

General Histological Preparations

- Most fresh tissue specimens are colorless and squishy. They provide little useful information. For scientific or diagnostic purposes, tissue specimens must undergo substantial alteration in preparation for viewing under a microscope.

1. Fixation

- **Tissue must be fixed:**
 1. To avoid tissue digestion by enzymes present within the cells (**autolysis**) or by bacteria
 2. To preserve the structure and molecular composition.
- ✓ This treatment- fixation can be done by **chemical** or **physical methods**.
- ✓ the specimens were fixed in **10% neutral buffered formalin** for twenty four hour (24 hour)

2. Dehydration, 3. Clearing and 4. Embedding

- **Dehydration:** After fixation , the specimens were passed through graded series of **ethanol alcohols**
 - 30% for 2-3hours,
 - 50% for 2-3hours,
 - 70% for 24 hours,
 - 90% for 9 hours,
 - 99% for 2-3 hours.
- **Clearing:** was done in **Xylene** for 15-30 minutes till specimens become transparent.
- **Embedding:** with **paraffin**, the specimens were transferred from the clearing agent to a bath of melted paraffin, and the specimens were left for three hours at 65 C° in oven.
- ❖ The blocking was Melted paraffin poured into (**L-shape**) metal mold,
- ❖ Each block were cut at (5μ) micrometer by using **microtome**, and stained with Harris haematoxyline and eosin (**H&E**)

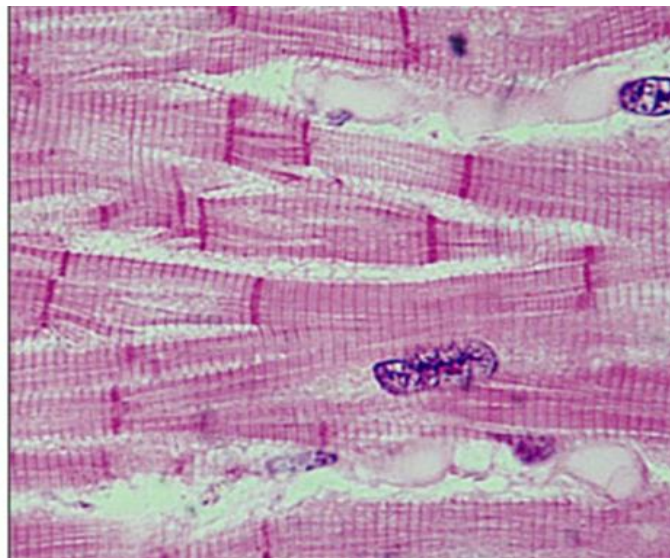
The whole procedure, from fixation to observing a tissue in a light microscope, may 12– 48h depending on:

1. The size of the tissue
 2. The fixative
 3. The embedding medium
- ❖ Tissue components that stain more readily with basic dyes are termed **basophilic**,
 - ❖ Tissue components that stain more readily with acid dyes are termed **acidophilic**,

- ❖ Examples of basic dyes are **Methylene blue, Hematoxylin** , and example of acid dyes is **eosin**

Haematoxylin and Eosin staining

1. The section were dewaxed in Xylene for 30 minutes.
2. The section were rehydrated in ethanol alcohol
 - 99% for 5 minute,
 - 90% for 5 minute,
 - 70% for 5 minute
- Then passed to distilled water for 5 minute.
3. The sections were stained with Haematoxylin for 1 minute then passed to tap water
4. Bluing was done by using running tap water.
5. Slides stained with eosin (few dips).
6. The sections were dehydrate in ascending concentration of
 - Ethanol alcohol 70% for 5 minute
 - 90% for 5 minute,
 - 99% for 5 minute.
7. Clearing in xylene was done for 10 minutes.
8. The sections were mounted with Eukitta mounted media.
9. After completed the histological preparation, the slides examined under Light microscope, to evaluation of histological changes consistent with the experiment.



Epithelial tissue

Epithelial tissue (epithelium) forms a continuous layer or sheet over the entire body surface and most of body's inner cavities. Externally it forms a covering layer that protects the animal from infection, injury and drying out. Some epithelial tissue produce and release secretions, others absorb nutrient

We can classify the epithelial tissue according to:-

- Function
- Number of cell layers
- Shape of cells

According to function we have:-

- Covering and lining epithelial tissues.
- Glandular epithelial tissue

According to the number of cells layers we have:-

- Simple epithelial tissue.
- Stratified epithelial tissue.

And According to the shape of cells we have:-

1. Squamous epithelial tissue.
2. Cuboidal epithelial tissue.
3. Columnar epithelial tissue.
4. Pseudostratified epithelial tissue.

A-Covering and lining epithelial tissue:-

In which the cells are organized in layers that cover the external surface or lines the cavities of the body.

It can be classified according to the number of cell layers in to:-

1-Simple epithelial tissue

*contain one layer of cell all of them based on the basement membrane

2- Stratified epithelial tissue

*contain more than one layer placed on top of each other,

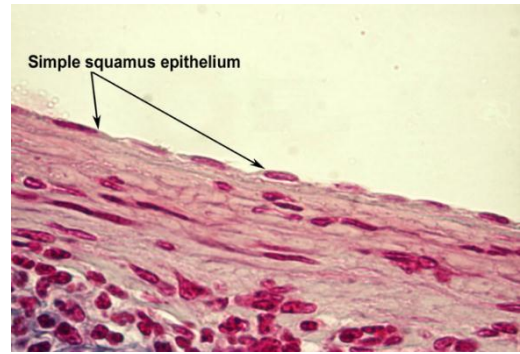
*only the inner moist layer based on the basement membrane.

Simple epithelial tissue can be classified according to the cell shape:-

- Simple Squamous epithelial tissue

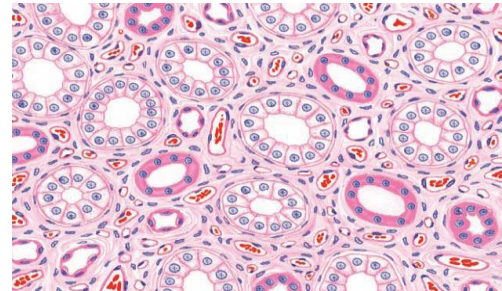
It's a single layer of thin, flat, polygonal cells, each with a central nucleus.

EX: - **endothelium** that lines blood vessels, **mesothelium** that lines certain body cavities such as peritoneal cavities.



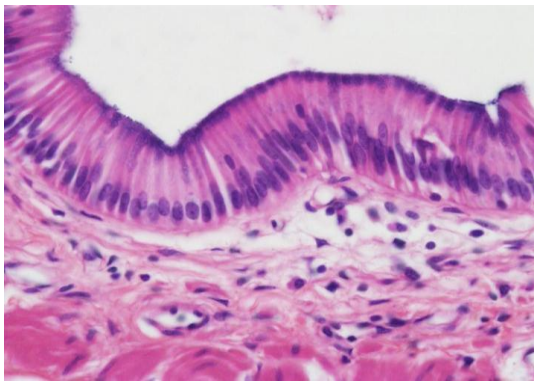
- Simple cuboidal epithelial tissue.

It's a single layer of cube-shaped cells, each with a central spherical nucleus. It's found **in kidney tubules and in ducts of many glands** where it has a protective function. It also occurs in **secretory portions of some glands** where the tissue produces and releases secretions.

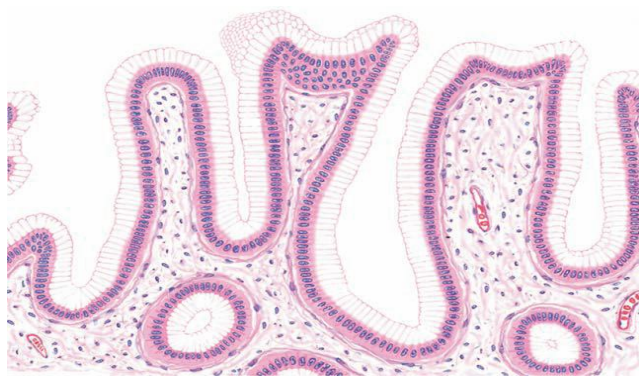


- Simple Columnar epithelial tissue.

It's a single layer of tall, cylindrical cells, each with a nucleus near the base. This tissue **lines the digestive tract from the stomach to the anus**, protects, secretes, and allows absorption of nutrients.



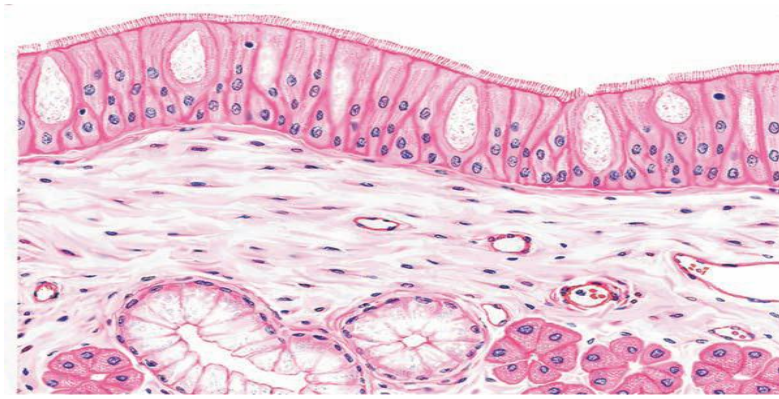
Simple Columnar epithelial tissue



Simple columnar epithelium: surface of stomach

- Pseudo stratified epithelial tissue.

It's so called because the nuclei appear to lie in various layers, but in fact, all cells are attached to the basal lamina "basement membrane" although some don't reach to the surface. EX: - **ciliated Pseudostratified columnar epithelial tissue in the respiratory tract.**



- **Pseudostratified columnar ciliated epithelium: respiratory passages—trachea**

Stratified epithelial tissue:-

It can be divided according to the shape of cells in the outer most layers in to

- Stratified Squamous epithelial tissue

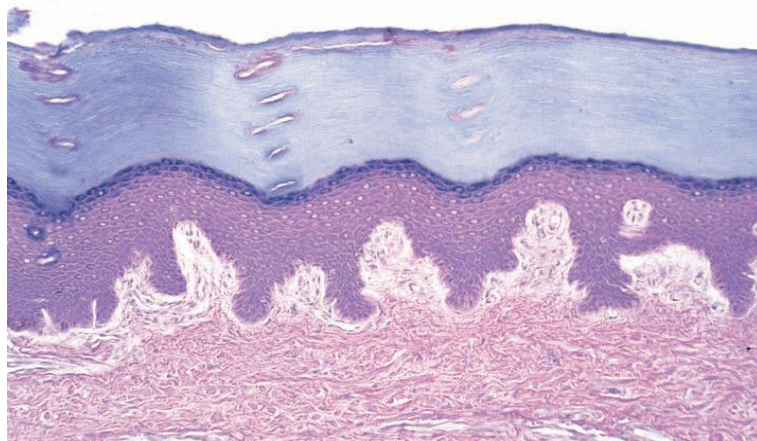
It's found primarily in places subject to attrition (**skin, mouth, esophagus, and vagina**). There cells from many layers:

The cells closer to the underlying connective tissue are cuboidal or columnar in shape while the cells closer to the surface are irregular in shape and flatten, becoming very thin and squamous

This tissue is divided in to:-

1-Keratinized stratified Squamous epithelial tissue:-

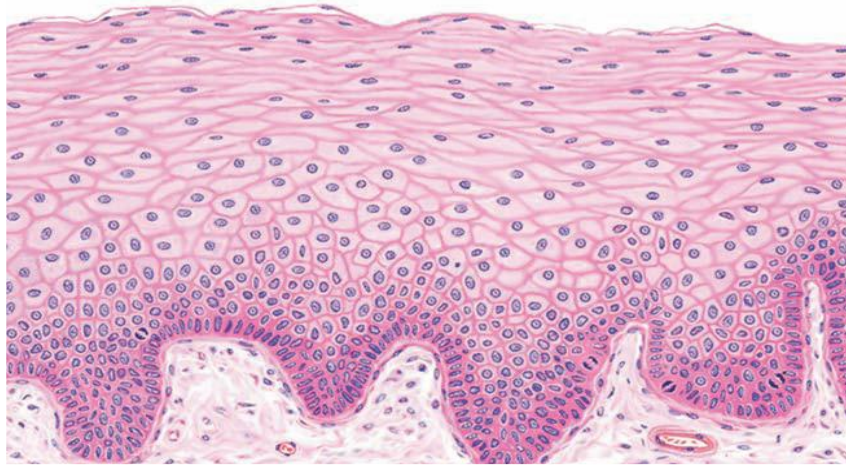
Cover dry surfaces such as the skin, the cells on the surface layer are transmitted in to dead scales without nuclei.



