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Practical Histology

for

Medical Students

As per the Revised Competency-based Medical Education Curriculum (NMC)

5th Edition



A Practical Approach to Understanding Tissues



SR Prasad

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Section 1

Tissues

- History of Histology
- Study of Cell
- Study of Simple Epithelium
- Study of Stratified Epithelium
- Glandular Tissue
- Study of Connective Tissue: Cells
- Study of Connective Tissue: Fibers and Matrix
- Study of Cartilage
- Study of Bones
- Study of Muscle Tissue
- Study of Nervous Tissue I
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CHAPTER

1 History of Histology

Histology is the science to study the finer structures of animals and plant kingdom with the help of microscope. The histology was coming as biological and medical science in early 19th century. In 1628–1694, Marcello Malpighi started observing tissues by naked eye examination and with the help of magnifying glasses therefore, he is regarded as the founder of microscopic anatomy. He described the small air sacs (alveoli) and network of minute blood vessels joining arteries to veins.

Three German scientists namely Johannes Petrus Muller (1801–1858), a physiologist and comparative anatomist, Rudolf Albert Kolliker (1817–1905), embryologist and histologist and Jan Evangelista Purkyne (1787–1869), a physiologist and anatomists had worked in the field of histological research and described tissues, protoplasm, mitochondria and smooth muscles. JP Muller encouraged his students to use a microscope and due to his work later on anatomy, physiology and pathology were separated. He was recognized as one of the most outstanding biologists of all time. Jacob Henle (1809–1885) discovered tubules of kidney. Theodor Schwann (1810–1882) and Matthias Jacob Schleiden (1864–1881) had formulated that plants and animals are made up of myriads of individual cells.

Robert Remak (1815–1865) in 1841 reported that the red cells of chicken are formed by division of pre-existing cells in contrary to prevailing opinion of new cells arose from surrounding fluids.

Book on Human Histology was published in 1841, "Henles Allgemeine Anatomie". Koelliker completed his two volumes of books in 1852 and 1854 "Handbuch der Gewebelehre des Menschen für Äertze und Studierende". In 1873, silver stain revolutionized the study of neurons by Cajal and Camillo Golgi and for this work they were nominated for Nobel Prize for physiology or medicine. Robert Brown in 19th century discovered and coined the term nucleus.

HISTORY OF HISTOLOGICAL ESSENTIALS

A well stained slide can be visualized and studied in a good microscope, means proper processing and staining procedure of sections. Process consists of fixation, mounting, cutting and staining. Many scientists worked since 16th century.

Fixation

Duke Frederico Cesi (1585–1630) studied tissue in his microscope by teasing the tissues. Giovanni Battista Hodiema (1597–1660) studied insects after decomposition and separated the eye of a fly into four layers. In 1666, Robert Boyle (1627–1691) studied tissue after immersing the tissues in spirits of wine and reported that by this method tissues were in natural state and becomes sufficiently hard to cut in a thin section by sharp knife. In 1850s, Franze Schulze (1815–1921) introduced osmic acid as preservative. In 1865, Alexander Butlerov (1828–1886) used formaldehyde (formalin) to preserve the tissue. Tissues fixed by this method can be cut in very fine sections.

Accidently in 1842, Benedict Stilling found that soft tissue can be hardened to cut in section by freezing. Later on ice and salt, ether and carbon dioxide was used as freezing agents.

Mounting

In 19th century many scientists started using substances in which tissues can be hardened before cutting. Rudolf Heidenhain (1834–1897) used Gum Arabic whereas Salomon Striker (1834–1898) used mixture of wax and oil. In 1869, Edwin Kiebs used paraffin wax for embedding tissues.

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Microtome

For sectioning tissue, razor was used by assistant of Purkinje. Sectioning of tissue from razor to microtome was claimed to be done in England since 1770 and but in 1857 Wilhelm His Sr. developed techniques of using microtome for fine section of the tissue. Later on brain tissue was cut in section by Stilling using razor dipped in alcohol.

Staining

In 1781, Felice Fontana observed nucleus and nucleolus of an animal cell. Joseph Gerlach (1820–1896) used Carmine (bright red) as stain. Many scientists worked on carmine. Hematoxylin was extracted from logwood and used as stain by Friedrich Bohmer in 1865. Accidently aniline dye was found during quinine synthesis by William Henry Perkin in 1853, which was used as commercial dyeing industry. Paul Ehrlich used methylene blue to stain blood film and be able to identify different types of white blood cells. Ziehl-Neelsen developed acid fast stain to identify the tubercle bacilli and was announced by Robert Koch on 24 March 1882. Counterstaining was introduced in 1870 using hematoxylin and eosin. Hematoxylin stained nuclei in blue color whereas eosin stained cytoplasm, connective tissues etc. as pink or red. In 1873, Camillo Golgi used silver stain for nervous tissues.

Microscope

In ancient time glasses of different shapes were used to magnify the objects. Lens was formed to see the objects very closely. Giovanni Faber (1574–1629) named the instrument microscopio using lenses–derived from Greek micron (small) and skopein (to look at) in 1625. Compound microscope was developed by Robert Hooke in 1665. Optical glasses were manufactured in England. By the end of 19th century reliable microscopes were made in America, France, Germany, UK, etc. therefore, microscopic study was possible by this time.

Unit of Measurement

For light microscopy: 1μ or 1μm = 10 μm or 0.001mm.
For electron microscopy: 1A* = 10 μm or 0.1 nm
: 1nm = 10 μm or 0.001 micron.
*Nowaday nanometer is used in place of Angstrom.

