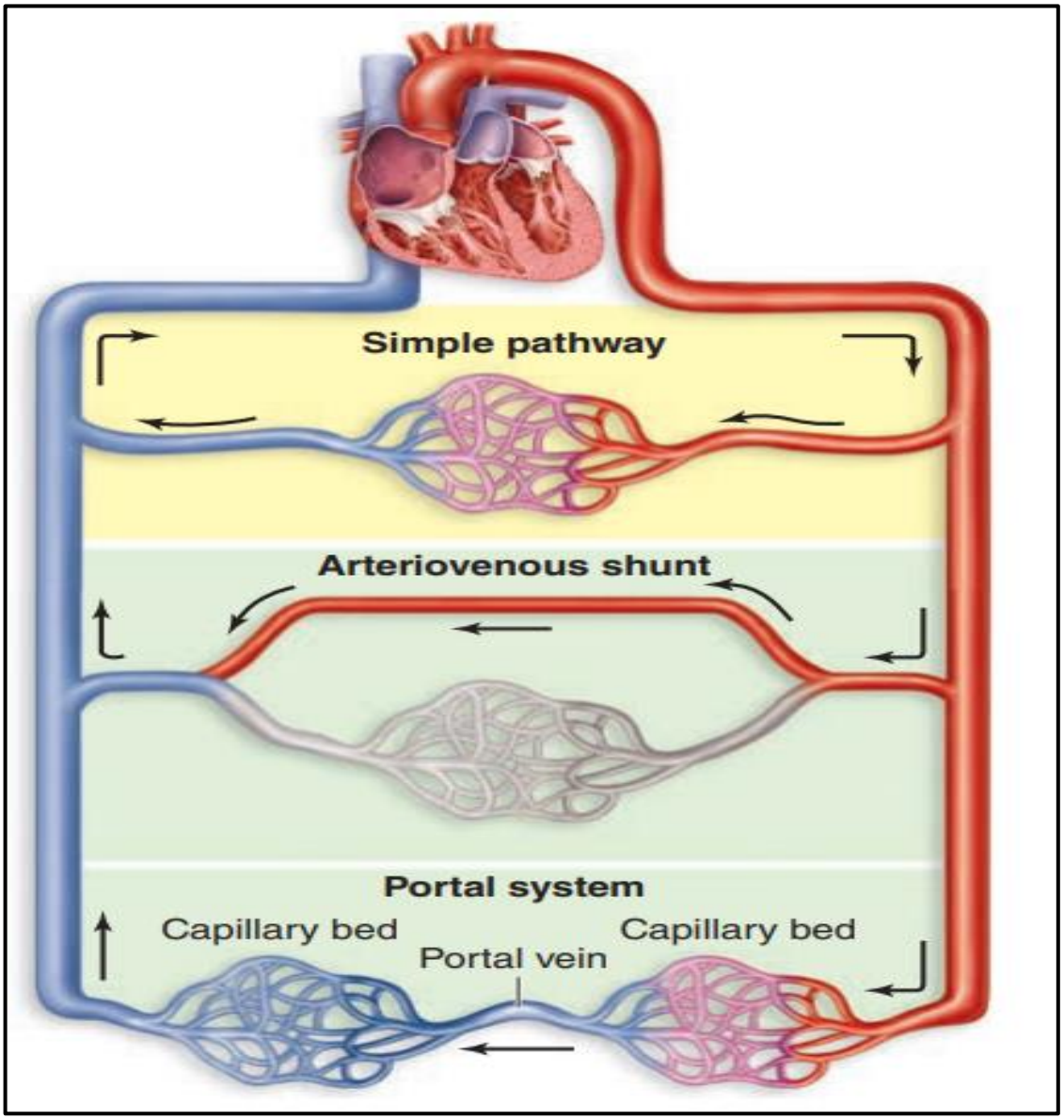


Figure 7: Comparison of the simple microvascular pathway with arteriovenous shunts and portal systems.

➢ Capillaries

Capillaries permit and regulate metabolic exchange between blood and surrounding tissues. These smallest blood vessels always function in networks called capillary beds, whose size and overall shape conforms to that of the structure supplied. The density of the capillary bed is related to the metabolic activity of the tissues. Tissues with high metabolic rates, such as the kidney, liver, and cardiac and skeletal muscle, have abundant capillaries; the opposite is true of tissues with low metabolic rates, such as smooth muscle and dense connective tissue.

Intima: Endothelium only. **Media**: A few pericytes only. **Adventitia**: None. **Roles in Circulatory System**: Exchange metabolites by diffusion to and from cells.

Capillaries are classified as **three structural and functional types**, with features that allow different degrees of molecular or even cellular exchange:

(1) continuous capillaries with many tight junctions so that all exchange must occur through the cells. All molecules exchanged across the endothelium must cross the cells by diffusion or transcytosis.

(2) fenestrated capillaries also have tight junctions, but perforations through the endothelial cells allow greater exchange across the endothelium. The basement membrane is continuous in both these capillary types. Fenestrated capillaries are found in organs where molecular exchange with the blood is important, such as endocrine organs, intestinal walls, and choroid plexus.

(3) sinusoids, or discontinuous capillaries, usually have a wider diameter than the other types and have discontinuities between the endothelial cells, large fenestrations through the cells, and a partial, discontinuous basement membrane. Sinusoids are found in organs where exchange of macromolecules and cells occurs readily between tissue and blood, such as in bone marrow, liver, and spleen (figure 8).

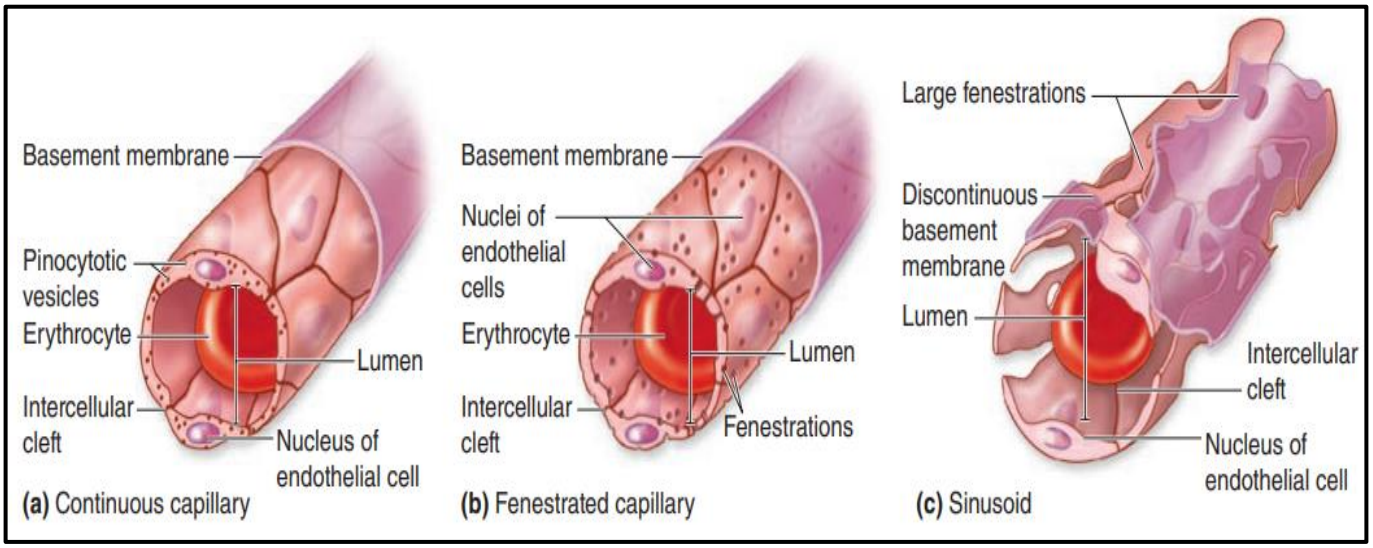


Figure 8: Types of capillaries.

At various locations along continuous capillaries and postcapillary venules are mesenchymal cells called pericytes, with long cytoplasmic processes partly surrounding the endothelial layer. Pericytes secrete many ECM components and form their own basal lamina, which fuses with the basement membrane of the endothelial cells (Figure 9). Well-developed cytoskeletal networks of myosin, actin, and tropomyosin indicate that pericytes also dilate or constrict capillaries, helping to regulate blood flow in some organs. After injuries pericytes proliferate and differentiate to form smooth muscle and other cells in new vessels as the microvasculature is reestablished. In many organs the pericyte population also includes mesenchymal stem cells important for regeneration of other tissues.

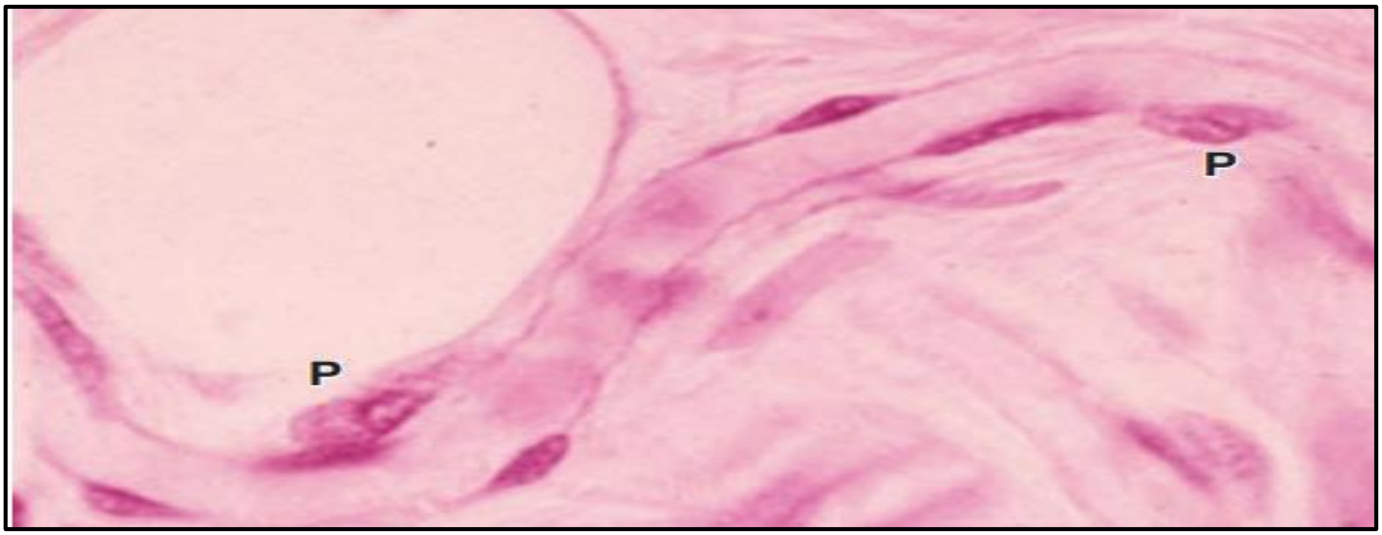


Figure 9: Capillary with pericytes. Capillaries are normally associated with perivascular contractile cells called pericytes (P). The more flattened nuclei belong to endothelial cells. (X400; H&E).

➢ Venules

The transition from capillaries to venules occurs gradually. Postcapillary venules (Figure 10a) are similar to capillaries with pericytes but larger, ranging in diameter from 15 to 20 μm. Postcapillary venules are the primary site at which white blood cells adhere to endothelium and leave the circulation at sites of infection or tissue damage.

Postcapillary venules converge into larger collecting venules that have more distinct contractile cells. With increasing size venules become surrounded by a recognizable tunica media with two or three smooth muscle layers and are called muscular venules. A characteristic feature of all venules is the large diameter of the lumen compared to the overall thinness of the wall (Figure 10).

Intima: Endothelium; no valves. **Media**: Pericytes and scattered smooth muscle cells. **Adventitia**: None. **Roles in Circulatory System**: Drain capillary beds; site of leukocyte exit from vasculature.

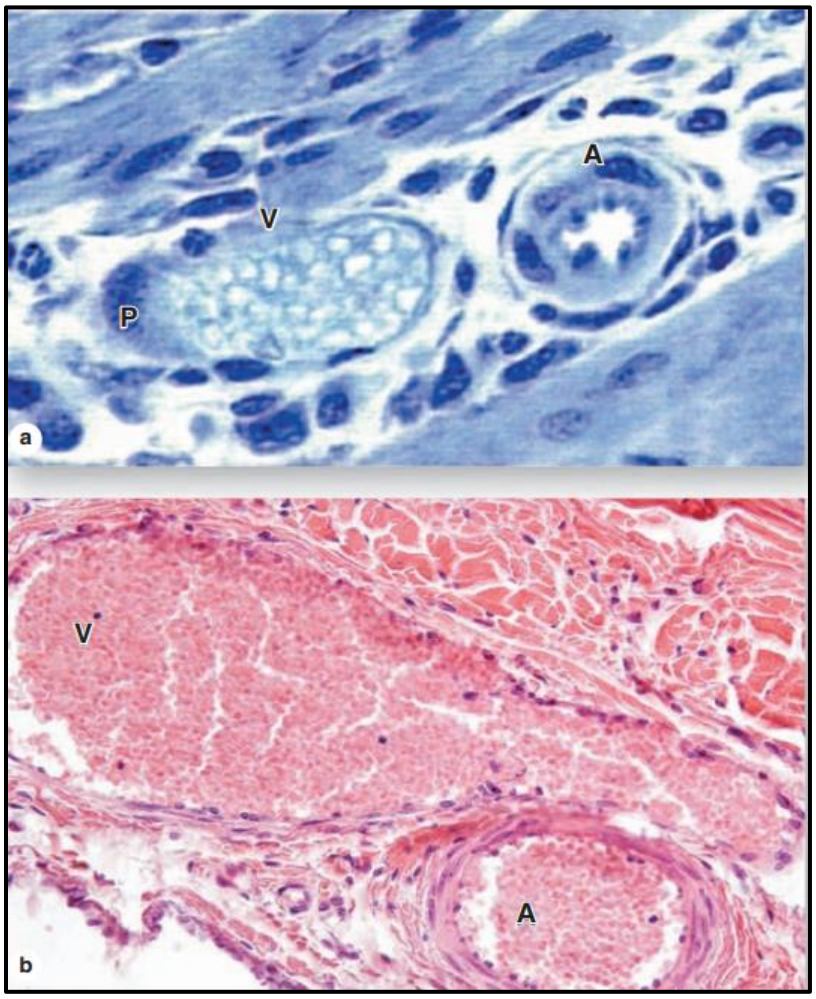


Figure 10: Venules. (a) Compared to arterioles (A), postcapillary venules (V) have large lumens, with occasional pericytes (P). (X400; Toluidine blue). (b) Larger collecting venules (V) have much greater diameters than arterioles (A), (X200; H&E).

➢ Veins

Veins carry blood back to the heart from microvasculature all over the body. Blood entering veins is under very low pressure and moves toward the heart by contraction of the smooth muscle fibers in the media and by external compressions from surrounding skeletal muscles and other organs. Most veins are classified as small or medium veins (Figure 11).

- **Small veins**

Intima: Endothelium; connective tissue with scattered smooth muscle fibers. **Media**: Thin, 2-3 loose layers of smooth muscle cells. **Adventitia**: Connective tissue, thicker than media. **Roles in Circulatory System**: Collect blood from venules.

- **medium veins**

Intima: Endothelium; connective tissue, with valves. **Media**: 3-5 more distinct layers of smooth muscle. **Adventitia**: Thicker than media; longitudinal smooth muscle may be present. **Roles in Circulatory System**: Carry blood to larger veins, with no backflow.

The big venous trunks, paired with elastic arteries close to the heart, are the large veins (Figure 4b). These have well-developed intimal layers, but relatively thin media with alternating smooth muscle and connective tissue. The tunica adventitia is thicker than the media in large veins and frequently contains longitudinal bundles of smooth muscle. Both the media and adventitia contain elastic fibers, and an internal elastic lamina like those of arteries may be present.

An important feature of large and medium veins are valves, which consist of thin, paired folds of the tunica intima projecting across the lumen, rich in elastic fibers and covered on both sides by endothelium (Figures 11and 12). The valves help keep the flow of venous blood directed toward the heart.

- **Large Vein**

Intima: Endothelium; connective tissue, smooth muscle cells; prominent valves. **Media**: > 5 layers of smooth muscle, with much collagen. **Adventitia**: Thickest layer, with bundled longitudinal smooth muscle. **Roles in Circulatory System**: Return blood to heart.

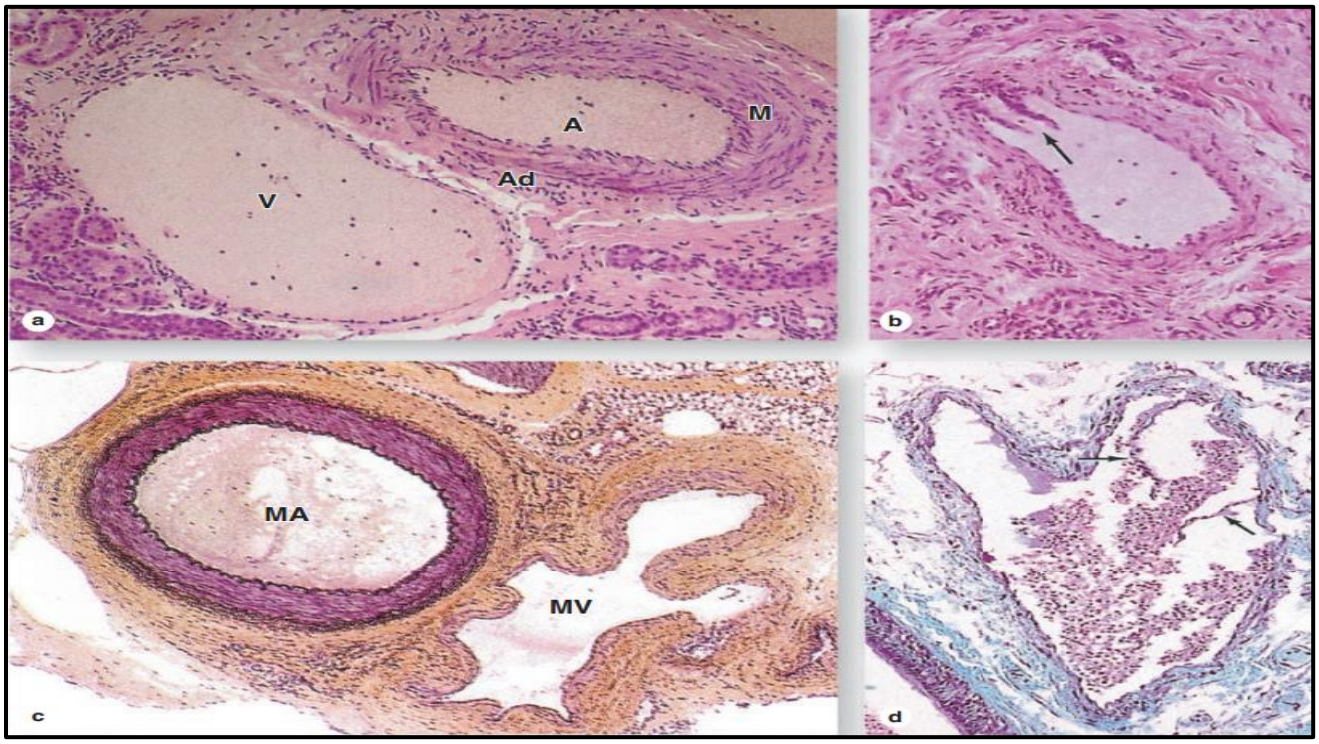


Figure 11: Veins. (a) Micrograph of small vein (V) shows a relatively large lumen compared to the small muscular artery (A) with its thick media (M) and adventitia (Ad) (X200; H&E). (b) Micrograph showing valve in an oblique section of a small vein (arrow). Valves are thin folds of

intima projecting well into the lumen (X200; Aldehyde fuchsin & van Gieson). (c) Micrograph of a medium vein (MV) shows a thicker wall but still less prominent than that of the accompanying muscular artery (MA). Both the media and adventitia are better developed, but the wall is often folded around the relatively large lumen (X100; Aldehyde fuchsin & van Gieson). (d) Micrograph of a medium vein contains blood and shows valve folds (arrows). (X200; Masson trichrome).

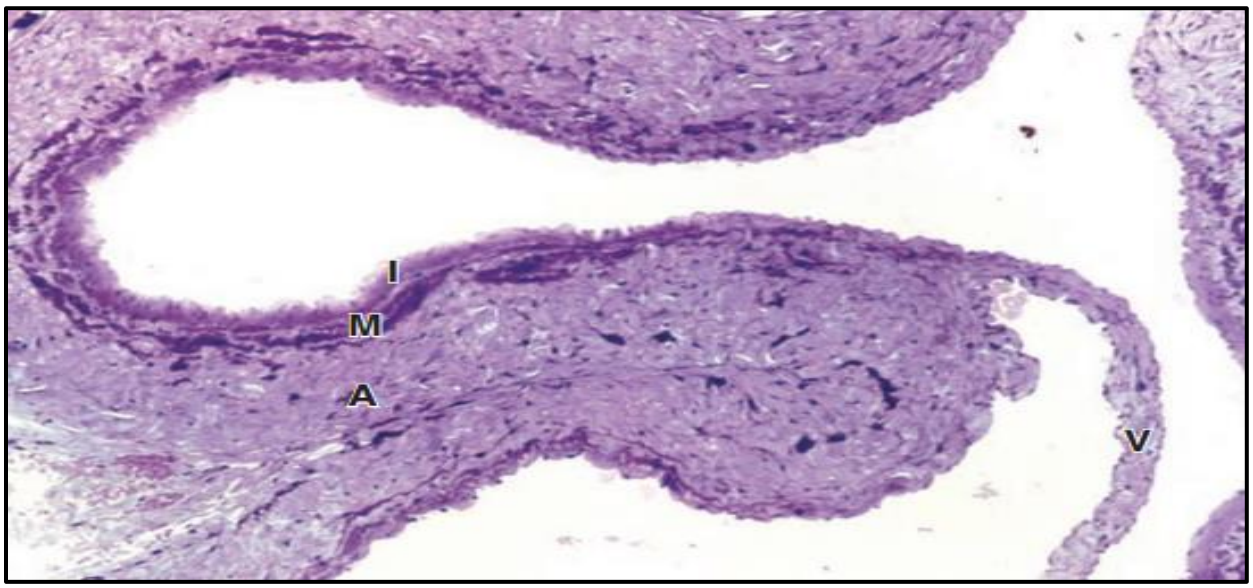


Figure 12: Wall of large vein with valve. Large veins have a muscular media layer (M) which is very thin compared to the surrounding adventitia (A) of dense irregular connective tissue. The wall is often folded as shown here, with the intima (I) projecting into the lumen as a valve (V) composed of the subendothelial connective tissue with endothelium on both sides. (X100; PT).

Lecture 8,9: Lymphatic Vascular System And Lymphoid System

In addition to the blood vasculature, the body has a system of very thin-walled channels, the **lymphatic capillaries**, which collect excess interstitial fluid from the tissue spaces as **lymph** and return it to the blood. Like the interstitial fluid, lymph is usually rich in lightly staining proteins but does not normally contain red blood cells, although lymphocytes and other white blood cells may normally be present (Figure 1a). With exceptions such as **the bone marrow** and most of **the Central Nervous System (CNS)**, most tissues with blood microvasculature also contain lymphatic capillaries.

Lymphatic capillaries originate locally as tubes of very thin endothelial cells which lack **tight junctions** and rest on a discontinuous basal lamina. Interstitial fluid enters lymphatic capillaries by flowing between endothelial cells and by **transcytosis**. Specific domains of adjacent endothelial cells also lack **hemidesmosome** connections to the basal lamina and extend into the lumen to form leaflets of valves facilitating fluid entry and preventing most backflow of lymph (Figure 1b).

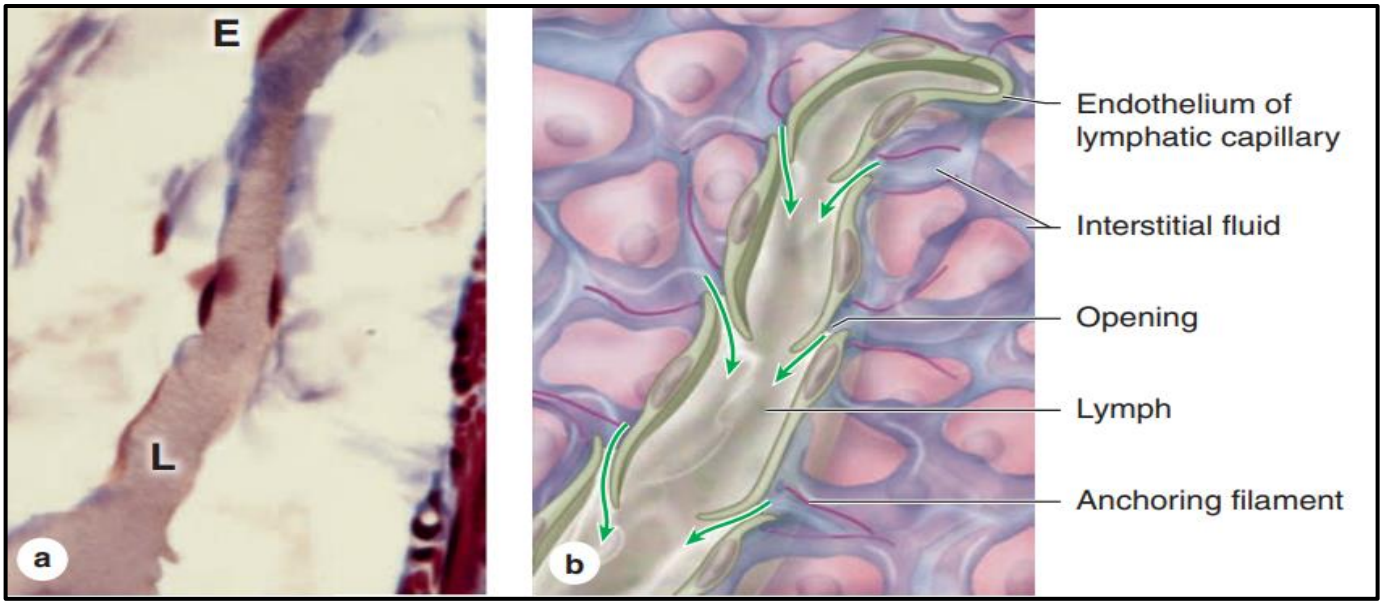


Figure 1: Lymphatic capillary. (a) Micrograph shows a lymphatic capillary filled with lymph (L). Lymphatics have a wall of very thin endothelial cells. (X200; Mallory trichrome) (b) Diagram indicating more details about lymphatics, including the openings between the endothelial cells. The openings are held in place by anchoring filaments containing elastin and are covered by extensions of the endothelial cells. Lymphatic endothelial cells are typically larger than those of blood capillaries.

Lymphatic capillaries converge into larger **lymphatic vessels** with thin walls and increasing amounts of connective tissue and smooth muscle which never form a distinct outer tunics (Figure 2). Like veins lymphatic vessels have valves comprised of complete intimal folds. Interposed in the path of these larger lymphatic vessels are **lymph nodes**,

where lymph is processed by cells of the immune system. In histological sections lymphatic vessels are often dilated with lymph. As in veins, lymphatic circulation is aided by external forces (eg, contraction of surrounding skeletal muscle) with the valves keeping lymph flow unidirectional.

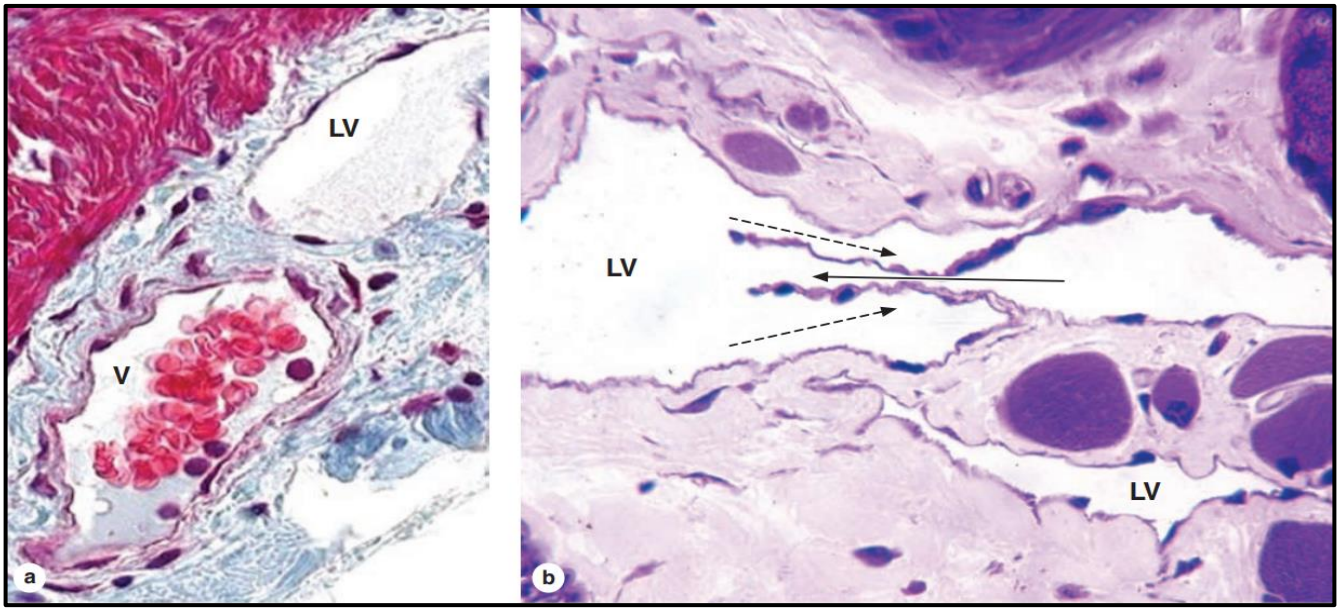


Figure 2: Lymphatic vessels and valve. (a) Cross section shows a lymphatic vessel (LV) near a venule (V), whose wall is thick by comparison. Lymphatic vessels normally do not contain red blood cells, which provide another characteristic distinguishing them from venules (X200; Mallory trichrome). (b) Lymphatic vessel (LV) in muscle cut longitudinally shows a valve. The solid arrow shows the direction of the lymph flow, and the dotted arrows show how the valves prevent lymph backflow. The lower small lymphatic vessel is a lymphatic capillary with a wall consisting only of endothelium (X200; PT).

Lymphatic vessels ultimately converge as two large trunks: **the thoracic duct** and **the right lymphatic duct**, which empty lymph back into the blood. The structure of these largest lymphatic vessels is similar to that of small veins. The adventitia is relatively underdeveloped, but contains vasa vasorum and a neural network.

Besides gathering interstitial fluid as lymph and returning it to the blood, the lymphatic vascular system is a major distributor of lymphocytes, antibodies, and other immune components that are carried through many organs to and from lymph nodes and other lymphoid tissues.

Lymphoid System

The **immune system** provides defense or **immunity** against infectious agents ranging from viruses to multicellular parasites. Histologically this system consists of a

large, diverse population of leukocytes located within every tissue of the body and **lymphoid organs** interconnected only by the blood and lymphatic circulation.

Immunologists recognize two lines of defense against invaders and/or other abnormal, harmful cells: **innate immunity and adaptive immunity**. The first of these is nonspecific, involves a wide variety of effector mechanisms. Among the cells mediating innate immunity are most of the granulocytes and other leukocytes. Conversely, adaptive immunity aims at specific microbial invaders, is mediated by lymphocytes and **antigen-presenting cells (APCs)**, and produces memory cells that permit a similar, very rapid response if that specific microbe appears again.

The lymphocytes and APCs for adaptive immunity are distributed throughout the body in the blood, lymph, and epithelial and connective tissues. Lymphocytes are formed initially in **primary lymphoid organs (the thymus and bone marrow)**, but most lymphocyte activation and proliferation occur in **secondary lymphoid organs** (the lymph nodes, the spleen, and diffuse lymphoid tissue found in the mucosa of the digestive system, including the tonsils, Peyer patches, and appendix).

The immune cells located diffusely in the digestive, respiratory, or urogenital mucosae comprise what is collectively known as **mucosa-associated lymphoid tissue (MALT).** Proliferating B lymphocytes in the secondary structures of MALT are arranged in small spherical **lymphoid nodules**. The wide distribution of immune system cells and the constant traffic of lymphocytes through the blood, lymph, connective tissues, and secondary lymphoid structures provide the body with an elaborate and efficient system of surveillance and defense.

Cells of Adaptive Immunity

As we explained previously, lymphocytes and the monocyte-derived cells specialized for antigen presentation to lymphocytes are the major players in adaptive immune responses.

➢ Antigen-Presenting Cells (APCs)

Most specialized APCs are part of the mononuclear phagocyte system, including all types of macrophages and specialized **dendritic cells** in lymphoid organs. Features common to all APCs are an active endocytotic system and expression of **major histocompatibility complex** (MHC class II) molecules for presenting peptides of exogenous antigens. During inflammation transient expression of MHC class II is induced by interferon-γ in certain local cells, including fibroblasts and vascular endothelial cells.

➢ Lymphocytes

Lymphocytes both regulate and carry out adaptive immunity. In adults stem cells for all lymphocytes are located in the red bone marrow, but cells of the major lymphoid lineages mature and become functional in two different central or **primary lymphoid organs**. Cells destined to become **B lymphocytes** remain and differentiate further in the bone marrow. Progenitors of **T lymphocytes** move via the circulation into the developing **thymus**. After maturation in these primary structures, **B and T cells** circulate to the peripheral secondary lymphoid organs, which include the **MALT**, **the lymph nodes**, and **the spleen**. **Lymphocytes** do not stay long in the lymphoid organs; they continuously recirculate through the body in connective tissues, blood, and lymph. Because of the constant mobility of lymphocytes and APCs, the cellular locations and microscopic details of lymphoid organs differ from one day to the next. However, the relative percentages of T and B lymphocytes in these compartments are relatively steady.

Lymphoid tissue is usually reticular connective tissue filled with large numbers of **lymphocytes**. It can be either diffuse within areas of loose connective tissue or surrounded by capsules, forming **secondary lymphoid organs**. Because lymphocytes have prominent basophilic nuclei and very little cytoplasm, lymphoid tissue packed with such cells usually stains dark blue in hematoxylin and eosin (H&E) -stained sections. In all secondary lymphoid tissue the lymphocytes are supported by a rich reticulin fiber network of type III collagen (Figure 3). Besides **lymphocytes** and **reticular cells**, lymphoid tissue typically contains various **APCs** and **plasma cells**.

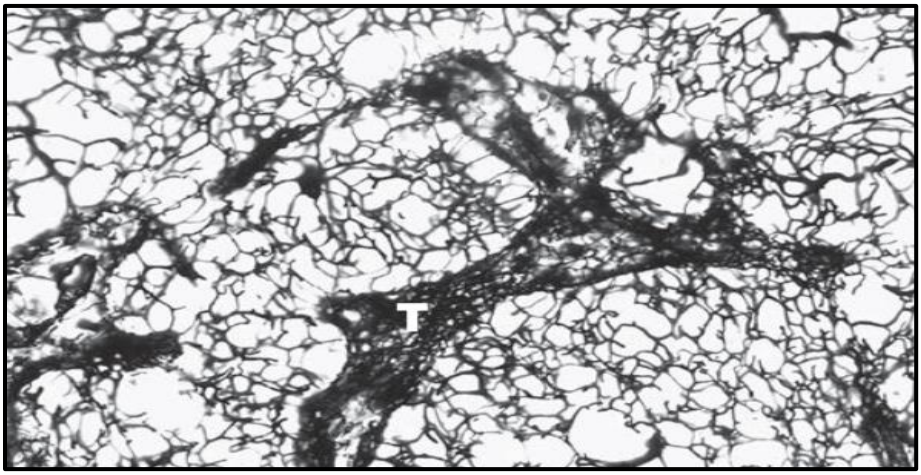


Figure 3: Reticular fibers and cells of lymphoid tissue. A three-dimensional framework of reticular fibers (collagen type III) supports the cells of most lymphoid tissues and organs. Areas with larger spaces between the fibers offer more mobility to cells than areas in which the fiber meshwork is denser, such as in trabeculae (T) where fewer lymphocytes are aggregated and cells are generally more stationary. (X140; Silver impregnation).

➢ T Lymphocytes

T cells are long-lived lymphocytes and constitute nearly **75%** of the circulating lymphocytes. They recognize antigenic epitopes via surface protein complexes termed **T-cell receptors (TCRs)**. Most **TCRs** include two glycoproteins called the **α and β chains**, each with variable regions produced similarly to those of **immunoglobulins**. Because **TCRs** only recognize antigenic peptides when presented as part of **MHC molecules** (interacting with both the MHC and the peptide it presents), T lymphocytes are said to be MHC restricted.

➢ B Lymphocytes

B-cells are a type of white blood cell that makes infection-fighting proteins called **antibodies**. **B-cells** are an important part of the immune system, the body's defense against harmful pathogens (viruses, bacteria and parasites) that enter the body and cause the disease. Most of the new, specific B lymphocytes differentiate into **plasma cells** secreting **antibodies** that will bind the same epitope recognized by the **activated B cell**. Because the antibodies specified by B cells circulate in lymph and blood throughout the body, B cells are said to provide **humoral immunity**.

Lymphoid Organs

➢ Thymus

Primary lymphoid organ, **the thymus**, a bilobed structure in the mediastinum (Figure 4). A main function of the thymus is induction of **central tolerance**, which along with **regulatory T cells** prevents **autoimmunity**. The organ originates from **the endoderm**, with precursor lymphoblasts circulating from the bone marrow to invade and proliferate in this unique thymic epithelium during its development. Fully formed and functional at birth, the thymus remains large and very active in T-cell production until puberty during which it normally undergoes **involution**, with decreasing lymphoid tissue mass and cellularity and reduced T cell output (Figure 4).

The thymus has **a vascularized connective tissue capsule** that extends septa into the parenchyma, dividing the organ into many incompletely separated lobules. Each lobule has an outer darkly basophilic cortex surrounding a more lightly stained medulla. The staining differences reflect the much greater density of lymphoblasts and small lymphocytes in the cortex than the medulla (Figure 4b).

The thymic cortex contains an extensive population of T lymphoblasts, some newly arrived via venules, located among numerous macrophages and associated with

the unique **thymic epithelial cells (TECs)** that have certain features of both epithelial and reticular cells. These cells usually have large euchromatic nuclei but are morphologically and functionally diverse.

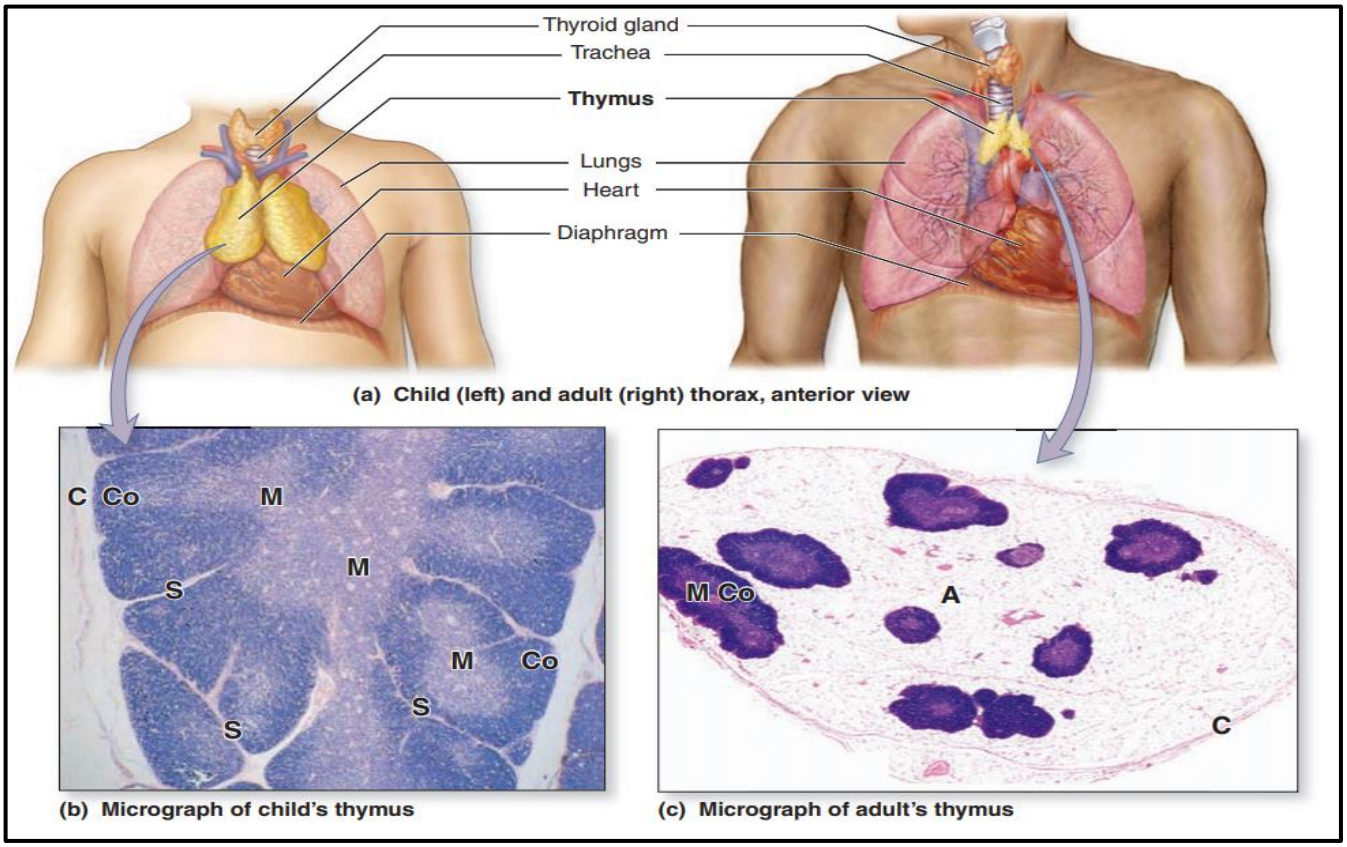


Figure 4: Thymus. (a) The thymus, a bilobed organ located in the superior mediastinum. (b) A child's thymus, showing connective tissue of the capsule (C) and septa (S) between thymic lobules, each having an outer cortex (Co) and incompletely separated medulla (M) of lymphoid tissue. (H&E; X40) (c) After involution the thymus shows only small regions of lymphoid tissue, here still with cortex (Co) and medulla (M), and these are embedded in adipose tissue (A) (H&E; X24).

There are three major types of TECs in the cortex of the thymus:

■ **Squamous TECs** form a layer, joined by desmosomes and occluding junctions, line the connective tissue of the capsule and septa and surround the microvasculature. This creates an isolated cortical compartment and, together with the vascular endothelial cells and pericytes, forms **a blood-thymus barrier** preventing unregulated exposure of thymocytes to antigens.

■ Throughout this compartment **another population of stellate TECs**, with processes containing keratin tonofilaments joined by desmosomes, form a **cytoreticulum** to which macrophages and developing lymphocytes attach instead of to reticulin fibers (Figure 5). Importantly, these cells are **APCs**, expressing **MHC class II molecules** in addition

to **MHC class I**. They also secrete numerous cytokines for **T-cell development** and other immune functions, justifying this organ's inclusion among endocrine glands.

■ **Other squamous cortical TECs** also express **MHC class II molecules** but form a sheetlike structure contributing to a functional corticomedullary barrier between these two regions of each lobule.

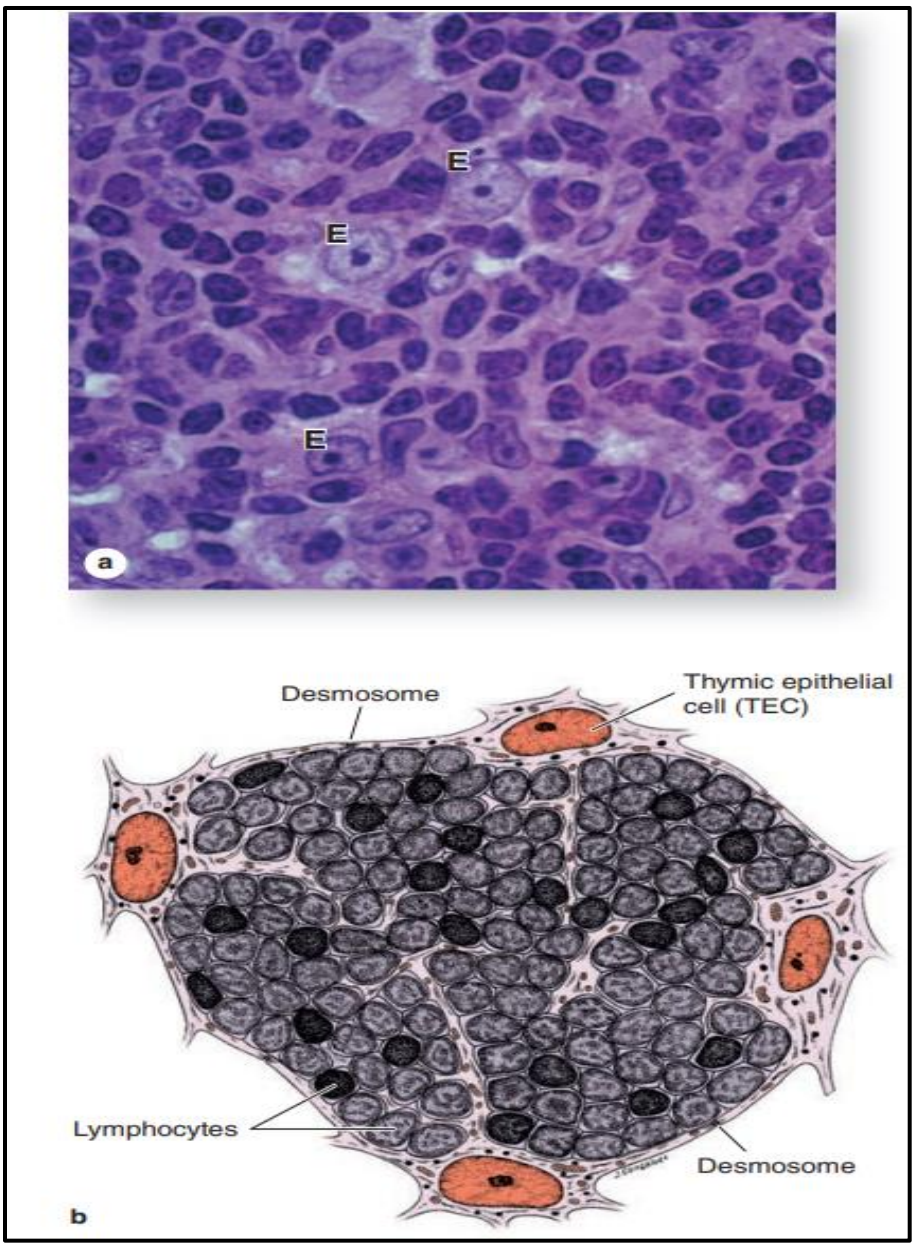


Figure 5: Cortex of the thymus. (a) The cortical zone of an active thymus is packed with small, highly basophilic lymphoblasts. The lymphoblasts are supported on a meshwork of unusual thymic epithelial cells (E)(X400; PT). (b) The epithelial reticular cells throughout the cortex are APCs and extend long processes bound together by desmosomes to make the framework for the lymphoblasts, having a cytoreticulum consisting of APC cellular processes, allows regulated specificity of lymphocyte binding via the changing antigens on MHC proteins.

The more lightly stained thymic medulla contains fewer and larger, more mature lymphocytes. Three related types of medullary TECs form the following:

■ A second layer of the boundary between cortex and medulla.

■ A cytoreticulum that (1) supports T lymphocytes, dendritic cells, and macrophages (all less densely packed than in the cortex), and (2) expresses many specialized proteins specific to cells of other organs.

■ Large aggregates of TECs, sometimes concentrically arranged, called **Hassall corpuscles** (Figure 6). Thymic corpuscles are unique to the medulla. Their cells secrete several cytokines that control activity of local dendritic cells, including factors promoting development of regulatory T cells for peripheral tolerance.

The microvasculature of the medulla is not surrounded by a tight layer of **TECs**, and **mature T lymphocytes** exit the thymus by passing through the walls of venules and efferent lymphatics in this region.

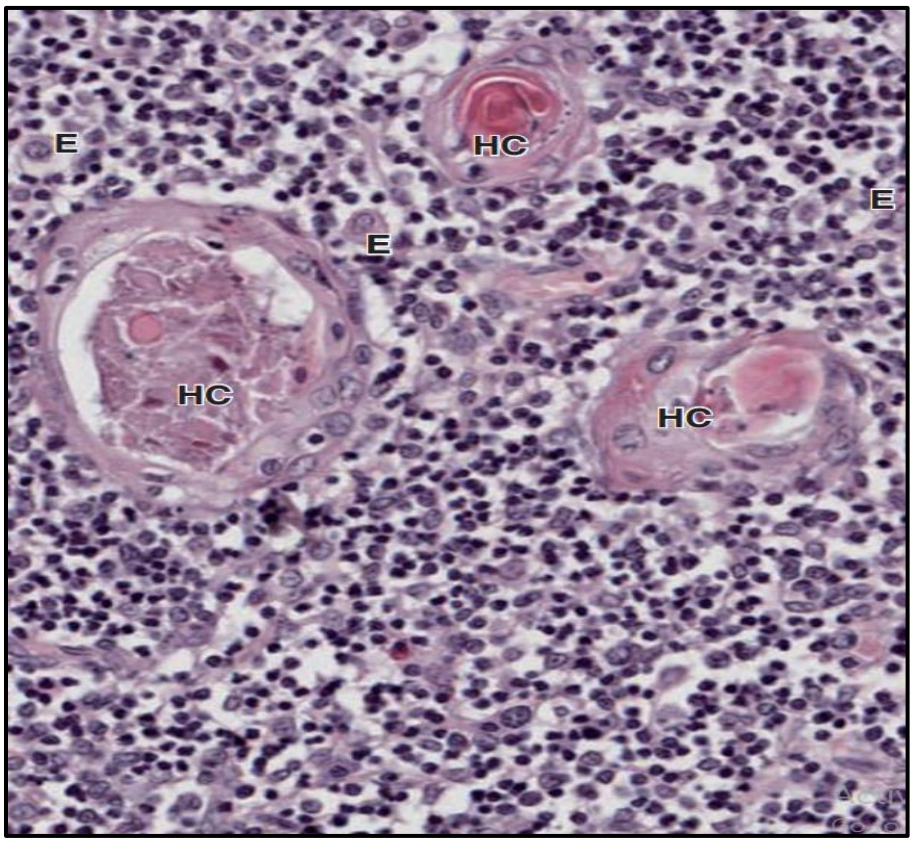


Figure 6: Medulla of the thymus with Hassall corpuscles. The thymic medulla contains fewer lymphocytes than the cortex, and the epithelial cells (E) located here have different morphology and function. The most characteristic feature of the medulla in humans is the presence of thymic (Hassall) corpuscles (HC) (X200; H&E).

➢ Mucosa-Associated Lymphoid Tissue

Secondary lymphoid structures, where most lymphocytes are activated by antigen presentation, include the **MALT**, **the lymph nodes**, and **the spleen**.

The **mucosa or inner lining** of the **digestive**, **respiratory**, and **genitourinary tracts** is a common site of invasion by pathogens because their lumens open to the external environment. To protect against such invaders mucosal connective tissue of these tracts contains large and diffuse collections of **lymphocytes**, **IgA-secreting plasma cells**, **APCs**, and **lymphoid nodules**, all of which comprise the **MALT**. **Lymphocytes** are also present within the epithelial lining of such mucosae. Most of the immune cells in MALT are dispersed diffusely in the connective tissue; others are found in aggregates forming large, conspicuous structures such as **the tonsils**, the **Peyer patches** in the **ileum**, and the **appendix**. Collectively the **MALT** is one of the largest lymphoid organs, containing up to 70% of all the body's immune cells. Most of the lymphocytes here are B cells; among T cells, helper T cells predominate.

Tonsils are large, irregular masses of lymphoid tissue in the mucosa of the posterior oral cavity and nasopharynx where their cells encounter antigens entering the mouth and nose. Named by their location these masses are the **palatine**, **lingual**, and **pharyngeal tonsils** (Figure 7a). In all tonsils the lymphoid tissue is closely associated with the surface epithelium. Other features include the following:

■ **Palatine tonsils**, located posteriorly on the **soft palate**, are covered by **stratified squamous epithelium**. The surface area of each is enlarged with 10-20 deep invaginations in which the epithelial lining is densely infiltrated with lymphocytes and other leukocytes (Figure 7). **The lymphoid tissue** is filled diffusely with lymphocytes, with many secondary lymphoid nodules around the crypts. This tissue is underlain by dense connective tissue acting as a partial capsule.

■ **Lingual tonsils** are situated along the base of the **tongue**, are also covered by **stratified squamous epithelium** with crypts, and have many of the same features as palatine tonsils but lack distinct capsules.

■ The single **pharyngeal tonsil** is situated in the posterior wall of the **nasopharynx**, is covered by **pseudostratified ciliated columnar epithelium**, and has a thin underlying capsule.

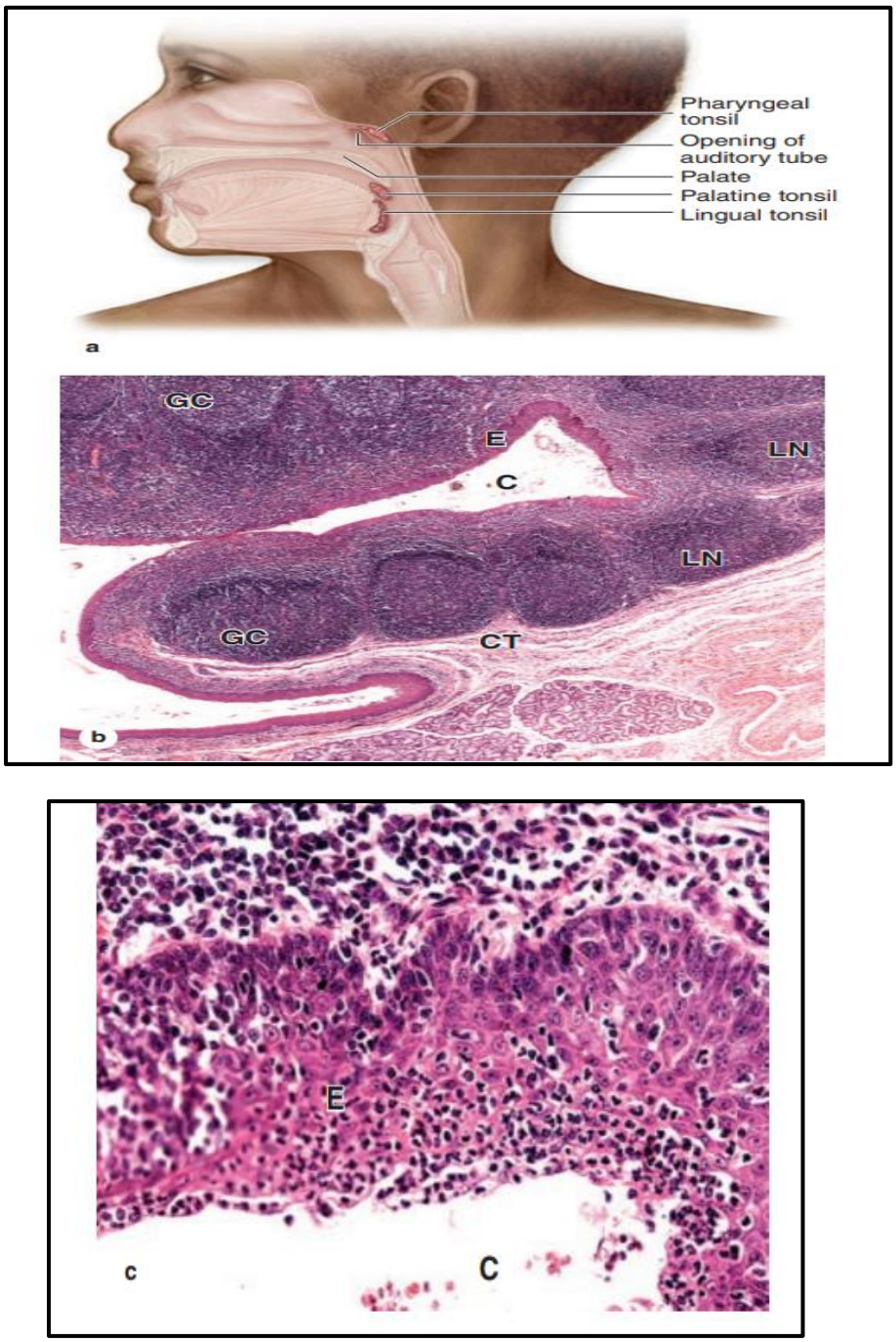


Figure 7: Tonsils. (a) Palatine tonsils , lingual tonsils and the pharyngeal tonsil locations. (b) A section showing several lymphoid nodules (LN), covered by stratified squamous epithelium (E) on one side and a connective tissue capsule (CT) on the other. Some nodules show lighter staining germinal centers (GC). Infoldings of the mucosa in some tonsils form crypts (C) (X140; H&E). (c) Epithelium (E) surrounding tonsillar crypts (C) often becomes infiltrated with lymphocytes and other leukocytes and can become difficult to recognize histologically. Adjacent connective tissue at the top of the photo also contains numerous lymphocytes (X200; H&E).

Diffuse **MALT** extends from the **pharynx** along the **entire gastrointestinal tract** but becomes very well-developed again in the **mucosa** and **submucosa** of the

ileum. Here large aggregates of lymphoid nodules comprise **the Peyer patches**, each containing dozens of nodules with no underlying connective tissue capsule (Figure 8a).

The simple columnar epithelium that covers the **lymphoid nodules** of **Peyer patches** includes scattered, large epithelial **M cells** with **apical microfolds** rather than the **brush border**. **M cells** are a unique epithelial cell type specialized for uptake of particles and intact microorganisms. The basolateral surface of each **M cell** is deeply invaginated to form a large "pocket" open to the underlying lymphoid tissue through a uniquely **sieve-like basement membrane** and containing a transient population of **lymphocytes** and **dendritic cells** (Figure 8b).

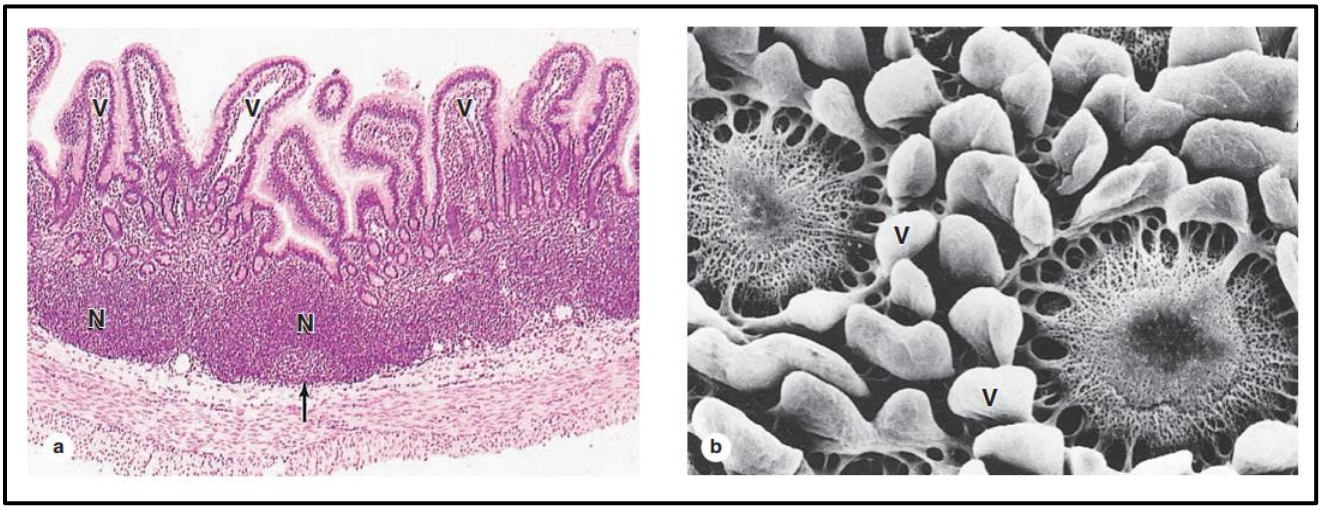


Figure 8: Peyer's patch and M cells. (a) A section through a Peyer patch shows a few lymphoid nodules (N), some with germinal centers (arrow). The mucosa of the small intestine is folded into many projecting villi (V). (X100; H&E) (b) With the surface epithelial cells removed, scanning electron microscopy (SEM) shows typical basement membrane over the villi (V) but reveals a highly porous covering over lymphoid nodules of the Peyer patch.

Another significant collection of **MALT** occurs in **the mucosa of the appendix**, a short, small-diameter projection from the cecum. Typically the **mucosa** of the **appendix** is almost completely filled with **lymphoid tissue**, effacing the glands otherwise found in the large intestine wall (Figure 9). The lumen contains the normal bacterial flora of the large intestine and may serve to retain some of these beneficial bacteria there during diarrheal illnesses.

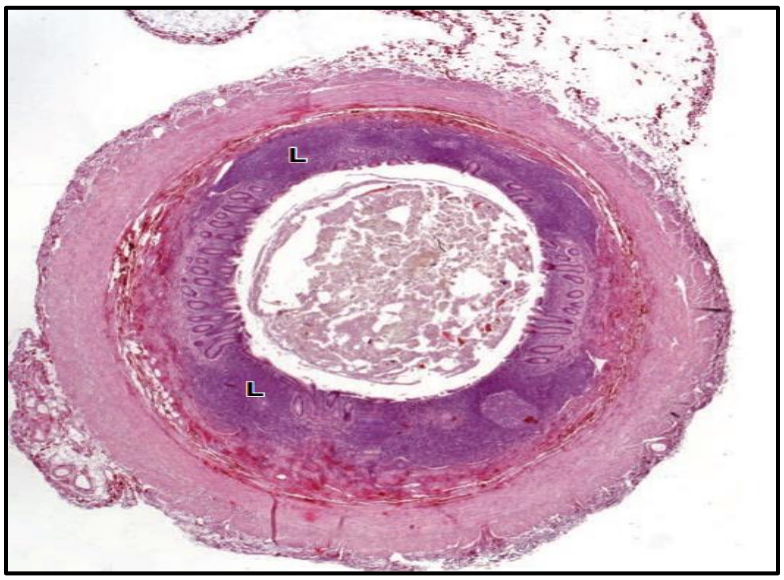


Figure 9: Appendix. the appendix with its lamina propria and submucosa filled with lymphocytes and lymphoid follicles (L) (X20; H&E).

➢ Lymph Nodes

Lymph nodes are bean-shaped, encapsulated structures, distributed throughout the body along the **lymphatic vessels**. A total of **400-450 lymph nodes** are present in the **axillae** and **groin**, along the major vessels of the **neck**, and in the **thorax** and **abdomen**, and especially in the **visceral mesenteries**. The nodes constitute a series of in-line filters of lymph that defend against the spread of microorganisms and tumor cells and provide enclosed environments for **antigen presentation** and **development of plasma cells**. Before merging with the bloodstream, all lymph is filtered and has antibodies added by at least one lymph node.

A **lymph node** has a **convex surface** where afferent lymphatics enter and **the hilum**, where an **efferent lymphatic** leaves and where an **artery**, **vein**, and **nerve** penetrate the organ (Figure 10). A **dense connective tissue** capsule surrounds the lymph node, extending trabeculae internally through which the blood vessels branch. **Valves** in the lymphatics ensure unidirectional lymph flow.

