Ministry of Higher Education and Scientific Research

University of Baghdad

College of Science Department of Biology

Practical Histology

2022-2023

المرحلة الثالثة/الدراستين الصباحية والمسائية

الفصل الدراسي الاول

د عبد حسن براج د امال خضير عباس د بان جاسم محمد

ا. بسام كريم عبد الامير د عمر فاضل عبد الرحمن ا. مصطفى عبد الخالق

LAB 1: The Science of Tissue Processing

•Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections(slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures with the aid of the light microscope.

•Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections.

•These sections are precisely cut from tissues previously prepared for sectioning using fine cutting instrument called microtome.

•Most fresh tissues are very delicate and easily distorted and damaged, and thus, it is impossible to prepare thin sections from it unless it is chemically preserved or "fixed" and supported in some way whilst it is being cut.

•The ideal microscope tissue preparation should be preserved so that the tissue on the slide has the same structure and molecular composition as it had in the body. **What is Tissue Processing?**

•A procedure of removing water from cells, and replacing it with a medium which solidifies allowing thin sections to be cut on a microtome.

•Tissue processing can be performed manually (hand processing), but where multiple specimens must be dealt with, it is more convenient and much more efficient to use an automated tissue processing machine called a "tissue processor".

The procedure of tissue processing involves the following steps:

A. Gross examination:

means describing the specimen: weight, dimensions, color, texture, type, followed by photography.

B. Tissue dissection:

•Placing all or parts of specimen via cutting by a sharp knife in case of soft tissue, or by a saw in case of hard tissue (bones or cartilage), into a small plastic cassette which hold the tissue. Cassettes hold and protect the specimen while it undergoes processing.

• Purpose: Because the fixative needs some time to fully diffuse into the tissues, the tissues are usually cut into small fragments before fixation to facilitate the penetration of the fixative and to guarantee preservation of the tissue.

C. Fixation:

•To avoid tissue digestion by enzymes existing within the cells (autolysis) or by bacteria, and to preserve the structure and molecular composition, pieces of organs should be promptly and adequately treated immediately after removal from the body.

•This treatment is called fixation and can be done by chemical or, less frequently, physical methods.

•In chemical fixation, the tissues are usually immersed in solutions of stabilizing or crosslinking agents called fixatives.

•Fixative: a chemical agent that preserves the architecture of tissue near normal via stopping enzymatic degradation by cellular enzymes.

•Fixative will slowly penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing steps.

•Ideal fixation should take place at the site of removal, perhaps in the operation theatre, or, if this is not possible, immediately following transport to the lab.

•Ideally specimens should remain in fixative for long enough to allow the fixative to penetrate into every part of the tissue, and then for an additional period to allow the chemical reactions of fixation to reach equilibrium.

•Data shows that optimal time for formalin fixation for most stains is 37 days.

•One of the best fixatives for routine light microscopy is a buffered isotonic solution of 4% formaldehyde.

Formalin vs. Formaldehyde

•We have occasionally encountered some confusion about the difference between formaldehyde and formalin.

•It is incorrect to use the two words interchangeably. The concentrations of chemical fixative that the two names represent are quite different.

•A fixative labeled as 10% buffered formalin is actually only a 4% solution of formaldehyde. This is because 10% buffered formalin is an example of old-time histologist's jargon describing a 10% solution made from a stock bottle of 3.7-4% solution of formaldehyde.

D: Dehydration

•Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax.

•The water is extracted from the fragments to be embedded by bathing them successively in a graded series of mixtures of ethanol and water (usually from 70% to 100% ethanol).

•Ethanol is miscible with water in all proportions, so that the water in the specimen is progressively replaced by the alcohol. **E. Clearing:** •Involves the replacement of ethanol with a solvent miscible with the embedding medium (wax), since wax and ethanol are largely immiscible.

•In paraffin embedding, the solvent used is usually xylene; an intermediate solvent that is fully miscible with both ethanol and paraffin wax.

•This solvent will displace the ethanol in the tissue, and this in turn, will be displaced by molten paraffin wax.

•Multiple changes are required to completely displace ethanol.

•As the tissues are infiltrated with the solvent, they generally become transparent.

F. Wax infiltration

•Includes the exchange of xylene with an embedding agent.

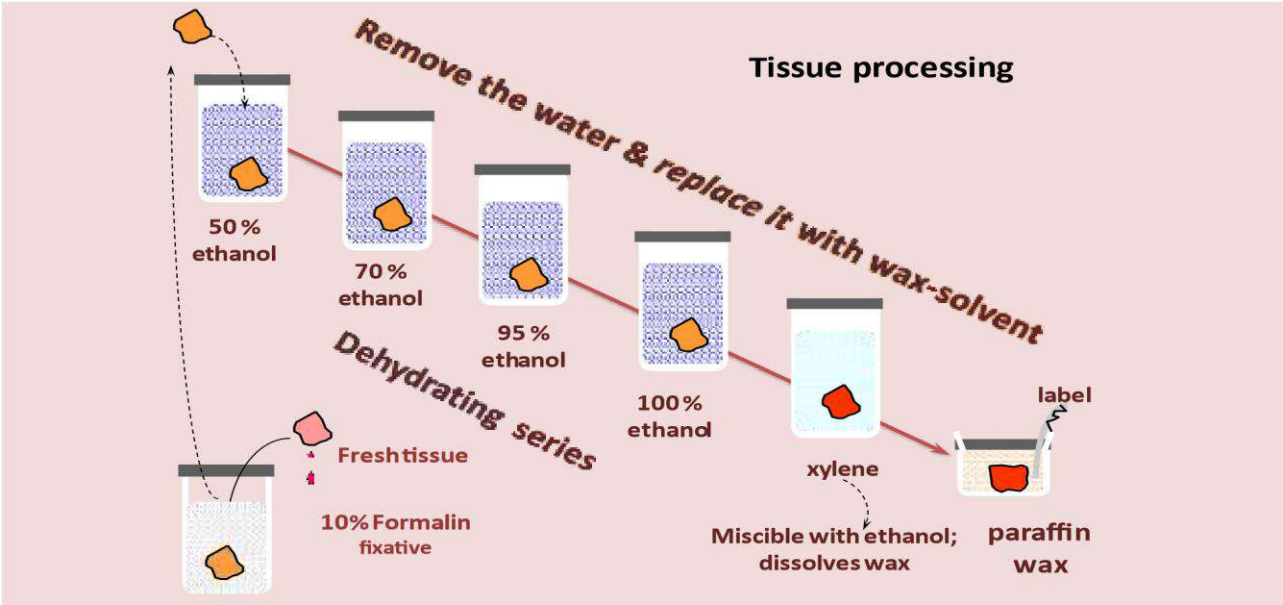
•Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven, typically at 58–60°C.

•The heat causes the solvent to evaporate, and the spaces within the tissues become filled with paraffin.

•A typical wax is liquid at 58-60°C, and can be infiltrated into tissue at this temperature then allowed to cool to 20°C where it solidifies to a consistency that allows sections to be consistently cut.

•Although many different reagents have been used for this purpose over many years, the paraffin wax-based histological waxes are the most popular.

•Paraffin wax-based histological waxes are mixtures of purified paraffin wax and various additives that may include resins such as styrene or polyethylene.



G. Embedding

•After tissue has been dehydrated, cleared and infiltrated with paraffin, it must be formed into a "block" which can be clamped into a microtome for section cutting.

•This is achieved by placing tissue in a mold filled with molten paraffin wax and the specimen placed into it.

•The specimen is very carefully orientated in the mold because its placement will determine the "plane of section", an important consideration in both diagnostic and research histology.
•Then, the block is allowed to solidify on a cold surface and when set, the mold is removed.
•The hard blocks containing the tissues are then taken to a microtome and are sectioned by the microtome's steel or glass blade to a thickness of 1–10 µm.
•The sections are floated on water and transferred to glass slides to be stained.

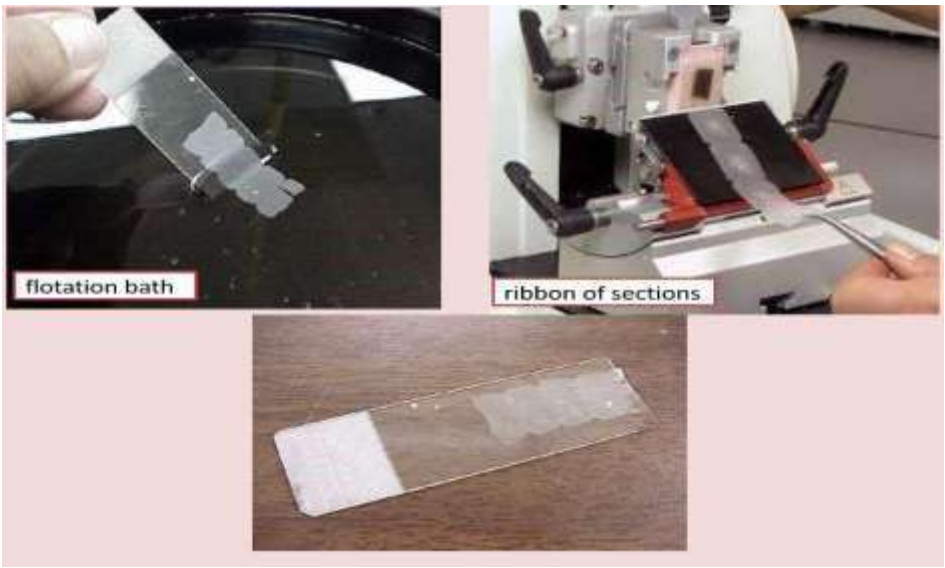
H. Labeling:

•For indefinite storage of a block, an identification number is written on a small piece of paper and attached to one side of the block.
•Labeling of samples embedded in paraffin wax can also be done directly on the wax block or the tissue cassette, with a chemical-resistant printout that will not fade or smear, using labels with an extra-permanent adhesive that allows the labels to adhere to hard to stick surfaces.
•For labeling tissue cassettes, a cassette printer is the preferable and most reliable option.

I. Sectioning:

•To stain samples, paraffin embedded tissues must be melted, and the blocks need to be thinly sectioned using a sharp blade, making thin cross sections in which to view the cells.
•The blocks are chilled in a try of ice because the cold wax makes a clean cut compared to paraffin wax cut at room temperature.
• The paraffin block is then cut by using a microtome with 4-5 µm thickness.
•One of the advantages of paraffin wax as an embedding agent is that as sections are cut they will stick together edge-to-edge, forming a "ribbon" of sections. This makes handling easier.

•Sections are then "floated out" on the surface of warm water in a flotation bath to flatten them and then picked up onto microscope slides. After thorough drying they are ready for staining.



J. Staining

•In order to reveal structural details some form of staining is required. •Here, the use of labels designed to fit microscope slides, with a strong resistance to both direct contact and immersion in harsh chemicals and stains is essential.

•Slide printers are available as well, though durable stain resistant labels still remain the more reliable choice.

- The routine stain used universally as a starting point in providing essential structural information, is the hematoxylin & eosin (H&E) stain.
- When it is properly performed, it has the ability to demonstrate a wide range of normal and abnormal cell and tissue components, and yet, it is a relatively simple stain to carry out on paraffin sections.
- With this method, cell nuclei are stained blue, and cytoplasm and many extracellular components in shades of pink.

Steps of H&E stain:

1. Dewaxing in xylene.
2. Rehydration in descending concentrations of ethanol alcohol. Then rinsed in D.W.
3. Apply hematoxylin (nuclear stain).
4. Apply eosin counter stain.
5. Dehydrate, clear, and mount.

Basophilic and acidophilic staining:

• Acidic dyes react with cationic or basic components in cells. Proteins and other components in the cytoplasm are basic, and will bind to acidic dyes. Another way of saying this is that cytoplasmic proteins are acidophilic (acid liking - i.e. bind to acidic dyes).

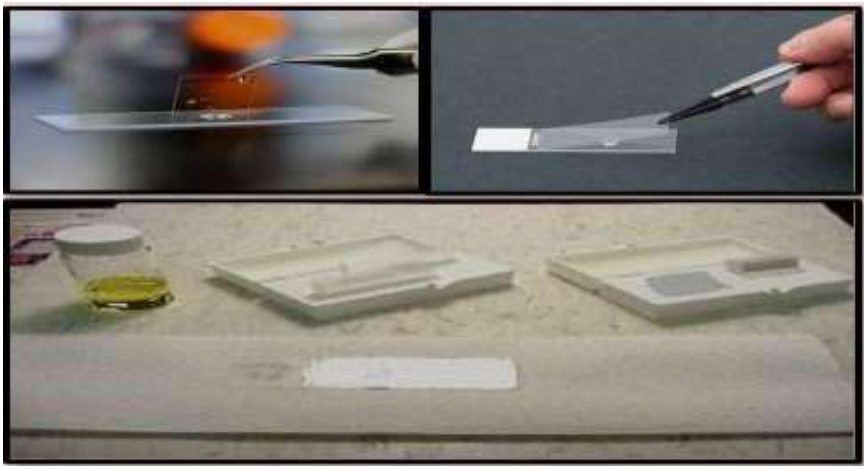
• Basic dyes react with anionic or acidic components in cells. Nucleic acids are acidic, and therefore bind to basic dyes. Another way of saying this is that nucleic acids are basophilic (basic liking).

Basic dyes		Acidic dyes	
Methyl green	Green	Acid fuchsin	Red
Methylene blue	Blue	Aniline Blue	Blue
Pyronin G	Red	Eosin	Red
Toluidine blue	Blue	Orange G	Orange

K. Cover-slipping:

The stained section on the slide must be covered by a thin piece of plastic or glass with DPX to:

1. protect the tissue from being scratched.
2. provide better optical quality for viewing under the microscope.
3. preserve the tissue section for years to come.



L. Drying:

Finally, the slide dried in hot air oven for 5 minutes.

Common orientations of tissue sections:

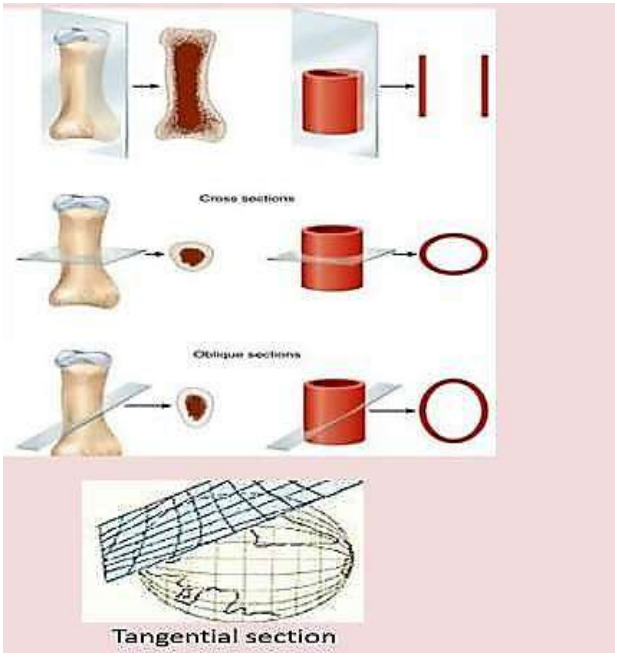
1. Longitudinal section: a cut that is parallel to the longest dimension of the organ or specimen.

2. Cross, or transverse section: a cut that is perpendicular to the main plane of the organ or specimen.

3. Oblique section: a cut that is at any angle between the longitudinal and transverse planes.

4. Tangential section: a section that only grazes the surface of a spherical structure.

Tangential section



Lab 2: Epithelial tissues

•The organs of the human body are composed of four basic tissue types: **epithelial, connective, muscular, and nervous tissues.**

•These tissues are formed by **cells** and **extracellular matrix (ECM)**

Epithelial tissue or "Epithelium":

Composed of closely aggregated cells adhering strongly to one another, and to a thin layer of extracellular matrix- the basement membrane- forming cellular sheets that line the cavities of organs and cover the body surfaces.