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CHAPTER

2 Study of Cell

The cells are structural and functional unit of body. Different types of cells are present having different functions but their basic structures are common to all. Cytoplasmic organelles differ depending upon their functions. Each cell has cell wall enclosing the cytoplasm with nucleus and organelles.

Cell membrane: It is formed by double-layered phospholipids molecule; having membrane protein in between with selective permeability. Oxygen, carbon dioxide, water, steroids, etc. are permeable whereas glucose, ions or protein pass by specific transport mechanism. Thickness of the cell membrane is about 7.55 nm.

The cell membrane encloses protoplasm. It has a nucleus covered by nuclear membrane. The cytoplasm contains a number of organelles and inclusion bodies.

- *Cellular organelles:* For the maintenance of proper homeostasis and specialized metabolic function, different organelles are present in cytoplasm of each cell. Common organelles are mitochondria, endoplasmic reticulum, Golgi apparatus, ribosomes, and lysosomes, etc.
- *Mitochondria:* Spread all over cell as granules or round, oval, or elongated rods. Size varies from 0.5–2 μm. It has outer and inner membranes with cristae. Cristae are folds of inner membrane. They provide energy.
- *Endoplasmic reticulum:* It is present as extensive network of sacs and cisternae. It is two types—rough and smooth. Rough endoplasmic reticulum is seen as many flattened interconnected cisternae studded with ribosomes. They are concerned with protein synthesis. Smooth endoplasmic reticulum is devoid of ribosomes. They are concerned with further processing of proteins synthesized by rough endoplasmic reticulum.
- *Golgi apparatus:* It consists of smooth, curved cisternae present near the nucleus for transport of proteins. It adds sugar to form glycoprotein, lipoprotein and glycolipids.
- *Ribosomes:* These are small, dark and dense granules. It may be free or attached to the endoplasmic reticulum, concern with protein synthesis.
- *Lysosomes:* Lysosomes are membrane-bound vesicle or dense bodies of various sizes and shapes, containing, lysosomal enzymes with property of phagocytosis (intracellular digestion). They are rich in glycoprotein, and are abundant in phagocytic cells.
- *Peroxisomes:* They are also membrane bound vesicles smaller than lysosomes containing several types of oxidases and catalase. They are present mostly in liver and kidney to remove toxic materials.

CYTOSKELETON OF THE CELLS

It gives the structural supporting framework and facilitates cell motility. They are microfilaments, intermediate filaments and microtubules formed by proteins.

- *Microfilament:* They are formed by protein actin, present near the cell wall and maintain the shape of the cells. It also forms the core of microvilli.
- *Intermediate filament:* Different types of thick intermediate filaments are present in different types of cells having different names due to its different types of protein present. They are cytokeratin in skin, vimentin in mesenchymal cells, desmin in muscles, neurofilament in nervous cells, glial filament in glial cells (astrocytes), and lamin in inner layer of nuclear membrane.
- *Microtubules:* It is the largest filament formed by α and β tubulin originating from centrosome in the cytoplasm which contains a pair of centriole. They are visible in cilia and flagella. They are present in almost all types of cells except red blood cells.

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- *Centrosome and centriole:* They are present near nucleus having two centrioles lying perpendicular to one another. Centriole has nine microtubules arranged in group of three. They replicate before mitosis and form the spindles.
- *Cytoplasmic inclusions:* They are lipids, glycogen, pigments and crystals.
- *Nucleus:* Except RBC all living cells have nucleus, covered by double layers of nuclear membranes containing ribosome on its outer layer. Nucleus has chromatin, nucleoli, and DNA. Nuclear membrane has nuclear pores to control the movement of metabolites between nucleus and cytoplasm.

Epithelial cells have the followings functional surfaces:

1. Basal surface—forms basement membrane.
2. Side surface—lying in contact with side surface of other cells.
3. Free surface—exposed to either air or fluid in body cavity of system.

Basal surface: It rests on basement membrane formed by basal lamina and reticular lamina. At some places basal surface shows infolding of plasma membrane to increase the surface area for transport of fluid and ions.

Side surface: Specialized for cell to cell attachment. They are:

- *Interlocking projections*
- *Junctional complexes*: The important Junctional complexes are:
  - Zonula occludens or tight junction
  - Zonula adherens or loose junction
  - Macula adherens or desmosomes

Free surface: It shows many types of specialization.

- *Microvilli*: Microvilli are ultramicroscopic finger-like projections from free surface of the cell. It consists of a core of apical cytoplasm covered by plasma membrane. It increases the absorptive surface.
- *Cilia*: Cilia are hair-like processes. It may be motile or nonmotile (stereocilia). Function of motile cilia is to propel liquid, mucus, or foreign bodies. They are present in respiratory tract, uterine tube, and uterus, etc. Function of nonmotile cilia (stereocilia) is absorptive, or act as sensory receptors. They are present in central canal of spinal cord.

DIFFERENCE BETWEEN CILIA AND MICROVILLI

Cilia	Microvilli
1. Cilia are small hair-like projection arising from cell surface, 10 µm in length and 0.25 µm in diameter	Microvilli are minute finger-like projection of cell surface having 1–2 mm in length and about 73–90 µm in diameter
2. Cilia may be motile or nonmotile	Microvilli are nonmotile
3. Cilia are rich in microtubules having 9+2 pattern of microtubules	Contains numerous microfilaments (actin filaments)
4. Cilia are present over lining epithelium of respiratory tract and uterine tubes	Microvilli are present in intestinal epitheliumand proximal convoluted tubules
5. Functions: Movements of secretions and ova	Functions: Absorptive

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CHAPTER

3 Study of Simple Epithelium

Competencies Covered

AN65.1	Identify epithelium under the microscope and describe the various types that correlate to its function.
AN65.2	Describe the ultrastructure of epithelium.