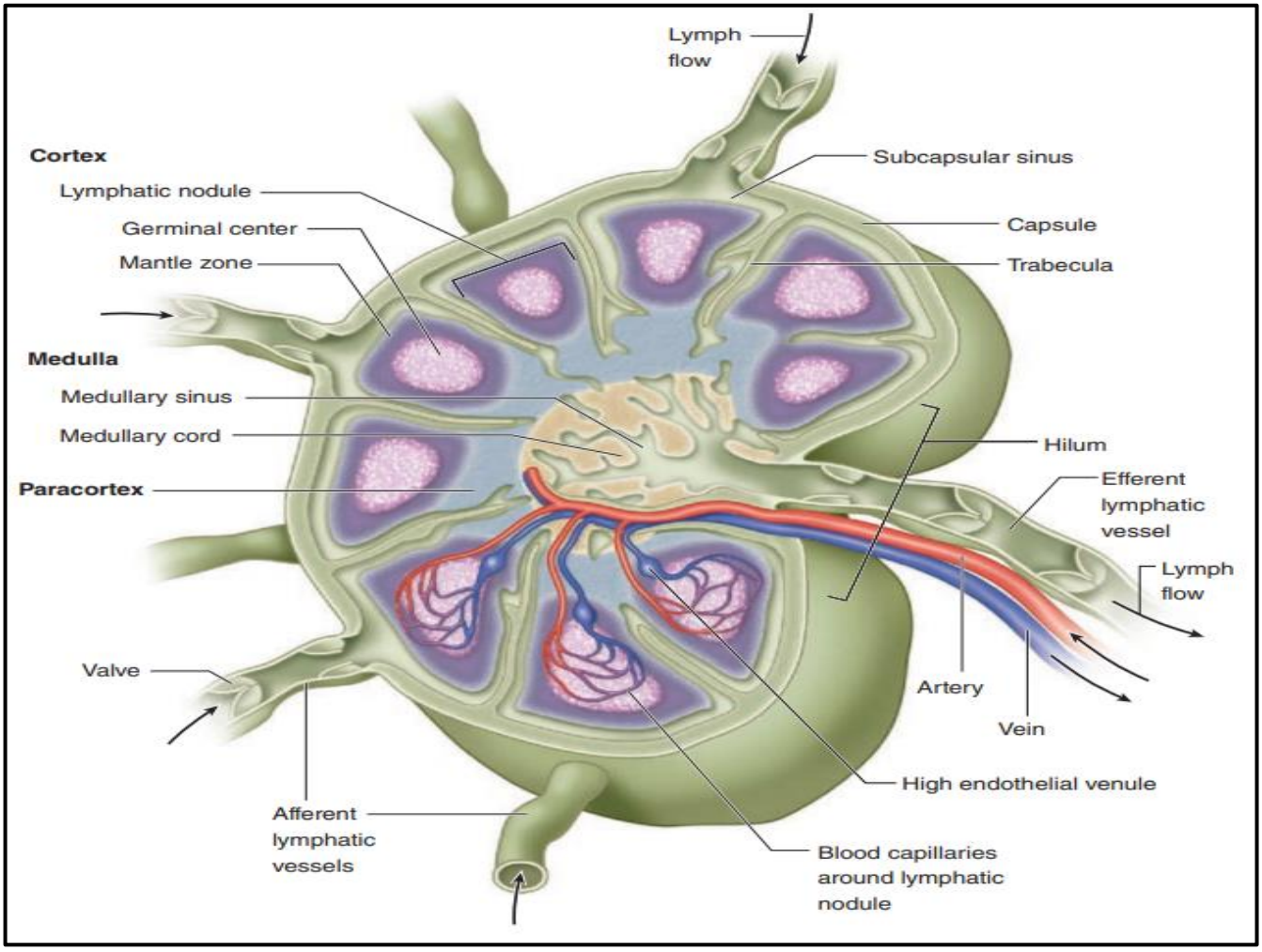


Figure 10: Lymph node.

The most abundant cells of lymph nodes are **lymphocytes of all types**, **plasma cells, dendritic cells, macrophages,** and other **APCs**. All of these cells are arranged in a stroma of reticulin fibers and reticular cells to form three major regions **within each lymph node:** an **outer** cortex containing the **nodules**; a **deeper** extension of cortex called the **paracortex**, which lacks nodules; and a **medulla** with prominent draining sinusoids adjacent to the hilum (Figures 10 and 11).

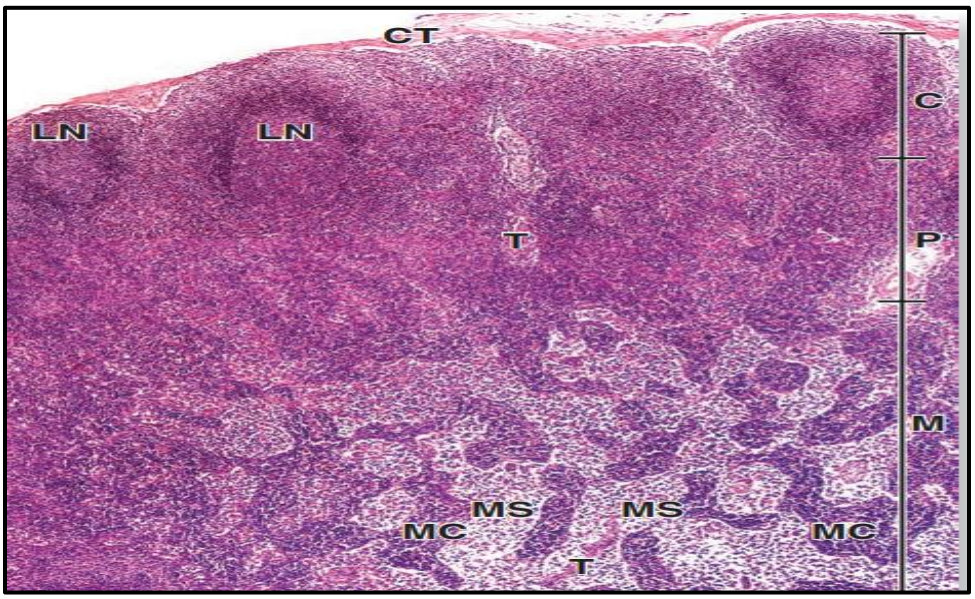


Figure 11: Regions of a lymph node. section of a lymph node shows the three functional regions: the cortex (C), the paracortex (P), and the medulla (M). Connective tissue of the capsule (CT) completely surrounds each lymph node and extends as several trabeculae (T) throughout the lymphoid tissue. Major spaces for lymph flow are present in this tissue under the capsule and along the trabeculae. Lymphoid nodules (LN) are normally restricted to the cortex, and the medulla is characterized by sinuses (MS) and cords (MC) of lymphoid tissue (X40; H&E).

Unlike the thymus these regions of lymph nodes are not compartmentalized by epithelium. **The cortex includes the following components:**

■ **A subcapsular sinus**, immediately inside the **capsule**, receives lymph from the **afferent lymphatics** (Figure 12). From this space cortical sinuses branch internally among the lymphoid nodules along trabeculae. These sinuses are lined by a very thin, discontinuous endothelium penetrated by reticulin fibers and processes of dendritic cells. **Lymph** containing **antigens**, **lymphocytes**, and **APCs** passes through these sinuses and percolates easily into the surrounding lymphoid tissue.

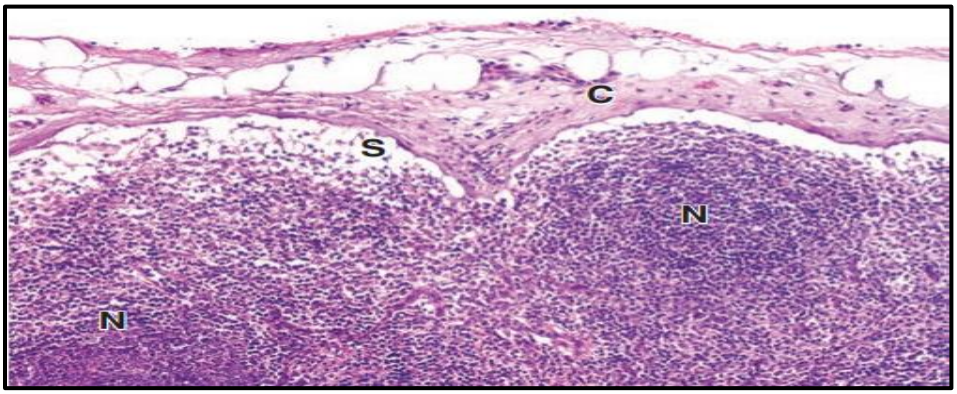


Figure 12: Lymph node cortex. The outer regions on the convex sides of a lymph node include the capsule (C), subcapsular sinuses (S), and diffuse lymphoid tissue with lymphoid nodules (N) (X140; H&E).

The medulla of a lymph node has two major components (Figures 10):

■ **Medullary cords** are branched cordlike masses of lymphoid tissue extending from the paracortex. They contain **T and B lymphocytes** and many **plasma cells**.

■ **Medullary sinuses** are dilated spaces lined by discontinuous endothelium that separate the medullary cords. **The lumens** of medullary sinuses include a meshwork of processes from reticular cells, which represent a final lymph filter.

➢ SPLEEN

The spleen contains the largest single accumulation of lymphoid tissue in the body and is the only lymphoid organ involved in filtration of blood, making it an important organ in defense against **blood-borne antigens**. It is also the main site of **old erythrocyte destruction**. As is true of other secondary lymphoid organs, the spleen is a production site of antibodies and activated lymphocytes, which here are delivered directly into the blood.

Located high in the left upper quadrant of the abdomen, the spleen's volume varies with its content of blood and tends to decrease very slowly after puberty. The organ is surrounded by a **capsule of dense connective tissue** from which emerge trabeculae to penetrate the splenic pulp (Figure 11). Large trabeculae originate at the **hilum**, on the medial surface of the spleen, and carry branches of the **splenic artery, vein, lymphatics, and nerves** into the splenic pulp.

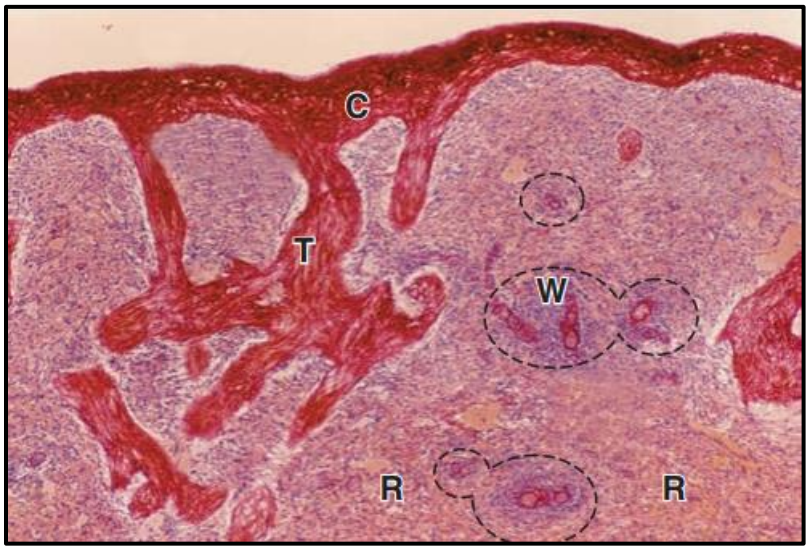


Figure 11: Spleen. The capsule (C) of the spleen connects to trabeculae (T) extending into the pulp-like interior of the organ. The red pulp (R) occupies most of the parenchyma, with white pulp (W) restricted to smaller areas, mainly around the central arterioles. Names of these splenic areas refer to their color in

the fresh state: red pulp is filled with blood cells of all types, located both in cords and sinuses; white pulp is lymphoid tissue. Large blood vessels and lymphatics enter and leave the spleen at a hilum (X20; Picro-Sirius-hematoxylin).

The spleen is filled with reticular tissue containing reticular cells and fibers, many lymphocytes and other blood cells, macrophages, and APCs. **This splenic pulp has two components: the white pulp** (20% of the spleen) and **the red pulp** (Figure 11). The small masses of white pulp consist of **lymphoid nodules** and the **periarteriolar lymphoid sheaths (PALS)**, while the red pulp consists of **blood-filled sinusoids and splenic cords**. The splenic sinusoids are lined by unusual endothelial cells called **stave cells**, which are elongated and aligned parallel to the blood flow, with open slits between the cells.

Blood flow through the splenic red pulp can take either of two routes (Figure 12):

■ **In the closed circulation**, capillaries branching from the penicillar arterioles connect directly to the sinusoids and the blood is always enclosed by endothelium.

■ **In the open circulation**, capillaries from about half of the **penicillar arterioles** are uniquely open-ended, dumping blood into the stroma of the splenic cords. In this route plasma and all the formed elements of blood must reenter the vasculature by passing through narrow slits between the stave cells into the sinusoids. These small openings present no obstacle to platelets, to the motile leukocytes, or to thin flexible erythrocytes. However stiff or effete, swollen RBCs at their normal life span of 120 days are blocked from passing between the stave cells and undergo selective removal by macrophages.

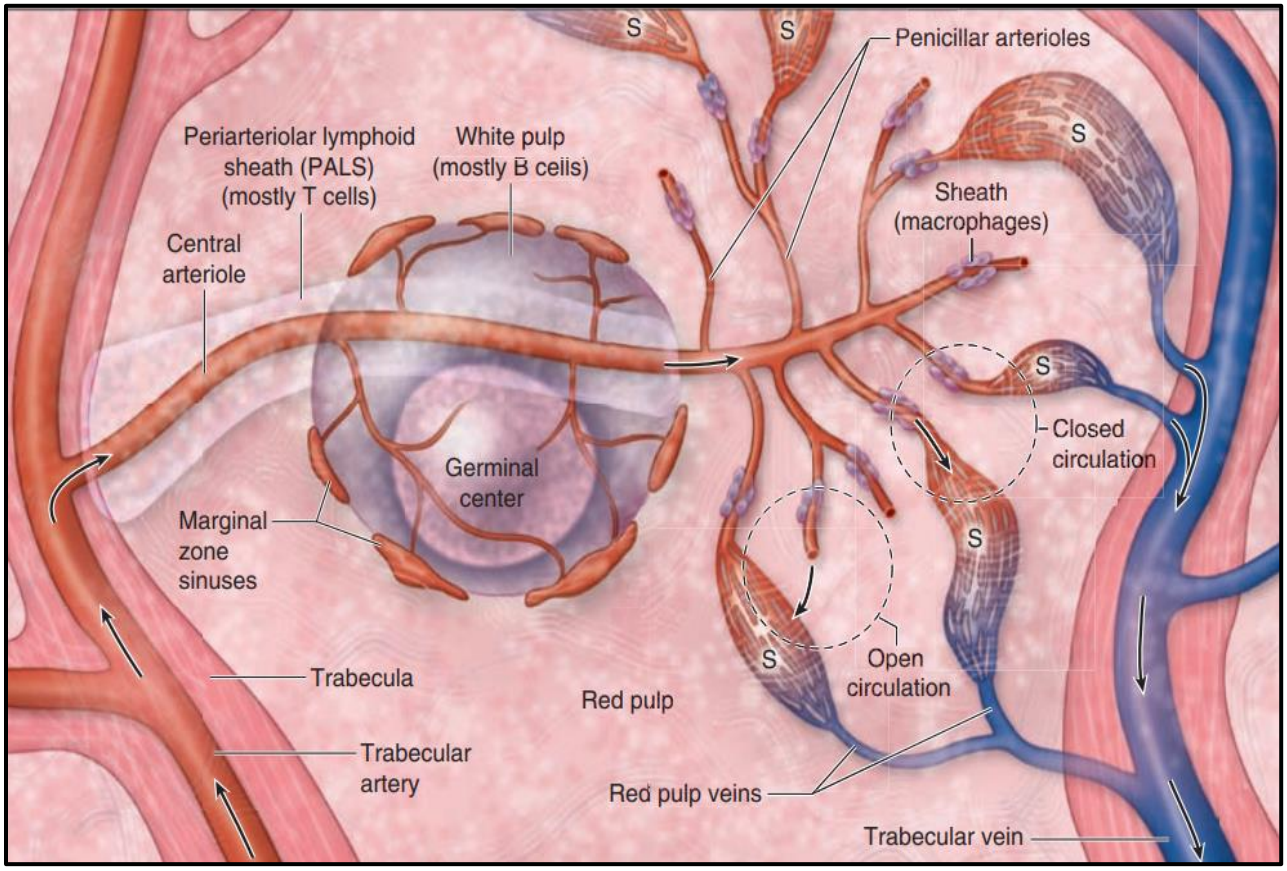


Figure 12: Blood flow in the spleen. * (s) indicates the splenic sinuses.

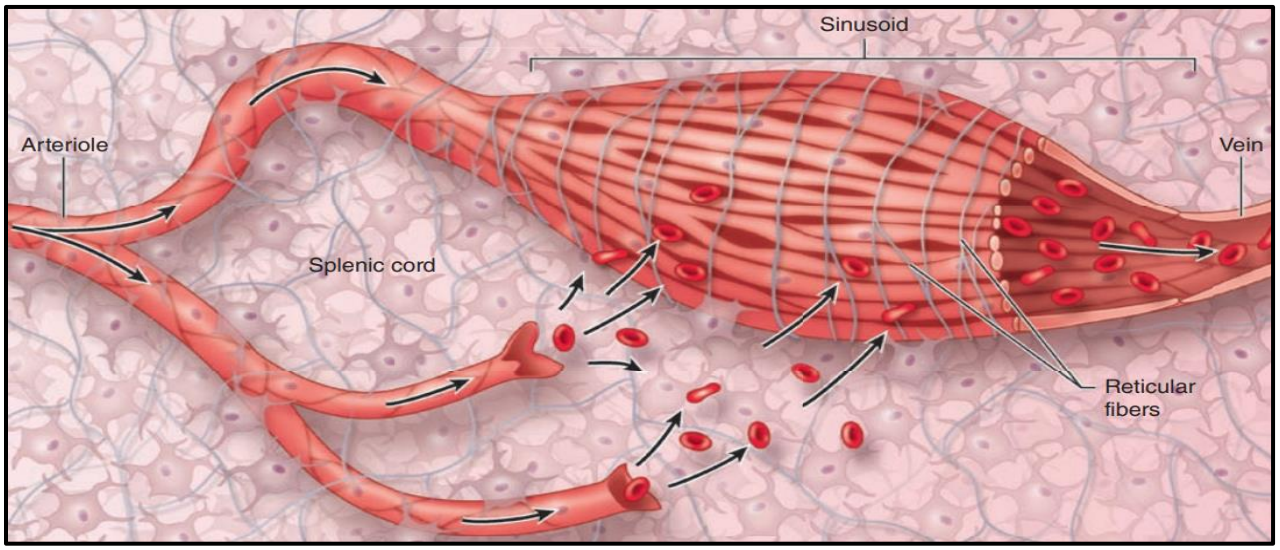


Figure 13: Structure of splenic sinusoids.

Lecture 10-12.. Endocrine System

Secretory cells of endocrine glands release their products, signaling molecules called **hormones**, into the neighboring vascularized compartment for uptake by capillaries and distribution throughout the body. There is no secretory duct as in **exocrine glands**. **Endocrine cells** are typically **epithelial**, and aggregated as cords or clusters. Besides the specialized endocrine glands discussed in this lecture, many other organs specialized for other functions, such as the **heart, thymus, gut, kidneys, testis,** and **ovaries**, contain various **endocrine cells**.

Hormones target their targets in three ways:

- **Paracrine secretion**: endocrine cells produce hormones that act on target cells only a short distance away. With localized dispersal in interstitial fluid or through short loops of blood vessels, as when gastrin made by **pyloric G cells** reaches target cells in the **fundic glands**.
- **autocrine secretion**, cells may produce molecules that act on themselves or on cells of the same type. For example, **insulin-like growth factor (IGF)** produced by several cell types may act on the same cells that produced it.
- **Endocrine glands** are often also target organs for other hormones that can establish a feedback mechanism to control hormone secretion and keep blood hormonal levels within strict limits.

Hormones, are frequently **hydrophilic molecules** such as **proteins**, **glycoproteins**, **peptides**, or **modified amino acids** with receptors on the surface of target cells. Alternatively, **hydrophobic steroid and thyroid hormones** must circulate on transport proteins but can diffuse through the cell membranes and activate cytoplasmic receptors in target cells.

Pituitary Gland (Hypophysis)

The pituitary gland, or hypophysis. It lies below the brain in a small cavity on the sphenoid bone, the sella turcica (Figure 1). The pituitary is formed in the embryo **partly** from **the developing brain** and **partly** from the **developing oral cavity**. Because of its dual origin, the pituitary actually consists of two glands: **the posterior (neurohypophysis) and the anterior (adenohypophysis)**, united anatomically but with different functions. **The neurohypophysis** retains many histologic features of brain tissue and consists of a large part, **the pars nervosa**, and the smaller **infundibulum stalk** attached to the hypothalamus at the median eminence (Figures 1 and 2). **The adenohypophysis**, derived from the oral ectoderm, has three parts: **a large pars distalis**

or anterior lobe; the pars tuberalis, which wraps around the infundibulum; and **the thin pars intermedia** adjacent to the posterior pars nervosa (Figures 1 and 2).

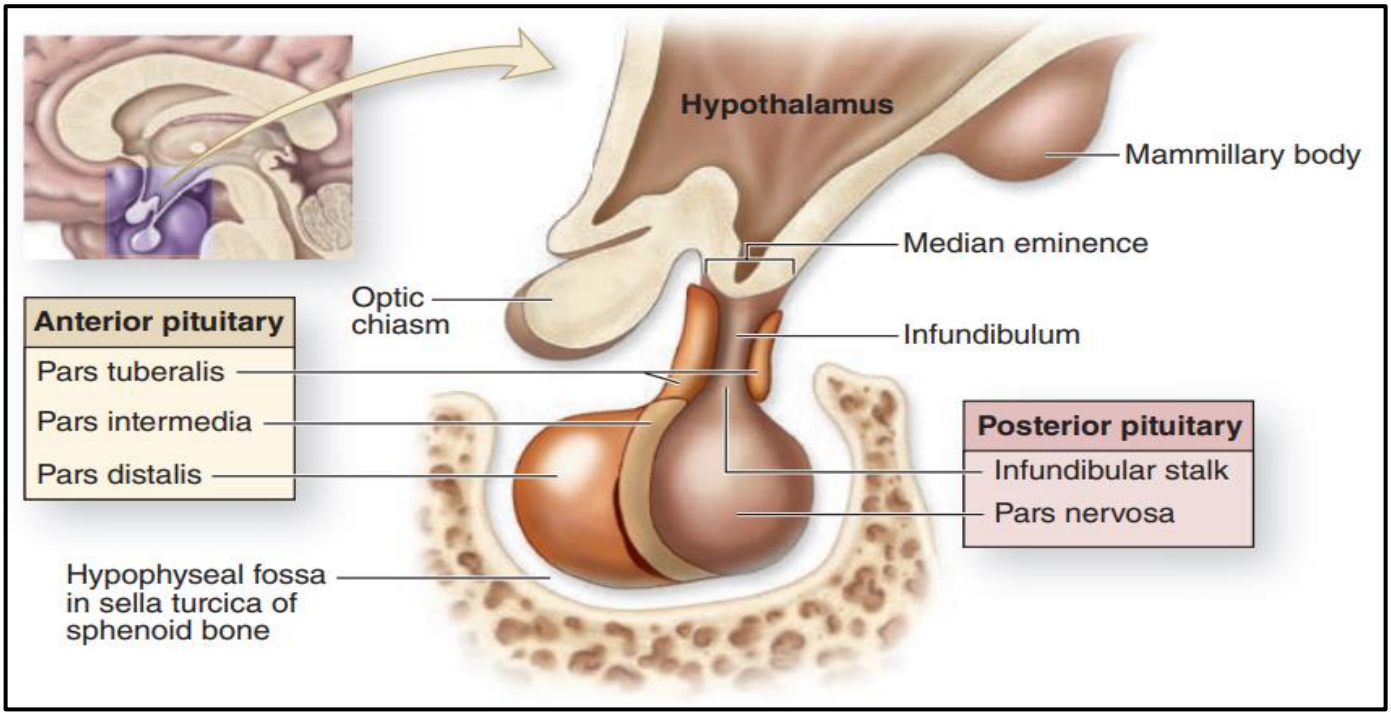


Figure 1: Pituitary gland. * The gland occupies a fossa of the sphenoid bone called the sella turcica.

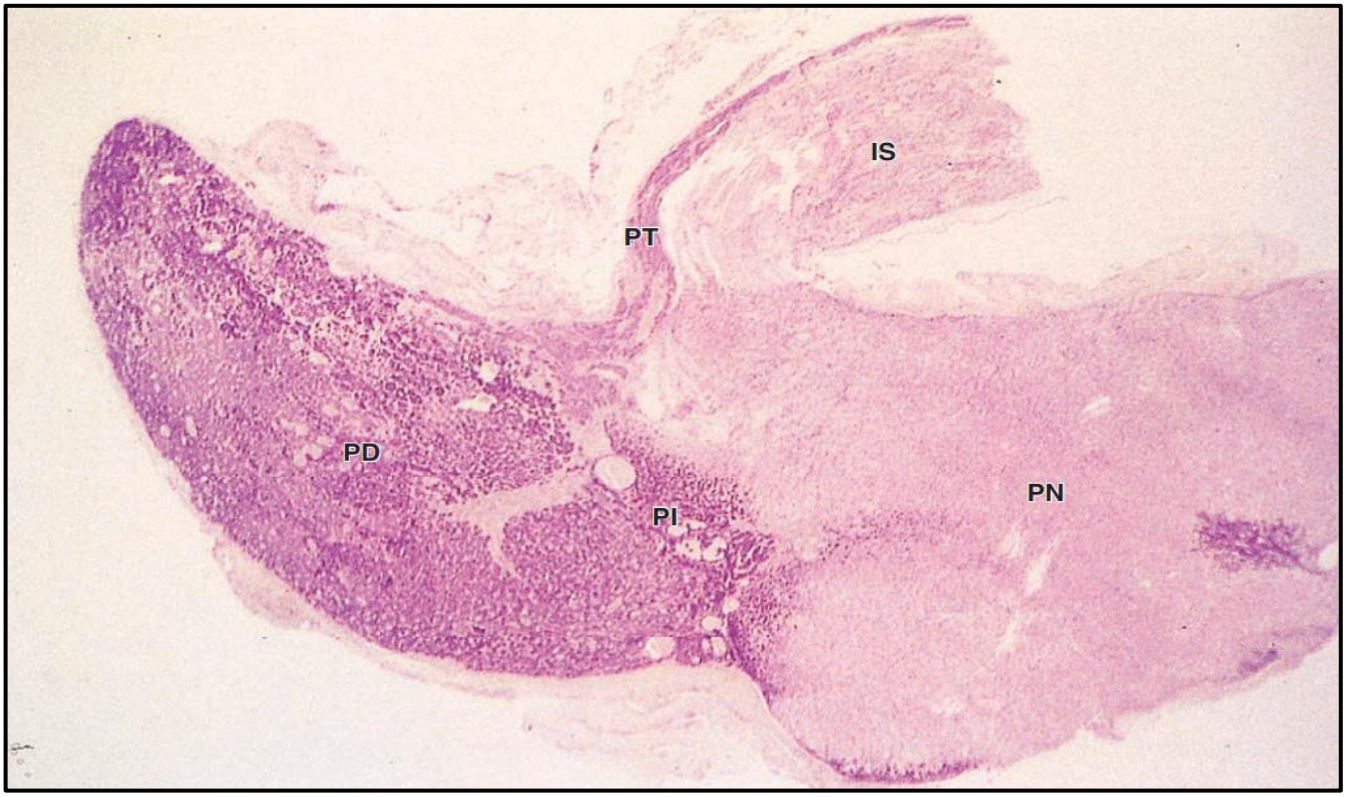


Figure 2: Pituitary gland. Histologically the two parts of the pituitary gland reflect their origins, as seen in this low-magnification section of an entire gland. The infundibular stalk (IS) and pars nervosa (PN) of the neurohypophysis resemble CNS tissue, while the adenohypophysis' pars distalis (PD), pars intermediate (PI), and pars tuberalis (PT) are typically glandular in their level of staining (X30; H&E).

Embryologically, anatomically, and functionally, **the pituitary gland** is connected to **the hypothalamus** at the base of the brain. In addition to the vascular portal system carrying small regulatory peptides from the hypothalamus to the adenohypophysis, a bundle of axons called **the hypothalamic-hypophyseal tract** courses into **the neurohypophysis** from two important hypothalamic nuclei. The peptide hormones **ADH (antidiuretic hormone)** and **oxytocin** are synthesized by large neurons in the supraoptic and the paraventricular nuclei, respectively. * **The supraoptic and paraventricular nuclei** contain **neurosecretory cells**. Both hormones undergo **axonal transport** and accumulate temporarily in the axons of **the hypothalamic-hypophyseal tract** before their release and uptake by capillaries branching from the inferior arteries.

Adenohypophysis (Anterior Pituitary)

The three parts of the adenohypophysis are derived embryonically from the hypophyseal pouch.

➢ Pars Distalis:

The pars distalis accounts for 75% of the adenohypophysis and has a thin fibrous capsule. The main components are cords of well-stained endocrine cells interspersed with fenestrated capillaries and supporting reticular connective tissue (Figures 2 and 3). Common stains suggest two broad groups of cells in the pars distalis with different staining affinities: **chromophils and chromophobes**. **Chromophils** are secretory cells in which hormone is stored in cytoplasmic granules. They are also called **basophils and acidophils**, based on their affinities for basic and acidic dyes, respectively (Figure 3). **Chromophobes** stain weakly, with few or no secretory granules, and also represent a heterogeneous group, including stem and undifferentiated progenitor cells as well as any degranulated cells present.

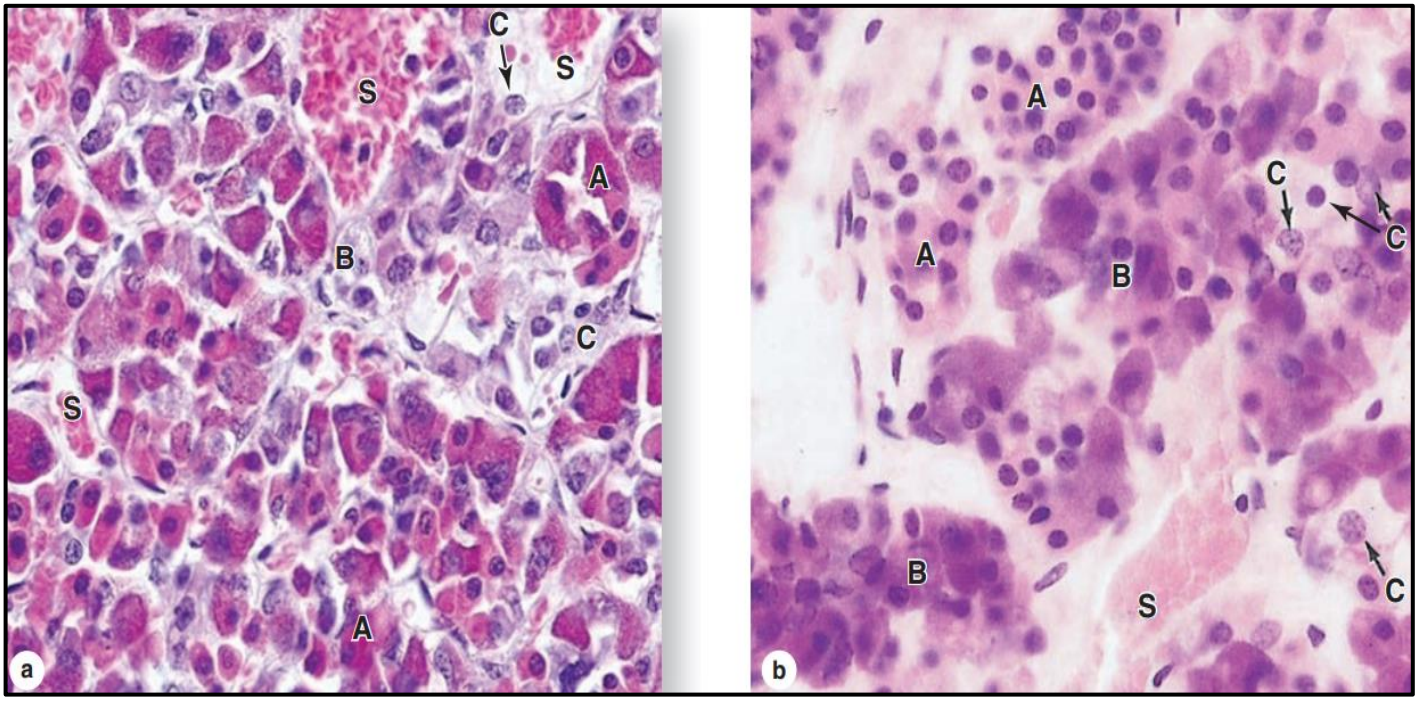


Figure 3: Pars distalis: Acidophils, basophils, and chromophobes. (a, b) Most general staining methods simply allow the parenchymal cells of the pars distalis to be subdivided into acidophil cells (A), basophils (B), and chromophobes (C) in which the cytoplasm is poorly stained. Also shown are capillaries and sinusoids (S) in the second capillary plexus of the portal system (X400; H&E).

Specific cells are usually named according to their hormone's target cells (Table 1).

Cell Type	Hormone Produced	Major Function
Somatotrophs	Somatotropin (growth hormone, GH)	Stimulates growth in epiphyseal plates of long bones via insulin-like growth factors (IGFs) produced in liver
Lactotrophs (mammotrophs)	Prolactin (PRL)	Promotes milk secretion
Gonadotrophs	Follicle-stimulating hormone (FSH) and luteinizing hormone (LH; interstitial cellstimulating hormone [ICSH] in men), both glycoprotein dimers, secreted from the same cell type	FSH promotes ovarian follicle development and estrogen secretion in women and spermatogenesis in men; LH promotes ovarian follicle maturation and progesterone secretion in women and interstitial cell androgen secretion in men
Thyrotrophs	Thyrotropin (TSH), a glycoprotein dimer	Stimulates thyroid hormone synthesis, storage, and liberation

Corticotrophs	Adrenal corticotropin (ACTH), polypeptide Lipotropin (LPH)	Stimulates secretion of adrenal cortex hormones Helps regulate lipid metabolism

➢ Pars Tuberalis

The pars tuberalis is a smaller funnel-shaped region surrounding the infundibulum of the neurohypophysis (Figures 1 and 2). Most of the cells of the pars tuberalis are **gonadotrophs**.

➢ Pars Intermedia

A narrow zone lying between the pars distalis and the pars nervosa, the pars intermedia contains **basophils (corticotrophs), chromophobes, and small, colloid-filled cysts** derived from the lumen of the embryonic hypophyseal pouch (Figure 4). **Corticotrophs** of the pars intermedia express **Pro-opiomelanocortin** (**POM)** which is then cleaved again to form smaller peptide hormones, including two forms of **melanocyte-stimulating hormone (MSH), γ- Lipotropin (LPH), and β-endorphin**.

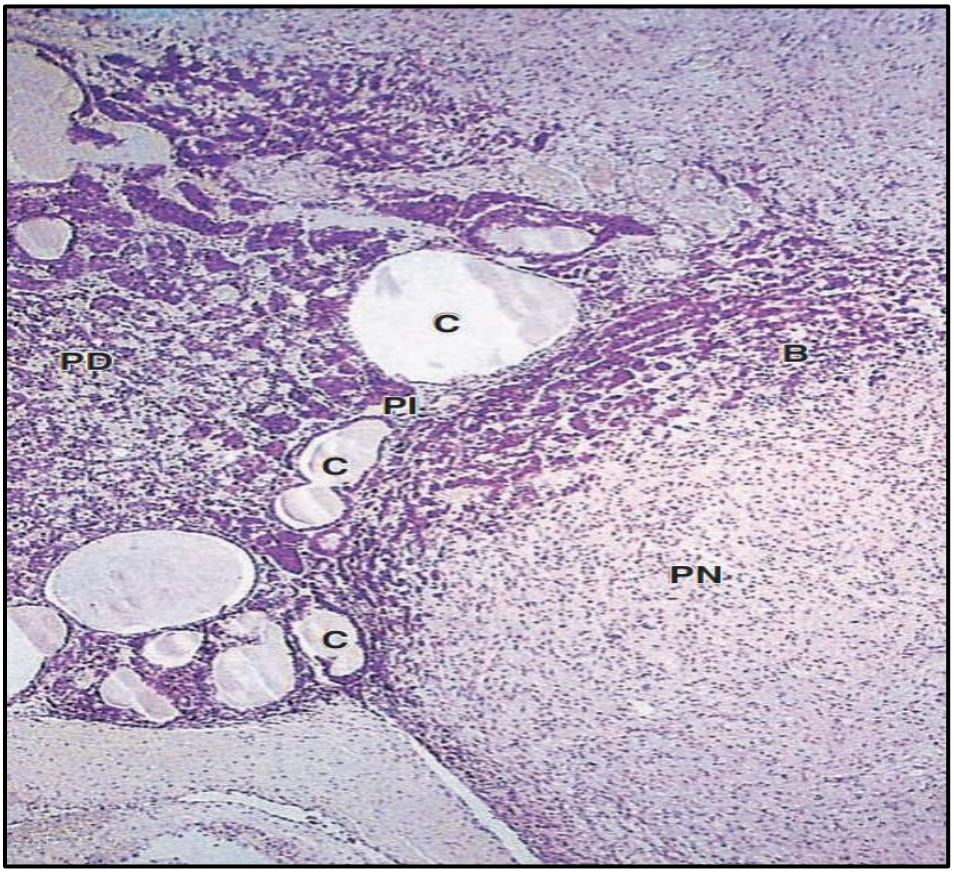


Figure 4: Pars intermedia. The pars intermedia (PI) is a narrow region lying between the pars distalis (PD) and the pars nervosa (PN), with many of its basophils (B). Remnants of the embryonic hypophyseal pouch's lumen are usually present in this region as colloid-filled cysts (C) of various sizes (X56; H&E).

Neurohypophysis (Posterior Pituitary)

The neurohypophysis consists of **the pars nervosa** and **the infundibular stalk** (Figures 1 and 2) and, unlike the **adenohypophysis**, does not contain the cells that synthesize its two hormones. It is composed of neural tissue, containing some 100,000 **unmyelinated axons** of large secretory neurons with cell bodies in **the supraoptic and paraventricular nuclei** of the hypothalamus. Also present are highly branched glial cells called **pituicytes** that resemble **astrocytes** and are the most abundant cell type in **the posterior pituitary** (Figure 5).

The secretory neurons have all the characteristics of typical neurons, including the ability to conduct an action potential, but have larger-diameter axons and well-developed synthetic components related to the production of the 9-amino acid peptide hormones **antidiuretic hormone (ADH)**, also called **arginine vasopressin**, and **oxytocin**. Transported axonally into the pars nervosa, these hormones accumulate in axonal dilations called **neurosecretory bodies** or **Herring bodies**, visible in the light microscope as faintly eosinophilic structures (Figure 5). **The neurosecretory bodies** contain **membrane-enclosed granules** with either **oxytocin or ADH** bound to carrier proteins called **neurophysin I and II**, respectively. Nerve impulses along the axons trigger the release of the peptides from **the neurosecretory bodies** for uptake by the fenestrated capillaries of **the pars nervosa**, and the hormones are then distributed to the general circulation. Axons from **the supraoptic and paraventricular nuclei** mingle in **the neurohypophysis** but are mainly concerned with ADH and oxytocin secretion, respectively.

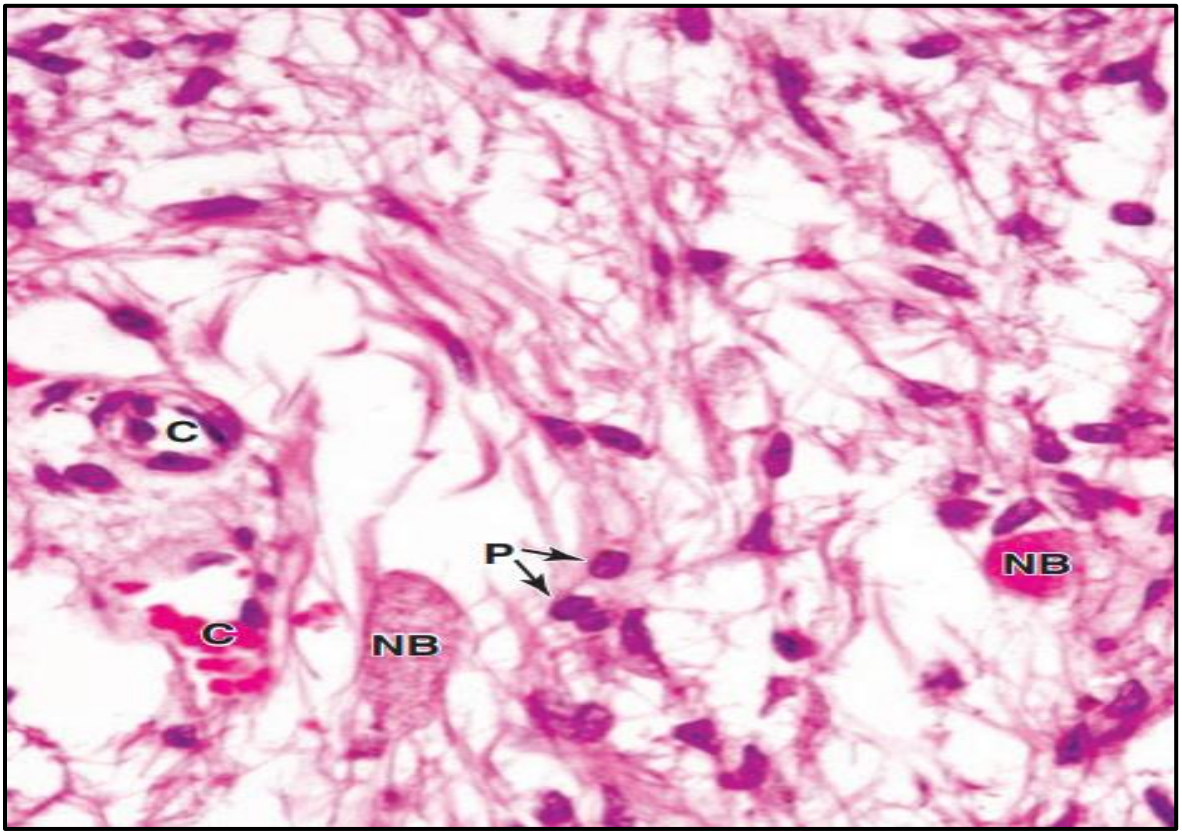


Figure 5: Pars nervosa: neurosecretory bodies and pituicytes. The pars nervosa consists of modified neural tissues containing unmyelinated axons supported by pituicytes (P), the most numerous cell present. The axons run from the supraoptic and paraventricular hypothalamic nuclei, and have swellings called neurosecretory (Herring) bodies (NB). The released hormones are picked up by capillaries (C) (X400; H&E).

Adrenal Glands

The adrenal (or suprarenal) glands are paired organs lying near the superior poles of the kidneys, embedded in the pararenal adipose tissue and fascia (Figures 6). They are flattened structures with a half-moon shape. Adrenal glands are each covered by a dense connective tissue capsule that sends thin trabeculae into the gland's parenchyma. The stroma consists mainly of reticular fibers supporting the secretory cells and microvasculature. **Each gland has two concentric regions**: **a yellowish adrenal cortex and a reddish-brown central adrenal medulla**.

The adrenal cortex and medulla can be considered two different organs with distinct embryonic origins, functions, and morphologic characteristics that become united during embryonic development. **The cortex** arises from **mesoderm** and **the medulla** from **the neural crest**. The general histologic appearance of the adrenal gland

is typical of an endocrine gland in which cells of both cortex and medulla are grouped in cords along wide capillaries.

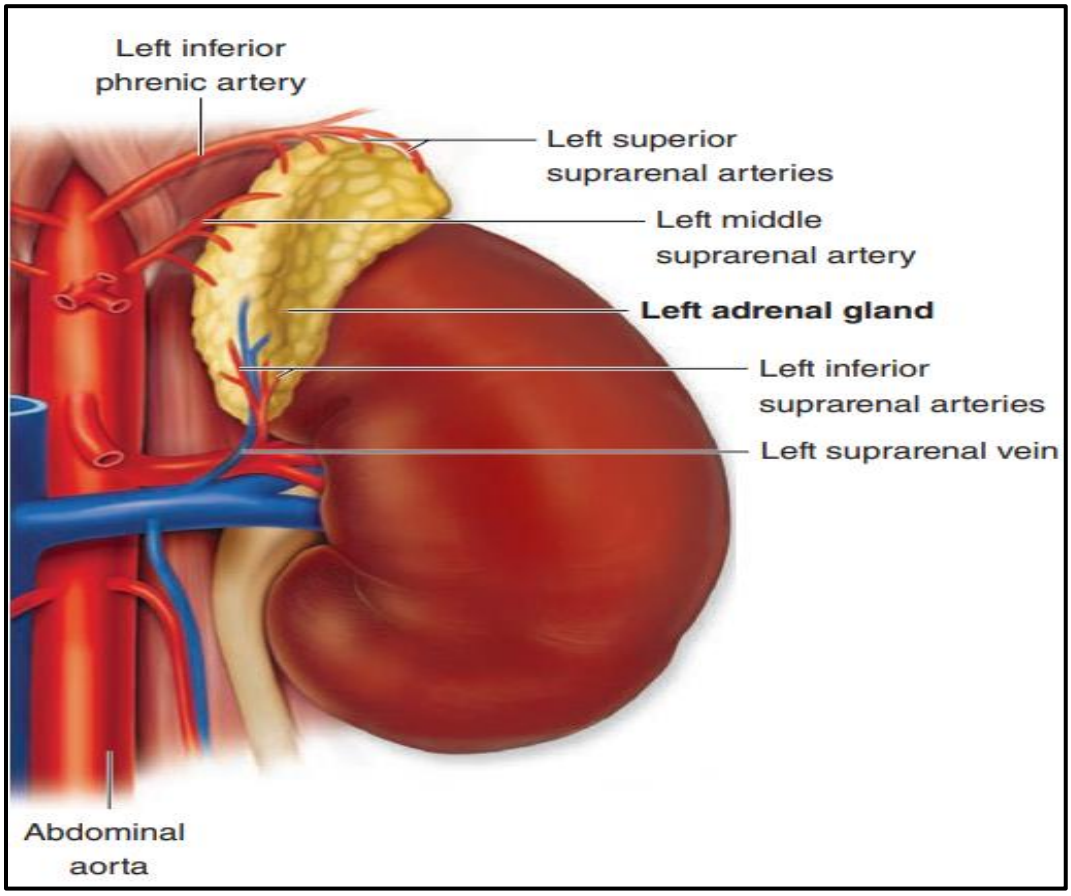


Figure 6: Location and blood supply of the adrenal glands.

➢ Adrenal Cortex

Cells of the adrenal cortex have characteristic **features of steroid-secreting cells**: acidophilic cytoplasm rich in lipid droplets, with central nuclei. Ultrastructurally their cytoplasm shows an exceptionally **profuse smooth ER (SER)** of interconnected tubules, which contain the enzymes **for cholesterol synthesis** and conversion of the steroid **prohormone pregnenolone** into specific active **steroid hormones**. **The mitochondria** are often spherical, with tubular cristae (Figure 7). These mitochondria not only synthesize ATP but also contain the enzymes for converting **cholesterol** to **pregnenolone**. The function of **steroid-producing cells** involves close collaboration between SER and mitochondria.

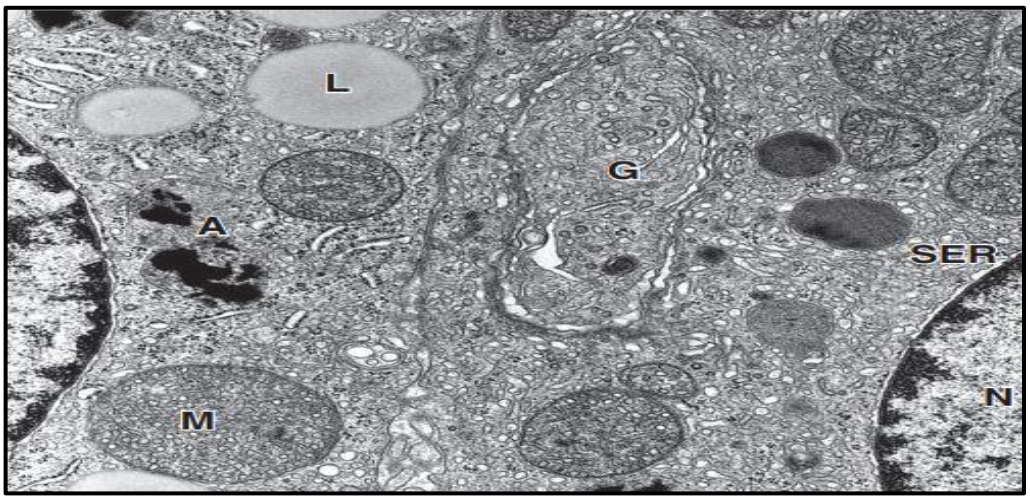


Figure7: Ultrastructure of cortical adrenalocytes. TEM of two adjacent steroid-secreting cells shows features typical of steroid-producing cells: lipid droplets (L), mitochondria (M) with tubular cristae, abundant SER, and autophagosomes (A), which remove mitochondria and SER between periods of active steroid synthesis. Also seen are the euchromatic nuclei (N), a Golgi apparatus (G) (X25,700).

Steroid hormones are not stored in granules like proteins nor undergo **exocytosis**. As **small lipid-soluble molecules**, steroids diffuse freely from cells through the plasma membrane.

The adrenal cortex has three concentric zones in which the cords **of epithelial steroid-producing cells** are arranged somewhat differently and which synthesize different classes of steroid hormones:

- **The zona glomerulosa**

inside the capsule and comprising about **15%** of the cortex, consists of closely packed, rounded cords of columnar or pyramidal cells with many capillaries (Figure 8). The steroids made by these cells are called **mineralocorticoids** because they affect uptake of **Na+, K+**, and **water** by cells of **renal tubules**. The principal product is **aldosterone**, the major regulator of salt balance, which acts to stimulate **Na+** reabsorption in the distal convoluted tubules.

- **The middle zona fasciculata**

occupies **65%-80%** of the cortex and consists of long cords of large polyhedral cells, one or two cells thick, separated by fenestrated sinusoidal capillaries (Figure 8). **The cells** are filled with lipid droplets and appear vacuolated in routine histologic preparations. These cells secrete **glucocorticoids**, especially **cortisol**, which affect carbohydrate metabolism by stimulating **gluconeogenesis** in many cells and **glycogen synthesis** in the liver. Small amounts of weak **androgens** are also produced here.

- **The innermost zona reticularis**

comprises about **10%** and consists of smaller cells in a network of irregular cords interspersed with wide capillaries (Figure 8). The cells are usually more heavily stained because they contain fewer lipid droplets and more lipofuscin pigment. The Cells here also produce **cortisol** but primarily secrete the weak **androgens**, including **dehydroepiandrosterone (DHEA)** that is converted to **testosterone** in both men and women.

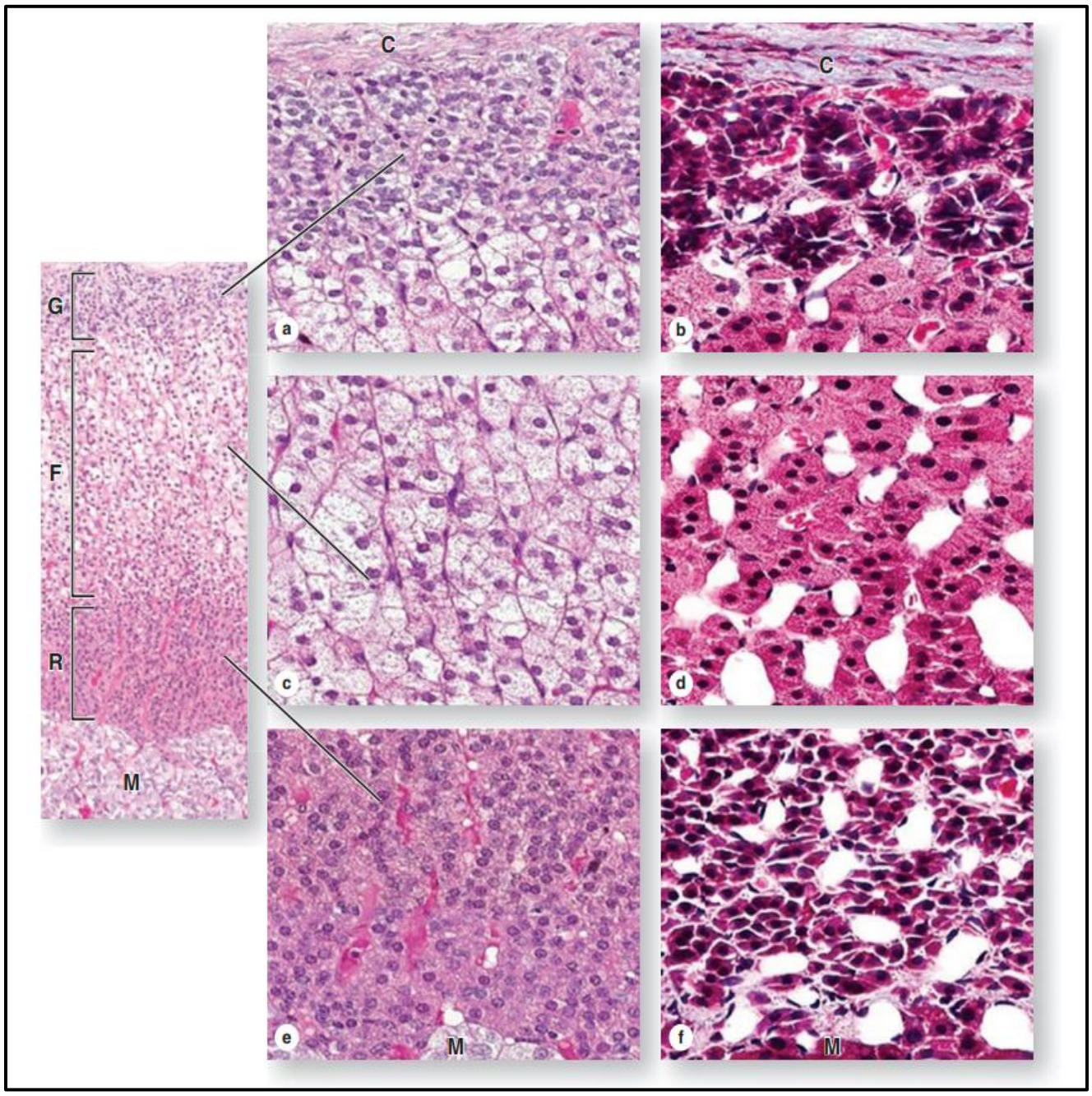


Figure 8: Adrenal cortex. (a, b) Immediately beneath the capsule (C), the zona glomerulosa consists of rounded clusters of columnar or pyramidal cells. Blood-filled regions are parts of the subcapsular arterial plexus. (c, d) The zona fasciculata, consists of long cords of large, spongy-looking cells mainly. (e, f) Cells of the innermost zona reticularis, next to the medulla (M), are small, have fewer lipid droplets and are therefore better stained, arranged in a close network. Cells of all the layers are closely associated with capillaries and sinusoids. Left: (X20); a–f: (X200).

➢ Adrenal Medulla

The adrenal medulla is composed of large, pale-staining polyhedral cells arranged in cords or clumps and supported by a reticular fiber network (Figure 9). A

supply of sinusoidal capillaries intervenes between adjacent cords and a few parasympathetic ganglion cells are present. **Medullary parenchymal cells**, known as **chromaffin cells**, arise from **neural crest cells**, as do **the postganglionic neurons of sympathetic and parasympathetic ganglia**. **Chromaffin cells** can be considered modified sympathetic postganglionic neurons, lacking axons and dendrites and specialized as secretory cells.

Unlike cells of the adrenal cortex, **chromaffin cells** contain many electron-dense granules, for storage and secretion of **catecholamines**, either **epinephrine or norepinephrine**. Medullary chromaffin cells are innervated by **preganglionic sympathetic neurons**, which trigger **epinephrine and norepinephrine** release during stress and intense emotional reactions. **Epinephrine** increases heart rate and dilates arteries of cardiac and skeletal muscle. **Norepinephrine** constricts vessels of the digestive system and skin, increasing blood flow to the heart, muscles, and brain. **Both hormones** stimulate **glycogen breakdown**, elevating blood glucose levels.

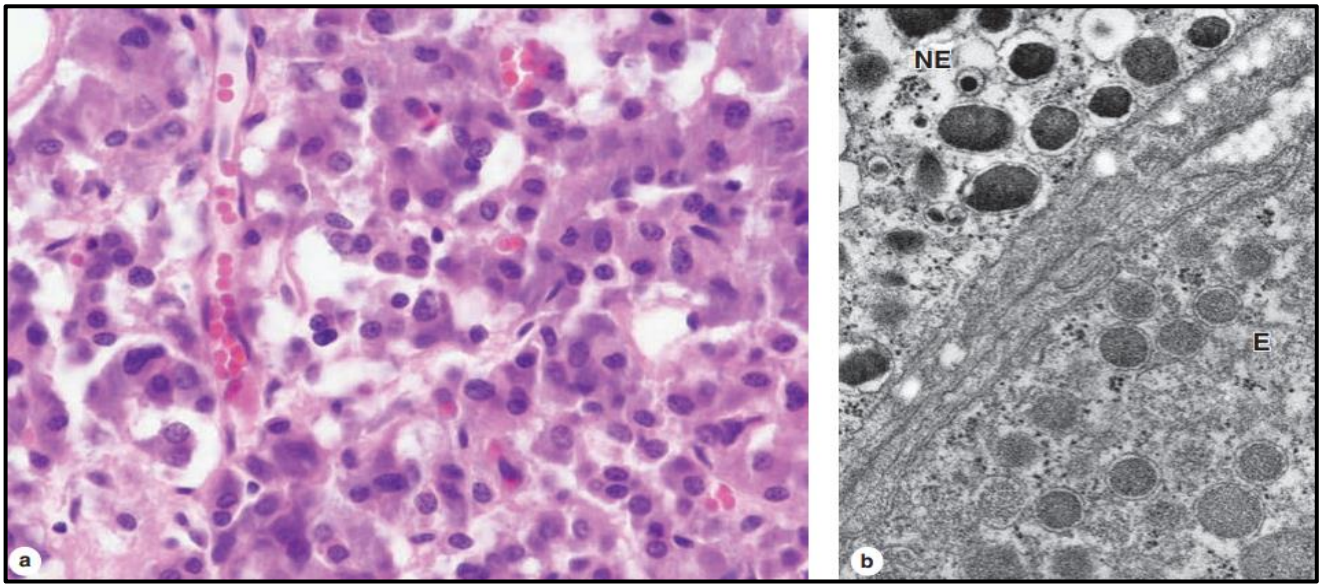


Figure 9: Adrenal medulla. (a) The micrograph shows that they are large pale-staining cells, arranged in cords interspersed with wide capillaries. Faintly stained cytoplasmic granules can be seen in most chromaffin cells. (X200; H&E) (b) TEM reveals that the granules of norepinephrine-secreting cells (NE) are more electron-dense than those of cells secreting epinephrine (E) (X33,000).

Pancreatic Islets

The pancreatic islets (islets of Langerhans) are compact spherical or ovoid masses of **endocrine cells** embedded within the acinar exocrine tissue of the pancreas (Figure 10). Most islets contain several hundred cells, but some have only a few cells.

The pancreas has more than 1 million islets, mostly in the gland's narrow tail region, but they only constitute 1%-2% of the organ's total volume. A very thin reticular capsule surrounds each islet, separating it from the adjacent acinar tissue. **Pancreatic islets** have the same embryonic origin as the pancreatic acinar tissue: in epithelial outgrowths from **endoderm** of the developing gut.

The cells of islets are polygonal or rounded, smaller, and more lightly stained than the surrounding acinar cells, arranged in cords separated by fenestrated capillaries (Figure 10). **Routine stains or trichrome stains** show that most islet cells are acidophilic or basophilic with fine cytoplasmic granules (Figure 10). Ultrastructural features are those of active polypeptide-secreting cells, with secretory granules that vary in size, morphology, and electron density from cell to cell. The major islet cells are most easily identified and studied by **immunohistochemistry**: **α or A cells**, **β or B cells and δ or D cells**.

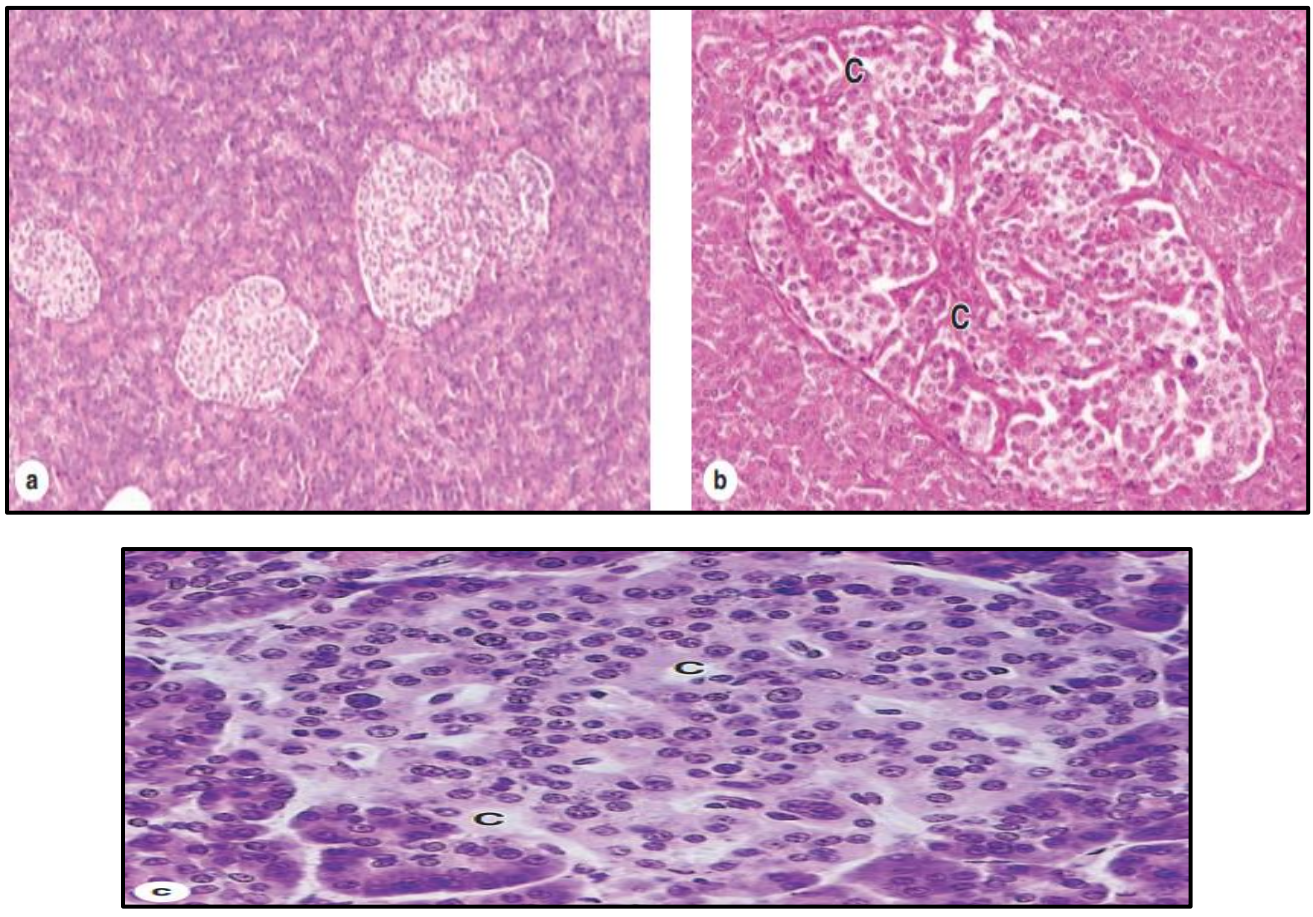


Figure 10: Pancreatic islets. (a) The islets are clusters of cells smaller and lighter staining than cells of the surrounding tissue (X12.5; H&E). (b) At higher magnification an islet's capillary system can be seen. Several arterioles enter each islet, branch into fenestrated capillaries (C) among the peripheral islet cells, then converge centrally before leaving the islet as efferent capillaries carrying blood to the acini surrounding the islet (X40; H&E). (c) With H&E staining all cells of an islet appear similar, although differences in cell size and basophilia may be apparent. Capillaries (C) are also apparent (X55; H&E).

