General Histology

The Cell

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Introduction :

Histology:(histo=tissue ,logia=branch of learning) is the science that deals with microscopic study of components of humans ,animal and plants which formed of cells ,tissues ,organs and systems.

Cells:

The cells is the structural and functional unit in the body.it is the smallest unit of living tissues in and animals that can exist independently

- **Tissues**:
- Cells are bound together with extracellular substances to form different types of tissues
- **There are 4 basic types of tissues:**
  - Epithelial tissue
  - Connective tissue
  - Muscular tissue
  - Nervous tissue

- **organs**:
- Two or more tissues are combined to give larger functional units , which are the organs e.g kidney , liver ,and spleen
- **Systems**: several organs having interrelated functions collect to form systems e.g digestive system , urinary system
- For scientific or diagnostic purposes , microscopic slide must be prepared from tissue and organs and stained to be viewed under the microscope

LIGHT MICROSCOPY

Conventional bright-field microscopy, as well as more specialized applications like fluorescence, phase-contrast, confocal, and polarizing microscopy, are all based on the interaction of light with tissue components and are used to reveal and study tissue features.

CONVENTIONAL MICROSCOPY

- Are standard optical instruments for examination of histological specimens. The basic type is the light microscope, the other types are modifications of the original light microscope. Their usefulness depends on their magnification power and resolution power
- The magnification power : is the degree of enlargement.
- The resolution power :is the least distance between two points or objects that can be seen as two and not as one point .

N.B : The limit of resolution of naked human eye = 0.2mm .

The limit of resolution of the light microscope =0.2µm.

The limit of resolution of electron microscope =0.2nm .

N.B : Milimeter (mm) =1000 micrometer (µm).

Micrometer (µm) = 1000 nanometers (nm) .

Nanometer (nm) = 10 angstroms(A) .

Fluorescence Microscopy

When certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength—a phenomenon called fluorescence. In fluorescence microscopy, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear bright on a dark background. For fluorescent microscopy the instrument has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.

Phase-Contrast Microscopy

Unstained cells and tissue sections, which are usually transparent and colorless, can be studied with these modified light microscopes. Cellular detail is normally difficult to see in unstained tissues because all parts of the specimen have roughly similar optical densities. Phase-contrast microscopy, however, uses a lens system that produces visible images from transparent objects and, importantly, can be used with living, cultured cells

Phase-contrast microscopy is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices.

These changes are used by the phase-contrast system to cause the structures to appear lighter or darker in relation to each other. Because they allow the examination of cells without fixation or staining, phase-contrast microscopes are prominent tools in all cell culture laboratories.