Stratified Squamous keratinized epithelium: palm of hand

2-Non- Keratinized stratified Squamous epithelial tissue:-

Cover wet surfaces such as the **lining of mouth, throat, anal canal, vagina, esophagus**, the cells in the surface layer remain soft, moist and live.



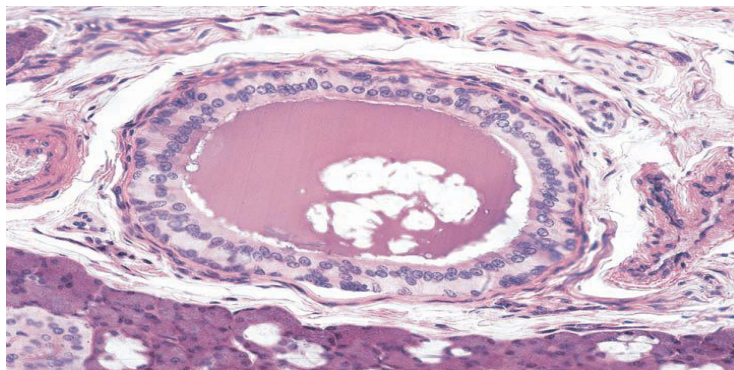
Stratified Squamous non- keratinized epithelium: esophagus

- Stratified columnar epithelial tissue

The cell in the surface layer are columnar in shape. It is present in human body in small areas, such as the **ocular conjunctive and large duct of salivary glands.**

- Stratified cuboidal epithelial tissue

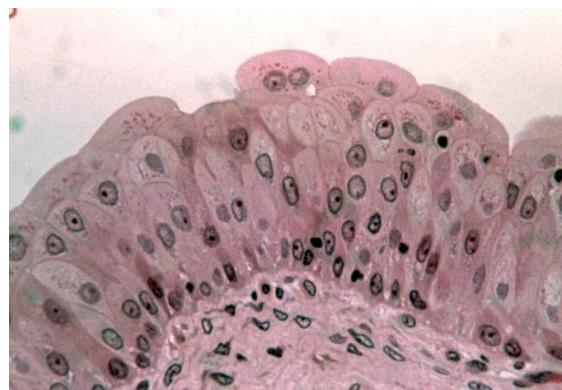
In which, the cells in the outer most layers are cubical in shape its present in the **duct of sweat gland**

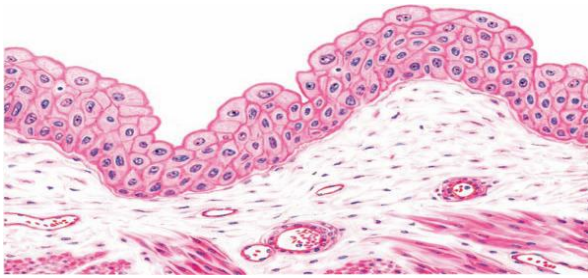


Stratified cuboidal epithelium: excretory duct in salivary gland.

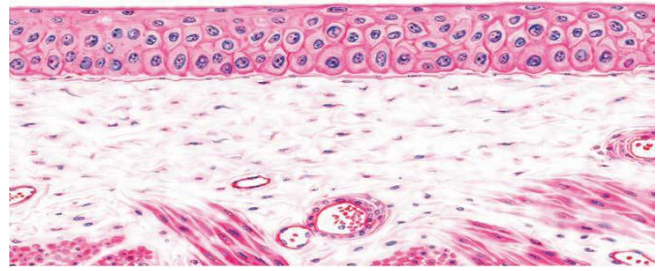
- Transitional epithelial tissue

It is characterized by the surface layer of dome like cells that is neither Squamous nor columnar , which **lines the urinary bladder and the ureter** , the form of these cells changes according to the degree of distention of the bladder .



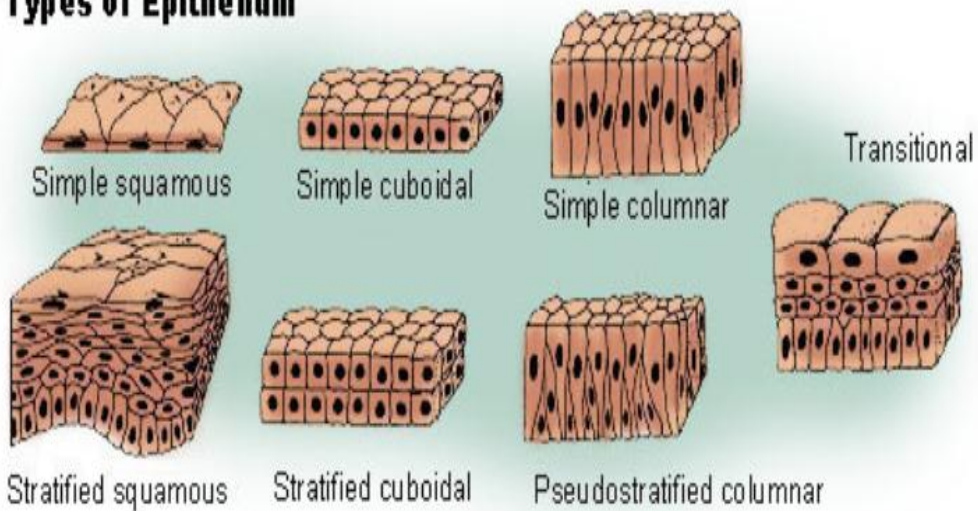


Transitional epithelium: bladder
(unstretched or relaxed).



Transitional epithelium: bladder
(Stretched).

Types of Epithelium



Specilization of the cell surfaces

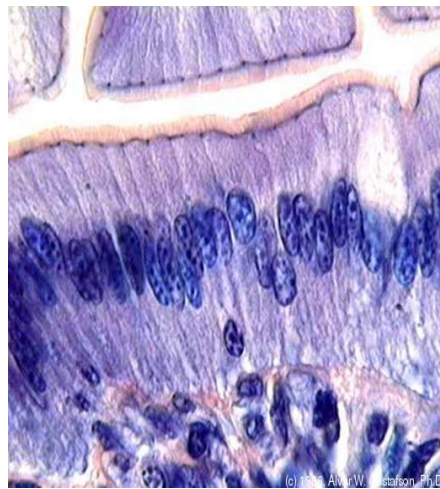
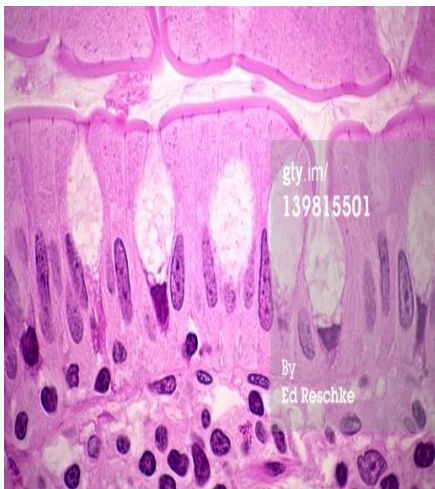
The surfaces of many types of epithelial cells contain specialize structures that increase the cell surface area or move substances to the epithelium.

Surfaces

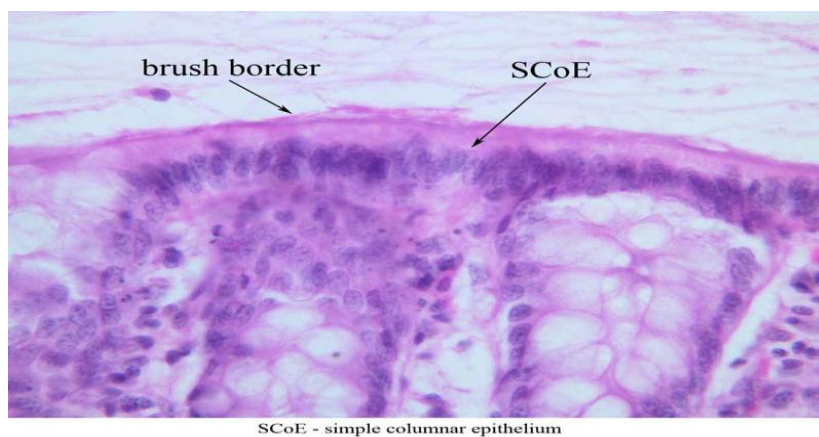
- **Lateral** → desmosome
- **Free** → Microvillus → 1- stereocilia
2- Brush border
3- Striated border

***Microvilli**: are finger like extensions found in the free cell surface.

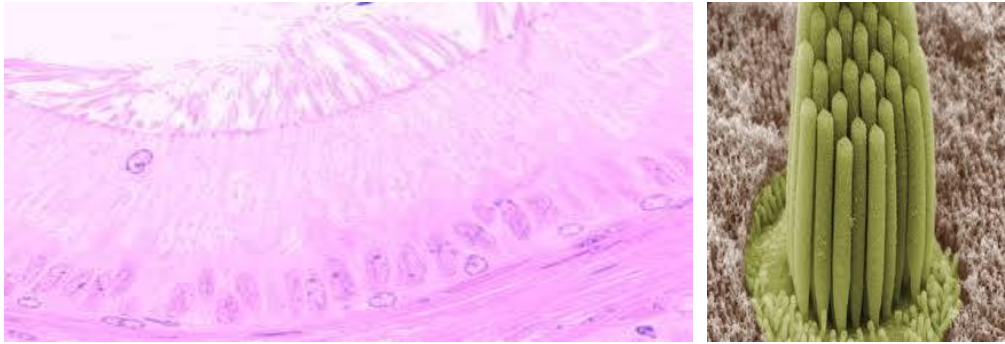
- 1- Striated border:** - in the lining of **small intestine (ileum)**, their function is to increase the surface area of absorptive cell.



- 2- Brush border:** - in the **proximal convoluted kidney tubules**.



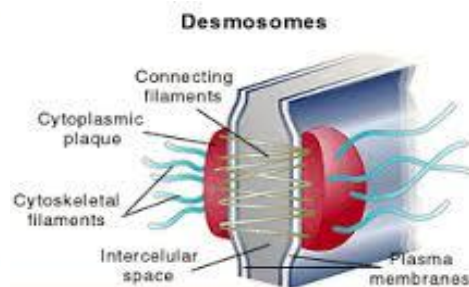
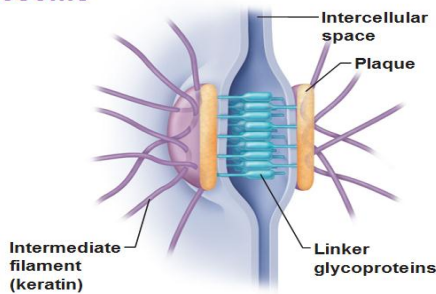
3- Stereocilia: - are long, non-motile extension found in the **epididymis**.



***the desmosome:** - is a complex disk-shaped structure on the surface of a one cell that matches an identical structure on the adjacent cell surface,

EX:- spinosum stratum in the epidermis of the skin .

osome



Glandular epithelial tissue

It is an epithelial tissue specialized for secretion. Classification of glands:

According to the way of secretion, it can be:

- 1- **Exocrine gland.**
- 2- **Endocrine gland.**
- 3- **Mixed gland**

According to the number of cell, we have:

Unicellular gland: - consist of one isolated cell ex: goblet cell in the lining of small intestine and in respiratory tract.

Multicellular gland: - composed of groups of cell, it consist of two portion: -

- 1-Secretory portion
- 2- Duct

According to the branched of the duct, gland can be classified in to:

Simple gland: have one un branched gland.

Compound gland: have more than one branched duct.