The functions of the hormones secreted by these glands can be summarized in the following table:

Cell Type	Hormone Produced	Hormone Structure	Hormone Function
A	**Glucagon**	Polypeptide	Acts on several tissues to make energy stored in glycogen and fat available through glycogenolysis and lipolysis; increases blood glucose content
β	**Insulin**	Dimer of α and β chains with S-S bridges	Acts on several tissues to cause entry of glucose into cells and promotes decrease of blood glucose content
δ or D;	**Somatostatin**	Polypeptide	Inhibits release of other islet cell hormones through local paracrine action; inhibits release of GH and TSH in anterior pituitary and HCl secretion by gastric parietal cells
PP(Rare)	**Pancreatic polypeptide**	Polypeptide	Stimulates activity of gastric chief cells; inhibits bile secretion, pancreatic enzyme and bicarbonate secretion, and intestinal motility

Thyroid Gland

The thyroid gland, located anterior and inferior to **the larynx**, consists of two lobes united by an **isthmus** (Figure 11). It originates in early embryonic life from the foregut **endoderm** near the base of the developing tongue. It synthesizes the thyroid hormones **thyroxine (tetra-iodothyronine or T4) and tri-iodothyronine (T3)**, which help control the basal metabolic rate in cells throughout the body, as well as the polypeptide hormone **calcitonin**.

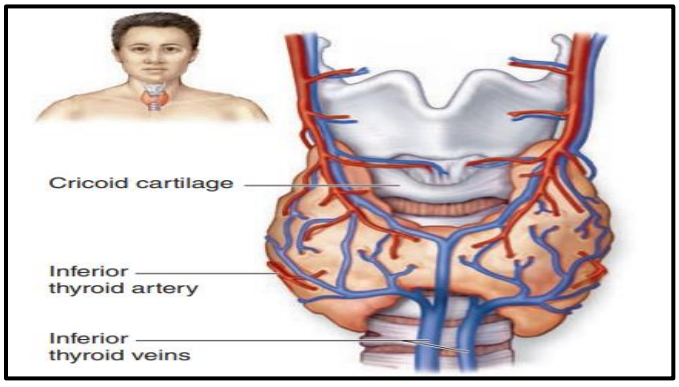
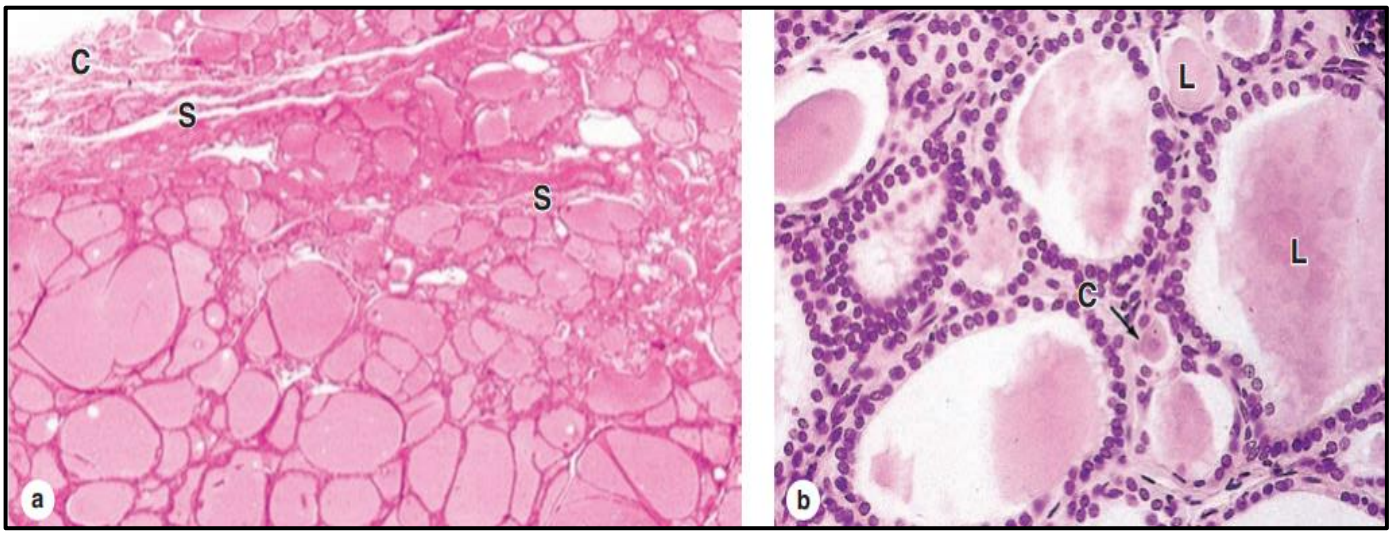


Figure 11: Thyroid gland.

The parenchyma of the thyroid is composed of millions of rounded epithelial thyroid follicles, each with simple epithelium and a central lumen densely filled with gelatinous acidophilic **colloid** (Figure 12). **The thyroid** is the only endocrine gland in which a large quantity of secretory product is stored. Moreover, storage is outside the cells, in the colloid of the follicle lumen, which is also unusual. There is sufficient hormone in follicles to supply the body for up to 3 months with no additional synthesis.

The thyroid gland is covered by a **fibrous capsule** from which septa extend into the parenchyma, dividing it into lobules and carrying blood vessels, nerves, and lymphatics. Follicles are densely packed together, separated from one another only by sparse reticular connective tissue (Figure 12), although this stroma is very well vascularized with fenestrated capillaries for transfer of released hormone to the blood.

The follicular cells, or **thyrocytes**, range in shape from squamous to low columnar (Figure 12), their size and other features varying with their activity, which is controlled by **thyroid-stimulating hormone (TSH)** from the anterior pituitary. **Active glands** have more follicles of low columnar epithelium; glands with mostly squamous follicular cells are **hypoactive**.



20–19 Thyroid follicular cells and parafollicular cells. (a) A low-power micrograph of thyroid gland shows the thin capsule (C), from which septa (S) with the larger blood vessels, lymphatics, and nerves enter the gland. The parenchyma of the organ is distinctive, consisting of colloid-filled epithelial follicles of many sizes. The lumen of each follicle is filled with a lightly staining colloid of a large gelatinous protein called thyroglobulin. (X12; H&E) (b) The lumen (L) of each follicle is surrounded by a simple epithelium of thyrocytes in which the cell height ranges from squamous to low columnar. Also present are large pale-staining parafollicular or C cells (C) secreting calcitonin (X200; H&E).

Thyrocytes have apical junctional complexes and rest on a basal lamina (Figure 13). The cells exhibit organelles indicating active protein synthesis and secretion, as well as phagocytosis and digestion. The nucleus is generally round and central. Basally the cells are rich in rough ER and apically, facing the follicular lumen, are Golgi complexes, secretory granules, numerous phagosomes and lysosomes, and microvilli.

Another endocrine cell type, **the parafollicular cell**, or **C cell**, is also found inside the basal lamina of the follicular epithelium or as isolated clusters between follicles (Figure 13). Derived from **the neural crest**, parafollicular cells are usually somewhat larger than follicular cells and stain less intensely. They have a smaller amount of rough ER, large Golgi complexes, and numerous small granules containing **calcitonin** (Figure 13).

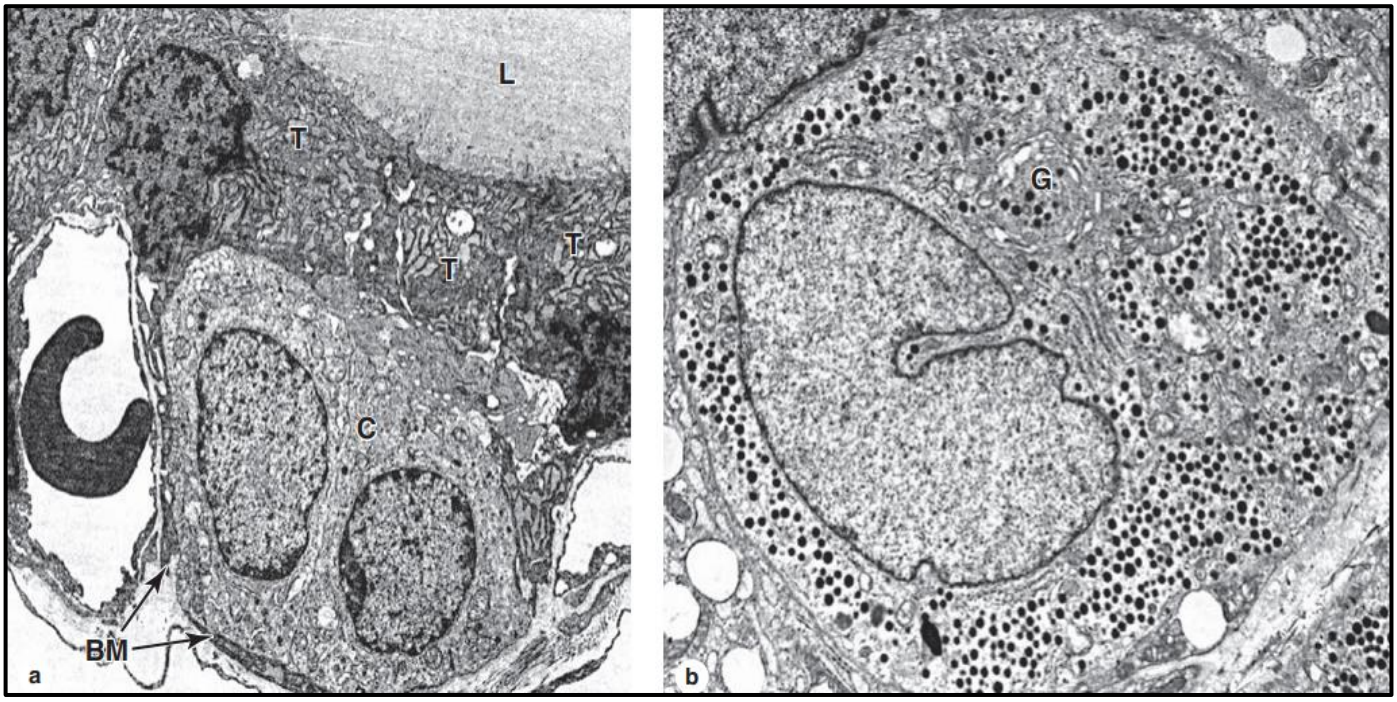


Figure 13: Ultrastructure of thyroid follicular and parafollicular cells. (a) TEM of the follicular epithelium shows pseudopodia and microvilli extending from the follicular thyrocytes (T) into the colloid of the lumen (L). The cells have apical junctional complexes, much RER, well-developed Golgi complexes, and many lysosomes. Inside the basement membrane (BM) of the follicle, but often not contacting the colloid in the lumen, are occasional C cells (C). To the left and right of the two C cells seen here are capillaries intimately associated with the follicular cells, but outside the basement membrane (X2000). (b) A TEM of a C cell, with its large Golgi apparatus (G), extensive RER, and cytoplasm filled with small secretory granules containing calcitonin (X5000).

* **Thyroid cells and their main hormones:**

- **Follicular cells**: Thyroid hormones (T3 and T4) which Increases metabolic rate.
- **Parafollicular or C cells**: Calcitonin which Lowers blood Ca2+ levels by inhibiting osteoclast activity.

Parathyroid Glands

The parathyroid glands are four small ovoid masses. They are located on the back of the thyroid gland, usually embedded in the larger gland's capsule (Figure 14). The microvasculature of each arises from the inferior thyroid arteries. Each parathyroid gland is contained within a thin capsule from which septa extend into the gland. A sparse reticular stroma supports dense elongated clusters of **secretory cells**.

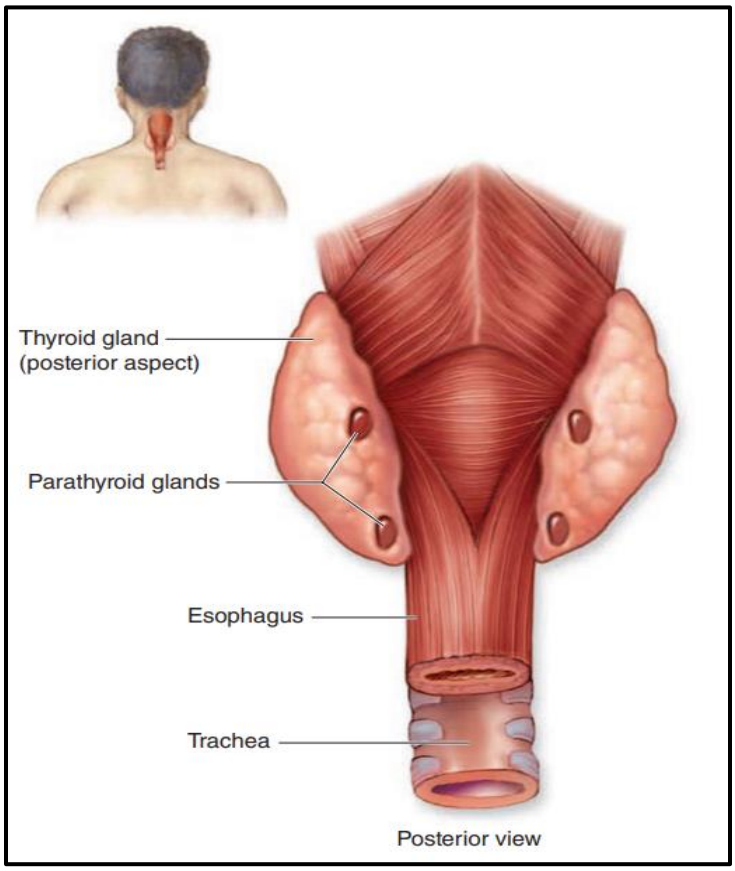


Figure 14: Parathyroid glands.

The parathyroid glands are derived from the embryonic pharyngeal pouches. Their migration to the developing thyroid gland is sometimes misdirected so that the number and locations of the glands are somewhat variable. Up to **10%** of individuals may have parathyroid tissue attached to **the thymus**, which originates from the same pharyngeal pouches.

The endocrine cells of the parathyroid glands, called **principal (chief) cells**, are small polygonal cells with round nuclei and pale-staining, slightly acidophilic cytoplasm (Figure15). Irregularly shaped cytoplasmic granules contain the polypeptide **parathyroid hormone (PTH)**, an important regulator of blood calcium levels.

With increasing age, many **secretory cells** are replaced with **adipocytes**, which may constitute more than 50% of the gland in older people. Much smaller populations of **oxyphil cells**, often clustered, are sometimes also present in parathyroid glands, more commonly in older individuals. These are much larger than the principal cells and are characterized by very acidophilic cytoplasm filled with abnormally shaped mitochondria.

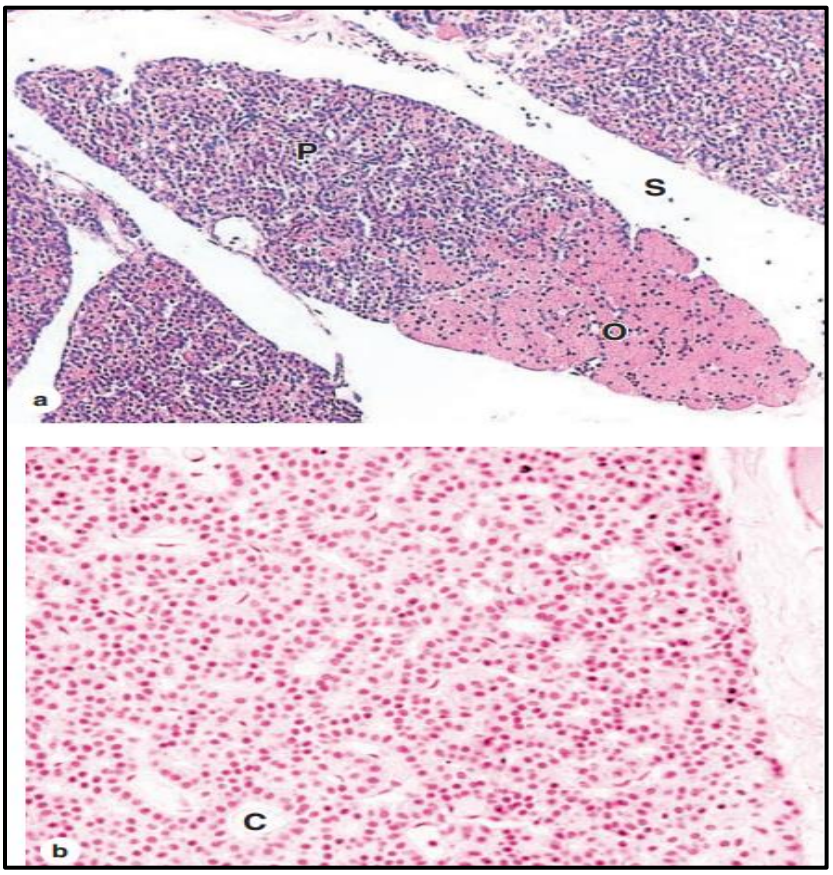


Figure 15: Parathyroid principal cells. (a) A small lobe of parathyroid gland, surrounded by connective tissue septa (S), shows densely packed cords of small principal cells (P). Older parathyroid glands show increasing numbers of much larger and acidophilic nonfunctional oxyphil cells (O) (X60; H&E). (b) Higher magnification shows that principal cells have round central nuclei and pale-staining cytoplasm. Cords of principal cells secreting PTH surround capillaries (C) (X200; H&E).

* Parathyroid cells and their main hormones:

- **Chief cells**: Parathyroid hormone (PTH) which Raises blood **Ca^{2+}** levels by stimulating osteoclast activity.

Pineal Gland

The pineal gland, also known as the **epiphysis cerebri**, regulates the daily rhythms of bodily activities. A small, pinecone-shaped organ. The pineal gland develops from **neuroectoderm** in the posterior wall of the third ventricle and remains attached to

the brain by a short stalk. **The pineal gland** is covered by connective tissue from which septa containing small blood vessels emerge and subdivide variously sized lobules.

Prominent and abundant secretory cells called **pinealocytes** have slightly basophilic cytoplasm and irregular euchromatic nuclei (Figure 16). Ultrastructurally **pinealocytes** are seen to have secretory vesicles, many mitochondria, and long cytoplasmic processes extending to the vascularized septa, where they end in dilatations near capillaries, indicating an endocrine function. These cells produce **melatonin**, a low-molecular-weight tryptophan derivative. Unmyelinated sympathetic nerve fibers enter the pineal gland and end among **pinealocytes**, with some forming synapses. Melatonin release from **pinealocytes** is promoted by darkness and inhibited by daylight.

The pineal gland also has interstitial glial cells that are modified **astrocytes**, staining positively for glial fibrillary acidic protein, which represent about **5%** of the cells. These have elongated nuclei more heavily stained than those of **pinealocytes** and are usually found in perivascular areas and between the groups of **pinealocytes**.

A characteristic feature of the pineal gland is the presence of variously sized concretions of **calcium and magnesium salts** called **corpora arenacea**, or **brain sand**, formed by mineralization of extracellular protein deposits. Such concretions may appear during childhood and gradually increase in number and size with age, with no apparent effect on the gland's function.

* pineal cells and their main hormones:

- **Pinealocytes**: Melatonin which regulates circadian rhythms.

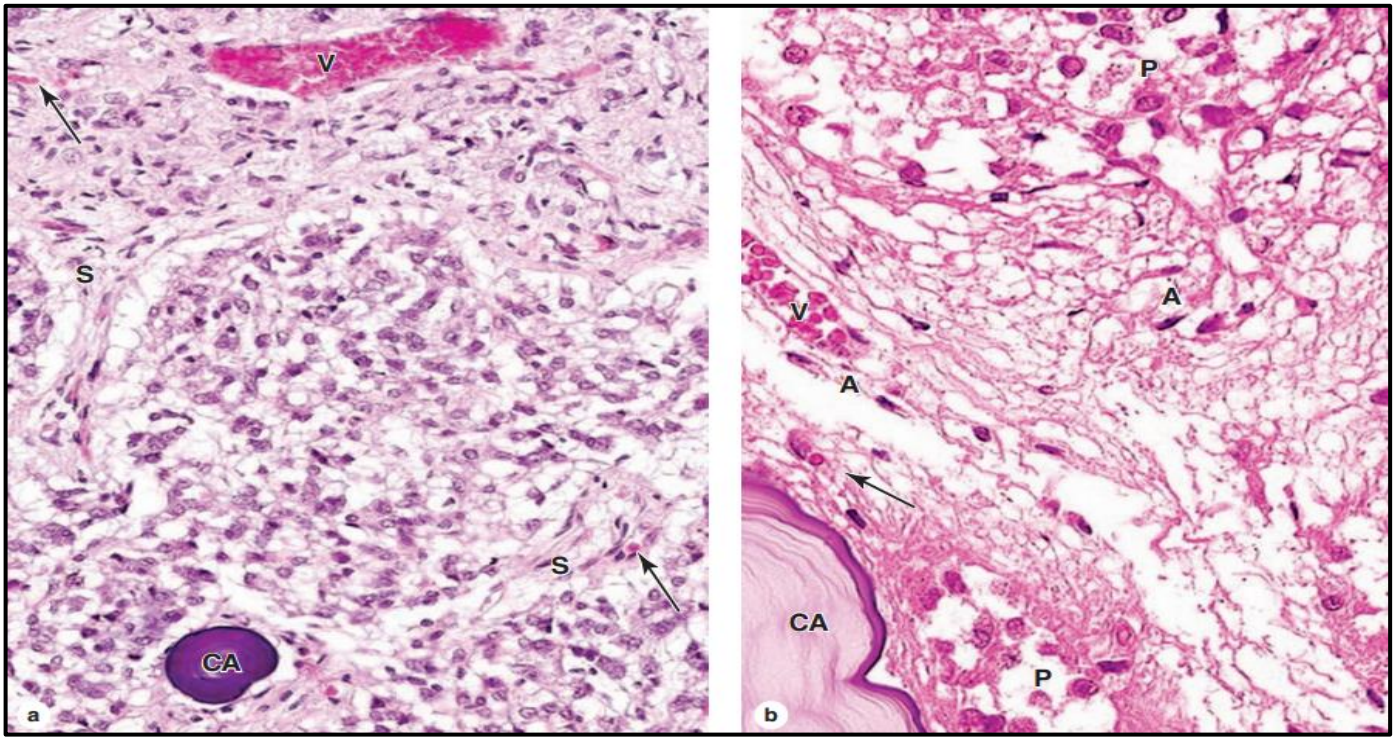


Figure 16: Pineal gland. (a) The micrograph shows a group of pinealocytes surrounded by septa (S) containing venules (V) and capillaries (arrows). Also seen is an extracellular mineral deposit called a corpus arenaceum (CA) (X200; H&E). (b) At higher magnification the numerous large pinealocytes (P) with euchromatic nuclei can be compared to much fewer astrocytes (A) that have darker, more elongated nuclei and are located mainly within septa and near small blood vessels (V). Capillaries (arrow) are not nearly as numerous as in other endocrine glands. At the lower left is a part of a very large corpus arenaceum (CA) (X400; H&E).

Lecture 13-14: The Nervous System

The human nervous system, the most complex system in the body, is formed by a network of many billion nerve cells (**neurons**) which typically have numerous long processes, all assisted by many supporting cells called **glial cells**, which also participate in many neural activities, neural nutrition, and defense of cells in the **CNS**. Each neuron has hundreds of interconnections with other neurons, forming a very complex system for processing information and generating responses.

Nerve tissue is distributed throughout the body as an integrated communications network. Anatomically, the general organization of the system has two major divisions:

- **Central nervous system (CNS)**, consisting of the brain and spinal cord.
- **Peripheral nervous system (PNS)**, composed of the cranial, spinal, and peripheral nerves conducting impulses to and from the **CNS** (sensory and motor nerves, respectively) and ganglia that are small aggregates of nerve cells outside the **CNS**.

Neurons respond to environmental changes (**stimuli**) by altering **the ionic gradient** that exists across their plasma membranes. All cells maintain such a gradient, also called **an electrical potential**, but cells that can rapidly change this potential in response to stimuli are said to be excitable or irritable. Neurons react promptly to stimuli with a reversal of the ionic gradient (membrane depolarization) that generally spreads from the place that received the stimulus and is propagated across the neuron's entire plasma membrane. This propagation, called **the action potential** or **the nerve impuls**e, is capable of traveling long distances along neuronal processes, transmitting such signals to other neurons, muscles, and glands.

By collecting, analyzing, and integrating information in such signals, the nervous system continuously stabilizes the intrinsic conditions of the body (eg, blood pressure, $\mathbf{O_2}$ and $\mathbf{CO_2}$ content, **pH**, blood glucose levels, and hormone levels) within normal ranges and maintains behavioral patterns (eg, feeding, reproduction, defense, and interaction with other living creatures).

Development of Nerve Tissue

The nervous system develops from the outermost of the three early embryonic layers, **the ectoderm**, beginning in the third week of development. With signals from the underlying axial structure, **the notochord**, ectoderm on the mid-dorsal side of the

embryo thickens to form the epithelial neural plate. The sides of this plate fold upward and grow toward each other medially, and within a few days fuse to form the neural tube. Cells of this tube give rise to the entire **CNS**, including **neurons** and **most glial cells**.

As the folds fuse and the neural tube separates from the now overlying surface ectoderm that will form **epidermis**, a large population of developmentally important cells, **the neural crest**, separates from the **neuroepithelium** and becomes **mesenchymal**. Neural crest cells migrate extensively and differentiate as all the cells of the **PNS**, as well as a number of other **non-neuronal** cell types.

Neurons

The functional unit in both the **CNS** and **PNS** is the **neuron**. Most neurons have three main parts:

- **The cell body** (also called **the perikaryon** or **soma**), which contains the nucleus and most of the cell's organelles and serves as the synthetic or trophic center for the **entire neuron**.
- **The dendrites**, which are the numerous elongated processes extending from the perikaryon and specialized to receive stimuli from other neurons at unique sites called **synapses**.
- **The axon**, which is a single long process ending at synapses specialized to generate and conduct nerve impulses to other cells (eg, nerve, muscle, and gland cells). Axons may also receive information from other neurons, information that mainly modifies the transmission of action potentials to those neurons.

Neurons can be classified according to the number of processes extending from the cell body (**Figure 1**):

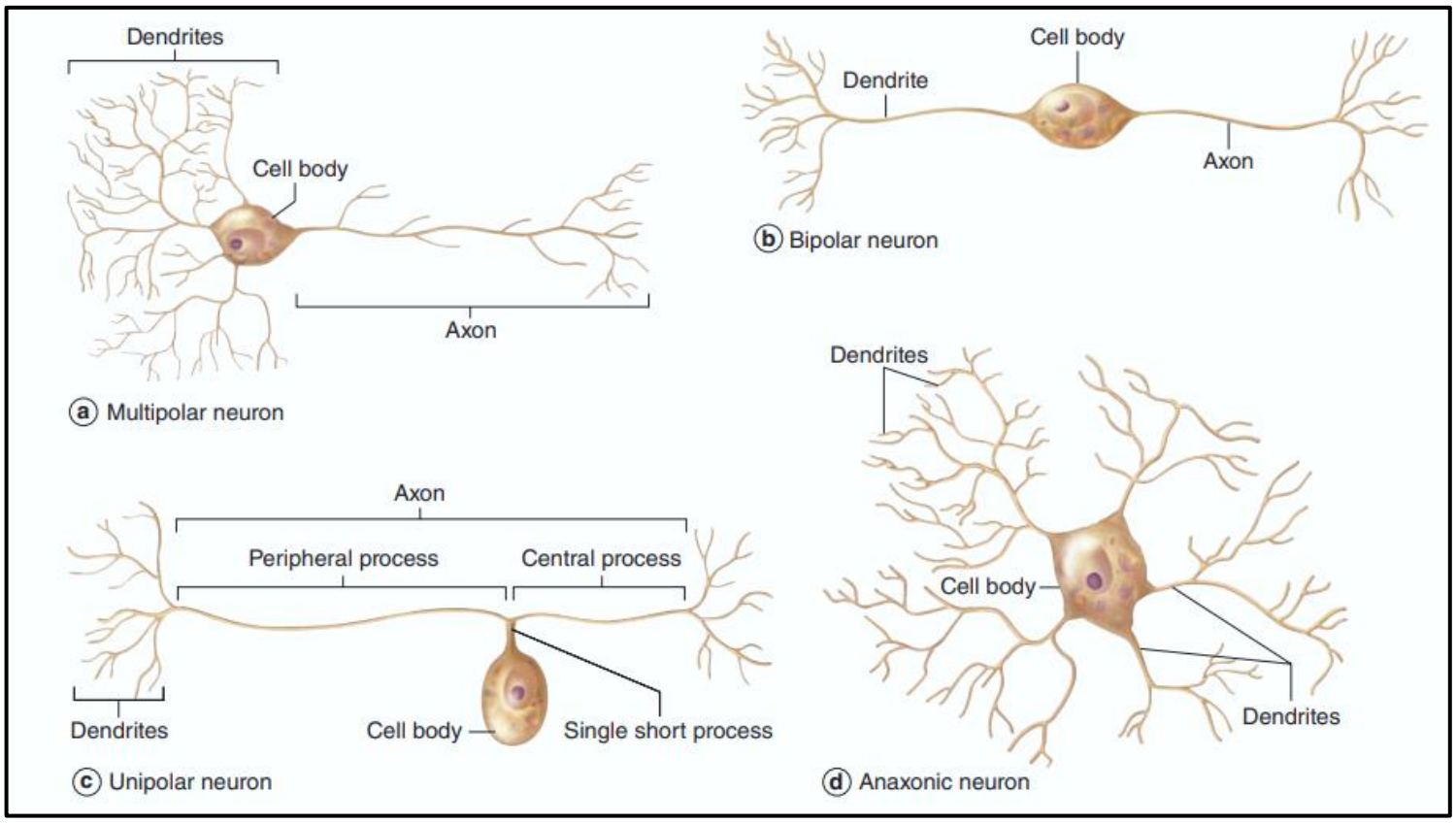


Figure 1: Structural classes of neurons.

- **Multipolar neurons**, each with one axon and two or more dendrites, are the most common.
- **Bipolar neurons**, with one dendrite and one axon, comprise the sensory neurons of the retina, the olfactory epithelium, and the inner ear.
- **Unipolar or pseudounipolar neurons**, which include all other sensory neurons, each have a single process that bifurcates close to the perikaryon, with the longer branch extending to a peripheral ending and the other toward the **CNS**.
- **Anaxonic neurons**, with many dendrites but no true axon, do not produce action potentials, but regulate electrical changes of adjacent CNS neurons.

Because the fine processes emerging from cell bodies are seldom seen in sections of nervous tissue, it is difficult to classify neurons structurally by microscopic inspection.

Nervous components can also be subdivided functionally . **Sensory neurons** are afferent, receiving stimuli from receptors throughout the body. **Motor neurons** are efferent, sending impulses to effector organs such as muscle fibers and glands. **Somatic motor** nerves are under voluntary control and typically innervate skeletal muscle;

autonomic motor nerves control the involuntary activities of glands, cardiac muscle, and most smooth muscle.

Interneurons establish relationships among other neurons, forming complex functional networks or circuits in the CNS. **Interneurons** are either **multipolar** or **anaxonic** and comprise 99% of all neurons in adults.

In the CNS most neuronal perikarya occur in **the gray matter**, with their axons concentrated in the **white matter**. These terms refer to the general appearance of unstained CNS tissue caused in part by the different densities of nerve cell bodies. In the **PNS** cell bodies are found in ganglia and in some sensory regions, such as the olfactory mucosa, and axons are bundled in nerves.

Glial Cells & Neuronal Activity

Glial cells support neuronal survival and activities, and are 10 times more abundant than neurons in the **mammalian brain**. Like neurons most glial cells develop from progenitor cells of the embryonic neural plate. In the **CNS glial cells** surround both the neuronal cell bodies, which are often larger than the glial cells, and the processes of axons and dendrites occupying the spaces between neurons. The fibrous intercellular network of **CNS** tissue superficially resembles collagen by light microscopy, but is actually the network of fine cellular processes emerging from neurons and glial cells. Such processes are collectively called **the neuropil** (Figure 2).

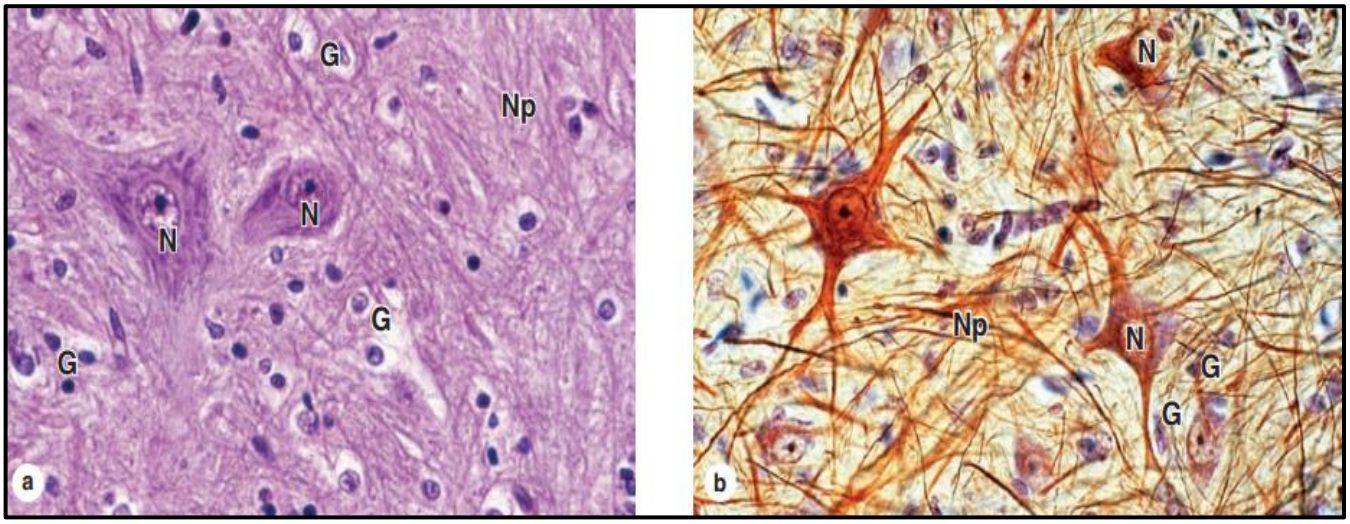


Figure 2: Neurons, neuropil, and the common glial cells of the CNS. (a) Most neuronal cell bodies (N) in the CNS are larger than the much more numerous glial cells (G) that surround them. The various types of glial cells and their relationships with neurons are difficult to distinguish by most routine light microscopic methods (X200; H&E). (b) With the use of gold staining for neurofibrils, neuropil (Np) is more apparent. (X200; Gold chloride and hematoxylin).

There are six major kinds of glial cells, as shown schematically in Figure 3, **four** in the **CNS** and two in the **PNS**.

- **Oligodendrocytes,** wrap processes around portions of axons in the **CNS**, forming myelin sheaths that insulate the axons and facilitate nerve impulses.
- **Astrocytes**, the most numerous cell of the **CNS**, all produce hundreds of processes to cover and provide regulated microenvironments for neuronal perikarya, synapses, and capillaries (Figure 4).

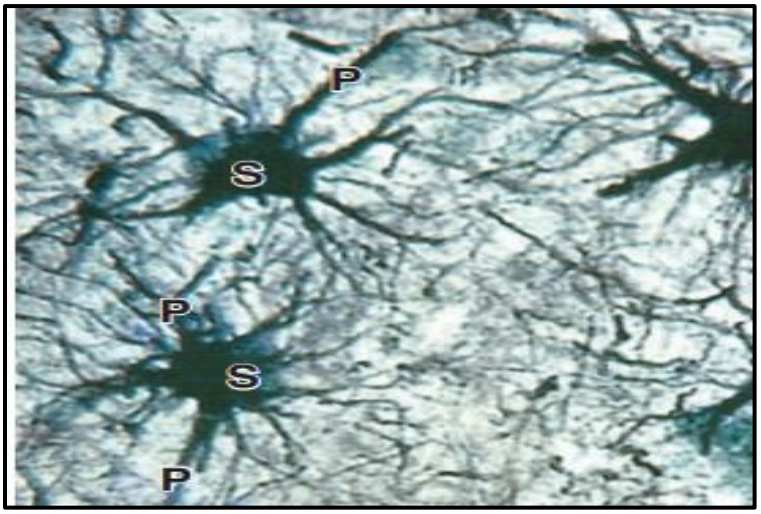


Figure 4: Astrocytes. Astrocytes are the most abundant glial cells of the CNS and are characterized by numerous cytoplasmic processes (P) radiating from the glial cell body or soma (S). Astrocytic processes are not seen with routine light microscope staining but are easily seen after gold staining (X500; Gold chloride).

- **Ependymal cells** are epithelial-like cells, lacking basement membranes, which line the fluid-filled cerebral ventricles and central canal of the spinal cord (Figure 5).

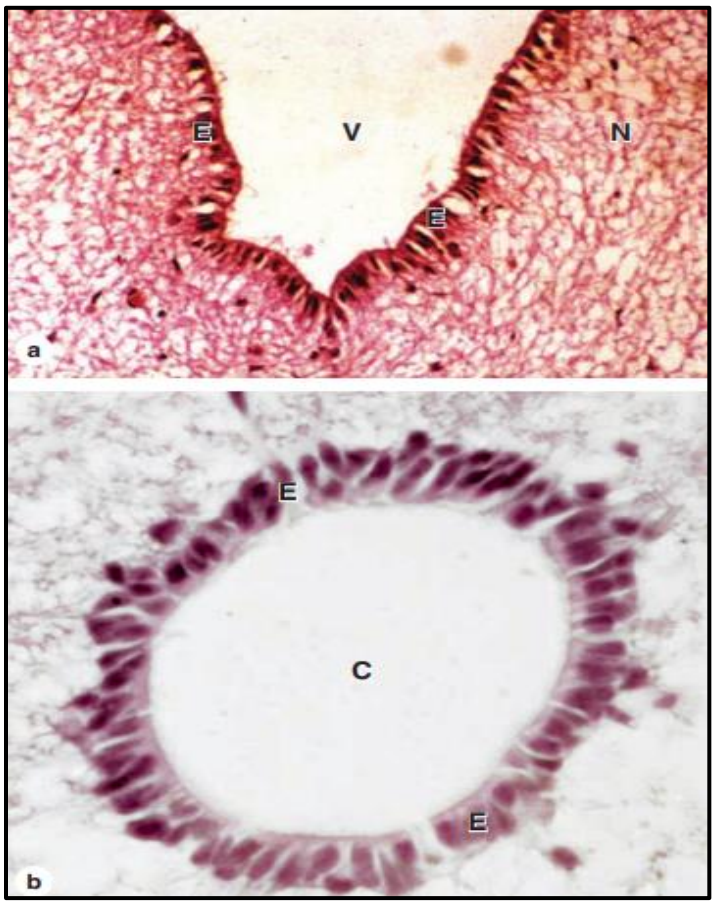


Figure 5: Ependymal cells. (a) Lining the ventricles of the cerebrum, columnar ependymal cells (E) extend cilia and microvilli from the apical surfaces into the ventricle (V). These modifications help circulate the CSF and monitor its contents. The cells' basal ends are tapered, extending processes that branch and penetrate some distance into the adjacent neuropil (N). Other areas of ependyma are responsible for production of CSF (X100; H&E). (b) Ependymal cells (E) lining the central canal (C) of the spinal cord help move CSF in that CNS region (X200; H&E).

- **Microglia** differs from all other glial cells in originating from blood monocytes, not from neural tissue precursors; they mediate immune defense activity within the **CNS** (figure 6).

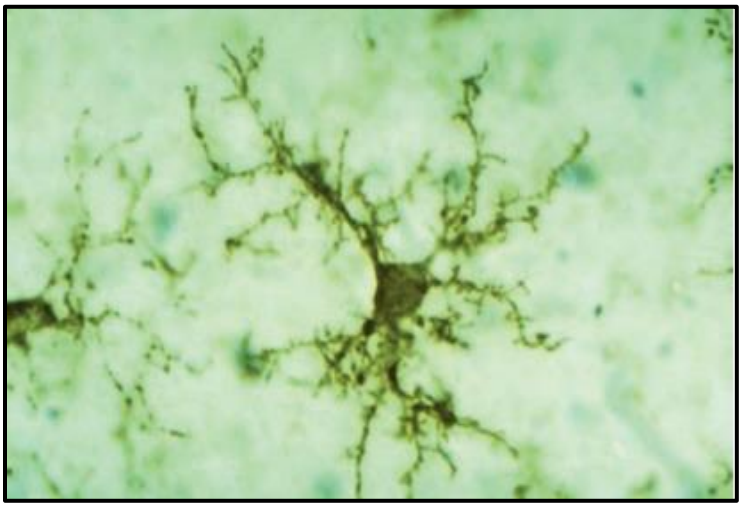


Figure 6: Microglial cells. Microglia are monocyte-derived, antigen-presenting cells of the CNS. By immunohistochemistry, here using a monoclonal antibody against human leukocyte antigens (HLA) of immune-related cells, the short branching processes of microglia can be seen. Routine staining demonstrates only the small dark nuclei of the cells (X500; Antibody against HLA-DR and peroxidase).

- **Schwann cells (neurolemmocytes)** enclose all axons in nerves of the PNS, producing myelin sheaths around large-diameter axons, whose impulse conductivity is augmented at the nodes of Ranvier between successive Schwann cells.
- **Satellite cells** are located within PNS ganglia, aggregated sensory or autonomic neuronal cell bodies, where they enclose each perikaryon and regulate its microenvironment (Figure 7).

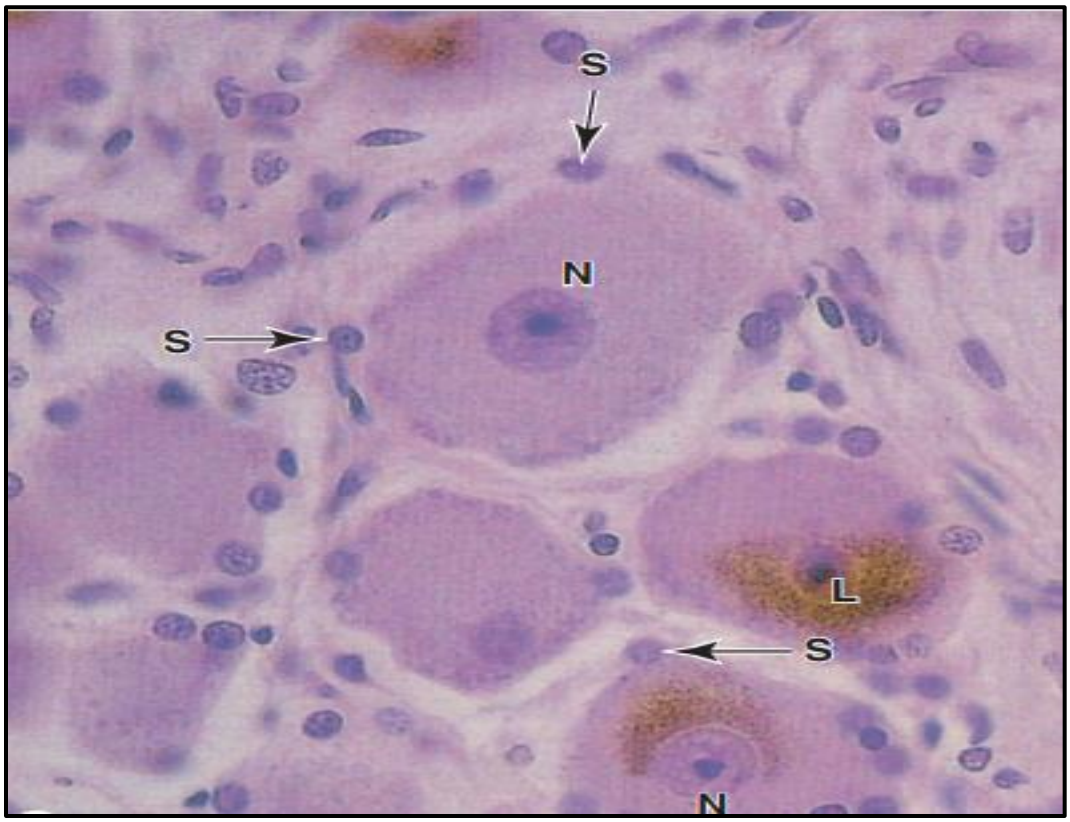
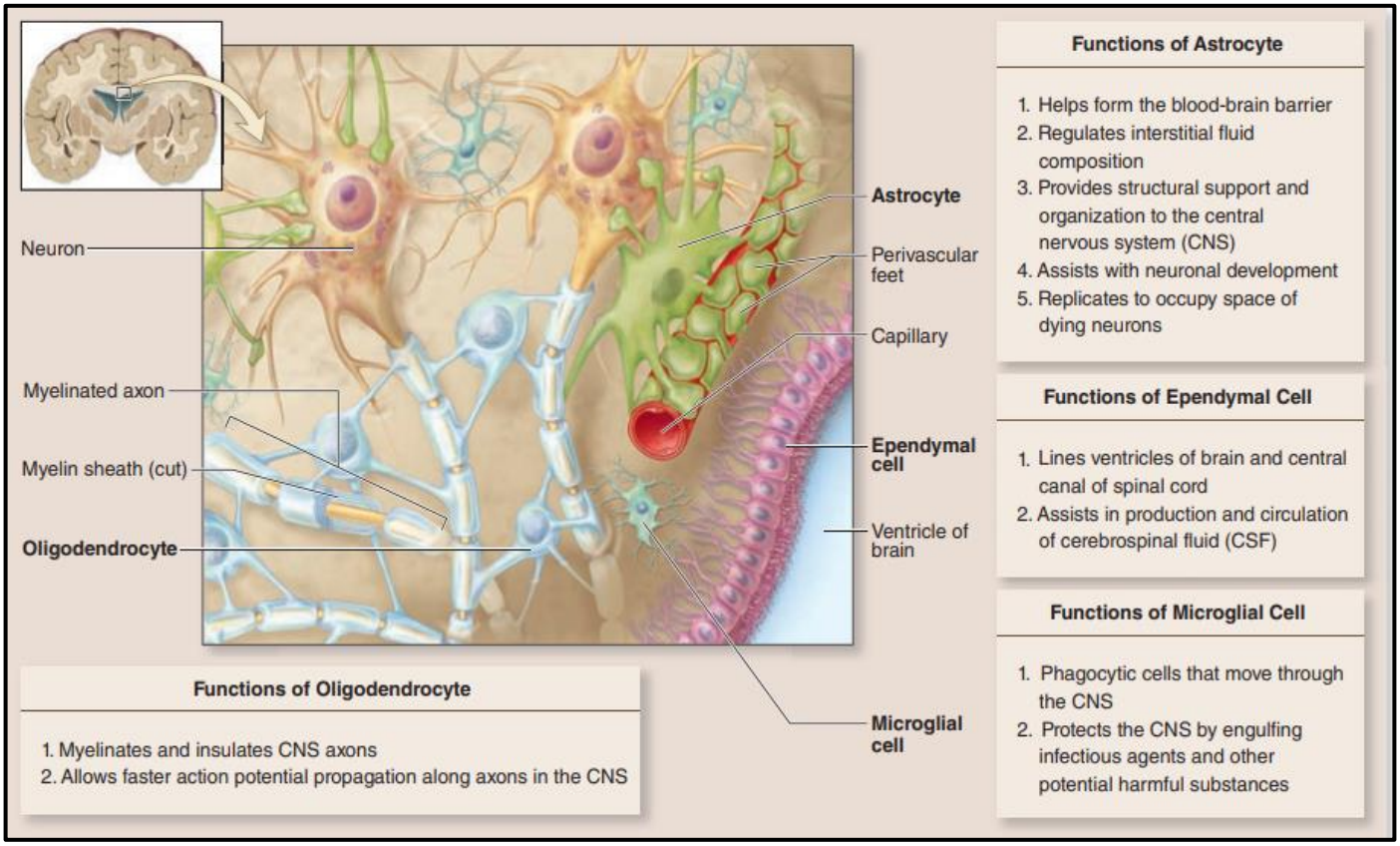
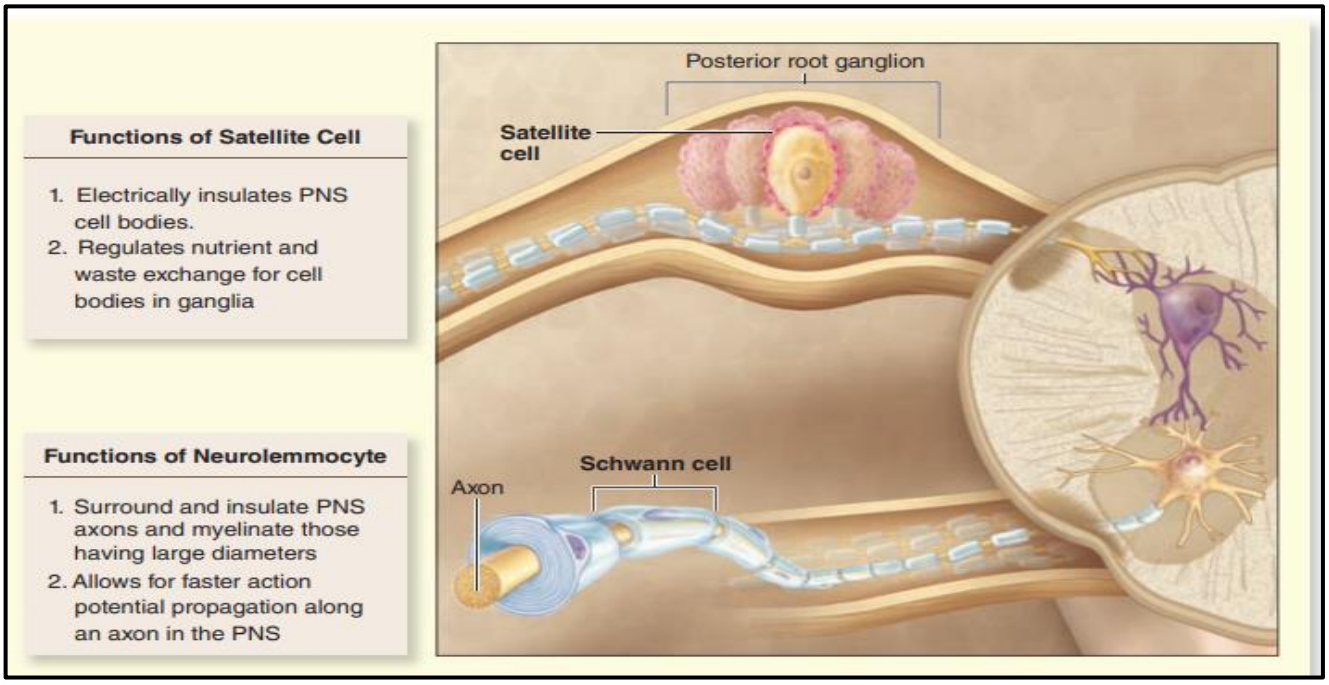


Figure 7: Satellite cells around neurons of ganglia in the PNS. Nuclei of the many satellite cells (S) surrounding the perikarya of neurons (N) in an autonomic ganglion can be seen by light microscopy, but their cytoplasmic extensions are too thin to see with H&E staining. These long-lived neurons commonly accumulate brown lipofuscin (L) (X560; H&E).



(a) four major kinds of glial cells in the CNS.



(b) Two glial cells occur in the PNS.

Figure 3: Glial cells of the CNS and PNS.

Central Nervous System

The major structures comprising the **CNS** are the **cerebrum**, **cerebellum**, and **spinal cord**. The **CNS** is completely covered by connective tissue layers, the **meninges**, but CNS tissue contains very little collagen or similar material, making it relatively soft and easily damaged by injuries affecting the protective skull or vertebral bones.

Many structural features of CNS tissues can be seen in unstained, freshly dissected specimens. Many regions show organized areas of white matter and gray matter, differences caused by the differential distribution of lipid-rich myelin. The main components of white matter are **myelinated axons** (Figure 8), often grouped together as tracts, and the **myelin-producing oligodendrocytes**. Astrocytes and microglia are also present, but very few neuronal cell bodies. Gray matter contains abundant neuronal cell bodies, dendrites, astrocytes, and microglial cells, and is where most synapses occur. Gray matter makes up the thick cortex or surface layer of both the **cerebrum** and the **cerebellum**; most white matter is found in deeper regions. Deep within the **brain** are localized, variously shaped darker areas called **the cerebral nuclei**, each containing large numbers of aggregated neuronal cell bodies.

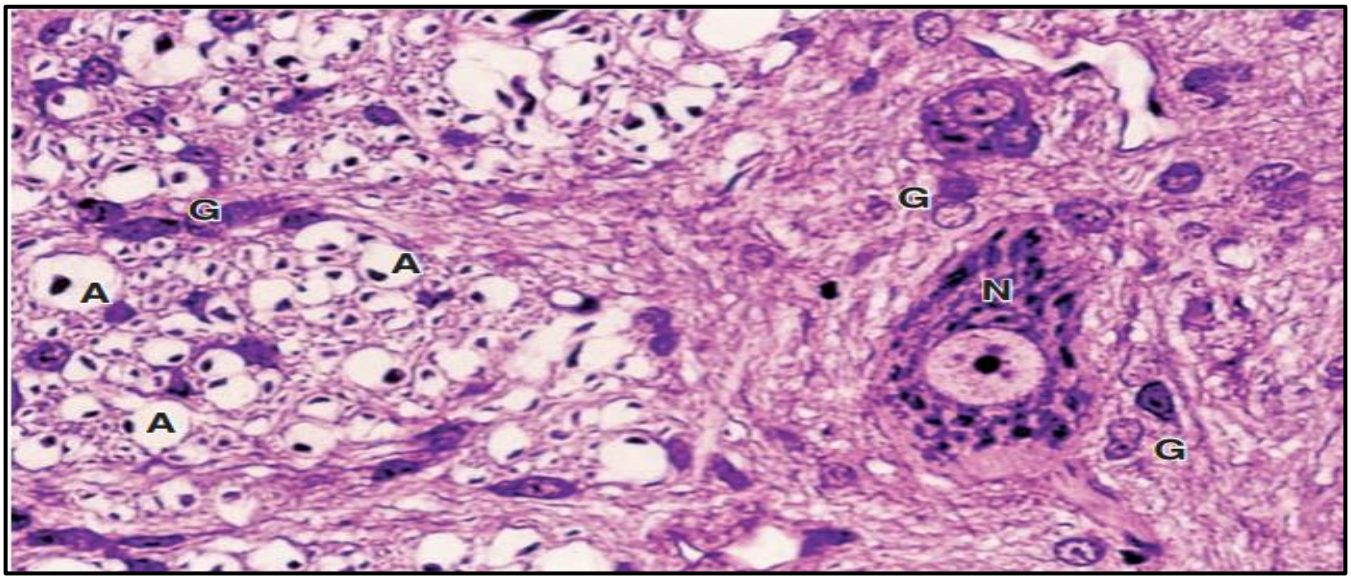


Figure 8: White versus gray matter. A cross section of spinal cord shows the transition between white matter (left region) and gray matter (right). The gray matter has many glial cells (G), neuronal cell bodies (N), and neuropil; white matter also contains glia (G) but consists mainly of axons (A) whose myelin sheaths were lost during preparation, leaving the round empty spaces shown. Each such space surrounds a darkstained spot that is a small section of the axon (H&E, X400).

In the folded cerebral cortex, neuroscientists recognize six layers of neurons with different sizes and shapes. **The most conspicuous** of these cells are the efferent

pyramidal neurons (Figure 9). **Neurons of the cerebral cortex** function in the integration of sensory information and the initiation of voluntary motor responses.

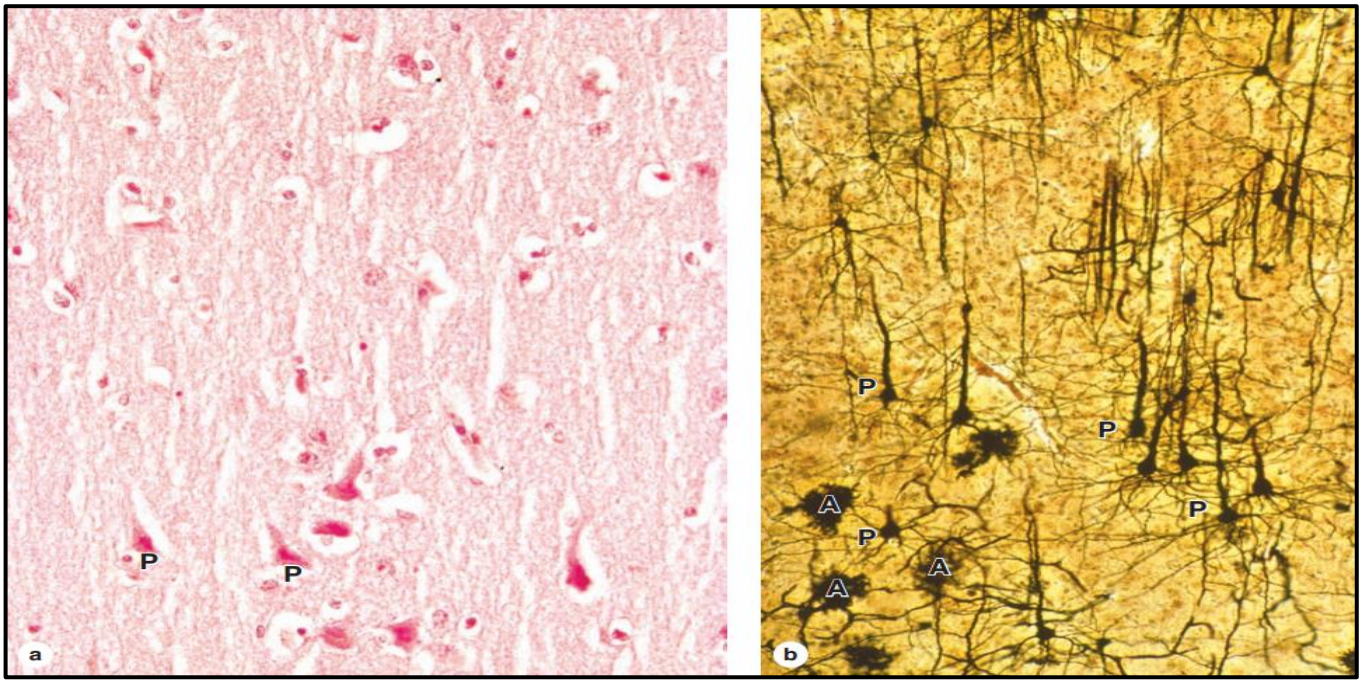


Figure 9: Cerebral cortex. (a) Important neurons of the cerebrum are the pyramidal neurons (P), which are arranged vertically in the eosinophilic neuropil (X200; H&E). (b) From the apical ends of pyramidal neurons (P), long dendrites extend in the direction of the cortical surface, which can be best seen in thick silver-stained sections in which only a few other protoplasmic astrocytes (A) cells are seen (X200; Silver).

The sharply folded cerebellar cortex coordinates muscular activity throughout the body and is organized with **three layers** (Figure 10):

- **A thick outer** molecular layer has much neuropil and scattered neuronal cell bodies.
- **A thin middle** layer consists only of very large neurons called **Purkinje cells**.
- **A thick inner** granular layer contains various very small, densely packed neurons and little neuropil.

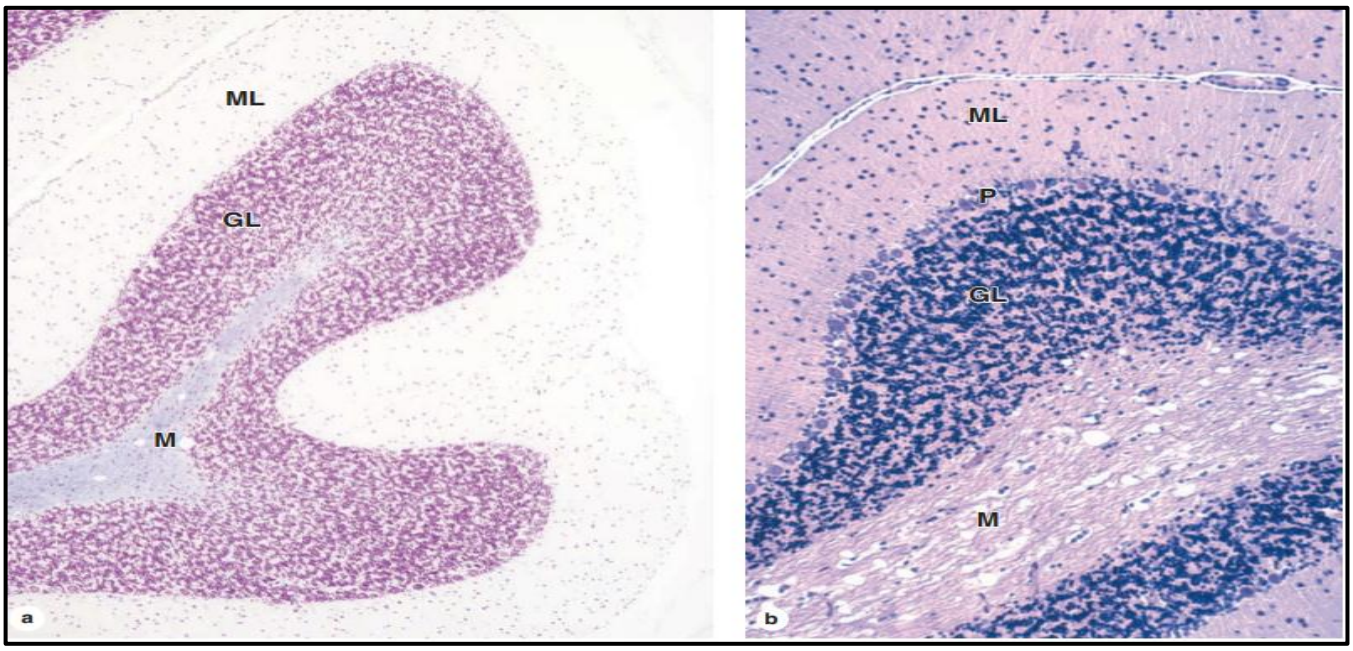


Figure 10: Cerebellum. (a) The cerebellar cortex is convoluted with many distinctive small folds, each supported at its center by tracts of white matter in the cerebellar medulla (M). Each fold has distinct molecular layers (ML) and granular layers (GL) (X6; Cresyl violet). (b) Higher magnification shows that the granular layer (GL) immediately surrounding the medulla (M) is densely packed with several different types of very small rounded neuronal cell bodies. The outer molecular layer (ML) consists of neuropil with fewer, much more scattered small neurons. At the interface of these two regions a layer of large Purkinje neuron (P) perikarya can be seen. (X20; H&E).

In cross sections of the **spinal cord**, the **white matter** is peripheral and the **gray matter** forms a deeper, H-shaped mass (Figure 11). The two anterior projections of this gray matter, **the anterior horns**, contain cell bodies of very large motor neurons. The two posterior horns contain interneurons which receive sensory fibers from neurons in the **spinal ganglia**. Near **the middle of the cord** the gray matter surrounds a small central canal, which develops from the lumen of the neural tube, is continuous with the ventricles of the **brain**, is lined by ependymal cells, and contains CSF.