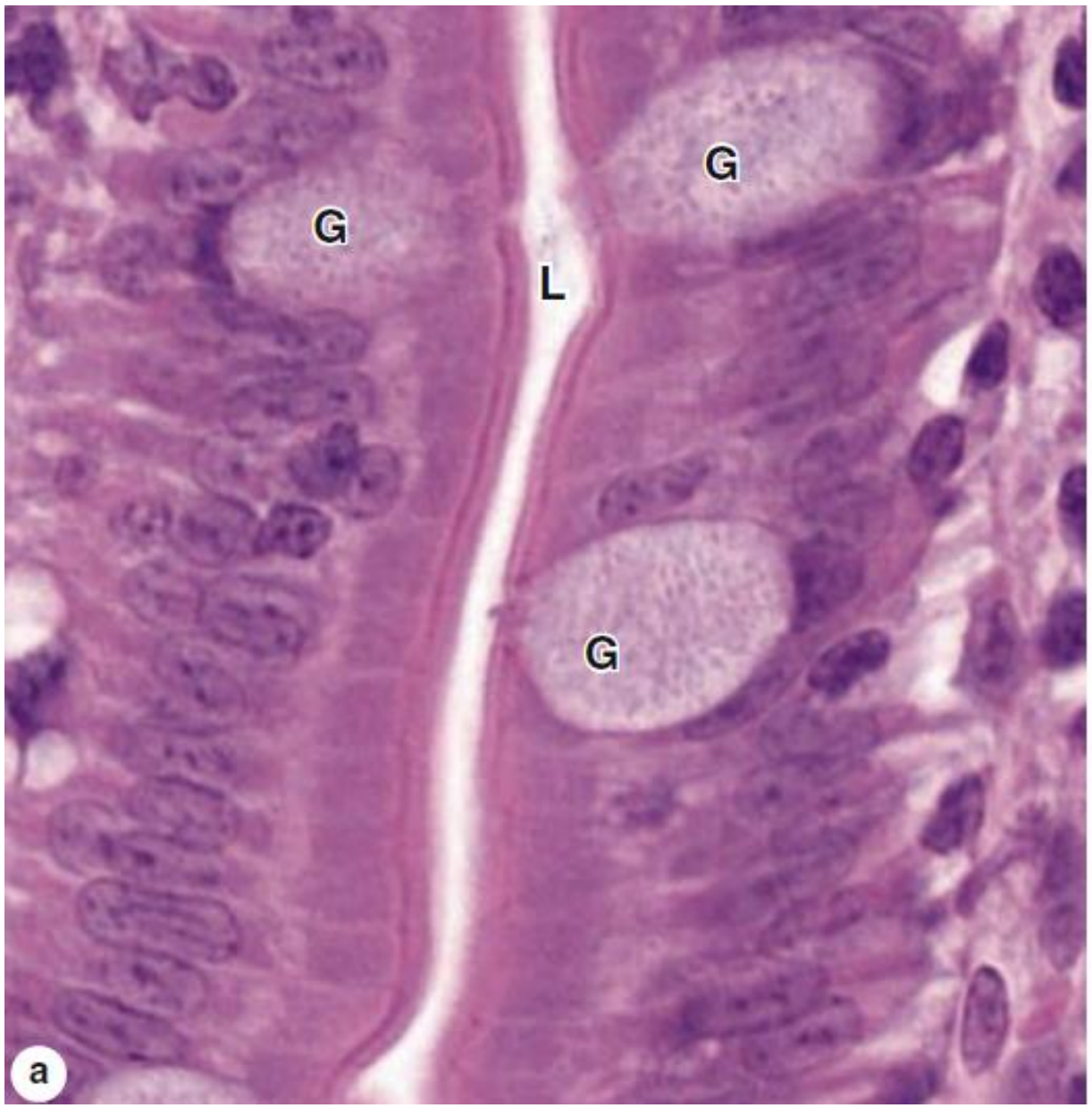
Polarizing Microscopy

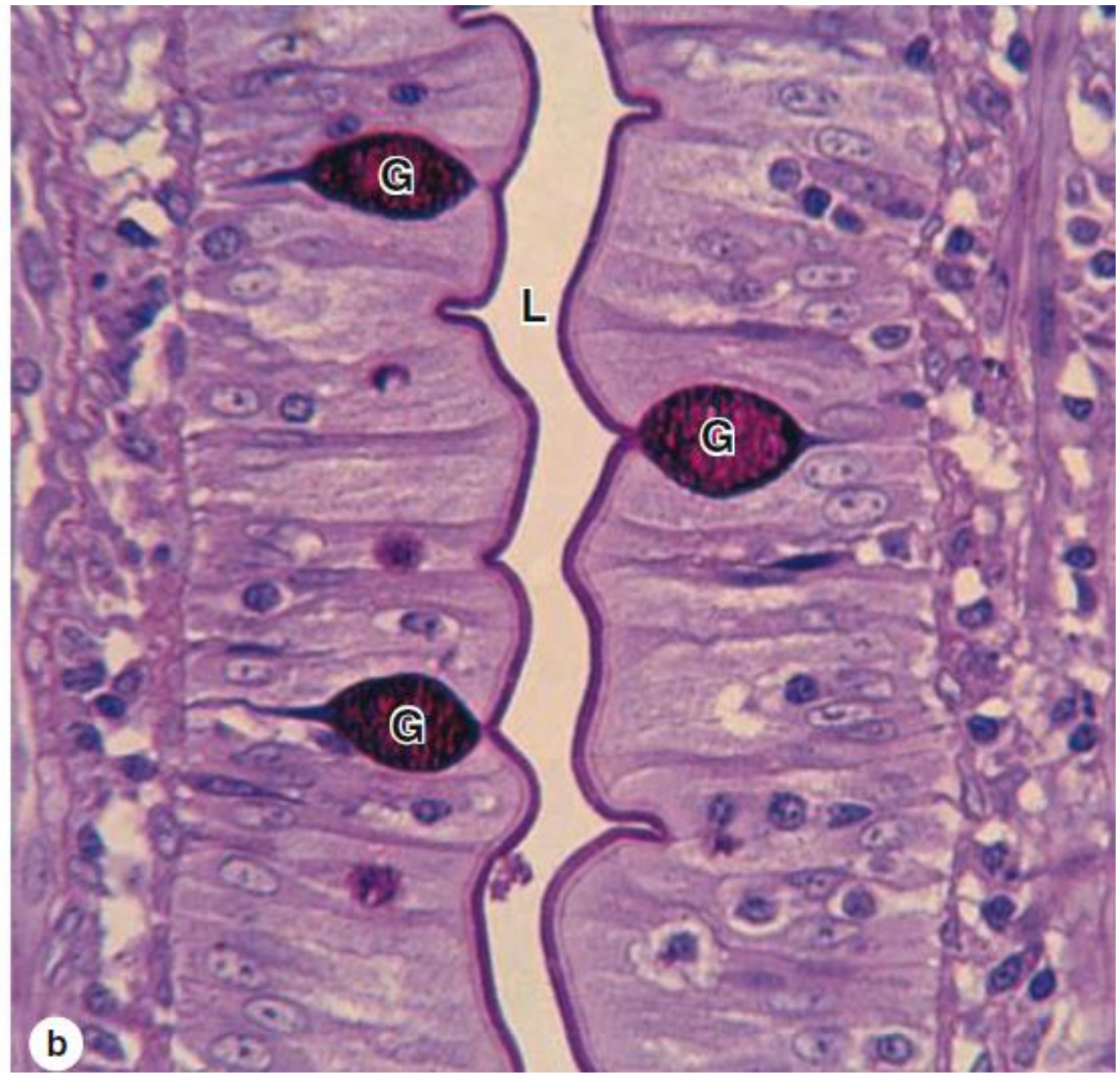
allows the recognition of stained or unstained structures made of highly organized subunits. When normal light passes through a polarizing filter, it exits vibrating in only one direction. If a second filter is placed in the microscope above the first one, with its main axis perpendicular to the first filter, no light passes through. If tissue structures containing oriented macromolecules are located between the two polarizing filters, their repetitive structure rotates the axis of the light emerging from the polarizer and they appear as bright structures against a dark background . The ability to rotate the direction of vibration of polarized light is called birefringence

Preparation of tissues for microscopic examination

- There are four steps in tissue preparation:
- 1-Fixation:stabilizes and preserves the tissue
- 2-Embedding: converts the tissue into a solid form which can be sliced.
- 3-Sectioning(slicing):providing the very thin specimens needed for microscopy.
- 4-Staining:provides visual contrast and may help to identify specific tissue components.


52°-60°C
(a)
Fixation
Dehydration
Clearing
Infiltration
Embedding


G
G
L
G
a


G
L
G
G
b

The basic techniques used for preparation of tissues:

- 1-microtechniques
- 2-tissue culture
- 3-spreading of blood films , bone marrow and tissue smear

- **1-microtechniques**
  - The paraffin technique (the most common used method)
  - The celloidin technique (the most perfect used method)
  - The freezing technique(the most rapid used method)

Enzyme Histochemistry

Histochemical methods are also used to identify and localize enzymes in cells and tissues.

To localize enzymes in tissue sections, special care must be taken in fixation to preserve the enzyme activity. Usually, mild aldehyde fixation is the preferred method. In these procedures, the reaction product of the enzyme activity, rather than the enzyme itself, is visualized. In general, a capture reagent, either a dye or a heavy metal, is used to trap or bind the reaction product of the enzyme by precipitation at the site of reaction.