Epithelium is a collection of adherent cells, with very little intercellular material, covering external and internal surfaces of the body; it forms various organs and glands, and lines their ducts. Epithelium may be simple or stratified. Classification of epithelium depends on the number of the cells and type of the cells. Surface of epithelium may have microvilli and cilia.

ORIGIN OF EPITHELIUM

Types of epithelium	Ectoderm	Mesoderm	Endoderm
Squamous	Epidermis of skin, lip, ocular conjunctiva, anal canal, fossa navicularis, oral cavity	Vagina and part of cervix	Pharynx, esophagus, part of larynx, vocal cord, and epiglottis
Cuboidal	-	Sweat gland	Thyroid gland
Columnar a. Nonciliated b. Ciliated	 Palpebral conjunctiva olfactory mucosa Respiratory part of nasal cavity	 Part of ductus deferens -	 Part of larynx Part of auditory tube
Transitional	-	Pelvis of kidney, ureter, and part of bladder	Part of bladder and part of urethra

FUNCTIONS OF EPITHELIUM

- Protection: Epithelium of skin
- Absorption: Kidney and intestine
- Secretion: Goblet cells, gastric glands and intestinal glands
- Excretion: Nephrons of kidney
- Act as barrier: Skin, and urinary bladder
- Exchange of gases: Alveoli of the lungs
- Detection of sensations: Hearing with the help of ear, vision with the help of eye, and different types of sensations from skin.
- Transport of materials.

SIMPLE EPITHELIUM

- **Simple squamous epithelium:** They are flat, scale-like cells having length and breadth but negligible thickness. Cytoplasm is finely granular with small, rounded or oval, central nucleus producing bulging over the surface.
  Examples: Alveoli of lungs, parietal layer of Bowman's capsule, loop of Henle, endothelium and mesothelium, etc.
  Function: Reduction of friction, filtration, secretion, and transport by pinocytosis.

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- **Simple cuboidal epithelium**: Cells are cubical in shape having equal length, breadth and thickness. Nucleus is centrally placed and spherical in shape.
  Examples: Follicles of thyroid gland, proximal and distal convoluted tubules, and cuboidal epithelium of proximal convoluted tubule have brush border consisting of microvilli, respiratory bronchioles, pigment layer of retina, germinal epithelium of ovary, ducts of many glands, etc.
  Function: Absorption, transport and protection of ducts lined by it.
- **Simple columnar epithelium**: Cells are cylindrical or rod-shaped and tall in appearance. Cytoplasm is reticulated and looks vacuolated due to mucinogen content. Nucleus is oval in shape. Types of simple columnar epithelium are:
  i. *Nonciliated:* Absence of cilia over free surface.
  Example: Lining of stomach, small and large intestine, gallbladder and larger ducts of gland. Absorptive cells of small intestine have microvilli.
  ii. *Ciliated:* Cells have cilia over its free surface. Cytoplasm is finely granular. Oval nucleus is placed in basal portion.
  Example: Uterus, uterine tube and central canal of spinal cord, etc.
  Function: It protects the stomach by secreting mucus, absorption of nutrients from intestines, helps to propel ovum, etc.
- **Goblet cell:** Cells are modified columnar cells, look-like inverted flask. Upper dilated portion appears vacuolated because cytoplasm contains mucinogen, which is lost during histological preparation. Nucleus is pushed in narrow basal portion.
  Example: Small intestine, large intestine, lining of respiratory tract, etc.
  Function: Secretion of mucus.
- **Pseudostratified columnar ciliated epithelium:** Some cells are tall columnar and some cells are short columnar. All cells touch the basal lamina but all of them do not reach the surface, therefore nuclei are placed at different levels giving rise to stratified look.
  Example: Trachea, bronchi and larger bronchioles (motile cilia) and epididymis, vas deferens (stereocilia), some parts of auditory tube, membranous and penile parts of urethra.
  Function: Filtration of air in respiratory passage, absorption of testicular fluid in vas and epididymis, helps in transportation of sperms.

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CHAPTER

4 Study of Stratified Epithelium

Competencies Covered

AN65.1	Identify epithelium under the microscope and describe the various types that correlate to its function.
AN65.2	Describe the ultrastructure of epithelium.

Stratified epithelium has multiple layers of epithelial cells. Stratified epithelium is subclassified according to their shape and presence or absence of keratin material over the cells. Superficial layer of the cells decides the type of the epithelium.