Classification of Epithelial Tissue:

Epithelia are divided into two main groups according to their **structure and function**:

1. **Covering & lining epithelia**
2. **Glandular epithelia.**

Covering & lining Epithelia:

- In which the cells are organized in layers that cover the external surfaces or line the cavities of the body.
- They can be classified according to **the number of cellular layers** into:
  - **A. Simple epithelium**
  - **B. Stratified epithelium**
  - **C. Pseudostratified epithelium**

Simple Epithelial Tissues:

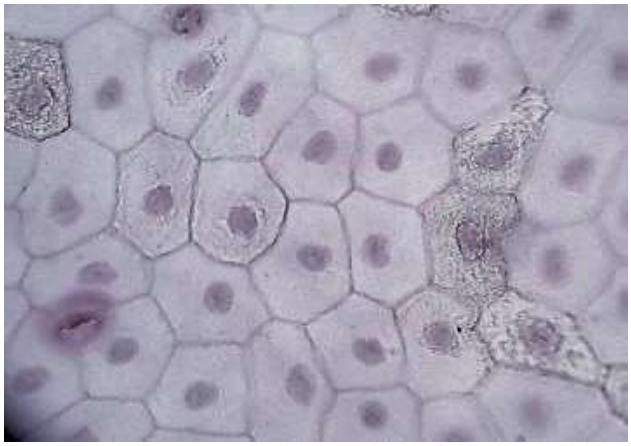
Contain one layer of cells, all of them based on the basement membrane. It can be classified according to the **shape of cells** into:

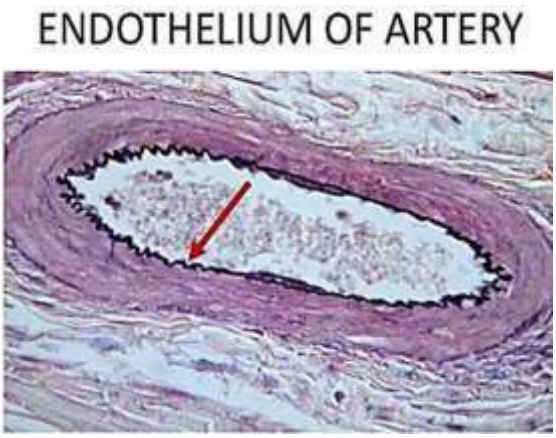
1- Simple Squamous Epithelium:

•The cells are flat in shape (look like scales) and arranged in a single layer.

•Each cell is irregular in shape, and has a disk-shaped flattened nucleus.

Ex: Endothelium, and Mesothelium.



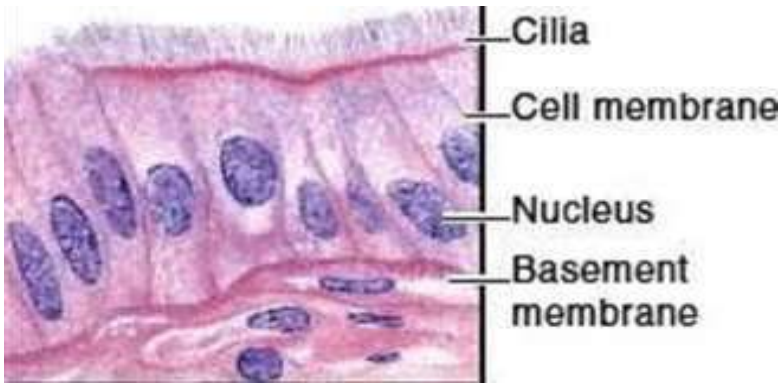


2-Simple Columnar Epithelial Tissue:

- A single layer of tall, closely packed cells, aligned in a row.
- Each cell has an oval nucleus that located close to the basal region of the cell.
- This epithelium is adapted for secretion and/or absorption, and can also be protective.
- This tissue lines the digestive tract from the stomach to the anus as well as uterus and other organs.
- Simple Columnar Epithelial Tissue is further divided into two categories: **ciliated and non-ciliated.**

•**Non ciliated columnar epithelium** often contains cellular extensions (microvilli) and scattering unicellular glands (goblet cells) as in the small intestine.

•**Ciliated columnar epithelium** contains cilia and found within bronchioles of the respiratory tract and in the oviduct of the female reproductive tract.



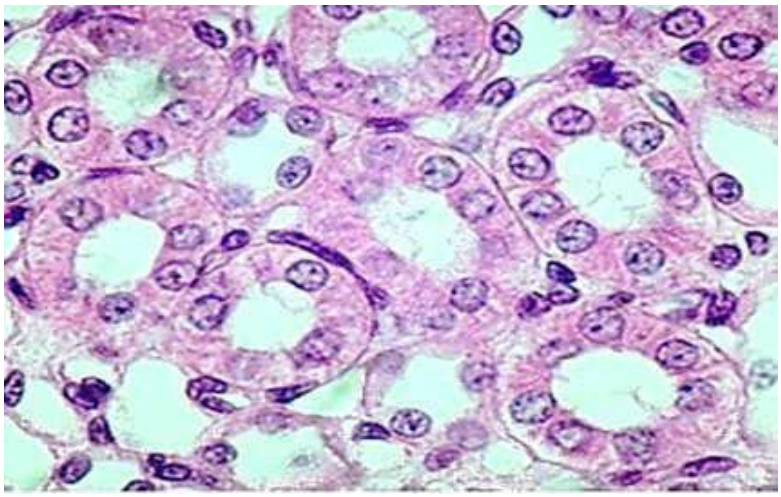
3-Simple Cuboidal Epithelial Tissue:

•It is a single layer of cells that are as tall as they are wide (appear to be square-shaped in cross section).

•Each cell has a large, rounded, centrally located nucleus.

•The important functions of this epithelium are covering and secretion.

•It's found in kidney tubules and in ducts of many glands, and secretory portion of salivary glands.



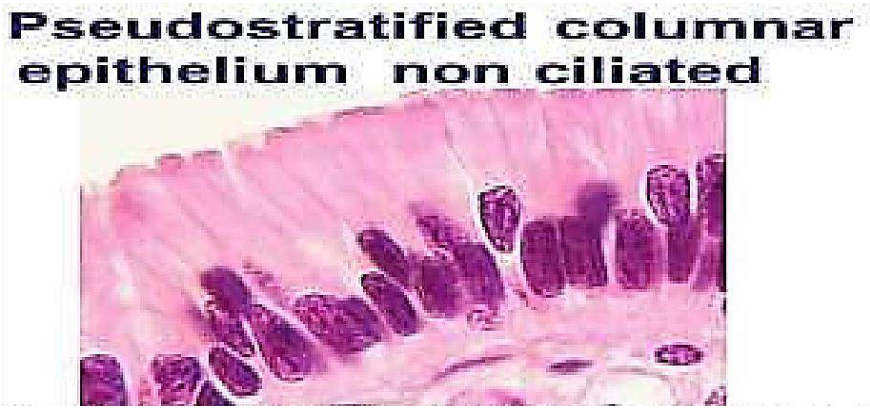
4-Pseudostratified Columnar Epithelial Tissue:

• It looks layered (stratified) because the nuclei of the cells are distributed at different levels between the apical and basal surfaces. Although all of these epithelial cells are attached to the basement membrane, though some of them do not reach its apical surface. Therefore, this tissue has been classified as a type of simple epithelium.

•It includes two subtypes:

a) **Ciliated pseudostratified columnar epithelium:** founds in the lining of the trachea and upper respiratory tract.

b) **Non-ciliated pseudostratified columnar epithelium:** lacks cilia and goblet cells, occurs primarily in part of the male urethra and epididymis.



Stratified Epithelial Tissues:

Contain more than one layer of cells placed on top of each other, only the inner most layer based on the basement membrane. It can be classified according to the shape of cells of its superficial layer into:

1) Stratified Squamous Epithelial Tissue:

•Found primarily in places subjected to attrition (skin, mouth, esophagus, vagina).

•This tissue has multiple cell layers, and only the deepest layer of cells is in direct contact with the basement membrane.

•The cells closer to the underlying connective tissue are usually cuboidal or columnar.

•As they move progressively closer to the surface, the cells become irregular in shape and flatten, becoming very thin and squamous.

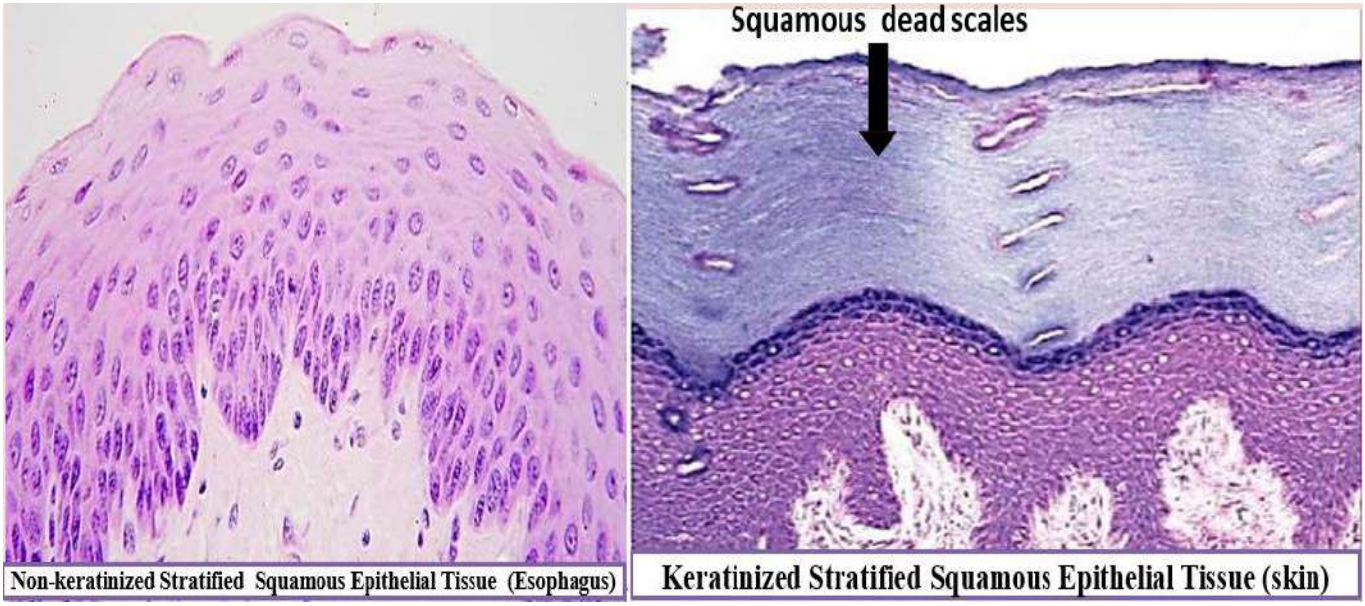
Stratified Squamous Epithelial Tissue is divided into: -

A. Keratinized stratified Squamous Epithelial Tissue

- Covers dry surfaces such as the skin.
- The cells on the surface layer squamous epithelium are transmitted into dead scales without nuclei.

B. Non-keratinized stratified squamous epithelium

- Covers wet surfaces such as the lining of mouth, throat, anal canal, vagina, and esophagus.
- The cells in the surface layer remain soft, moist and alive.



2) Stratified Columnar Epithelial Tissue:

•It consists of two or more layers of cells, but only the cells at the apical surface are columnar in shape.

•This type of epithelium protects and secretes.

•It is a rare tissue, presents in human body in small areas, such as the ocular conjunctive, membranous segment of the male urethra, and large duct of salivary glands.

3) Stratified Cuboidal Epithelial Tissue:

•This epithelium contains two or more layers of cells, and the superficial cells tend to be cuboidal in shape.

•It forms the walls of the large ducts of most exocrine glands, such as the ducts of the sweat glands in the skin.

•The function of this epithelium is mainly protective.

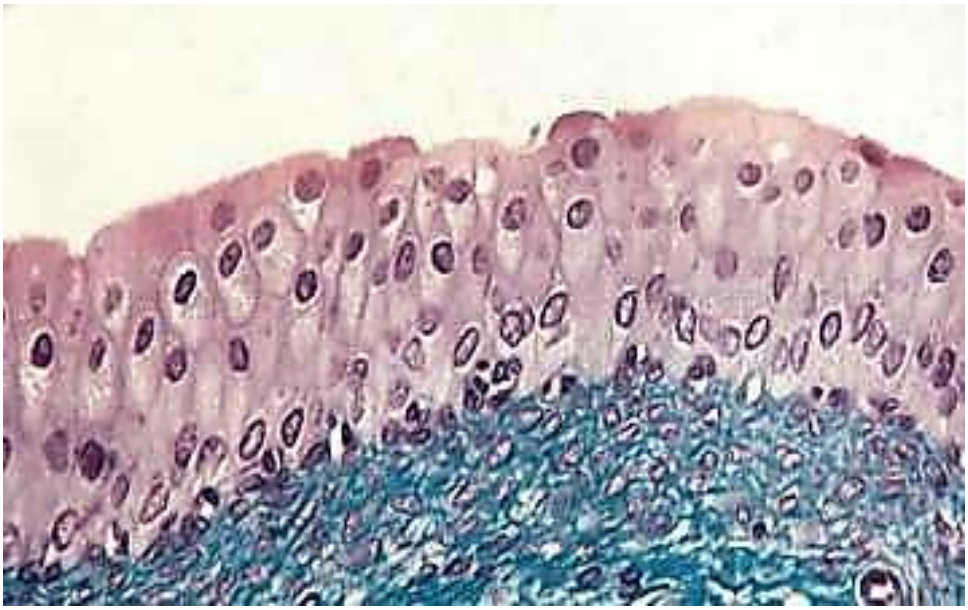
4) Transitional Epithelial Tissue:

•This epithelium is limited to the urinary tract (urinary bladder, ureters, and part of the urethra).

•It varies in appearance, depending upon whether it is in a relaxed state or a stretched state.

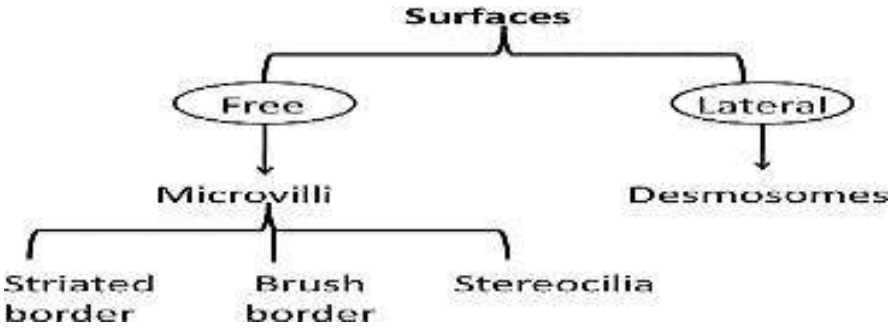
•In a relaxed state, the basal cells appear cuboidal or polyhedral, and the apical cells are large and rounded (dome-like cells).

•When transitional epithelium stretches, it thins and the apical cells flatten and become almost squamous in shape.



Specializations of the cell surfaces in epithelial tissues:

The free or apical surface of many types of epithelial cells contain specialized structures that increase the cell surface area or move substances or particles stuck to the epithelium.

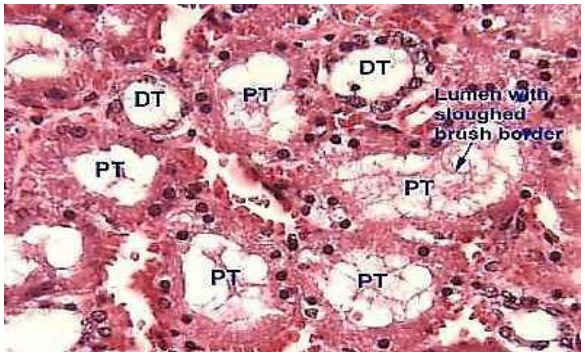


A. Microvilli:

- Fine, finger-like projections that increase the apical surface area in cells.
- Most developed in cells specialized for absorption.
- They may occur as:

1-**Striated border:** in the lining of small intestine (ileum), their function is to increase the surface area of absorptive cells.

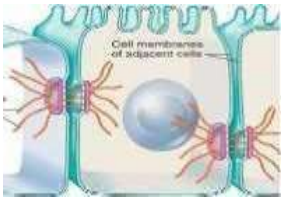
2-**Brush border**: A specialization of the free surface of a cell consisting of minute cylindrical processes (microvilli) that greatly increase the surface area. It presents in the proximal convoluted kidney tubules.

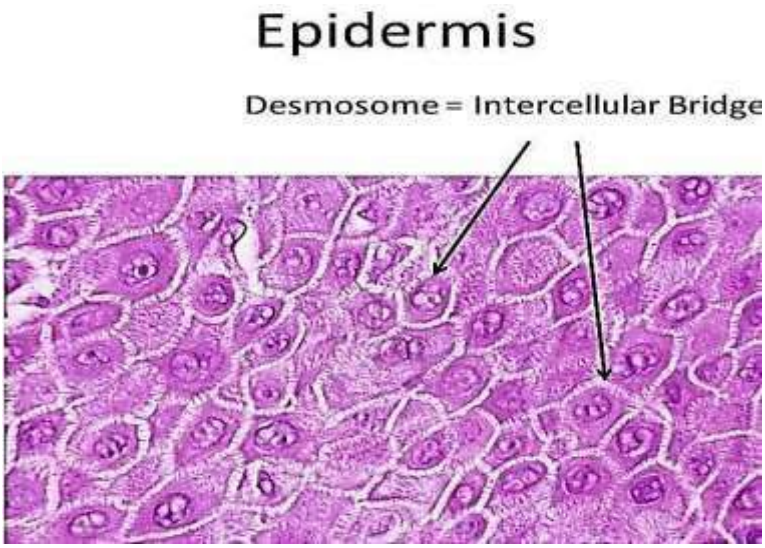


3-**Stereocilia:** long, non-motile extensions in cells of the epididymis and vas deferens that are actually long and branched microvilli, and should not be confused with true cilia. Stereocilia increase the cell surface area, facilitating the movement of molecules into and out of the cell.