Immunocytochemistry

The specificity of a reaction between an antigen and an antibody is the underlying basis of immunocytochemistry. Antibodies, also known as immunoglobulins, are glycoproteins that are produced by specific cells of the immune system in response to a foreign protein, or antigen. In the laboratory, antibodies can be purified from the blood and conjugated (attached) to a fluorescent dye. In general, fluorescent dyes (fluorochromes) are chemicals that absorb light of different wavelengths (e.g., ultraviolet light) and then emit visible light of a specific wavelength (e.g., green, yellow, red).

Fluorescein, the most commonly used dye, absorbs ultraviolet light and emits green light. Antibodies conjugated with fluorescein can be applied to sections of lightly fixed or frozen tissues on glass slides to localize an antigen in cells and tissues.

Two types of antibodies are used in immunocytochemistry: polyclonal antibodies that are produced by immunized animals and monoclonal antibodies that are produced by immortalized (continuously replicating) antibody-producing
cell lines.

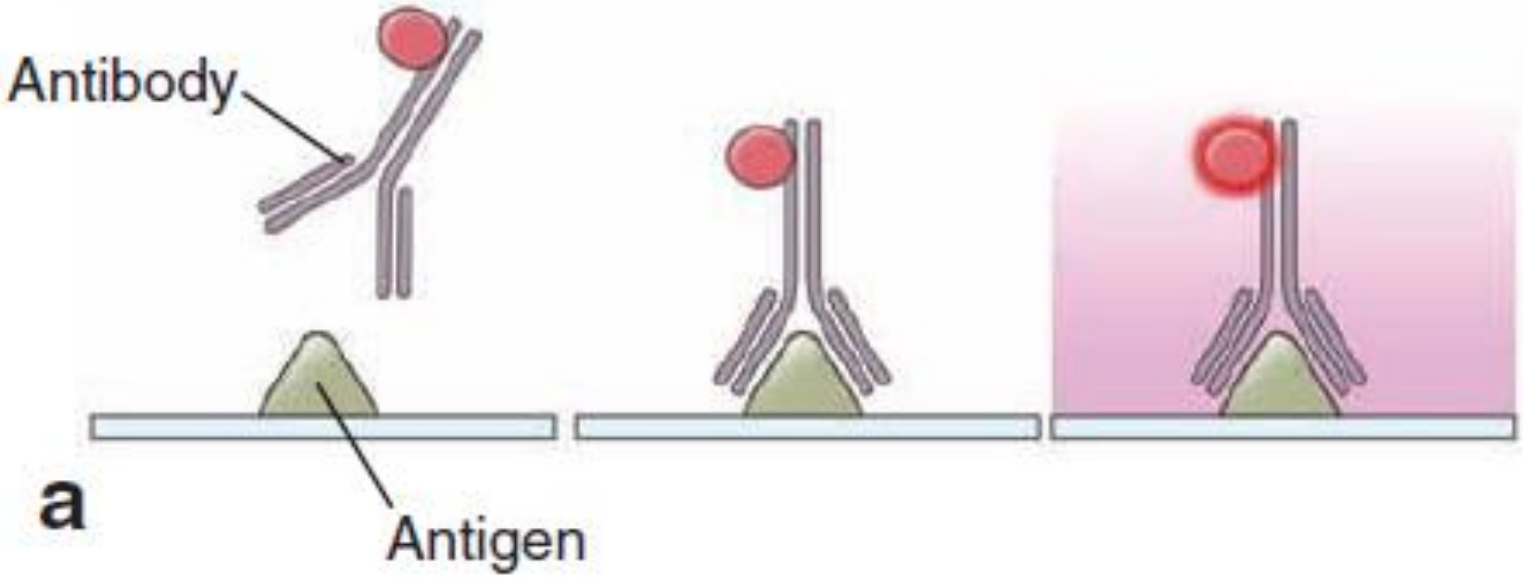
In a typical procedure, a specific protein, such as actin, is isolated from a muscle cell of one species, such as a rat, and injected into the circulation of another species, such as a rabbit. In the immunized rabbit, the rat's actin molecules are recognized by the rabbit immune system as a foreign antigen. This recognition triggers a cascade of immunologic reactions involving multiple groups (clones) of immune cells called B lymphocytes. The cloning of B lymphocytes eventually leads to the production of anti-actin antibodies.

Collectively, these polyclonal antibodies represent mixtures of different antibodies produced by many clones of B lymphocytes that each recognize different regions of the actin molecule.

The antibodies are then removed from the blood, purified, and conjugated with a fluorescent dye. They can now be used to locate actin molecules in rat tissues or cells.

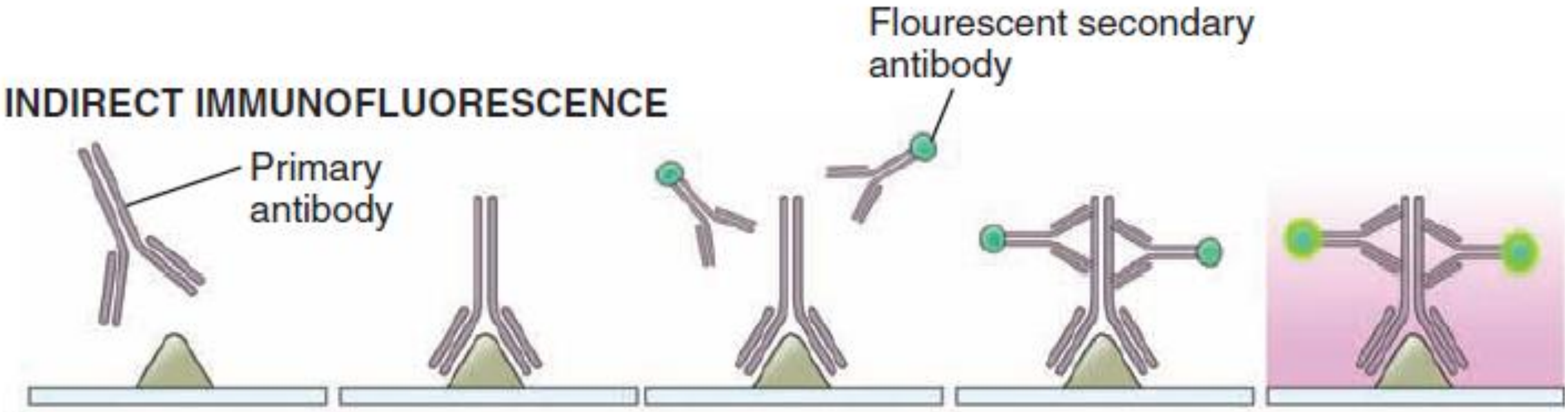
Monoclonal antibodies are those produced by an antibody-producing cell line consisting of a single group (clone) of identical B lymphocytes. The single clone that becomes a cell line is obtained from an individual with multiple myeloma,