Simple gland: - can be classified according to the shape of secretory unit to: -

a-Tubular: -

1-Straight tubular. **ex: crypt of lieberkuhn**

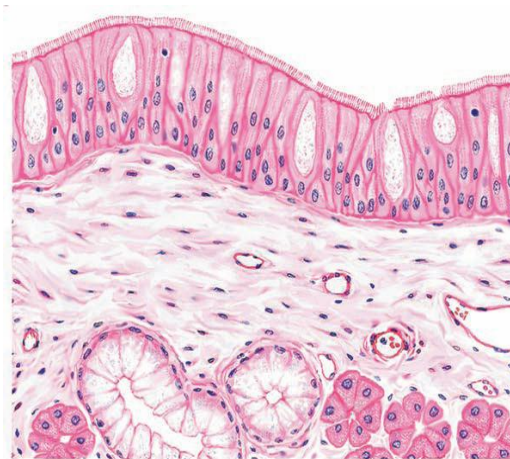
2-Coiled tubular . **sweat gland**

3-Branched tubular . **ex : pyloric gland of stomach**

b- Alveolar :

1-Unbranched alveolar . **ex : mucous gland in frog skin**

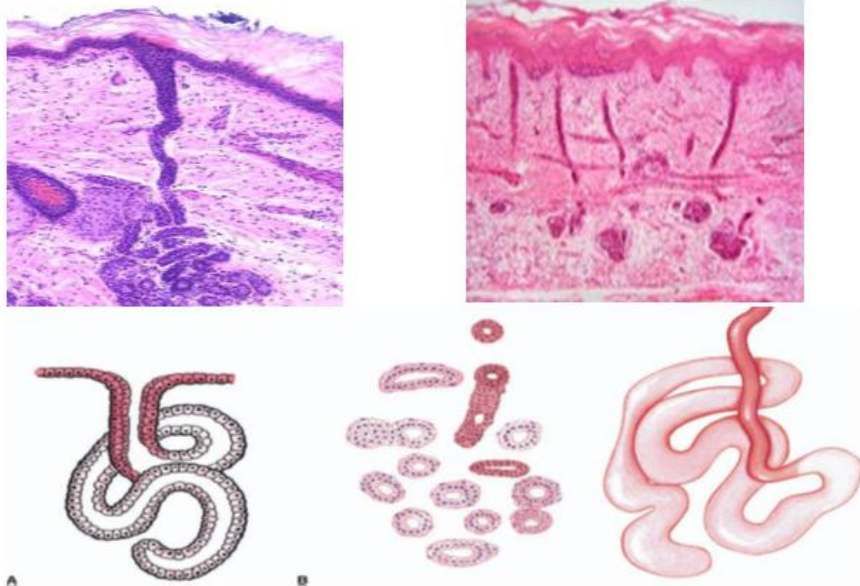
2-Branched alveolar . **ex : sebaceous gland in skin .**



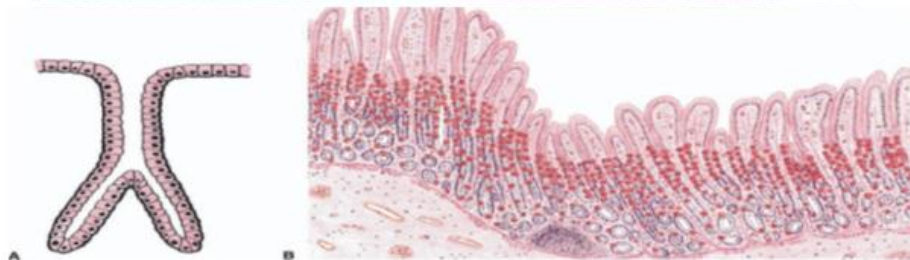
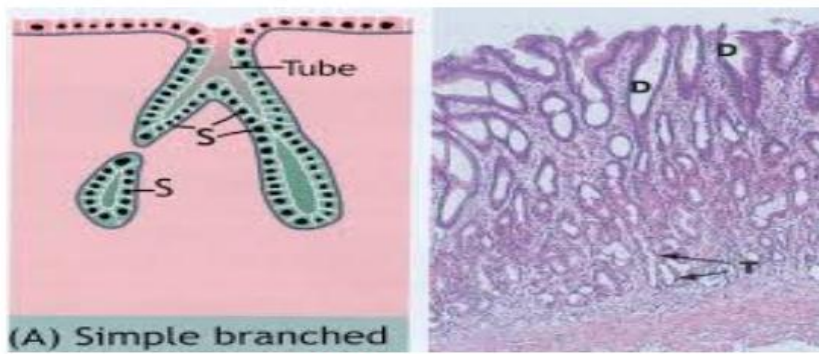
Unicellular gland



Straight tubular



Coiled tubular



Branched tubular



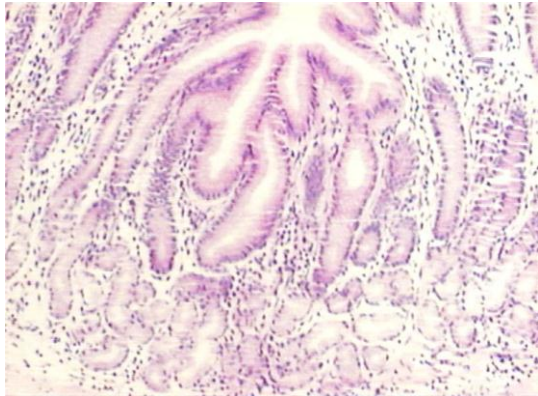
Branched alveolar. Ex: sebaceous gland in skin

Compound gland: - can be classified according to the shape of secretory unit to: -

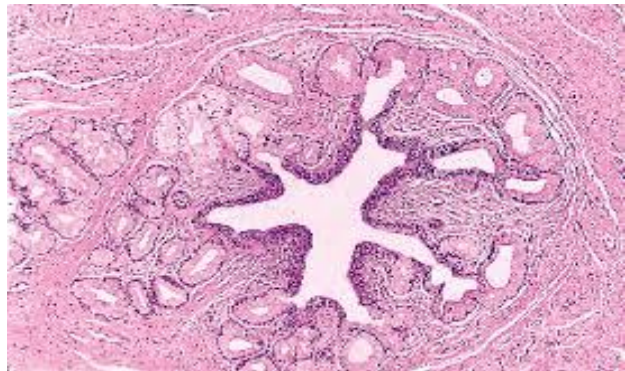
1-Compound tubular: **kidney, testes**

2-Compound alveolar: **mammary gland**

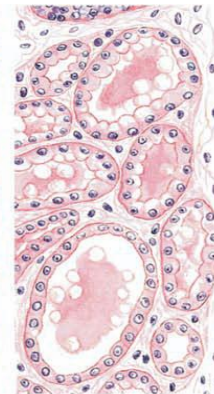
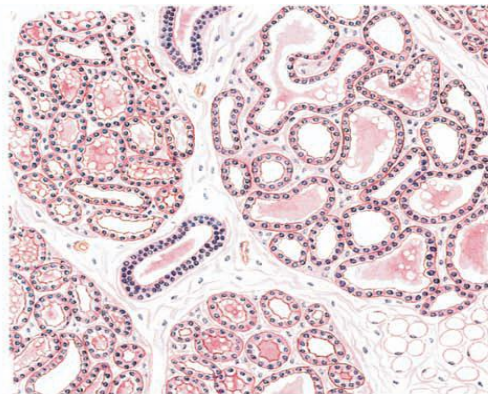
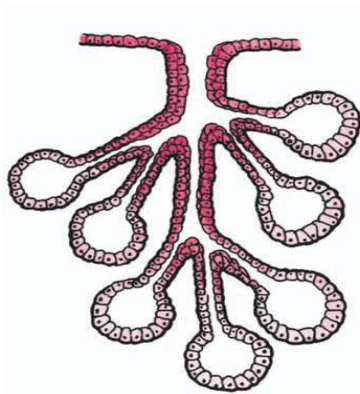
3-Compound tubulo alveolar: **salivary gland**



Compound tubular



Compound alveolar

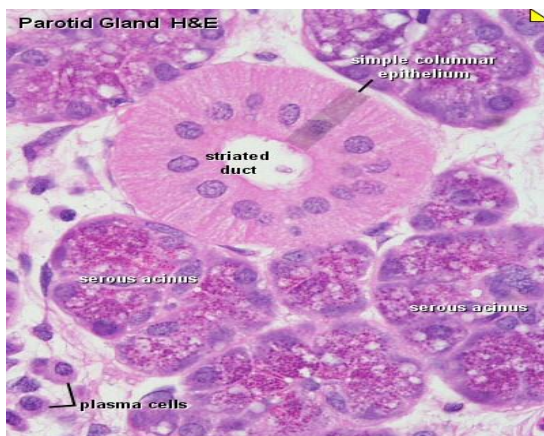


Tubulo – alveolar or Tubulo –acinar

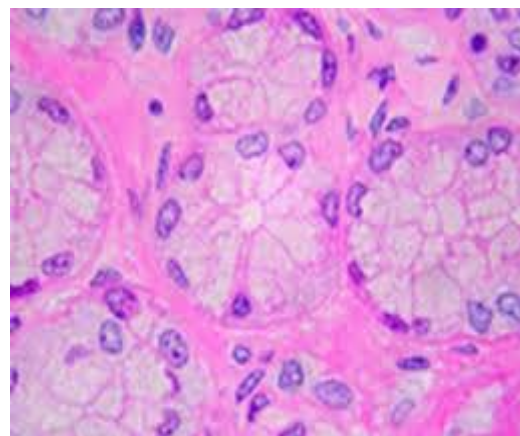
Based on the type secretory products glands can be classified in to:-

1-Serous gland: - pyramidal in shape with central rounded nuclei cytoplasm is alkaline, the cavity of gland is large **ex: parotid gland**

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



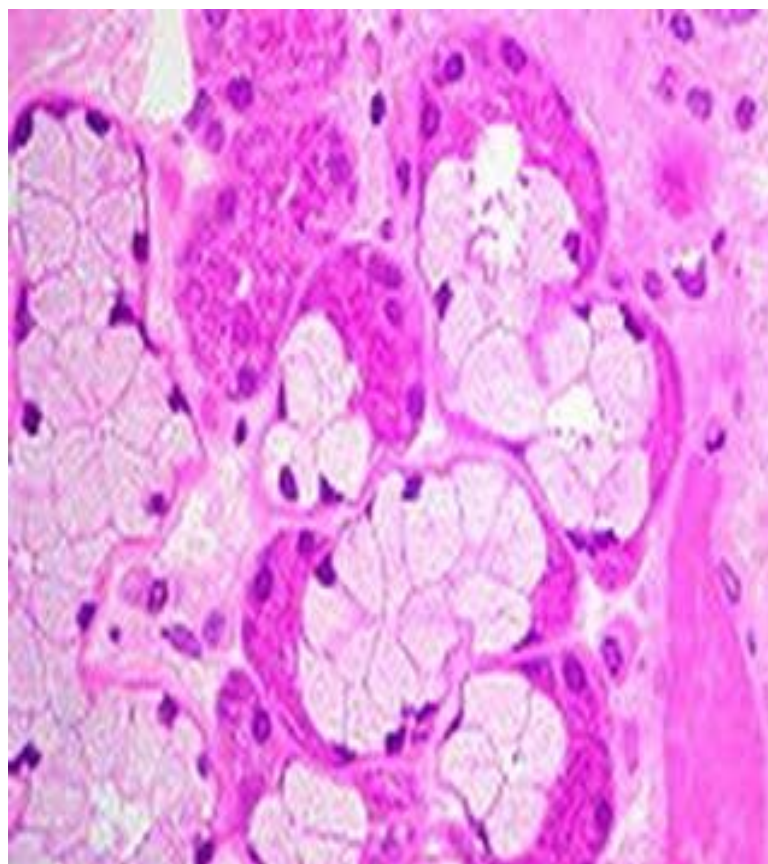
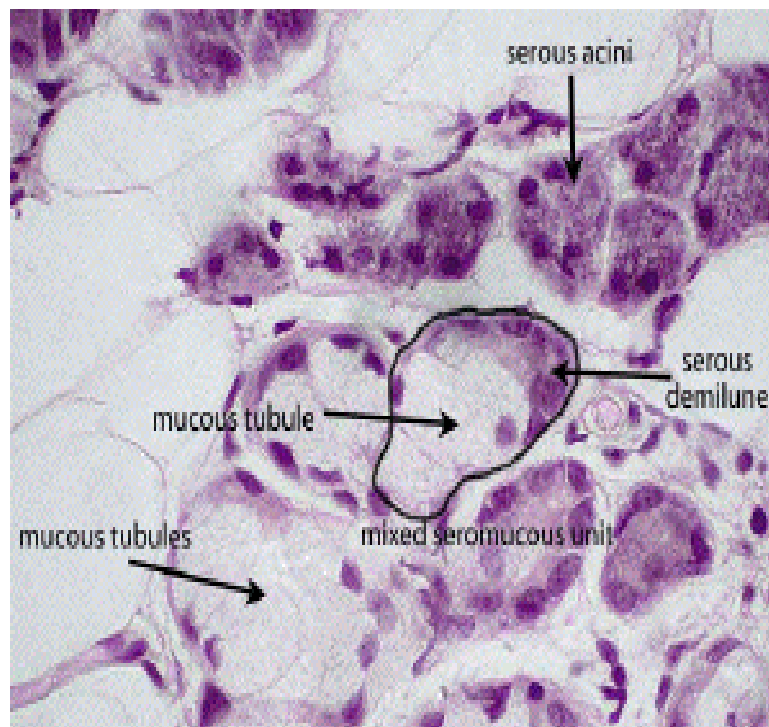
Serous gland



Mucous gland

3-Sero mucous gland: - this gland consist 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 **serous demilune**

Ex : sub mandibular gland , sub maxillary gland .