B. Desmosomes:

The desmosome is a complex disk-shaped structure at the lateral surface of one cell, that is match with an identical structure at the surface of the adjacent cell. It can be seen in spinosum stratum in the epidermis of the skin.





Lab 3: Glandular Epithelial Tissues

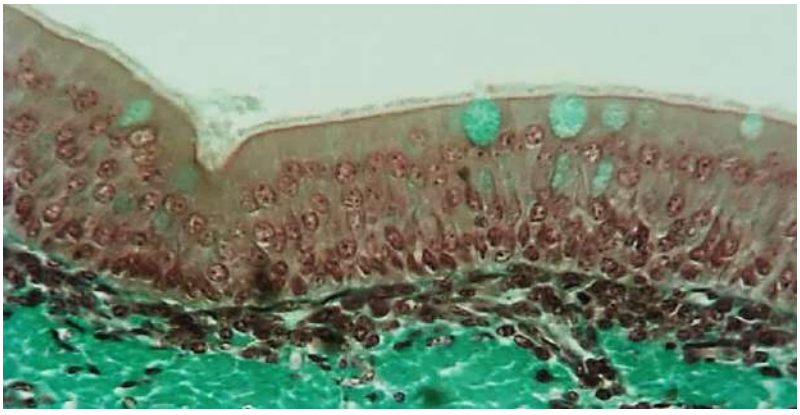
- Glandular epithelia are formed by cells specialized to produce secretion.
- Glands are classified as **endocrine and exocrine**
- **Endocrine glands** lack ducts and secrete their products directly into the interstitial fluid and bloodstream.
- **Exocrine glands** these glands usually maintain their contact with the epithelial surface by means of a duct.

Exocrine gland:

Can be classified according to the **number of cells** into:

1.Unicellular gland:

- An individual exocrine cell located among non-secretory epithelial cells.
- These glands typically do not contain a duct, and they are located close to the surface of the epithelium in which they reside.
- Ex: goblet cell in the lining of small intestine and in respiratory tract.



2. Multicellular gland:

composed of clusters of cells, it consists of two portions:

A. secretory portion

B. duct

Based on the **structure and complexity of their ducts**, exocrine glands are considered either **simple or compound**.

A. **Simple gland:** has one unbranched duct.

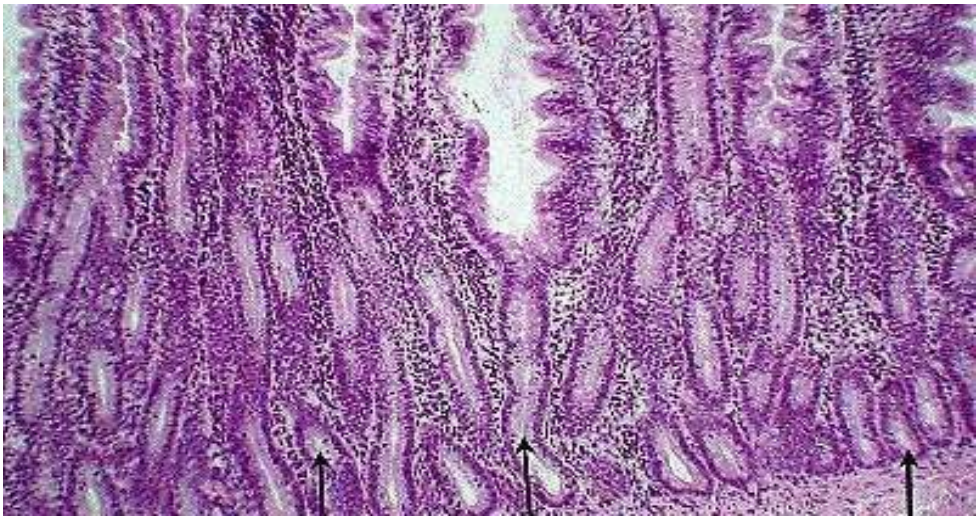
B. **Compound gland:** has ducts that branch repeatedly

A. Simple exocrine glands are classified **according to the shape or organization of their secretory portions:**

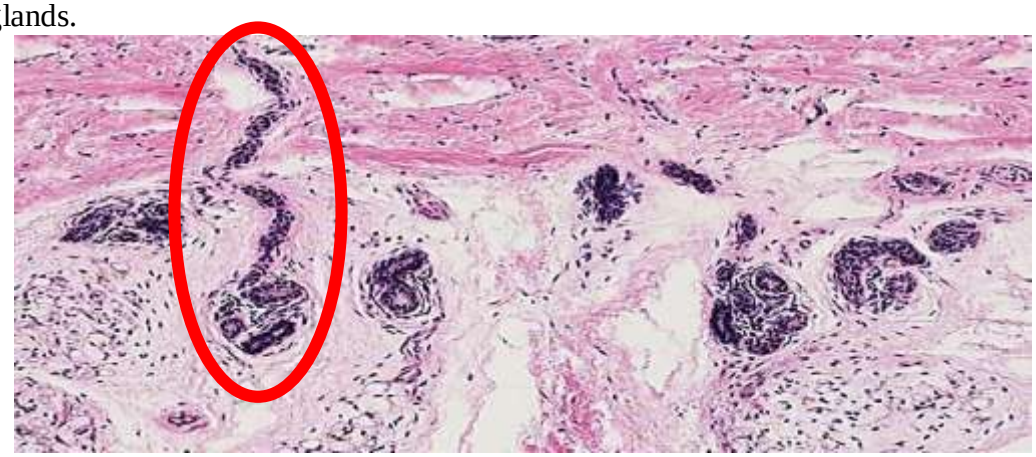
1-Tubular:

In which the secretory portion and the duct are of uniform diameter. It can be further subdivided into:

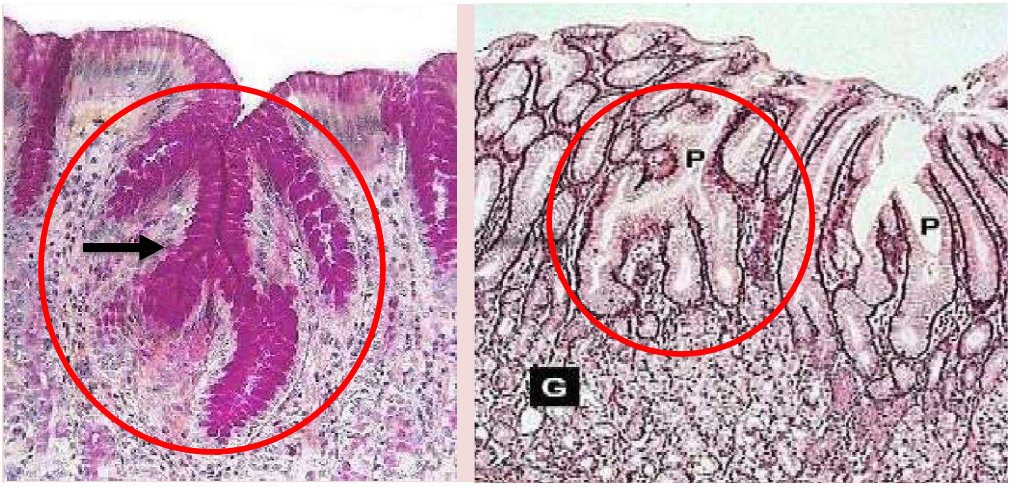
I. **simple(straight) tubular**: the gland looks like a straight tube Ex: **crypt of Lieberkühn's**



II. **simple coiled tubular:** They basically look like a hose tangled or coiled around itself at one end. **The sweat glands** in your skin are example of simple coiled tubular glands.



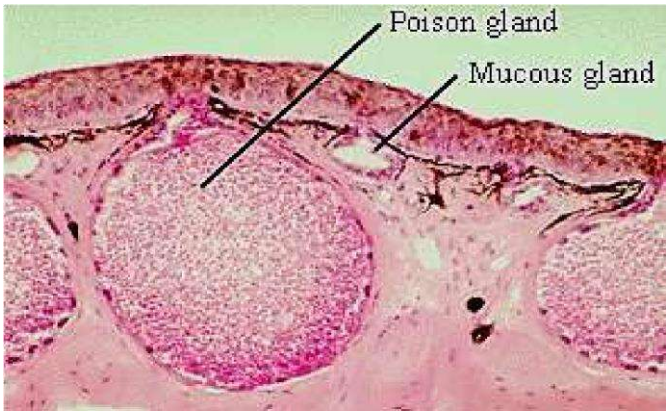
III. **simple branched tubular**: have a straight duct opening with branched clusters of secretory units. **Ex: pyloric gland of stomach**.



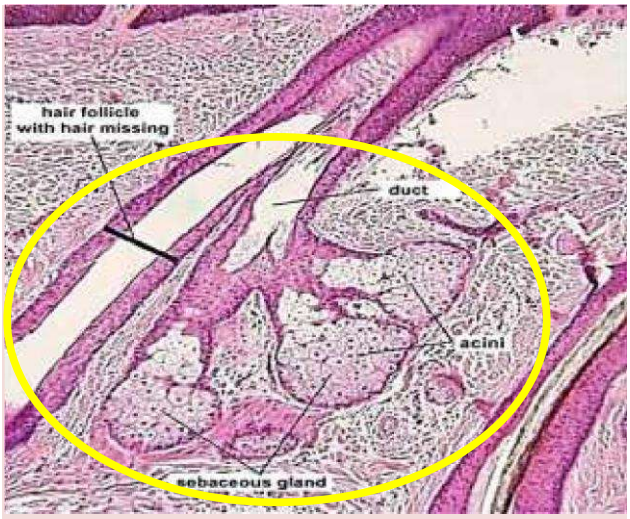
2- Alveolar (Acinar):

Here, the secretory cells form an expanded sac. It can be further subdivided into:

I. **Simple(unbranched)alveolar**: has a simple duct and a sac like base. **Ex: mucous gland in frog skin.**



II. **Simple branched alveolar**: has a simple duct and branched alveolar sacs (acini). **Ex: sebaceous gland in skin.**



B. Compound glands:

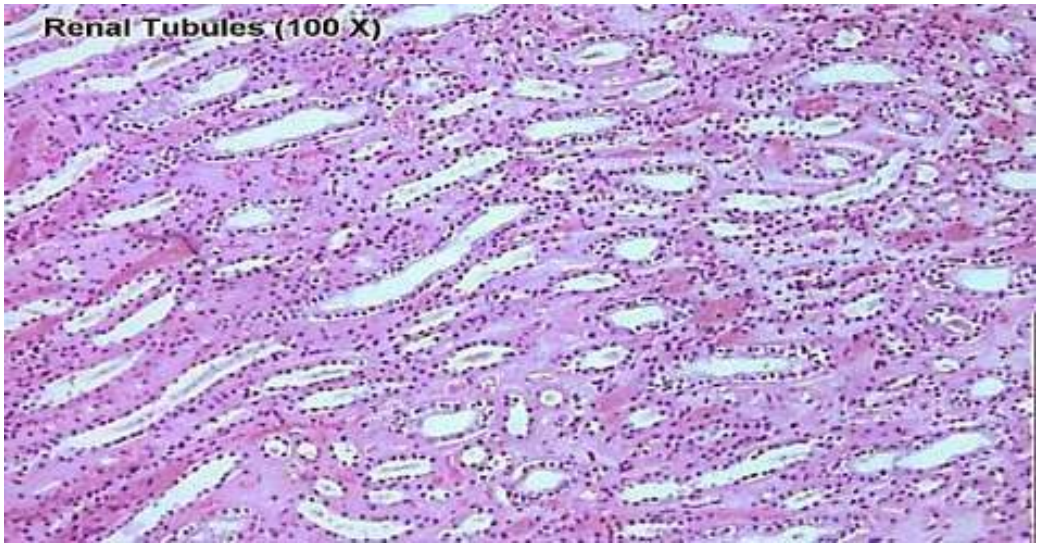
•Consist of a varying number of simple glands whose small excretory ducts join to form larger and larger ducts which carry the secretion onto an epithelial surface.

•They are also classified according to the **shape of their secretory portions**:

I. Compound tubular glands:

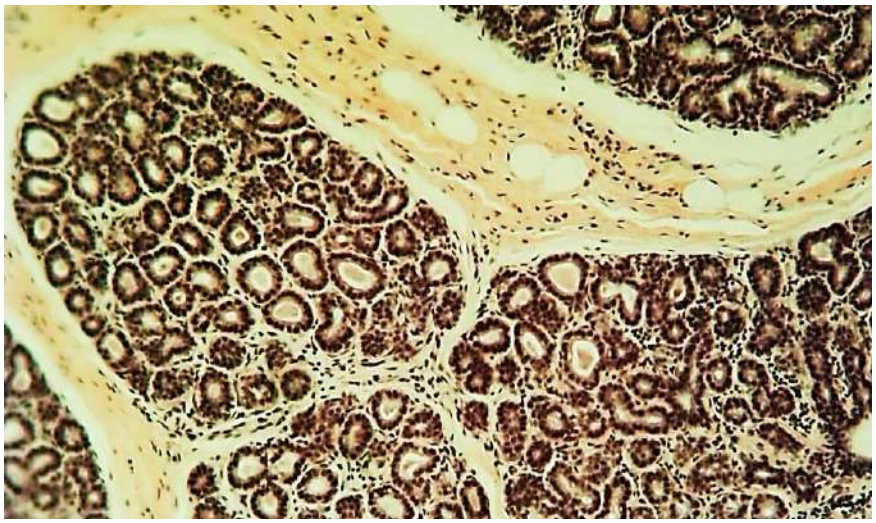
•Consist of a number of distinct duct systems that open into a main excretory duct.

•The secretory portions are in the form of long branching tubules which are coiled or convoluted. **Ex: kidney, testis.**



II.Compound Alveolar Glands:

These glands have a large number of duct systems. The terminal excretory ducts end in dilated sac-like alveoli. **Ex: mammary gland**



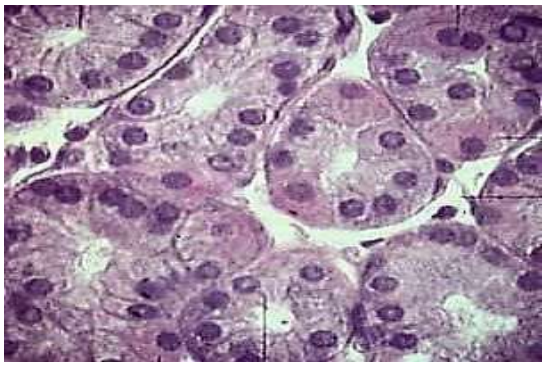
III.Compound Tubulo- alveolar Glands:

In which the secretory portions are in the form of irregularly branched tubules with numerous acini. **Ex: salivary glands**

Based on the type of secretory products, glands can be classified into:

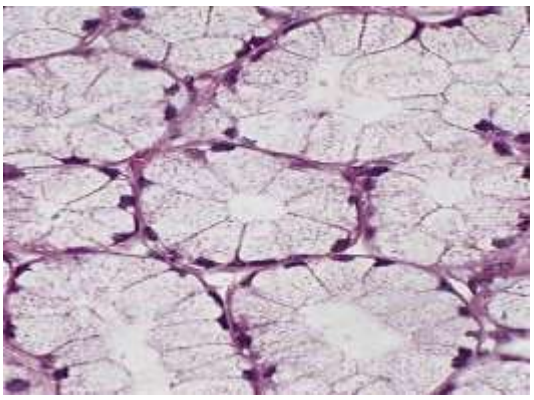
1- Serous gland:

- They are polyhedral or pyramidal in shape, with central rounded nuclei.
- Cytoplasm is alkaline, and the cavity of gland is small.
- **Pancreas and parotid salivary glands** are examples of serous glands.



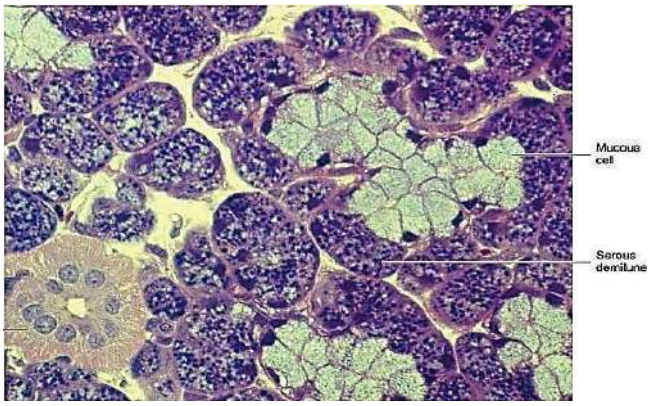
2-Mucous gland:

- Larger, and lightly staining.
- The nucleus is located in the base of the cell.
- The cavity is larger than serous gland. **Ex: palatine gland**



3- Mixed Gland(seromucous):

• This gland consists of mucous portion which has the same properties of mucous gland, and serous portion as cluster of cells located in one side of mucous gland and called sero-demilune. • **Ex: Submandibular gland, Sub maxillary gland**



Lab 4: Connective tissue

•The most diverse, abundant, widely distributed tissue.