DIRECT IMMUNOFLUORESCENCE



a

INDIRECT IMMUNOFLUORESCENCE



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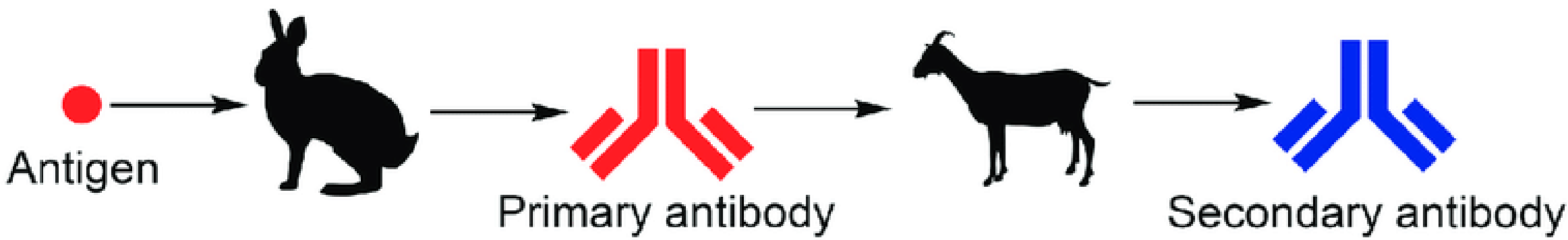
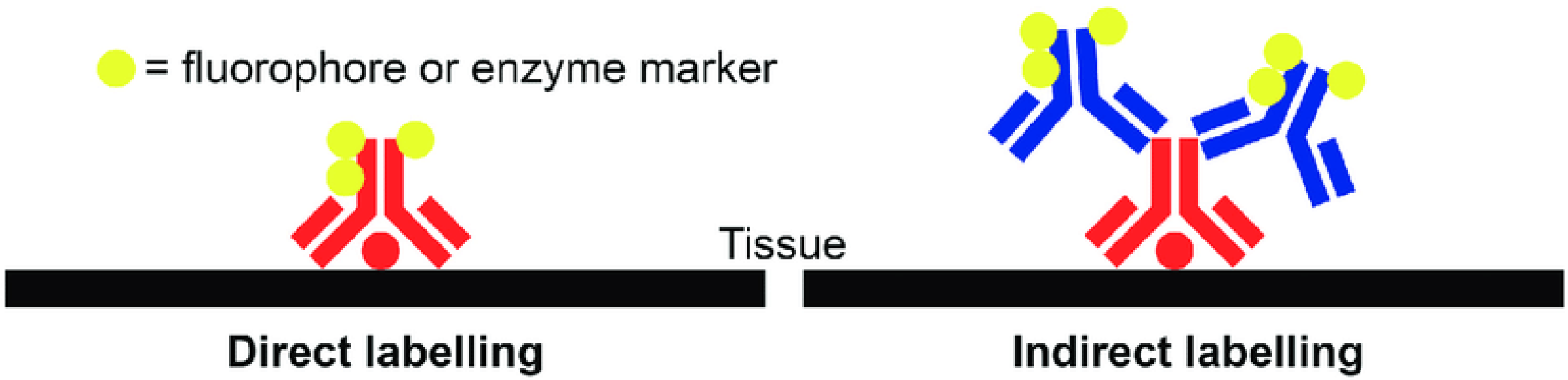
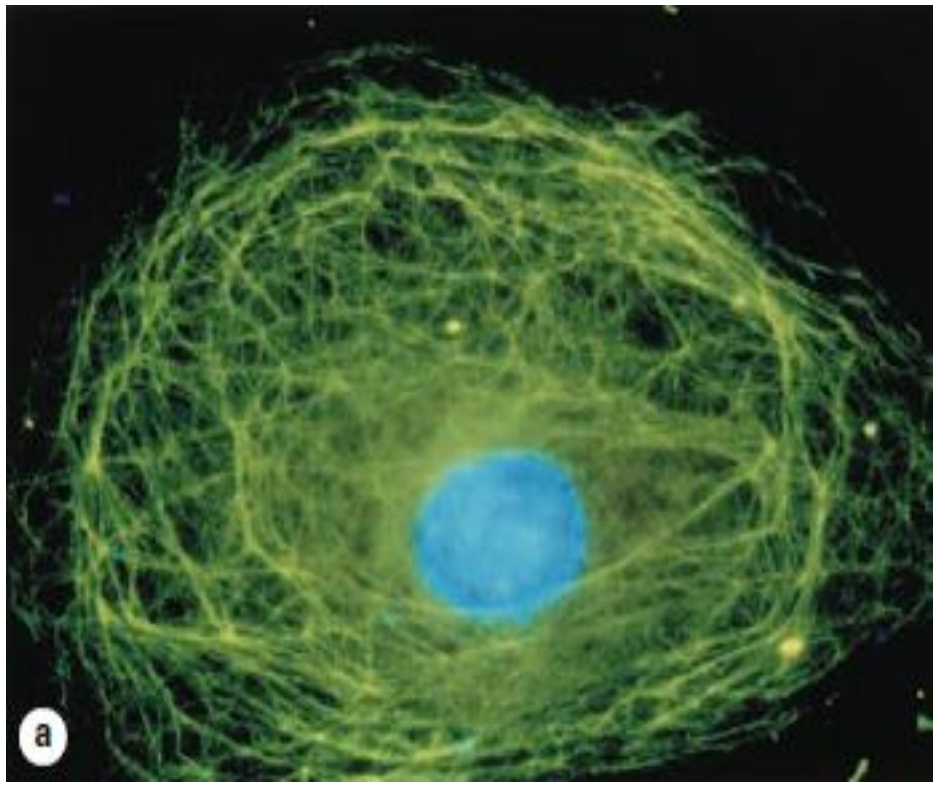