- **Stratified squamous epithelium:** It consists of numerous superimposed layers of cells. Basal layer consists of cuboidal or low columnar cells with mitotic activity. Intermediate layer consists of polygonal cells. Superficial cells are flattened and have several layers with flattened nuclei. The types includes:
  - *Nonkeratinized stratified squamous epithelium:* All cells are living and therefore have nuclei.
    Examples: Lining of mouth cavity, tongue, tonsil, pharynx, esophagus, epiglottis, vagina, cervix uteri, cornea and anal canal, etc.
    Function: It forms moist protective layer in oral cavity, esophagus and vagina.
  - *Keratinized stratified squamous epithelium:* Superficial cells are broken, and lose their nuclei and therefore become dead. They are converted into a horny material called keratin (scleroprotein) which takes acidophilic stain.
    Example: Epidermis of skin.
    Function: Protection from injury.
- **Stratified cuboidal epithelium:** It consists of several layers of cells, of which, the superficial cells are cuboidal and the rest are polyhedral in shape.
  Example: Ducts of sweat gland, sebaceous gland, mammary gland, seminiferous tubules of testis, developing ovarian follicles and larger excretory ducts, etc.
  Function: Protection.
- **Stratified columnar epithelium:** It consists of several layers of cells of which superficial cells are columnar in shape. Deeper cells are polyhedral or cuboidal in shape.
  Examples: Cavernous part of male urethra, transition zone of anal canal, fornix of conjunctiva and larger ducts, etc.
  Function: Protection.
- **Transitional epithelium:** All epithelial cells are similar, soft and to a certain extent plastic, being able to adjust themselves to influences like increase or decrease the general surface area. Basement membrane is not indented by connective tissue papillae, and it exhibits an even contour.
  - In dilated condition it consists of two or three layers of cells. Superficial cells are large cells (umbrella cells); deeper cells are cuboidal in shape.
  - In contracted condition epithelium becomes five or six layered. Superficial cells are large and cuboidal, deeper cells are flask or pear-shaped.

  Examples: Major and minor calyces, pelvis of ureter, ureter, urinary bladder and proximal part of urethra.
  Function: Changes shapes during stretching without destruction of cells and acts as barrier between urine and underlying tissue.

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CHAPTER

5 Glandular Tissue

Competencies Covered

AN65.1	Identify epithelium under the microscope and describe the various types that correlate to its function.
AN65.2	Describe the ultrastructure of epithelium.

Glandular tissues are specialized epithelial tissue suitably modified to produce secretions and can change the tissue environment by transport of macromolecules, water and ions.

DEVELOPMENT OF EXOCRINE GLAND

- **Exocrine gland** develops from diverticulum of epithelium which appears as bud and elongates as solid cord. Later on canalization occurs which becomes divided into secretory and conducting part.
- **Endocrine gland** also appear as bud from epithelium but soon become detached and forms clumps of cells. Soon it becomes surrounded by blood vessels in which cells pour their secretions.

CLASSIFICATION

1. **According to method of transport of secretion:** They are classified as **exocrine** gland or **endocrine** gland. Exocrine glands discharge their secretions into the ducts, whereas, endocrine glands discharge their secretions directly into the blood or circulatory system.
2. **According to mode of secretion:** Exocrine glands are classified according to their mode of secretions. They are:
   - *Merocrine:* Secretory granules are discharged by exocytosis without any loss of cellular material.
     Example: Pancreas, eccrine sweat gland, etc.
   - *Apocrine:* Apical part of gland is discharged therefore, loss of apical part of cytoplasm.
     Example: Mammary gland, apocrine sweat gland, etc.
   - *Holocrine:* The entire cell disintegrates and is discharged as secretion.
     Example: Sebaceous gland.
3. **According to secretory product**: Depending upon the viscosity of secretion. They are:
   - *Serous gland:* Secretion is watery and often rich in enzymes.
     Example: Parotid gland, pancreas, etc.
   - *Mucous gland:* Secretion is viscous which lubricates and protect the inner lining of organs.
     Example: Goblet cells, palatine glands, lingual glands, etc.
   - *Mixed gland:* Secretion is mixed as in submandibular and sublingual salivary glands.
4. **Morphological classification**:
   i. *Unicellular gland*: Single cell act as gland, usually lie within the thickness of the gland.
   Example: Goblet cell—cells are inverted flask-shaped cells with neck and belly. Neck contains nucleus and belly contains mucinogen. They are distributed in trachea, bronchi, and intestines, etc.
   ii. *Multicellular gland:* They are divided into epithelial and subepithelial glands.
   - *Epithelial gland:* They are present as epithelial secretary sheets.
     Examples:
     - » Surface epithelium of gastric mucosa and mucosa of uterus in secretory phase.
     - » Intraepithelial gland lying within the thickness of epithelium.
       Example: Glands present in nasal mucosa, ductus deferens, and urethra.

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- *Subepithelial gland:* It may be simple or compound exocrine glands.
  - » Simple exocrine gland: They have only one duct. They are:
    - ◊ Simple tubular gland: Crypt of Lieberkuhn.
    - ◊ Simple coiled tubular gland: Lower portion of tube become coiled as in sweat gland.
    - ◊ Simple branched tubular gland: Terminal portion of gland is branched.
      Example: Gastric gland, uterine gland, etc.
    - ◊ Simple branched alveolar gland: Terminal portion is expanded to form alveoli.
      Example: Sebaceous gland, meibomian glands of eyelid.
- *Compound gland:* They have repeated branching pattern. They have minor and major ducts. They are:
  - » Compound tubular gland: Brunner's gland.
  - » Compound alveolar gland: Mammary gland.
  - » Compound tubuloalveolar gland: Salivary gland, and pancreas.

STRUCTURAL ORGANIZATION OF GLAND

Whole gland may be encapsulated in a connective tissue stroma, which divides the gland into lobes and lobules. Blood vessels follow the septa and form a capillary network around the secretory unit.

- ❖ **Exocrine glands:** In this alveoli open into the intercalated duct which joins to form intralobular duct then interlobular duct. Interlobular duct unites to form interlobar and larger ducts, which drain the secretion usually into the lumen.
  Lining of the ducts:
  - – Intercalated duct: Low cuboidal or squamous epithelium
  - – Intralobular duct: Simple cuboidal epithelium
  - – Interlobular duct: Simple columnar epithelium
  - – Lobar and interlobar ducts: Simple columnar epithelium
  - – Larger duct: Stratified columnar epithelium. Terminal part of a duct passing through stratified squamous epithelium acquires similar lining.
- ❖ **Endocrine gland:** They are ductless, highly vascularized glands. They release secretory products directly into the bloodstream. Endocrine gland may be:
  - – *Unicellular gland:* Enteroendocrine cells of gastrointestinal tracts.
  - – *Mixed gland:* Organs having both exocrine as well as endocrine portion of the gland.
    Example: Pancreas, and reproductive organs.
  - – *Separate endocrine organs:* They are pituitary gland, thyroid gland, parathyroid gland, and adrenal gland.