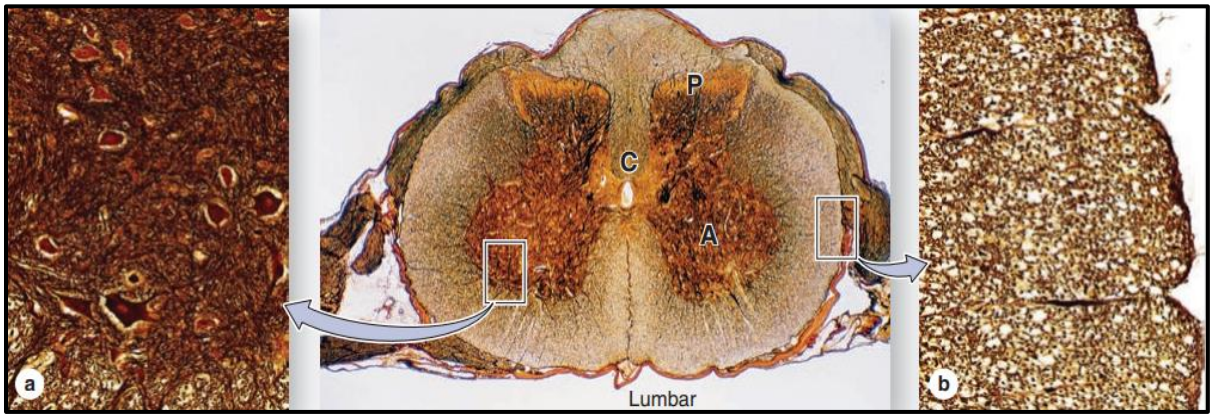
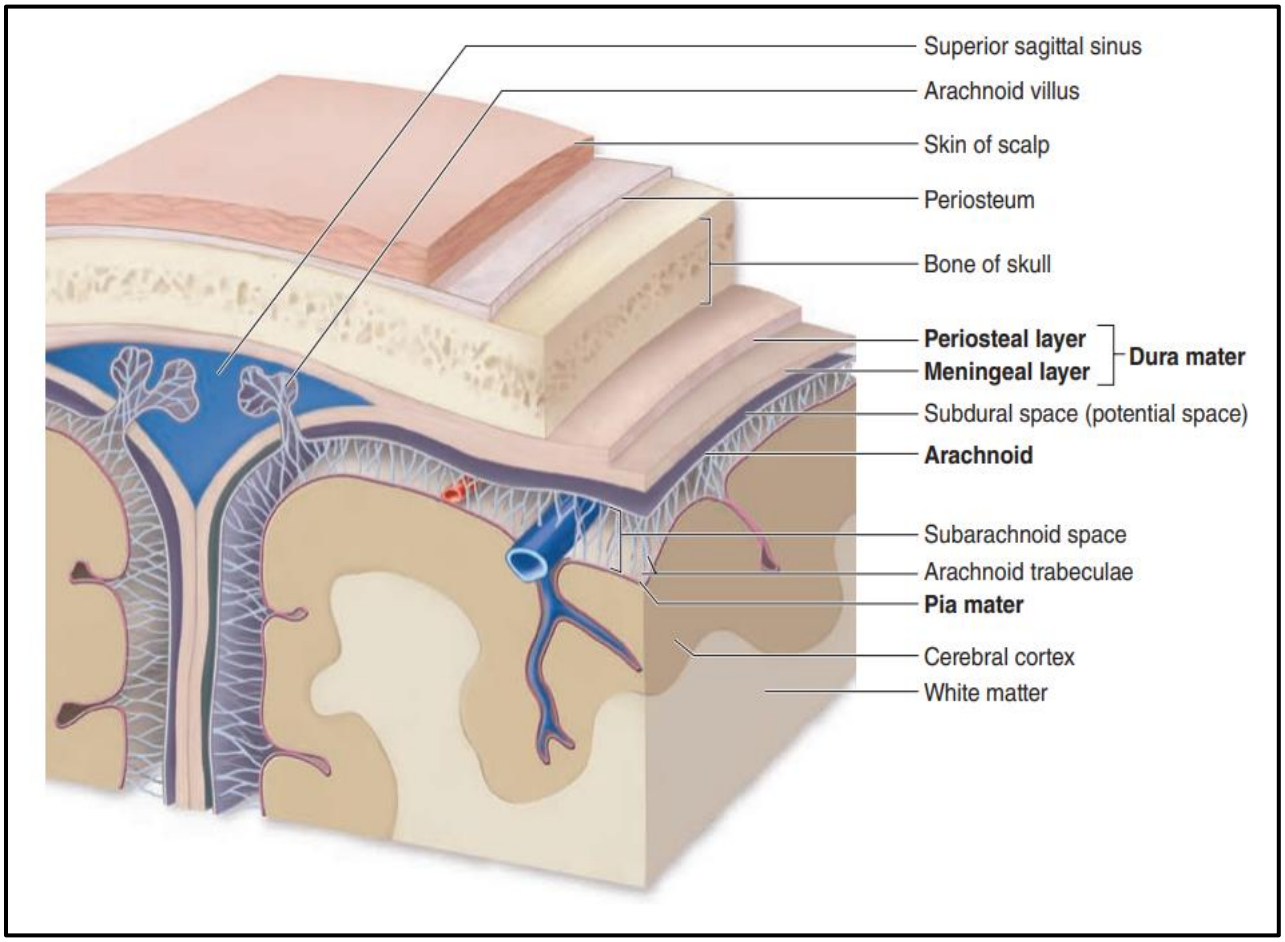


Figure 11: Spinal cord. The spinal cord shows bilateral symmetry around the small, CSF-filled central canal (C). the gray matter is forming a roughly H-shaped structure that consists of two posterior (P)

horns (sensory) and two anterior (A) (motor) horns, all joined by the gray matter around the central canal. (a) The gray matter contains abundant astrocytes and large neuronal cell bodies, especially those of motor neurons in the ventral horns. (b) The white matter surrounds the gray matter and contains primarily oligodendrocytes and tracts of myelinated axons running along the length of the cord (Center X5, a, b X100; All silver-stained).

The **CNS** is completely enclosed by three connective tissue layers called **meninges**: **(1) the tough external dura mater; (2) the middle arachnoid layer; and (3) the delicate pia mater that directly contacts neural tissue.**

*__The arachnoid layer__ contains much **CSF**, which helps cushion the **CNS** within its bony enclosure.



Meninges around the brain.

Blood-Brain Barrier

The blood-brain barrier (BBB) is a functional barrier that allows much tighter control in most tissues over the passage of substances moving from blood into the **CNS** tissue. The main structural component of the **BBB** is the capillary endothelium, in which the cells are tightly sealed together with well-developed occluding junctions, with little

or no transcytosis activity, and surrounded by the basement membrane. The limiting layer of perivascular astrocytic feet that envelops the basement membrane of capillaries in most **CNS** regions contributes to the **BBB** and further regulates passage of molecules and ions from blood to brain. The **BBB** protects neurons and glia from bacterial toxins, infectious agents, and other exogenous substances, and helps maintain the constant balance of ions in the interstitial fluid required for normal neuronal function.

Choroid Plexus

The choroid plexus consists of highly vascular tissue, elaborately folded and projecting into the large ventricles of the **brain** (Figure 12a). It is found in the roofs of the third and fourth ventricles and in parts of the two lateral ventricular walls.

Each villus of **the choroid plexus** contains a thin layer of well-vascularized pia mater covered by cuboidal ependymal cells (Figure 12b). The function of the choroid plexus is to remove water from blood and release it as the **CSF**. **CSF** is clear, contains **Na+, K+,** and **Cl^-** ions but very little protein, and its only cells are normally very sparse lymphocytes. It is produced continuously and it completely fills the **ventricles**, the central canal of the **spinal cord**, and other special regions. It provides the ions required for **CNS** neuronal activity and in **the arachnoid** serves to help absorb mechanical shocks.

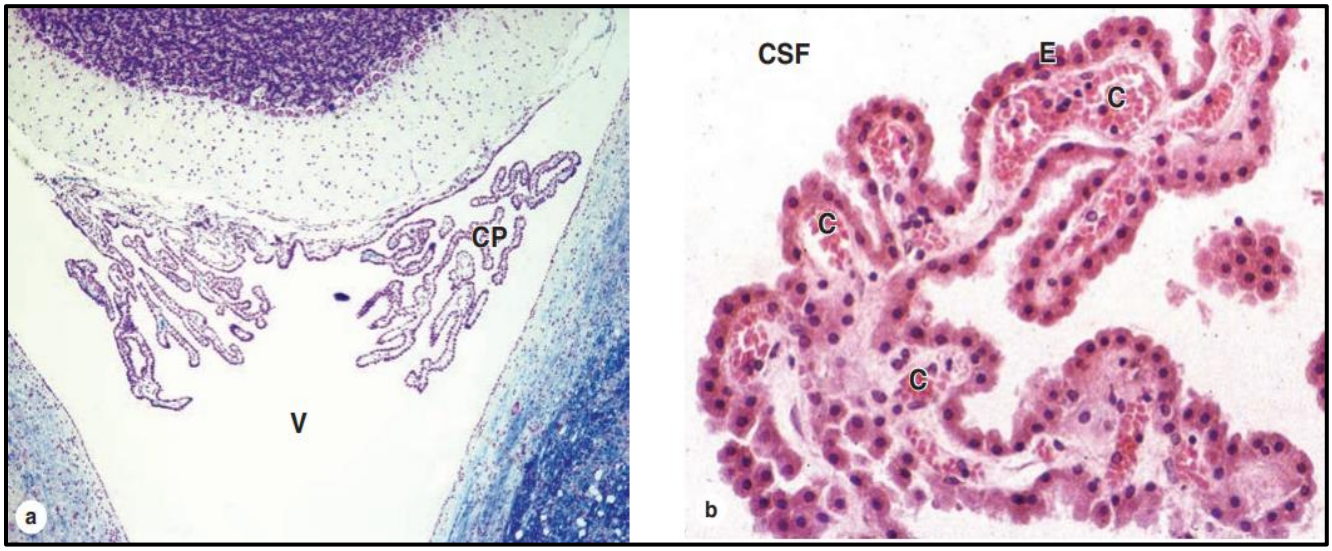


Figure 12: Choroid plexus. (a) Section of the bilateral choroid plexus (CP) projecting into the fourth ventricle (V) near the cerebellum (X12; Kluver-Barrera stain). (b) At higher magnification each fold of choroid plexus is seen to be well-vascularized with large capillaries (C) and covered by a continuous layer of cuboidal ependymal cells (E) (X150).

Peripheral Nervous System

The main components of the **peripheral nervous system (PNS)** are the **nerves**, **ganglia**, and **nerve endings**. Peripheral nerves are bundles of nerve fibers (axons) individually surrounded by **Schwann cells** and **connective tissue**.

➢ Nerve Fibers

Nerves are analogous to tracts in the CNS, containing axons enclosed within sheaths of glial cells specialized to facilitate axonal function. In peripheral nerves, axons are sheathed by Schwann cells or neurolemmocytes. The sheath may or may not form myelin around the axons, depending on their diameter.

❖ Myelinated Fibers

As axons of large diameter grow in the **PNS**, they are engulfed along their length by a series of differentiating neurolemmocytes and become myelinated nerve fibers. The figure 13 shows the steps for the **myelination of PNS axons**.

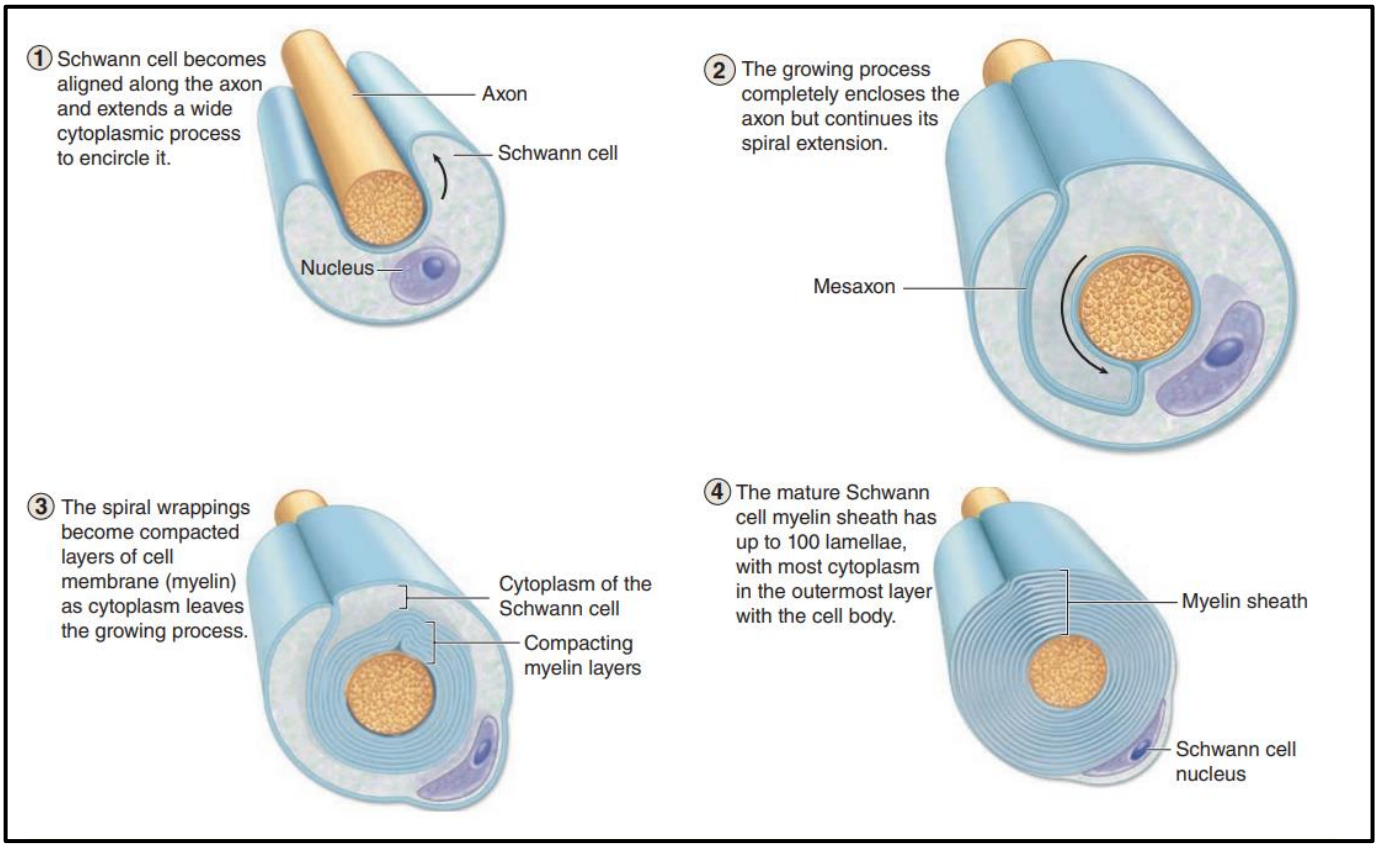


Figure 13: Myelination of large-diameter PNS axons.

❖ Unmyelinated Fibers

The smallest diameter axons of peripheral nerves are still enveloped within simple folds of Schwann cells (Figure 14). These very small unmyelinated fibers do not however undergo multiple wrapping to form a myelin sheath. The following figure shows the steps for the **dysmyelination of PNS axons**.

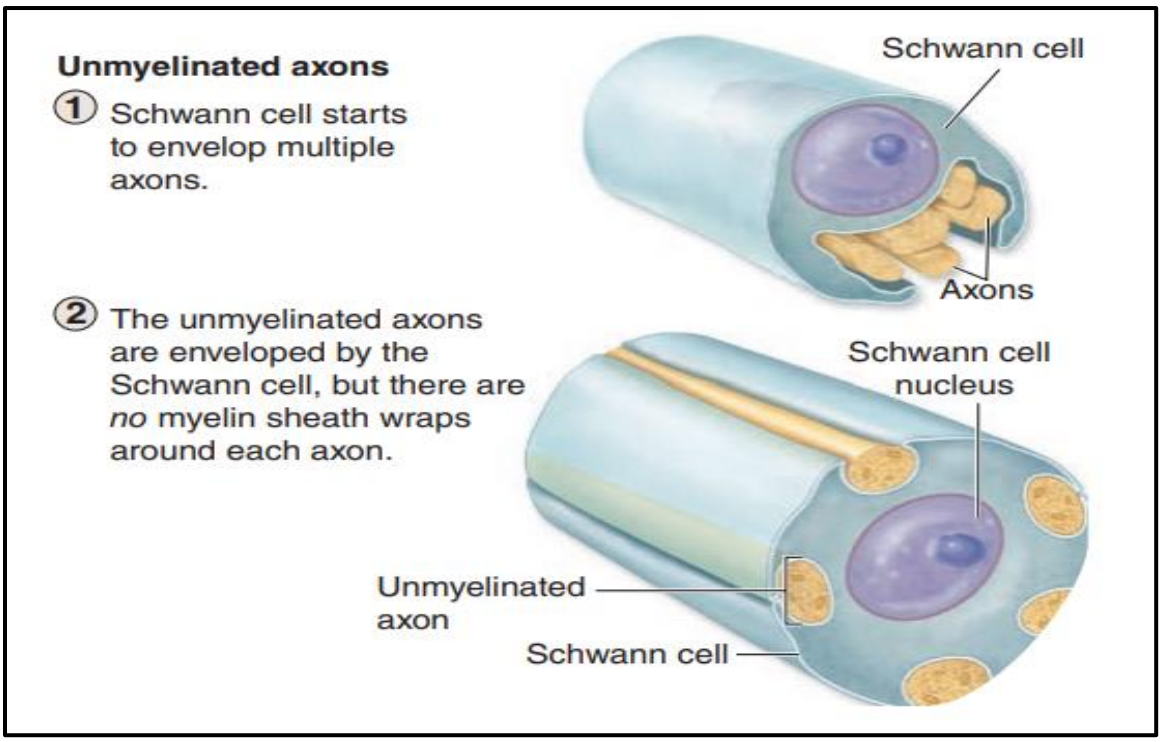


Figure 14: Unmyelinated nerves.

Nervous regulation in the peripheral nervous system includes the following, As shown in the figures 15 and 16:

- Peripheral nerves consist of axons from motor neurons (in **the spinal cord**), sensory neurons, and autonomic neurons (in ganglia); all the axons are enclosed within a series of Schwann cells, but only large (myelinated) axons have myelin sheaths and nodes of Ranvier.
- Endoneurium is a thin connective tissue layer immediately surrounding Schwann cells in peripheral nerves, containing a few nonfenestrated capillaries and much reticulin.

- Groups of axons (with **Schwann cells and endoneurium**) are surrounded by perineurium, consisting of layered, squamous fibroblastic cells joined by tight junctions to make a blood-nerve barrier.
- In large peripheral nerves, groups of axons are subdivided as **fascicles**, each of which is surrounded by **perineurium**.
- Surrounding the **perineurium** is a thick, outermost layer of dense irregular connective tissue, the **epineurium**.

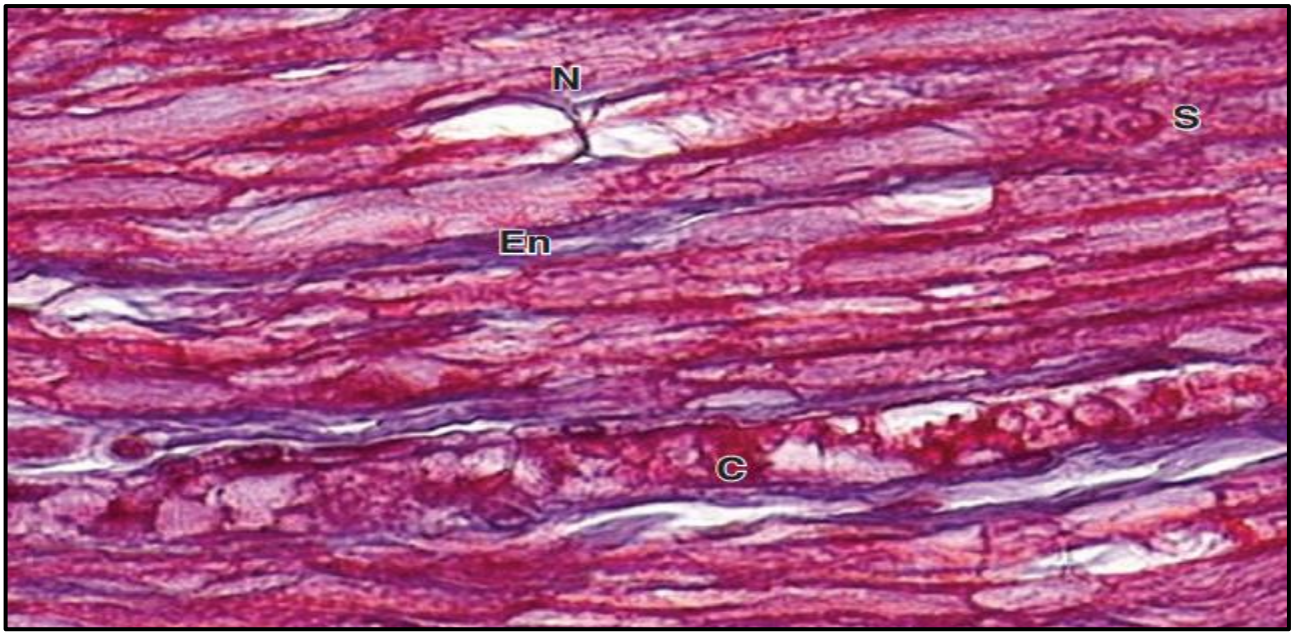
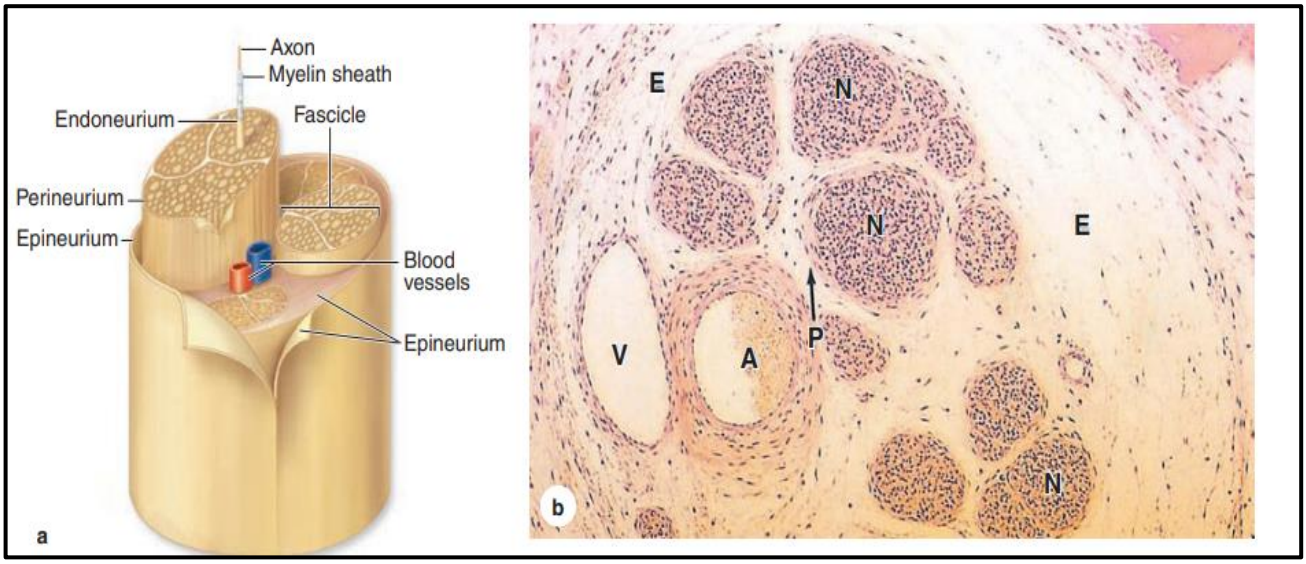


Figure 15: Node of Ranvier and endoneurium. A longitudinally oriented nerve shows one node of Ranvier (N). Collagen of the sparse endoneurium (En), blue in this trichrome stain, surrounds the Schwann cells and a capillary (C). At least one Schwann cell nucleus (S) is also clearly seen (X400; Mallory trichome).



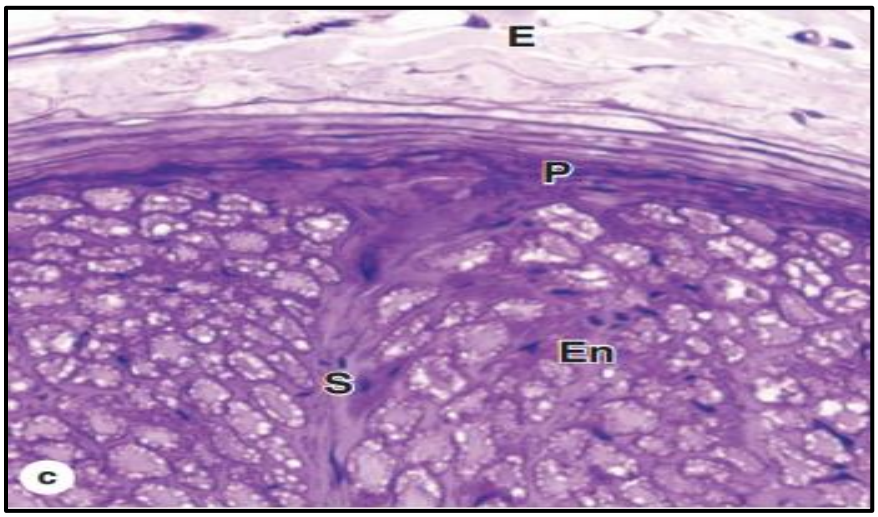


Figure 16: Peripheral nerve connective tissue: Epi-, peri-, and endoneurium. (a) The diagram shows the relationship among these three connective tissue layers in large peripheral nerves. (b) The micrograph shows a small vein (V) and artery (A) in the deep epineurium (E). Nerve fibers (N) are bundled in fascicles. Each fascicle is surrounded by the perineurium (P), consisting of a few layers of unusual squamous fibroblastic cells that are all joined at the peripheries by tight junctions. The resulting blood-nerve barrier helps regulate the microenvironment inside the fascicle. Axons and Schwann cells are in turn surrounded by a thin layer of endoneurium (X140; H&E). (c) As shown here and in the diagram, septa (S) of connective tissue often extend from the perineurium into larger fascicles. The endoneurium (En) and lamellar nature of the perineurium (P) are also shown at this magnification, along with some adjacent epineurium (E) (X200; PT).

Ganglia

Ganglia are typically ovoid structures containing neuronal cell bodies and their surrounding glial satellite cells supported by delicate connective tissue and surrounded by a denser capsule. Because they serve as relay stations to transmit nerve impulses, at least one nerve enters and another exits from each ganglion. The direction of the nerve impulse determines whether the ganglion will be a sensory or an autonomic ganglion.

➢ Sensory Ganglia

Sensory ganglia receive afferent impulses that go to the **CNS**. Sensory ganglia are associated with both cranial nerves (cranial ganglia) and the dorsal roots of the spinal nerves (spinal ganglia). The large neuronal cell bodies of ganglia (Figure 17) are associated with thin, sheetlike extensions of small glial satellite cells (Figures 3b and 7). Sensory ganglia are supported by a distinct connective tissue capsule and an internal framework continuous with the connective tissue layers of the nerves.

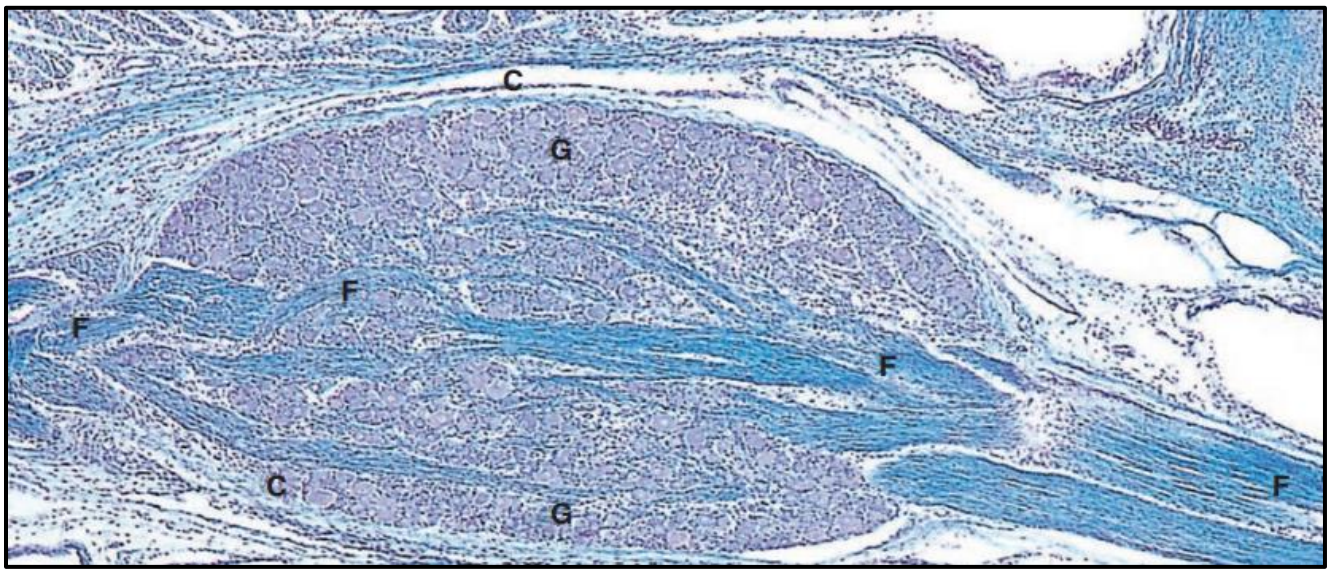


Figure 17: Ganglia. A sensory ganglion (G) has a distinct connective tissue capsule (C) and internal framework continuous with the epineurium and other components of peripheral nerves, except that no perineurium is present and that there is no blood-nerve barrier function. Fascicles of nerve fibers (F) enter and leave these ganglia (X56; Kluver-Barrera stain).

➢ Autonomic Ganglia

Autonomic nerves effect the activity of smooth muscle, the secretion of some glands, heart rate, and many other involuntary activities by which the body maintains a constant internal environment (**homeostasis**).

Autonomic ganglia are small bulbous dilations in autonomic nerves, usually with multipolar neurons. Some are located within certain organs, especially in the walls of the digestive tract, where they constitute the intramural ganglia.

The capsules of these ganglia may be poorly defined among the sacral portion of the spinal cord. Sympathetic second neurons are located in small ganglia along the vertebral column, while second neurons of the parasympathetic series are found in very small ganglia always located near or within the effector organs, for example in the walls of the stomach and intestines. **Parasympathetic ganglia** may lack distinct capsules altogether, **perikarya** and associated **satellite cells** simply forming a loosely organized plexus within the surrounding connective tissue.

Neural Plasticity & Regeneration

Certain regions of the **CNS**, such as near the **ependyma**, retain rare neural stem and progenitor cells that allow some replacement of neurons throughout life; **neural plasticity** involving formation and remodeling of synaptic connections is also prevalent throughout life. The complexity and distances of the neuronal and glial interconnections

with the **CNS** make regeneration and restoration of function within this tissue after major injury very difficult. The more simply organized peripheral nerves have better capacity for axonal regeneration, a process involving reactivation of the **perikaryon**, **Schwann cells, and macrophages**.

Lecture 15-18: The Digestive System and Organs Associated with the Digestive Tract

Part I: The Digestive System

The digestive system consists of **the digestive tract**-oral cavity, esophagus, stomach, small and large intestines, and anus, and its associated glands, salivary glands, liver, and pancreas. Also called the **gastrointestinal (GI) tract** or **alimentary canal**, its function is to obtain molecules from the ingested food that are necessary for the maintenance, growth, and energy needs of the body. During digestion proteins, complex carbohydrates, nucleic acids, and fats are broken down into their small molecule subunits that are easily absorbed through the small intestine lining. Most water and electrolytes are absorbed in the large intestine. In addition, the inner layer of the entire digestive tract forms an important protective barrier between the content of the tract's lumen and the internal milieu of the body's connective tissue and vasculature.

Structures within the digestive tract allow the following:

- **Ingestion**, or introduction of food and liquid into the oral cavity.
- **Mastication**, or chewing, which divides solid food into digestible pieces.
- **Motility**, muscular movements of materials through the tract.
- **Secretion** of lubricating and protective mucus, digestive enzymes, acidic and alkaline fluids, and bile.
- **Hormone** release for local control of motility and secretion.
- **Chemical digestion** or enzymatic degradation of large macromolecules in food to smaller molecules and their subunits.
- **Absorption** of the small molecules and water into the blood and lymph.
- **Elimination** of indigestible, unabsorbed components of food.

General Structure of The Digestive Tract

All regions of the GI tract have certain structural features in common. The GI tract is a hollow tube with a lumen of variable diameter and a wall made up of four main layers: the **mucosa, submucosa, muscularis, and serosa**. Figure 1 shows a general overview of these four layers; key features of each layer are summarized here.

- The **mucosa** consists of an **epithelial lining**; an underlying **lamina propria** of loose connective tissue rich in blood vessels, lymphatics, lymphocytes, smooth muscle cells, and often containing small glands; and a thin layer of smooth muscle called the **muscularis mucosae** separating mucosa from submucosa and

allowing local movements of the mucosa. The mucosa is also frequently called a **mucous membrane**.

- The **submucosa** contains denser connective tissue with larger blood and lymph vessels and the **submucosal (Meissner) plexus** of autonomic nerves. It may also contain glands and significant lymphoid tissue.
- The thick **muscularis** (or muscularis externa) is composed of smooth muscle cells organized as two or more sublayers.

closer to the lumen, the fiber orientation is generally circular; in the external sublayer it is longitudinal. The connective tissue between the muscle sublayers contains blood and lymph vessels, and the **myenteric (Auerbach) nerve plexus** of many autonomic neurons aggregated into small ganglia and interconnected by pre- and postganglionic nerve fibers. This and the submucosal plexus together comprise the **enteric nervous system** of the digestive tract. Contractions of the muscularis, which mix and propel the luminal contents forward, are generated and coordinated by the myenteric plexus.

- The **serosa**, a thin sheet of loose connective tissue, rich in blood vessels, lymphatics, and adipose tissue, and covered with a simple squamous covering **epithelium** or **mesothelium**, is the outermost layer of the digestive tract located within the abdominal cavity. The serosa of the small and large intestines is continuous with portions of the mesentery, a large fold of adipose connective tissue, covered on both sides by mesothelium, that suspends the intestines and is continuous with the **peritoneum**, the serous membrane lining the abdominal cavity. The esophagus is not suspended in a cavity but bound directly to adjacent structures and therefore lacks a serosa, having instead a thick **adventitia**, a layer of connective tissue continuous with that of surrounding tissues.

The numerous free immune cells and lymphoid nodules in the mucosa and submucosa constitute the MALT. The digestive tract normally contains thousands of microbial species, including both useful inhabitants of the gut as well as potential pathogens ingested with food and drink. The mucosa-associated immune defense system provides an essential backup to the thin physical barrier of the epithelial lining. Located just below the epithelium, the lamina propria is rich with macrophages and lymphocytes, many for production of IgA antibodies. Such antibodies undergo transcytosis into the intestinal lumen bound to the secretory protein produced by the epithelial cells. This IgA complex resists proteolysis by digestive enzymes and provides important protection against specific viral and bacterial pathogens.

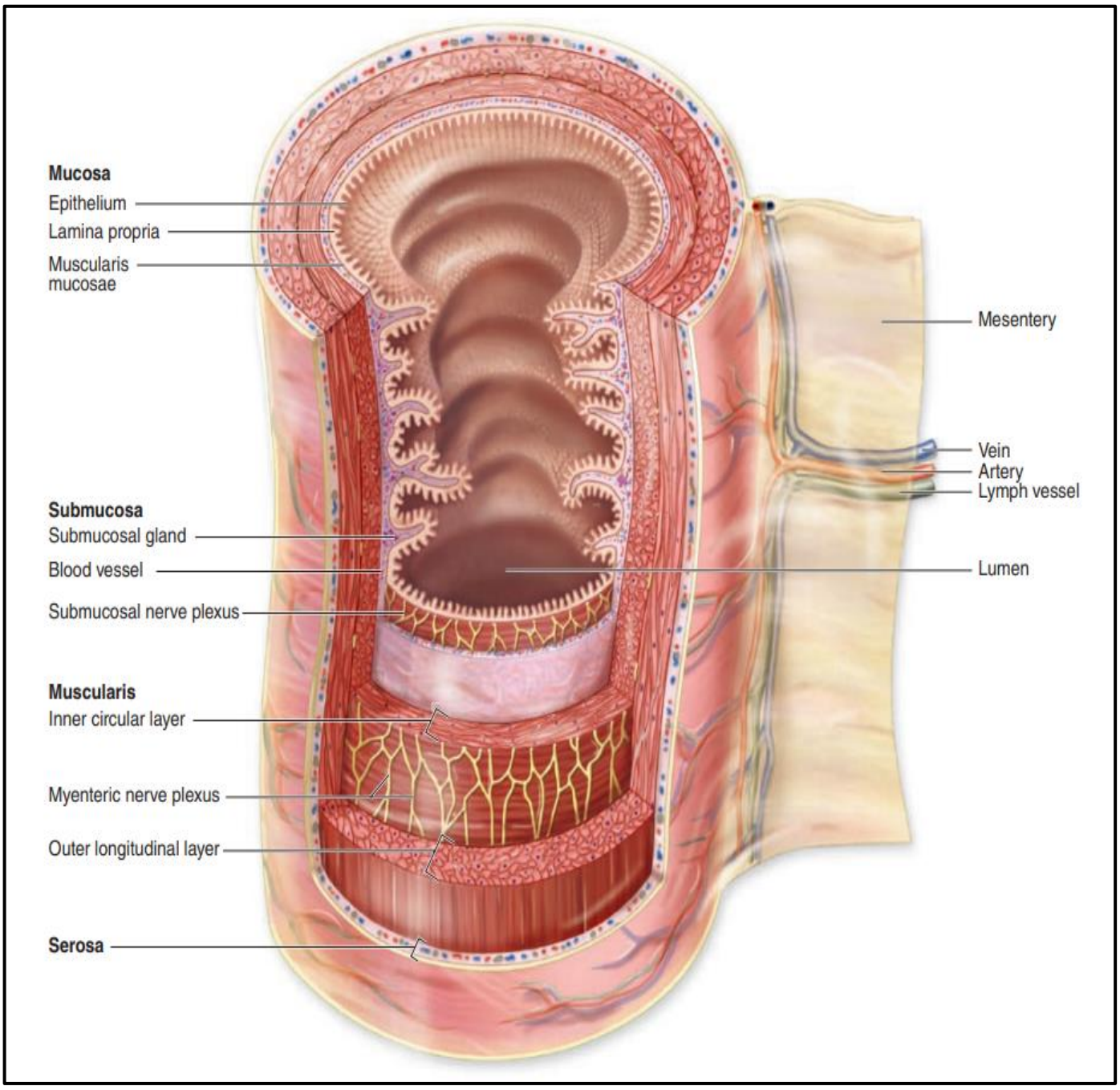


Figure 1: Major layers and organization of the digestive tract.

ORAL CAVITY

The oral cavity is lined with stratified squamous epithelium, which may be keratinized, partially keratinized, or nonkeratinized depending on the location. The keratinized cell layers resist damage from abrasion and are best developed in the **masticatory mucosa** on the gingiva (gum) and hard palate. The lamina propria in these regions rests directly on the periosteum of underlying bone. Nonkeratinized squamous epithelium predominates in the lining mucosa over the soft palate, cheeks, the floor of

the mouth, and the pharynx, the posterior region of the oral cavity leading to the esophagus. submucosa containing many minor salivary glands, which secrete continuously to keep the mucosal surface wet, and diffuse lymphoid tissue. Throughout the oral cavity, the epithelium contains transient antigen-presenting cells and rich sensory innervation.

The well-developed core of striated muscle in the **lips**, or labia, (Figure 2) makes these structures highly mobile for ingestion, speech, and other forms of communication. Both lips have three differently covered surfaces:

- The internal mucous surface has lining mucosa with a thick, nonkeratinized epithelium and many minor labial salivary glands.
- The red **vermilion zone** of each lip is covered by very thin keratinized stratified squamous epithelium and is transitional between the oral mucosa and skin. This region lacks salivary or sweat glands and is kept moist with saliva from the tongue.
- The outer surface has thin skin, consisting of epidermal and dermal layers, sweat glands, and many hair follicles with sebaceous glands.

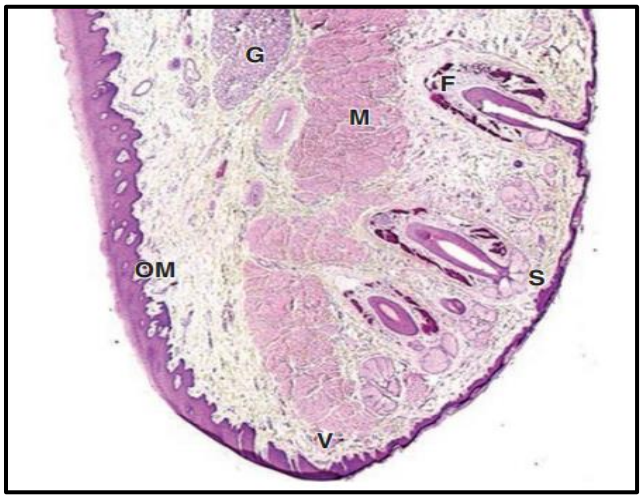


Figure 2: Lip. Low-magnification micrograph of a lip section showing one side covered by typical oral mucosa (OM), the opposite side covered by skin (S) containing hair follicles (F) and associated glands. Between the oral portion of the lips and normal skin is the vermilion zone (V). Internally, the lips contain much striated muscle (M) and many minor salivary glands (G) (X10; H&E).

Tongue

The tongue is a mass of striated muscle covered by mucosa, which manipulates ingested material during mastication and swallowing. The muscle fibers are oriented in all directions, allowing a high level of mobility. Connective tissue between the small fascicles of muscle is penetrated by the lamina propria, which makes the mucous

membrane strongly adherent to the muscular core. The lower surface of the tongue is smooth, with typical lining mucosa. The dorsal surface is irregular, having hundreds of small protruding **papillae** of various types on its anterior two-thirds and the massed lingual tonsils on the posterior third, or root of the tongue (Figure 3).

The papillary and tonsillar areas of the lingual surface are separated by a V-shaped groove called the **sulcus terminalis**. The lingual papillae are elevations of the mucous membrane that assume various forms and functions. There are four types (Figure 3):

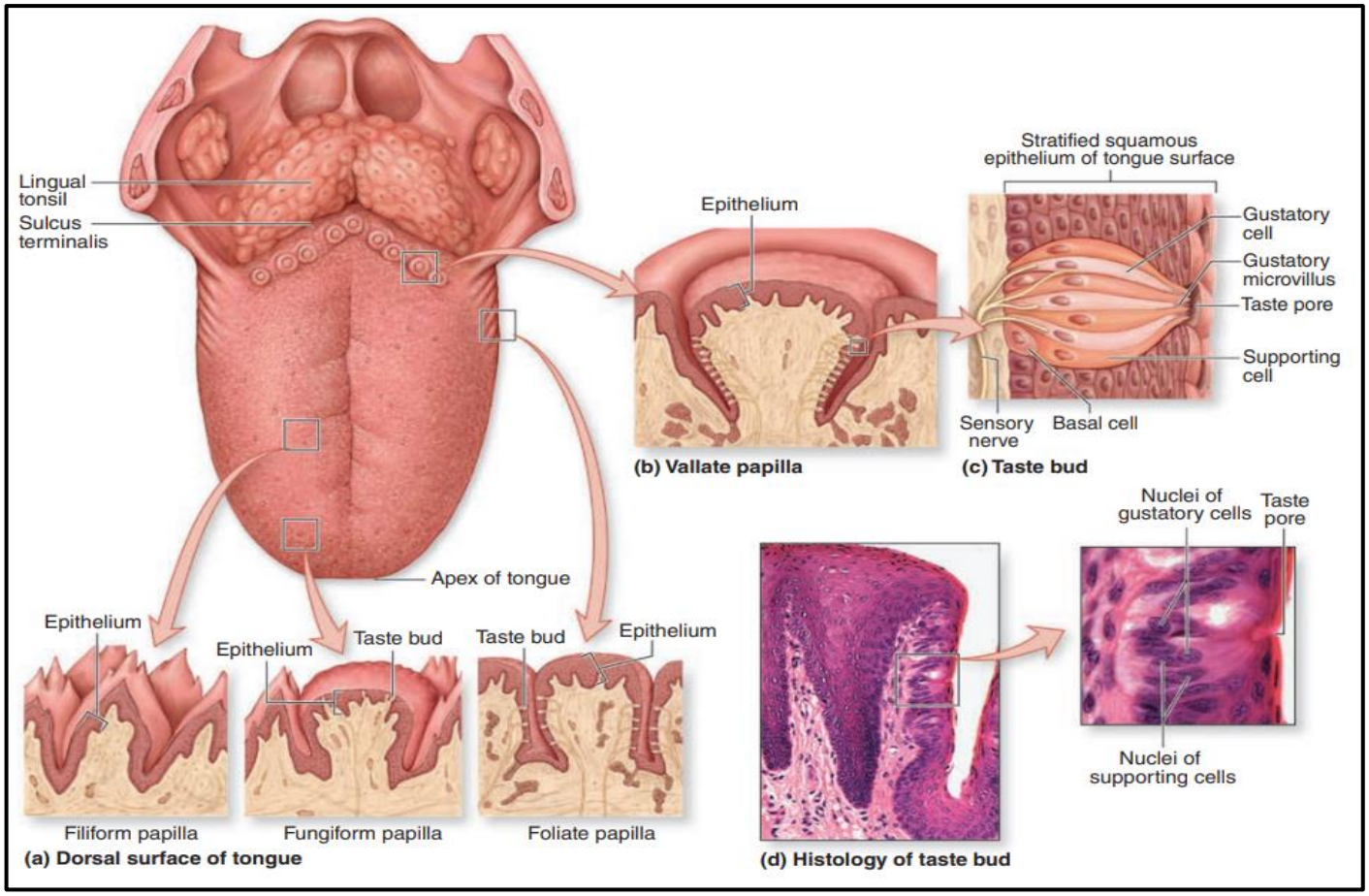


Figure 3: Tongue, lingual papillae, and taste buds.

- **Filiform papillae** are very numerous, have an elongated conical shape, and are heavily keratinized, which gives their surface a gray or whitish appearance. They provide rough surface that facilitates movement of food during chewing.
- **Fungiform papillae** are much less numerous, lightly keratinized, and interspersed among the filiform papillae. They are mushroom-shaped with well-vascularized and innervated cores of lamina propria.

- **Foliate papillae** consist of several parallel ridges on each side of the tongue, anterior to the sulcus terminalis, but are rudimentary in humans, especially older individuals.
- **Vallate (or circumvallate) papillae** are the largest papillae, with diameters of 1-3 mm. Eight to twelve vallate papillae are normally aligned just in front of the terminal sulcus. Ducts of several small, serous **salivary (von Ebner) glands** empty into the deep, moatlike groove surrounding each vallate papilla.

Taste buds are ovoid structures within the stratified epithelium on the tongue's surface (Figure 3) Approximately 250 taste buds are present on the lateral surface of each vallate papilla, with many others present on fungiform and foliate (but not the keratinized filiform) papillae. They are not restricted to papillae and are also widely scattered elsewhere on the dorsal and lateral surfaces of the tongue, where they are also continuously flushed by numerous minor salivary glands.

A taste bud has 50-100 cells, about half of which are elongated **gustatory (taste) cells**, which turn over with a 7- to 10-day life span. Other cells present are slender **supportive cells**, immature cells, and slowly dividing basal **stem cells** that give rise to the other cell types. The base of each bud rests on the basal lamina and is entered by afferent sensory axons that form synapses with the gustatory cells. At the apical ends of the gustatory cells, microvilli project toward a 2-μm-wide opening in the structure called the **taste pore**. Molecules (tastants) dissolved in saliva contact the microvilli through the pore and interact with cell surface taste receptors (Figure 3). Taste buds detect at least five broad categories of tastants: sodium ions (salty); hydrogen ions from acids (sour); sugars and related compounds (sweet); alkaloids and certain toxins (bitter); and amino acids such as glutamate and aspartate (umami; Jap. umami, savory).

Esophagus

The **esophagus** is a muscular tube, about 25-cm long in adults, which transports swallowed material from the pharynx to the stomach. The four layers of the GI tract (Figure 4) first become well-established and clearly seen in the esophagus.

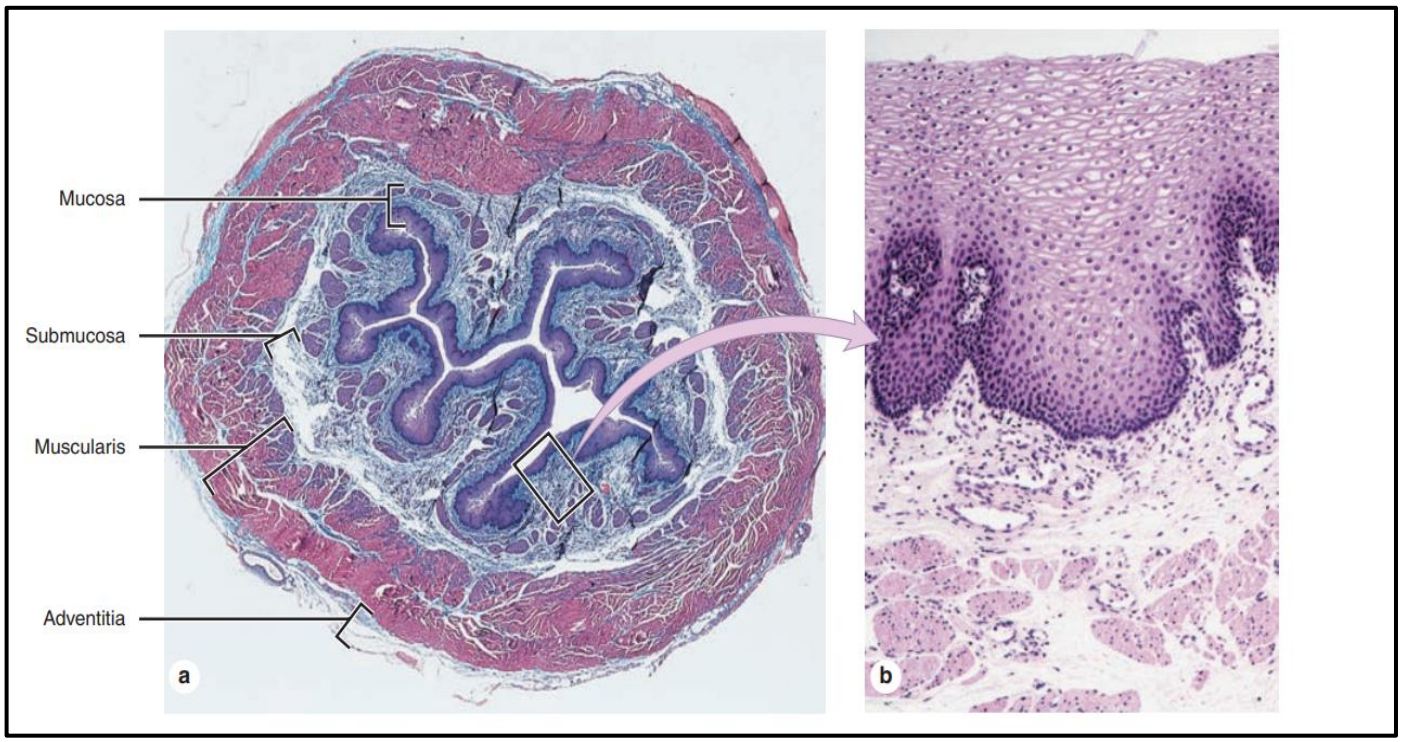


Figure 4: Esophagus. (a) In cross section the four major layers of the GI tract are clearly seen. The esophageal mucosa is folded longitudinally, with the lumen largely closed (X10; H&E). (b) Higher magnification of the mucosa shows the stratified squamous epithelium (E), the lamina propria (LP) with scattered lymphocytes, and strands of smooth muscle in the muscularis mucosae (MM) (X65; H&E).

The esophageal mucosa has nonkeratinized stratified squamous epithelium, and the submucosa contains small mucus secreting glands, **the esophageal glands**, which lubricate and protect the mucosa (Figure 5a). Near the stomach the mucosa also contains groups of glands, the **esophageal cardiac glands**, which secrete additional mucus.

Swallowing begins with voluntary muscle action but finishes with involuntary peristalsis. In approximately the upper one-third of the esophagus, the muscularis is exclusively skeletal muscle like that of the tongue. The middle portion of the esophagus has a combination of skeletal and smooth muscle fibers (Figure 5b), and in the lower third the muscularis is exclusively smooth muscle. Only the distal 1-2 cm of the esophagus, in the peritoneal cavity, is covered by serosa; the rest is enclosed by the loose connective tissue of the adventitia, which blends into the surrounding tissue.

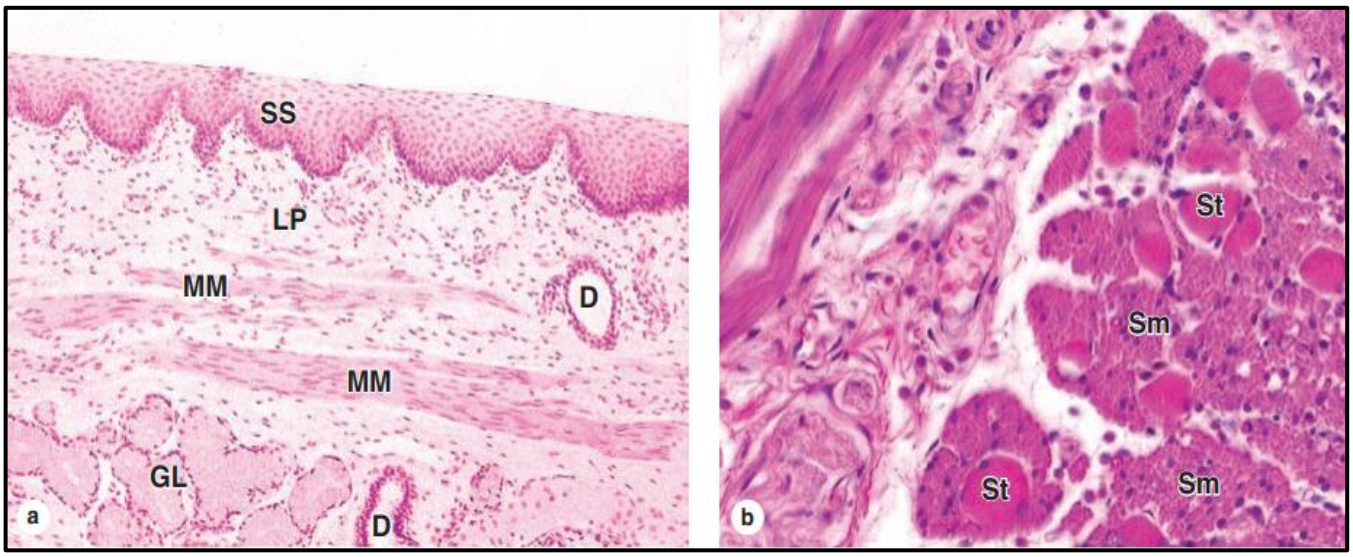


Figure 5: Esophagus. (a) Longitudinal section of esophagus shows mucosa consisting of nonkeratinized stratified squamous epithelium (SSE), lamina propria (LP), and smooth muscles of the muscularis mucosae (MM). Beneath the mucosa is the submucosa containing esophageal mucous glands (GL) that empty via ducts (D) onto the luminal surface (X40; H&E). (b) Transverse section showing the muscularis halfway along the esophagus reveals a combination of large skeletal or striated muscle fibers (St) and smooth muscle fibers (Sm) in the outer layer, which is cut transversely here (X200; H&E).

Stomach

The stomach is a greatly dilated segment of the digestive tract whose main functions are: **1.** to continue the digestion of carbohydrates initiated by the amylase of saliva **2.** to add an acidic fluid to the ingested food and mixing its contents into a viscous mass called **chyme**. **3.** to begin digestion of triglycerides by a lipase, and **4.** to promote the initial digestion of proteins with the enzyme **pepsin**.

Four major regions make up the stomach: the cardia, fundus, body, and pylorus (Figure 6). The **cardia** is a narrow transitional zone, 1.5-3 cm wide, between the esophagus and the stomach; the **pylorus** is the funnel-shaped region that opens into the small intestine. Both these regions are primarily involved with mucus production and are histogically similar. The much larger **fundus** and **body** regions are identical in microscopic structure and are the sites of gastric glands releasing acidic gastric juice. The mucosa and submucosa of the empty stomach have large, longitudinally directed folds called **rugae**, which flatten when the stomach fills with food. The wall in all regions of the stomach is made up of all four major layers (Figures 6 and 7).

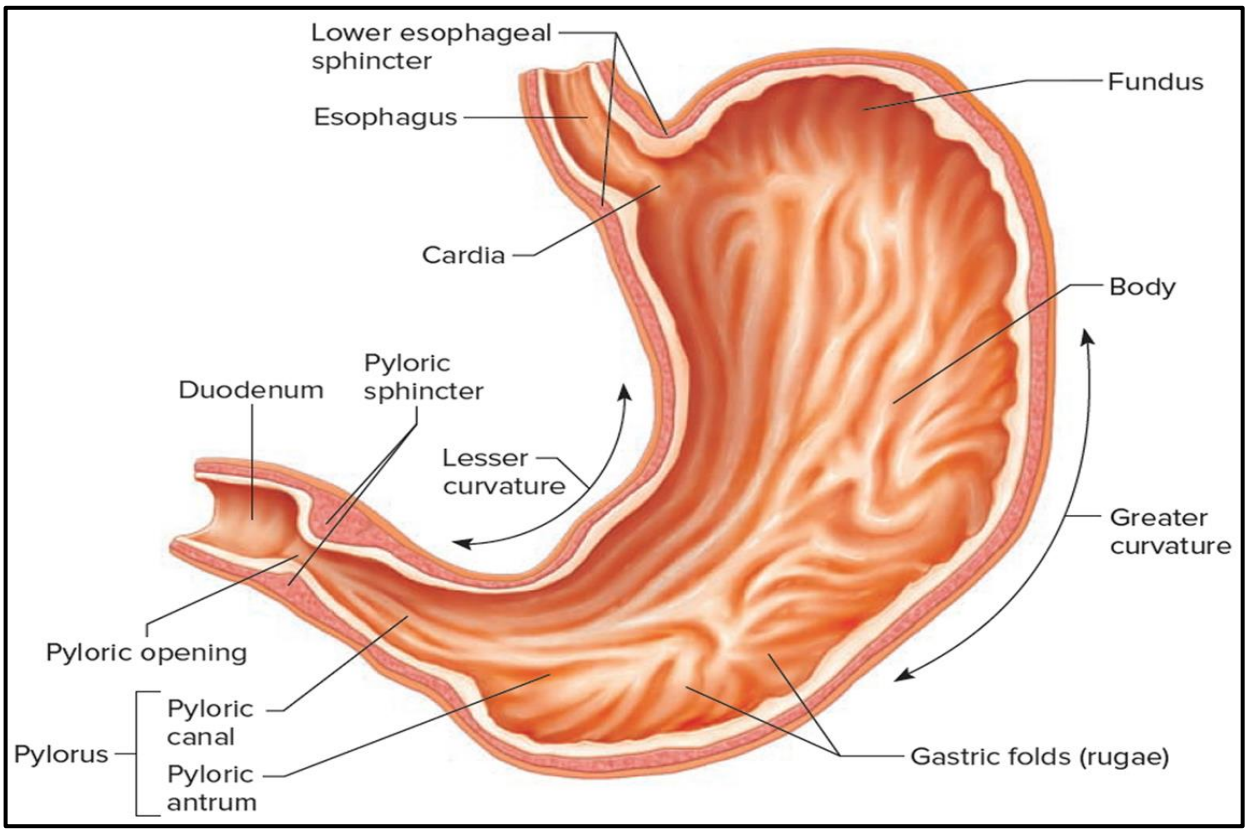


Figure 6: Stomach. Stomach regions, anterior view.

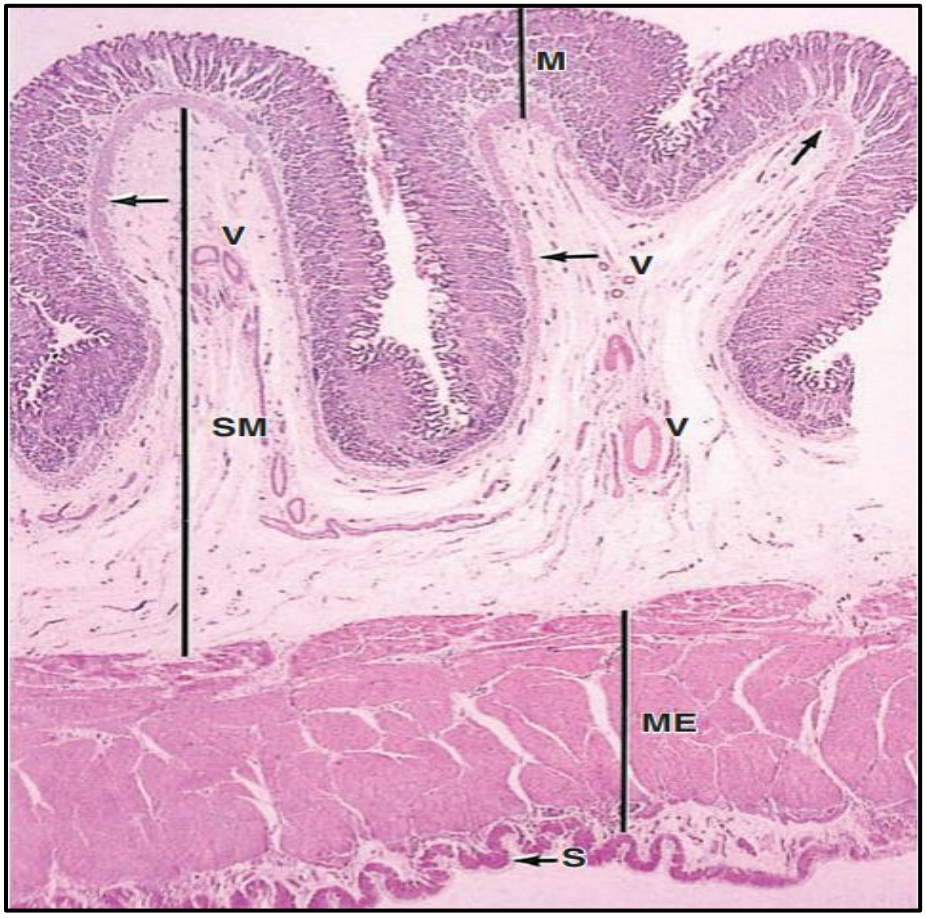


Figure 7: Wall of the stomach with rugae. A low-magnification micrograph of the stomach wall at the fundus shows the relative thickness of the four major layers: the mucosa (M), the submucosa (SM), the muscularis externa (ME), and the serosa (S). Two rugae (folds) cut transversely and consisting of mucosa and submucosa are included. The mucosa is packed with branched tubular glands penetrating the full thickness of the lamina propria so that this sublayer cannot be distinguished at this magnification. The muscularis mucosae (arrows), immediately beneath the basal ends of the gastric glands, is shown. The submucosa is largely loose connective tissue, with blood vessels (V) and lymphatics (X12; H&E).

➢ Mucosa

Changing abruptly at the esophagogastric junction, the mucosal surface of the stomach is a simple columnar epithelium that invaginates deeply into the lamina propria. The invaginations form millions of **gastric pits**, each with an opening to the stomach lumen (figure 8). The **surface mucous cells** that line the lumen and gastric pits secrete a thick and highly viscous mucous layer, which protects the mucosa from both effects of intraluminal food and the effects of stomach acid.

The gastric pits lead to long, branched, tubular glands that extend through the full thickness of the lamina propria. **Stem cells** for the epithelium lining the glands, pits, and stomach lumen are found in a narrow segment between each gastric pit and the gastric glands. The pluripotent stem cells divide asymmetrically, producing progenitor cells for

all the other epithelial cells. Some of these move upward to replace surface mucous cells, which have a turnover time of 4-7 days.