Antigen
Primary antibody
Secondary antibody


= fluorophore or enzyme marker
Tissue
Direct labelling
Indirect labelling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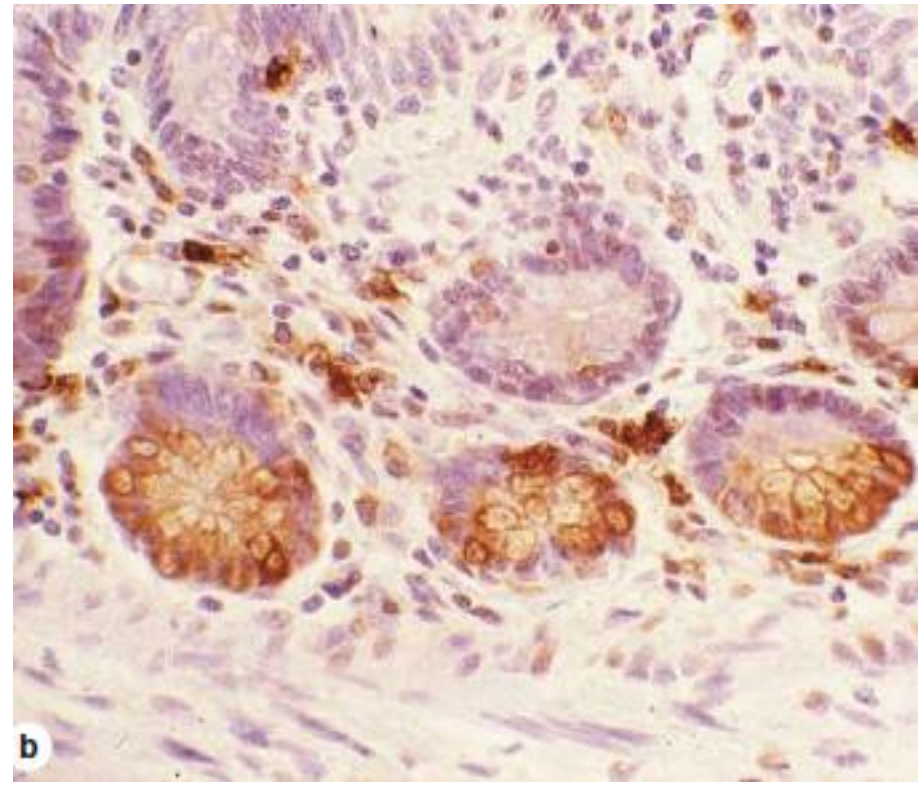
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Hybridization Techniques

-FISH is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity.

-Is used to detect and localize the presence or absence of specific DNA sequences on chromosomes affixed to a microscope slide .

-Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.