Connective tissue

Its support and protect, consist of:-

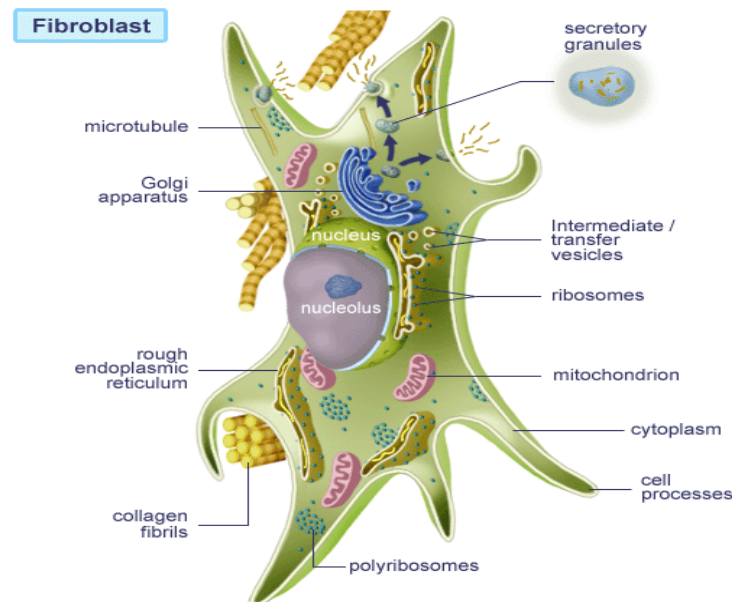
* **cells**

* **Fibers**

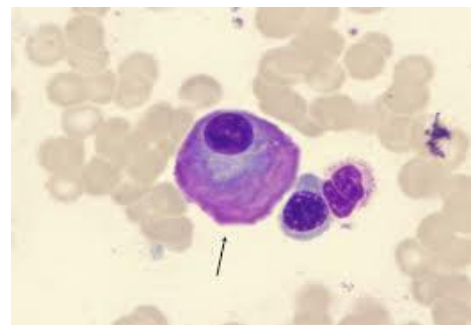
* **Ground substance:** its transparent, homogenous , random , in shape may be viscous , semisolid or solid

Cells

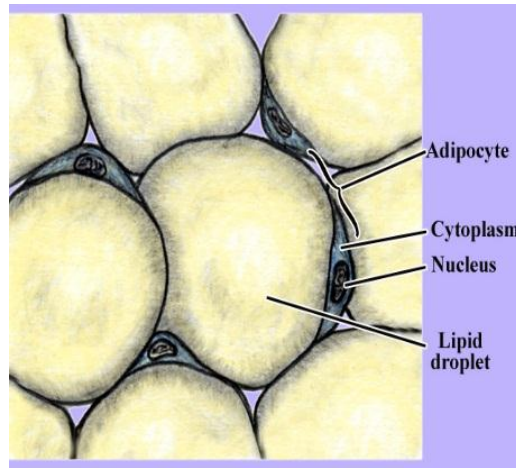
Fibroblast : they are large , flat , branching cell which appear spindle shape inside view , the nucleus is oval and appear pale and has one or two nucleoli , cytoplasm is very pale so that the out line of the cells are indistinct . **Fibroblasts are responsible for formation the fibers.** We can see in **Areolar connective tissue.**



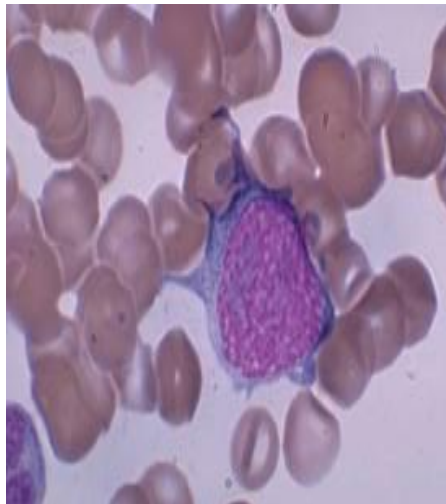
Plasma cell :-are rare in connective tissue but are found in lymphoid tissue . its small in size . spherical or ovoid within the nucleus chromatin occur in course clumps peripherally and arranged in pattern like wheel or clock face , **plasma cell responsible for antibodies production**



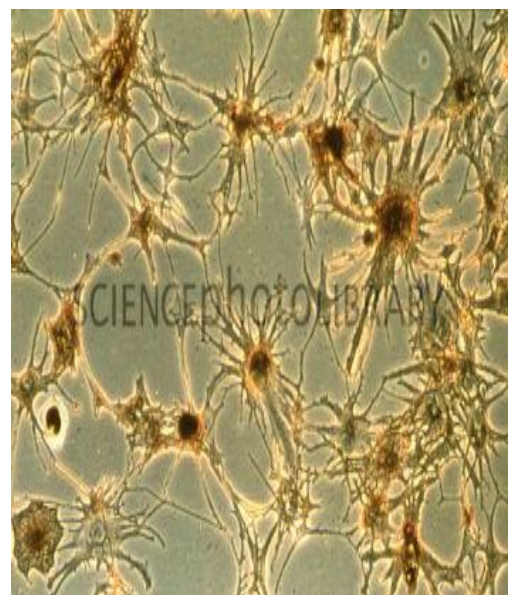
Adipose cell: - their shape spherical to ovoid contains a single large droplet of oil and thin rim cytoplasm contains in one area the flattened nucleus.



Melanocyte and pigment cell: - cells have irregular cytoplasmic processes like the general cytoplasm contain small granules of pigment called melanosomes which contain melanin. It has a role in absorption light rays pigment cell found in dermis of skin.



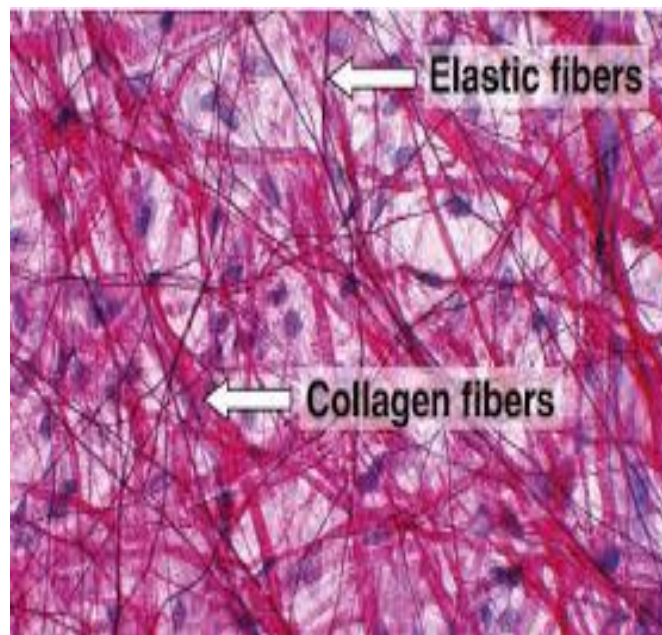
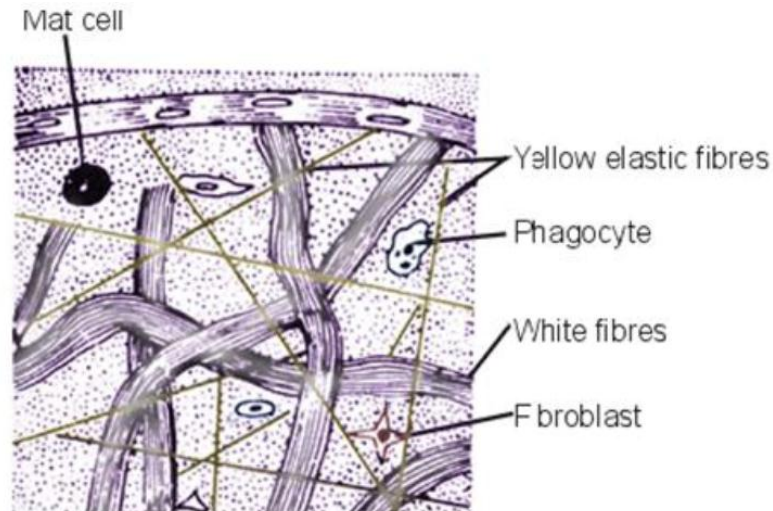
Reticular cells: - are stellate they have long cytoplasmic extension, which appear to join with other cell extensions .they have pale, large nuclei, and basophile cytoplasm. **it's found in lymph node**



Fibers

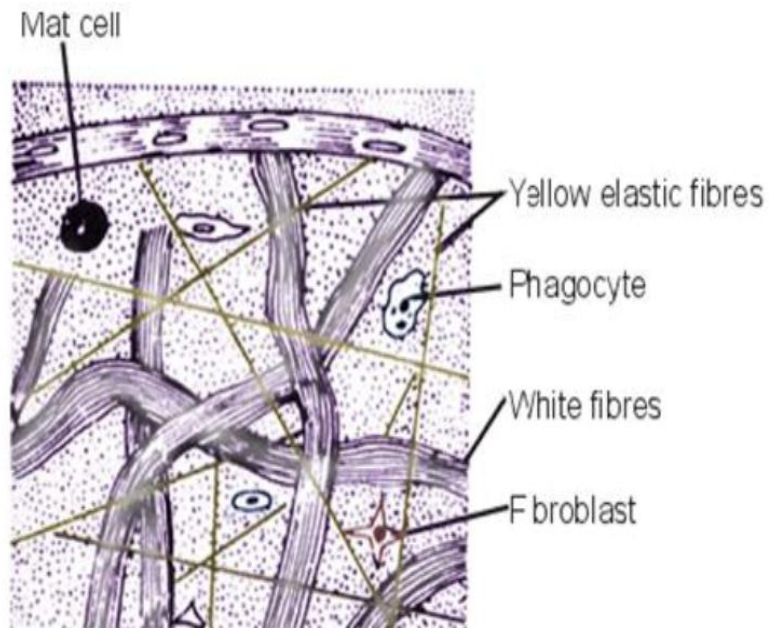
1- White (collagenous) fibers:-

Are seen as straight wavy bundle, each bundle consist of fibrils, which appear white in fresh state. White fiber is soft, flexible and inelastic that gives the tissue strength. **We can see in dermis.**



2- Yellow (elastic) fibers

Are seen as along, than and single threads and branched bundle, in fresh state has a yellowish color. Yellow fibers are elastic and easily to stretching. **We can see in cross section in aorta.**



3- Reticular fibers:-

Are very fine threads arrange to form a net colored brown when staining them by silver impregnation. **They are found in lymphoid organs.**



TYPES OF CONNECTIVE TISSUE

- Proper connective tissue
- Special connective tissue

Proper connective tissue

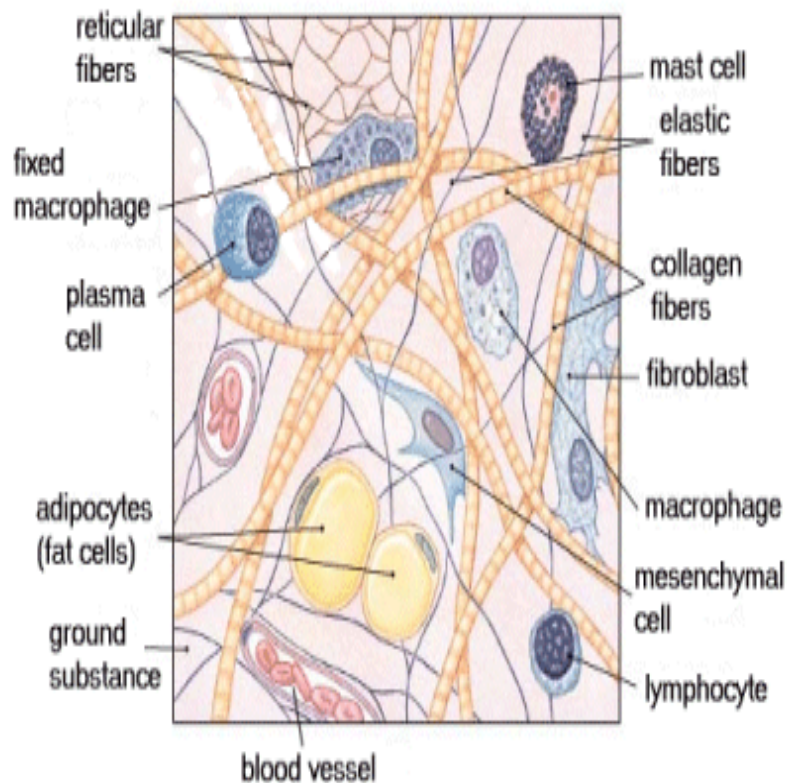
- * Loose connective tissue
- * Dense connective tissue