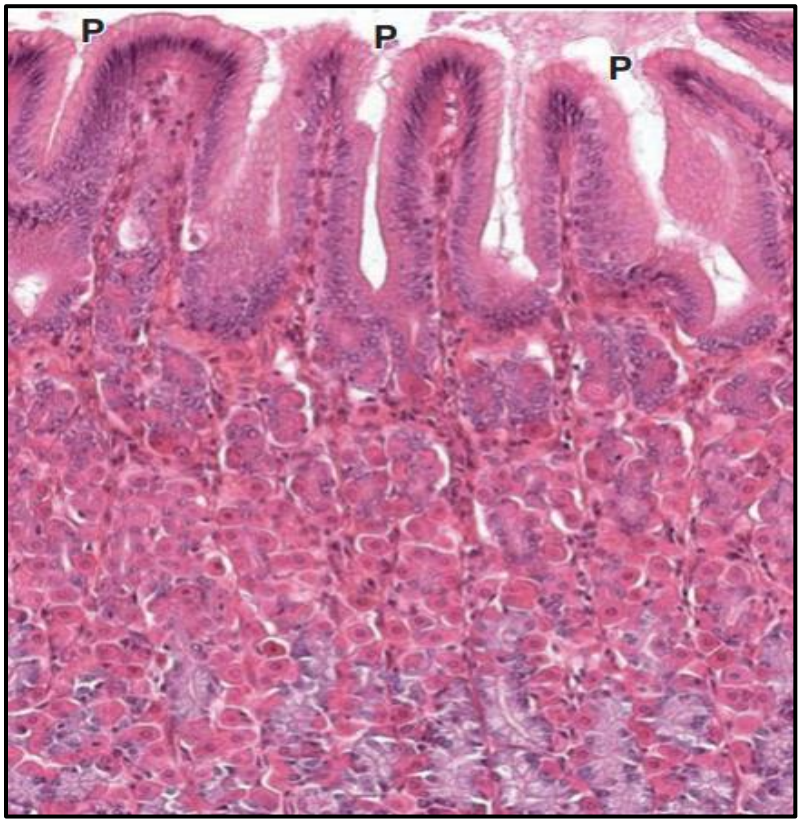


Figure 8: Gastric pits and glands. A section of the same lining shows that these surface mucous cells are part of a simple columnar epithelium continuous with the lining of the pits (P). Each pit extends into the lamina propria and then branches into several tubular glands. These glands coil and fill most of the mucosa. Around the various cells of the closely packed gastric glands are cells, capillaries, and small lymphatics of the connective tissue lamina propria (X200; H&E).

In the fundus and body the **gastric glands** themselves fill most of the mucosa, with several such glands formed by branching at the isthmus or neck of each gastric pit. Secretory epithelial cells of the gastric glands are distributed unevenly and release products that are key to the stomach's functions.

These cells are of four major types and important properties of each are as follows Figure 9:

- **Mucous neck cells** include immature precursors of the surface mucous cells but produce less alkaline mucus while migrating up into the gastric pits.
- **Parietal cells** are large cells with many mitochondria and large intracellular canaliculi for production of HCl in the gastric secretion; they also secrete intrinsic factor for vitamin B12 uptake.

- **Chief (zymogenic) cells**, clustered mainly in the lower half of the gastric glands, secrete the protein pepsinogen that is activated by the low pH in the lumen to form the major protease pepsin.
- **Enteroendocrine cells** are scattered epithelial cells of the diffuse neuroendocrine system, which release peptide hormones to regulate activities of neighboring tissues during food digestion.

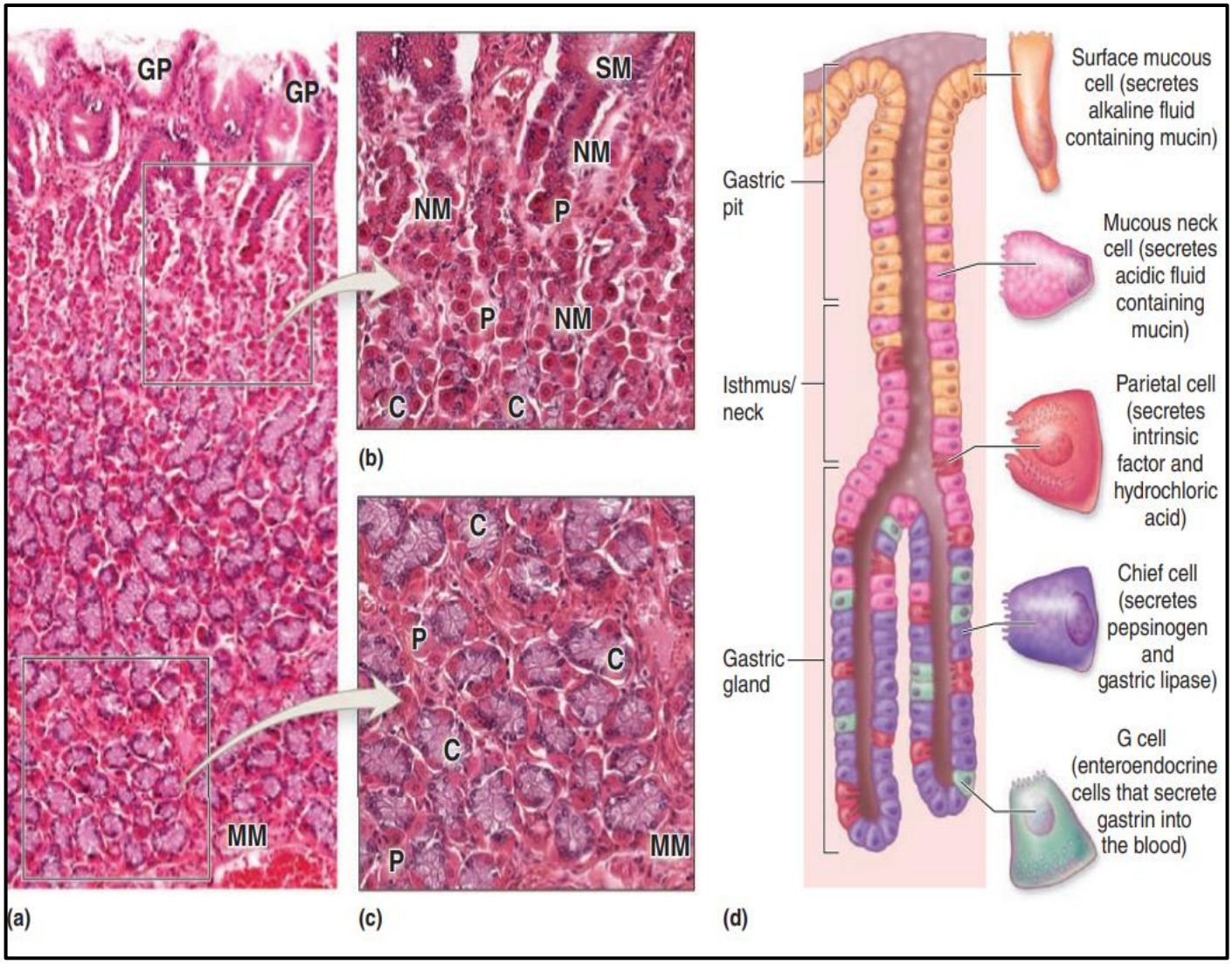


Figure 9: Gastric glands. Throughout the fundus and body regions of the stomach, the gastric pits lead to gastric glands with various cell types.

(a) The long, coiled gastric glands penetrate the complete thickness of the mucosa, from the gastric pits (GP) to the muscularis mucosae (MM).

(b) In the neck of a gastric gland, below the surface mucous cells (SM) lining the gastric pit, are small mucous neck cells (MN), scattered individually or clustered among parietal cells (P) and stem cells that give rise to all epithelial cells of the glands. The numerous parietal cells (P) are large distinctive cells often bulging from the tubules, with central nuclei surrounded by intensely eosinophilic cytoplasm with unusual ultrastructure. These cells produce HCl, and the numerous mitochondria required for this

process cause the eosinophilia. Chief cells (C) begin to appear in the neck region. Around these tubular glands are various cells and microvasculature in connective tissue.

(c) Near the muscularis mucosae (MM), the bases of these glands contain fewer parietal cells (P) but many more zymogenic chief cells (C). Chief cells are found in clusters, with basal nuclei and basophilic cytoplasm. From their apical ends chief cells secrete pepsinogen, the zymogen precursor for the major protease pepsin. Zymogen granules are often removed or stain poorly in routine preparations (Both X200; H&E).

(d) Diagram showing general morphology and functions of major gastric gland cells.

➢ Other Layers

The other major layers of the stomach wall are summarized in Figure 7. In all stomach regions the **submucosa** is composed of connective tissue with large blood and lymph vessels and many lymphoid cells, macrophages, and mast cells. The **muscularis** has three poorly defined layers of smooth muscle: an outer longitudinal layer, a middle circular layer, and an innermost oblique layer. Rhythmic contractions of the muscularis thoroughly mix ingested food and chyme with mucus, HCl, and digestive enzymes from the gastric mucosa. At the pylorus the middle layer is greatly thickened to form the **pyloric sphincter**. The stomach is covered by a thin **serosa**.

Small Intestine

The small intestine is the site where the digestive processes are completed and where the nutrients (products of digestion) are absorbed by cells of the epithelial lining. The small intestine is relatively long -approximately 5m- and consists of three segments: the **duodenum, jejunum**, and **ileum**. These segments have most histologic features in common.

➢ Mucosa

Viewed macroscopically, the lining of the small intestine shows a series of permanent circular folds (**plicae circulares**), consisting of mucosa and submucosa (Figures 10a and 11), which are best developed in the jejunum. Densely covering the entire mucosa of the small intestine are short mucosal outgrowths called villi that project into the lumen. These finger- or leaflike projections are covered by a simple columnar epithelium of absorptive cells called **enterocytes**, with many interspersed **goblet cells**. Each villus has a core of loose connective tissue that extends from the lamina propria and contains fibroblasts, smooth muscle fibers, lymphocytes and plasma cells, fenestrated capillaries, and a central lymphatic called a **lacteal**.

Between the villi are the openings of short tubular glands called **intestinal glands (or crypts of Lieberkühn)** and the epithelium of each villus is continuous with that of the intervening glands (Figure 10).

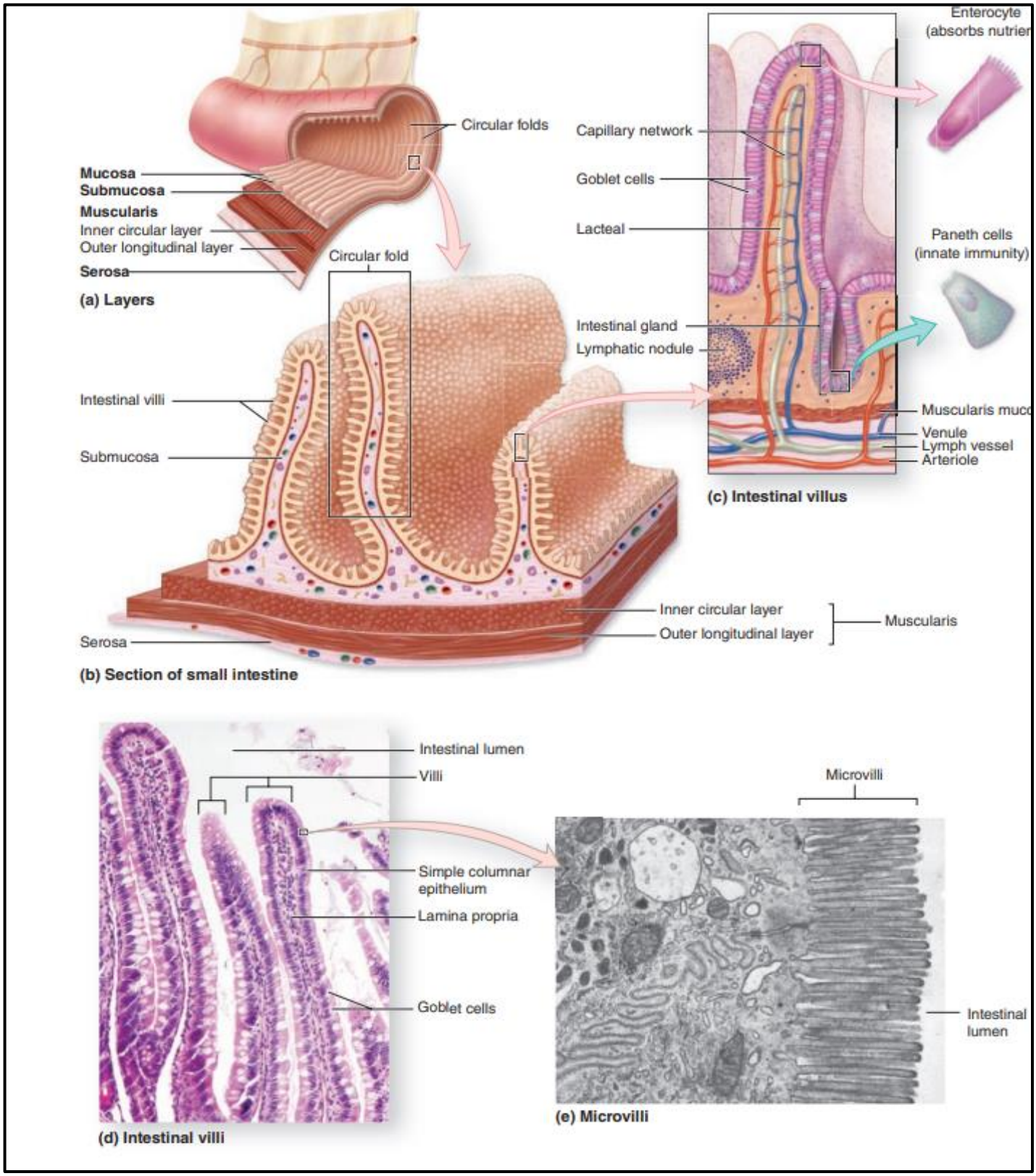


Figure 10: Absorptive surface of the small intestine.

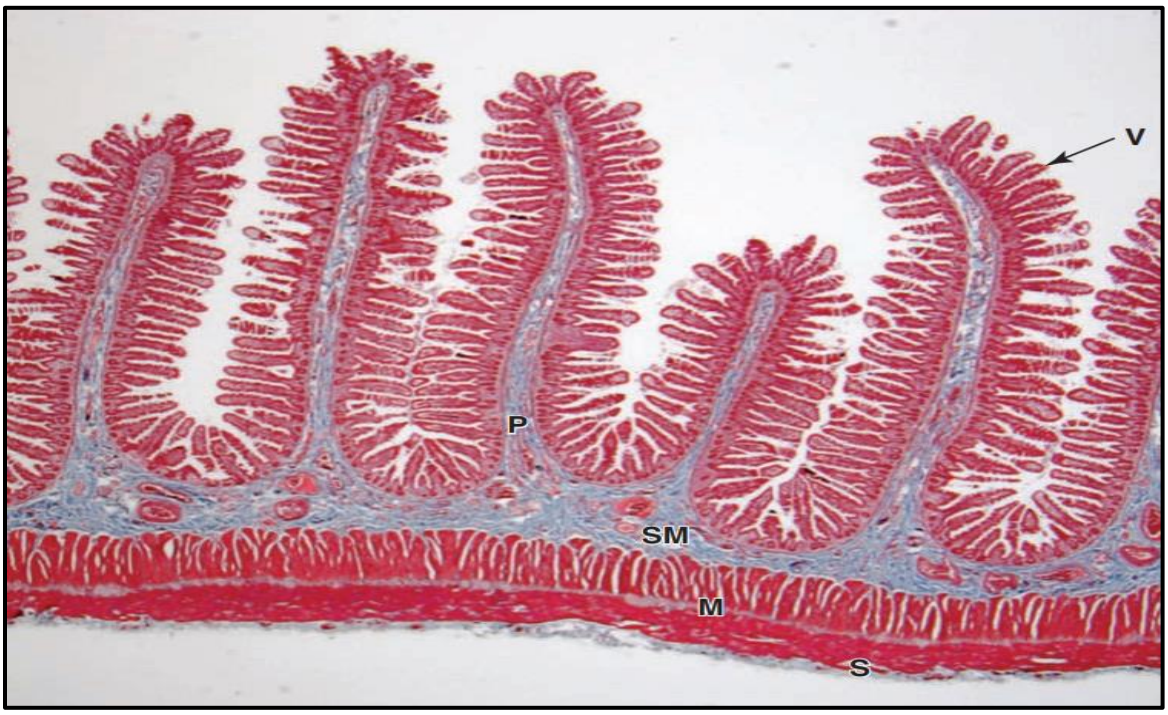


Figure 11: Circular folds (plicae circulares) of the jejunum. The mucosa and submucosa (SM) of the small intestine form distinct projecting folds called plicae (P), which encircle around the inner circumference and are best developed in the jejunum. On each fold the mucosa forms a dense covering of projecting structures called villi (V). In this longitudinal section the two layers of the muscularis (M) are clearly distinguished. The inner layer has smooth muscle encircling the submucosa; the outer layer runs lengthwise just inside the serosa (S), the gut's outer layer (X12; Masson trichrome).

The epithelium of the intestinal glands includes differentiating cells and pluripotent stem cells for all the cell types of the small intestine. These include the following:

- **Enterocytes**, the absorptive cells, are tall columnar cells, each with an oval nucleus located basally (Figure 12). The apical end of each enterocyte displays a prominent ordered region called the striated (or brush) border. Ultrastructurally the striated border is seen to be a layer of densely packed microvilli covered by glycocalyx through which nutrients are taken into the cells (Figure 10e).
- **Goblet cells** are interspersed among the absorptive enterocytes (Figures 10d and 12a,b). They secrete glycoprotein mucins, which are then hydrated to form mucus, whose main function is to protect and lubricate the lining of the intestine.

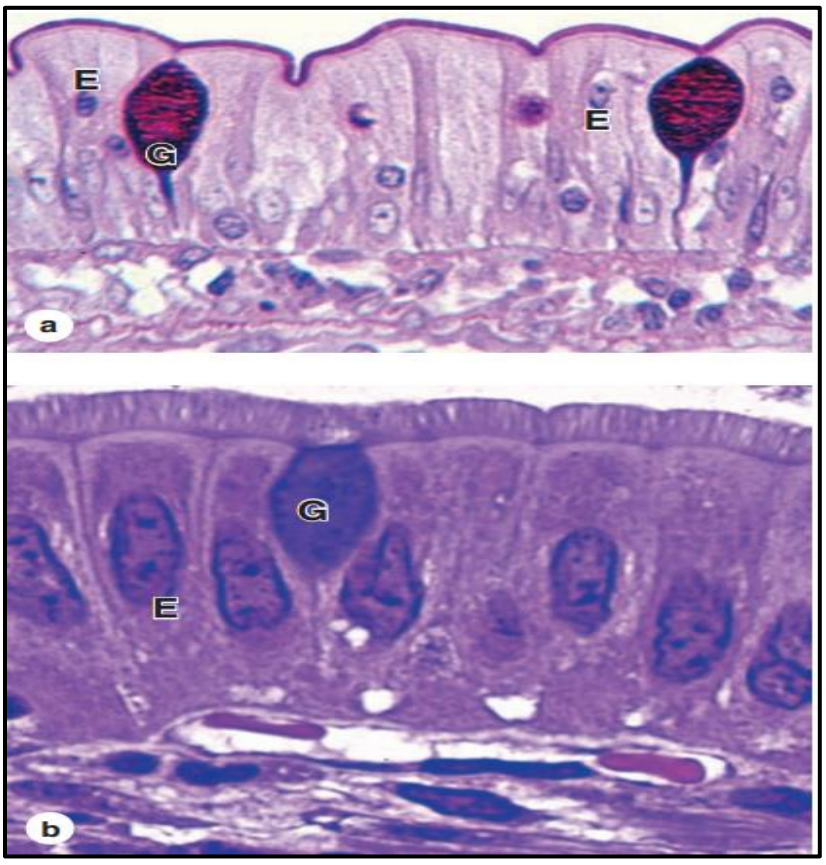


Figure 12: Cells covering the villi. (a) The columnar epithelium that covers intestinal villi consists mainly of the tall absorptive enterocytes (E). The apical ends of these cells are joined and covered by a brush border of microvilli. Covered by a brush border, along with the mucus-secreting goblet cells (G), stains with carbohydrate staining methods. Other cells of the epithelium are scattered enteroendocrine cells, which are difficult to identify in routine preparations, and various immune cells such as intraepithelial lymphocytes(X250; PAS-hematoxylin). (b) At higher magnification individual microvilli of enterocytes are better seen and the striated appearance of the border is apparent (X500).

- **Paneth cells**, located in the basal portion of the intestinal crypts below the stem cells, are exocrine cells with large, eosinophilic secretory granules in their apical cytoplasm (Figure 13). Paneth cell granules release lysozyme, phospholipase A2 , and hydrophobic peptides called **defensins**, all of which bind and break down membranes of microorganisms and bacterial cell walls. Paneth cells have an important role in innate immunity and in regulating the microenvironment of the intestinal crypts.

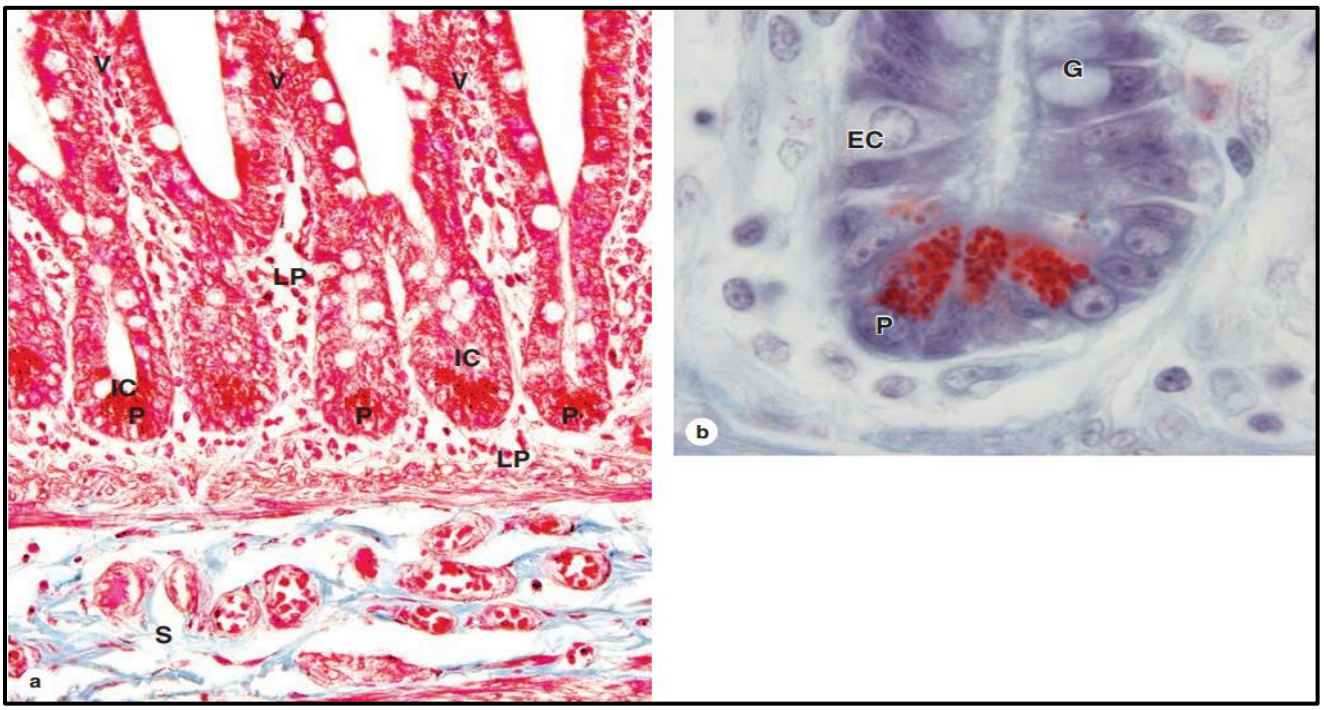


Figure 13: Intestinal crypts or glands, with Paneth cells. (a) Between villi (V) throughout the small intestine, the covering epithelium invaginates into the lamina propria (LP) to form short tubular glands called intestinal glands or intestinal crypts (IC). At the base of the crypts are many Paneth cells (P) with an innate immune function. The submucosa (S) has many lymphatics draining lacteals (X200; H&E). (b) Higher magnification at the base of an intestinal gland shows the typical eosinophilic granules of Paneth cells (P), along with an open-type enteroendocrine cell (EC) and a differentiating goblet cell (G) (X400; H&E).

- **Enteroendocrine cells** are present in varying numbers throughout the length of the small intestine, secreting various peptide hormones. Many of these are of the "open" type, in which the constricted apical end of the cell contacts the intestinal lumen and has chemoreceptors similar to those of taste buds, sampling levels of certain nutrients such as sugars to regulate hormone release basally (Figure 14).

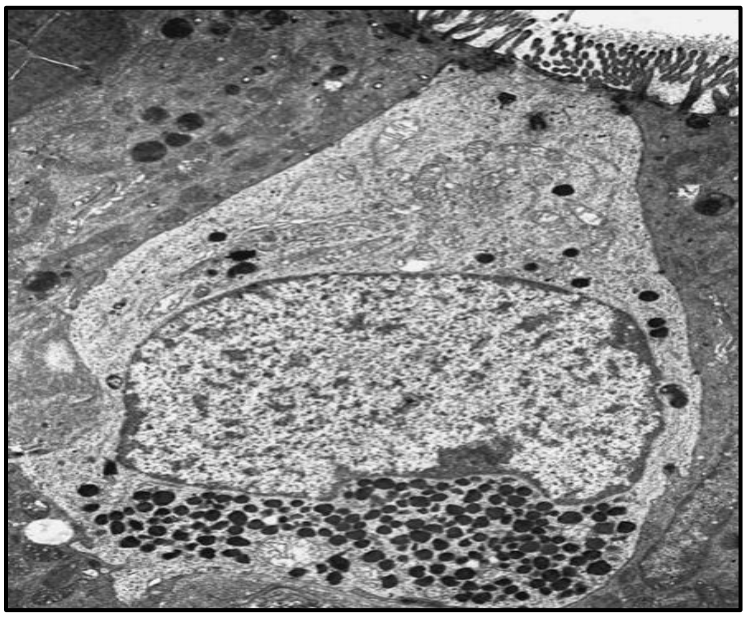


Figure 14: Enteroendocrine cell. TEM of an open-type enteroendocrine cell in the epithelium of the duodenum shows microvilli at its apical end in contact with the lumen. The microvilli have components of nutrient-sensing and signal transduction systems similar in some components to those of taste bud gustatory cells.

- **M (microfold) cells** are unique epithelial cells specialized for transepithelial transport of particles and microorganisms, located mainly in the ileum's mucosa overlying the lymphoid follicles of Peyer patches. each M cell has a pocket formed by the deeply invaginated basolateral membrane and containing lymphocytes and macrophages.

➢ Other Layers

Along the entire small intestine loose connective tissue of the mucosal lamina propria contains extensive blood and lymph microvasculature, nerve fibers, smooth muscle cells, and diffuse lymphoid tissue. The lamina propria penetrates the core of each intestinal villus, bringing with it microvasculature, lymphatics, and nerves (Figures 10c and 15). Smooth muscle fibers extending from the muscularis mucosae produce rhythmic movements of the villi that increase the absorption efficiency. Fibers of the muscularis mucosae also produce local movements of plicae circulares that help propel lymph from the lacteals into submucosal and mesenteric lymphatics.

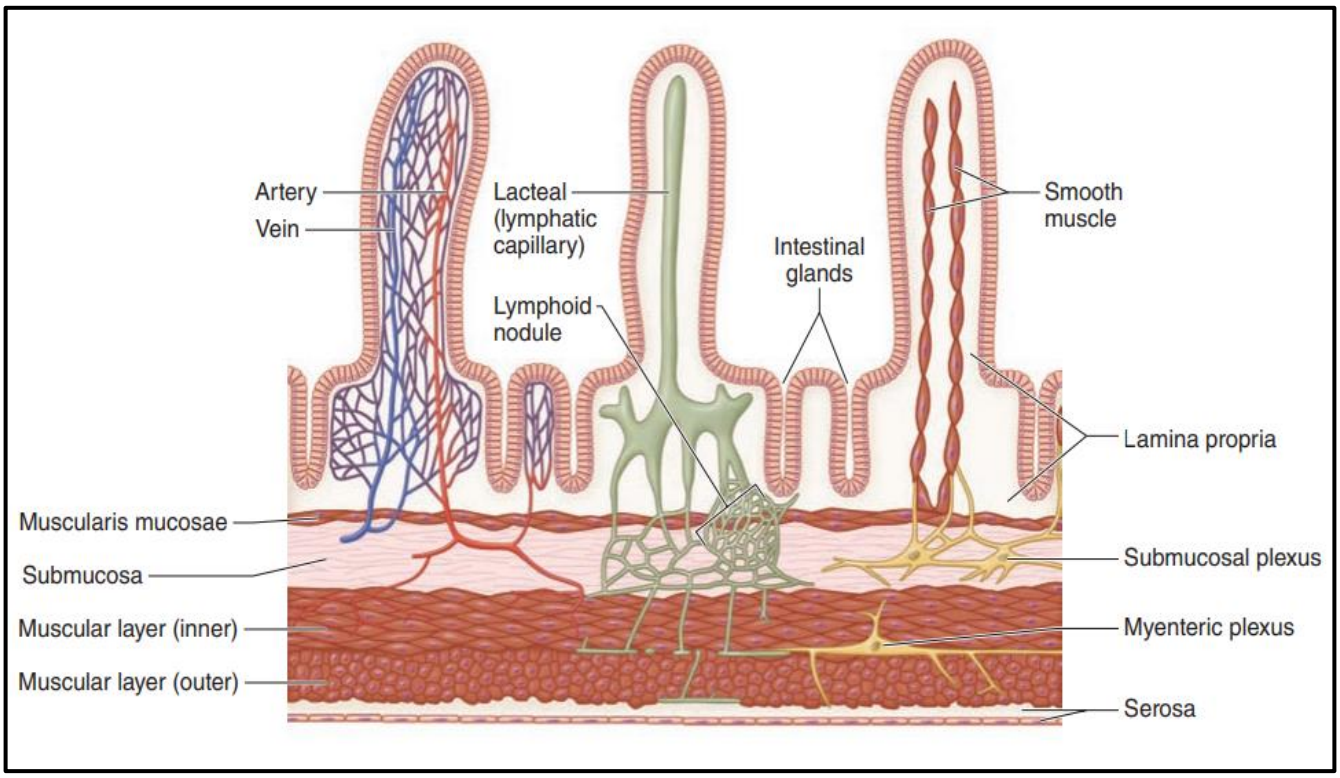


Figure 15: Microvasculature, lymphatics, and muscle in villi. The villi of the small intestine contain blood microvasculature (left), lymphatic capillaries called lacteals (center), and both innervation and smooth muscle fibers (right).

The submucosa has larger blood and lymph vessels and interconnected neurons of the **submucosal (Meissner) nerve plexus**. The proximal part of the duodenum has in the submucosa and mucosa large clusters of branched tubular mucous glands, **the Brunner glands**, with small excretory ducts opening among the intestinal crypts (Figure 16). Mucus from these glands is distinctly alkaline (pH 8.1-9.3), which neutralizes chyme entering the duodenum from the pylorus, protecting the mucous membrane, and bringing the intestinal contents to the optimum pH for pancreatic enzyme action. In the ileum both the lamina propria and submucosa contain well-developed mucosa-associated lymphoid tissue (MALT), consisting of the large lymphoid nodule aggregates known as Peyer patches underlying the epithelial M cells.

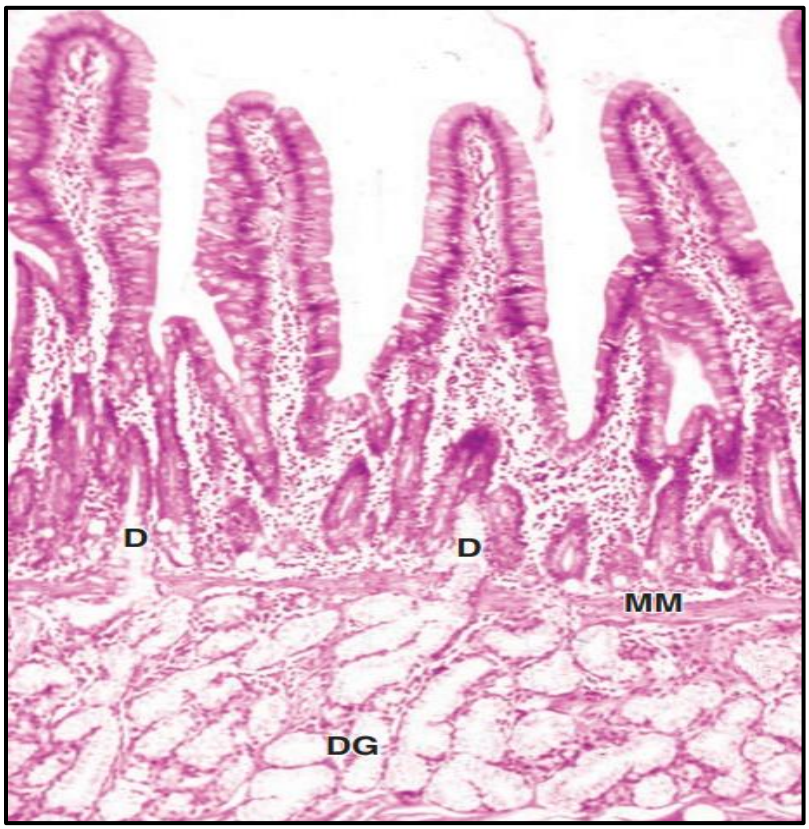


Figure 16: Duodenal (Brunner) glands. Concentrated in the upper duodenum are large masses of compound tubular mucous glands, the duodenal glands (DG), with many lobules that occupy much of the submucosa and may extend above the muscularis mucosae (MM) into the mucosa. Many small excretory ducts (D) extend from these lobules through the lamina propria and empty into the lumen among the small intestinal crypts (X100; H&E).

Smooth muscle of the lamina propria and muscularis mucosae, under the control of the **autonomic submucosal (Meissner) plexus**, moves the villi and helps propel lymph through the lacteals. Smooth muscle in the inner circular layer and the outer longitudinal layer of the muscularis, under the control of the **autonomic myenteric (Auerbach) plexus**, produces strong peristalsis (Figure 17).

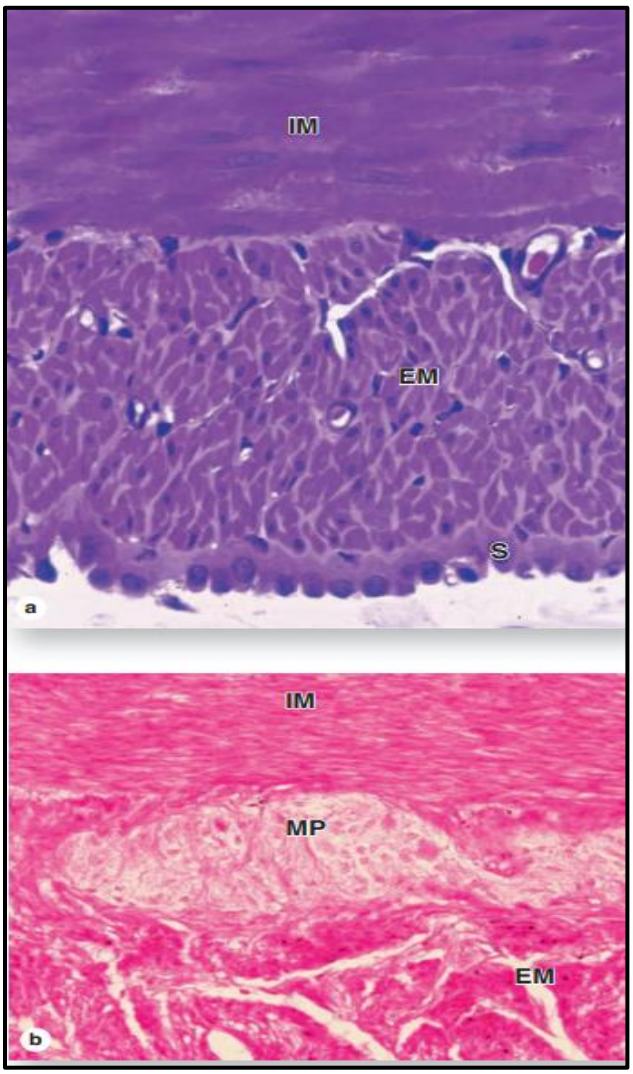


Figure 17: Small intestine muscularis and myenteric plexus. (a) Transverse sections of the small intestinal wall show the orientation of the internal (IM) and external (EM) smooth muscle layers. The inner layer is predominantly circular, while the outer layer is longitudinal. The serosa (S) is a thin connective tissue covered here by a mesothelium of cuboidal or squamous cells (X200; PT). (b) Between the internal and external layers of muscularis (IM and EM) are ganglia of pale-staining neurons and other cells of the myenteric plexus (MP) (X100; H&E).

Large Intestine

The large intestine or bowel, which absorbs water and electrolytes and forms indigestible material into feces, has the following regions: the short **cecum**, with the **ileocecal valve** and the **appendix**; the ascending, transverse, descending, and sigmoid **colon**; and the **rectum**, where feces are stored prior to evacuation (Figure 18).

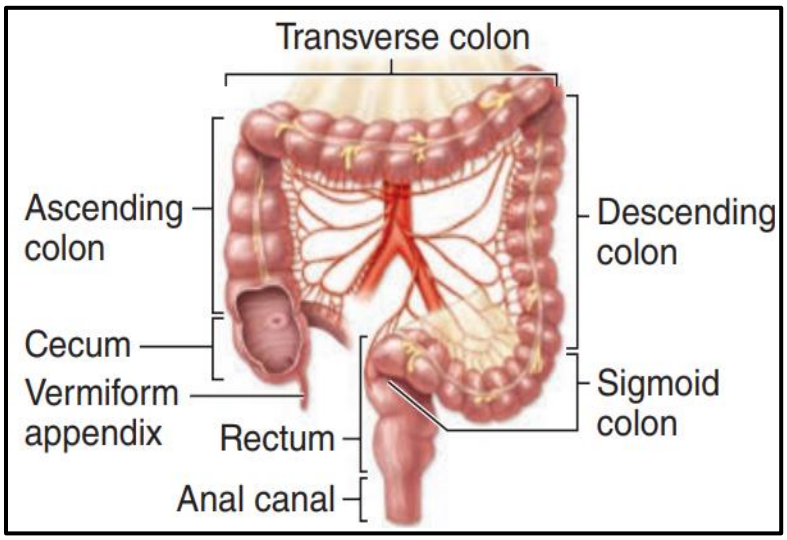


Figure 18: Large intestine.

The mucosa lacks villi and except in the rectum has no major folds. Less than one-third as long as the small intestine, the large intestine has a greater diameter (6-7 cm). The wall of the colon is puckered into a series of large sacs called **haustra**.

The mucosa of the large bowel is penetrated throughout its length by tubular **intestinal glands**. These and the intestinal lumen are lined by goblet and absorptive cells, with a small number of enteroendocrine cells (Figures 19 and 20). The columnar absorptive cells or **colonocytes** have irregular microvilli and dilated intercellular spaces indicating active fluid absorption (Figure 20d). Goblet cells producing lubricating mucus become more numerous along the length of the colon and in the rectum. Epithelial stem cells are located in the bottom third of each gland.

The lamina propria is rich in lymphoid cells and in lymphoid nodules that frequently extend into the submucosa (Figure 19). The richness in MALT is related to the large bacterial population of the large intestine. The appendix has little or no absorptive function but is a significant component of MALT.

The muscularis of the colon has longitudinal and circular layers but differs from that of the small intestine, with fibers of the outer layer gathered in three separate longitudinal bands called **teniae coli** (Figure 19a). Intraperitoneal portions of the colon are covered by serosa, which is characterized by small, pendulous protuberances of adipose tissue.

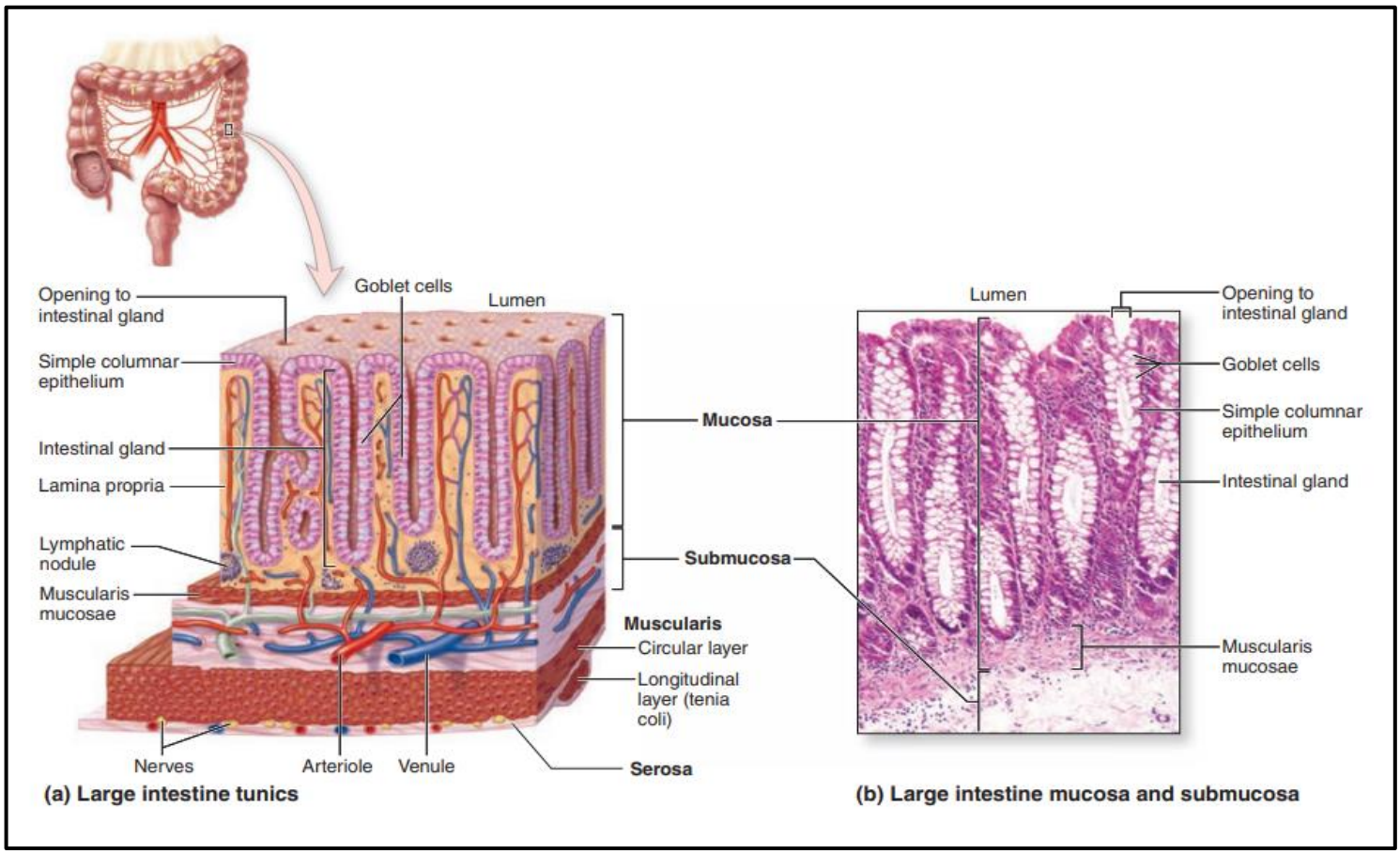


Figure 19: Wall of the large intestine. (a) Diagram shows the wall of the large intestine composed of the four typical layers. The submucosa is well vascularized. The muscularis has a typical inner circular layer. (b) The mucosa is occupied mostly by tubular intestinal glands extending as deep as the muscularis mucosae and by lamina propria rich in MALT (X80; H&E).

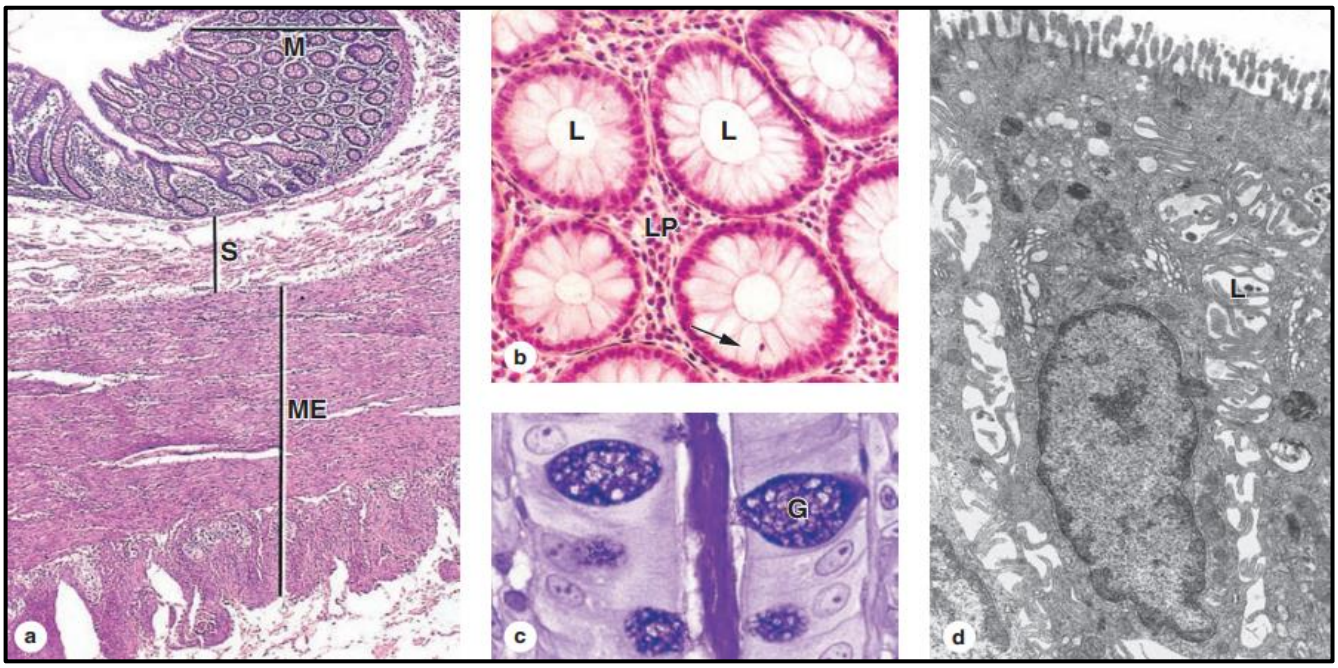


Figure 20: Colon mucosa. (a) Transverse section of the colon shows the muscularis externa (ME), including a tenia coli cut transversely in the lower part of the figure, the submucosa (S), the mucosa (M)

filled with tubular intestinal glands. Some of these glands are cut longitudinally, but most seen here are cut transversely. (X14; H&E).

(b) Transversely cut glands are seen to consist of simple columnar epithelium surrounded by a tubular lumen (L) and embedded in lamina propria (LP) with many free lymphocytes. Lymphocytes can also be seen penetrating the epithelium (arrow) (X200; H&E).

(c) Longitudinal section of one intestinal gland stained for glycoproteins shows mucus in the lumen and two major cell types in the epithelium: goblet cells (G) and the neighboring columnar cells specialized for water absorption (X400; PAS).

(d) TEM of the absorptive cells, or colonocytes, reveals short microvilli at their apical ends and dilated intercellular spaces with interdigitating leaflets of cell membrane (L), a sign of active water transport (X2500).

The distal end of the GI tract is the **anal canal**, 3-4 cm long. At the **rectoanal junction** the simple columnar mucosal lining of the rectum is replaced by stratified squamous epithelium (Figure 21). The mucosa and submucosa of the anal canal form several longitudinal folds, the **anal columns**, in which the lamina propria and submucosa include sinuses of the rectal venous plexus. Near the anus the circular layer of the rectum's muscularis forms the **internal anal sphincter**.

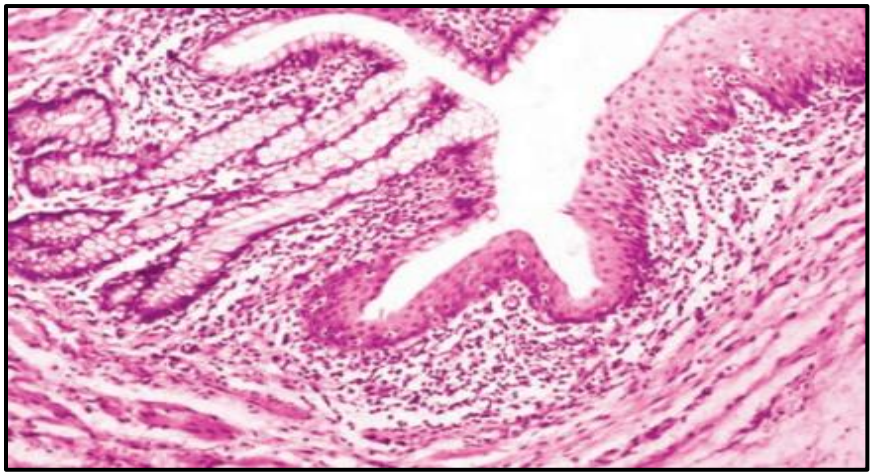


Figure 21: Mucosa of the rectoanal junction. The simple columnar epithelium with tubular intestinal glands in the rectum (left side of photo) changes abruptly to stratified squamous epithelium in the anal canal (right side of photo), as seen in this longitudinal section. The connective tissue of the lamina propria is seen to contain many free lymphocytes (X40; H&E).

Lecture 15-18: The Digestive System and Organs Associated with the Digestive Tract.

Part II: Organs Associated with the Digestive Tract.

The organs associated with the digestive tract include the major **salivary glands**, **the pancreas**, **the liver**, and **the gallbladder**. Products of these organs facilitate transport and digestion of food within **GI tract**. The main functions of **the salivary glands** are to moisten and lubricate ingested food and the oral mucosa, to initiate the digestion of carbohydrates and lipids with amylase and lipase, and to secrete innate immune components such as **lysozyme** and **lactoferrin**.

The pancreas secretes digestive enzymes that act in **the small intestine** and hormones important for the metabolism of the absorbed nutrients. **Bile**, whose components are necessary for digestion and absorption of fats, is made in **the liver** but stored and concentrated in **the gallbladder**.

Salivary Glands

Exocrine glands in the mouth produce saliva, which has important functions. With a normal **pH of 6.5-6.9**, saliva also has an important buffering function and in some species is also important for evaporative cooling. There are three pairs of large salivary glands: **the parotid, submandibular, and sublingual glands** (Figure 1), in addition to the numerous intrinsic salivary glands located throughout most of the oral mucosa which secrete about 10% of the total saliva volume.

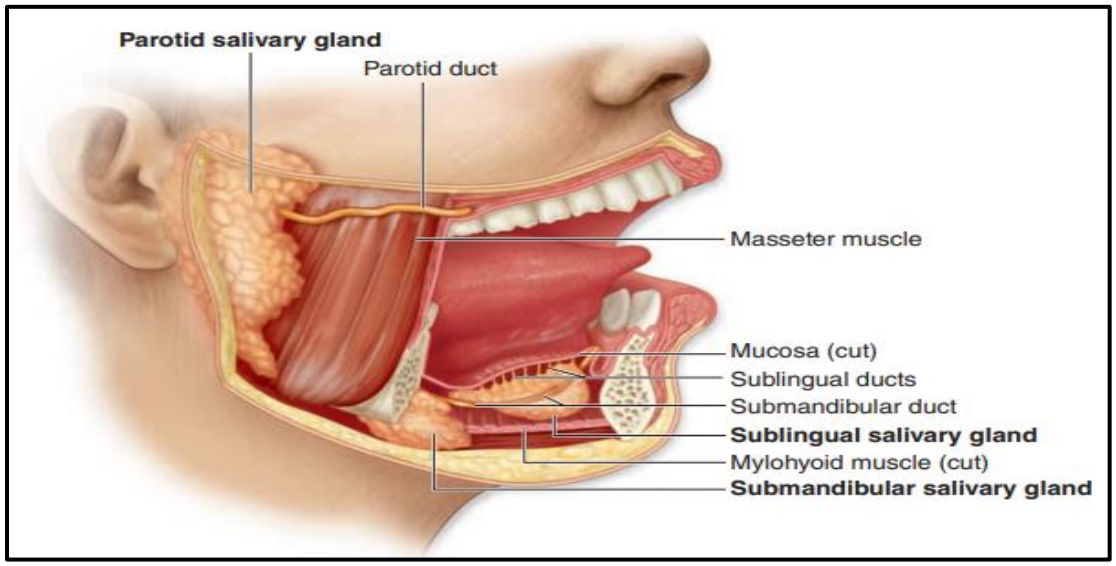


Figure 1: Major salivary glands.

A connective tissue capsule surrounds each major salivary gland. The parenchyma of each consists of secretory units on a branching duct system arranged in lobules, separated by septa of connective tissue. The secretion of each gland is either **serous, seromucous, or mucous**, depending on its content of the glycoprotein mucin. Saliva from the parotids is serous and watery. The submandibular and sublingual glands produce a seromucous secretion, while that of the minor glands is mostly mucous.

Three epithelial cell types comprise the salivary secretory units:

- **Serous cells** are polarized protein-secreting cells, usually pyramidal in shape (Figure 2 and 3). Serous cells form spherical unit called an **acinus**, with a very small central lumen. Serous acinar cells secrete enzymes and other proteins.
- **Mucous cells** are more columnar in shape (Figure 2 and 3). Mucous cells contain apical granules with hydrophilic mucins that provide lubricating properties in saliva but cause poor cell staining in routine preparations (Figure 5).
- **Myoepithelial cells** are found inside the basal lamina surrounding acini, tubules, and the proximal ends of the duct system (Figure 2 and 3). These small, flattened cells extend several contractile processes around the associated secretory unit.

In the intralobular duct system, secretory acini and tubules empty into short **intercalated ducts**, lined by cuboidal epithelial cells, and several of these ducts join to form striated duct (Figure 2). Striated ducts reabsorb **Na+** ions from the initial secretion and their folded cell membranes present a large surface area with ion transporters, facilitating rapid ion transcytosis and making the secretion slightly hypotonic.

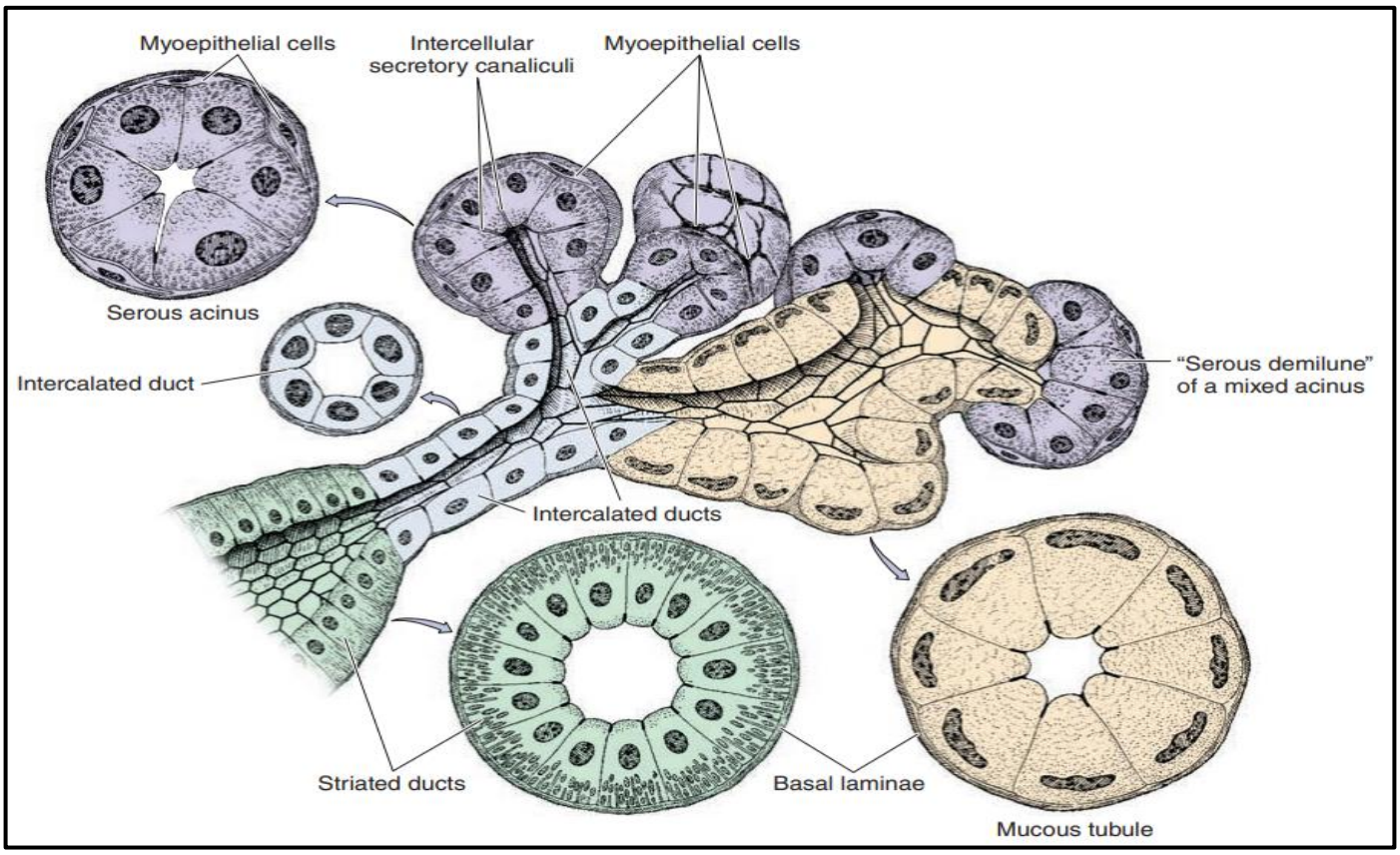


Figure 2: Epithelial components of a submandibular gland lobule.

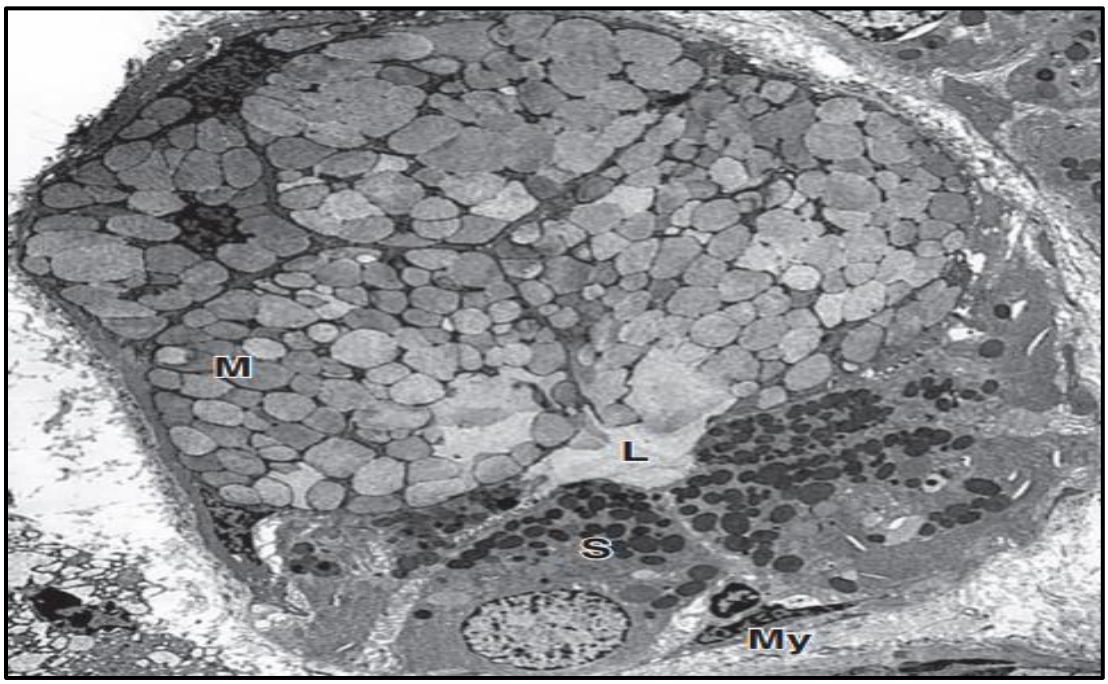


Figure 3: Ultrastructure of serous and mucous cells.

Plasma cells in the connective tissue surrounding the small intralobular ducts release IgA, which forms a complex with the secretory component synthesized by the epithelial cells of the serous acini and intralobular ducts. Transferred into the saliva, the

IgA complex released into the saliva provides defense against specific pathogens in the oral cavity.

Ducts from each lobule converge and drain into interlobular excretory ducts with increasing size and thicker connective tissue layers. The lining of these ducts is unusual, combining various epithelial types, including simple **cuboidal or columnar, stratified cuboidal or columnar, and pseudostratified epithelia**. These atypical epithelia may reflect their composition of cells with many diverse functions, including cells for ion reabsorption, cells for secretion of mucin and other proteins, enteroendocrine cells, and basal stem cells. Before emptying into the oral cavity, the main duct of each gland is lined with **nonkeratinized stratified squamous epithelium.**

Vessels and nerves enter the large salivary glands at a hilum and gradually branch into the lobules. **The capillaries** surrounding the secretory units provide fluid important for saliva production, which is stimulated by the **autonomic nervous system**. **Parasympathetic** stimulation, usually elicited through the smell or taste of food, activate a watery secretion with relatively little organic content. **Sympathetic** stimulation inhibits such secretion and produces the potential for dry mouth often associated with anxiety.

Features specific to each group of major salivary glands include the following:

- **Parotid glands**, located in each cheek near the ear, are branched acinar glands with exclusively serous acini (Figure 4). Serous cells secrete abundant **α-amylase** that initiates hydrolysis of carbohydrates and **proline-rich proteins** with antimicrobial and other protective properties.

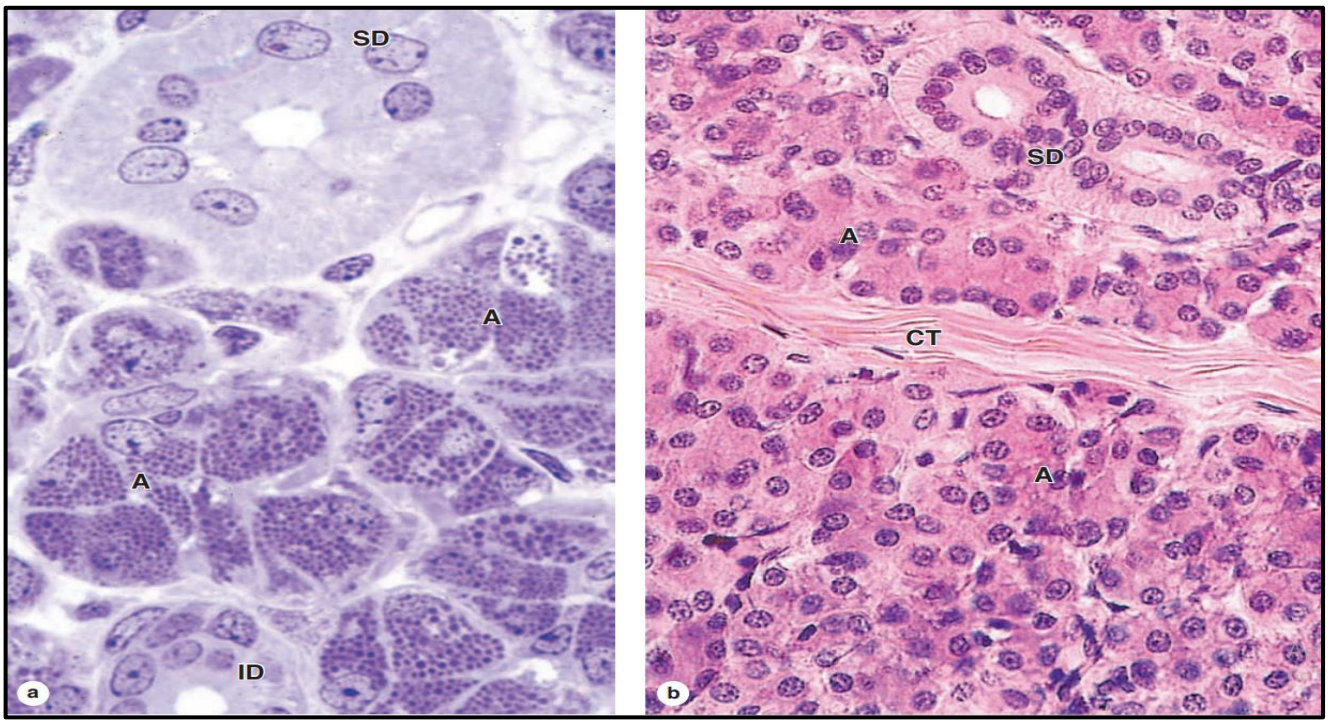


Figure 4: Parotid gland. (a) Micrograph of a parotid gland shows densely packed serous acini (A) with ducts. Secretory granules of serous cells are clearly shown in this plastic section, as well as an intercalated duct (ID) and striated duct (SD), both cut transversely (X400; PT). (b) Striations of a duct (SD) are better seen here, along with a septum (CT) and numerous serous acini (A) (X200; H&E).