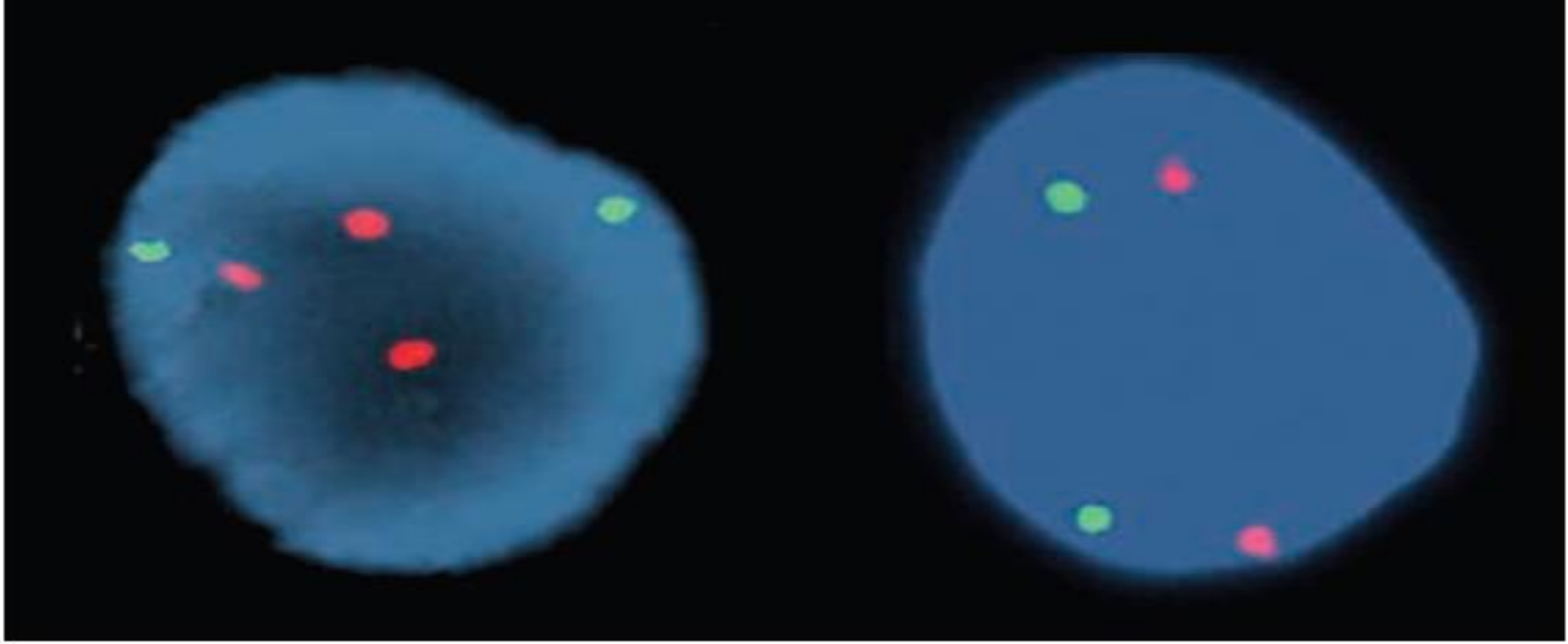


FIGURE 1.7 ▲ Example of the FISH technique used in a prenatal screening test. Interphase nuclei of cells obtained from amniotic fluid specimens were hybridized with two specific DNA probes. The *orange* probe (LSI 21) is locus specific for chromosome 21, and the *green* probe (LSI 13) is locus specific for chromosome 13. The *right* nucleus is from a normal amniotic fluid specimen and exhibits two *green* and two *orange* signals, which indicates two copies of chromosomes 13 and 21, respectively. The nucleus on the *left* has three *orange* signals, which indicate trisomy 21 (Down syndrome). DNA has been counterstained with a nonspecific blue stain (DAPI stain) to make the nucleus visible. ×1,250. (Courtesy of Dr. Robert B. Jenkins.)

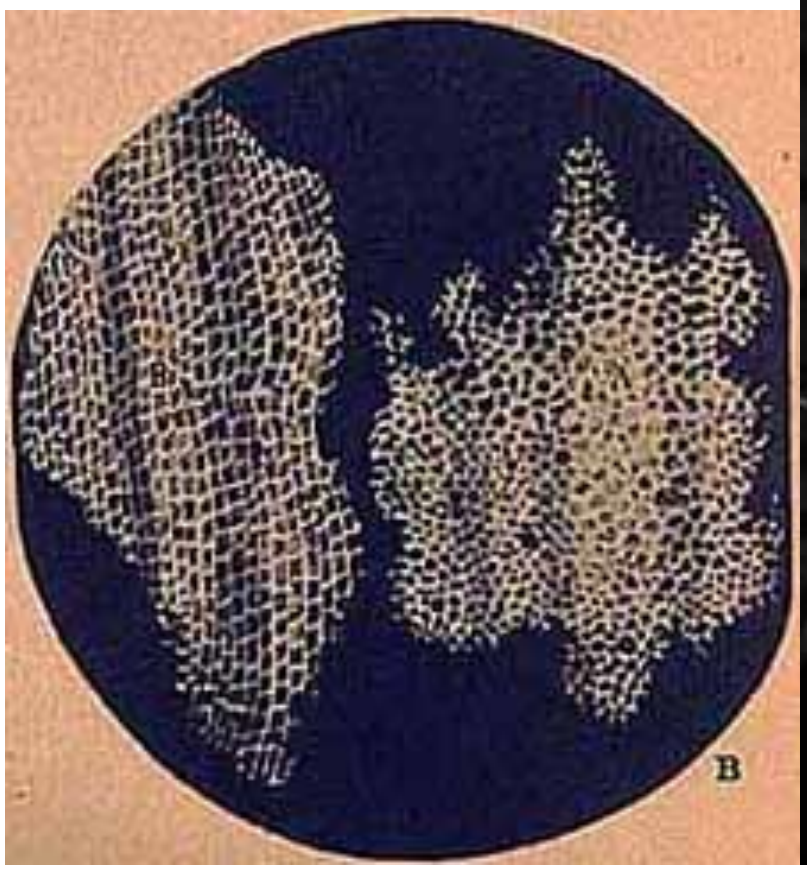
The Cell

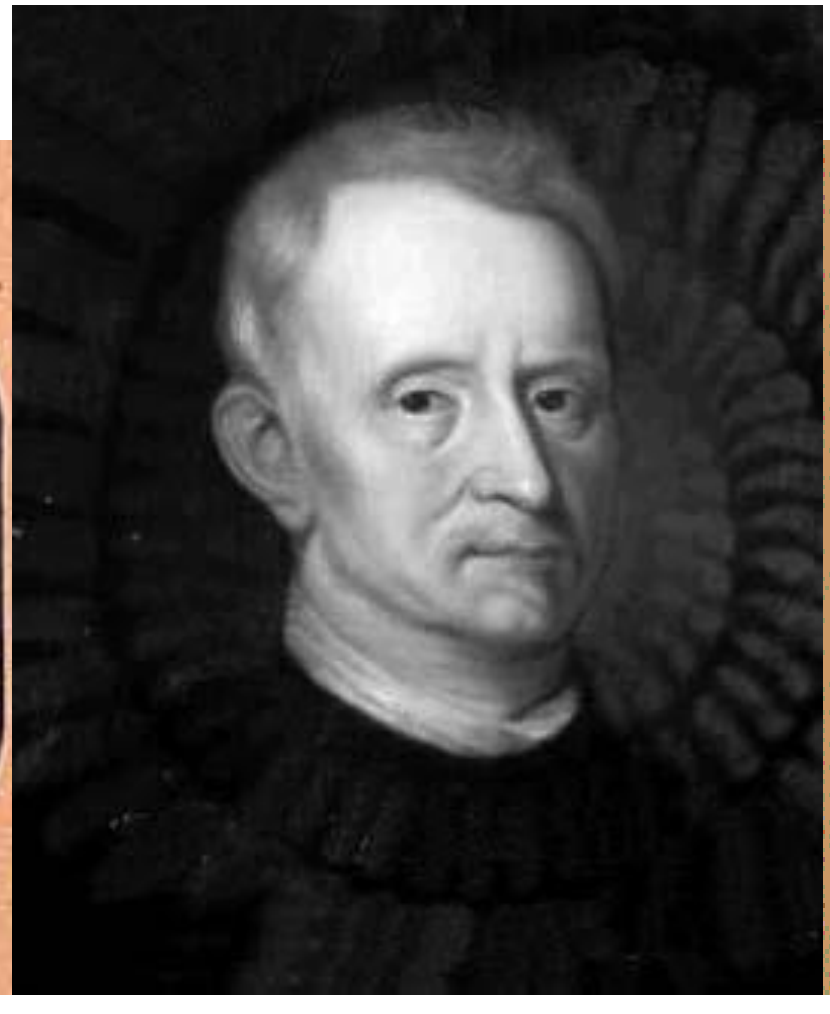
Definition: the smallest unit of living material that can live independently and perform vital functions. It is considered the structural & functional unit of all living tissues.