•It is designed to support, protect, and bind organs.

•Connective tissue is formed by three components:

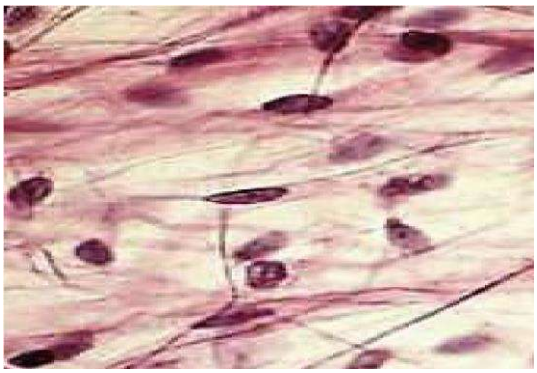
A. Cells

B. Fibers

C.Ground Substance: the major constituent of connective tissue. It is a transparent, homogenous substance, random in shape may be viscous, semisolid or solid.

A. Cells:

1.Fibroblast:



•The most abundant cell in connective tissue proper.

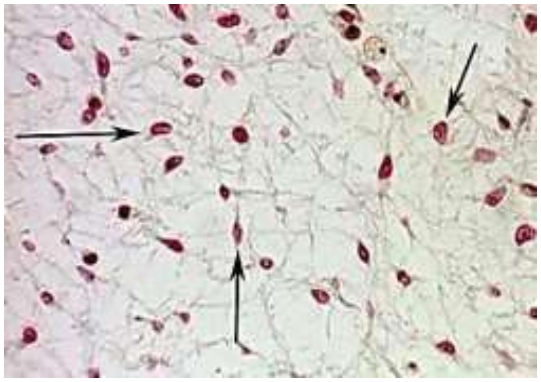
•Originates from undifferentiated mesenchymal cells.

•The active fibroblast has an abundant and irregularly branched cytoplasm.

•The nucleus is ovoid, large, and pale staining, with fine chromatin and a prominent nucleolus.

•They produce the fibers and ground substance components.

•It is found in Areolar connective tissue.



2.Mesenchymal cell:

•A type of embryonic stem cells contained within connective tissue.

•An elongated cell, similar to fibroblasts but smaller in size.

•Characterized by an oval nucleus with prominent nucleolus and fine chromatin.

•They possess many thin cytoplasmic processes.

•It can be seen in embryo sections.

3.Macrophage: •They are irregular in shape with processes which usually are short.

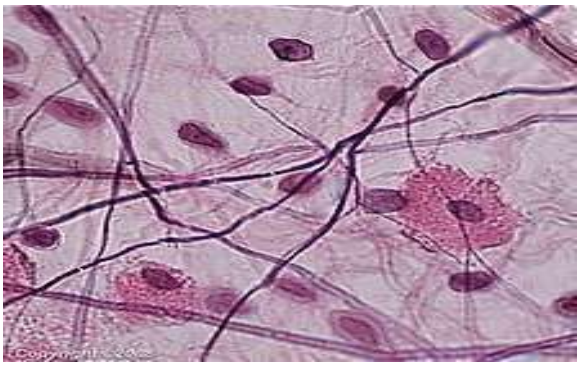
•The nucleus is ovoid, small and heterochromatic.

•Cytoplasm fills with granules and vacuoles.

•Macrophages are important agents of defense because of their phagocytic activity.

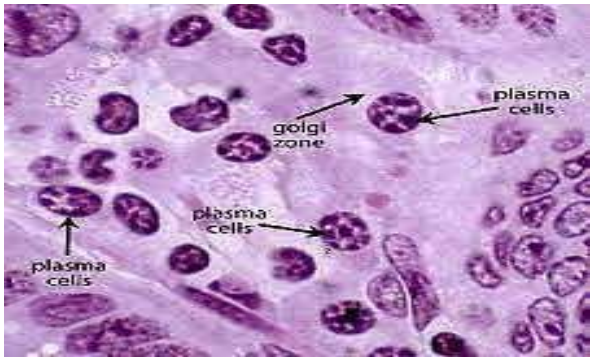
•We can observe it in section of the lung.

4.Mast cell:



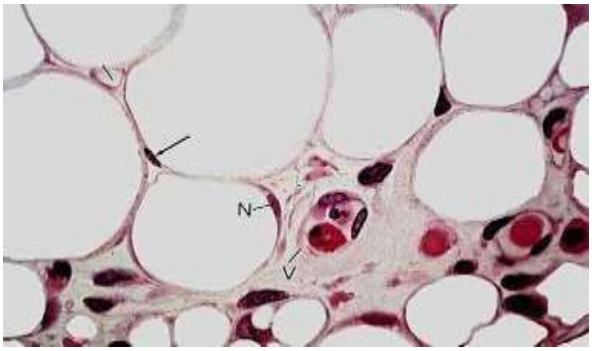
•An oval to round connective tissue cells whose cytoplasm is filled with basophilic secretory granules.
•Mast cells are identified easily by their contents of cytoplasmic granules.
•The nucleus is rather small, spherical, centrally situated; and is frequently covered by the cytoplasmic granules.
•Their functions are the formation of heparin (anticoagulant) and histamine for vasodilatation.
•We can see it in Areolar connective tissue.

5.Plasma cells: •Found mainly in lymphoid tissue.



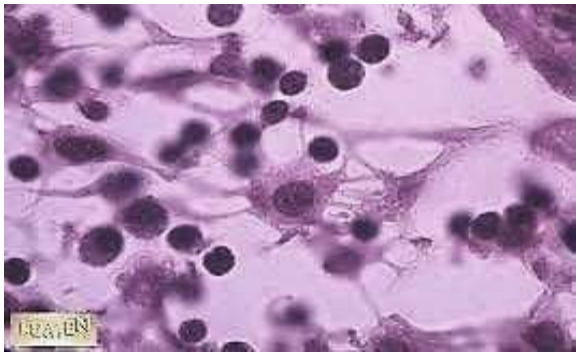
•They are large, ovoid cells that have a basophilic cytoplasm due to their richness in rough endoplasmic reticulum.
•The nucleus is spherical and eccentrically placed, with chromatin occurs in course clumps peripherally, and arrange in pattern like wheel or clock face. •Plasma cell is responsible for the production of antibodies.

6.Adipose cells: •Also called fat cells.



•Spherical to ovoid in shape.
•Contain a single large droplet of fat and thin rim of cytoplasm which contains in one area the flattened nucleus.
•They are specialized for storage of fat.

7.Reticular cells: •Stellate in shape, have long cytoplasmic extensions, which appear to join with other cells' extensions. •The cell has a pale, large nucleus, and basophilic cytoplasm.
•It is found in lymph node.

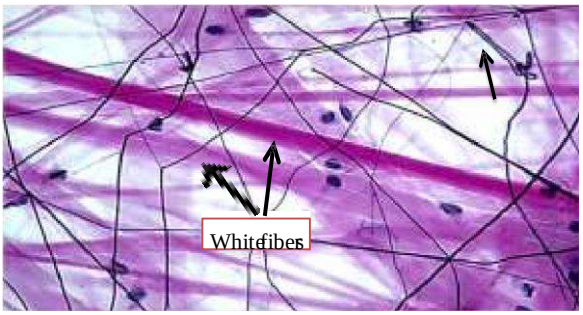


C. **Fibers:**

There are 3 types of protein fibers found in connective tissue: **collagenous, elastic, and reticular fibers.**

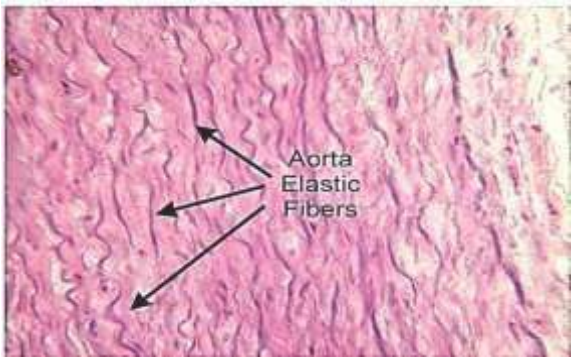
1.White (collagenous) fibers:

- Long, wavy, unbranched fibers composed of collagen.
- They are strong, flexible, inelastic, and gives the tissue strength. • In fresh tissue, collagen fibers appear white, and thus, they are often called white fibers.
- In tissue sections stained with hematoxylin and eosin, collagen fibers appear pink.
- We can see it in dermis of skin.



2. Yellow (elastic) fibers:

- They are made of the protein elastin and are thinner than collagen fibers.
- Long, thin, branched single threads, in fresh state they have a yellowish color.
- Yellow fibers are elastic and easily to stretching, branch then rejoin.
- We can notice them in cross section in aorta.

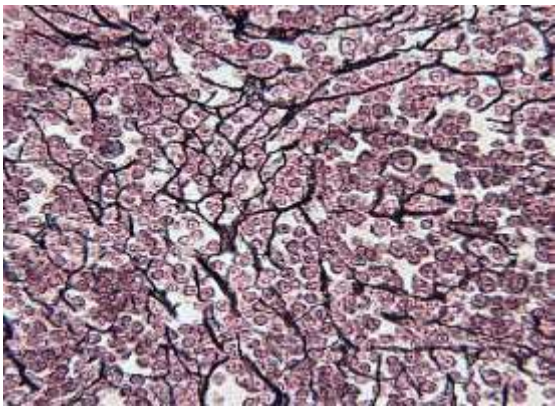


3. Reticular fibers: •They are extremely thin, and they form an extensive network in certain organs.

•They are not visible in H&E preparations, but can be easily stained black by impregnation with silver salts.

•These fibers form a branching interwoven framework that is tough but flexible.

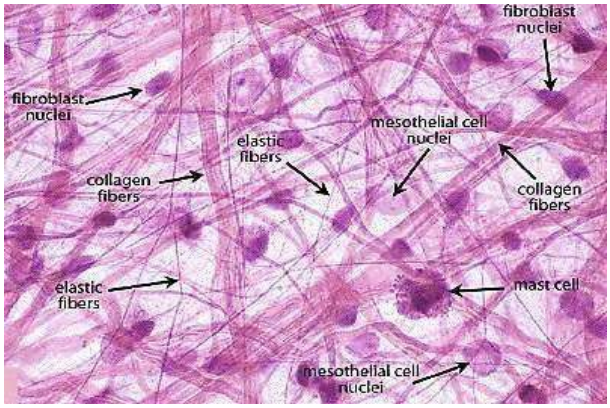
•Can be seen in lymph nodes, spleen & red bone marrow.



Loose connective tissue:

- Loose connective tissue comprises all the main components of connective tissue proper.
- It fills spaces between groups of muscle cells, supports epithelial tissue, and forms a layer that sheathes the lymphatic and blood vessels
- Characterized by loose arrangement of collagen, elastic, and reticular fibers.
- It can be subdivided as follows:

1. Areolar connective tissue:



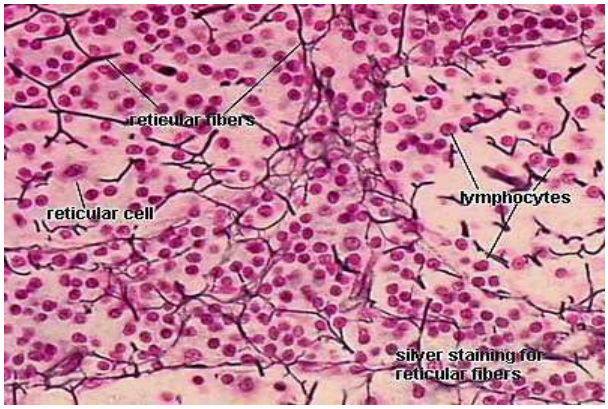
• Highly variable in appearance and the least specialized connective tissue in the body.
• Contains vacuoles (intercellular spaces) in the ground substance which remain unstained during tissue preparations. • The ground substance is a viscous, contains yellow & white fibers, with little number of reticular fibers.
• Contains all the cell types of connective tissue proper; although, the predominant cell is the fibroblast.
• It surrounds nerves, blood vessels, and individual muscle cells. It is also a major component of the subcutaneous layer deep to the skin.

2.Mucoid connective tissue:

•It is a jelly-like tissue, has an abundance of ground substance containing fibroblasts with few collagen fibers.

•It is found in umbilical cord.

3.Reticular connective tissue:

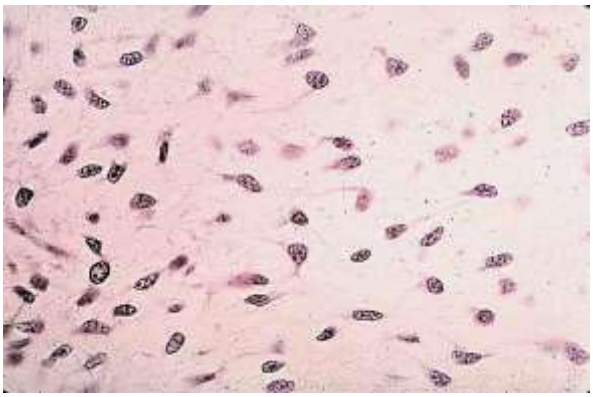


•Characterized by the presence of network of reticular fibers, associated with reticular cells.

•Also, it contains lymphocytes which have a dark nucleus that occupied most of the cell volume.

•This connective tissue forms the stroma of many lymphatic organs such as the spleen, thymus, lymph nodes, and bone marrow.

4.Mesenchymal connective tissue:



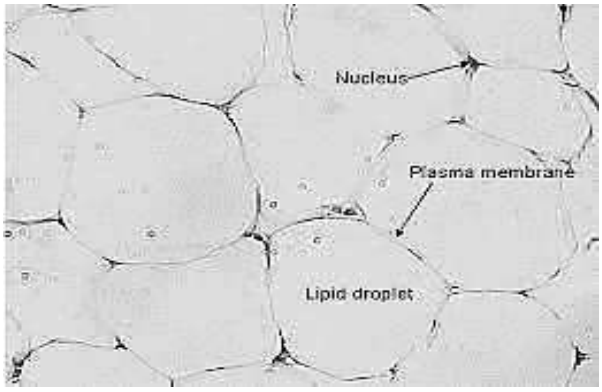
•An embryonic tissue formed by elongated cells, the mesenchymal cells immersed in an abundant and viscous extracellular substance containing few fine sparse fibers.

•This tissue gives rise to all other connective tissue types.

•It is found in embryo sections.

5. Adipose connective tissue:

•A large aggregation of adipose cells (adipocytes).
•Each fat cell is surrounded by a web of different fibers and fibroblasts.
•Adipose connective tissue is commonly found throughout the body in such diverse locations as a fat capsule surrounding kidney, pericardial and abdomino-pelvic cavities and subcutaneous layer.



LAB 5: Dense Connective Tissue

• It is adapted to offer resistance and protection.

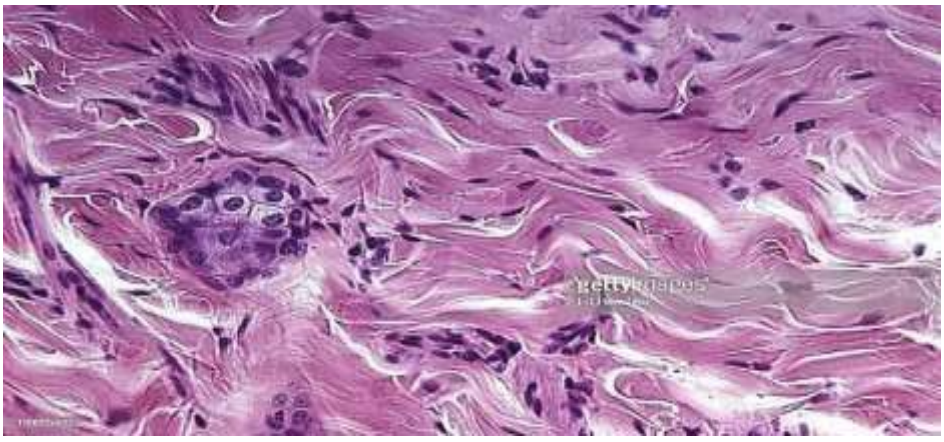
•Less flexible and far more resistant to stress than is loose connective tissue.

•It consists of the same components found in loose connective tissue, but there are fewer cells, less ground substance, and a clear predominance of collagen fibers.

•There are categories of dense connective tissues, dense irregular, dense regular, and elastic connective tissue.

I. Dense irregular connective tissue •In this tissue the collagen fibers are arranged in bundles extended in all directions without a definite orientation comprising a three-dimensional network and provide resistance to stress from all directions.

•It can be seen in the dermis of skin.