- **Submandibular glands**, which produce two-thirds of all saliva, are branched tubuloacinar glands, having primarily serous acini, but with many mixed tubuloacinar secretory units (Figure 4 and 5a). Within the mixed units grouped serous cells form a crescent-shaped arrangement called **a serous demilune** (Figure 5a). Serous cells of this gland secrete **lysozyme** for hydrolysis of bacterial walls.
- **Sublingual glands**, the smallest of the major glands, are also considered branched tubuloacinar glands, but here secretory tubules of mucous cells predominate and the main product of the gland is **mucus** (Figure 5b). The few serous cells present add **amylase** and **lysozyme** to the secretion.

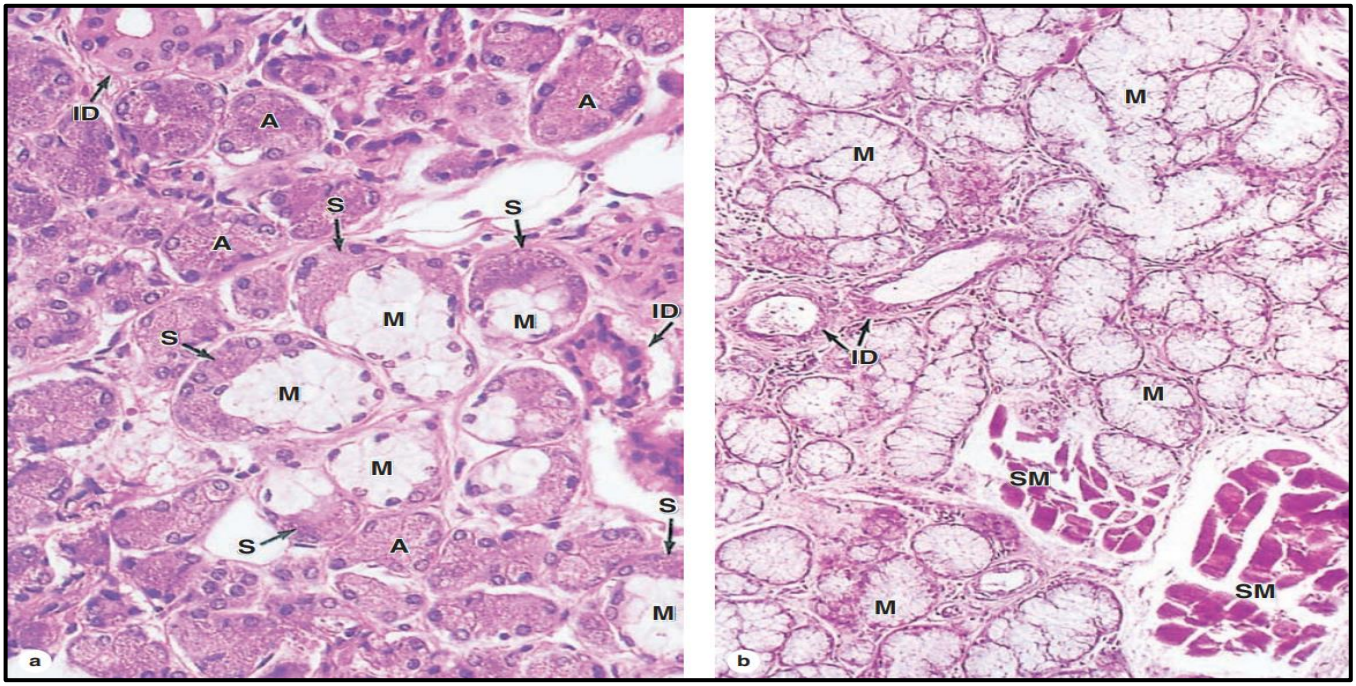


Figure 5: Submandibular gland and sublingual gland. (a) The submandibular gland is a mixed serous and mucous gland (serous cells predominate), and shows well-stained serous acini (A) and "serous demilunes" (S) and pale-staining mucous cells (M) grouped as tubules in this tubuloacinar gland. (The crescentshaped "serous demilunes" arise at least in part artifactually due to disproportionate swelling of the adjacent mucous cells during slide preparation.) Small intralobular ducts (ID) drain each lobule (X340; H&E). (b) The sublingual gland is a mixed but largely mucous gland with a tubuloacinar arrangement of poorly stained mucous cells (M). Small intralobular ducts (ID) are seen in connective tissue, as well as small fascicles of lingual striated muscle (SM) (X140; H&E).

Pancreas

The pancreas is a **mixed exocrine-endocrine gland** that produces both digestive enzymes and hormones. It is an elongated organ, with a large head near the duodenum and more narrow body and tail regions that extend to the left (Figure 6).

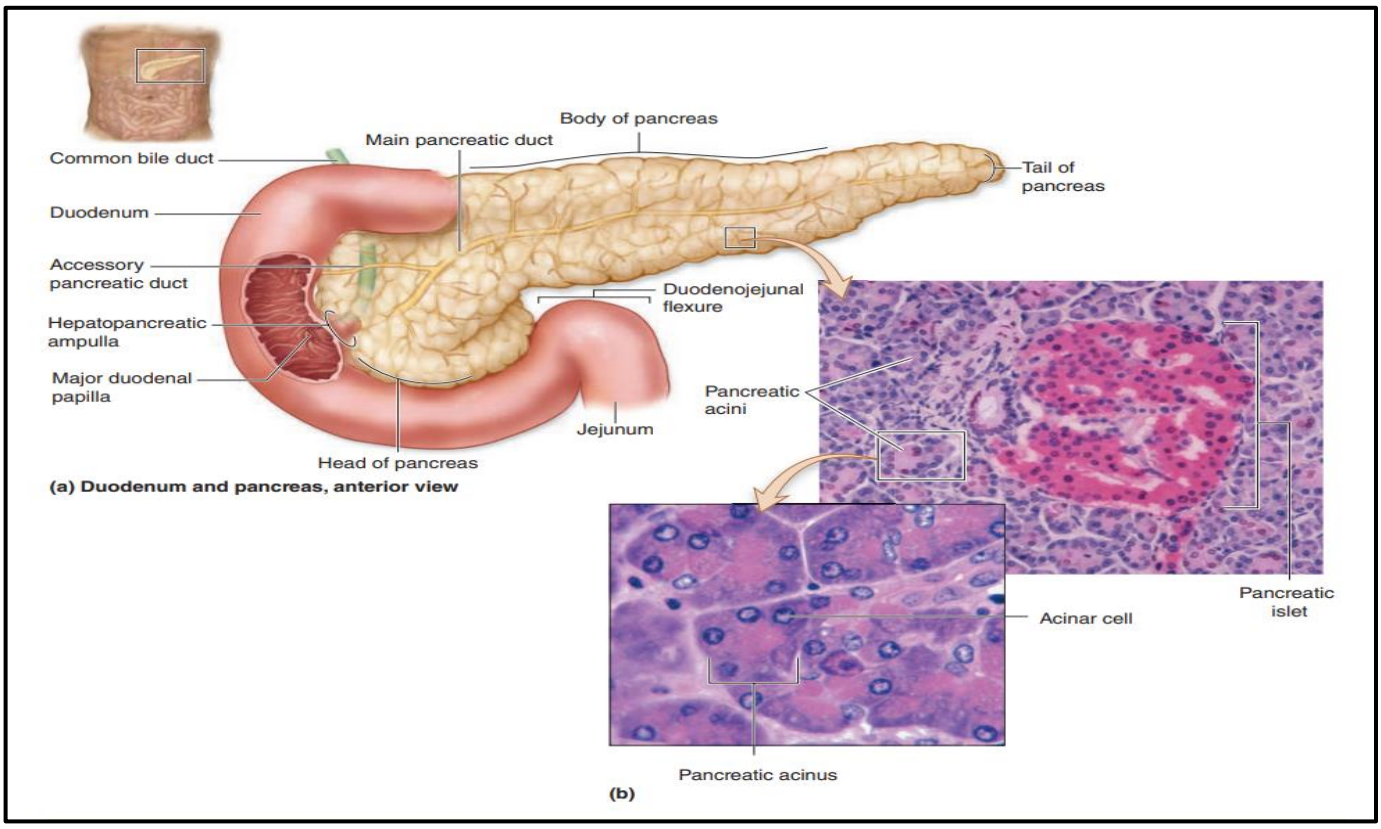


Figure 6: Pancreas and duodenum. (a) The main regions of the pancreas are shown in relation to the two pancreatic ducts and the duodenum. (b) Micrographs show a pancreatic islet and several pancreatic acini. (X75 and X200; H&E)

The pancreas has a thin capsule of connective tissue, from which septa extend to cover the larger vessels and ducts and to separate the parenchyma into lobules (Figure 7). The secretory acini are surrounded by a basal lamina that is supported only by a delicate sheath of reticular fibers with a rich capillary network. Endocrine function of it involves primarily smaller cells located in variously sized clusters called the pancreatic islets (islets of Langerhans).

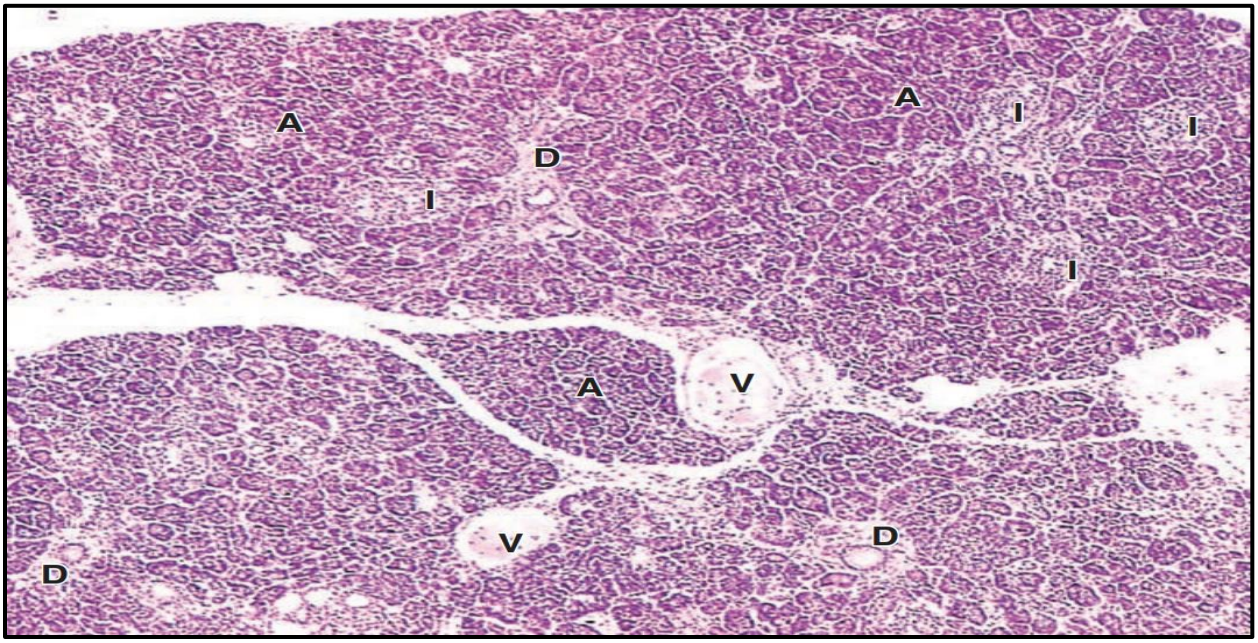


Figure 7: Pancreas. Low-power view of pancreas includes several islets (I) surrounded by many serous acini (A). The larger intralobular ducts (D) are lined by simple columnar epithelium. The ducts and blood vessels (V) are located in connective tissue, which also provides a thin capsule to the entire gland and thin septa separating the lobules of secretory acini (X20; H&E).

The digestive enzymes are produced by cells of serous acini (Figure 8a). Each pancreatic acinus consists of several serous cells surrounding a very small lumen, without myoepithelial cells (Figure 8). Each acinus is drained by a short intercalated duct. The initial cells of these small ducts extend into the lumen of the acinus as small pale-staining **centroacinar cells** that are unique to the pancreas. Cells of the intercalated ducts secrete a large volume of fluid, rich in **$HCO3^-$** (bicarbonate ions), which alkalinizes and transports hydrolytic enzymes produced in the acini.

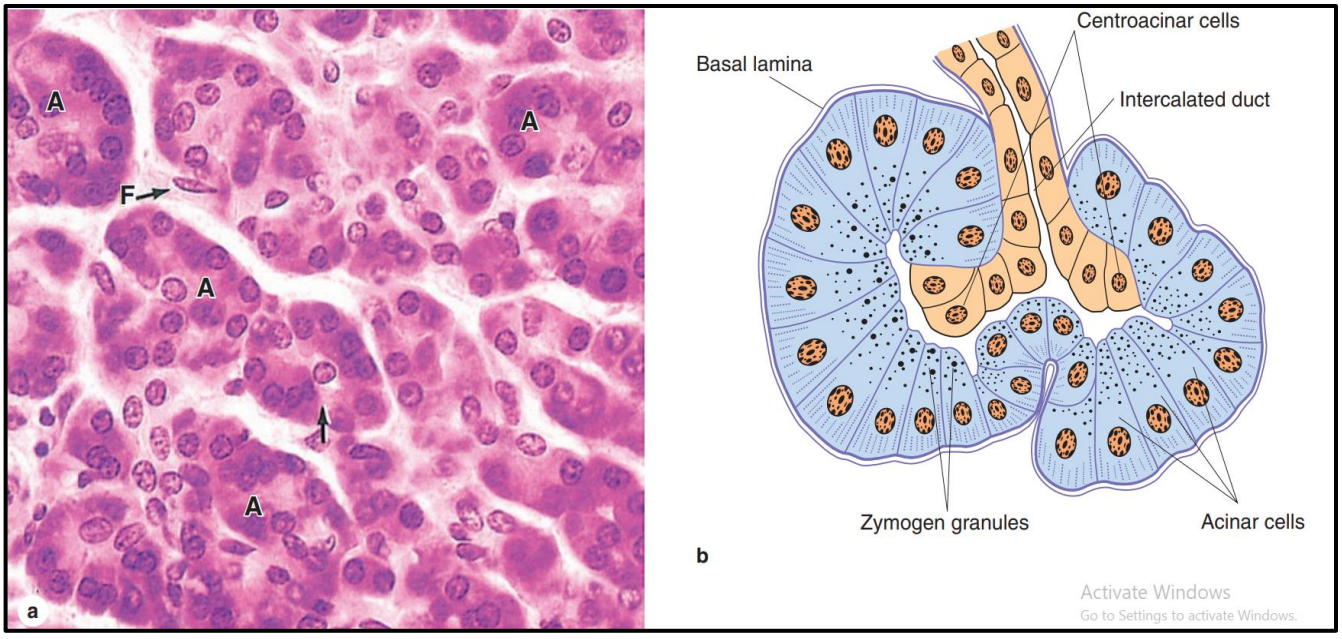


Figure 8: Pancreatic acini. (a) Micrograph of exocrine pancreas shows the serous, enzymeproducing cells arranged in small acini (A) with very small lumens. Acini are surrounded by only small amounts of connective tissue with fibroblasts (F). Each acinus is drained by an intercalated duct with its initial cells, the centroacinar cells (arrow), inserted into the acinar lumen (X200; H&E). (b) The diagram shows the arrangement of cells more clearly.

The exocrine pancreas secretes about **1.5 L** of alkaline pancreatic juice per day and delivers it directly into the duodenum where the **$HCO3^-$** ions neutralize the acidic chyme entering there and establish the pH for optimal activity of the pancreatic enzymes. These digestive enzymes include several **proteases**, **α-amylase**, **lipases**, and **nucleases** (**DNAase and RNAase**).

Pancreatic tissue is protected against autodigestion by the following:

- Restricting protease activation to **the duodenum**.
- Trypsin inhibitor, which is copackaged in the secretory granules with **trypsinogen**.
- The higher pH in the acini and duct system due to **$HCO3^-$** secreted by the centroacinar and intercalated duct cells, which helps keep all the enzymes inactive.

Exocrine secretion in the pancreas is regulated mainly through two polypeptide hormones produced by enteroendocrine cells of the small intestine:

- **Cholecystokinin** (CCK) stimulates enzyme secretion by the acinar cells.
- Secretin promotes water and **$HCO3^-$** secretion by the duct cells.

*Autonomic (parasympathetic) nerve fibers also stimulate secretion from both acinar and duct cells.

Liver

The liver is **the largest internal organ**, in adults averaging about 1.5 kg or 2% of the body weight. Located in the right upper quadrant of the abdomen just below the diaphragm, the liver has major left and right lobes with two smaller inferior lobes, most of which are covered by a thin capsule and mesothelium of the visceral peritoneum. The capsule thickens at the hilum on the inferior side, where the dual blood supply from the hepatic portal vein and hepatic artery enters the organ and where the hepatic vein, lymphatics, and common hepatic (bile) duct exit.

The main digestive function of the liver is production of **bile**, a complex substance required for the, hydrolysis and uptake of fats in the duodenum. The liver is also the major interface between the digestive system and the blood, as in which

nutrients absorbed in the small intestine are processed before distribution throughout the body. About 75% of the blood entering the liver is nutrientrich (but O2 poor) blood from the portal vein arising from the stomach, intestines, and spleen; the other 25% comes from the hepatic artery and supplies the organ's O2 .

Hepatocytes are the key cells of this organ, are among the most functionally diverse cells of the body. In addition to an exocrine function in the secretion of bile components, hepatocytes and other liver cells process the contents of blood, with many specific functions:

- Synthesis and endocrine secretion into the blood of the major plasma proteins, including **albumins, fibrinogen, apolipoproteins and transferrin**.
- Conversion of amino acids into glucose (**gluconeogenesis**).
- detoxification and conjugation of ingested toxins, including many drugs.
- Amino acid deamination, producing urea removed from blood in kidneys.
- Storage of glucose in glycogen granules and triglycerides in small lipid droplets.
- Storage of vitamin A (**in hepatic stellate cells**) and other fat-soluble vitamins.
- Removal of effete erythrocytes (by **Kupffer cells**).
- Storage of iron in complexes with the protein ferritin.

Hepatocytes & Hepatic Lobules

The liver's unique histologic organization and microvasculature allow hepatocytes to perform their diverse metabolic, exocrine, and endocrine functions. **Hepatocytes** are large cuboidal or polyhedral epithelial cells, with central nuclei and eosinophilic cytoplasm rich in mitochondria. The cells are frequently binucleated and about 50% of them are polyploid, with two to eight times the normal chromosome number. **The liver parenchyma** is organized as thousands of small hepatic lobules in which hepatocytes form hundreds of irregular plates arranged radially around a small central vein (Figure 9 through 11). The hepatocyte plates are supported by a delicate stroma of reticulin fibers (Figure 10b). **Peripherally each lobule has three to six portal areas with more fibrous connective tissue, each of which contains three interlobular structures that comprise the portal triad (Figure 9 and 10d):**

- **A venule** branch of the portal vein, with blood rich in nutrients but low in O2 .
- **An arteriole** branch of the hepatic artery, which supplies O2 .
- **One or two small bile ductules** of cuboidal epithelium, branches of the bile conducting system.

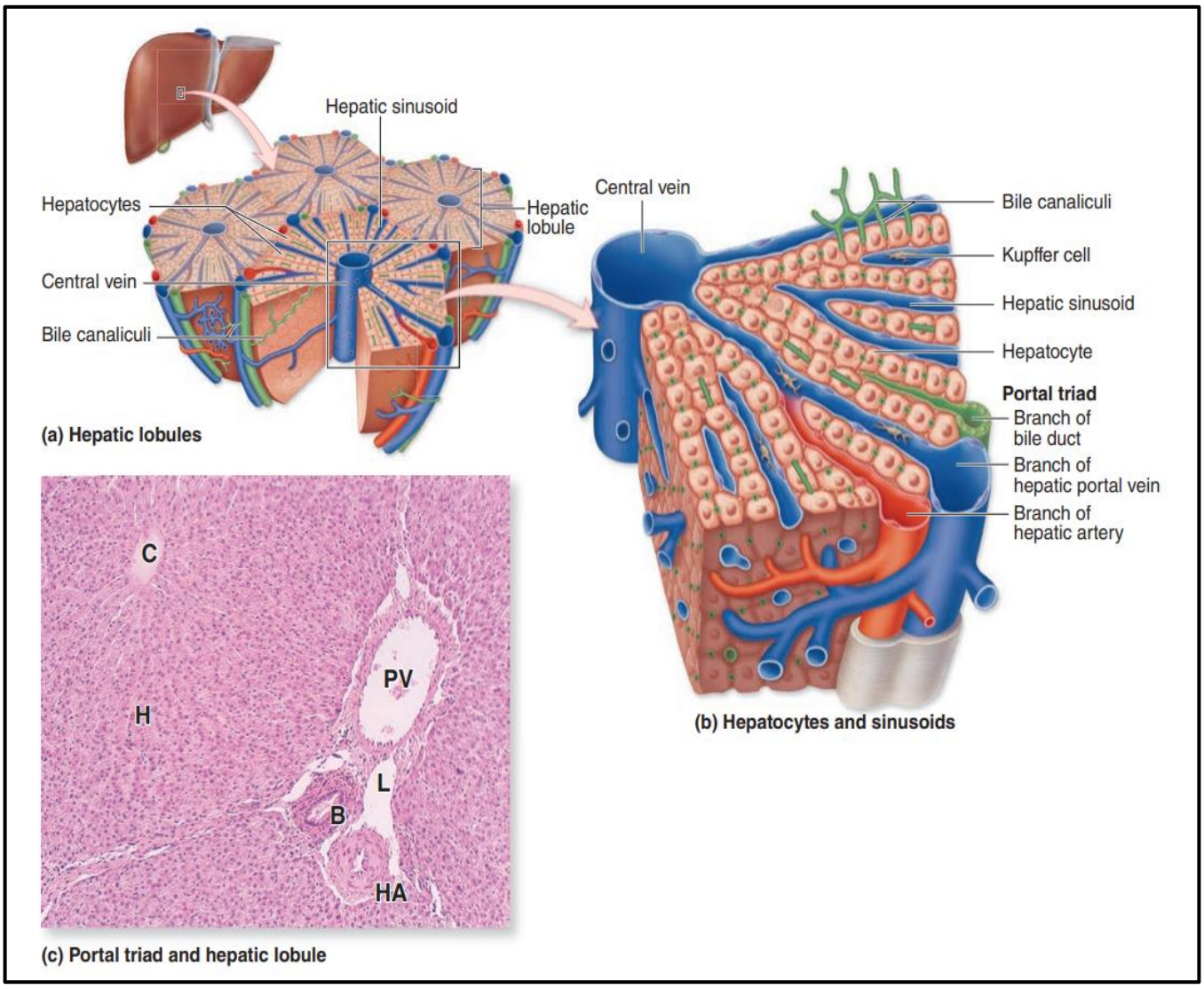


Figure 9: Liver. (a) Diagram showing a small central vein in the center of a hepatic lobule. (b) Both blood vessels in this triad branch as sinusoids, which run between plates of hepatocytes and drain into the central vein. (c) Micrograph of a lobule shows the central vein (C), plates of hepatocytes (H), and in an adjacent portal area a small lymphatic (L) and components of the portal triad: a portal venule (PV), hepatic arteriole (HA), and bile ductule (B) (X220; H&E).

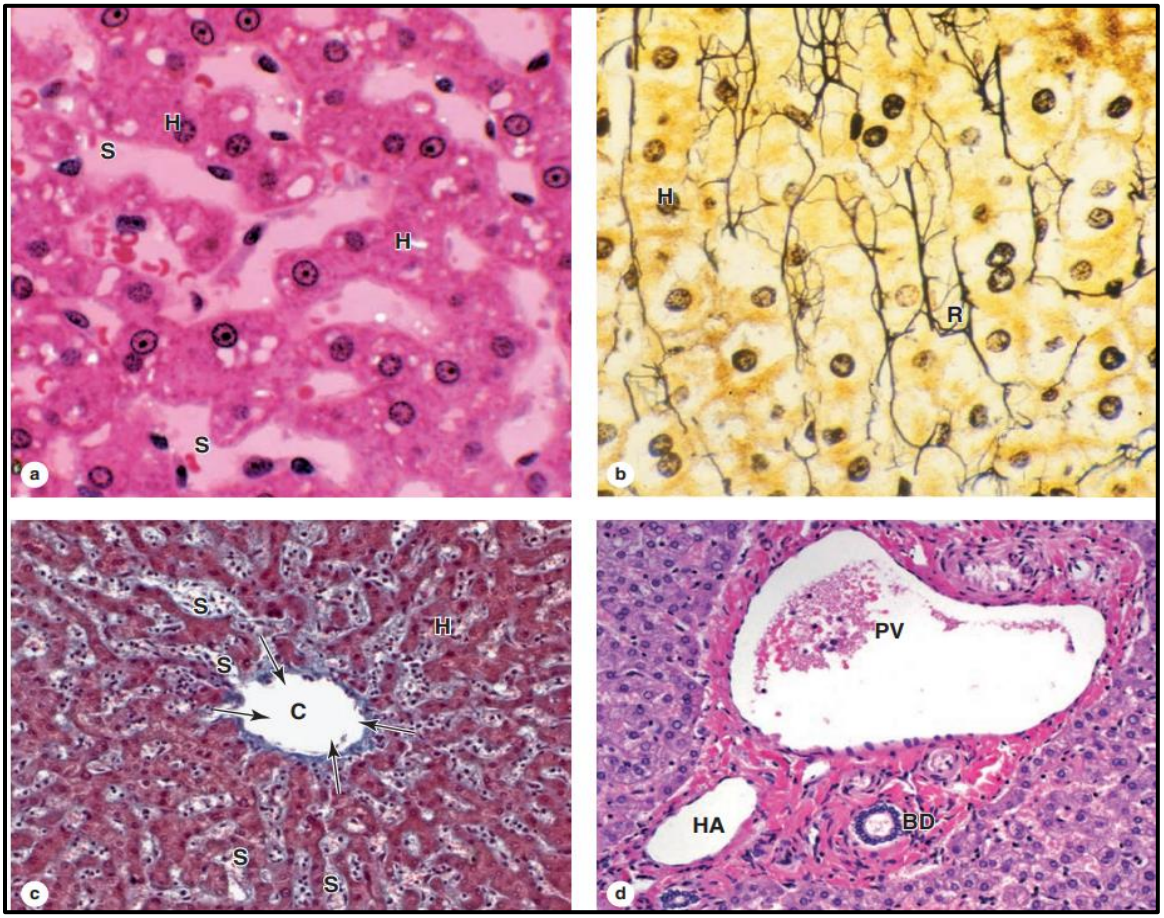


Figure 10: Hepatic lobule microvasculature. (a) Hepatocytes (H) are polygonal epithelial cells, which form branching, irregular plates separated by venous sinusoids (S) (H&E X400). (b) Reticulin (collagen type III) fibers (R) running along the plates of hepatocytes (H), supporting these and the intervening sinusoids (X400; Silver) (c) With plates of hepatocytes (H) appearing to radiate from it, the central vein (C) of the lobule has more collagen than the smaller sinusoids (S) that drain into it from all directions (arrows) (X200; Mallory trichrome). (d) Peripheral portal areas contain more connective tissue and are the sites of the portal triad: a portal venule (PV), an arteriole branching off the hepatic artery (HA), and one or two bile ductules (BD) (X200; H&E).

The General Structure of the Liver

In the lobules **the portal venule and hepatic arteriole** both branch into irregular sinusoids between the hepatic plates where the nutrient-rich and O2 -rich blood mixes, flows past hepatocytes, and drains to the central vein (Figure 11).

- The endothelium of the hepatic sinusoids is discontinuous and fenestrated; between it and the hepatocytes is the perisinusoidal space (of Disse) where exchange occurs between the hepatocytes and blood plasma (Figure 12).
- The sinusoidal endothelium includes many specialized stellate macrophages or Kupffer cells, which recognize and remove effete erythrocytes, releasing iron and bilirubin for uptake by hepatocytes (Figure 12).

- Also present in the perisinusoidal spaces are hepatic stellate cells (or Ito cells) containing many small lipid droplets for storage of vitamin A and other fat-soluble vitamins (Figure 12).
- Between adherent hepatocytes in the hepatic plates are grooves called bile canaliculi, sealed by tight junctions, into which hepatocytes secrete water and bile components, including bilirubin and bile acids
- In each hepatic lobule, all bile canaliculi converge on the bile canals, which join the bile ductules in the portal areas and eventually all merge to form the left and right hepatic ducts.

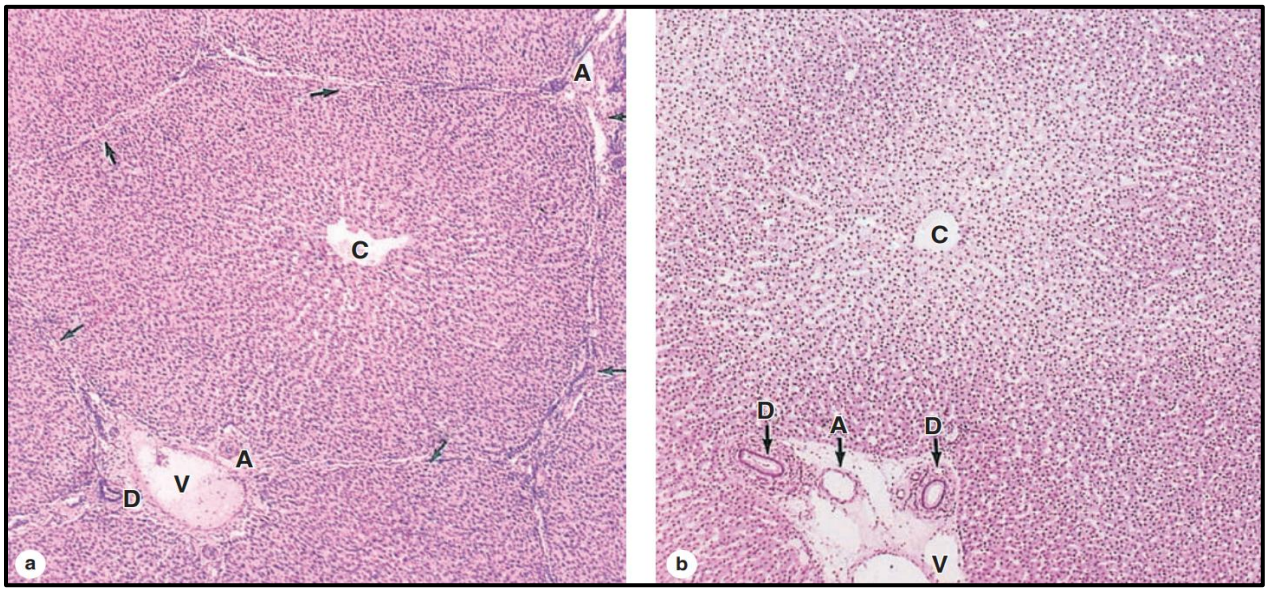


Figure 11: Hepatic lobule. Cut transversely, hepatic lobules are polygonal units showing plates of epithelial cells called hepatocytes radiating from a central venule (C). (a) Hepatic lobules of some mammals, such as the pig, are delimited on all sides by connective tissue. (b) In humans these lobules have much less connective tissue and their boundaries are more difficult to distinguish. In both cases peripheral connective tissue of portal areas contains the portal triad: small bile ductules (D), venule (V) branches of the portal vein, and arteriole (A) branches of the hepatic artery. (Both X150; H&E).

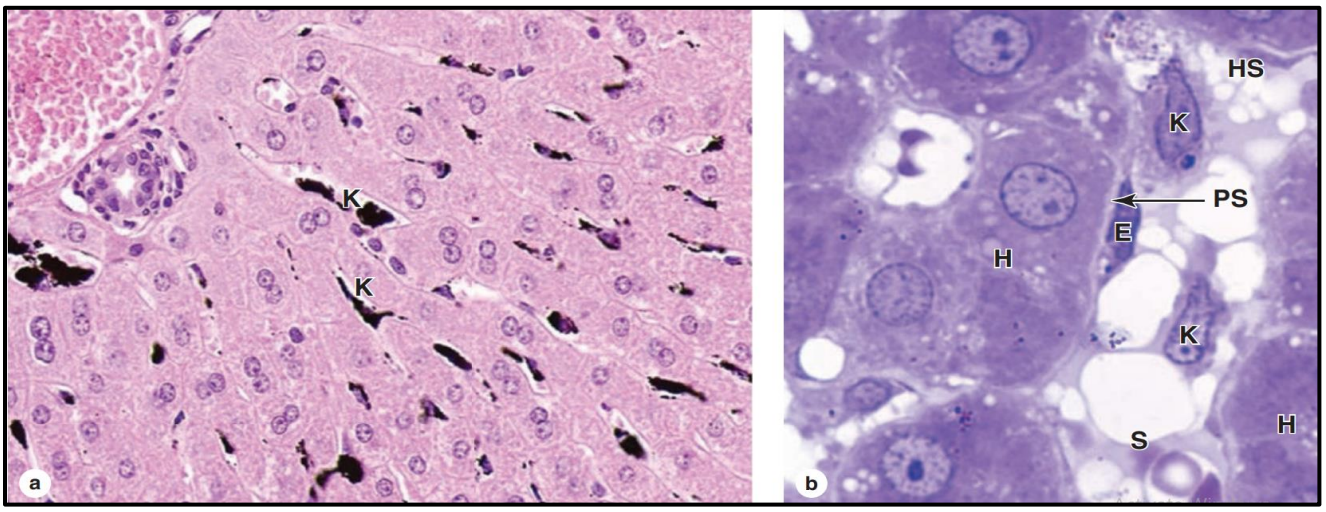


Figure 12: Hepatic sinusoids. (a) Kupffer cells (K) are seen as black cells in a liver lobule from a rat injected with particulate India ink. (X200; H&E) (b) In a plastic section, Kupffer cells (K) are seen in the sinusoid (S) between two groups of hepatocytes (H). They are larger than the flattened endothelial cells (E). Between the endothelium and the hepatocytes is a very thin space called the perisinusoidal space (PS) of Disse, in which are located small hepatic stellate cells (HS), or Ito cells. These cells are numerous but are difficult to demonstrate in routine histologic preparations. (X750; PT).

Abundant rough ER is focused on synthesis of plasma proteins and causes cytoplasmic basophilia. **Abundant smooth ER**, distributed more evenly throughout the cytoplasm, contains the enzyme systems for the biotransformation of substances in blood, which are then usually excreted with bile. These include enzymes responsible for oxidation, methylation, and conjugation of steroids.

Glycogen granules and small lipid droplets in hepatocytes, and very small electron-dense ferritin complexes (**hemosiderin**) primarily in the **Kupffer cells**, mediate temporary storage of glucose, triglycerides, and iron.

Hepatocyte peroxisomes are also abundant and important for oxidation of excess fatty acids, catalase-mediated breakdown of the hydrogen peroxide generated by fatty acid oxidation and conversion of excess purines to uric acid.

Many Golgi complexes are also present, involved in synthesis of both plasma proteins and bile components. The numerous mitochondria provide energy for all these activities.

Biliary Tract & Gallbladder

The bile produced by the hepatocytes flows through the bile canaliculi, bile ductules, and bile ducts. These structures gradually merge, forming a converging network that ultimately forms the common hepatic duct, which joins the cystic duct

from **the gallbladder** and continues to the duodenum as the common bile duct (Figure 13).

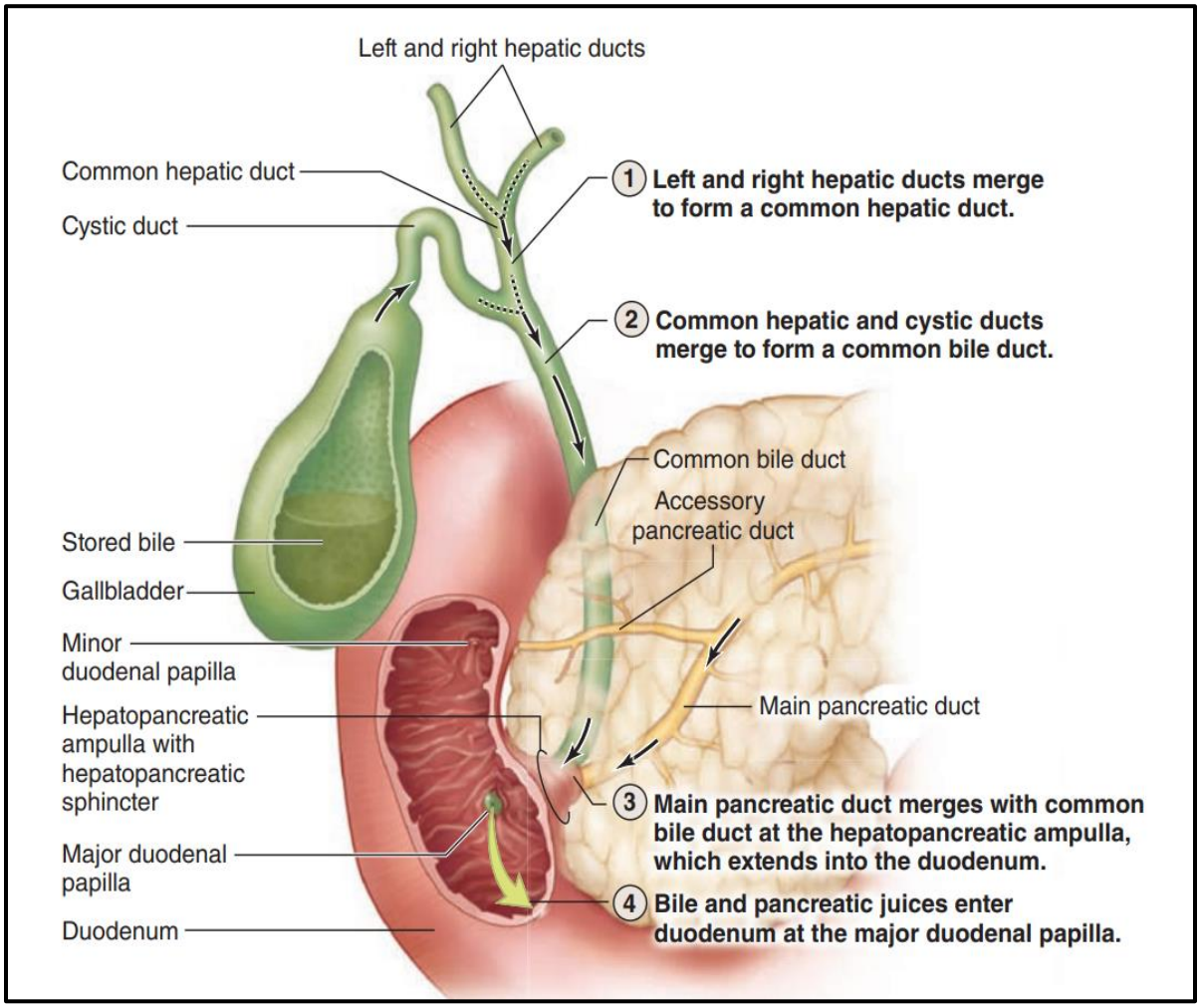


Figure 13: Biliary tract and gallbladder.

The hepatic, cystic, and common bile ducts are lined with a mucous membrane having a simple columnar epithelium of **cholangiocytes**. The lamina propria and submucosa are relatively thin, with mucous glands in some areas of the cystic duct, and surrounded by a thin muscularis. This muscle layer becomes thicker near the duodenum and finally, in the duodenal papilla, forms a sphincter that regulates bile flow into the small bowel.

The gallbladder is a hollow, pear-shaped organ (Figure 13) attached to the lower surface of the liver, capable of storing 30-50 mL of bile that is concentrated during storage. The wall of the gallbladder consists of a mucosa composed of simple columnar epithelium and lamina propria, a thin muscularis with bundles of muscle fibers oriented in several directions, and an external adventitia or serosa (Figure 14a). The mucosa has numerous folds that are particularly evident when the gallbladder is empty.

The lining epithelial cells of the gallbladder have prominent mitochondria, microvilli, and large intercellular spaces, all indicative of cells actively transporting water, in this case for concentrating bile (Figure 14b). The mechanism for this includes activity of **Na+ pumps** in the basolateral membranes, followed by passive movement of water from the bile. To move stored bile into the duodenum, contraction of the gallbladder muscularis is induced by **cholecystokinin (CCK**) released from enteroendocrine cells of the small intestine. Release of **CCK** is, in turn, stimulated by the presence of ingested fats in the small intestine. **Gallbladder** removal due to obstruction or chronic inflammation leads to the direct flow of bile from liver to gut, with few major consequences on digestion.

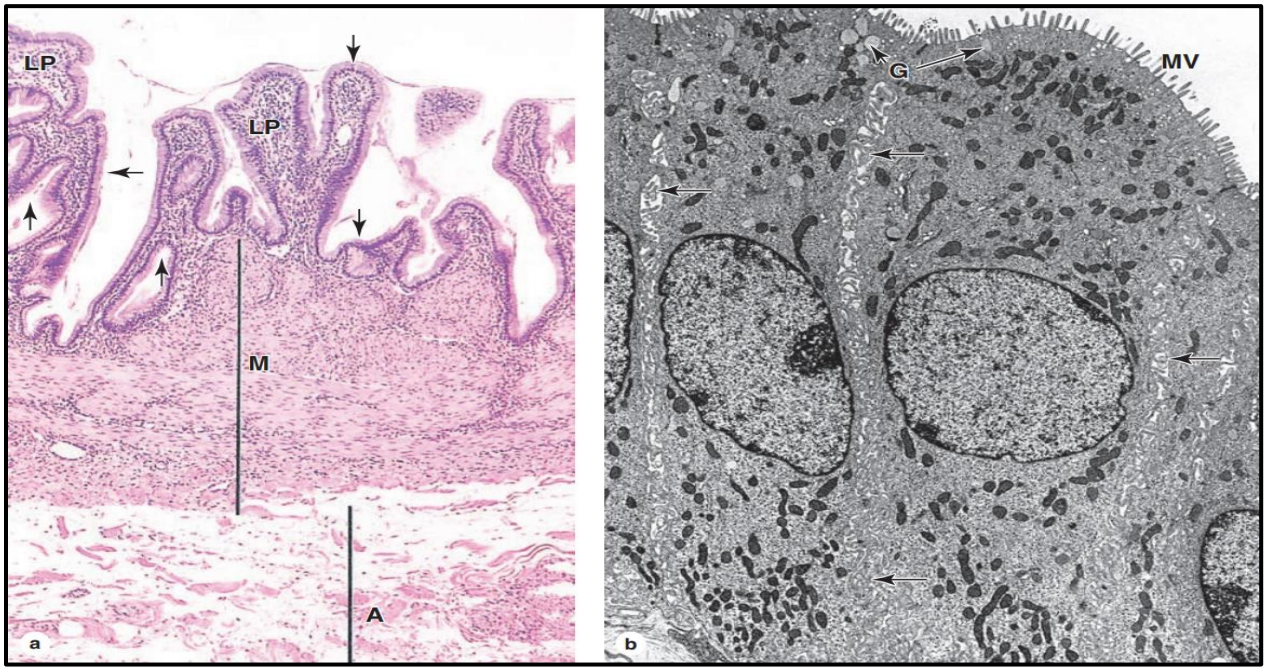


Figure 14: Gallbladder. (a) Its wall consists largely of a highly folded mucosa, with a simple columnar epithelium (arrows) overlying a typical lamina propria (LP); a muscularis (M) with bundles of muscle fibers oriented in all directions to facilitate emptying of the organ; and an external adventitia (A) where it is against the liver and a serosa where it is exposed (X60; H&E). (b) TEM of the epithelium shows cells specialized for water uptake across apical microvilli (MV) and release into the intercellular spaces (arrows) along the folded basolateral cell membranes. From these spaces water is quickly removed by capillaries in the lamina propria. Abundant mitochondria provide the energy for this pumping process. Scattered apical secretory granules (G) contain mucus. (X5600).

Lecture 19-20.. The urinary system

The urinary system consists of the **paired kidneys** and **ureters**, **the bladder**, and **the urethra**. This system's role is to ensure optimal properties of the blood, which the kidneys continuously monitor. This general role of the kidneys involves:

- Regulation of the balance between water and electrolytes and the acid-base balance.
- Excretion of metabolic wastes along with excess water and electrolytes in urine.
- Excretion of many bioactive substances, including many drugs.
- Secretion of renin, a protease important for regulation of blood pressure.
- Secretion of erythropoietin, a glycoprotein growth factor that stimulates erythrocyte production in red marrow.
- Conversion of the steroid prohormone vitamin D, initially produced in the skin, to the active form (**1,25-dihydroxyvitamin D3**).
- Gluconeogenesis during starvation, making glucose from amino acids.

All the major functions of the kidneys are performed by **specialized epithelial cells** of the nephrons and collecting systems.

Kidneys

Approximately 12-cm long, 6-cm wide, and 2.5-cm thick in adults, each kidney has a concave medial border, the hilum—where nerves enter, the ureter exits, and blood and lymph vessels enter and exit—and a convex lateral surface, both covered by a thin fibrous capsule (Figure 1). Within the hilum the upper end of the ureter expands as the **renal pelvis** and divides into **two or three major calyces**. The area surrounding the renal pelvis and calyces contains adipose tissue.

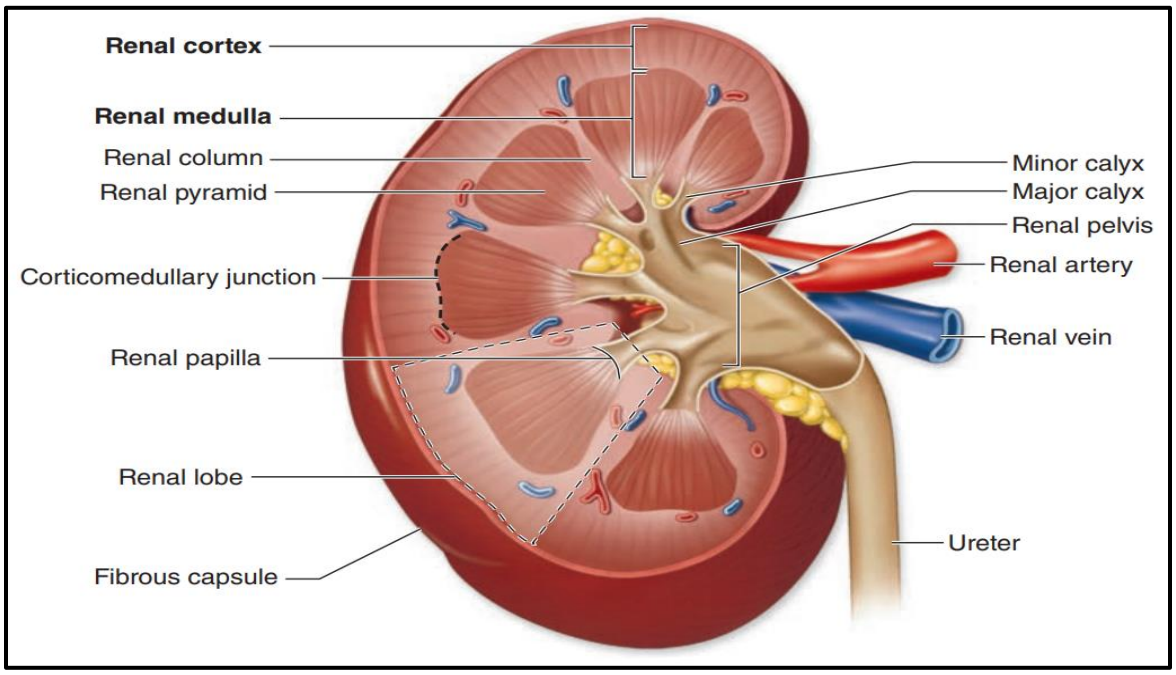


Figure 1: Kidney.

The parenchyma of each kidney has an outer renal cortex, a darker stained region with many round corpuscles and tubule cross sections, and an inner renal medulla consisting mostly of aligned linear tubules and ducts (Figure 1). **The renal medulla** in humans consists of 8-15 conical structures called **renal pyramids**, all with their bases meeting the cortex (at **the corticomedullary junction**) and separated from each other by extensions of the cortex called **renal columns**. Parallel ducts and tubules extending from the medulla into the cortex comprise **the medullary rays**; these plus their associated cortical tissue are considered **renal lobules**. The tip of each pyramid, called **the renal papilla**, projects into a minor calyx that collects urine formed by tubules in one renal lobe (Figure 1).

Each kidney contains **1-4 million** functional units called **nephrons** (Figure 2), each consisting of a corpuscle and a long, simple epithelial renal tubule with three main parts along its length. **The following are the major divisions of each nephron:**

- **Renal corpuscle**, an initial dilated part enclosing a tuft of capillary loops and the site of blood filtration, always located in the cortex.
- **Proximal tubule**, a long convoluted part, located entirely in the cortex, with a shorter straight part that enters the medulla.
- **Loop of Henle** (or nephron loop), in the medulla, with a thin descending and a thin ascending limb.
- **Distal tubule**, consisting of a thick straight part ascending from the loop of Henle back into the cortex and a convoluted part completely in the cortex.

- **Connecting tubule**, a short minor part linking the nephron to collecting ducts.

Connecting tubules from several nephrons merge to form collecting tubules that then merge as larger collecting ducts. These converge in the renal papilla, where they deliver urine to a minor calyx.

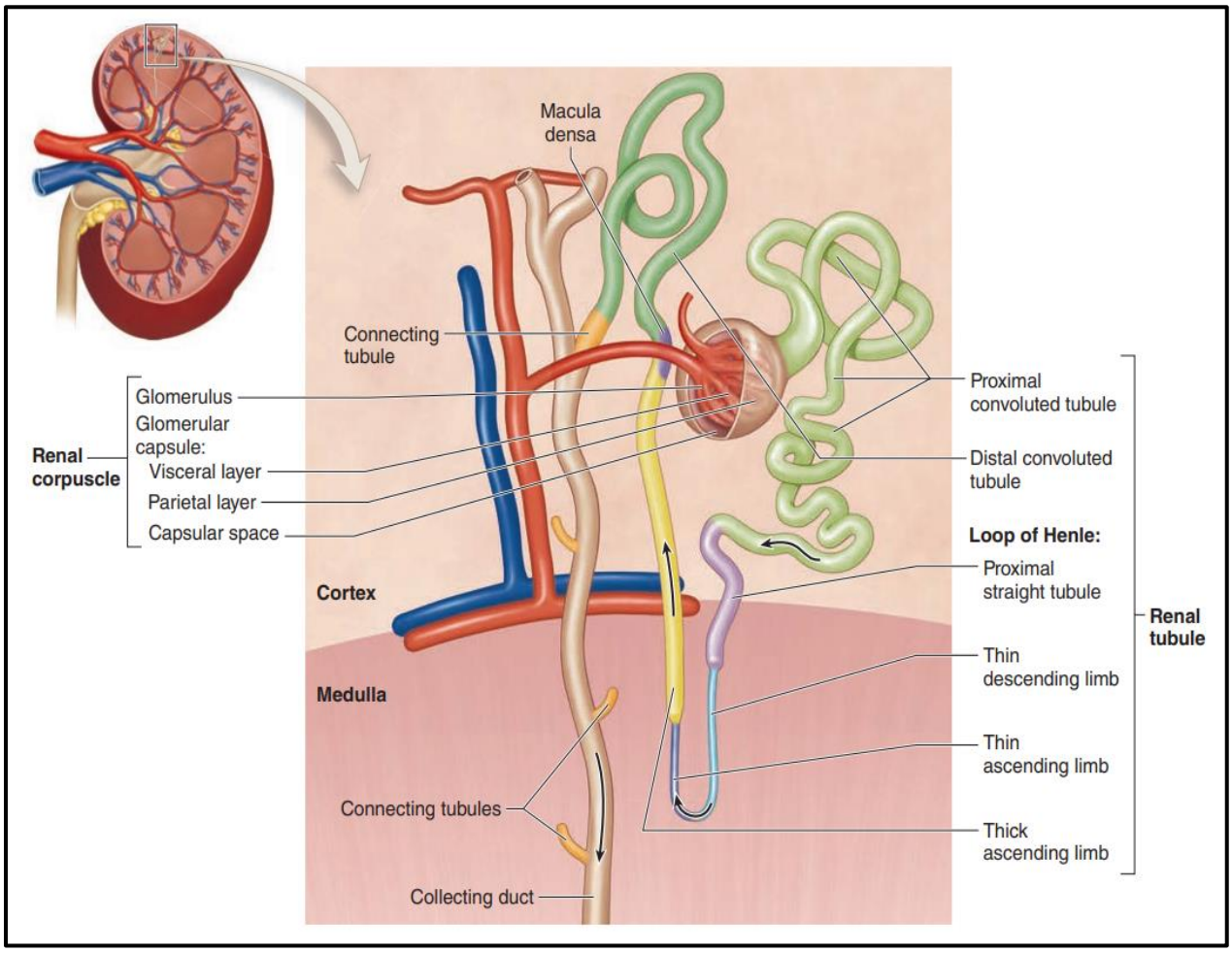


Figure 2: A nephron and its parts.

Renal Vasculature

- **Renal arteries** branch to form smaller arteries between **the renal lobes**, with **interlobular arteries** entering the cortex to form **the microvasculature**.
- **In the cortex** afferent arterioles enter capillary clusters called **glomeruli**, which are drained by efferent arterioles, instead of venules, an arrangement that allows higher hydrostatic pressure in the capillaries.
- **The efferent arterioles** from cortical glomeruli branch diffusely as **peritubular capillaries**, while those from **juxtamedullary glomeruli** branch as long microvascular loops called **vasa recta in the medulla**.

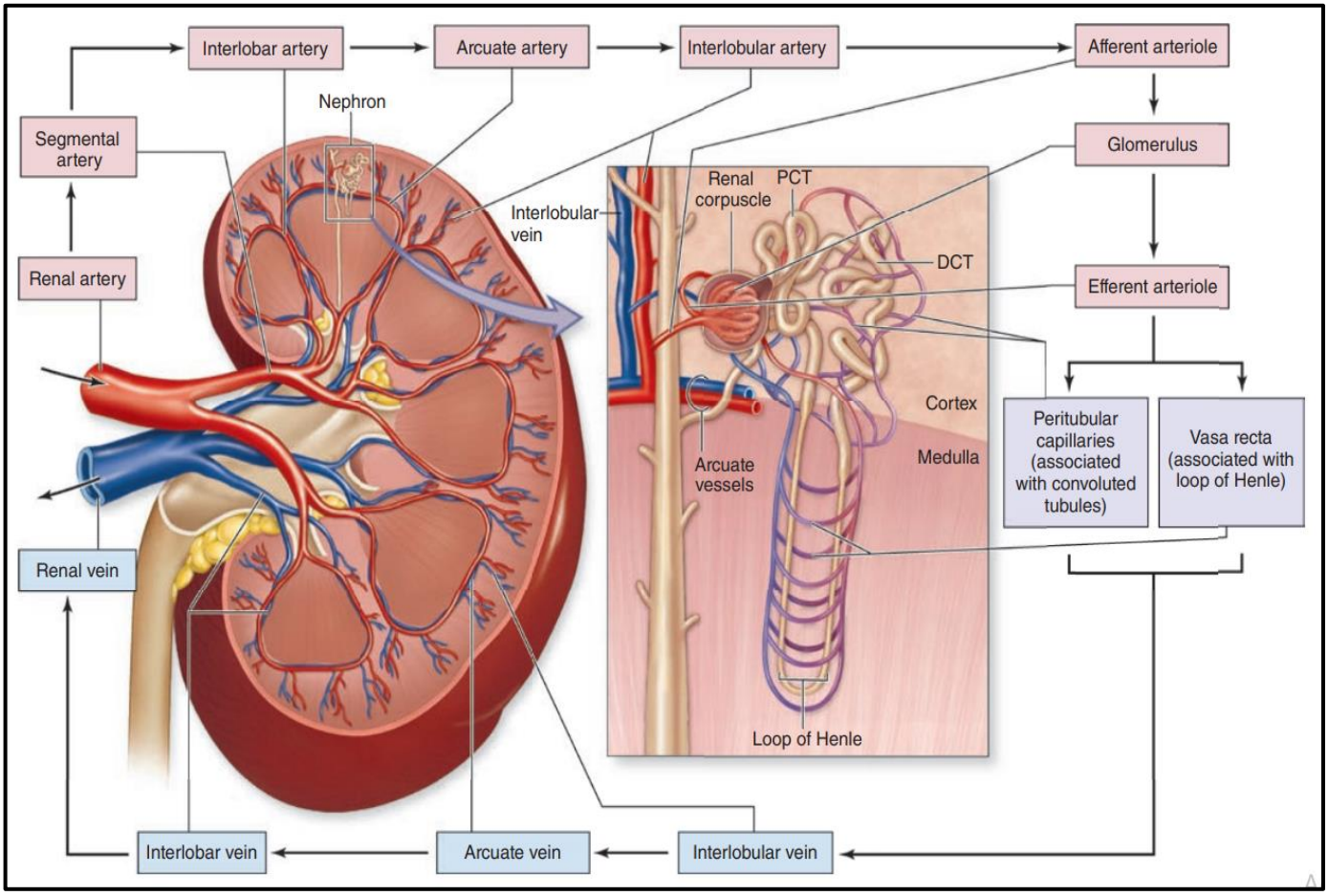


Figure 3: Blood supply to the kidneys.

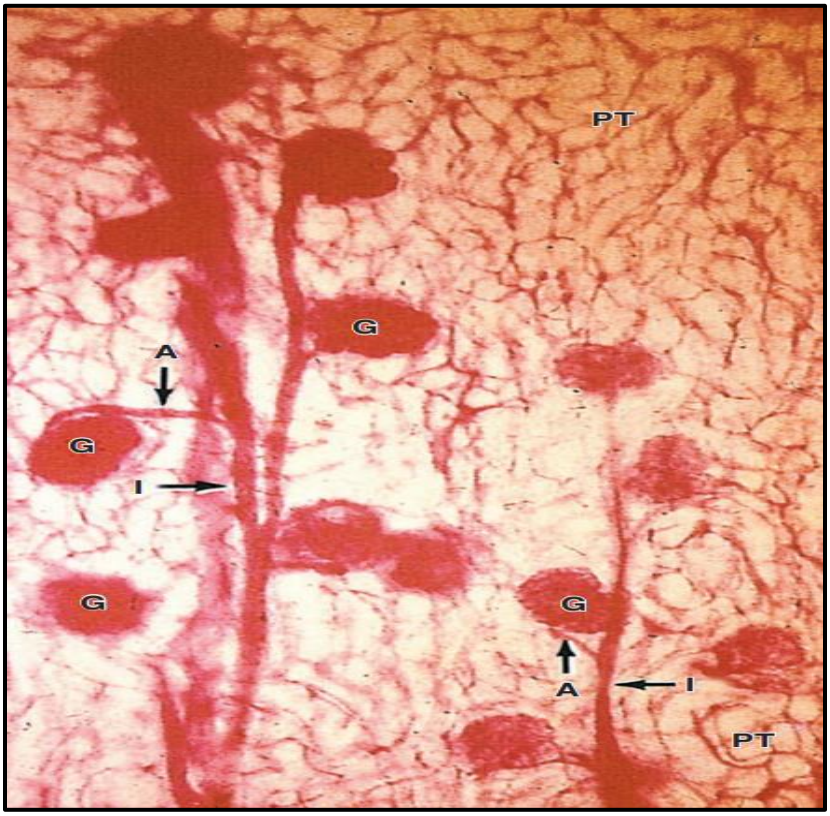


Figure 4: Microvasculature of the renal cortex. Cortical vasculature is revealed in a section of the kidney with the renal artery injected with carmine dye before fixation. Small interlobular arteries (I) branch from the arcuate arteries and radiate out through the cortex giving off the afferent arterioles (A) that bring blood to the glomerular capillaries. Each glomerulus (G) contains a mass of capillary loops that drain into an efferent arteriole. These then branches as a large, diffuse network of peritubular capillaries (PT) throughout the cortex. (X125).

Renal Corpuscles & Blood Filtration

At the beginning of each nephron is a **renal corpuscle**, containing a tuft of glomerular capilaries, surrounded by a double-walled epithelial capsule called **the glomerular (Bowman) capsule** (Figure 2 and 5).

The visceral layer of this capsule closely envelops the glomerular capillaries, which are finely fenestrated. Between the two capsular layers is the capsular space, which receives the fluid filtered through **the capillary wall** and **the visceral layer**. Each renal corpuscle has **a vascular pole**, where the afferent arteriole enters and the efferent arteriole leaves, and **a tubular pole**, where the proximal convoluted tubule (PCT) begins (Figure 5).

The outer parietal layer of a glomerular capsule consists of a simple squamous epithelium supported externally by a basal lamina. **At the tubular pole**, this epithelium

changes to the simple cuboidal epithelium that continues and forms **the proximal tubule** (Figure 5).

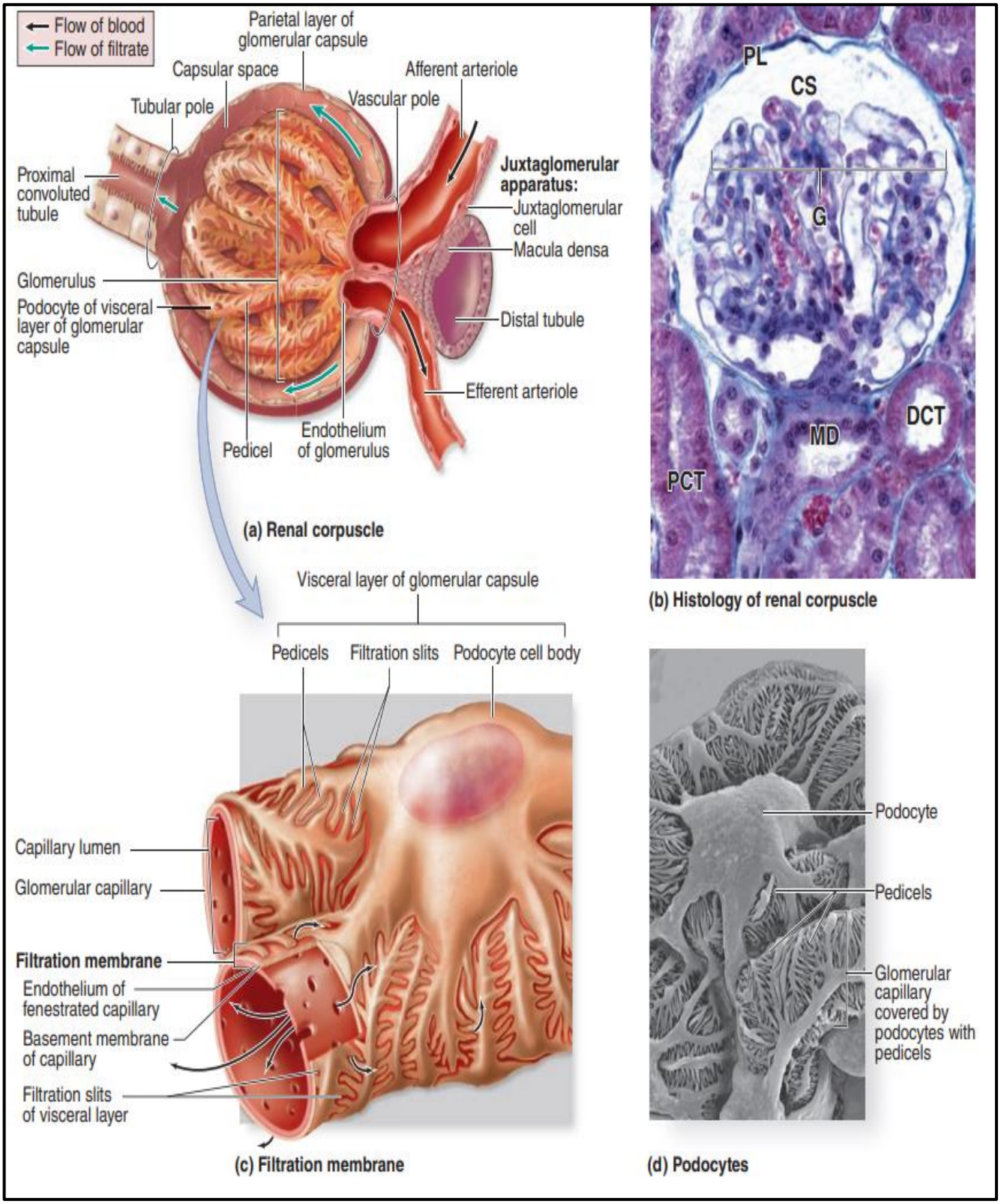


Figure 5: Renal corpuscles. (a) The renal corpuscle. (b) The micrograph shows the major histologic features of a renal corpuscle. The glomerulus (G) of capillaries is surrounded by the capsular space (CS) covered by the simple squamous parietal layer (PL) of Bowman capsule. Near the corpuscle is that

nephron's macula densa (MD) and sections of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). (H&E; X300). (c) The details of filtration membrane. (d) The scanning electron microscopy (SEM) shows the distinctive appearance of podocytes and their pedicel processes that cover glomerular capillaries. (X800).