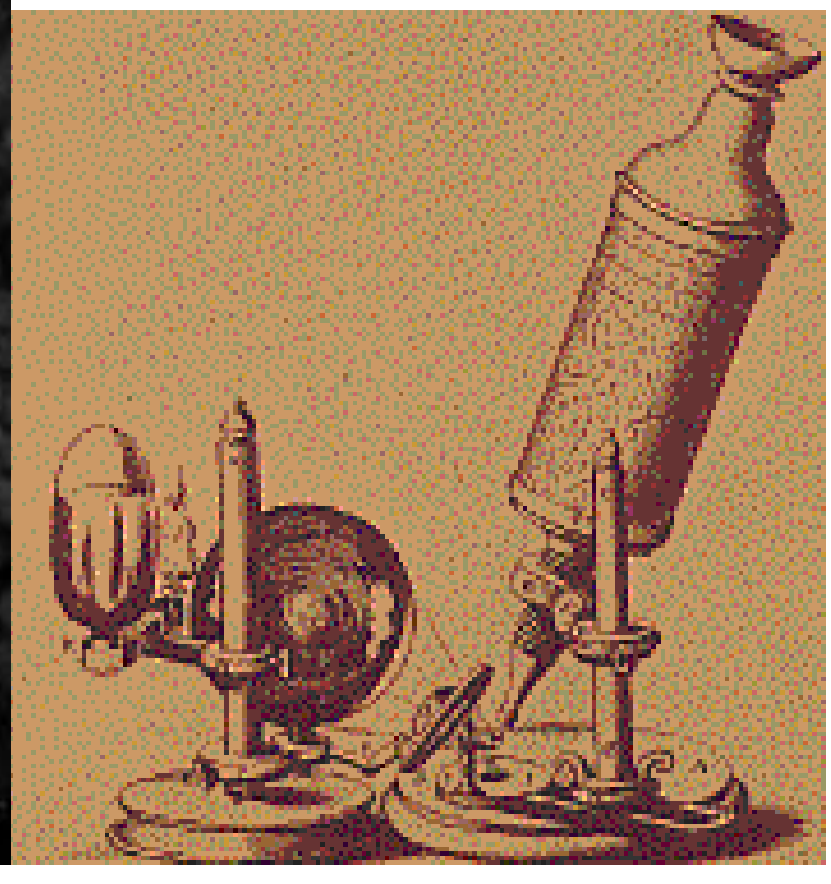
The term ‘CELL’ comes from the latin word cella meaning the small room

The Discovery of the Cell

- Robert Hooke – first to see and identify cork "cells."







Prokaryotic Cell

(in Greek. Pro= before, karyon= nucleus)

- Only in bacteria.
- Smaller in size.
- Have a cell wall
- No nuclear membrane or histones(specific basic proteins) bound to their DNA and usually no membranous organelles.