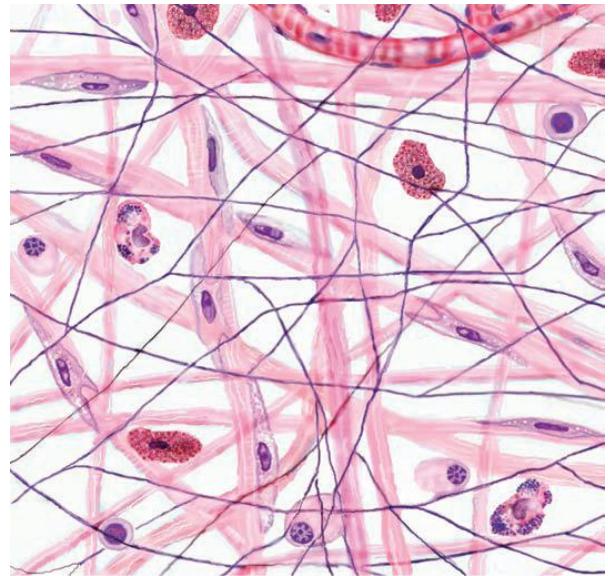
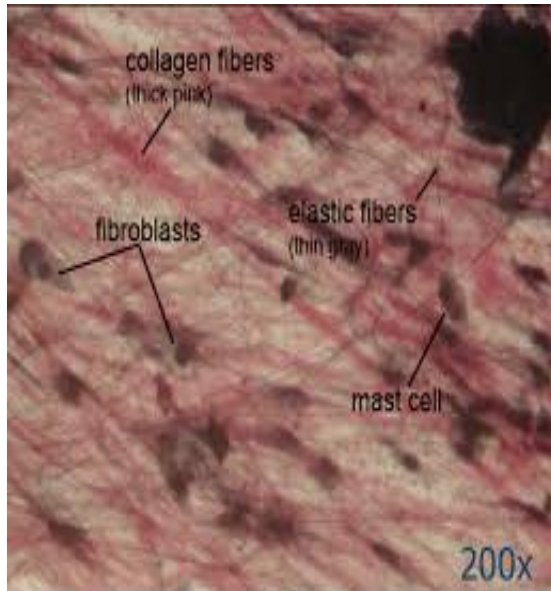
Loose connective tissue

Are characterized by loose arrangement of fibers, with low concentration of fibers.

1- Areolar con . t

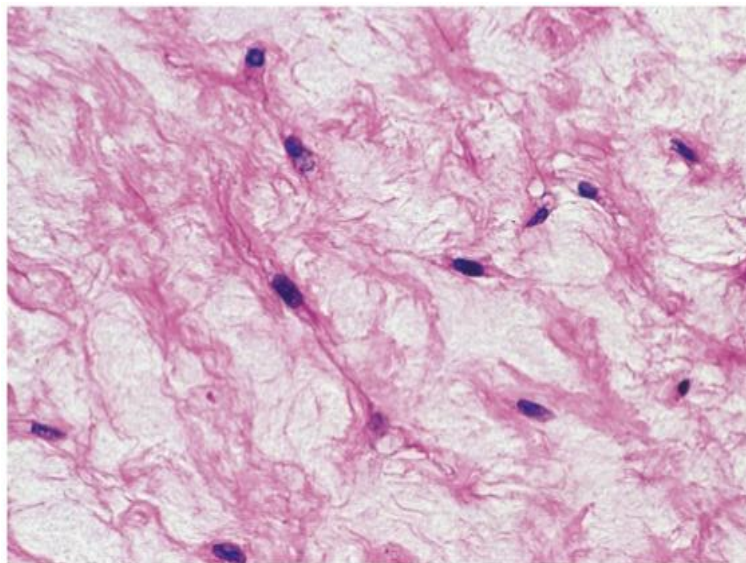
Contain vacuoles or intracellular distance .its found in the substance of lung ,heart and digestive trunk . the ground substance is semisolid which contain yellow and white fibers and little of reticular fibers . the cell which the most common in this tissue are : fibroblast , mast cell , macrophage , and plasma cell





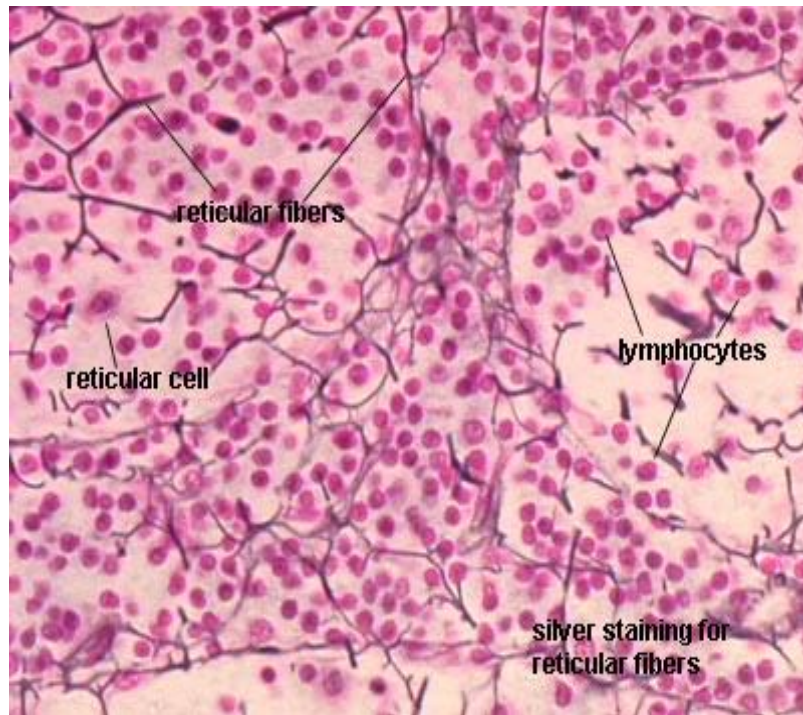
2- Muroid con. t

Contain fibroblast with ratio of white fibers and fewer ratio of yellow and reticular fibers. **It's found in umbilical cord.**



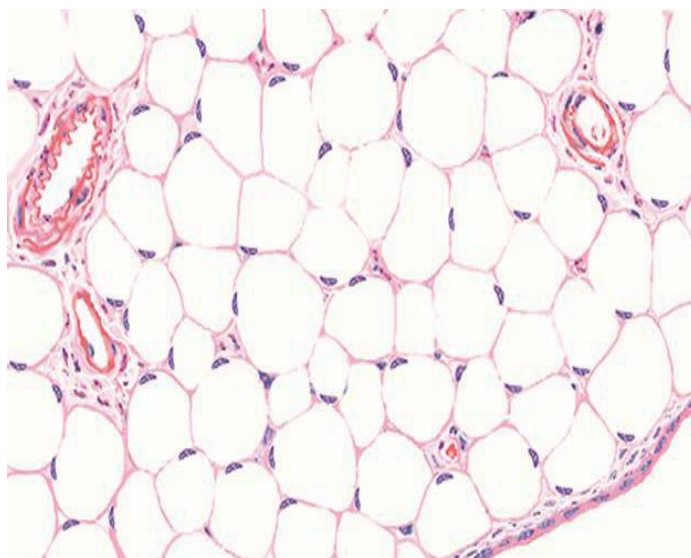
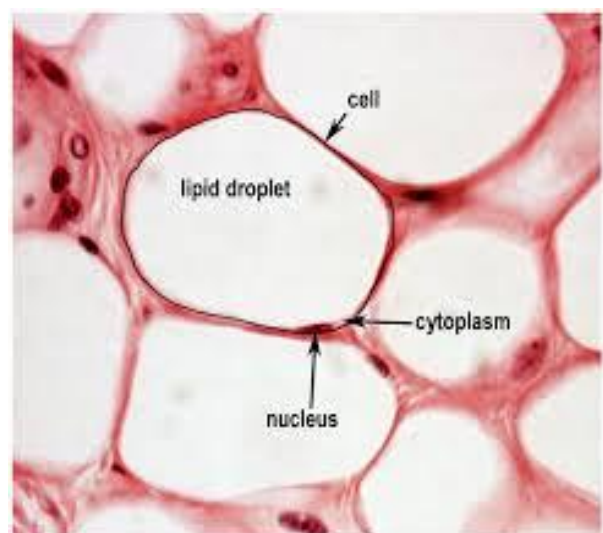
3- Reticular con. t

Which is characterize by presence of network of reticular fibers associated with reticular cells which are stellate and have long cytoplasm extensions which appear to join with extension of other cells . Also it is contain lymphocytes which have darkly nucleus and occupied most of the cell. **It's found in lymph node.**



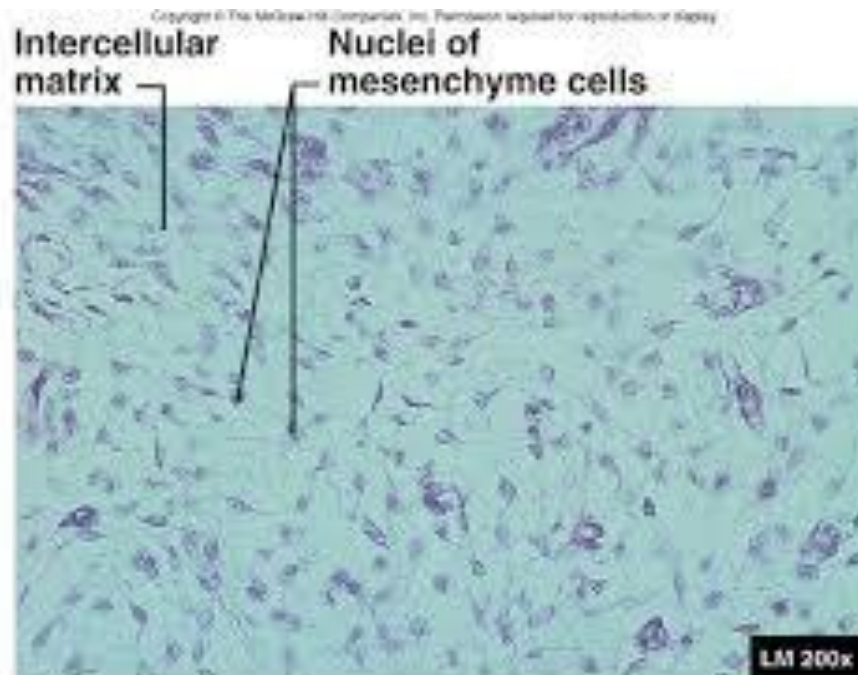
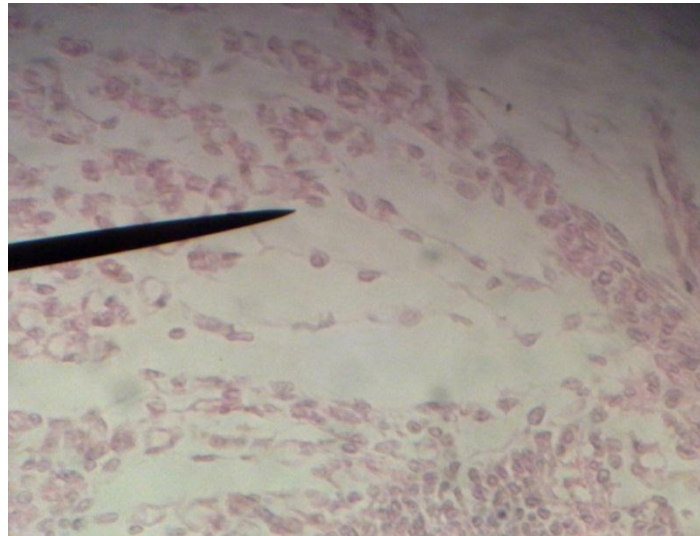
4- Adipose con. t.