The visceral layer of a renal corpuscle consists of **unusual stellate epithelial cells** called **podocytes** (Figure 5c and 5d), which together with **the capillary endothelial cells** compose **the apparatus for renal filtration**. From the cell body of each podocyte several primary processes extend and curve around a length of glomerular capillary. Each primary process gives rise to many parallel, secondary processes (Figure 5c and 5d). between which are narrow spaces called **slit pores**. The elevated pressure in the capillaries forces water and small solutes of blood plasma through the glomerular filter into the capsular space inside the glomerular capsule.

In addition to capillary endothelial cells and podocytes, renal corpuscles also contain **mesangial cells**. Mesangial cells are difficult to distinguish in routine sections from podocytes, but often stain more darkly. They and their surrounding matrix comprise **the mesangium** (Figure 6), which fills interstices between capillaries that lack podocytes. **Functions of the mesangium include**: Physical support of capillaries within the glomerulus, Adjusted contractions in response to blood pressure changes and Secretion of several cytokines, prostaglandins, and other factors important for immune defense and repair in the glomerulus.

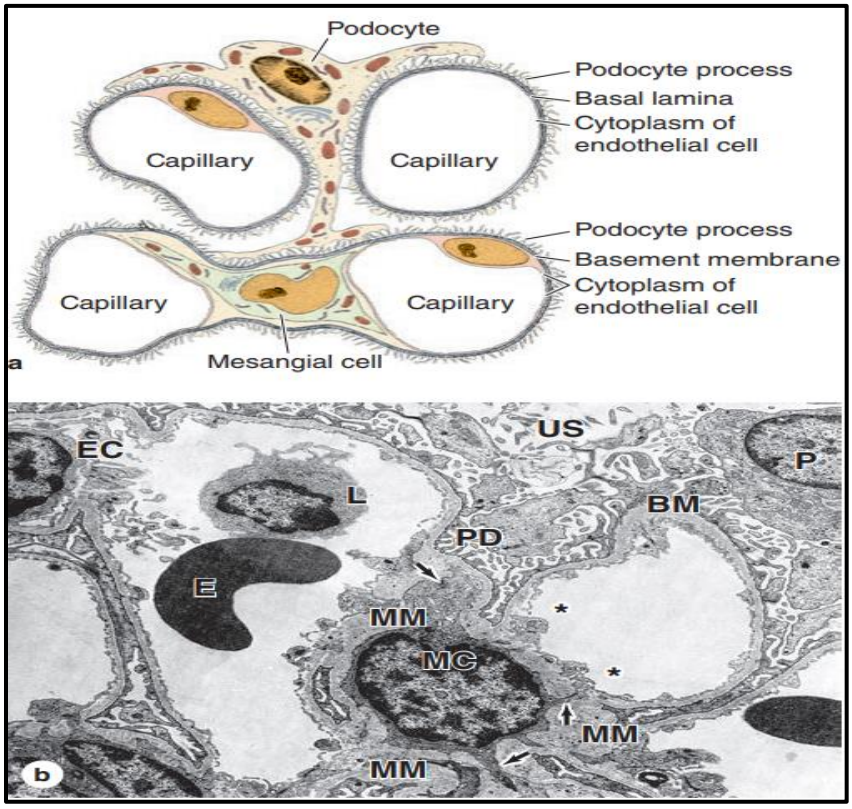


Figure 6: Mesangium. (a) Diagram shows that mesangial cells are located between capillaries. (b) The TEM shows one mesangial cell (MC) and the surrounding mesangial matrix (MM). This matrix appears similar to and in many places continuous with basement membrane (BM) and supports capillaries where podocytes are lacking. Mesangial cells extend contractile processes (arrows) along capillaries. Some mesangial processes appear to pass between endothelial cells (EC) into the capillary lumen (asterisks). The capillary at the left contains an erythrocyte (E) and a lymphocyte (L). Podocytes (P) and their pedicels (PD) open to the urinary space (US) and associate with the capillary surfaces not covered by mesangial cells (X3500).

Proximal Convoluted Tubule

Cells in many parts of the nephron tubule and collecting system reabsorb water and electrolytes. **At the tubular pole** of the renal corpuscle, the simple squamous epithelium of the capsule's parietal layer is continuous with the simple cuboidal epithelium of **the proximal convoluted tubule (PCT)** (Figure 7). These long, tortuous tubules fill most of the cortex. PCT cells are specialized for both reabsorption and secretion. Over half of the water and electrolytes, and all of the organic nutrients, filtered from plasma in the renal corpuscle.

The cells of the proximal tubules have central nuclei and very acidophilic cytoplasm (Figure 7) because of the abundant mitochondria. The cell apex has very many long microvilli that form a prominent brush border in the lumen that facilitates reabsorption (Figure 7 and 8). Because the cells are large, each transverse section of a PCT typically contains only three to five nuclei. (Figure 7).

Ultrastructurally the apical cytoplasm of these cells has numerous pits and vesicles near the bases of the microvilli, indicating active endocytosis and pinocytosis (Figure 8). These vesicles contain the small, reabsorbed proteins that will be degraded in lysosomes, with the amino acids released to the circulation. Proximal tubular cells also have many long basal membrane invaginations and lateral interdigitations with neighboring cells (Figure 8). Long mitochondria concentrated along the basal invaginations (Figure 8) supply ATP locally for the membrane proteins involved in active transport.

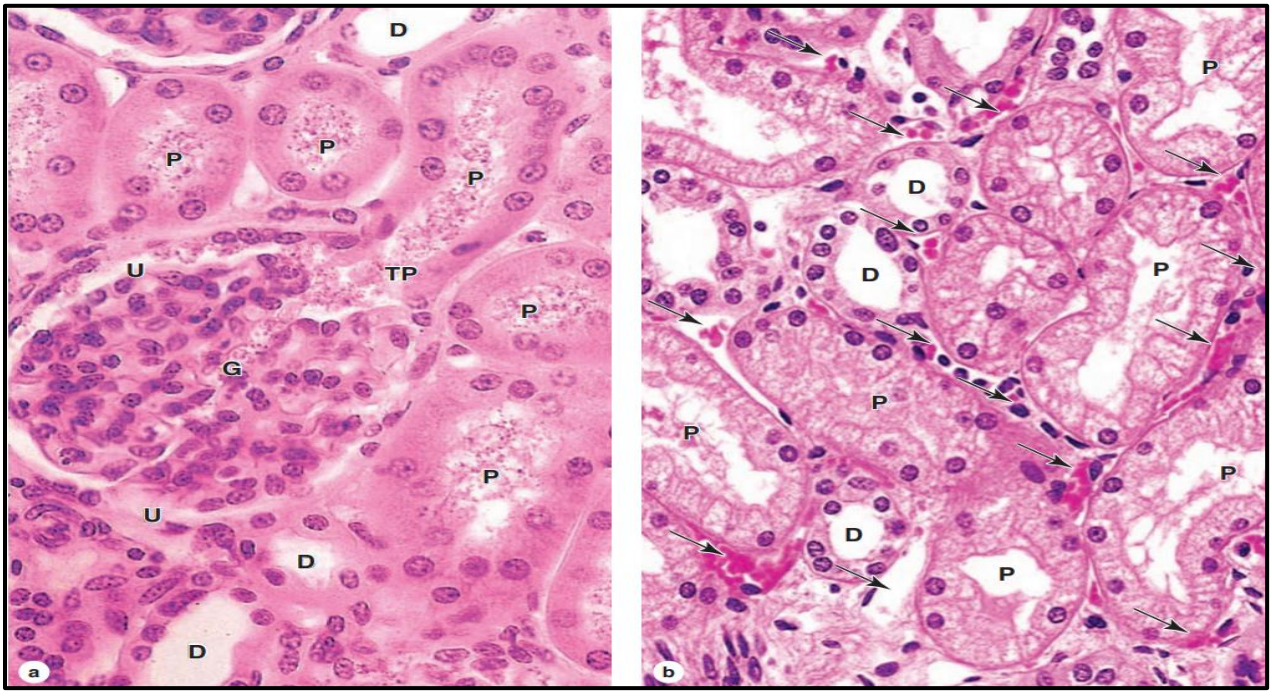


Figure 7: Renal cortex: proximal and distal convoluted tubules. (a) The micrograph shows the continuity at a renal corpuscle's tubular pole (TP) between the simple cuboidal epithelium of a proximal convoluted tubule (P) and the simple squamous epithelium of the capsule's parietal layer. The urinary space (U) between the parietal layer and the glomerulus (G) drains into the lumen of the proximal tubule. The lumens of the proximal tubules appear filled, because of the long microvilli of the brush border and aggregates of small plasma proteins bound to this structure. By contrast, the lumens of distal convoluted tubules (D) appear empty, lacking a brush border and protein. (b) Here the abundant peritubular capillaries and draining venules (arrows) surrounding the proximal (P) and distal (D) convoluted tubules are clearly seen. (Both X400; H&E).

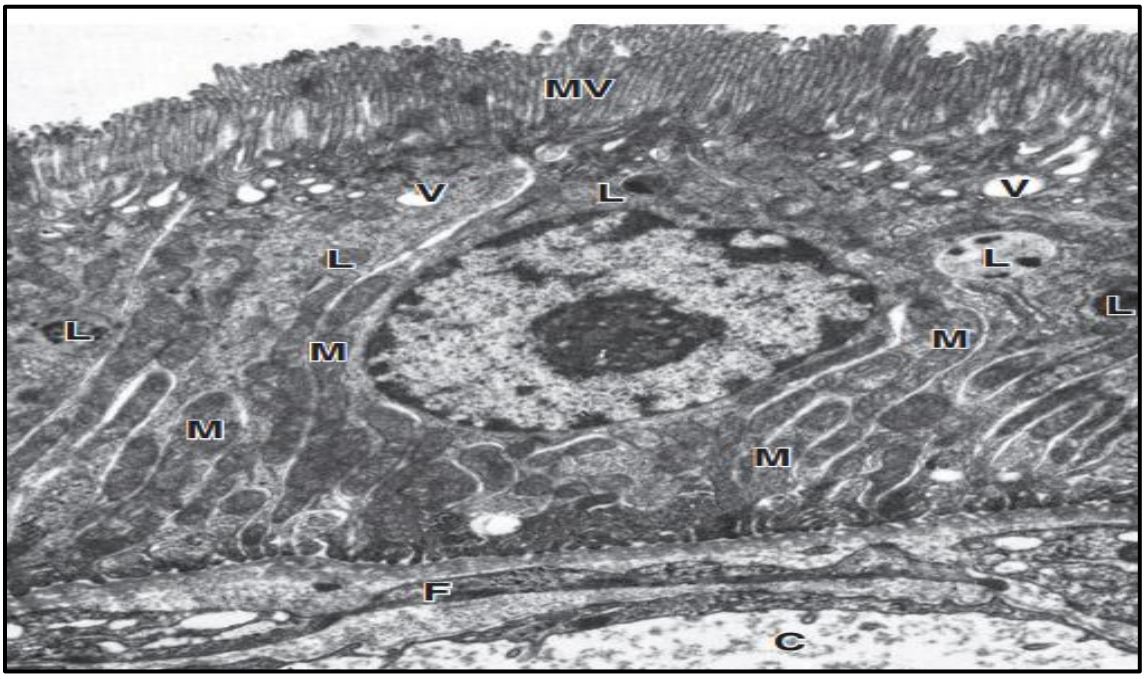


Figure 8: Ultrastructure of proximal convoluted tubule cells. TEM reveals important features of the cuboidal cells of the PCT: the microvilli (MV), the abundant endocytotic pits and vesicles (V) in the apical regions near lysosomes (L). The basolateral sides are characterized by long invaginating folds of membrane along which many long mitochondria (M) are situated. Water and the small molecules released from the PCTs are taken up immediately by the adjacent peritubular capillaries (C). Between the basement membranes of the tubule and the capillary shown here is an extension of a fibroblast (F) (X10,500).

Loop of Henle

The **PCT** continues with the **much shorter proximal straight tubule**, which enters the medulla and continues as **the nephron's loop of Henle** (Figure 2). This is a **U-shaped** structure with a thin descending limb and a thin ascending limb, both composed of simple squamous epithelia. The wall of the thin segments consists only of squamous cells (Figure 9). **The thin ascending limb** of the loop becomes **the thick ascending limb (TAL)**, with simple cuboidal epithelium, in the outer medulla and extends as far as the **macula densa** near the nephron's glomerulus.

The primary role of the loop of Henle is water and sodium chloride recovery from urine, by concentrating urine, it allows the body to excrete waste while minimizing water loss.

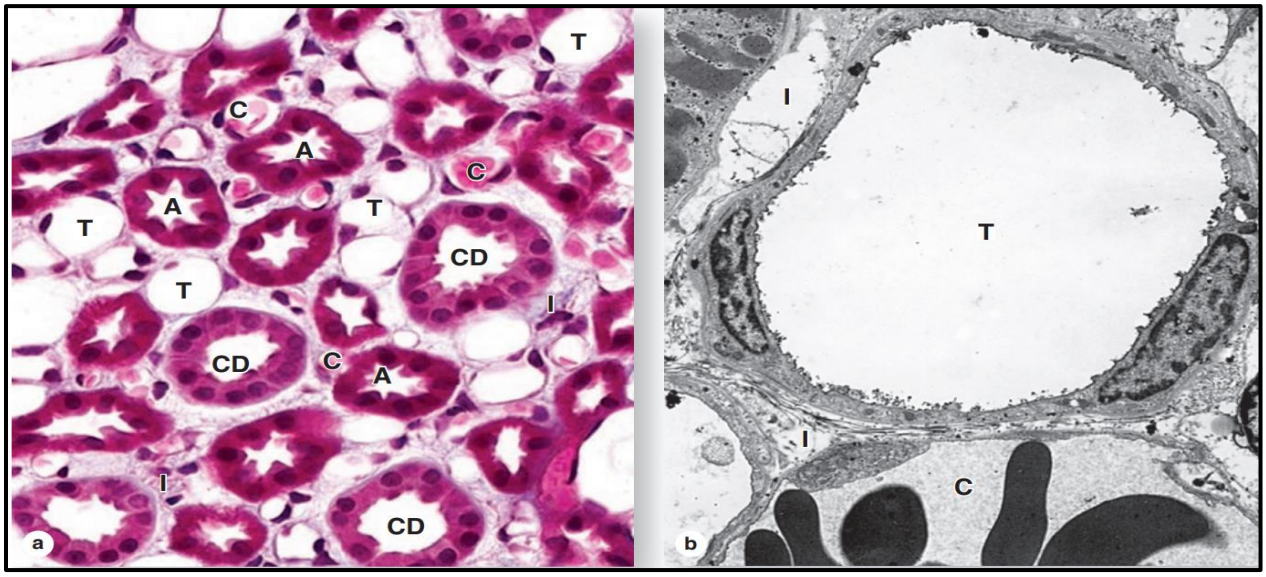


Figure 9: Renal medulla: nephron loops and collecting ducts. (a) A micrograph of a medullary renal pyramid cut transversely shows closely packed cross sections of the many nephron loops' thin descending and ascending limbs (T) and thick ascending limbs (A), intermingled with parallel vasa recta capillaries contaiing blood (C) and collecting ducts (CD). All these structures are embedded in the interstitium (I), which contains sparse myofibroblast-like cells in a matrix very rich in hydrophilic hyaluronate (X400; Mallory trichrome). (b) The TEM reveals the slightly fibrous nature of the interstitium (I) and shows that the simple squamous epithelium of the thin limbs (T) is slightly thicker than that of the nearby vasa recta capillaries (C). (X3300)

Distal Convoluted Tubule & Juxtaglomerular Apparatus

The ascending limb of the nephron is straight as it enters the cortex and forms the **macula densa**, and then becomes tortuous as **the distal convoluted tubule (DCT)** (Figure 2). Much less tubular reabsorption occurs here than in the proximal tubule. The simple cuboidal cells of the distal tubules differ from those of the proximal tubules in being smaller and having no brush border and more empty lumens (Figure 7). The rate of Na+ absorption here is regulated by aldosterone from the adrenal glands.

Where the initial, straight part of the distal tubule contacts the arterioles at the vascular pole of the renal corpuscle, its cells become more columnar and closely packed, forming the macula densa. This is part of a specialized sensory structure, the **juxtaglomerular apparatus (JGA)** that utilizes feedback mechanisms to regulate glomerular blood flow and keep the rate of glomerular filtration relatively constant. The JGA is shown in Figure 5 and 10.

Cells of **the macula densa** typically have apical nuclei, basal Golgi complexes, and a more elaborate and varied system of ion channels and transporters. Adjacent to the macula densa, **the tunica media** of the afferent arteriole is also modified. The smooth muscle cells are modified **as juxtaglomerular granular** (**JG**) cells, with a secretory phenotype including more rounded nuclei, rough ER, Golgi complexes, and granules with the protease renin (Figure 5 and 10). Also **at the vascular pole** are **lacis cells**, which are **extraglomerular mesangial cells** that have many of the same supportive, contractile, and defensive functions as these cells inside **the glomerulus**.

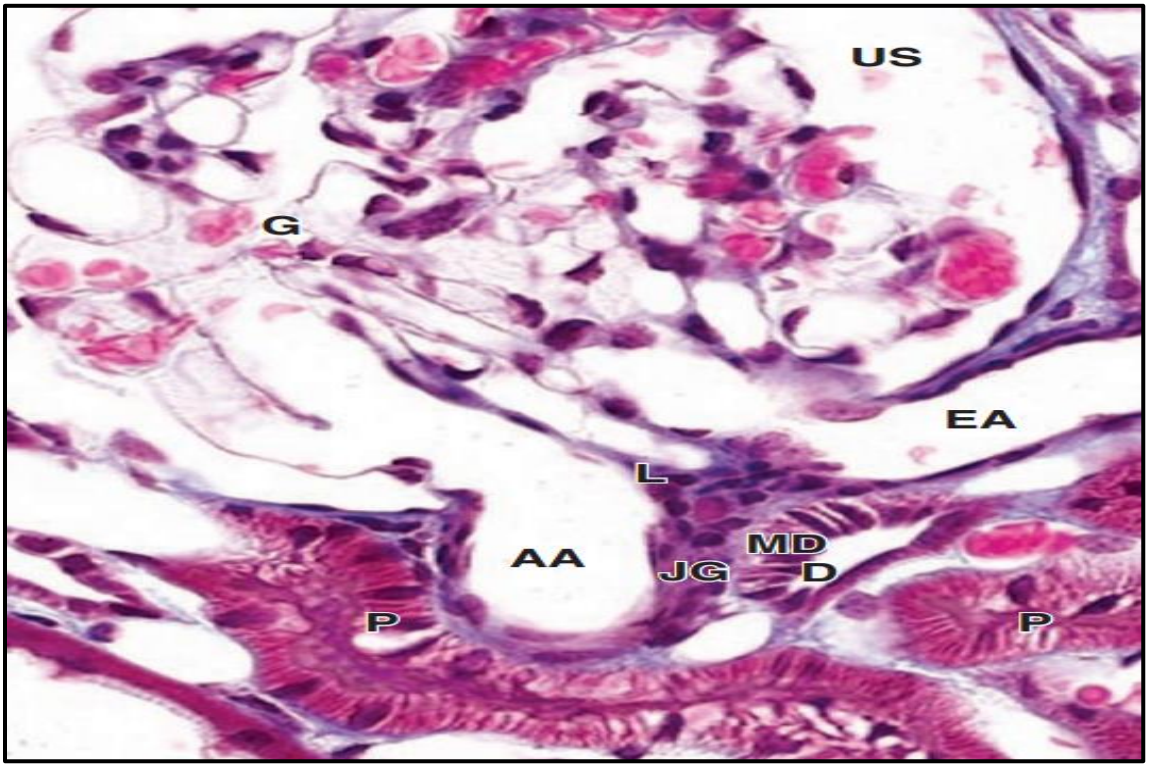


Figure 10: Juxtaglomerular apparatus. The JGA forms at the point of contact between a nephron's distal tubule (D) and the vascular pole of its glomerulus (G). At that point cells of the distal tubule become columnar as a thickened region called the macula densa (MD). Smooth muscle cells of the afferent arteriole's (AA) tunica media are converted from a contractile to a secretory morphology as juxtaglomerular granule cells (JG). Also present are lacis cells (L), which are extraglomerular mesangial cells adjacent to the macula densa, the afferent arteriole, and the efferent arteriole (EA). In this specimen the lumens of proximal tubules (P) appear filled and the urinary space (US) is somewhat swollen. (X400; Mallory trichrome).

Collecting Ducts

The last part of each **nephron**, **the connecting tubule**, carries the filtrate into a collecting system that transports it to a minor calyx and in which more water is reabsorbed if needed by the body. As shown in, a connecting tubule extends from each nephron and several join together in **the cortical medullary** rays to form collecting ducts of simple cuboidal epithelium. In the medulla these merge further, forming larger and straighter collecting ducts with increasingly columnar cells (Figure 9 and 11). Approaching the apex of each renal pyramid, several medullary collecting ducts merge again to form each papillary duct (or duct of Bellini), which deliver urine directly into the minor calyx. Running parallel with the descending and ascending limbs of **the loops of Henle and vasa recta**, medullary collecting ducts lie in the area with very high interstitial osmolarity (Figure 2 and 9).

Principal cells (Figure 11) of the collecting ducts are pale-staining, with relatively few mitochondria and distinct cell membranes that are rich in **aquaporins** for passive water reabsorption. The largest collecting ducts deliver filtrate into the minor calyces, where it undergoes no further modification and is called **urine**.

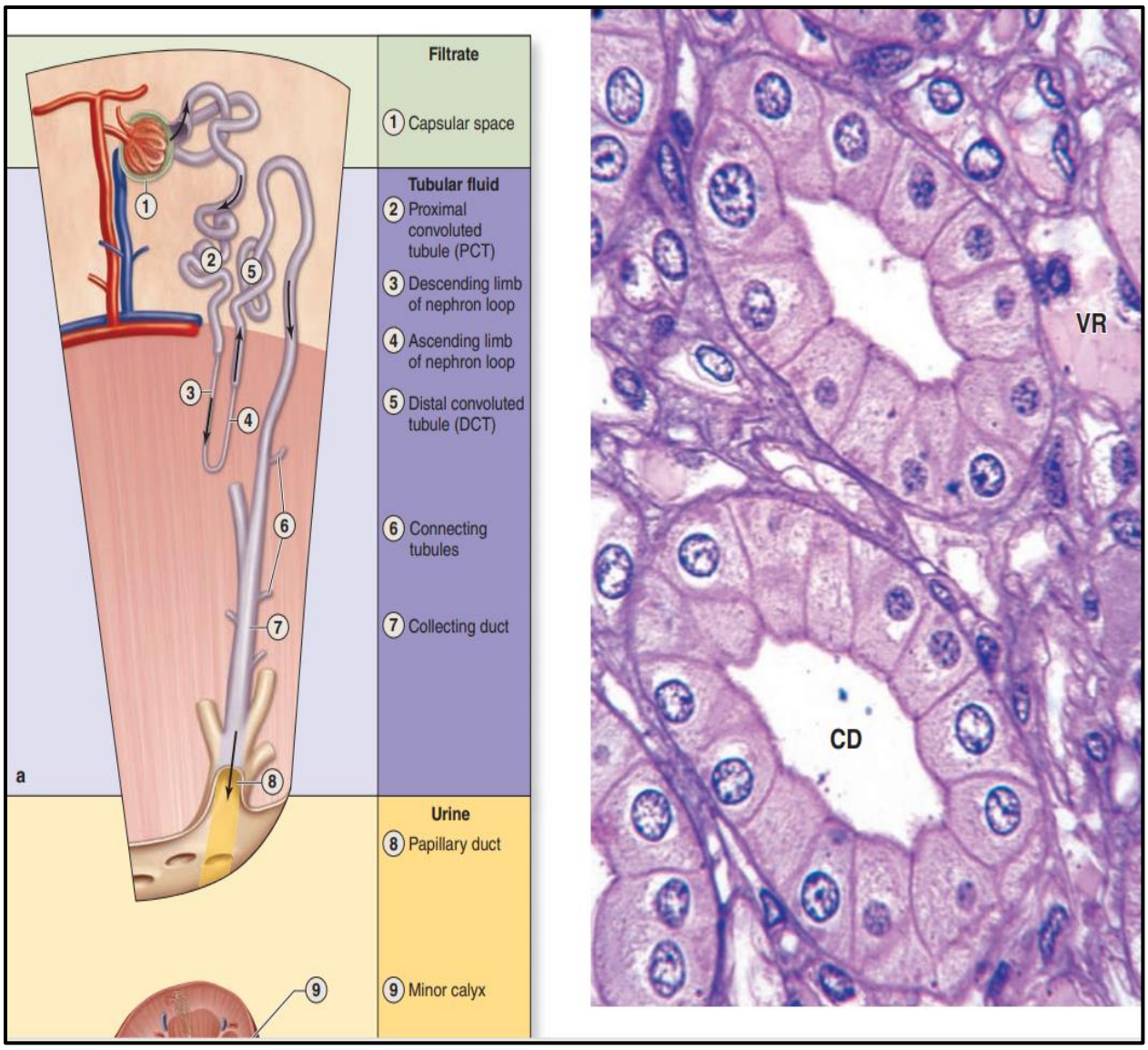


Figure 11: Collecting ducts. Pale-staining columnar principal cells, in which ADH-regulated aquaporins of the cell membrane allow more water reabsorption, are clearly seen in these transversely sectioned collecting ducts (CD), surrounded by interstitium with vasa recta (VR). (X600; PT).

Ureters, Bladder, & Urethra

Urine is transported by **the ureters** from **the renal pelvis** to **the urinary bladder** where it is stored until emptying by micturition via **the urethra**. The walls of the ureters are similar to that of the calyces and renal pelvis, with mucosal, muscular, and

adventitial layers and becoming gradually thicker closer to the bladder. The mucosa of these organs is lined by the **uniquely stratified urothelium** or **transitional epithelium** (Figure 12 and 13). Cells of this epithelium are organized as three layers:

- **A single layer** of small basal cells resting on a very thin basement membrane.
- **An intermediate region** containing from one to several layers of cuboidal or low columnar cells.
- **A superficial layer** of large bulbous or elliptical umbrella cells, sometimes binucleated, which are highly differentiated to protect the underlying cells against the potentially cytotoxic effects of hypertonic urine.

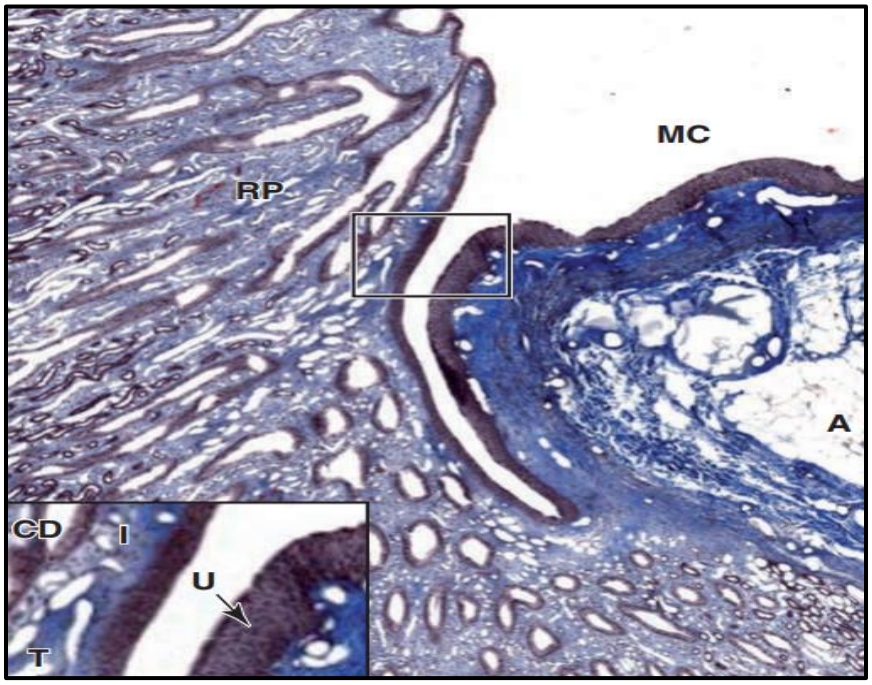


Figure 12: Renal papilla, collecting ducts, and minor calyx. A sagittal section of a renal papilla shows numerous collecting ducts converging at the end of the renal papilla (RP) where they empty into the minor calyx (MC). The mucosa of the calyx contains dense connective tissue stained blue here and adipose tissue (A) (X50; Mallory trichrome). Inset: An enlarged area shows the columnar epithelium of the collecting ducts (CD), the interstitium (I) and thin limbs (T), and the protective urothelium (U) that lines the minor calyx. (X200; Mallory trichrome).

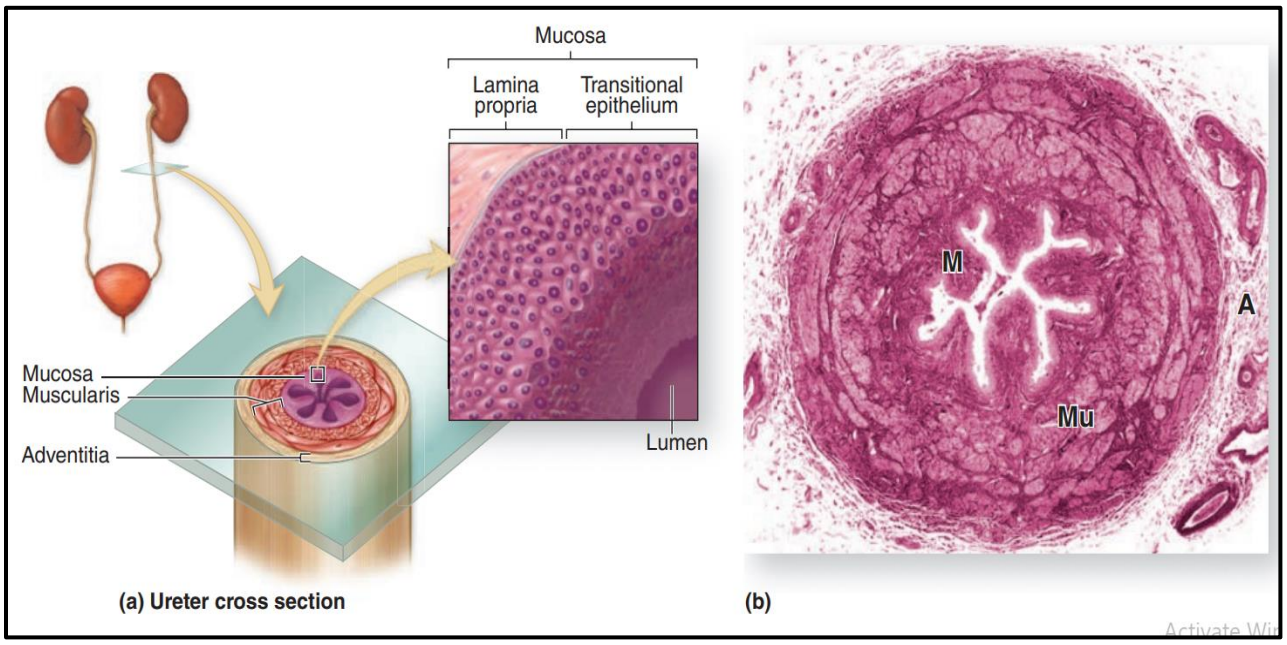


Figure 13: Ureters. (a) Diagram of a ureter in cross section. The lamina propria is lined by a unique stratified epithelium called transitional epithelium or urothelium that is resistant to the potentially deleterious effects of contact with hypertonic urine. (b) Histologically the muscularis (Mu) is much thicker than the mucosa (M) and adventitia (A) (X18; H&E).

The thick muscularis of the ureters moves urine toward **the bladder** by **peristaltic contractions** and produces prominent mucosal folds when the lumen is empty (Figure 13).

Umbrella cells are especially well developed in **the bladder** (Figure 14) where contact with urine is greatest. These cells have extensive intercellular junctional complexes surrounding unique **apical membranes**.

Urothelium is surrounded by a folded lamina propria and submucosa, followed by **a dense sheath** of interwoven smooth muscle layers and adventitia (Figure 13 and 14). **Urine** is moved from the **renal pelvises** to **the bladder** by peristaltic contractions of the ureters.

The bladder's lamina propria and dense irregular connective tissue of the submucosa are highly vascularized. The muscularis consists of three poorly delineated layers, collectively called **the detrusor muscle**, which contract to empty the bladder (Figure 14). **Three muscular layers** are seen most distinctly at the neck of the bladder near the urethra (Figure 14). **The ureters** pass through the wall of the bladder obliquely, forming a valve that prevents the backflow of urine into the ureters as the bladder fills. **All the urinary passages** are covered externally by an adventitial layer, except for the upper part of the bladder that is covered by **serous peritoneum**.

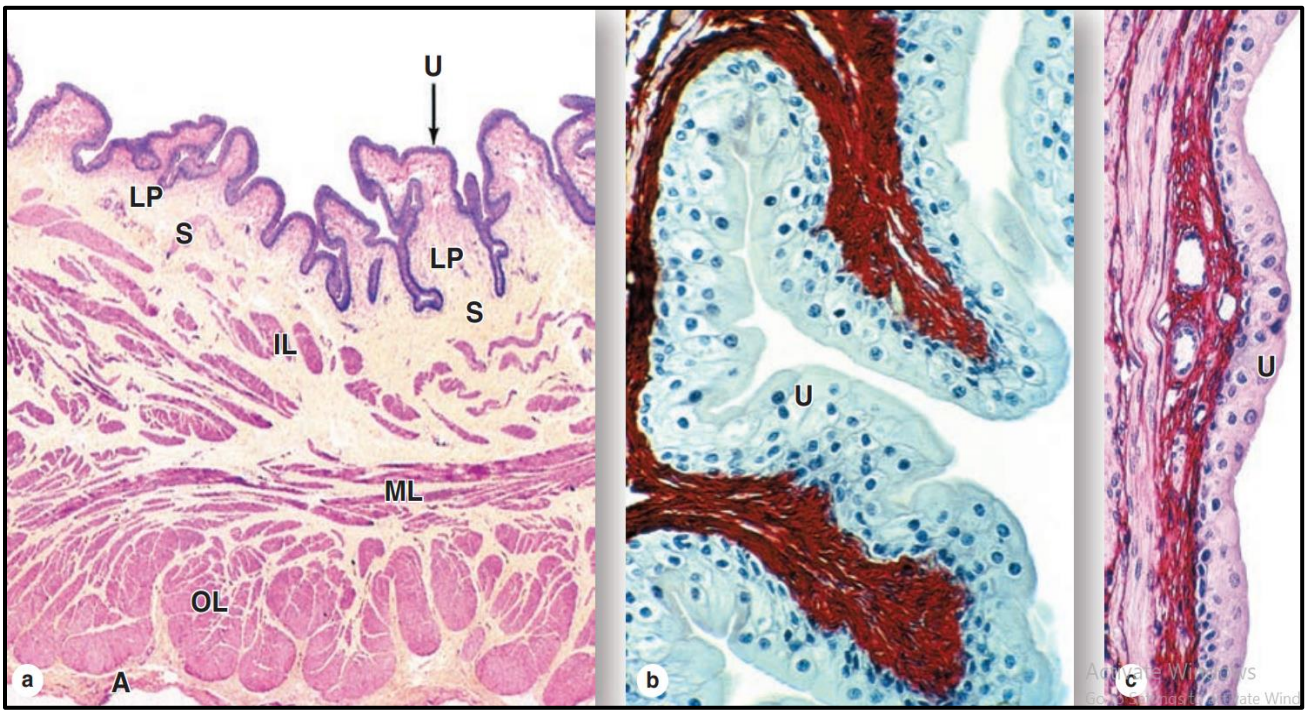


Figure 14: Bladder wall and urothelium. (a) In the neck of the bladder, near the urethra, the wall shows four layers: the mucosa with urothelium (U) and lamina propria (LP); the thin submucosa (S); inner, middle, and outer layers of smooth muscle (IL, ML, and OL); and the adventitia (A). (X15; H&E) (b) When the bladder is empty, the mucosa is highly folded and the urothelium (U) has bulbous umbrella cells. (X250; PSH) (c) When the bladder is full, the mucosa is pulled smooth, the urothelium (U) is thinner, and the umbrella cells are flatter. (X250; H&E).

The urethra is a tube that carries the urine from the bladder to the exterior (Figure 15). **The urethral mucosa** has prominent longitudinal folds, giving it a distinctive appearance in cross section.

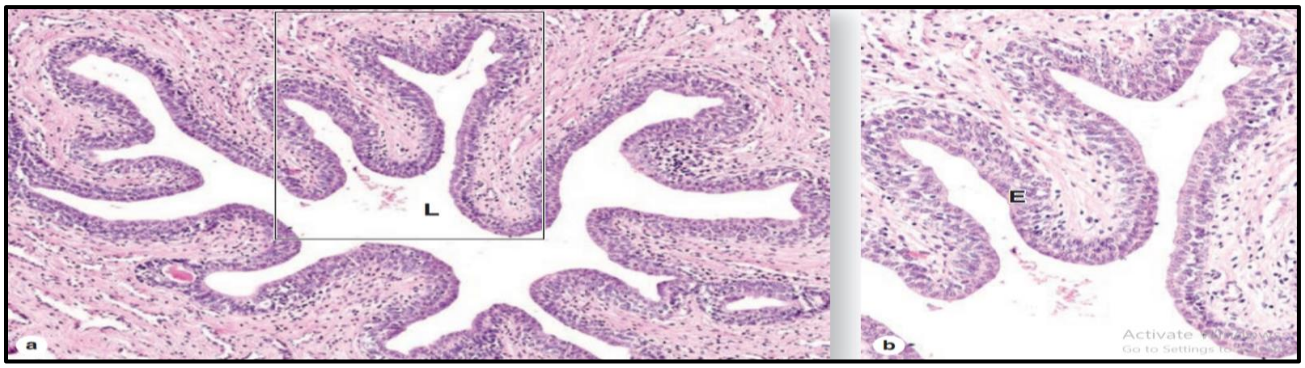


Figure 15: Urethra. (a) A transverse section shows that the mucosa has large longitudinal folds around the lumen (L) (X50; H&E). (b) A higher magnification of the enclosed area shows the unusual stratified columnar nature of the urethral epithelium (E) (X250; H&E).

Lecture 21-22.. The Respiratory System

The respiratory system provides for exchange of O_2 and CO_2 to and from the blood. **Respiratory organs** include **the lungs** and **a branching system of bronchial tubes**. Air is moved through the lungs by a ventilating mechanism, consisting of the thoracic cage, intercostal muscles, diaphragm, and elastic components of the lung tissue. **The system can be divided anatomically into the upper and lower respiratory tracts (Figure 1).**

■ **The conducting portion**, which consists of the nasal cavities, pharynx, larynx, trachea, bronchi, bronchioles, and terminal bronchioles.

■ **The respiratory portion**, where the system's main function of gas exchange occurs, consisting of respiratory bronchioles, alveolar ducts, and alveoli.

Alveoli, the cellular sites of the exchange of O_2 and CO_2 between inspired air and blood, are small, air-filled, saclike structures, which make up most of the lung structure. To ensure an uninterrupted supply of air, a combination of cartilage, collagen and elastic fibers, and smooth muscle provides the conducting portion with rigid structural support and the necessary flexibility and extensibility.

Nasal Cavities

The left and right nasal cavities each have two components: **the external, dilated vestibule and the internal nasal cavity**. Skin of the nose enters the nares (nostrils) partway into the vestibule and includes **sweat glands**, **sebaceous glands**, and **coarse, moist vibrissae (hairs)**, which filter out particulate material from inspired air. Within the vestibule, the epithelium loses its keratinized nature and undergoes a transition to typical pseudostratified columnar epithelium which also lines the nasal cavities.

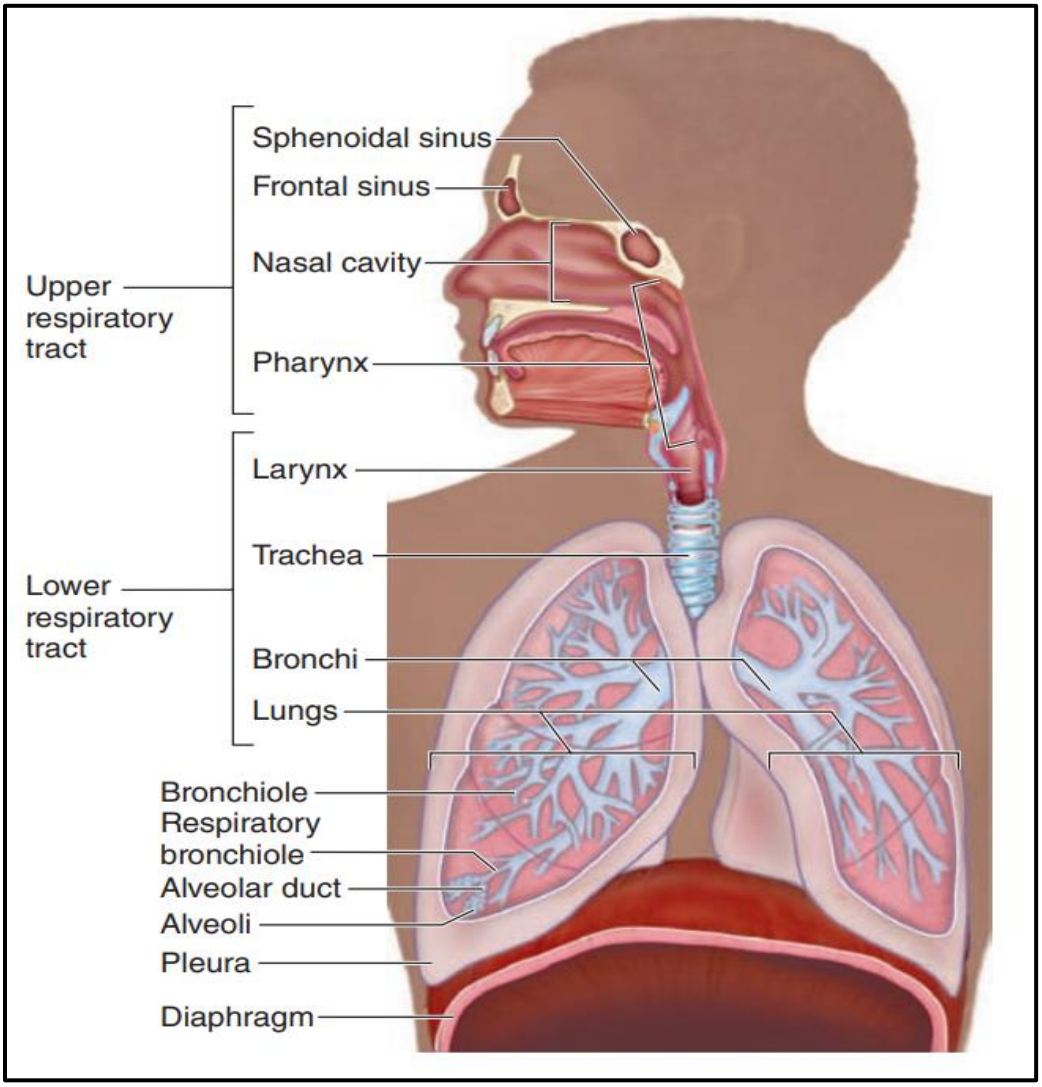


Figure 1: Anatomy of the respiratory system.

Respiratory Epithelium

Most of the nasal cavities and conducting portion of the system is lined with mucosa having ciliated pseudostratified columnar epithelium (Figure 2). This epithelium has five major cell types, all of which contact an unusually thick basement membrane:

- **Ciliated columnar cells** are the most abundant, each with 250-300 cilia on its apical surface (Figure 2).
- **Goblet cells** are also numerous and predominate in some areas (Figure 2), with basal nuclei and apical domains filled with granules of mucin glycoproteins.
- **Brush cells** are a much less numerous, columnar cell type, in which a small apical surface bears sparse microvilli. Brush cells are chemosensory receptors,

with signal transduction components and synaptic contact with afferent nerve endings on their basal surfaces.

- **Small granule cells** (or Kulchitsky cells) are difficult to distinguish in routine preparations but possess numerous dense core granules 100-300 nm in diameter. They are part of the diffuse neuroendocrine system (DNES).
- **Basal cells** are mitotically active stem and progenitor cells that give rise to the other epithelial cell types.

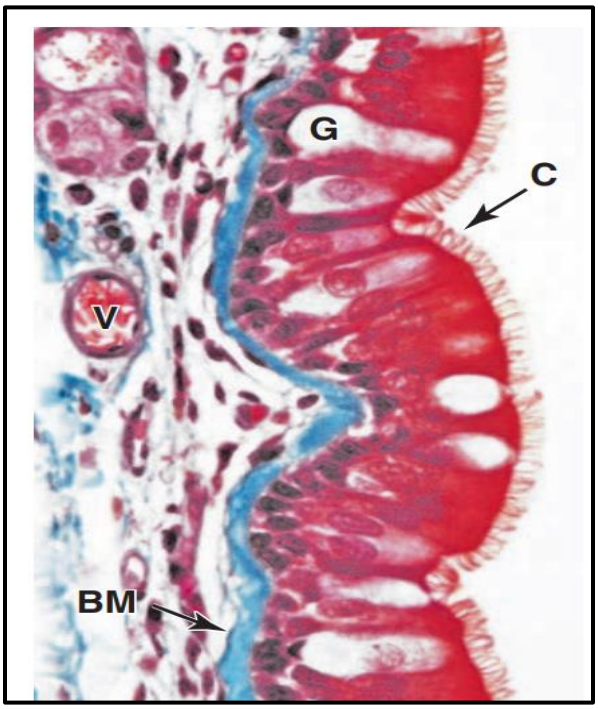


Figure 2: Respiratory epithelium. Details of its structure vary in different regions of the respiratory tract, but it usually rests on a very thick basement membrane (BM) and has several cell types, some columnar, some basal, and all contacting the basement membrane. Ciliated columnar cells are most abundant, with hundreds of long robust cilia (C) on each of their bulging apical ends that provide a lush cover of cilia on the luminal surface. Mucus-secreting goblet cells (G) and intraepithelial lymphocytes and dendritic cells are also present in respiratory epithelium. The lamina propria is well-vascularized (V) (X400; Mallory trichrome).

Olfactory Epithelium

The olfactory chemoreceptors for the sense of smell are located in **the olfactory epithelium**, a specialized region of the mucous membrane covering the superior conchae at the roof of the nasal cavity. This thick, pseudostratified columnar epithelium has three major cell types (Figure 3):

- **Olfactory neurons** are bipolar neurons present throughout this epithelium. The apical (luminal) pole of each cell has cilia project into the overlying aqueous layer. These cilia provide a large surface for transmembrane chemoreceptors. The

receptors respond to odoriferous substances by generating an action potential along the axons extending from the basal ends of these neurons (Figure 3).

- **Supporting cells** are columnar, cylindrical apexes containing the nuclei and extending microvilli into the fluid layer. The supportive role of these cells is not well understood, but they express abundant ion channels, which help maintain a microenvironment to olfactory function and survival.
- **Basal cells** are small, spherical, or cone-shaped cells near the basal lamina. These are the stem cells for the other two types, replacing the olfactory neurons every 2-3 months and support cells less frequently.

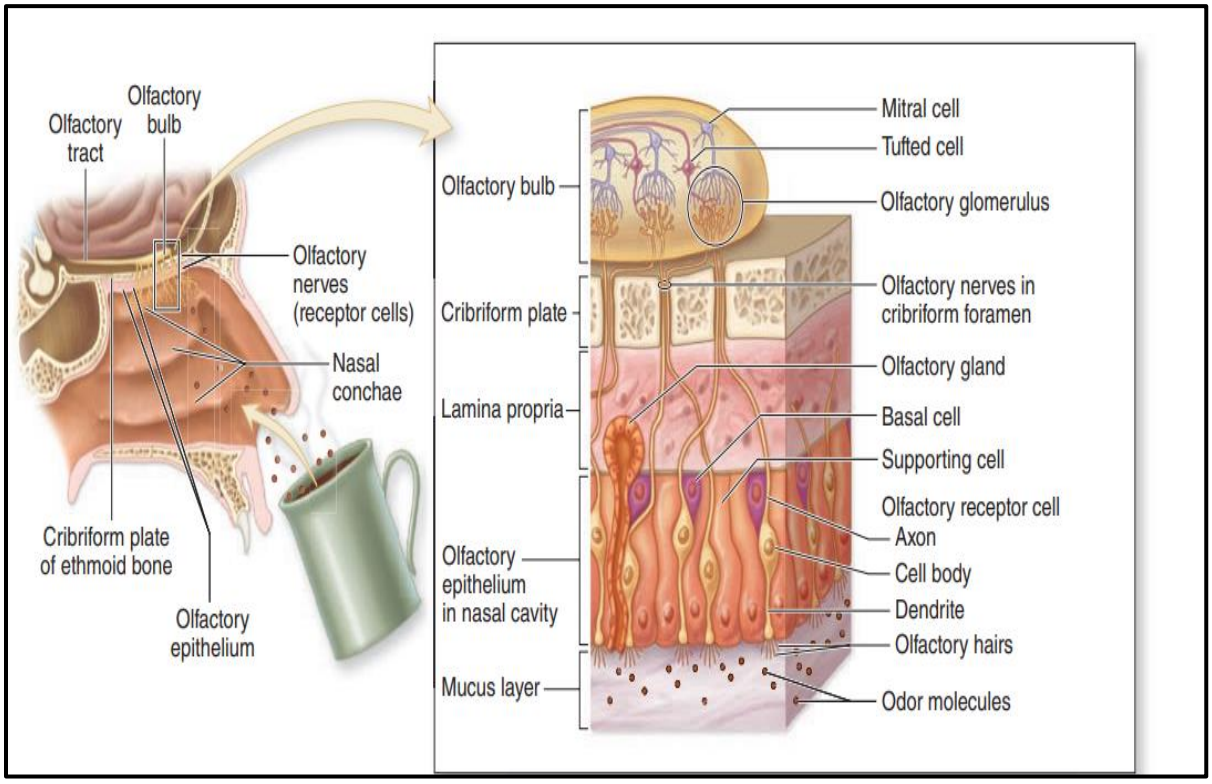


Figure 3: Olfactory mucosa.

The lamina propria of the olfactory epithelium possesses large **serous glands**, which produce a fluid surrounding the olfactory cilia and facilitating the access of new odoriferous substances.

Paranasal Sinuses

The paranasal sinuses are bilateral cavities in **the frontal, maxillary, ethmoid, and sphenoid bones of the skull** (Figure 1). They are lined with a thinner respiratory epithelium having fewer goblet cells. The lamina propria contains only a few small glands and is continuous with the underlying periosteum. The paranasal sinuses

communicate with the nasal cavities through small openings; mucus produced there is moved into the nasal passages by the activity of the ciliated epithelial cells.

Pharynx

The nasal cavities open posteriorly into **the nasopharynx**, the first part of the pharynx. The nasopharynx is continuous caudally with the oropharynx (throat), the posterior part of the oral cavity leading to the larynx and esophagus (Figure 1). Unlike the stratified squamous epithelium of the oropharynx, **the nasopharynx** lining is respiratory epithelium, and its mucosa contains the medial pharyngeal tonsil and the openings of the two auditory tubes which connect to each middle ear cavity.

Larynx

The larynx is a short passage for air between the pharynx and the trachea (Figure 1). Its rigid wall is reinforced by hyaline cartilage (in the thyroid, cricoid, and the inferior arytenoid cartilages) and smaller elastic cartilages (in the epiglottis, cuneiform, corniculate, and the superior arytenoid cartilages), all of which are connected by ligaments.

The epiglottis, a flattened structure projecting from the upper rim of the larynx, serves to prevent swallowed food or fluid from entering that passage. Its upper, or lingual, surface has stratified squamous epithelium; at variable points on its laryngeal surface this epithelium undergoes a transition to ciliated pseudostratified columnar (respiratory) epithelium. Mixed mucous and serous glands are found in the lamina propria beneath the epithelium.

Below the epiglottis and vestibule of the larynx, the mucosa projects bilaterally into the lumen with two pairs of folds separated by a narrow space or ventricle (Figure 4). The upper pair, the immovable vestibular folds, is partly covered with typical respiratory epithelium overlying numerous seromucous glands and occasional lymphoid nodules. **The lower pair of folds, the vocal folds , have features important for phonation or sound production**:

- Each is covered with nonkeratinized stratified squamous epithelium that protects the mucosa from abrasion.
- A dense regular bundle of elastic connective tissue, the vocal ligament, supports the free edge of each vocal fold.
- Deep to the mucosa are large bundles of striated fibers comprising the vocalis muscle that allow each vocal fold to be moved.

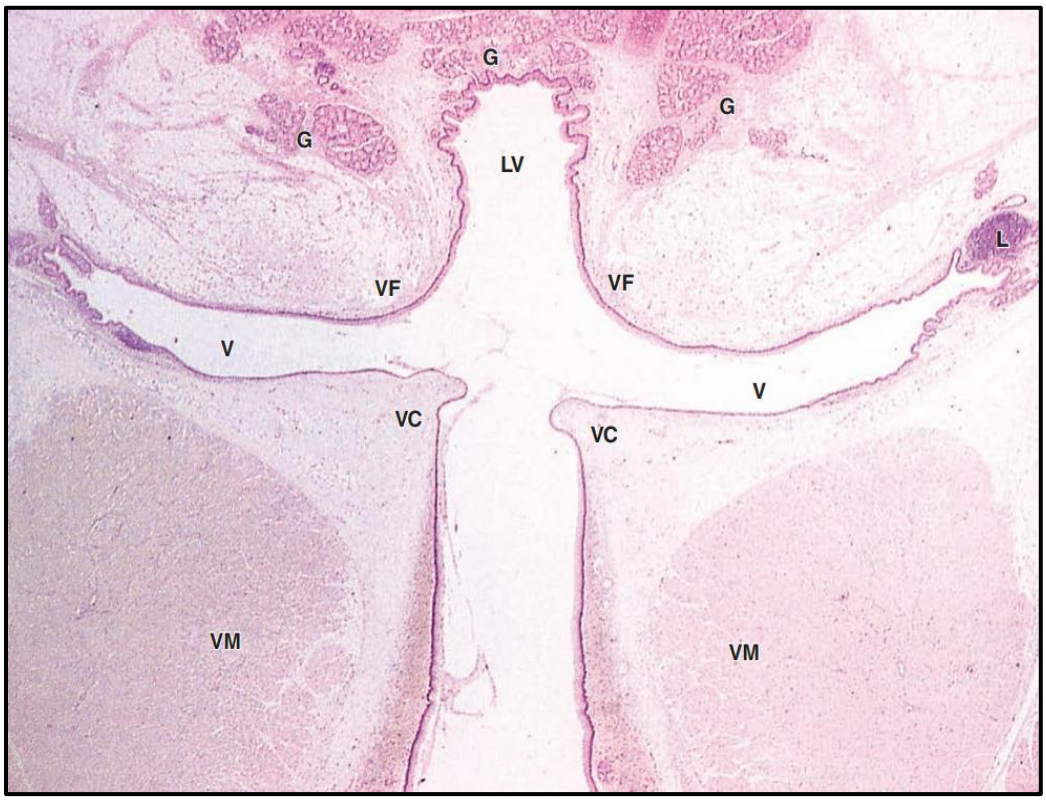


Figure 4: Larynx. This low-power micrograph shows the laryngeal vestibule (LV), which is surrounded by seromucous glands (G). The lateral walls of this region bulge as a pair of vestibular folds (VF). These also contain seromucous glands and areolar tissue with MALT, often with lymphoid nodules (L) and are largely covered by respiratory epithelium. Below each large vestibular fold is a narrow space or ventricle (V), below which is another pair of lateral folds, the vocal folds (VC). These are covered by stratified squamous epithelium and project more sharply into the lumen. Each contains a large striated vocalis muscle (VM) and nearer the surface a small ligament, which is cut transversely and therefore difficult to see here (X15; H&E).

Trachea

The trachea, is lined with typical respiratory mucosa in which the lamina propria contains numerous seromucous glands producing watery mucus (Figure 5). A series with about **a dozen C-shaped rings** of hyaline cartilage between the submucosa and adventitia reinforces the wall and keeps the tracheal lumen open. The open ends of the cartilage rings are on the posterior surface, against the esophagus, and are bridged by a bundle of smooth muscle called **the trachealis** muscle and a sheet of fibroelastic tissue attached to the perichondrium. The trachealis muscle relaxes during swallowing and strongly contracts in the cough.

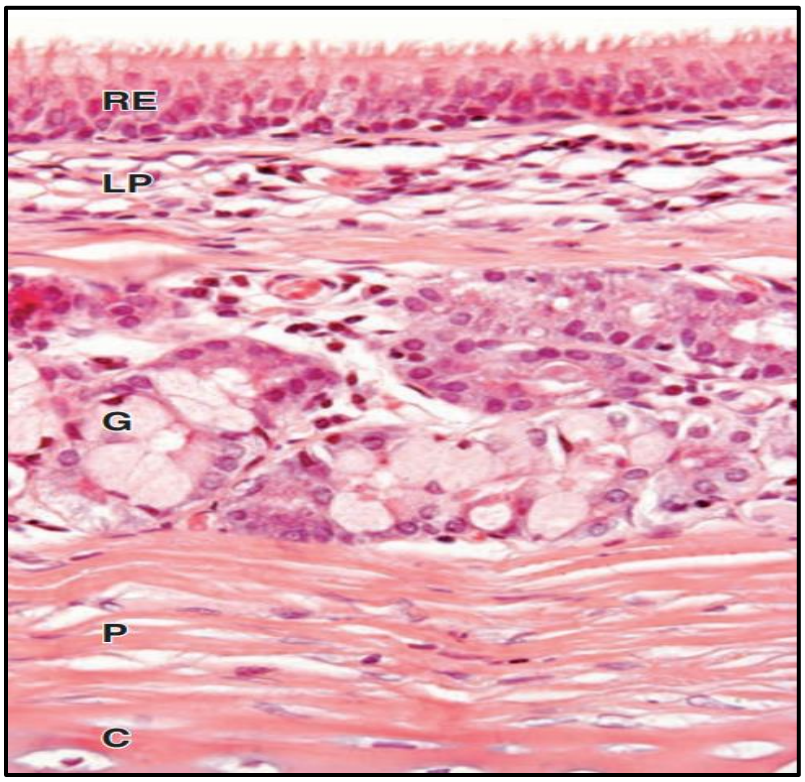


Figure 5: Tracheal wall. The trachea is lined by typical respiratory epithelium (RE) underlain by connective tissue of the lamina propria (LP) and seromucous glands (G) in the lamina propria and submucosa. Adjacent to the submucosa are the C-shaped rings of hyaline cartilage (C) covered by perichondrium (P) (X50; H&E).

Bronchial Tree & Lung

The trachea divides into two primary bronchi that enter each lung at the hilum, along with arteries, veins, and lymphatic vessels. After entering the lungs, the primary bronchi course downward and outward, giving rise to three secondary (lobar) bronchi in the right lung and two in the left lung (Figure 6), each of which supplies a pulmonary lobe. These lobar bronchi again divide, forming tertiary (segmental) bronchi. Each of the tertiary bronchi, together with the smaller branches it supplies, constitutes a bronchopulmonary segment. The tertiary bronchi give rise to smaller and smaller bronchi, whose terminal branches are called **bronchioles**. Each bronchiole enters a pulmonary lobule, where it branches to form five to seven terminal bronchioles. The pulmonary lobules are each pyramid-shaped, with the apex aimed at the pulmonary hilum, and each is delineated by a thin layer of connective tissue.

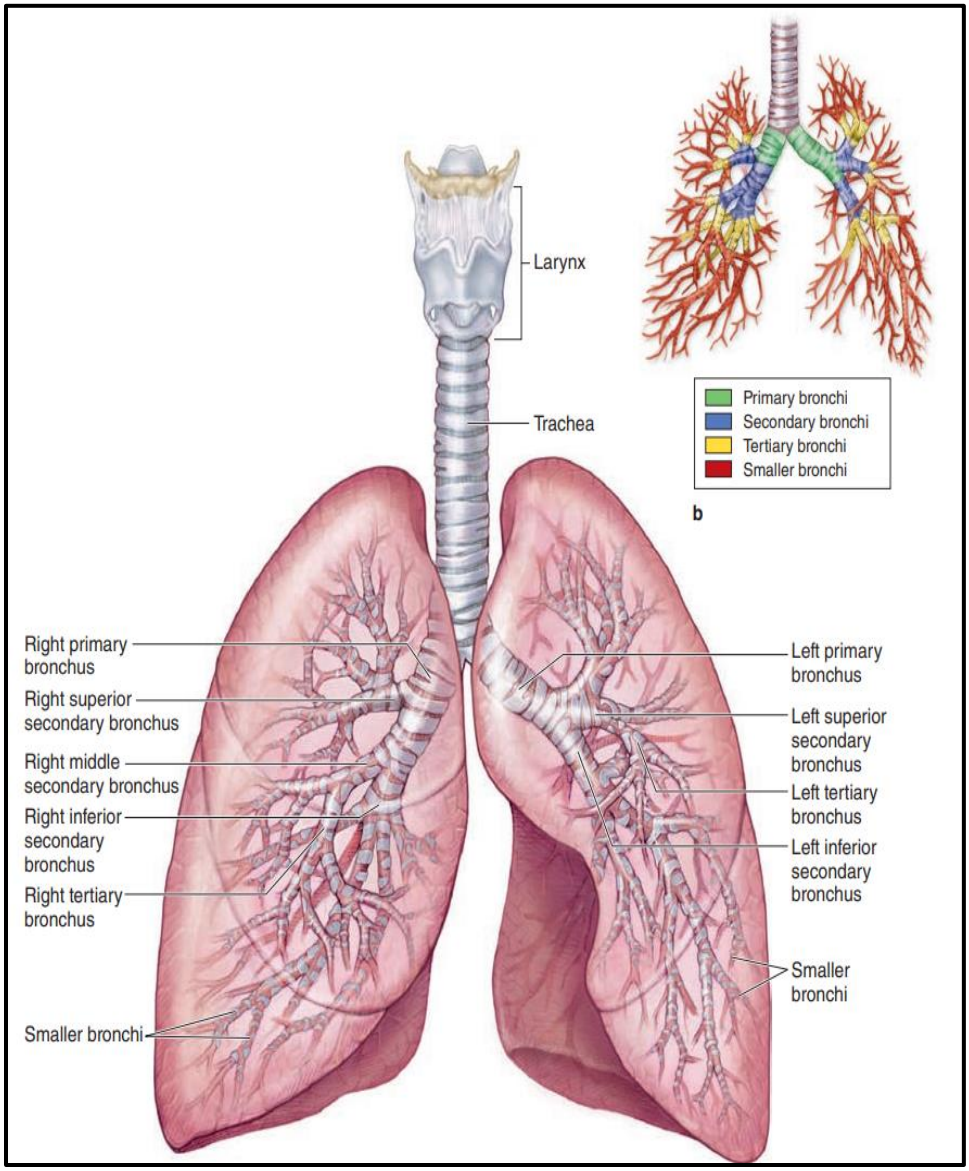


Figure 6: Bronchial tree. (a) Within each lung, bronchi subdivide further to form the bronchial tree, the last component of the air conducting system. (b) The small diagram shows the color-coded major branches of the bronchial tree.