II.Dense regular connective tissue

- Also called white fibrous connective tissue or collagenous tissue, because collagen fibers are the dominant type.
- Consists of collagen fibers aligned with the linear orientation of fibroblasts in response to prolonged stresses exerted in the same direction; consequently, they offer great resistance to traction forces.
- Tendons are the most common example of dense regular connective tissue: Function of tendons:

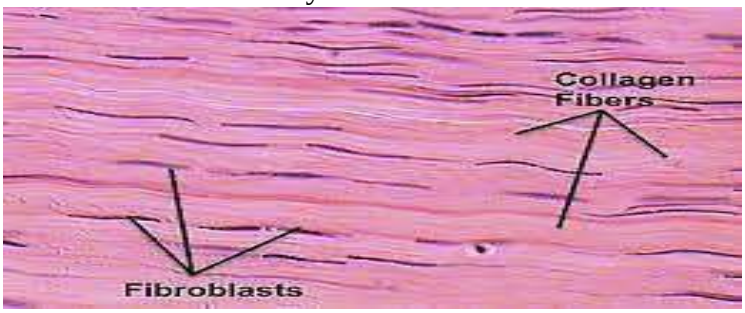
These elongated cylindrical structures attach striated muscle to bone. Characteristics of tendons:

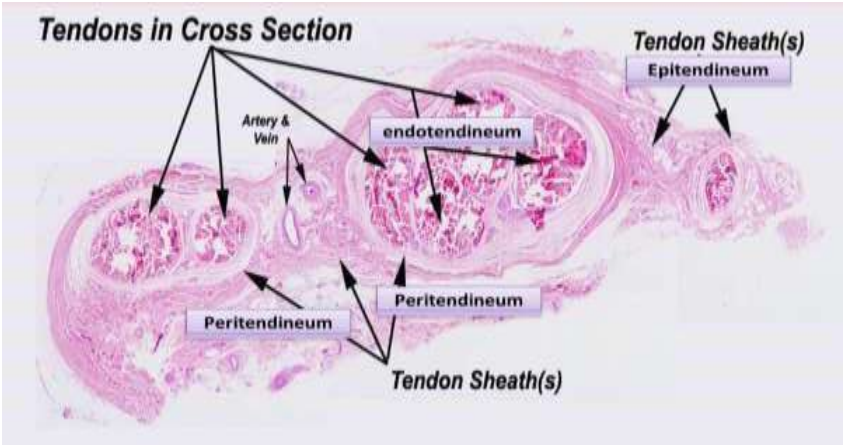
- They have parallel, closely packed bundles of collagen separated by a small quantity of intercellular ground substance.
- Their fibrocytes (called tendon cells) contain elongated nuclei parallel to the fibers. **C.S. of tendon:**

•The entire tendon is surrounded by a sheath of dense connective tissue called **Epitendineum**.

•The collagen bundles of the tendon (primary bundles) aggregate into larger bundles (secondary bundles) that are enveloped by loose connective tissue containing blood vessels and nerves called **Peritendineum**.

•Each primary bundle is surrounded by **Endotendineum**.



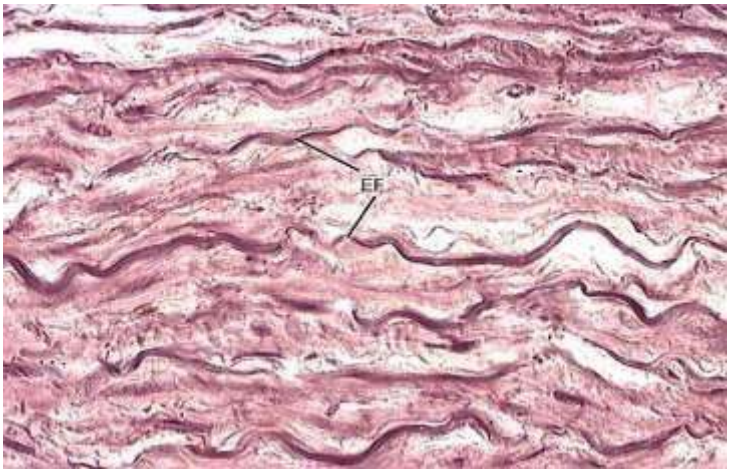


III. Dense elastic connective tissue

•Elastic tissue is composed of bundles of thick, parallel elastic fibers.

•The space between these fibers is occupied by thin collagen fibers and flattened fibroblasts.

•The abundance of elastic fibers in this tissue is the cause of its typical yellow color and great elasticity. •Elastic tissue is present in the yellow ligaments of the vertebral column and vocal cords.



Supporting connective tissues:

•Also called skeletal connective tissue

•Cartilage and bone are the types of supporting connective tissue, because they form a strong, durable framework that protects and supports the soft body tissues.

A. Cartilage: -

•Consists of cells called chondrocytes and an extensive extracellular matrix composed of fibers and gel-like ground substance contains chondroitin sulfates.

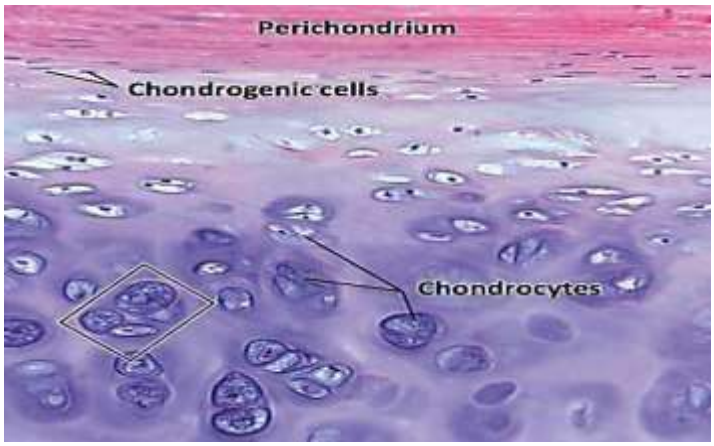
•Chondrocytes synthesize and secrete the extracellular matrix, and are located in matrix cavities called lacunae.

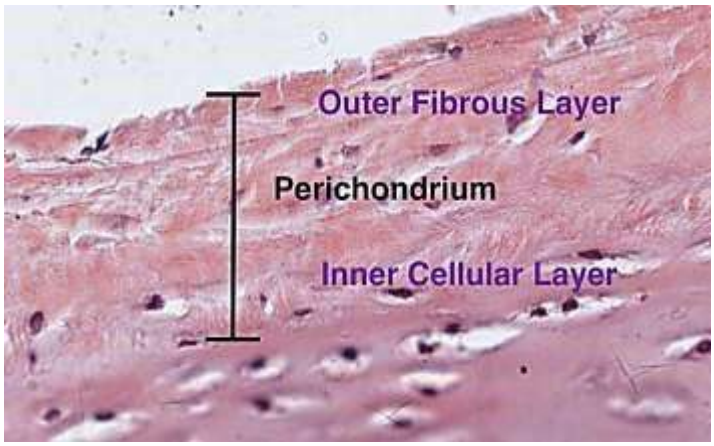
•There are three types of cartilage:

1. **Hyaline cartilage**
2. **Elastic cartilage**
3. **White fibro cartilage**

Hyaline cartilage: -

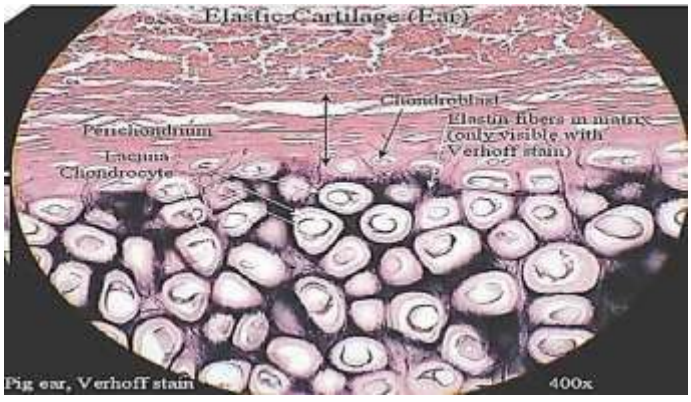
 It is found in the cartilage of nose, larynx, trachea & bronchi, and in ventral ends of ribs.
 The chondrocyte appears single or aggregates as groups called cell nest, it is surrounded with capsule and found within lacuna in the ground substance.
 Ground substance appears as hyaline (glassy) and contains little amounts of white fibers; therefore, it is called hyaline cartilage.
Hyaline cartilage is surrounded by perichondrium; a sheath of dense connective tissue that surrounds cartilage, consisting of two layers: fibrous layer & chondrogenic layer. Its function is to protect and repair the cartilage.



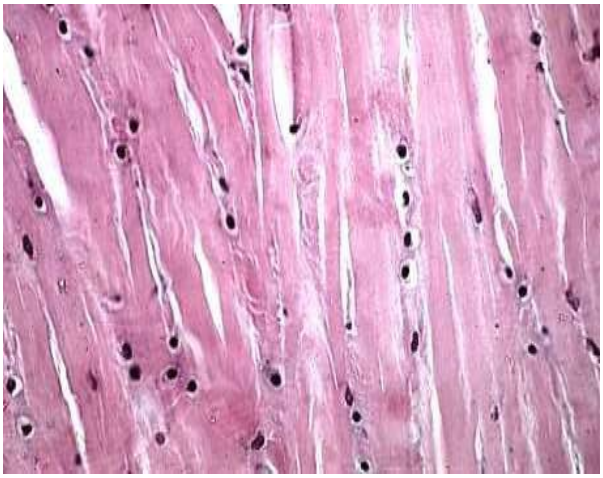


Elastic cartilage: -

It is found in auricle of the ear and Eustachian tube.
It is identical to hyaline cartilage except it contains bundles of elastic fibers in the ground substance.
It is surrounded with perichondrium.



Fibrocartilage: -



- It is found in intervertebral discs.
- Ground substance contains bundles of white fibers in parallel arrangement.
- It is never present alone, but associated with hyaline cartilage or dense fibrous con. tissue, because it lacks perichondrium.

B. Bone: -

A specialized connective tissue composed of intercellular calcified material called bone matrix, and three types of cells:

1.Osteocytes; which is found in lacunae within the bone matrix, situated between lamellae of matrix. These cells are actively involved in the maintenance of the bone matrix.

2.Osteoclasts; which is multinucleated giant cells lies within a depression or pit on the bone surface called Hawship's lacunae, and involved in the resorption and remodeling of bone tissue.

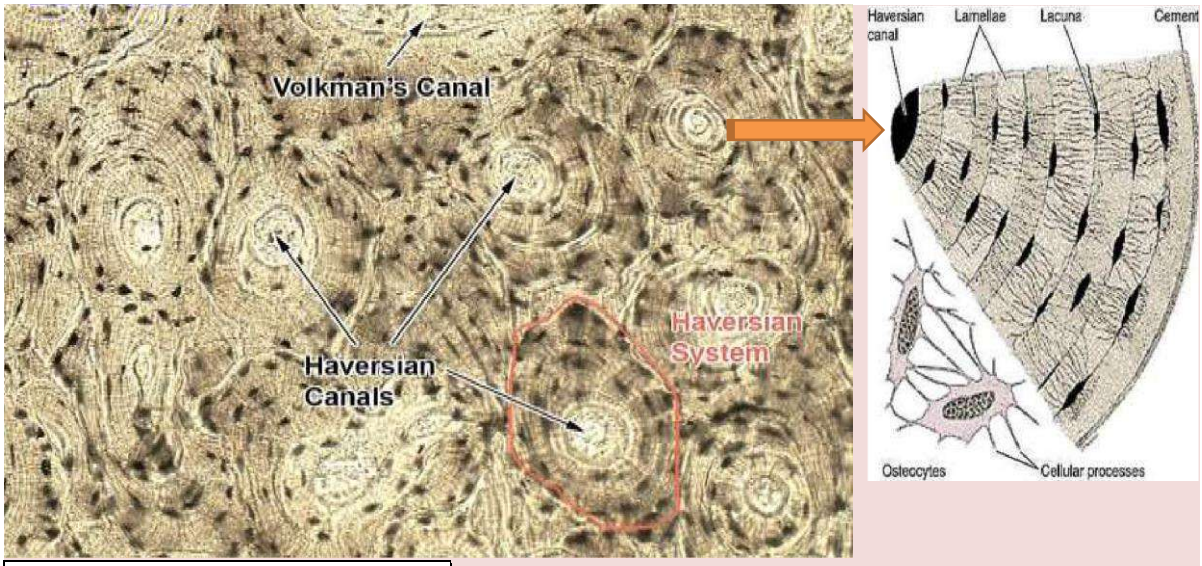
3.Osteoblasts; located at the surfaces of bone tissue, side by side, in a way that resembles simple epithelium. They synthesize the organic components of the matrix.

Types of Bone:

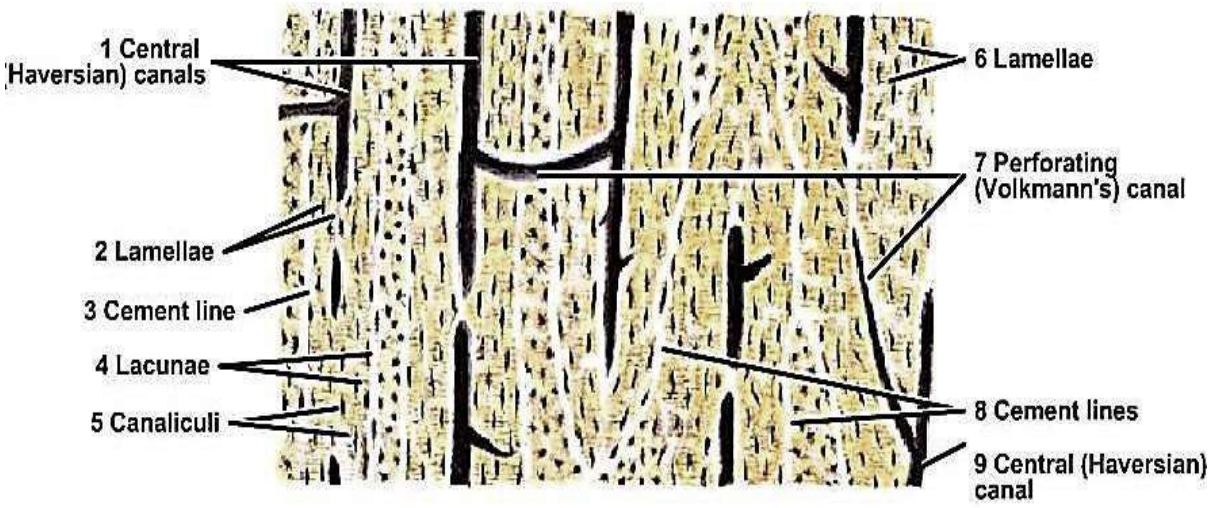
1. Compact bone:

- It usually forms the hard outer shell of the bone.
- It is shown in long bone diaphysis.
- In cross section of compact bone, bone lamellae are regularly arranged around Haversian's canal, and determined by blood vessels and nerves. This complex system is called Haversian's system.
- Haversian's canal connects with others by

Volkmann's canal, and between Haversian systems there are interstitial lamellae.



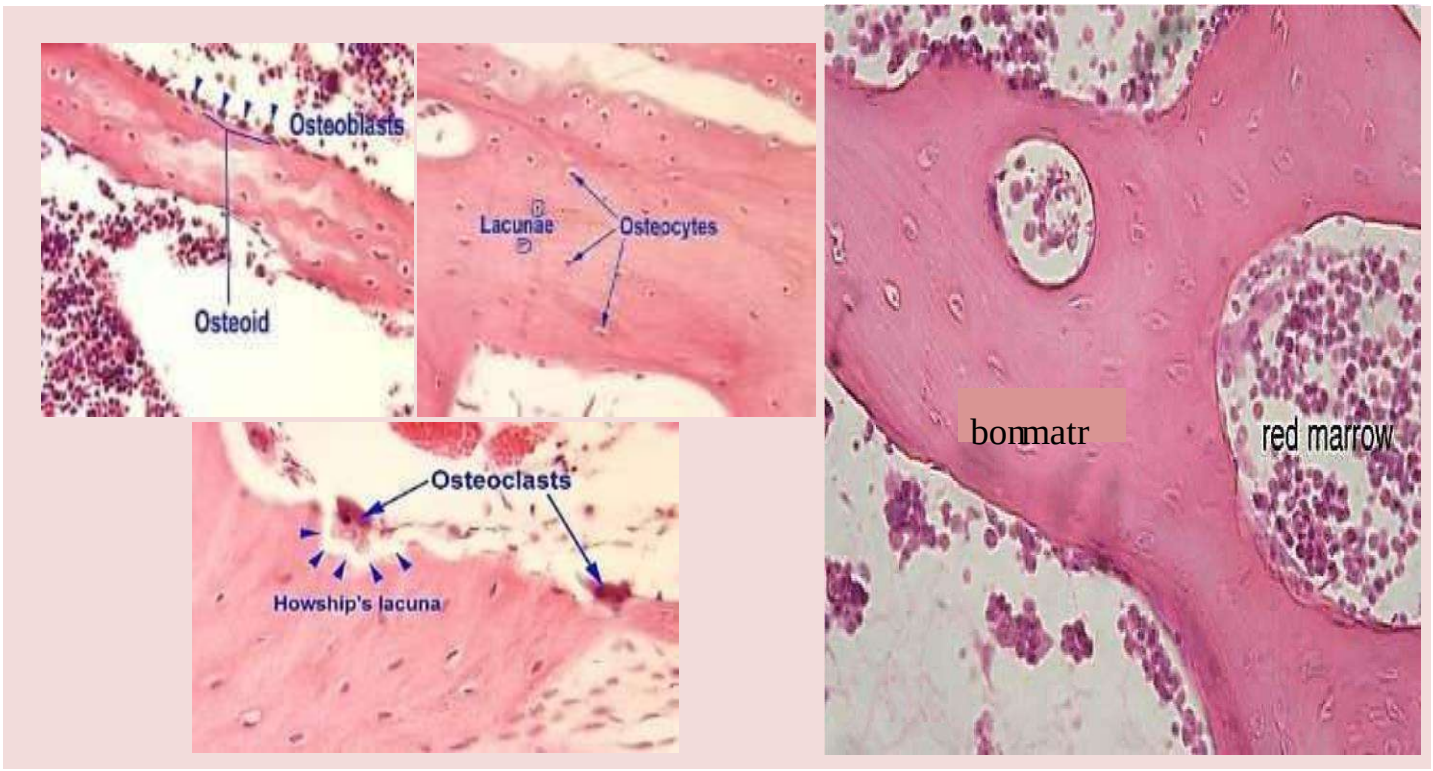
c.s compound bone



Compact bone (L.S.)