Fat cell from large aggregation.
Each fat cell is surrounded by
net of different fibers and
fibroblast. **It found in the skin,
mesenteries and bone marrow.**



5- Mesenchymal con. t

Its component of mesenchymal cell whose branching process that swimming in ground substance transparent without any fibers



(a) Mesenchyme

(Dense connective tissue)

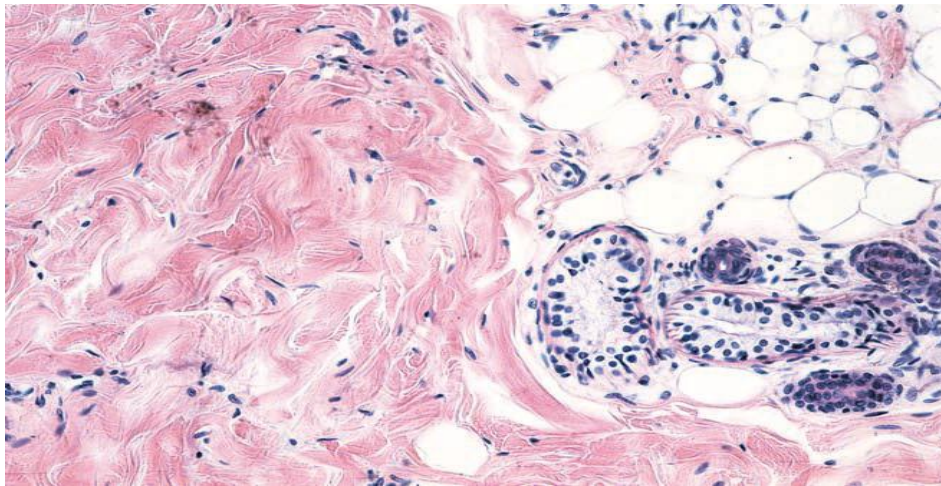
Contain of the same competent found in connective tissues, but there are fewer cells and ground substance.

Dense connective tissues can be classified according to the arrangement of fiber in to :-

1- Irregular connective tissues

In this section the collagen fibers are arrange in bundle , and form three

dimension network to provide resistance to stress from all directions , **it can be seen in the dermis of skin .**



2- Regulars connective tissues

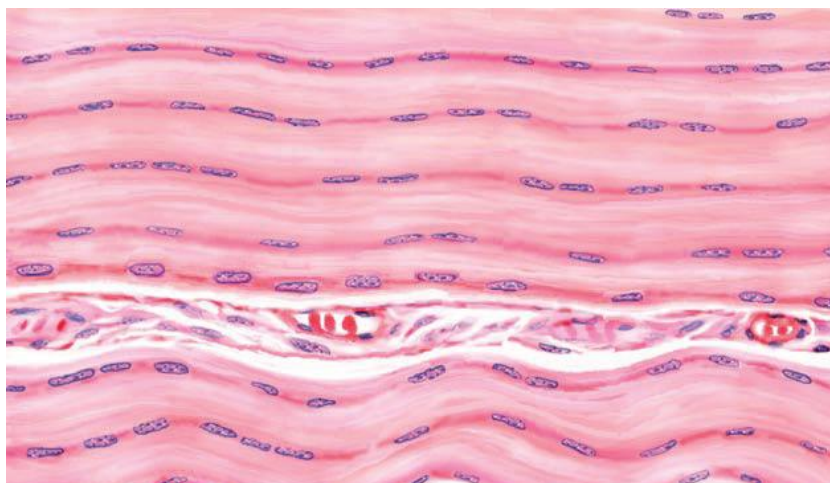
In this section the collagen fibers are arranged in regular bundles , its resistant to tension from all directions , **it can be classified according to the type of fibers to :-**

1-White fibrous connective tissue

Tendons are the most common of the white fibrous ,they have parallel , and closely packed bundles of collagens (the primary bundles) separated by small amount of ground substance . Their fibroblast called tendon cell, contain elongated nuclei parallel of fibers.

2-Elastic connective tissue

is composing of bundles of thick. And parallel elastic fibers the space between these fibers is occupied by thin collagen fibers and flattened fibroblast. It's called elastic because it's yellow color and great elastic. Its **present in ligaments of vertebral.**



Dense regular connective tissue: tendon (longitudinal section)

Specialize connective tissues

- 1- Cartilage
- 2- Bone
- 3- Blood

Cartilage

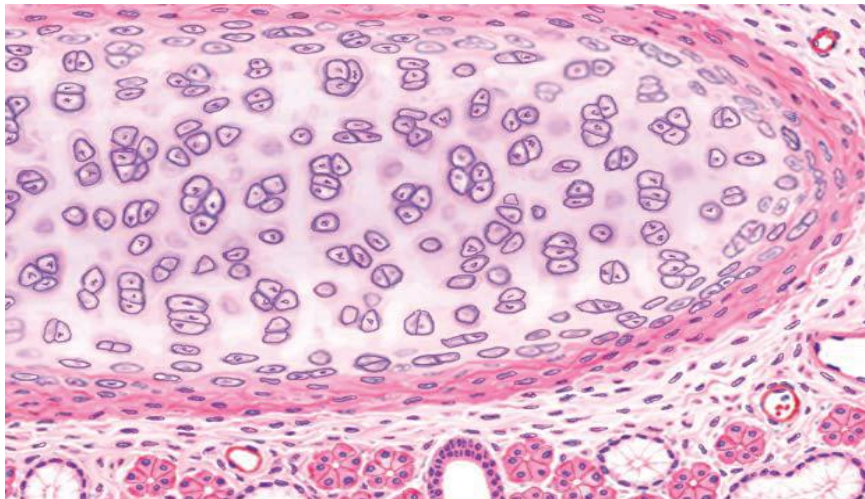
Consists of cell called chondrocyte and ground substance contain chondroitin sulfates.

There are three kinds of cartilage: -

- 1- Hyaline cartilage
- 2- Elastic cartilage
- 3- White cartilage

Hyaline cartilage

- * It's the present in the **cartilage of nose, larynx, trachea and bronchi also in vertebral ends of rib.**
- * Chondrocyte is single or aggregate as group called **cell nest**, it's surrounded with capsule and found with lacuna in ground substance
- * Ground substance appears as hyaline (glass) and contains fewer amounts of white fibers so it called **hyaline cartilage** .
- * Its surrounded with perichondrium which consist of two layers to **protect and repair the cartilage.**



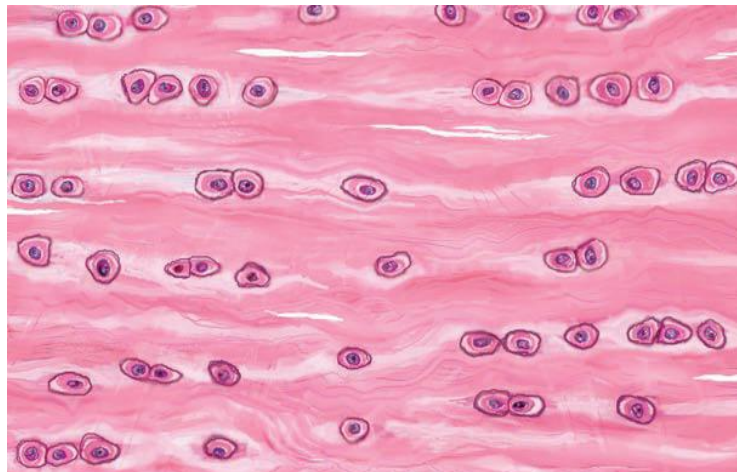
Elastic cartilage:-

- It's present in **auricle of the ear and Eustachian tube.**
- It's identical to hyaline cartilage except it contains bundles of elastic fiber.
- Its surrounded with perichondrium



White cartilage:-

- It's found in **intervertebral disc**
- Ground substance contains bundles of white fibers in parallel arrangement
- It's never present in alone but associated with hyaline cartilage or dense fibrous tissue , because , **it lack perichondrium**



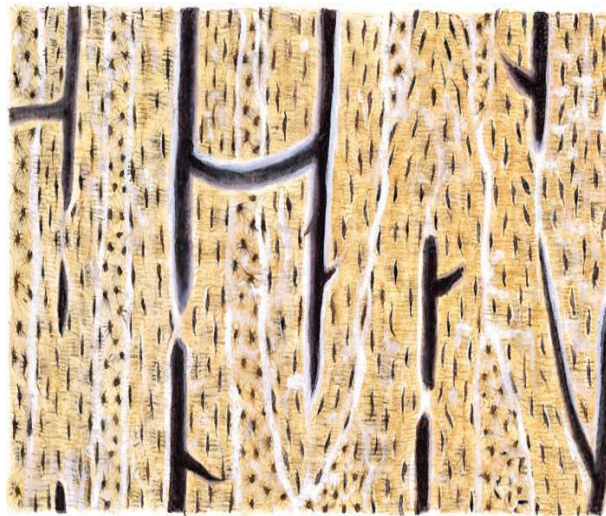
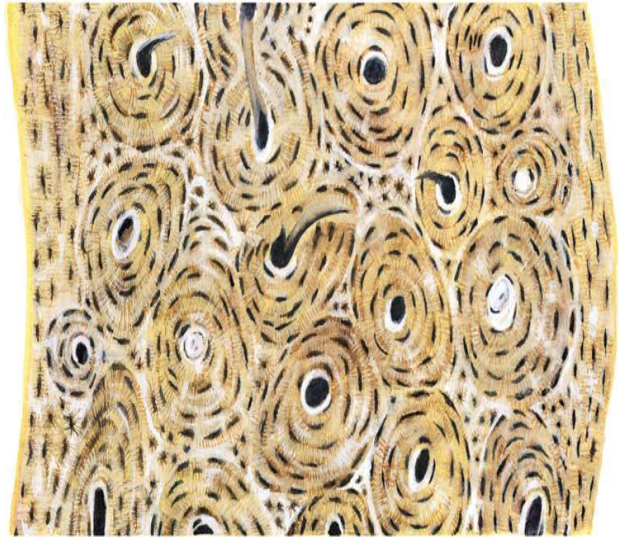
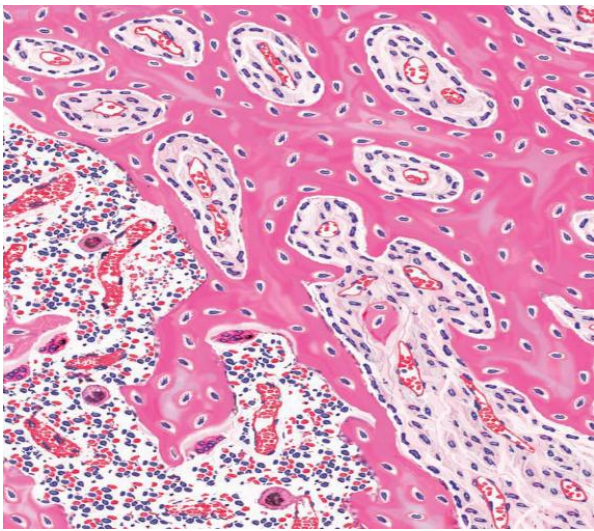
Bone

is specialized connective tissue composed of intercellular calcified material (bone matrix) and three types of cells:

- **Osteocyte**, which is found in lacunae within the matrix
- **Osteoclast**, which is multinucleated giant cell in the matrix called how ship lacunae.
- **Osteoblast**, which generate osteocyte

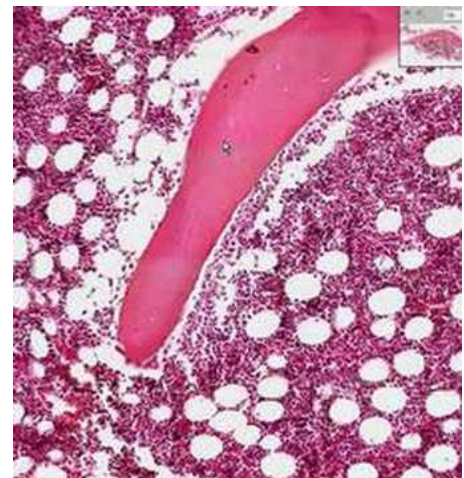
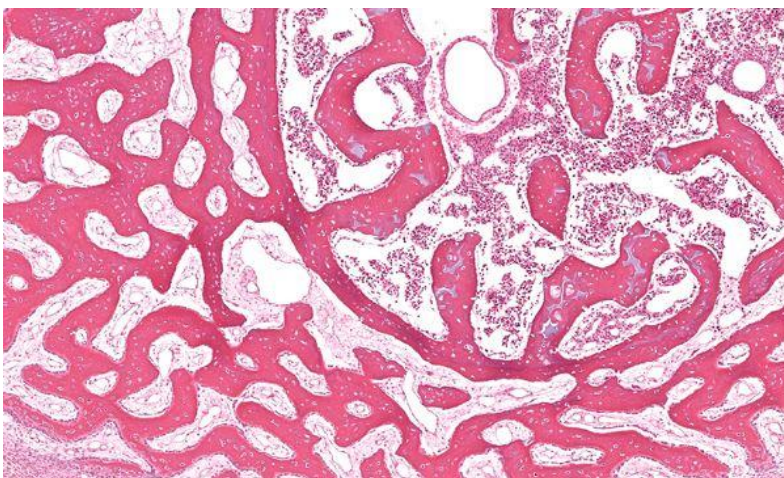
1- Compact bone

its show in long bone diaphesis, in this section, lamellae is regularly arrange around haversians canal and determined by blood vessels and nerves, this complex system called haversians system. Haversians canal connects with other by Volkmann's canal, and between haversian system there are interstitial lamellae.