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CHAPTER

6 Study of Connective Tissue: Cells

Competencies Covered

AN66.1	Describe and identify various types of connective tissue with functional correlation.
AN66.2	Describe the ultrastructure of connective tissue.

CONNECTIVE TISSUE

Connective tissue binds together and supports the functionally active structures. It provides nutrition and gives shape to the organs and body. Connective tissues are the medium of transport of waste product and the electrolytes between the cells and blood. They provide defense and repair mechanism. They are mesodermal in origin except pigment cells, reticular cells, and neuroglial cells.

Connective tissue has three components:

1. Cells
2. Fibers, and
3. Ground substance or matrix

CLASSIFICATION

According to the components connective tissue can be divided into the followings:

1. Connective tissue proper
   - Loose connective tissue
   - Reticular tissue
   - Adipose tissue
   - Dense connective tissue
     - Irregular dense connective tissue
     - Regular dense connective tissue
     - Elastic connective tissue
2. Specialized connective tissue
   - Cartilage: In cartilage ground substance is firm which can resist mechanical stress.
   - Bone: In bone ground substance contains minerals which gives hardness and provide framework. Bones gives protection to the vital organs.
   - Blood and lymph

CELLS

- **Mesenchyme cell:** It is small spindle-shaped cell with processes. Cytoplasm is deeply basophilic and granular.
  Example: They lie along the wall of blood vessels
  Function: They give rise to all varieties of connective tissue cells.
- **Fibroblast:** It is large, spindle-shaped cell with processes. Cytoplasm is lightly acidophilic. Nucleus is large, oval and centrally placed. It synthesizes connective tissues fibers and the surrounding ground substance.
  Example: Present in all types of connective tissue.

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Function: They synthesize collagen, reticular and elastic connective tissue fibers. It also forms protein tropocollagen of ground substance which forms collagen fibers. It helps in formation of scar tissues.

- **Fibrocyte**: They are derived from fibroblasts and are smaller than fibroblast. They are spindle-shaped cells without cytoplasmic processes. Cytoplasm is faintly basophilic or slightly acidophilic without granules. Nucleus is large, oval and centrally placed.
  Example: Present in all types of connective tissue.
- **Adipose cell (fat cell):** Cells are large, oval or spherical in shape containing large, colorless globule of fat with a thin rim of cytoplasm. Nucleus is pushed on to the periphery with accumulation of cytoplasm around it, giving rise to signet ring appearance.
  Example: Present in all types of connective tissue.
  Function: It stores lipids for energy and forms pads for protection of organs. It also secretes hormone leptin to enhance lipid metabolism.
- **Reticular cell:** Cells are stellate shaped and have processes. The types includes:
  - *Primitive reticular cells*: It resembles embryonic mesenchyme.
  - *Phagocytic reticular cells (fixed macrophages):* They may become free in inflammatory conditions.
- **Pigment cell:** They are stellate cells with branching processes. Cytoplasm contains numerous dark brown or black pigment granules which are usually melanin. The type include:
  - *Melanocytes:* They are concerned with production of pigments.
  - *Melanophores:* They are concerned with storage of pigment granules discharged by melanocytes.

  Function: It gives color to retina and skin.
- **Macrophage (histiocyte):** They are large and well stained irregular or rounded cells. Cytoplasm is coarsely granular, vacuolated and slightly acidophilic. Nucleus is relatively small and densely stained due to rich chromatin content; especially stained by vital stain.
  Example: Loose connective tissue and connective tissue of organs.
  Function: Ingestion of bacteria, dead cells, debris and foreign particles. It also produces antigen causing allergy.
- **Mast cell:** They are small rounded or oval cells. Cytoplasm is packed with large number of coarse granules which stain deep brick red with neutral red (stains metachromatically). Nucleus is small, pale and centrally placed and may not be visible as it is covered by granules.
  Example: Loose connective tissue, fibrous capsule of liver, serous membranes, and around blood vessels, etc.
  Function: It releases histamine, heparin and serotonin.
- **Plasma cell:** Cells are oval or rounded in shape, and smaller than macrophages. Cytoplasm is intensely basophilic and may contain round acidophilic bodies, known as 'Russel body'. Nucleus is relatively small, spherical and usually eccentrically placed and surrounded by a clear, 'Halo'. Chromatin is present in form of deeply staining granules, adherent to nuclear membrane, giving rise to a 'Cart wheel' appearance.
  Example: Mucosa and submucosa of gut, greater omentum and lymphoid tissue, etc.
  Function: Produce antibodies (IgG, and IgE) to neutralize specific antigen.

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CHAPTER

7

Study of Connective Tissue: Fibers and Matrix

Competencies Covered

AN66.1	Describe and identify various types of connective tissue with functional correlation.
AN66.2	Describe the ultrastructure of connective tissue.