Bronchi

Each primary bronchus branches repeatedly, with each branch becoming progressively smaller until it reaches a diameter of 1-2 mm. The mucosa of the larger bronchi is structurally similar to the tracheal mucosa except for the organization of cartilage and smooth muscle (Figure 7). In the primary bronchi most cartilage rings completely encircle the lumen, but as the bronchial diameter decreases, cartilage rings are gradually replaced with smaller isolated plates of hyaline cartilage. Small mucous and serous glands are abundant, with ducts opening into the bronchial lumen. The lamina propria also contains smooth muscle and elastic fibers (Figure 7 and 8), which become more prominent in the smaller bronchial branches. Contraction of this muscle

layer is responsible for the folded appearance of the bronchial mucosa observed histologically in cross sections. Numerous lymphocytes are found in the lamina propria and among the epithelial cells. Lymphatic nodules are also present.

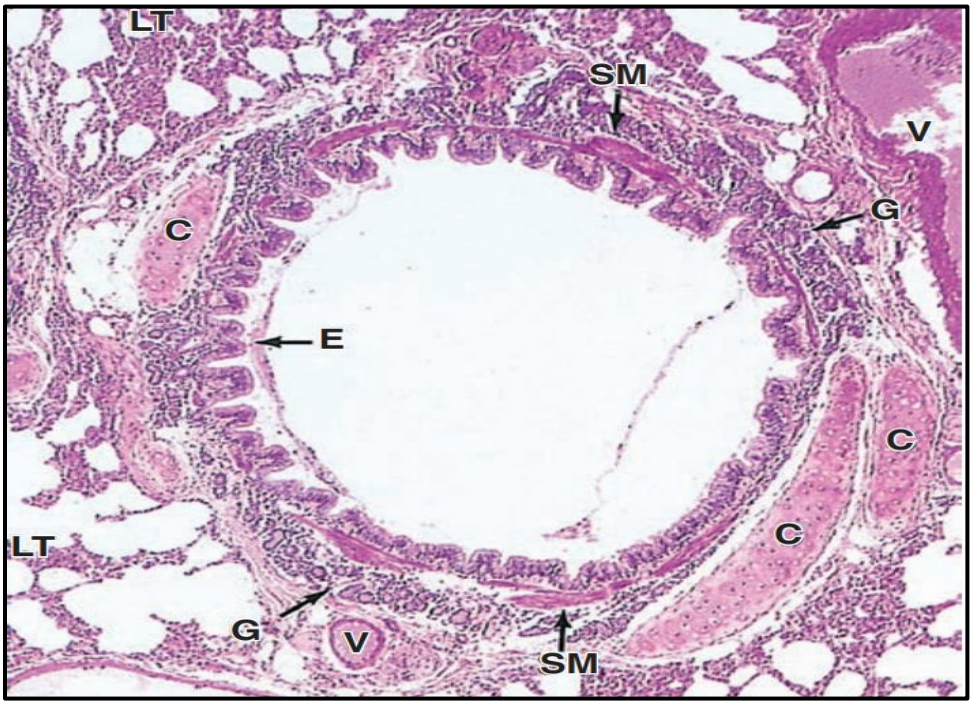


Figure 7: Tertiary (segmental) bronchus. In a cross section of a large bronchus, the lining of respiratory epithelium (E) and the mucosa are folded due to contraction of its smooth muscle (SM). At this stage in the bronchial tree, the wall is also surrounded by many pieces of hyaline cartilage (C) and contains many seromucous glands (G) in the submucosa. In the connective tissue surrounding the bronchi can be seen arteries and veins (V). All bronchi are surrounded by distinctive lung tissue (LT) showing the many empty spaces of pulmonary alveoli (X56; H&E).

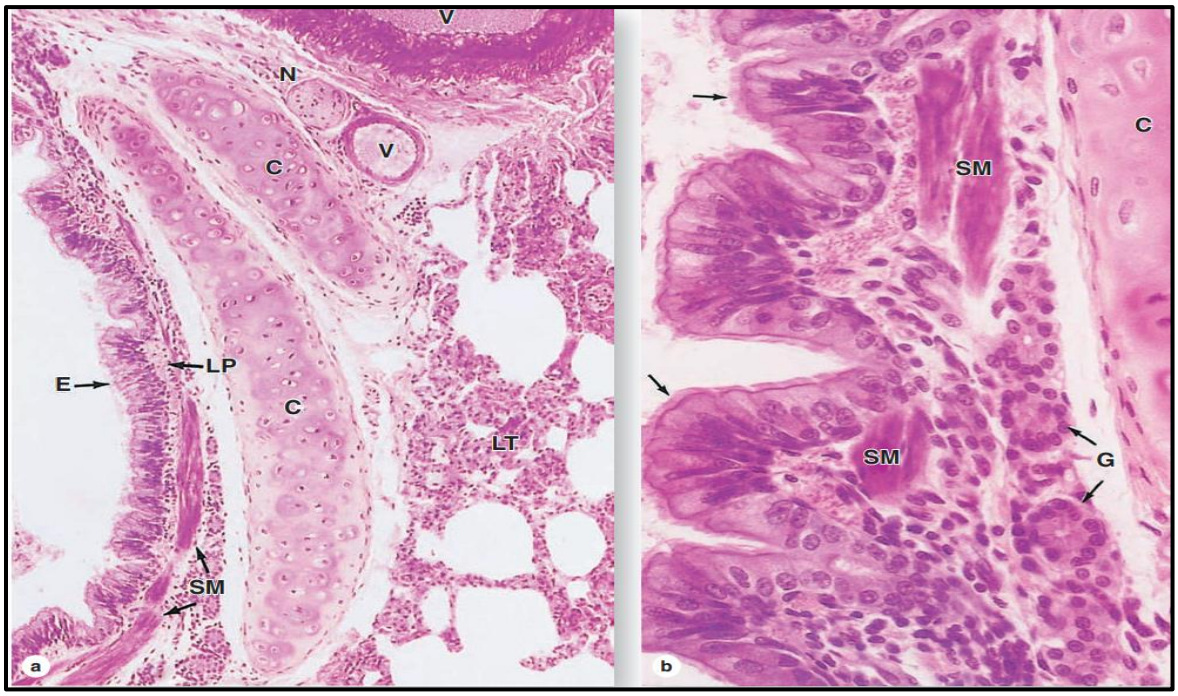


Figure 8: Bronchial wall. (a) The epithelial lining (E) of bronchi is mainly pseudostratified ciliated columnar cells with a few goblet cells. The lamina propria (LP) contains the distinct layer of smooth muscle (SM) surrounding the entire bronchus. The submucosa is the site of the supporting cartilage (C) and the adventitia includes blood vessels (V) and nerves (N). Lung tissue (LT) directly surrounds the adventitia of bronchi. (X140; H&E) (b) In the smaller bronchi the epithelium is primarily of columnar cells with cilia (arrows), with fewer goblet cells. The lamina propria has both smooth muscle (SM) and small serous glands (G) near cartilage (C). (X400; H&E).

Bronchioles

Bronchioles are the intralobular airways with diameters of 1 mm or less, formed after about the tenth generation of branching; they lack both mucosal glands and cartilage, although dense connective tissue is associated with the smooth muscle (Figure 9). In the larger bronchioles, the epithelium is still ciliated pseudostratified columnar, but this decreases in height and complexity to become ciliated simple columnar or simple cuboidal epithelium in the smallest terminal bronchioles, which are the last parts of the air conducting system.

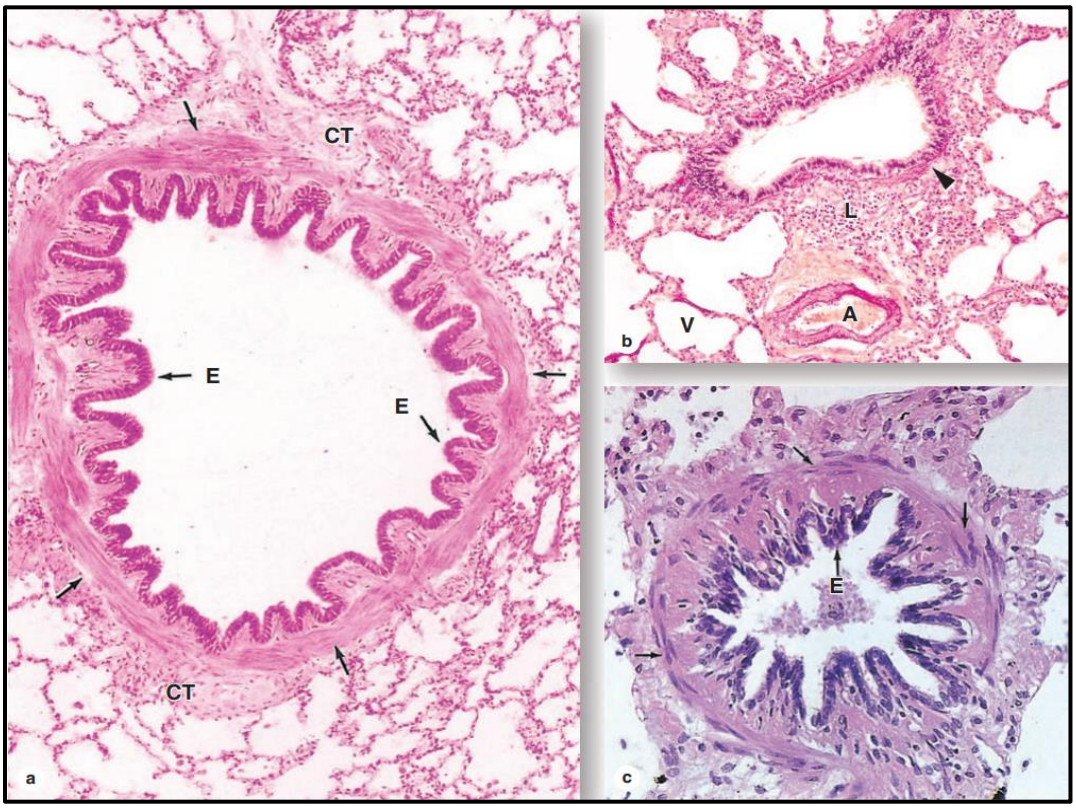


Figure 9: Bronchioles. (a) A large bronchiole has the folded respiratory epithelium (E) and prominent smooth muscle (arrows), but it is supported only by fibrous connective tissue (CT). (X140; H&E) (b) Staining for elastic fibers reveals the high elastic content of the smooth muscle (arrowhead) associated with the muscle of a smaller bronchiole in which the epithelium is simple columnar but still ciliated. Darkly stained elastic fibers are also present in the tunica media of a large arteriole (A) nearby and to a lesser extent in the accompanying venule (V). The connective tissue includes many lymphocytes (L) of diffuse MALT and lymphoid nodules. (X180; Aldehyde fuchsin) (c) In very small bronchioles the

epithelium (E) is reduced to simple cuboidal cells with cilia. Several layers of smooth muscle cells (arrows) comprise a high proportion of the wall. (X300; H&E).

The cuboidal epithelium of terminal bronchioles consists largely of club cells or bronchiolar exocrine cells (previously called **Clara cells**), with nonciliated, dome-shaped apical ends containing secretory granules (Figure 10). These exocrine cells have various functions, including the following: Secretion of surfactant lipoproteins and mucins, Detoxification of inhaled xenobiotic compounds by enzymes of the SER and Secretion of antimicrobial peptides and cytokines for local immune defense.

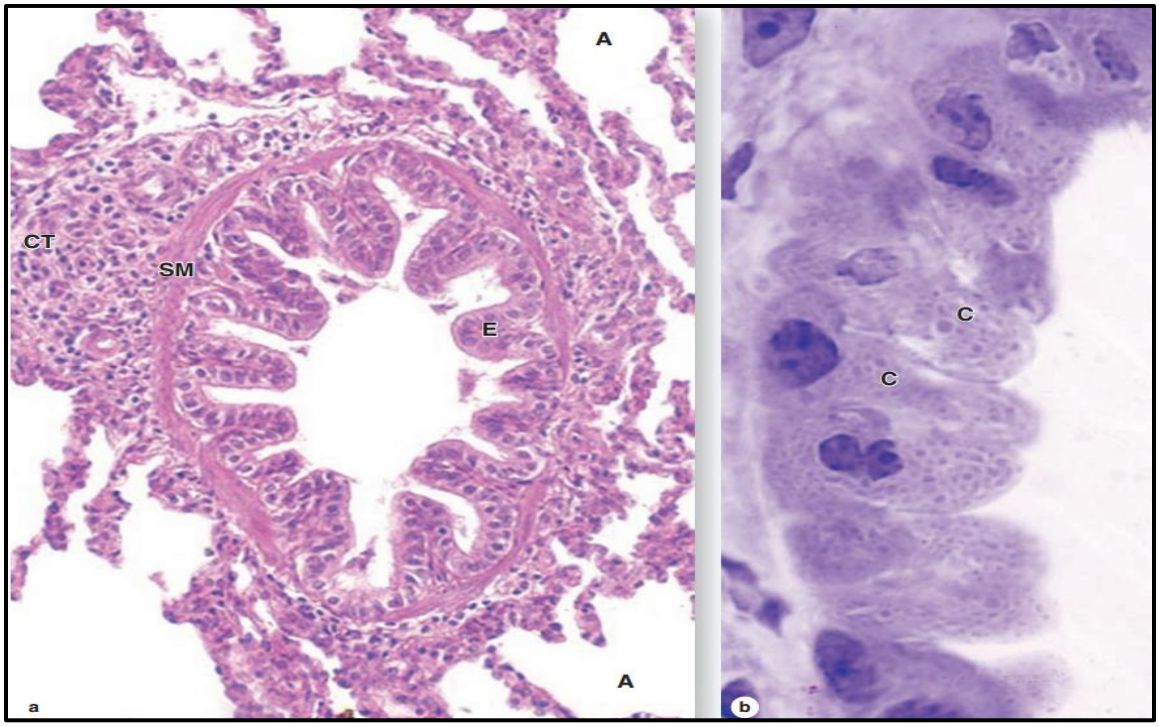


Figure 10: Terminal bronchiole and exocrine bronchiolar cells. (a) A terminal bronchiole has a mucosa with nonciliated cuboidal or low columnar epithelium (E), surrounded by only one or two layers of smooth muscle (SM) embedded in connective tissue (CT). Alveoli (A) are seen in the surrounding lung tissue. (X300; PT) (b) Most of the epithelium consists of exocrine club cells (C) with bulging domes of apical cytoplasm contain granules, as shown here in a plastic section.

Respiratory Region

- **Terminal bronchioles** subdivide into two or three respiratory bronchioles, lined by simple cuboidal epithelium and interrupted by scattered squamous evaginations called **alveoli**, the sites of gas exchange.
- **A respiratory bronchiole** leads to an alveolar duct, which is lined by a continuous series of alveoli and which ends in a cluster of alveoli called **the alveolar sac**.

- All alveoli are surrounded by sparse connective tissue in interalveolar septa consisting primarily of elastic and reticular fibers and a dense capillary network.
- The wall of each alveolus consists of **alveolar cells**, or **pneumocytes**, of two types: extremely **thin type I alveolar cells** and **cuboidal type II alveolar cells** with surfactant secreting and innate immune properties (figure 11).
- **Type II alveolar cells** are characterized ultrastructurally by unique cytoplasmic lamellar bodies, large granules with closely stacked layers of membrane involved in surfactant synthesis.
- **The blood-air barrier** allowing gas exchange at each alveolus consists of the **thin type I alveolar cell**, the thin capillary endothelial cells, and the fused basal laminae of these two cells.
- The surfactant material secreted by **exocrine club cells** and **type II alveolar cells** is an oily mixture of cholesterol, phospholipids and surfactant proteins, which forms a film and lowers surface tension in alveoli.

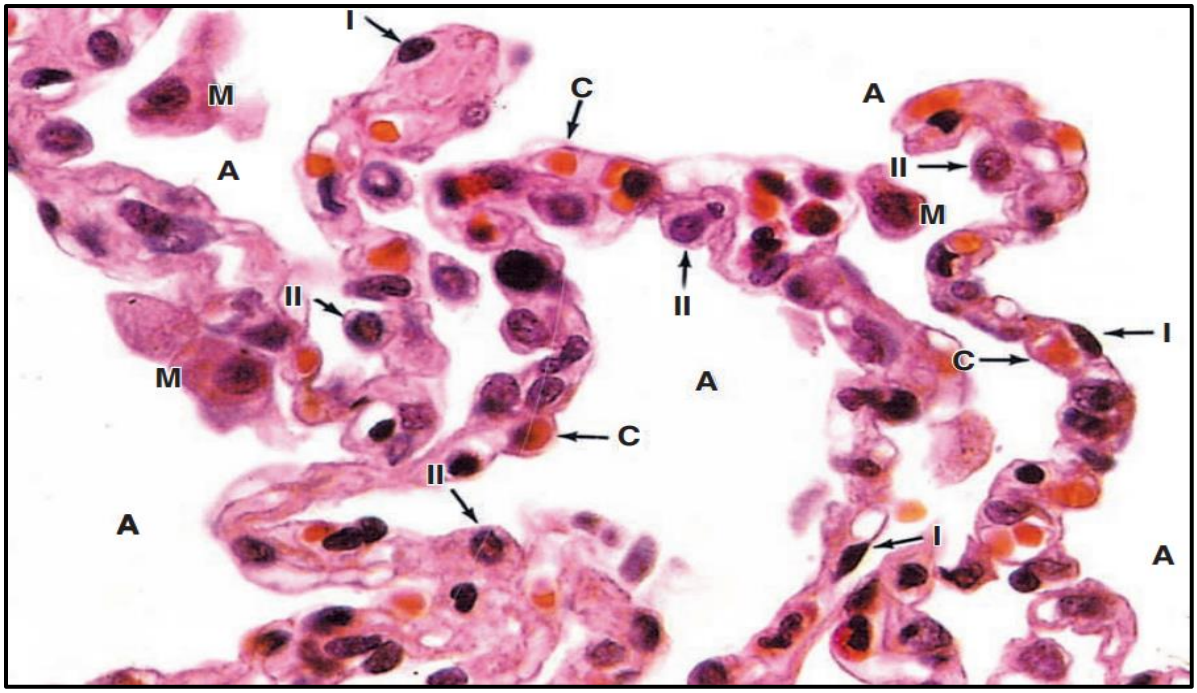


Figure 11: Alveolar walls. The septa between alveoli (A) contain several cell types. As seen here, the capillaries (C) include erythrocytes and leukocytes. The alveoli are lined mainly by squamous type I alveolar cells (I), which line almost the entire alveolus surface and across which gas exchange occurs. Type II alveolar cells line a bit of each alveolus and are large rounded cells, often bulging into the alveolus (II). These type II cells have many functions of club cells, including production of surfactant. Also present are alveolar macrophages (M), sometimes called dust cells, which may be in the alveoli or in the interalveolar septa. (X450; H&E).

- Each lung is covered by **visceral pleura**, a layer of thin connective tissue and **mesothelium**, and is continuous with **parietal pleura**, a similar tissue layer that lines **the pleural cavity** (figure 12).

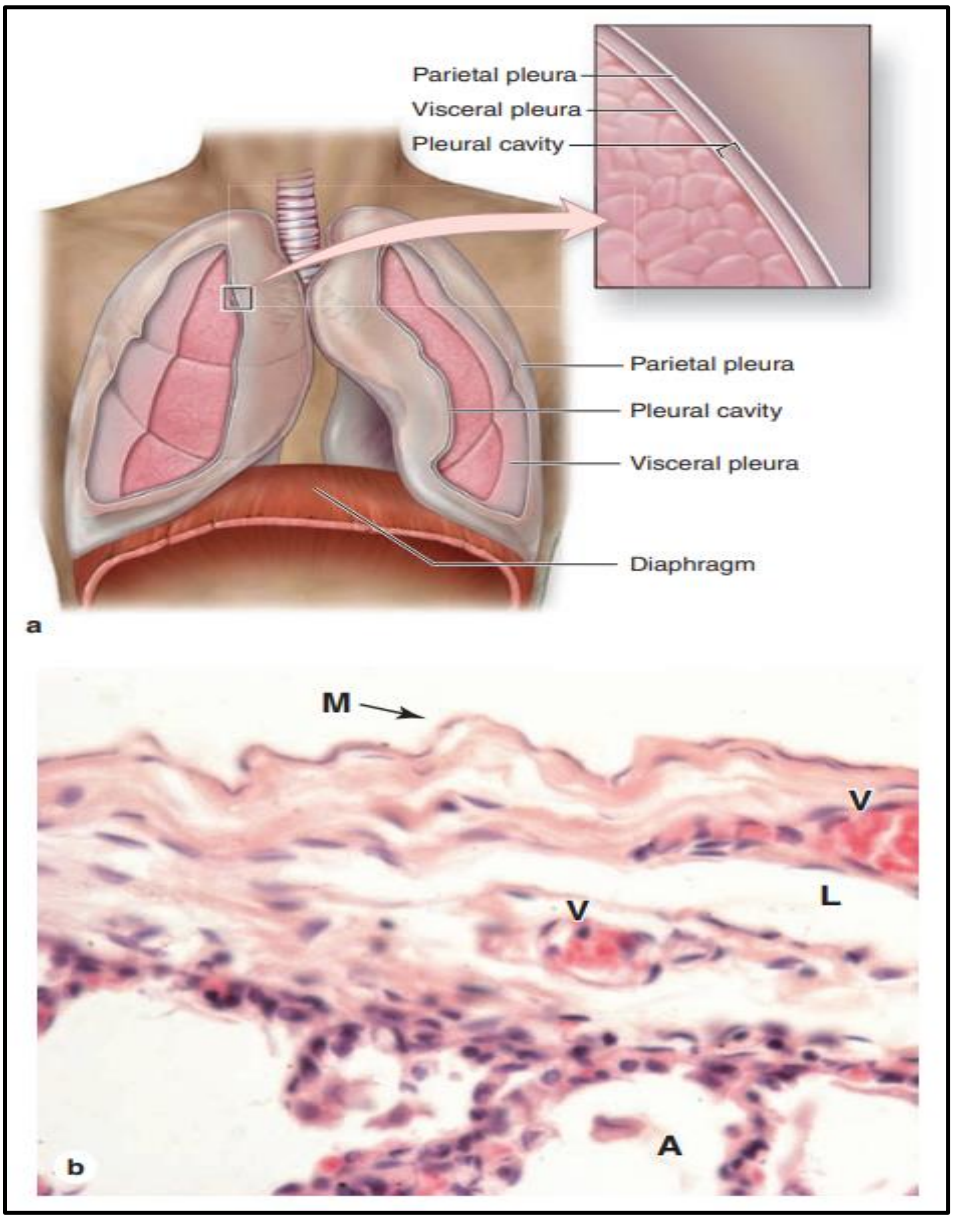


Figure 12: Pleura. (a) The diagram shows the parietal pleura lining the inner surface of the thoracic cavity and the visceral pleura covering the outer surface of the lung. Between these layers is the narrow space of the pleural cavity. (b) Both layers are similar histologically and consist of a simple squamous mesothelium (M) on a thin layer of connective tissue, as shown here for visceral pleura covering alveoli (A). The connective tissue is rich in both collagen and elastic fibers and contains both blood vessels (V) and lymphatics (L) (X140; H&E).

Lecture 23-24.. The Integumentary system

Skin consists mainly of a superficial stratified squamous epithelium, **the epidermis**, and a thicker layer of connective tissue, **the dermis**, which overlies a subcutaneous hypodermis **figure 1.**

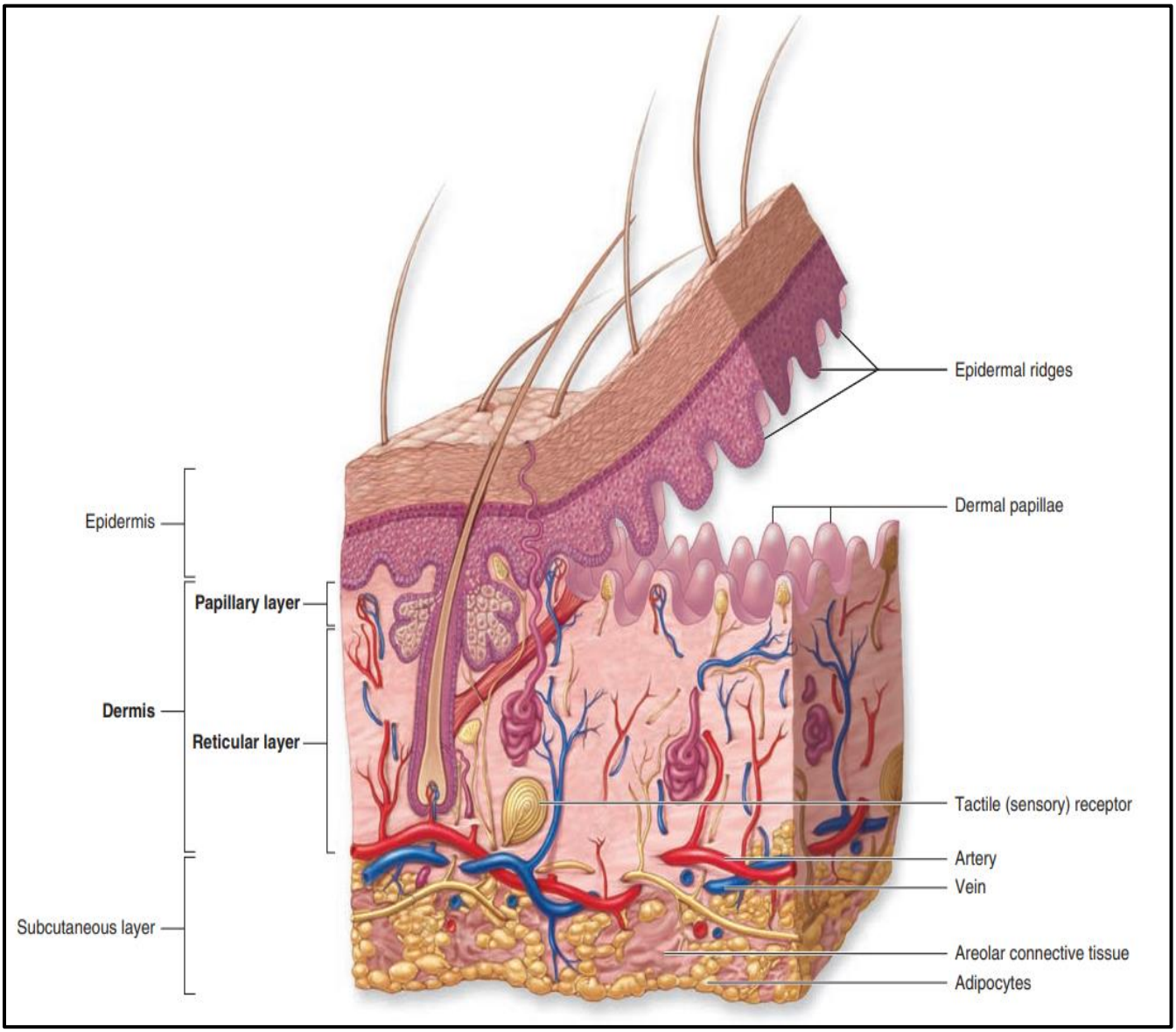


Figure 1: Layers and appendages of skin. Diagrammatic overview of skin, showing the major layers and epidermal appendages (hair follicles, sweat, and sebaceous glands), the vasculature, and the major sensory receptors.

Epidermis

The epidermis consists of keratinocytes that undergo a terminal differentiation process called **keratinization** in a series of steps that form distinct epidermal strata or layers Figure 2.

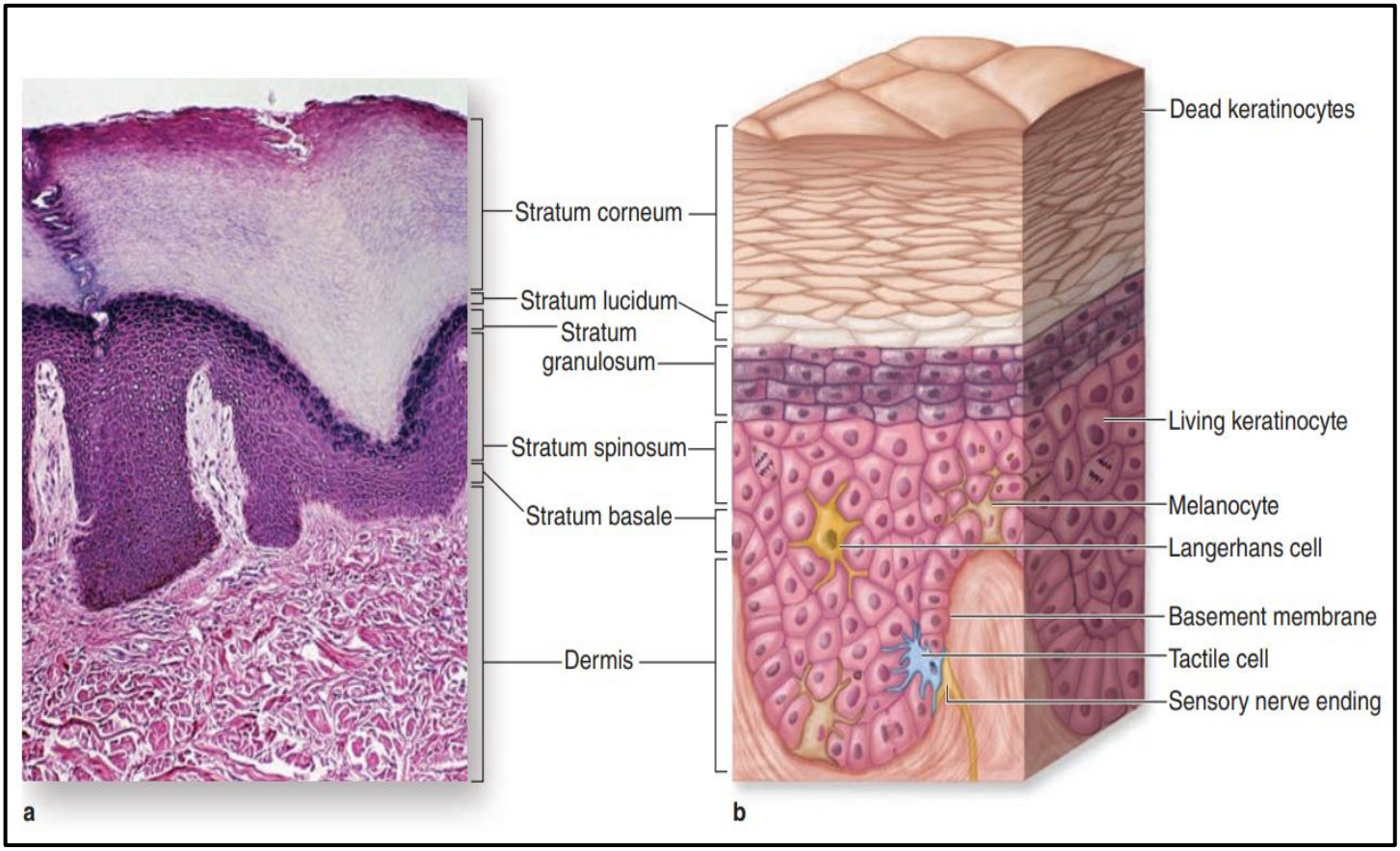


Figure 2: Layers (strata) of epidermis in thick skin. (a) Micrograph shows the sequence of the epidermal layers in thick skin and the approximate sizes and shape of keratinocytes in these layers. Also shown are the coarse bundles of collagen in the dermis and on the far left, the duct from a sweat gland entering the epidermis from a dermal papilla and coiling to a surface pore through all the strata. (X100; H&E) (b) Diagram illustrating the sequence of the epidermal layers also indicates the normal locations of three important nonkeratinocyte cells in the epidermis: melanocytes, a Langerhans cell, and a tactile Merkel cell.

Layers of The Epidermis

- **The stratum basale** is one layer of mitotically active cuboidal cells attached by hemidesmosomes and integrins to the basement membrane and to each other by desmosomes.
- **The stratum spinosum** has several layers of polyhedral cells attached to each other by desmosomes at the tips of short projections containing bundled keratin, or tonofibrils.
- **The stratum granulosum** is a thinner layer of keratinocytes, now flattened and filled densely with keratohyaline granules containing filaggrin and other proteins binding the tonofibrils.

- **The superficial stratum corneum** protects against water loss, friction, and microbial invasion, and consists of flattened, terminally differentiated cells, or squames, which are slowly lost.
- **The epidermis-dermis interface** is enlarged and strengthened by interdigitating epidermal ridges or pegs and dermal papillae in which microvasculature also supplies nutrients and $\mathbf{O_2}$ for the epidermis.

Cells of the Epidermis

- **Melanocytes** in the basal epidermis synthesize dark melanin pigment in melanosomes and transport these to adjacent keratinocytes, which accumulate them to protect nuclear DNA from UV damage Figure 3.

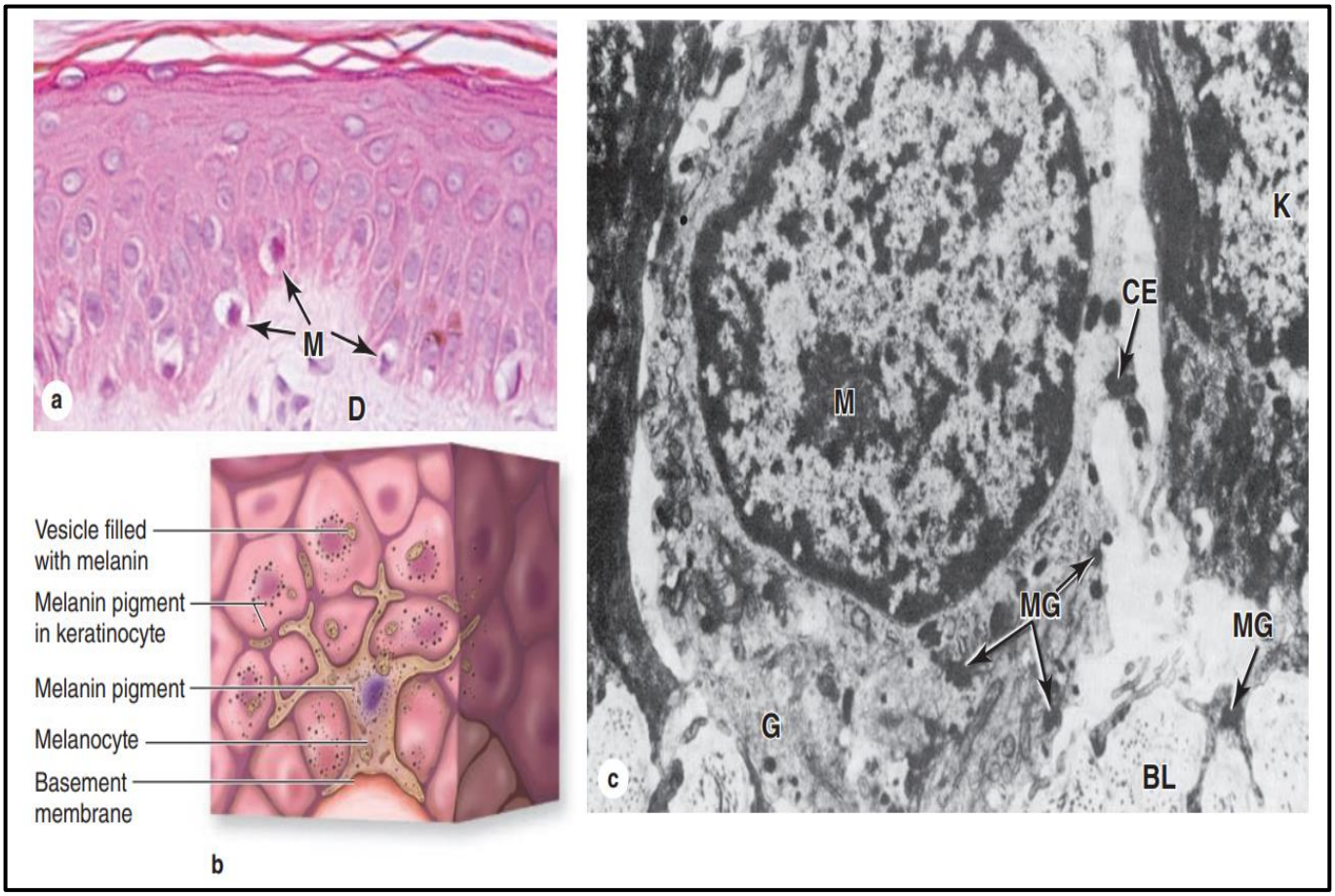


Figure 3: Melanocytes. (a) In light microscopy melanocytes (M) typically appear as rounded, pale-staining or clear cells just above the dermis (D). Melanocytes are difficult to distinguish from Merkel cells by routine microscopy. Langerhans cells are also rounded, poorly stained cells but are typically located more superficially than melanocytes, in the stratum spinosum. (X400; H&E) (b) Diagram of a melanocyte shows the irregular cytoplasmic processes between neighboring keratinocytes for transfer of melanin to those cells. (c) Ultrastructurally, a melanocyte is located on the basal lamina (BL) and has well-developed Golgi complexes (G) producing the vesicles in which melanin is synthesized. As they fill, these vesicles become melanin granules (MG), which accumulate at the tips of the dendritic cytoplasmic extensions (CE) before transfer to keratinocytes (K). (X14,000)

- **Antigen-presenting cells** called **Langerhans cells** form a network through the epidermis, intercepting and sampling microbial invaders before moving to lymph nodes in an adaptive immune response Figure 4.

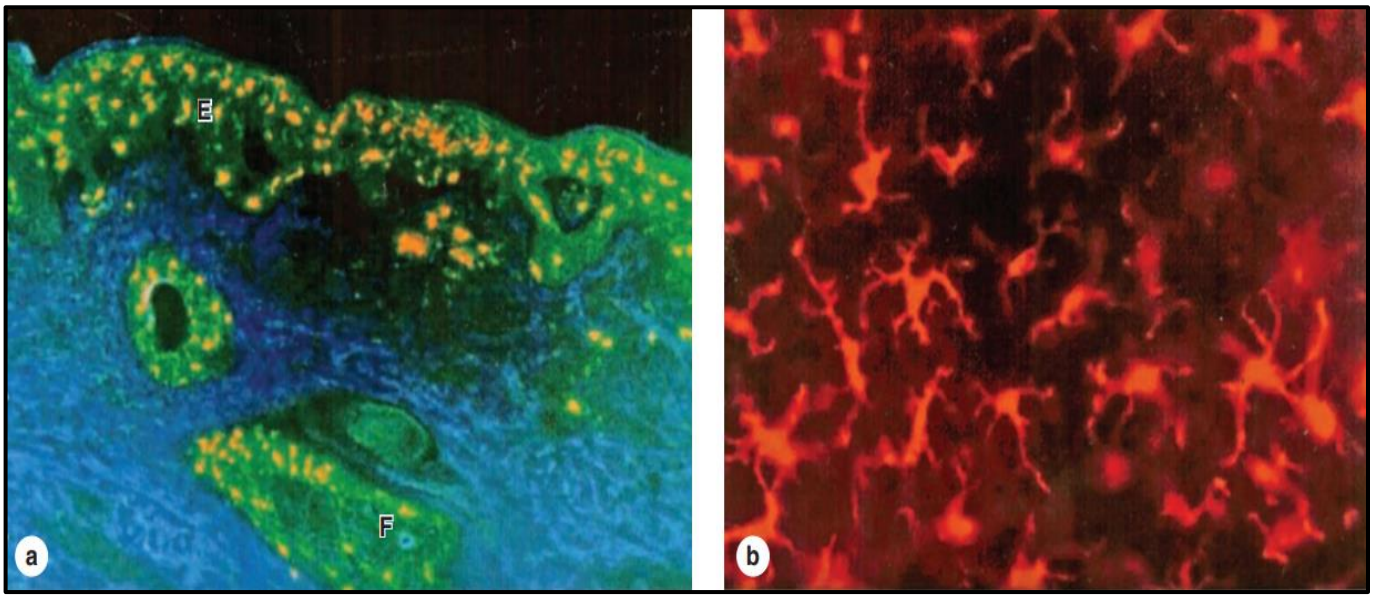


Figure 4: Langerhans cells. (a) Section of immunostained skin shows Langerhans cells (yellow) abundant in hair follicles (F), where many microorganisms live, and throughout the epidermis (E). Keratin of the epidermis and follicles is stained green. (X40) Antibodies against langerin/ CD207 and keratin. (b) Face-on view of an epidermal sheet stained using the same antibody showing the network of Langerhans cells among the other epidermal cells, which detects invading microorganisms. After sampling the invaders' antigens, Langerhans cells leave the epidermis and travel to the nearest lymph node to elicit lymphocytes that can mount a collective immune response. (X200; Anti-langerin/CD207)

Dermis

The dermis has two major layers: **a superficial papillary layer** or loose connective tissue with a microvascular plexus, and **a thicker dense irregular reticular layer** containing larger blood vessels (Figures 1 and 2).

Cutaneous Sensory Receptors

Sensory receptors in the epidermis include free nerve endings, which detect pain and temperature extremes, and **basal Merkel cells**, light-touch (tactile) receptors associated with **sensory fibers** Figure 5.

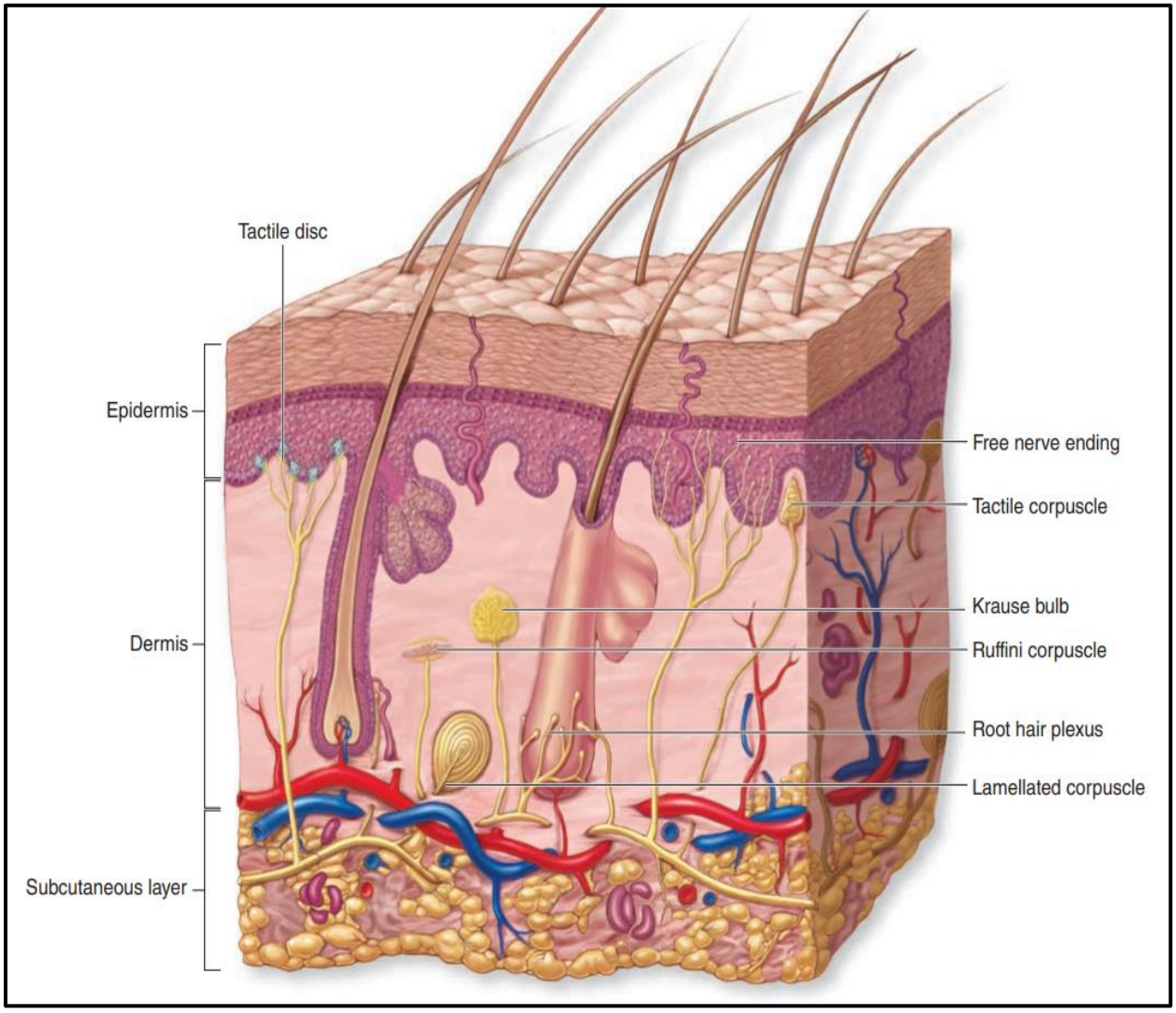


Figure 5: Tactile receptors.

Other cutaneous sensory structures include **Meissner corpuscles**, encapsulated elliptical mechanoreceptors that surround sensory axons and also detect light touch Figure 6. Deeper in the dermis and subcutaneous layer are **lamellated or pacinian corpuscles**, which are ovoid and much larger than Meissner corpuscles, for detection of pressure or firm touch Figure 6.

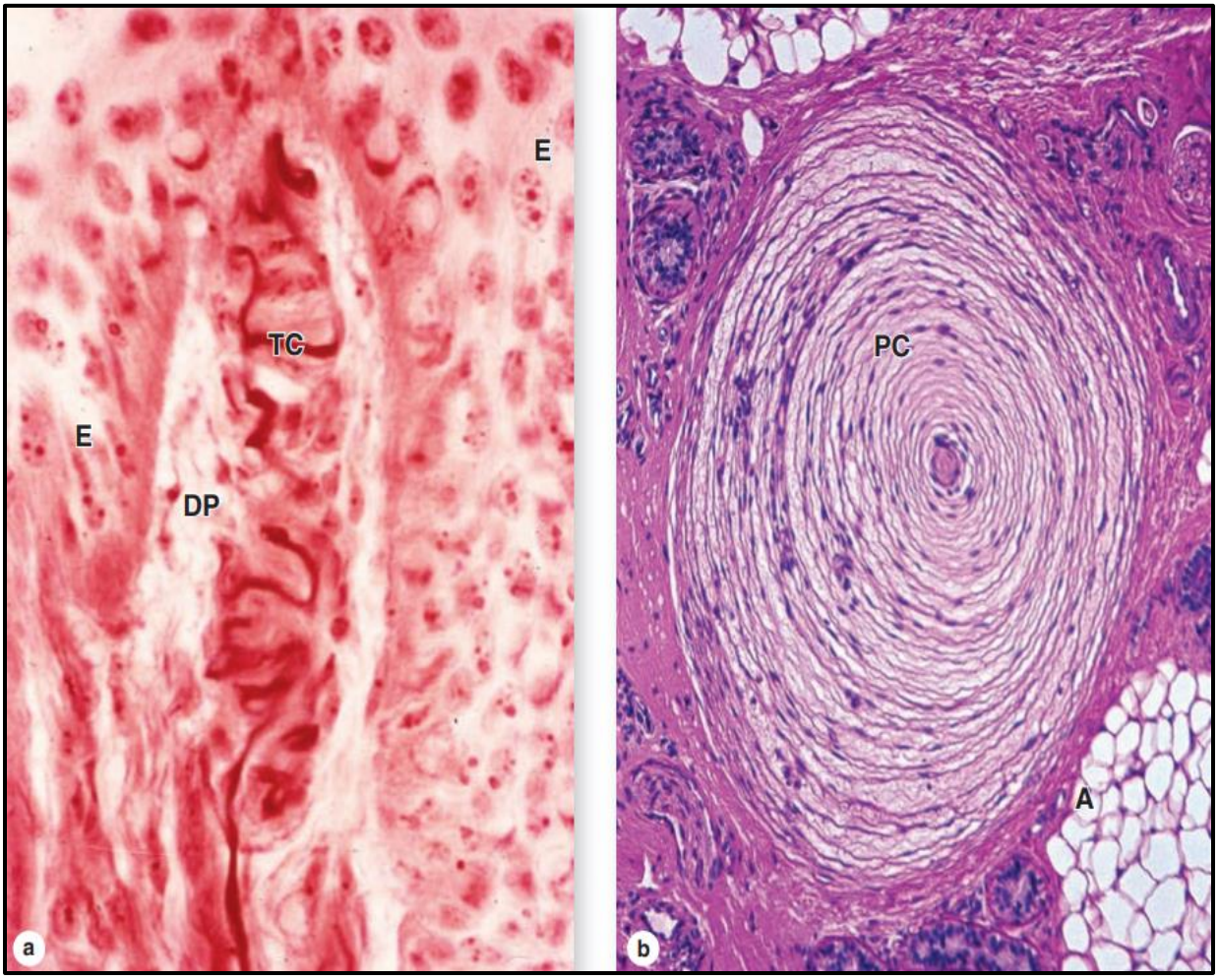


Figure 6: Meissner and lamellated (pacinian) corpuscles. (a) Meissner tactile corpuscles (TC) are specialized to detect light touch and are frequently located in dermal papillae (DP), partially surrounded by epidermis (E). They are elliptical, with an outer capsule (from the perineurium) and thin, stacked inner layers of modified Schwann cells, around which course nerve fibers (X400; H&E). (b) Lamellated (pacinian) corpuscles (PC) detect coarse touch or pressure and are large oval structures, found among adipose tissue (A) deep in the reticular dermis or in the subcutaneous tissue. Here the outer connective tissue capsule surrounds 15-50 thin, concentric layers of modified Schwann cells, each separated by slightly viscous interstitial fluid. Movement or pressure of this corpuscle from any direction displaces the inner core, leading to a nerve impulse. (X40; H&E)

Epidermal Appendages

Hairs form in hair follicles, in which keratinocytes comprising the matrix of the deep hair bulb proliferate rapidly and undergo keratinization to form **the medulla**, **cortex**, and **cuticle of a hair root** Figure 7.

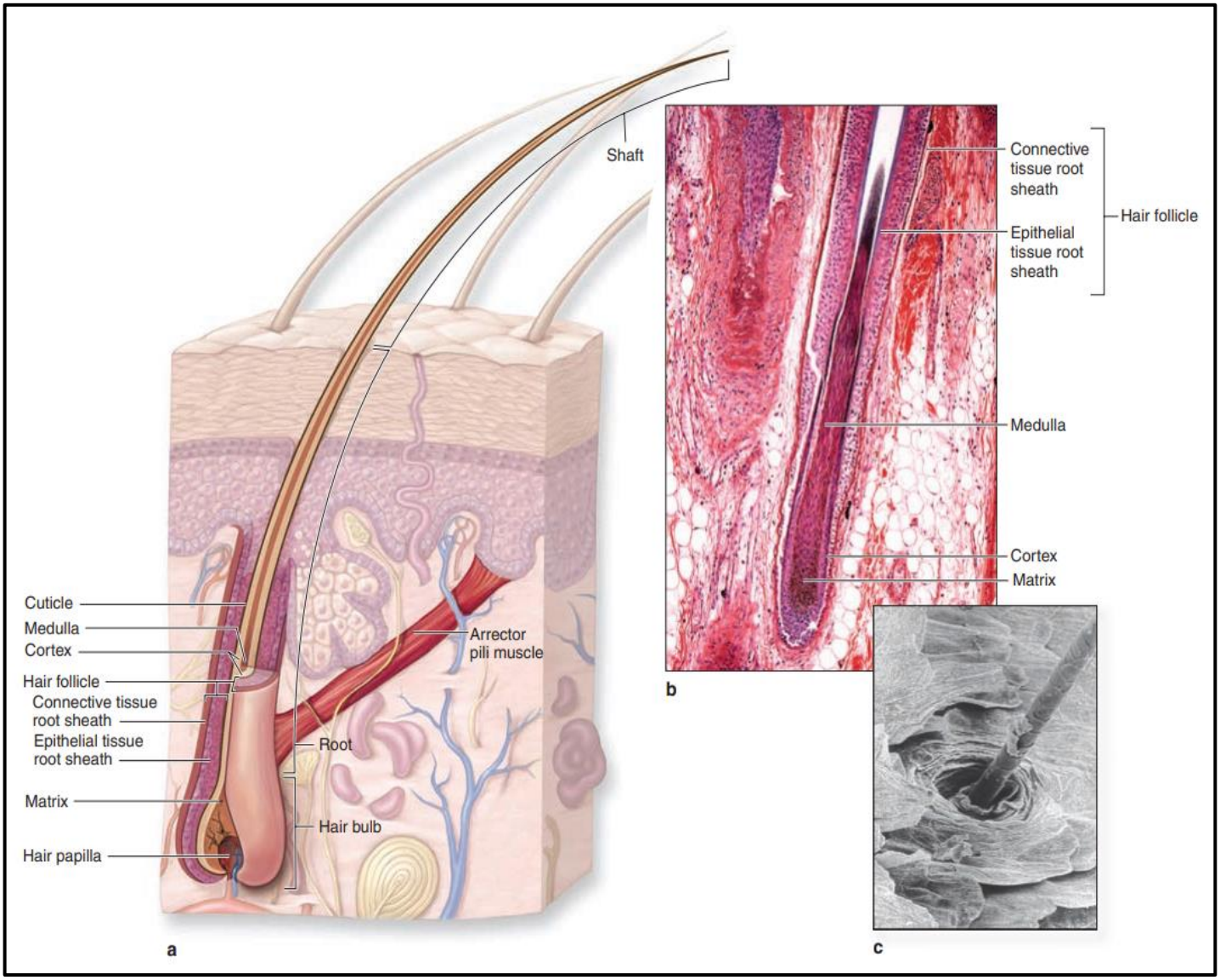


Figure 7: Hair. (a) The diagram shows major parts of a hair and its follicle, including vascularized, nutritive hair dermal papilla and the arrector pili muscle that pulls the hair erect. (b) A longitudinal section of a hair root and bulb shows the matrix, medulla, and cortex in the root and the surrounding epithelial and connective tissue sheaths. Cells of the hair bulb matrix proliferate, take up melanin granules, and undergo keratinization to differentiate as the three concentric layers of the hair (X70; H&E). (c) The outermost layer of the hair is the thin cuticle, composed of shingle-like cells, shown in this SEM of a hair shaft emerging at the stratum corneum (X260).

A large dermal hair papilla penetrates the base of the hair bulb, and its vasculature supplies nutrients and O_2 for proliferating and differentiating cells. The growing hair root is surrounded by internal and external root sheaths continuous with the epidermis, a glassy membrane formed in part by the basal lamina, and a connective tissue sheath.

Nails are formed in a manner similar to hairs: keratinocytes proliferate in the matrix of the nail root and differentiate with the formation of hard keratin as a growing nail plate with edges covered by skin folds Figure 8.

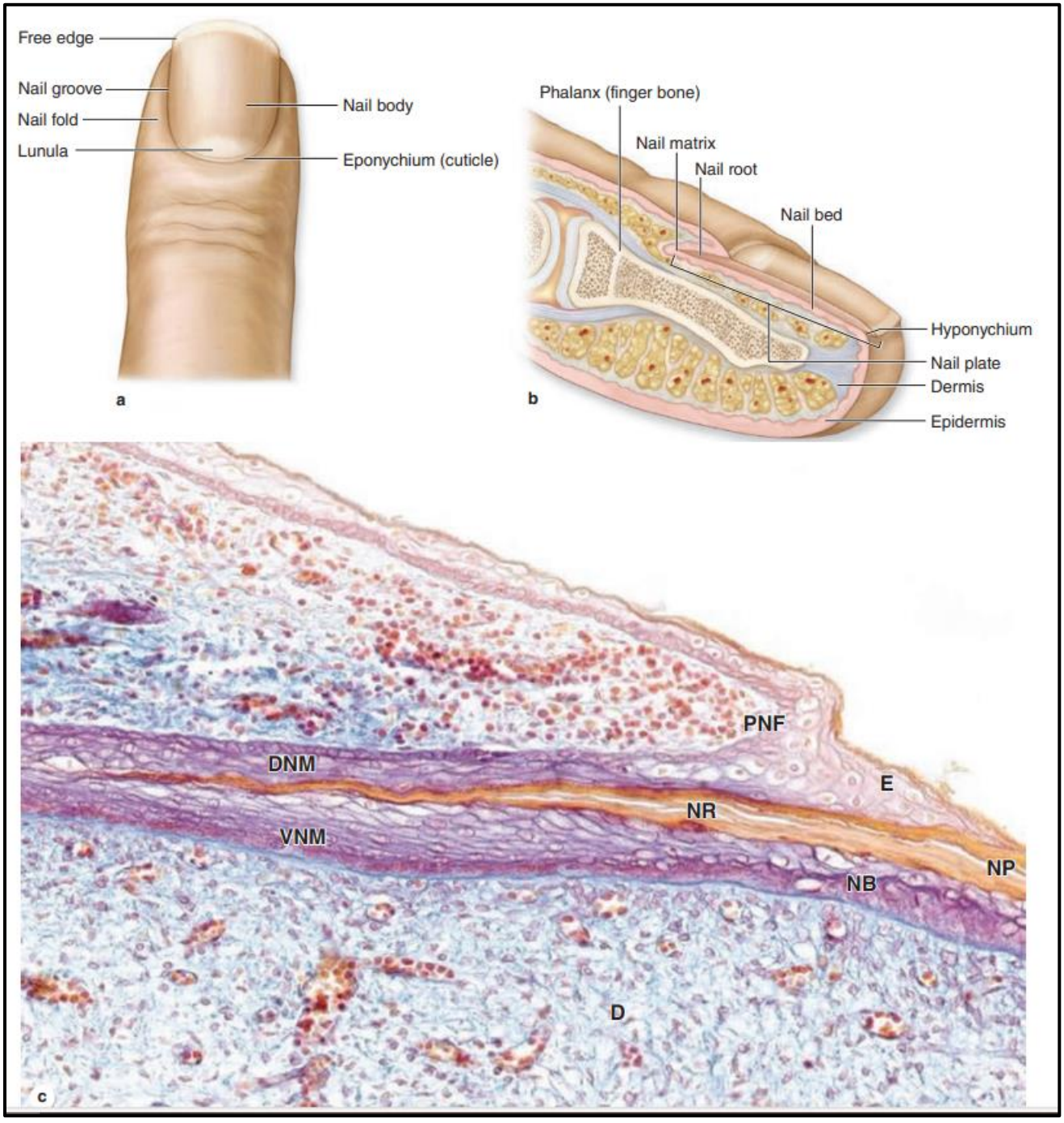


Figure 8: Nails. (a) Surface view of a finger shows the nail's major parts. (b) A diagrammatic sagittal section includes major internal details of the growing nail. (c) A sagittal section from a finger shows the proximal nail fold (PNF) and its epidermal extension, the eponychium (E) or cuticle. The nail root (NR), the most proximal region of the nail plate (NP), is formed like the hair root by a matrix of proliferating, differentiating keratinocytes. These cells make up the dorsal nail matrix (DNM) and ventral nail matrix (VNM), which contribute keratinized cells to the nail root. The mature nail plate remains attached to the nail bed (NB), which consists of basal and spinous epidermal layers over dermis (D), but is pushed forward on this bed by continuous growth in the nail matrix (X100; Mallory trichrome).

Glands of skin

- **Sebaceous glands** produce sebum by terminal differentiation of sebocytes, the classic example of holocrine secretion, secreting this oily substance onto hair in the follicles or pilosebaceous units.
- **Eccrine sweat glands** in the dermis produce sweat that is mostly water onto the skin surface, where its evaporation provides an important mechanism for cooling the body.
- **Apocrine sweat glands** are restricted to skin of the axillae and perineum, have much wider lumens than eccrine glands, develop after puberty, and secrete protein-rich sweat onto the hair of hair follicles Figure 9.

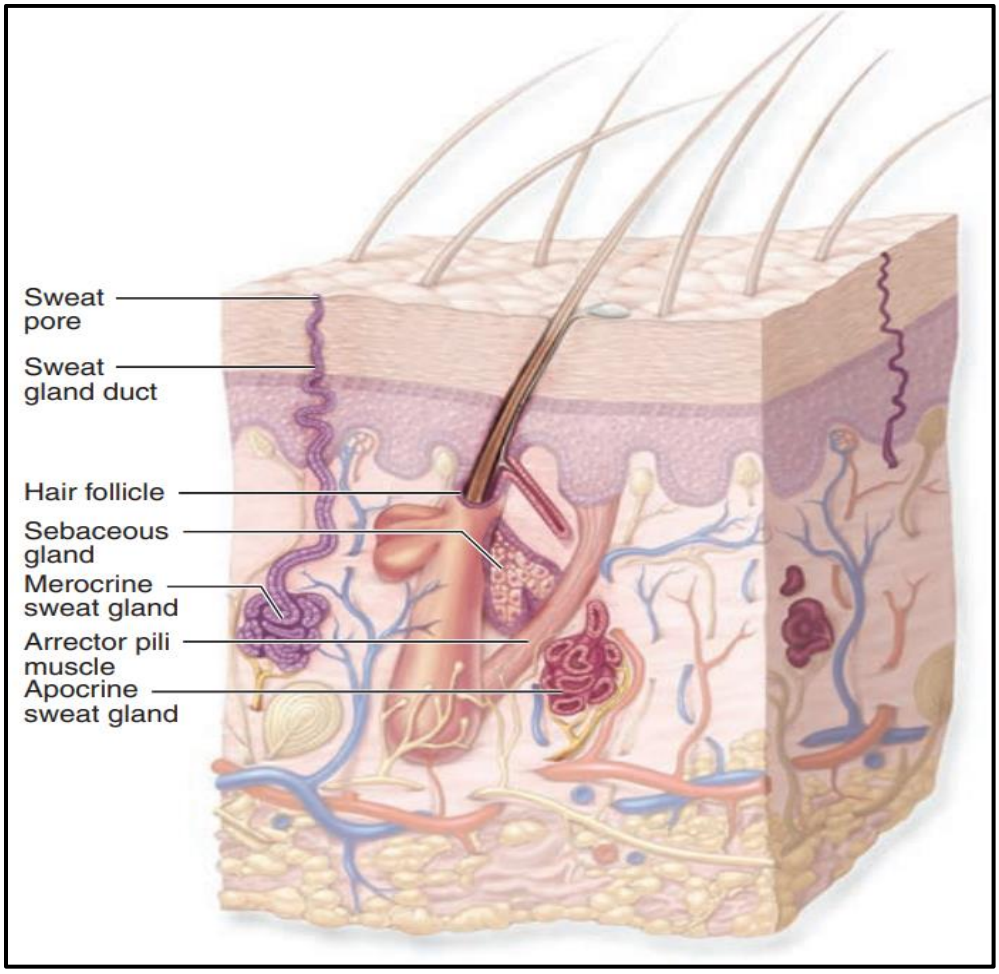


Figure 9: Glands of skin. Skin includes three major types of exocrine glands. Sebaceous glands are usually part of a pilosebaceous unit with a hair follicle and secrete oily sebum into the space around the hair root. Thermoregulatory eccrine sweat glands empty their secretion onto the skin surface via sweat pores. Apocrine sweat glands secrete a more protein-rich sweat into the follicles of hair in skin of the axillae and perineum.