2. Spongy bone:

- It's located internally, primarily within the bulbous ends of long bones which called epiphysis.
- Appears porous, like a sponge, forms an open lattice of narrow plates of bone, called trabeculae (bone matrix) contain three kinds of cells: osteocyte, osteoblast, and osteoclast.
- There are cavities between these trabeculae contain red bone marrow.



Stages of bone development by endochondral ossification:

- This type of ossification is responsible for formation of short and long bones.
- Epiphyseal cartilage is divided into five zones starting from the epiphyseal side of cartilage:

(1) **The resting zone** consists of hyaline cartilage without morphological changes in the cells.

(2) **The proliferative zone**, in which chondrocytes divide rapidly and form columns of stacked cells parallel to the long axis of the bone.

(3) **The hypertrophic cartilage** zone contains large chondrocytes whose cytoplasm has accumulated glycogen. The resorbed matrix is reduced to thin septa between the chondrocytes.

(4) **Calcified cartilage zone**, Simultaneous with the death of chondrocytes in this zone, the thin septa of cartilage matrix become calcified by the deposit of hydroxyapatite.

(5) **Ossification zone** in which:

- Endochondral bone tissue appears.
- Blood capillaries and osteoprogenitor cells formed by mitosis of cells originating from the periosteum invade the cavities left by the chondrocytes.
- The osteoprogenitor cells form osteoblasts, which are distributed in a discontinuous layer over the septa of calcified cartilage matrix.
- Ultimately, the osteoblasts deposit bone matrix over the calcified cartilage matrix.

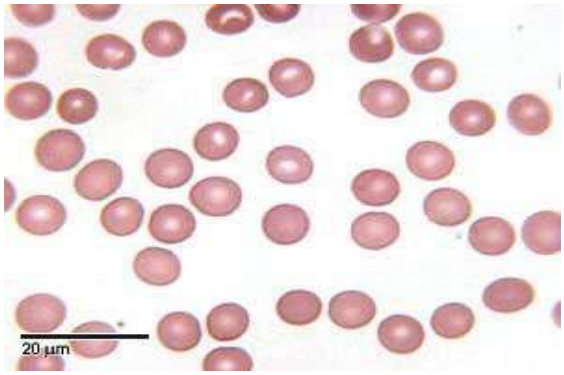
Stages Of Bone Development by endochondral ossification

LAB 6: Fluid connective tissue

- **Blood**: is a specializing connective tissue.
- It consists of formed elements which include **erythrocytes, leukocytes and platelets**, and an intercellular substance called plasma.
- The plasma is a liquid in which the formed elements are suspended.
- The fibers appear as **fibrin** when blood is clotted.

Blood components:

I. **Red blood Corpuscles (Erythrocytes):** are biconcave disks without nuclei, when we scan a blood smear, we can observe numerous amounts of R.B. C's.



II. **White blood cells (leukocytes):**

•Spherical nucleated cells

•According to the type of granules of their cytoplasm and the shape of their nuclei, leukocytes are divided into:

1)Agranulocytes:

•Have cytoplasm that appears homogenous.

•They do not have specific granules, but they do contain azurophilic granules (lysosomes) that bind the azure dyes of the stain.

•The nuclei are round or indented.

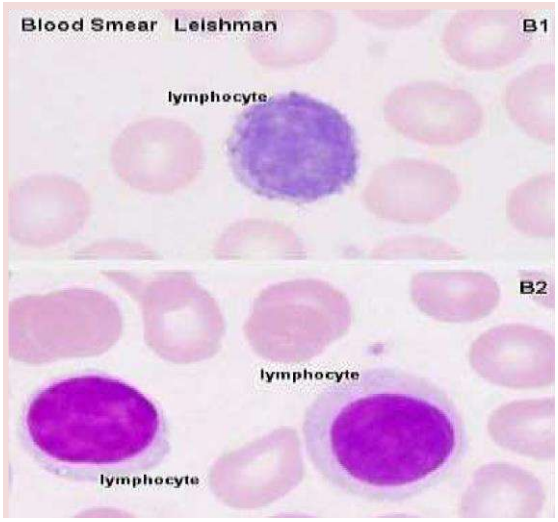
•This group includes lymphocytes and monocytes.

A. Lymphocytes:

•Spherical cells, most are little larger than erythrocytes.

•It has a large spherical nucleus surrounded by narrow rim of cytoplasm.

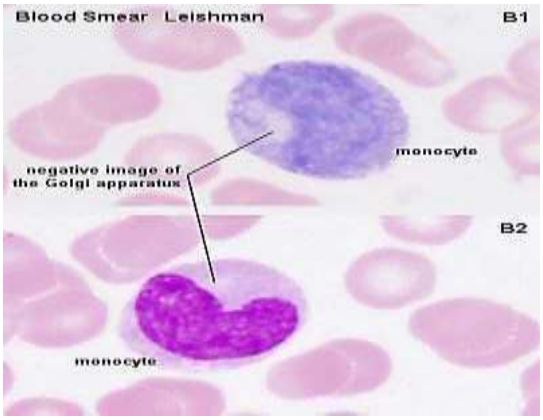
•The cytoplasm is basophilic.



B. Monocytes:

•Large cells in which the nucleus is oval, horseshoe, or kidney shaped, and is generally eccentrically placed.

•The cytoplasm is basophilic and grayish-blue in color.



2) Granulocytes:

•Possess two types of granules:

a. The specific granules that bind neutral, basic, or acidic components of the dye mixture and have specific functions.

b. The azurophilic granules which stain purple and are lysosomes.

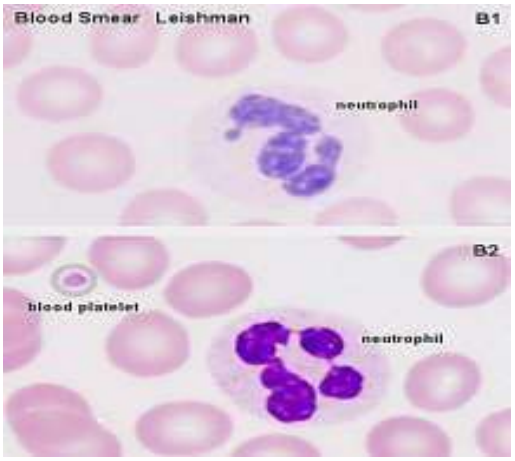
•Granulocytes have nuclei with two or more lobes, and include the **neutrophils, eosinophils, and basophils.**

1. Neutrophils:

•Polymorphonuclear leukocytes.

•Nucleus has from -5 irregular ovoid lobes connected by fine threads of chromatin.

•The cytoplasm filled with fine neutral granules.

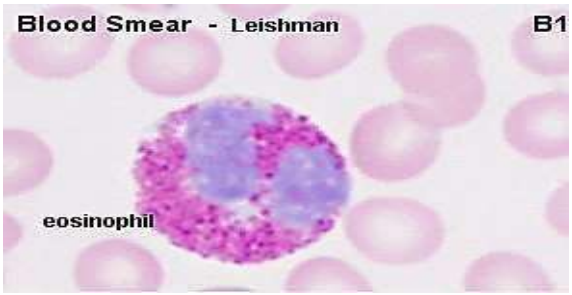


2. Eosinophils or (acidophils):

•Larger than neutrophils

•The nucleus is usually bilobed.

•The cytoplasm is filled with course granules and stain with acidic dyes.

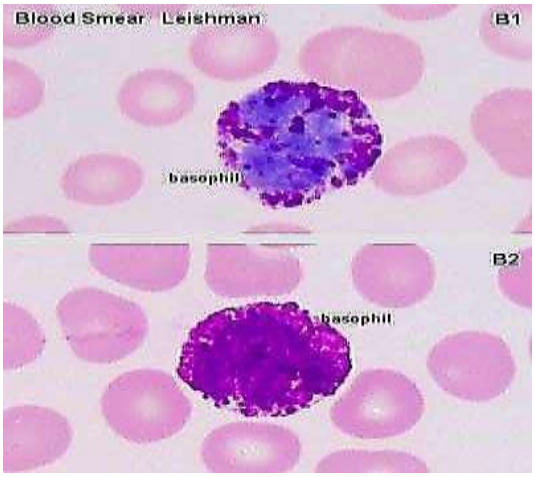


3. Basophiles:

•The same size as neutrophil.

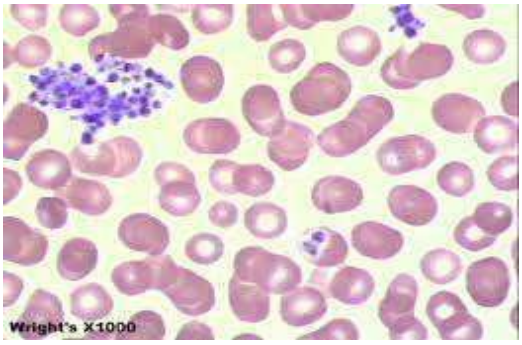
•Nucleus usually has irregular two lobes appearing as (S) shape.

•The cytoplasmic granules are course and variable in size, and stain with basic dyes.



III. Blood Platelets:

•Non-nucleated, disk- like cellular fragments.
•They are rounded or ovoid in shape.
•In stained blood smears, platelets often appear in clumps.
•Each platelet has a peripheral light blue- stained transparent zone called the hyalomere, and a central zone containing purple granules, called the granulomere.



Hematopoietic tissue

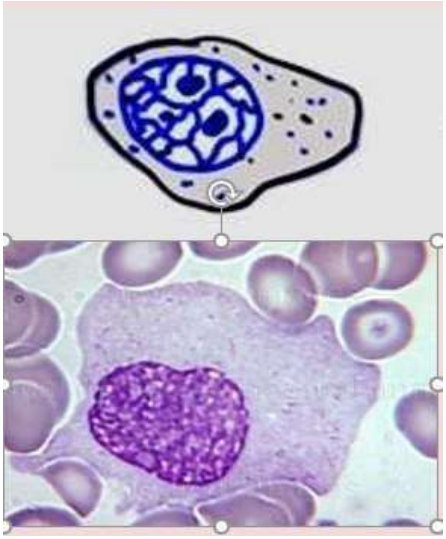
•Hematopoiesis is the process of blood cell formation, beginning with a pluripotential stem cell that subsequently goes through a series of cell division and differentiation to produce all the mature blood cells.

•Postnatal hematopoiesis occurs in red bone marrow located in the spongy bone region of long bones, vertebra, ribs, sternum, and skull.

Erythropoiesis – Red Blood Cell Development

Hemocytoblast :

•An immature blood stem cell considered as a pluripotent cell, gives rise to all formed elements. •It is an amoeboid large cell has a basophilic cytoplasm and a relatively large nucleus with a loose network of chromatin and several nucleoli.

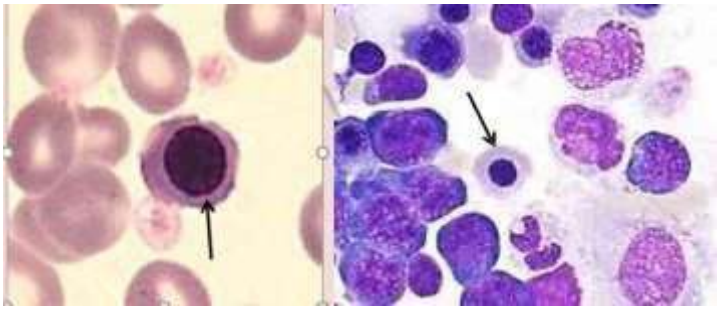


Normoblast:

•A small cell contains nucleus that is very dark in appearance.

•The cytoplasm is acidophilic.

•When it loses the nucleus, it converts to R.B.C.

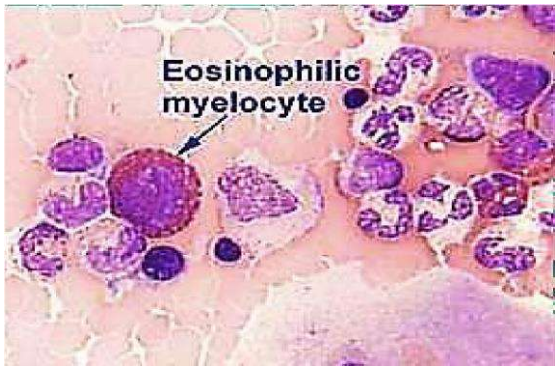


Acidophilic (or Eosinophilic) myelocyte:

•Has a rounded or oval nucleus.

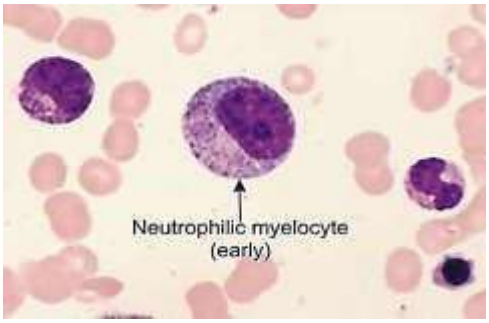
•Course granules of cytoplasm.

•It converts to acidophil.



Neutrophilic myelocyte:

•It has a small oval nucleus •The cytoplasm has fine granules and stains with neutral stains.



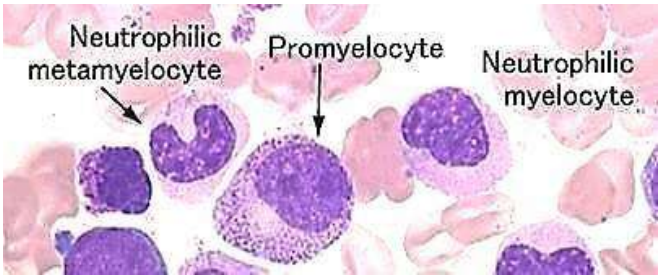
Neutrophilic metamyelocyte (juvenile neutrophil):

•It is smaller than myelocyte

•The nucleus is kidney shaped.

•The cytoplasm filled with fine granules.

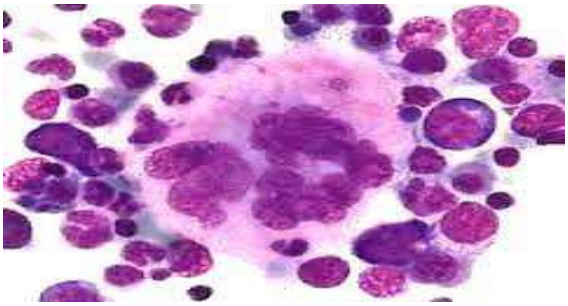
•It converts to neutrophil.



Megakaryocyte:

•A giant cell with an irregularly lobulated nucleus, course chromatin and no visible nucleoli.

•Its function is the formation of platelets.



Lab 7: Muscular tissue

•Muscle tissue is composed of differentiated cells which is called muscle fibers, responsible for producing movement of body.

•Some muscle cell organelles have names that differ from their counterparts in other cells:

a. The cytoplasm of muscle cell is called sarcoplasm.

b. The smooth endoplasmic reticulum is called sarcoplasmic reticulum.

c.The sarcolemma is the cell membrane, or plasmalemma. **Classification of Muscles:**

1- **Functional classification is based on the type of neural control:**

•Voluntary

•Involuntary

2- **Structural classification is based on the presence or absence of cross striations:**

•Striated

•Non striated smooth

3- **Combined functional and structural classification: Muscular tissue can be classified according to the function and structure to:**

- Skeletal muscle
- Cardiac muscle
- Smooth(visceral) muscle **Skeletal muscle:**

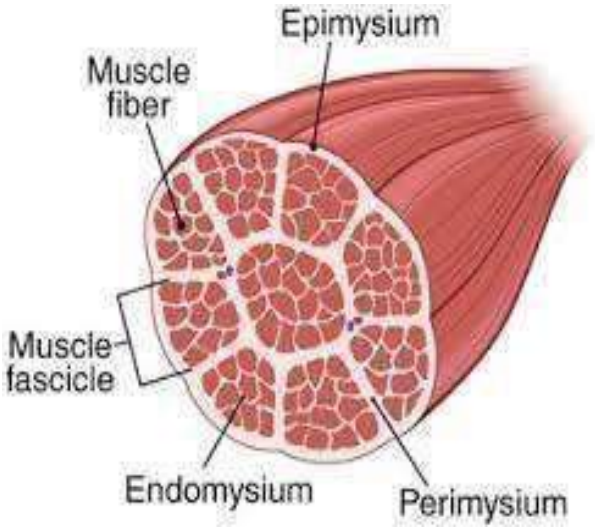
•It is a striated voluntary muscle attached to skeletal backbone.