There are three types of connective tissue fibers:

1. **Collagen fibers:** They are tough and most numerous and widely distributed formed by collagen fibers. They run in bundles which do not branch. Fibers are strong and flexible but not elastic, hence stretching is restricted. They are acidophilic in staining. On their molecular composition more than twentyfive types of collagen fibers are identified. Each has different distribution and function. Some of them are:
   - *Type I:* Found in skin, tendons, ligaments and bone.
   - *Type II:* Found in hyaline and elastic cartilage.
   - *Type III:* Found in liver, lymph node, spleen and hematopoietic organs.
   - *Type IV:* Found in basal lamina of basement membranes.
   - *Type V:* Found in blood vessels and fetal membranes.

   Distribution: All types of connective tissue proper, cartilage, bone, tendons, ligaments, aponeurosis and intervertebral disc and sclera of the eyeball, etc.
2. **Elastic fibers:** They are much less frequent than collagen fibers. They are fine and run singly. They branch and anastomose freely. They are elastic in nature and curl up when broken. They are yellow in color and stained by orcein and resorcin-fuchsin. They are formed by fibroblasts, but in the wall of arteries they are formed by smooth muscles.

   Distribution: Ligamentum nuchae, ligamentum flavum, internal elastic lamina of arteries, lungs, bladder and skin, etc.
3. **Reticular fibers:** They are of small length, branching and anastomosing and form a net-like supporting framework called 'reticulum'. They are especially stained by 'silver stains' and take black stain therefore they are known as argyrophilic.

   Distribution: Lymphoid organs, endocrine glands, liver, bone marrow and basal lamina of epithelium, etc.

MATRIX OR GROUND SUBSTANCE

Matrix is transparent, amorphous, viscous semifluid substance which occupies the space between cells and fibers. Its refractive index is same as water. Chemically, it is protein-polysaccharide. An enzyme 'hyaluronidase' hydrolyzes it and thus increases tissue permeability.

Function: Binding of the cells to the fibers by glycoproteins such as fibronectin. It forms barrier for pathogens but pathogens having hyaluronidase hydrolyses it and reduce the barrier viscosity.

LOOSE CONNECTIVE TISSUE

Loose connective tissue is soft and transparent containing a network of collagen and elastic fibers, different types of connective tissue cells and semifluid matrix. Loose connective tissue is distributed in submucosa of gastrointestinal tract muscle sheath, vessels, and subcutaneous tissue of eyelids, penis and labia, etc.

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DENSE CONNECTIVE TISSUE

Dense connective tissue: Dense connective tissue is irregular and regular connective tissue.

- **Dense irregular connective tissue:** It contains mainly thick bundles of collagen fibers arranged irregularly. Fibroblasts are few in number and distributed in periosteum, perichondrium, blood vessels, and sclera of the eyeball, etc.
- **Dense regular connective tissue:** It is also known as elastic tissue formed by regularly arranged bundles of collagen fibers and fibroblasts. They are mixed with elastic fibers and distributed in tendons, ligaments, ligamentum flavum and vocal folds, etc.

ELASTIC CONNECTIVE TISSUE

In elastic connective tissue large number of elastic fibers is present. The elastic fibers are freely branching. Fibroblasts and matrix are present in small intervening spaces. They are distributed in anterior abdominal wall, aorta, large blood vessels, trachea, bronchi, vocal cords, suspensory ligaments and penis, etc. due to presence of elastic fibers, it provides elasticity.

ADIPOSE CONNECTIVE TISSUE

There are two types of adipose connective tissue:

1. **White adipose tissue:** It is white or yellowish in color formed by aggregation of fat cells surrounded by reticular fibers and connective tissue septa. Adipose connective tissue is rich in blood vessels.
2. **Brown adipose tissue:** It is brown in color due to presence of large amount of cytochrome oxidase in the mitochondria. In brown adipose tissue nucleus is not flat. Fat contained in the cytoplasm is in the form of small drops therefore multilocular. Brown adipose tissue is present only in children not in adults. As the child grows the fat droplets coalesce together and form one droplet in white adipose tissue. It is present in posterior triangle of neck, scapular region and posterior abdominal wall of newborn.
   Function: It prevents the loss of temperature therefore maintains the body temperature of newborns. This is regulated by presence of thermogenin protein in the mitochondria of brown adipose cells.

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CHAPTER

8 Study of Cartilage

Competencies Covered

AN2.4	Describe various types of cartilage with its structure and distribution in body.
AN71.2	Identify cartilage under the microscope and describe various types and structure—function correlation of the same.

Cartilage is a special type of connective tissue having cells called chondrocytes and chondroblasts that synthesize connective tissue fibers and ground substance. It develops from mesenchyme. During development most of the skeleton of the body are in the form of cartilage. It is nonvascular and gets its nutrition via diffusion through ground substance. Articular cartilage receives its nutrition from synovial fluid. Components of cartilage are chondrocytes, fibers and matrix.

CLASSIFICATION

Cartilage is classified according to its amount and types of fibers. There are three types of cartilage:

1. **Hyaline cartilage:** The chondrocytes resides in a small space called lacunae, in groups of 2, 3 or 5, some are in state of division therefore hyaline cartilage has encapsulated cartilage cells, frequently forming cell nests. Ground substance appears homogenous and takes light blue stain. It contains dense network of fine collagen fibers mostly on periphery. The matrix between cells or group of cells is known as interterritorial matrix, more basophilic zone of matrix, around group of cells is known as territorial matrix. Most recently formed matrix surrounds lacunae which takes deeper basic stain and give an impression of a capsule. Perichondrium covers hyaline cartilage except articular surfaces.
   Distribution: Tracheal, bronchial, nasal, costal, articular, epiphyseal, thyroid, cricoid and basal part of arytenoid cartilages.
   Function: It forms skeletal model for most of the bones.
2. **Elastic cartilage:** Encapsulated cartilage cells are present either singly or in groups. Ground substance contains network of elastic fibers, which is visible in ordinary microscope. Perichondrium is present.
   Distribution: Epiglottis, external ear, external acoustic meatus, pharyngotympanic tube, arytenoid (except basal part), corniculate and cuneiform cartilages.
3. **Fibrocartilage:** Here encapsulated cartilage cells are fewer in number and lie in a row. Intercellular substance contains irregular and thick bundles of collagen fibers which run parallel to each other and are separated by narrow areas of nonfibrous matrix in which cartilage cells are present. Perichondrium is absent.
   Distribution: Intervertebral disc, pubic symphysis, articular disc of joints, cartilaginous lining of bony grooves, etc.
   Function: It provides tensile strength, bears weight, and resists compression.