2- Spongy bone

It's found in **bulbous ends of long bones called epiphysis**. Bone matrix appeared as irregular trabecular spongy. There is cavities between these trabecular contain red bone marrow and three kinds of cells: osteocyte, osteoblast, and osteoclast



Vascular connective tissue:-

Blood :- is specializing connective tissue it consist of erythrocyte , leukocyte , and intercellular substance is plasma , the fibers appear as fibrin when blood is clotted .

Red blood cells (erythrocytes):- are biconcave disks without nuclei, when we exam the blood smear, we can see several amount of R.B.C.

White blood cells (leukocytes):- are spherical in shape, according to the type granules of their cytoplasm and the shape of nuclei, leukocyte are divided in to:-

A granular leucocytes:- have cytoplasm that appear homogenous and nuclei are spherical shape

1- Lymphocytes:- are spherical cells , most are little larger than erythrocyte . it has larger spherical nucleus surrounded by narrow rim of cytoplasm . the cytoplasm is basophile .

lymphocytes

1- T-lymphocytes (T-cell) :manage and direct an immune response, some directly attack foreign cells and virus infected cells.

2- B-lymphocytes (B-cell): are stimulated to become plasma cells and produce antibodies

3- Natural killer cells (NK cells): attack abnormal and infected tissue cells

2- Monocytes:- are larger cell's nucleus is kidney in shape. The cytoplasm is grayish- blue in color.

Function:

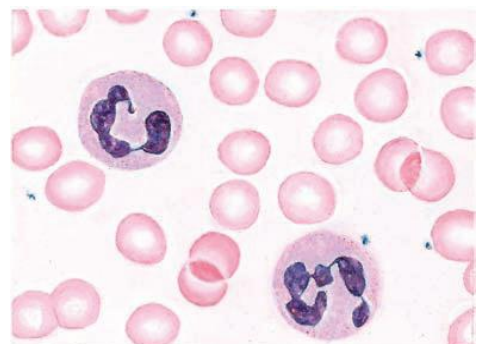
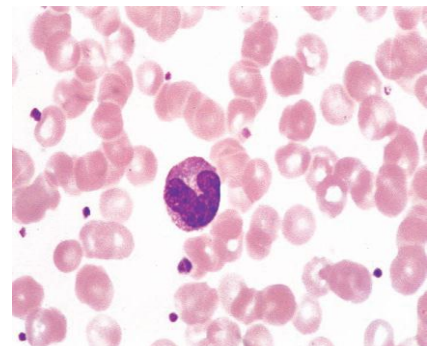
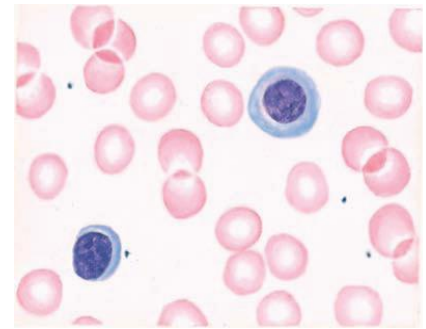
Where they change into large phagocytic cells called **macrophages**. Macrophages phagocytize bacteria, cell fragments, dead cells and debris.

Granular leucocytes:- contain specific granules and many loyed nucleus . they are three type of granular leucocytes :-

1- Neutrophils : polymorphous leucocytes , nucleus has from 3-5 irregular ovoid lobes connected by fine threads of chromatin . the cytoplasm filled with fine granular .

Function:

Specifically, neutrophils target and kill bacteria by secreting **lysozyme**, an enzyme that helps destroy component of bacteria cell walls.

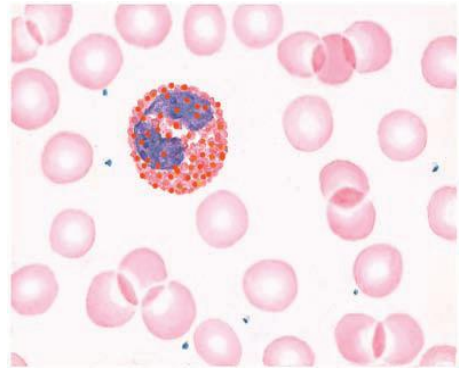


b – Eosinophil (acidophil):- are larger than neutrophils. The nucleus is usually bi-lobed. The cytoplasm is filled with coarse granules and stain with acidic dyes.

Function:

Eosinophils increase in number when they encounter and react to or phagocytize antigen-antibody complexes or **allergens** (antigens that initiate a hypersensitive or allergic reaction)

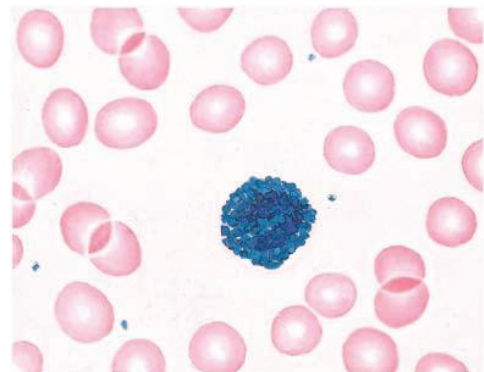
If the body is infected by parasitic worms, the eosinophils will release chemical mediators that attack the worms.



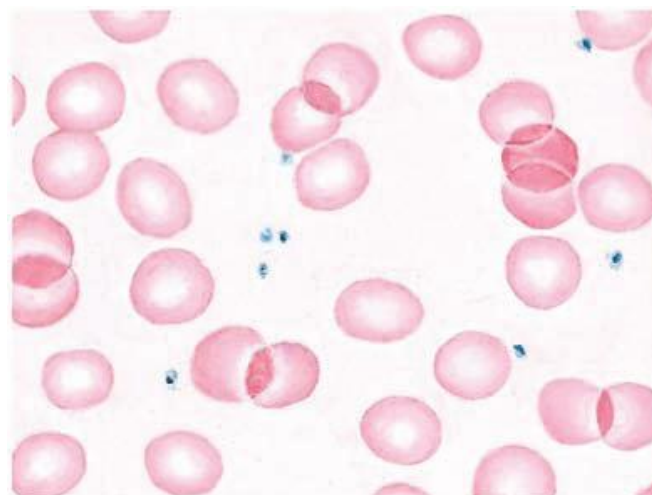
C–Basophiles: - are the same sizes as neutrophil, nucleus usually irregular two lobes appearing as (S) shape. The cytoplasmic granules are coarse and variable in size.

Function:

The primary components of basophile granules are **histamine and heparin**, the release of heparin from basophiles inhibits blood clotting



Blood platelets: - are small protoplasmic disks that are colorless in circulatory blood. Platelets are round or ovoid and aggregate as group.



Muscular Tissue

Muscular tissue composed of specialized cell (fiber) for producing movement body. We can classify muscular tissue according to the function and structure to: -

- *Smooth muscle
- *Skeletal muscle
- *Cardiac muscle

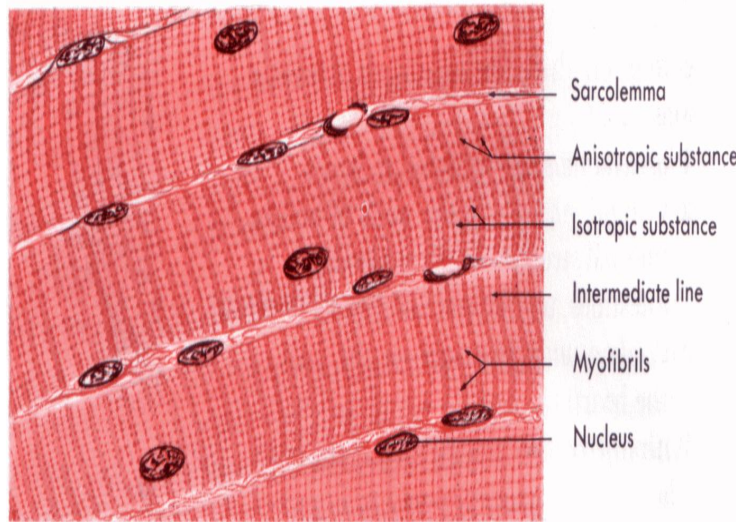
Smooth muscle

- *its non – striated , involuntary muscle , visceral distribution .
- *its present in wall of digestive tract from mid – esophagus to anus , urinary and genital system .
- *L.S in smooth muscle, fibers spindle shape, with flattened central nucleus, cytoplasm called sarcoplasm which contain many myofibrils.
- *C.S in smooth muscle appear different in size, its may appear wide and narrow and may be with nucleus or without nucleus.



Skeletal muscle

- *its striated voluntary muscle
- *it's attached to skeletal back bone.
- *L.S muscle fiber show alternating dark A band (anisotropic) , light I band (isotropic) , and Z line in the middle of I band . in general this section of skeletal muscle appear as cylindrical , parallel and filamentous bundle with multi-peripheral nucleus .
- *C.S the fibers section appear polygon or round shape with different diameter. The myofibrils appear as dots with clear.



Skeletal or striated voluntary muscle tissue.

Cardiac muscle

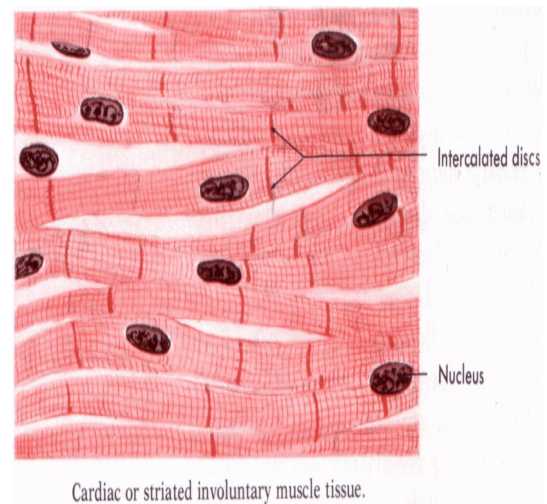
*its striated, involuntary muscle, contract rhythmically and automatically.

***it's found only in muscles layer of heart and the large vessels joining the heart.**

***A** cardiac muscle fiber is shown by light microscope to linear unit compose of several cardiac muscle cell joined end to end at specialized junction zone called **intercalated disks** the fiber to the cell units .

*** L.S** the myofibrils appear branched, striated similar to skeletal muscle. We can see the intercalated disk, the cardiac myofibrils have central, single nucleus.

*** C.S** the myofibrils irregular and smaller than the section skeletal myofibrils, and myofibrils rough than myofibrils, central and single nucleus in each fiber.



Cardiac or striated involuntary muscle tissue.