•Composed of bundles of long, cylindrical, multinucleated cells(fibers) that show cross-striations.

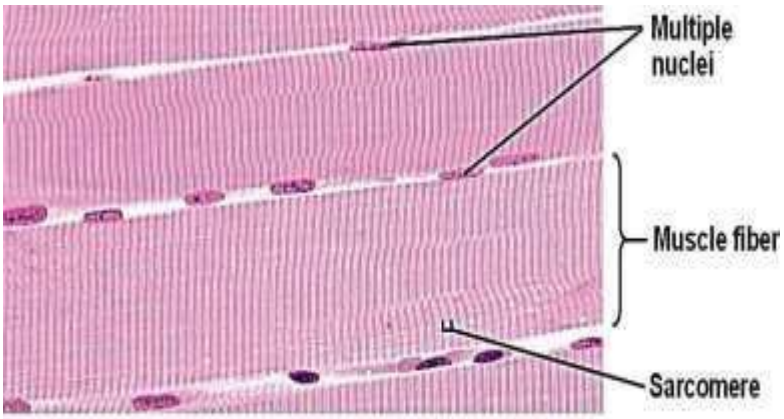
•Arranged in regular bundles surrounded by the epimysium.

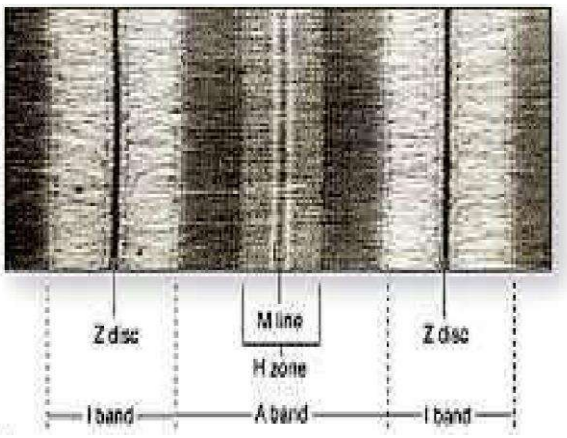
•From the epimysium, thin septa of connective tissue extend inward, surrounding the bundles of fibers within a muscle called the perimysium.

•Each muscle fiber is surrounded by a delicate layer of connective tissue, called the endomysium.



In L.S, the skeletal muscle appears as cylindrical, parallel bundles with multi-peripheral nuclei.

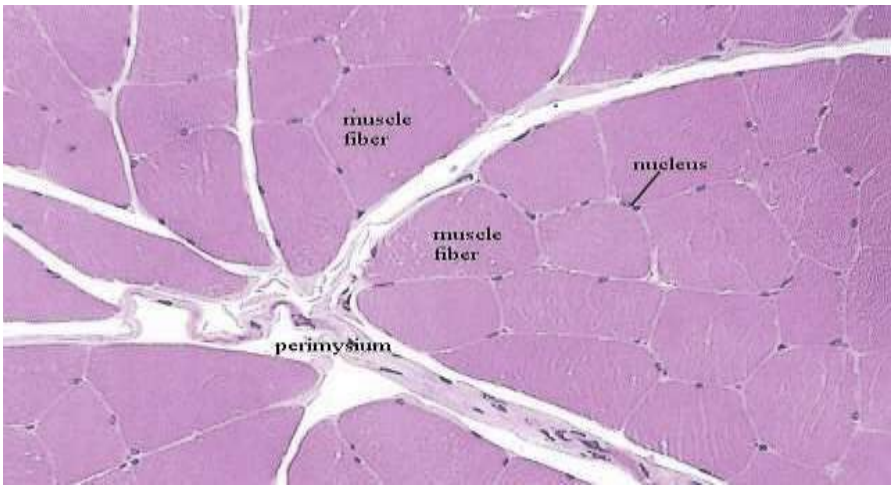




In C.S. of skeletal muscle:

•The fibers' sections appear polygonal to round in shape with different diameters.

•The multi-nuclei can be seen in the periphery of the fiber, just under the plasma membrane.



Cardiac muscle:

•It is striated, involuntary muscle.

•Found only in muscular layer of heart and the roots of large vessels joining the heart.

•Cardiac muscle cells (or fibers) have a branched shape.

•Unlike multinucleated skeletal muscle, each cardiac muscle cell possesses only one centrally located pale-staining nucleus.

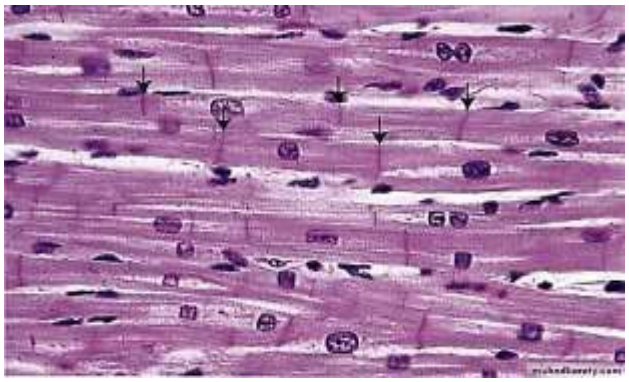
•A unique and distinguishing characteristic of cardiac muscle is the presence of dark staining transverse lines that cross the chains of cardiac cells at irregular intervals called intercalated disks.

In L.S cardiac muscle:

•The myofibers appear branched, and striated like skeletal muscle.

•The cardiac myofibers have central, single nucleus.

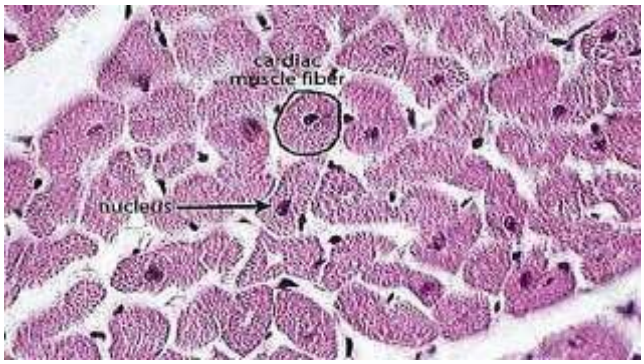
•The intercalated disks can be seen in this section.



In C.S cardiac muscle:

• Myofibers are irregular in shape and smaller than the sections of skeletal muscles fibers. • Myofibrils are rougher than myofibrils of skeletal muscle

• Central and single nucleus is seen in fiber.

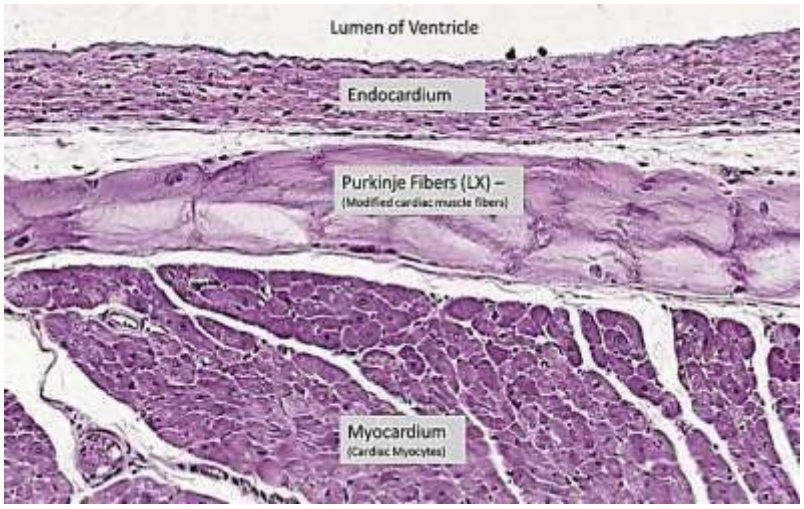


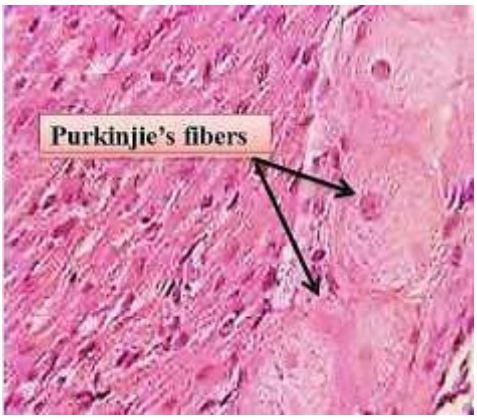
Purkinjie's fibers:

They are specialized cardiac muscles located just near the endocardium on the internal surface of the heart.

In L.S.: when comparing the Purkinjie's fibers with cardiac muscle fibers, they appear larger, shorter, wide thick and pale staining (lighter) than cardiac muscles, with central nucleus or bi-nucleated. Few myofibrils are usually found in peripheral position.

• In C.S, the Purkinjie's fibers appear as cell groups, of 3 -4) cells.





Smooth muscle:

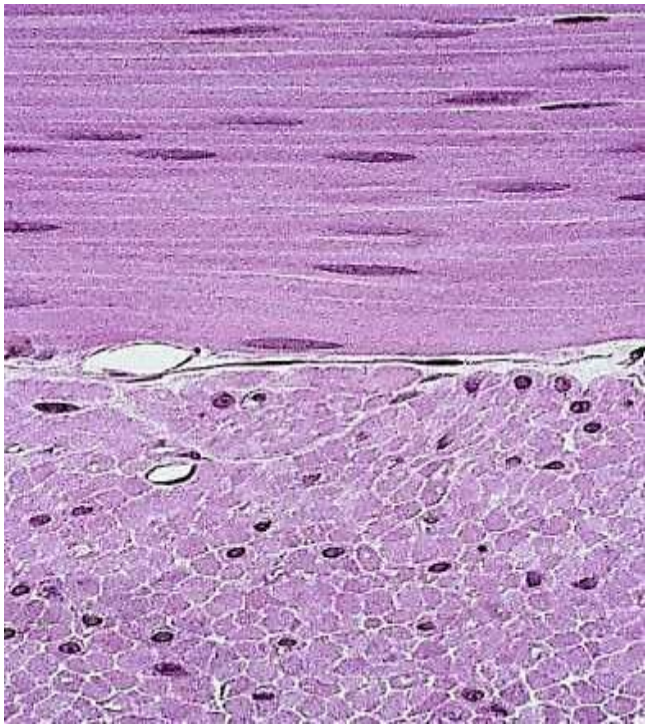
•It is non-striated, involuntary muscle.

•It has a visceral distribution; found in the wall of digestive tract from midesophagus to anus, urinary and genital system.

•Smooth muscle is composed of elongated, mononucleated, and non-striated cells.

L.S of smooth muscle:

•Fibers appear spindle shaped, without striation and with flattened central mononucleosis.



C.S of smooth muscle:

•The smooth muscle fibers appear different in size, it may appear wide and narrow, and may be with or without nucleus according to section's plane.

Lab 8: Nervous tissue

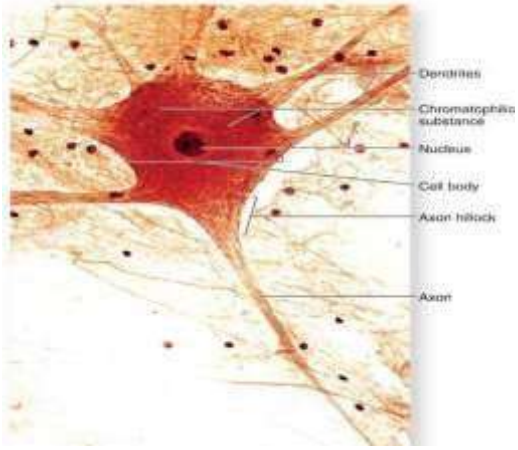
- It is responsible for transport of nerve impulses (motor and sensory impulses).
- It is formed by a network of more than 100 million of nerve cells assisted by many more glial cells, nerve fibers and nerve endings.

A. Nerve cells (neurons): - are responsible for reception, transmission and processing of stimuli. They are consisting of: -

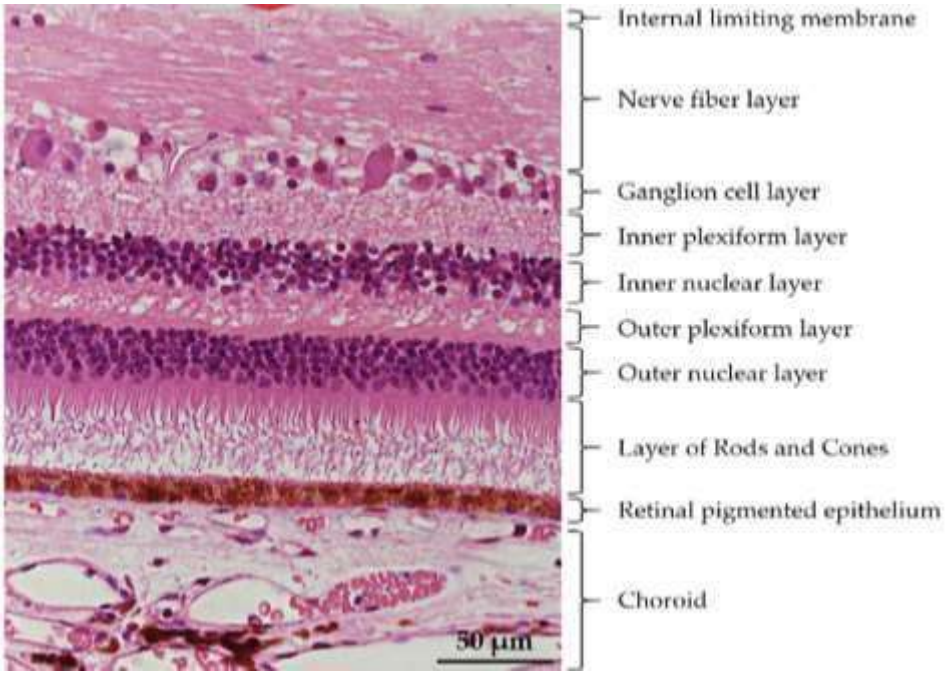
1.Dendrites: which are multiple elongated processes specialized for receiving stimuli from environment.
2.Cell body or (Perikaryon): contains nucleus, cytoplasm and Nissl's bodies which are the site of protein synthesis.
3.Axon: a single process specialized in generating or conducting nerve impulse to other cells (neuron, muscle, gland)

Based on the number of their processes, most neurons can be placed in one of the following categories:

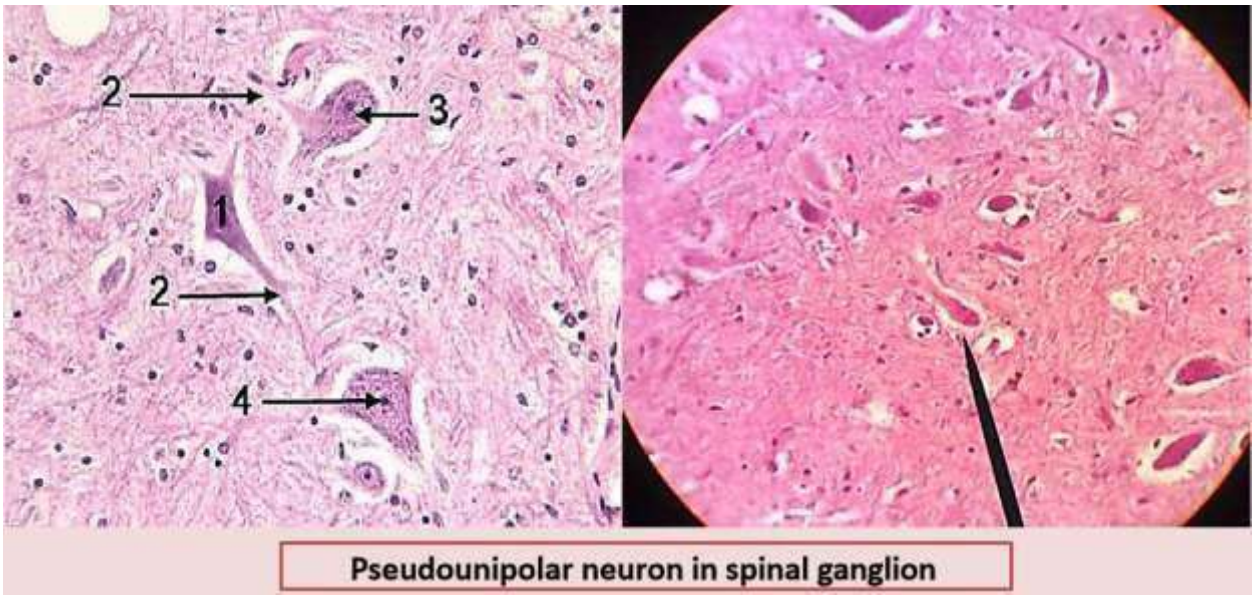
1.Multipolar neuron: which has more than 2 processes; one process being the axon, and the others are dendrites. Most neurons of the body are multipolar.