PERICHONDRIUM

It covers the outer surface of cartilage and has two layers:

1. Outer layer is dense vascular connective tissue with collagen fibers.
2. Inner layer is chondrogenic and gives rise to chondroblast that secrete cartilage matrix.

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CHAPTER

9 Study of Bones

Competencies Covered

AN71.1 Identify bone under the microscope; classify various types and describe the structure-function correlation of the same.

Bone is highly specialized connective tissue. Ground substance is impregnated with calcium salts and so becomes rigid. It has four types of bone cells: Osteogenic cells, osteoblasts, osteocytes, and osteoclasts. It has collagen fibers.

1. **Osteogenic cells:** Osteogenic cells are precursor of all types of bone cells. They are irregular, elongated, with pale cytoplasm. Nucleus is pale. Osteogenic cells are present at the periphery of mesodermal condensation. It gives rise to osteoblasts.
2. **Osteoblasts cells:** Osteoblast cells are large, low columnar with cytoplasmic processes. Cytoplasm takes basophilic stain. Nucleus is round with a prominent nucleolus. They are present at the places where active bone formation is taking place. Osteoblast cells synthesize collagen fibers and matrix.
3. **Osteocytes:** Osteocytes are flattened cells with less basophilic cytoplasm. Cells are in resting phase but become active in needs.
4. **Osteoclasts:** Osteoclasts cells are multinucleated giant cells with eosinophilic or faintly basophilic cytoplasm present at the site of bone resorption.

TYPES

There are three types of bones: Compact, spongy and sesamoid bones.

1. **Compact bone:** It consists of haversian system (osteon) centered on haversian canal. *Haversian canal* runs parallel to the long axis of the bone. They branch and communicate at short intervals and contain reticular connective tissue, blood vessels, lymphatics and nerves. Haversian system consists of concentric tubular layers known as lamellae. *Lamellae* contain oval spaces called *lacunae*, which contain cell bodies of osteocytes and osteoblasts. A number of minute channels radiate from the lacunae known as *canaliculi*, which contain the processes of bone cells. Canaliculi anastomose freely with each other, but the processes of bone cells do not anastomose. Lamellae consist of organic matter, collagen fibers and calcium salts.

   The outer boundary of osteon is separated by a cement line. Intervals between haversian system are occupied by interstitial lamellae. Outer and inner peripheries of cortex contain outer and inner circumferential lamellae respectively. Haversian canal communicates with each other, medullary cavity and outer surface of bone through oblique channels called *'Volkmann's canal'*. Bone is covered by periosteum.
2. **Spongy bone:** Characteristic features are:
   - Lamellae are irregularly arranged
   - Haversian system is absent
   - Bone tissues are arranged as thin plates known as trabeculae.
3. **Sesamoid bone:** It develops in tendons and ligaments. They do not have periosteum, haversian system and medullary cavity.

Bone marrow: Soft vascular tissue is present in medullary cavity of long bone and in the spaces between the trabeculae of spongy bone. Red marrow consists of blood cells in different stages of development. Yellow marrow consists of mainly fat. Yellow marrow is converted into red marrow during needs.

Periosteum: It has two layers:

1. Outer layer consists of dense collagenous connective tissue
2. Inner osteogenic layer consists of loose collagen fibers and osteogenic cells. Collagen fibers penetrate into the layer of outer circumferential lamellae to form 'Sharpeys fibers'.

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CHAPTER

10 Study of Muscle Tissue

Competencies Covered

AN67.1	Describe and identify various types of muscle under the microscope.
AN67.2	Classify muscle and describe the structure-function correlation of the same.
AN67.3	Describe the ultrastructure of muscular tissue.

All muscle tissues are composed of elongated cells called fibers. Cytoplasm is known as sarcoplasm, and cell membrane as sarcolemma. Each muscle fiber contains myofibrils which consists of actin and myosin. Actin and myosin are contractile protein element.

DEVELOPMENT

All muscle fibers develop from the mesoderm except the muscles of iris and myoepithelial cells which are ectodermal in origin. There are three types of muscle tissue:

1. **Skeletal muscle/somatic striped voluntary striated:** It is composed of muscle fibers held together in bundles (fasciculus) by connective tissue. Muscle fibers do not divide or anastomose. They are elongated cylindrical, multinucleated cell. These multiple nuclei are present on periphery under the sarcolemma. Cytoplasm contains nuclei, myofibrils and various cytoplasmic granules and organelles.