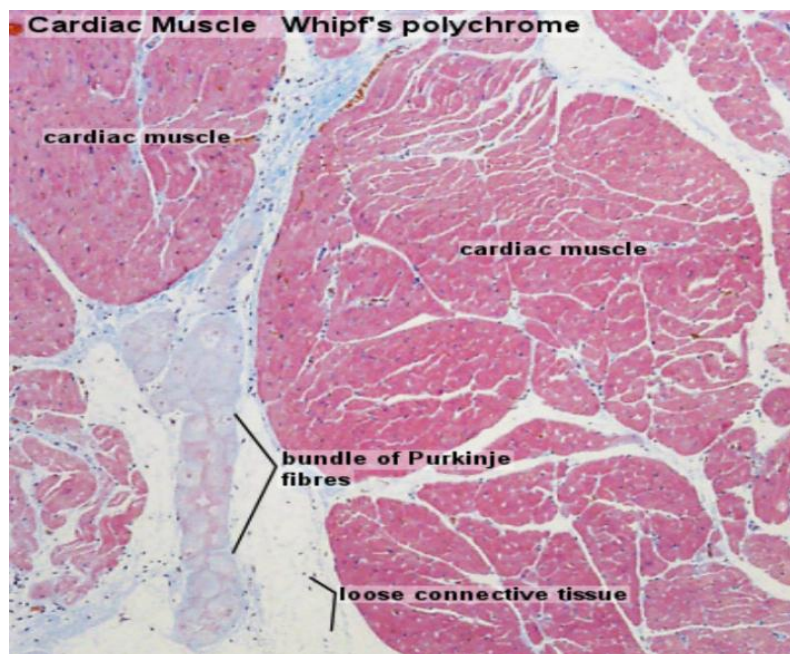
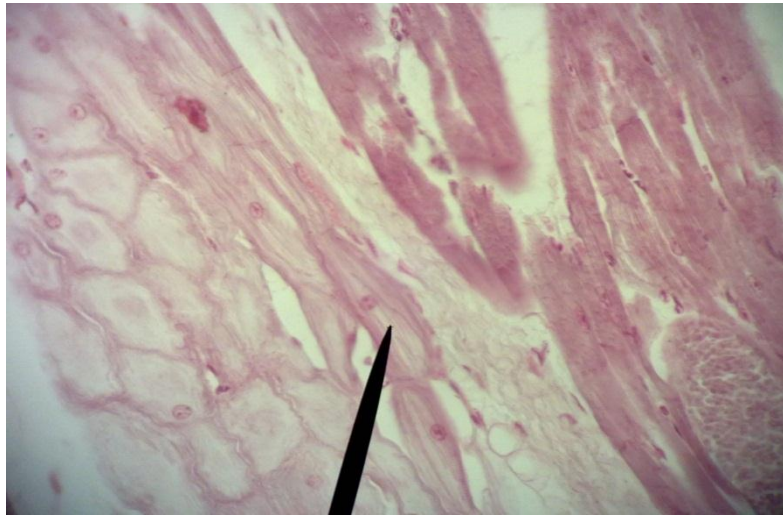
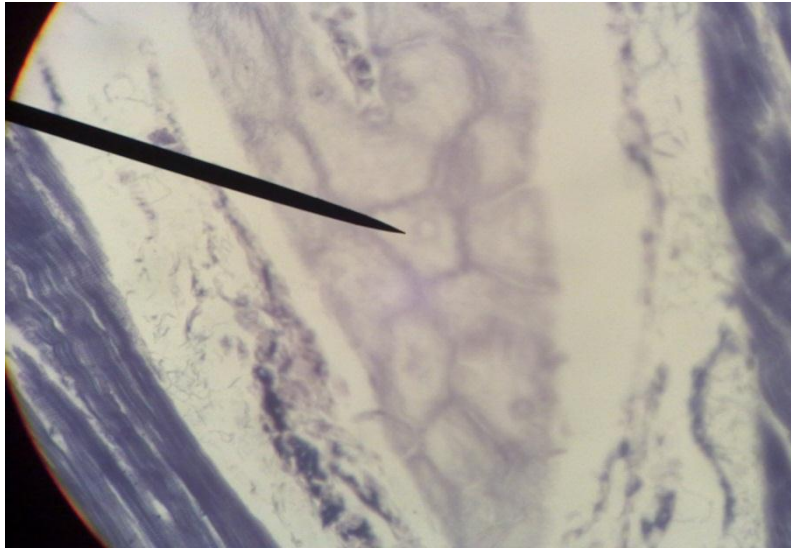
purkinje fibers

*they are specialized cardiac muscles .

*** they are located just near the endocardium on the internal surface of the heart .**

***L.S** the purkinje fibers compared with cardiac fibers appear shorted , wide thick and more palely staining with central nucleus and few myofibrils which usually are found peripheral position.

***C.S** the purkinje fibers appear as cell group (3-4) cells . the intercalated disks are present but not seen commonly .



Nervous tissue

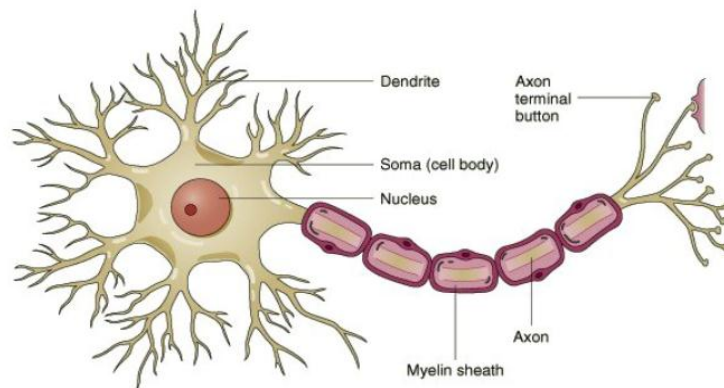
- Is responsible for transport nervous impulse (motor and sensory impulse) , and it is formed by network more 100 million nerve cell (neuron) nerve fiber and nerve ending , nerve tissue develop from ectoderm .
- **Nerve cell (neurons)** :- are responsible for reception transmission and processing of stimuli and release neurotransmitters and are consist of :-

- **Dendrites**
- **Cell body**
- **Axon**

Dendrites: - which are multiple elongated processes specialized for receiving stimuli from environment

Cell body: - perikaryon

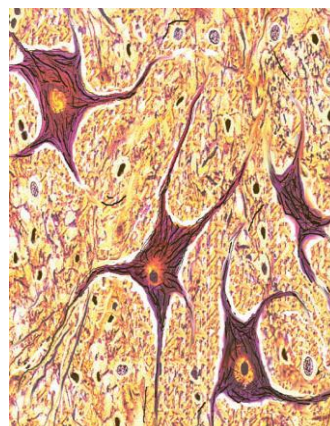
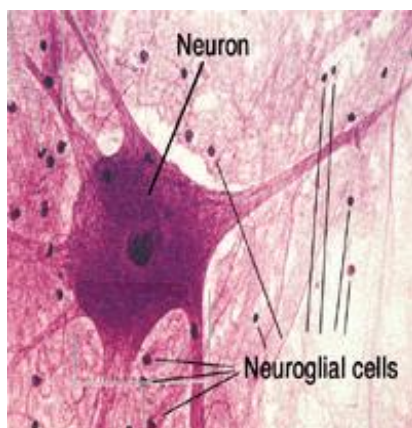
Axon: - single process specialized in generating or conducting nerve impulse to other cells (nerve, muscle, and gland).



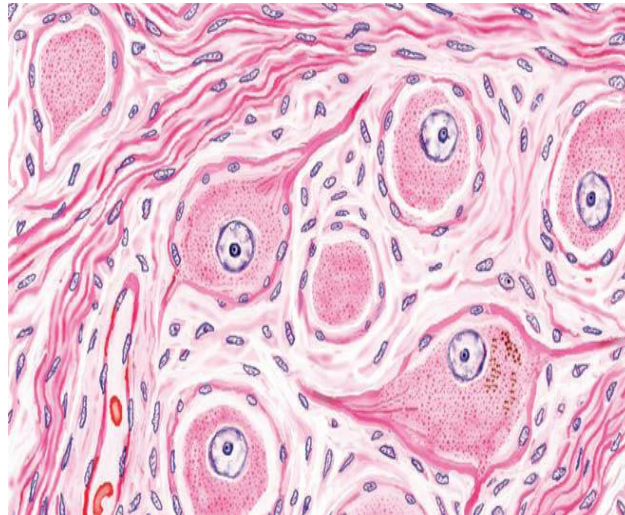
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Nerve cell classified to 3 types according to numbers of process

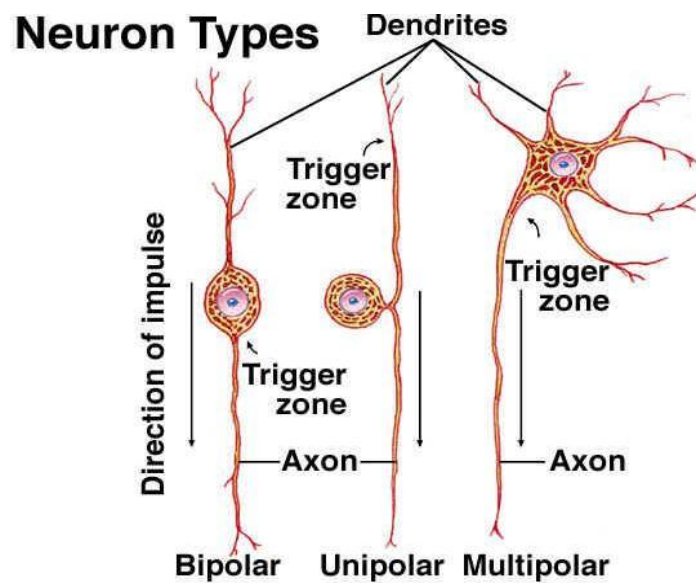
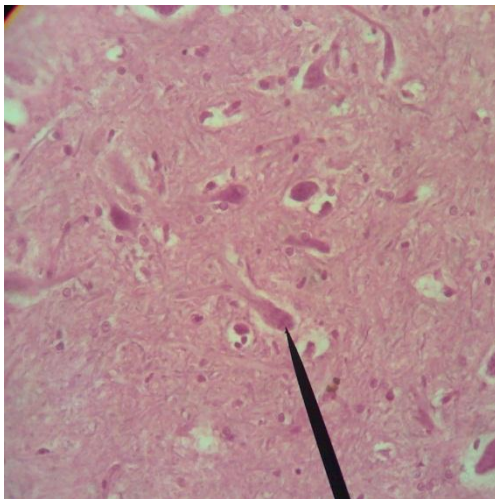
1-**Multipolar:** - which have more than 2 processes. Most neurons of the body are multipolar.



2- **Bipolar:** - which have 2 processes. **Found retina and olfactory mucosa**



3- **Pseudounipolar:** - which have single process and it divide to 2 branches. **Found spinal ganglia and cranial ganglia**

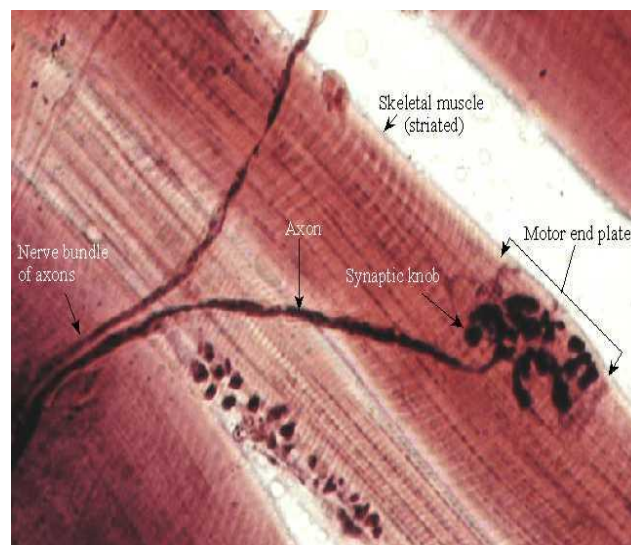


- **Fiber:** - consist of axons enveloped by special sheath of **Schwann cell**. and classified to :-
- **Myelinated fibers:** - are the fiber which enveloped with multilayer Schwann's plasmalemma and unite and form myelin sheath and the space between 2 Schwann cell is called **node of Ranvier**. Found mainly in **PNS**.
- **Un Myelinated fibers:** - the axons are enveloped within simple cleft of Schwann cells found in **CNS**.

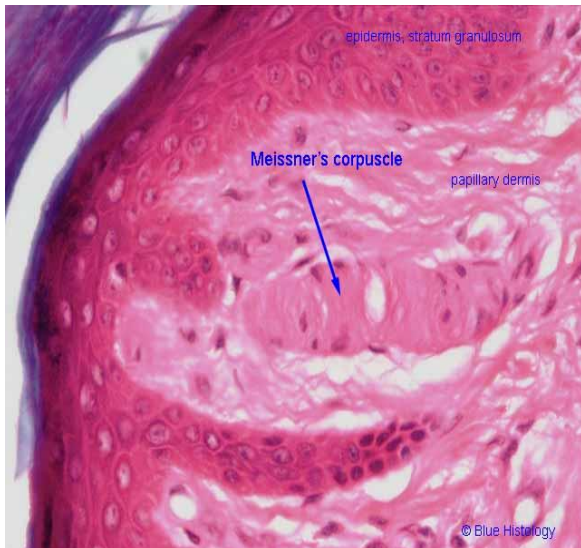


Myelinated nerve fibers (longitudinal and transverse sections)

Motor nerve end: - in which nerve fiber end in striated muscles and becomes unmyelinated and branch and end with dendrites .



Meissner corpuscles :- are small encapsulated sensory receptors **found in the dermis of skin (finger tip , foot , eyelid , lips)** meissner corpuscles are oval shape the receptors consist of delicate collagenous tissue capsule surrounding amass of plump , oval cells arranged transversely and representing specialized Schwann cells and non myelinated sensory fiber verify throughout the cell mass in helical meaner .



Pacinian corpuscles: - large encapsulated sensory responsive to pressure or coarse touch, vibration and tension **found in deep skin layer, ligament**. These organs consist of delicate capsule enclosing many concentric lamellae of flattened cell