2.Bipolar neuron: with one dendrite and one axon, found in retina of eye and olfactory mucosa.



3.Pseudounipolar neuron: has a single process that is rapidly divided into 2 branches. Found in spinal ganglia and cranial ganglia.



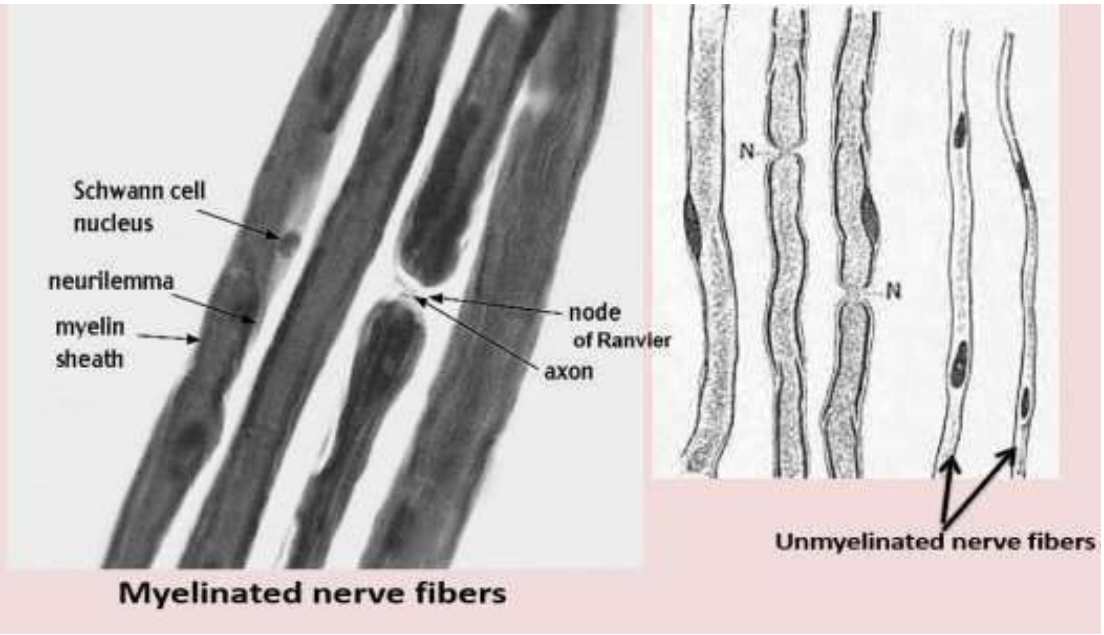
B. Fibers: - consist of axons enveloped by a special sheath of Schwann cell, and classified into: -

1.Myelinated fibers:

- The fibers which enveloped with multilayer of Schwann's plasmalemma that fuse to form myelin sheath
- The space between 2 Schwann cells is called the node of Ranvier.
- These fibers found mainly in peripheral nervous system (PNS).

2. Unmyelinated fibers:

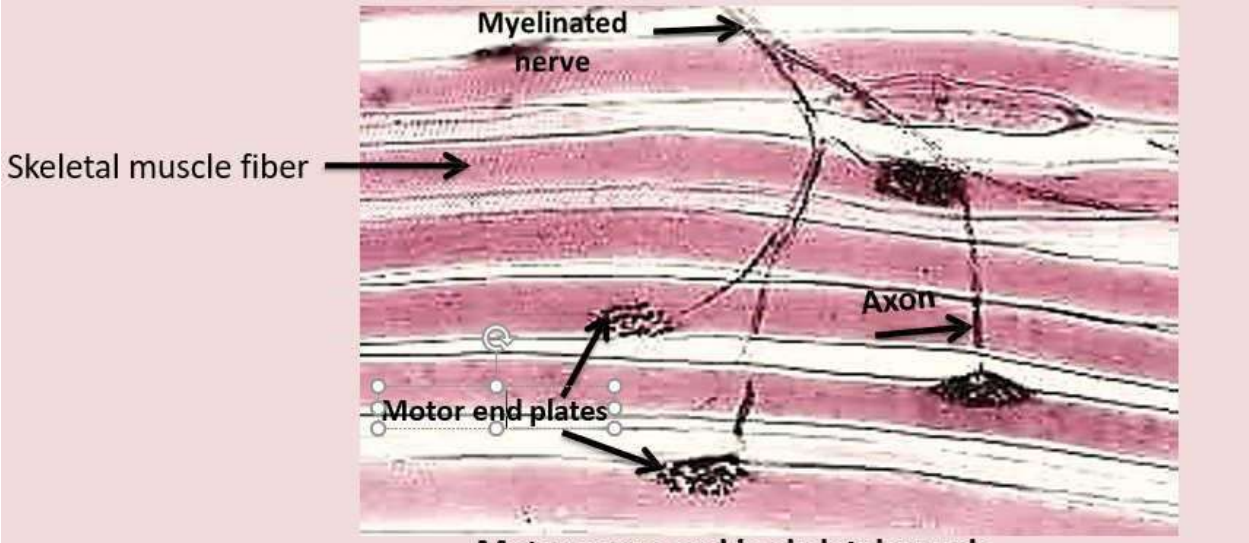
- The axons are enveloped with simple cleft of Schwann's cells
- They found in central nervous system (CNS).



Nerve endings:

•The nerves end either in muscle or connective tissue or epithelial tissue. Therefore, they are of two types; motor nerve ending and sensory nerve ending.

1.Motor nerve ending: in which nerve fiber ends in striated muscles, becomes unmyelinated, branched and ends with dents. Ex: motor end plate in skeletal muscle.



Motor nerve end in skeletal muscle

2.Sensory Nerve Ending:

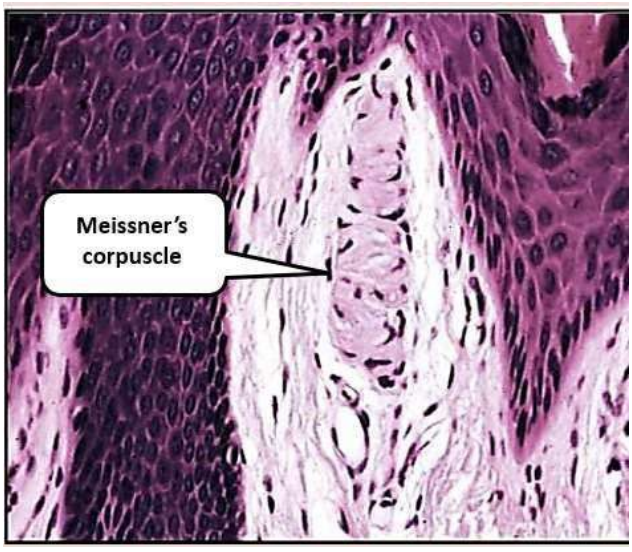
Meissner's corpuscles:

•Small, encapsulated, pressure-sensitive sensory receptors, responsible for detecting a light touch to the skin, found in the dermis of skin.

•They are most concentrated in thick hairless skin, especially at the finger pads as well as foot, eyelid and lips.

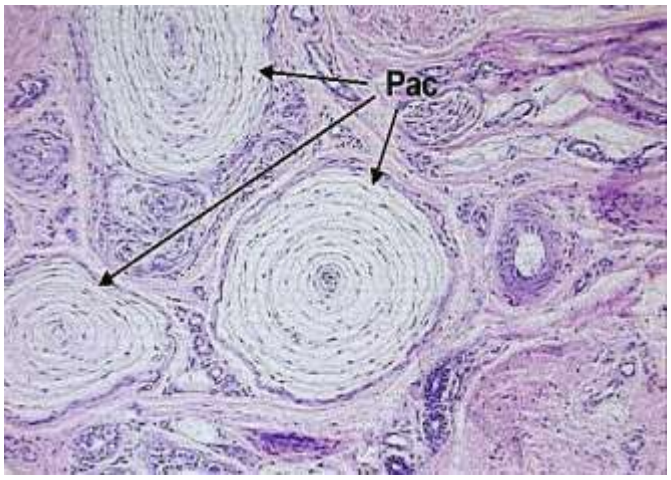
•Meissner's corpuscles are oval shaped, the receptors consist of flattened supportive cells arranged as horizontal lamellae and representing specialized Schwann cells, surrounded by a connective tissue capsule.

•A single unmyelinated nerve fiber meanders between the lamellae and throughout the corpuscle in a helical manner.



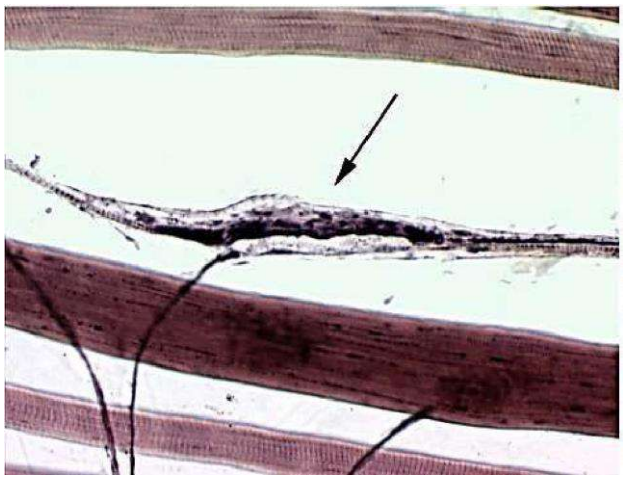
B. Pacinian corpuscles:

- A specialized bulb-like nerve ending located in the subcutaneous layer of the skin and scattered within the body, particularly around muscles and joints.
- Occurs abundantly in the skin of palms, soles, joints and genitals, and respond only to mechanical deformation.
- Larger and fewer in number than Meissner's.
- They contain a central single unmyelinated nerve fiber at the receptive region, surrounded by onion-like layers of connective tissue that behave like a shock absorber.



C. Neuromuscular spindle:

- A sensory receptor within the belly of a muscle.
- Detects changes in the length of the muscle.
- It is a fusiform end organ in skeletal muscle, at which the afferent and few efferent nerve fibers terminate.
- It contains from 3 to 10 modified striated muscle fibers that are much smaller than the ordinary muscle fibers.



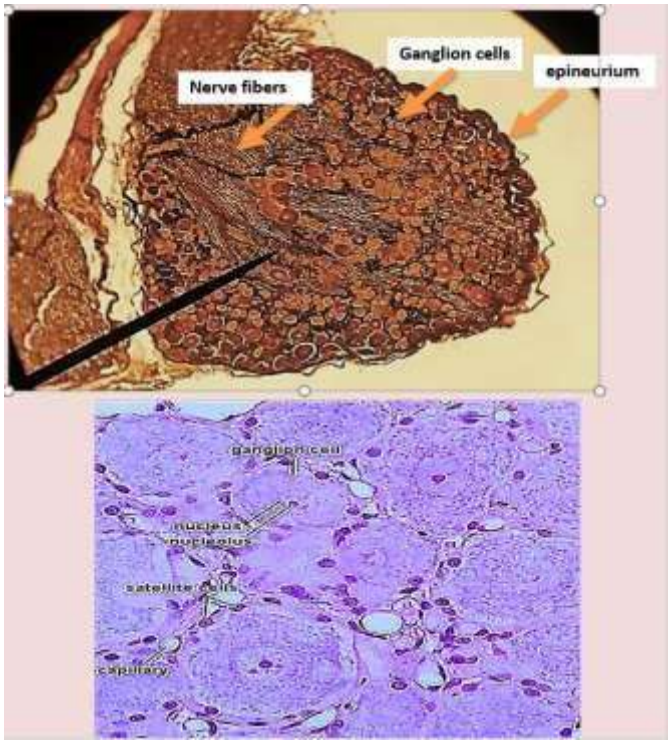
Examples of PNS:

A. Spinal ganglion (dorsal root ganglion):

- An aggregation of neuron's cell bodies located outside CNS.

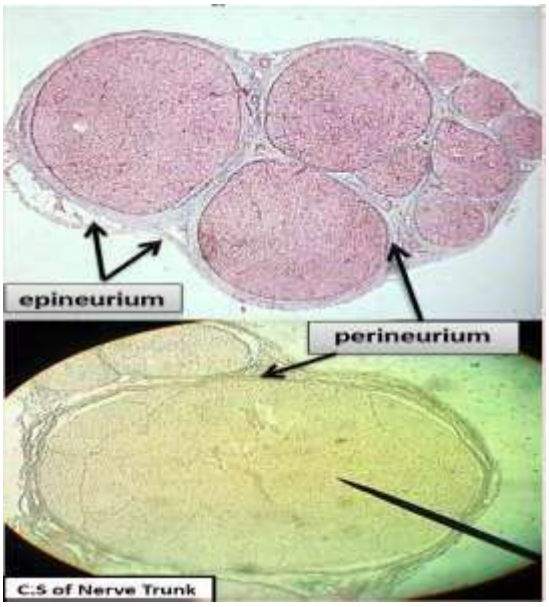
- Contains the cell bodies of primary sensory neurons which are Pseudounipolar, and they surrounded by satellite cells (provide structural and metabolic support).

- There is a fascicle of nerves passing to the center of ganglion, and the whole ganglion is encapsulated by a condensed supporting tissue which is continuous with perineural and epineural sheaths of the peripheral nerves.



B. Nerve trunk: -

- A collection of nerve fibers grouped into bundles to form the nerves.
- It is surrounded by a connective tissue sheath called epineurium which also fills the space between the bundles of nerve fibers.
- Each bundle is surrounded by the perineurium.
- The endoneurium consists of a thin layer of reticular fibers and envelops each axon.

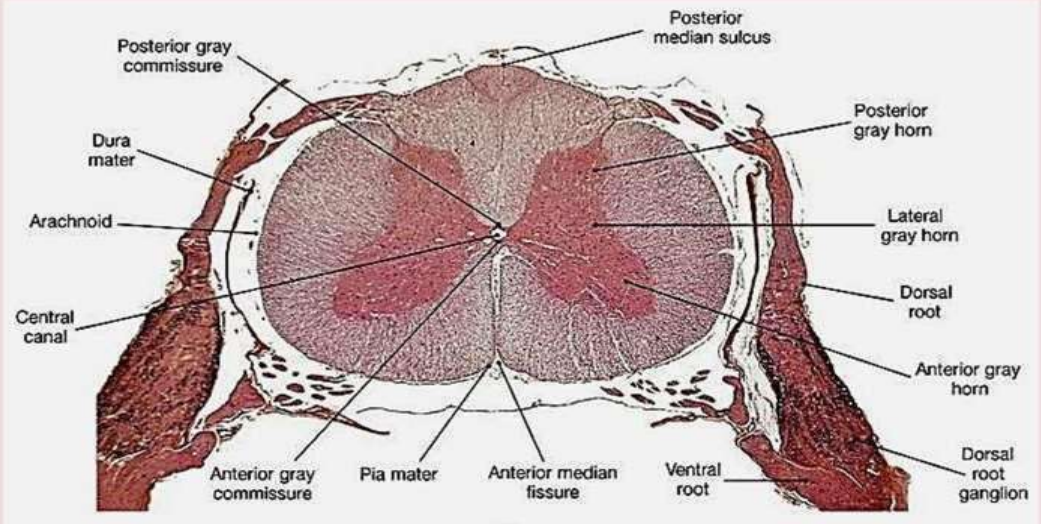


Example of CNS_ the spinal cord_

- The cord is sheathed with the pia mater, arachnoid and dura mater.
- The dura mater is a tough outer sheath, the arachnoid lies beneath it, and the pia closely adheres to the surface of the cord.

A cross section of the adult spinal cord shows:

- White matter in the periphery, gray matter inside assuming the shape of an H or a (butterfly).
- In the horizontal bar of this H is an opening, the central canal filled with CSF, which is a remnant of the lumen of the embryonic neural tube.
- Gray matter is a region containing nerve cell bodies, it is divided into dorsal or posterior horn, lateral horn and ventral or anterior horn.
- The legs of the H form the anterior horns which contain motor neurons whose axons make up the ventral roots of the spinal nerves.
- The arms of the H form the posterior horns which receive sensory fibers from neurons in the spinal ganglia (dorsal roots).
- The central cross bar of gray matter is called gray commissure. The part of gray commissure spanning posterior to the central canal called posterior gray commissure, while that spanning anterior to the central canal is called anterior gray commissure.



C.S in spinal cord