   Muscle fibers have longitudinal and transverse striations formed by actin and myosin. Transverse striations have alternate light I band and dark A bands. Whole skeletal muscles are surrounded by dense connective tissue known as epimysium. This epimysium sends septa of loose connective tissue to cover fasciculus called perimysium. The covering of individual muscle fiber is called endomysium. Neuromuscular spindles are located within skeletal muscle.
2. **Smooth muscle/unstriped involuntary unstriated:** They are spindle-shaped cells collected in bundles. They have single, oval nucleus placed centrally. They show faint longitudinal striations produced by longitudinally placed myofibrils. Myofibrils consist of myofilament. Transverse striation is absent. Areas of contact of muscle fibers are known as nexus. Actin and myosin are present but do not show regular arrangement hence any striations.
3. **Cardiac muscle:** Each muscle fiber branches and anastomoses. Transverse striations are present but are not as prominent as in skeletal muscle. Each muscle fiber has single large nucleus, oval in shape with a perinuclear sarcoplasm and is centrally placed. Individual muscle fibers are joined at their ends by junctional complex known as intercalated discs that contain gap junctions. Numerous blood vessels lie in interfascicular connective tissue (in between fasciculus). Gap junctions are for rhythmic contraction of muscle fibers.

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CHAPTER

11 Study of Nervous Tissue I

Competencies Covered

AN68.2	Describe the structure-function correlation of neuron.
AN68.3	Describe the ultrastructure of nervous tissue.

NEURONS

Nervous tissue consists of neurons. Neuron is structural and functional unit of nervous system. It has cell body or perikaryon and processes—axon and dendrites. Axon is one, but dendrites may be one or more. Cell body or perikaryon is irregular in shape and has nucleus, Nissl body, Golgi complex and mitochondria, etc. Nissl body is rough endoplasmic reticulum present in the cell bodies and dendrites but absent from axon hillock (junction of cell body with axon). Neurons receive and conduct impulses for analysis, integration, interpretation and response. Neurofibrils are thread-like structure present in cell body and extend to processes and can be stained by silver stains. The types include:

- **Unipolar:** Cell body has only one process, the axon.
  Example: Mesencephalic nucleus of trigeminal nerve.
- **Bipolar:** Spindle-shaped cell body has axon at one pole and dendrite at the other pole. They are sensory.
  Example: Olfactory neuroepithelium, bipolar cells of retina, spiral ganglia of cochlea and vestibular ganglion
- **Pseudounipolar:** A single process after originating from cell body bifurcates.
  Examples: Spiral ganglia, ganglions of cranial nerves except vestibulocochlear nucleus.
- **Multipolar:** They are most common. They have a single axon and numerous dendrites attached to the cell body.
  Examples: Motor neurons of central nervous system and autonomic ganglion and interneurons.

Nerve Fibers

Nerve fibers may be myelinated or nonmyelinated, as detailed below:

- **Myelinated:** It is laid down by specialized cells and extends along the length of axons to its terminal branches. Gaps between myelin sheaths are known as nodes of Ranvier.
  - *Peripheral nervous system:* Both myelin and Schwann sheath are present.
  - *Central nervous system:* Myelin sheath is present but Schwann sheath is absent.
- **Nonmyelinated:** They do not show nodes of Ranvier.
  - *Peripheral nervous system:* Myelin sheath is absent but Schwann sheath is present.
  - *Central nervous system:* Both myelin sheath and Schwann sheath are absent.

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CHAPTER

12 Study of Nervous Tissue II

Competencies Covered

AN68.1 Describe and identify multipolar and unipolar neuron, ganglia, peripheral nerve.

NERVE TRUNK

Nerve trunk is covered by epineurium which contains blood vessels, etc. Nerve trunk is formed by funiculus, which is covered by perineurium. Funiculus is collections of nerve fibers which are covered by endoneurium. Just inside the endoneurium is the sheath of Schwann cell. Just below this sheath, there is a thicker sheath composed principally of a fatty material called myelin. These two sheaths contain axon. The axon and its sheath constitute the nerve fiber.

Neuroglia

Neuroglia is highly branched non-neuronal cells which supports the neurons and holds them together. There are four types of neuroglia:

1. **Astrocytes:** It is star-shaped cell with branching processes. Nucleus is round or oval in shape. They are largest and present in gray matter. These cells support metabolic exchange and contribute to energy metabolism of central nervous system (CNS). They control chemical environment around neurons by clearing neurotransmitters. There are two types of astrocytes:
   i. *Protoplasmic astrocytes:* It has fairly thick processes with short secondary branches and perivascular (sucker's) feet. They are found within gray matter.
   ii. *Fibrous astrocytes:* It has long, slender and smooth processes, which branch frequently and have perivascular feet. They are found in white matter only.

   Function: It act as a blood-brain barrier. It gives support and nutrition. It acts as fibroblasts in inflammatory condition.
2. **Oligodendrocytes:** They are smaller than astrocytes and possesses deeply basophilic, heterochromatic nucleus, cytoplasms is scanty and has few processes. Perivascular foot is absent. They are found in both gray matter and white matter.
   Function: formation and maintenance of myelin sheath in CNS. It also gives support to the neurons and provides insulation and prevents random synapses.
3. **Microglia (gitter cell):** It is smallest neuroglial cell, mesodermal in origin and found in white matter. Its processes are short, slender, but tortuous and are covered with minute spines.
   Function: It has phagocytic action.
4. **Ependymal cells:** They are simple cuboidal or low columnar cells, apex have cilia and microvilli. They line the ventricles of brain and central canal of spinal cord. There are three types of ependymal cells: Ependymocytes, tanycytes and choroidal epithelial cells.
   Function:
   - Formation of cerebrospinal fluid (CSF)
   - Formation of germinal layer of primitive neural tube and give rise to development of neuroblast (neuron) and spongioblast (astrocytes and oligodendrocytes).

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