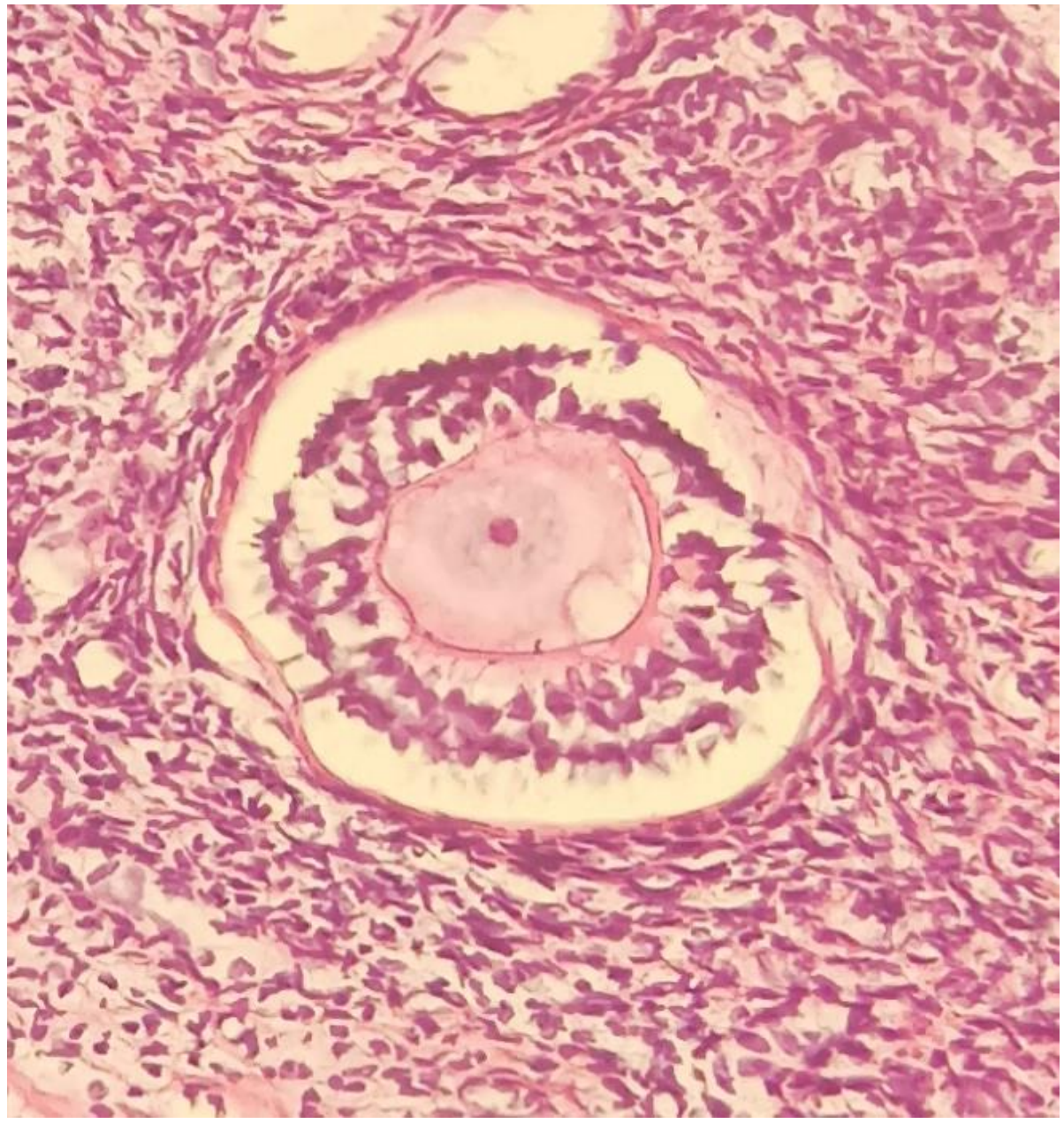
Eukaryotic Cell

(in Greek eu= good, karyon= nucleus)

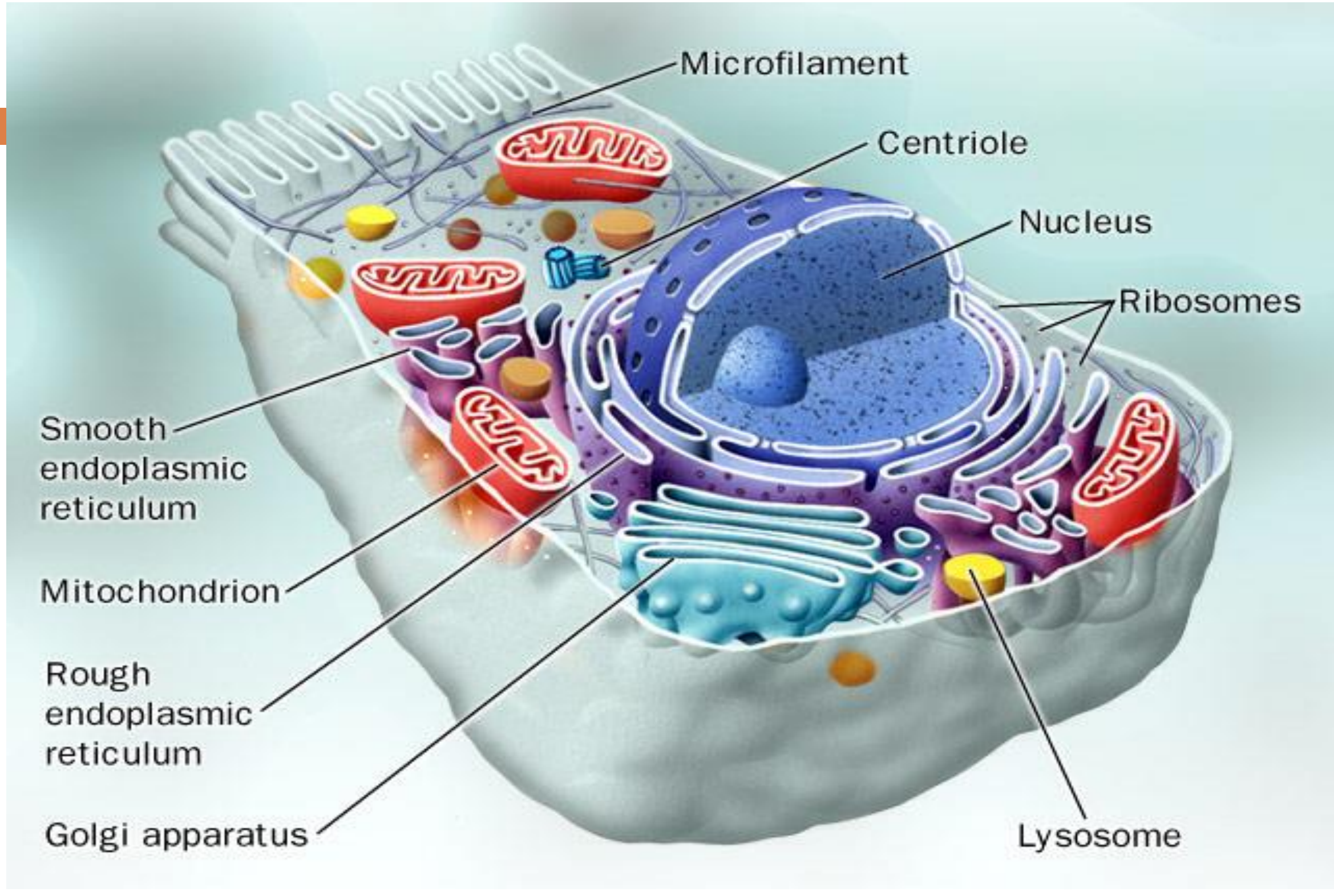
- Larger and have a distinct nucleus containing DNA & histones surrounded by a nuclear envelope.
- Humans have only eukaryotic cells

- **Functions:** secretion, respiration, absorption, reproduction, excretion, sensation, contraction, conduction, movements, and growth.

- **Size:** varies from **4** μm to **150** μm, e.g. small cells as lymphocyte **(6** μm),
- large cells as ovum **(150** μm).



- **Shape:** rounded, oval, flat, stellate, polygonal, cubical & columnar.
- **Structure:** two major components:
- 1- Cytoplasm.
- 2- Nucleus.


Microfilament
Centriole
Nucleus
Ribosomes
Smooth endoplasmic reticulum
Mitochondrion
Rough endoplasmic reticulum
Golgi apparatus
Lysosome

CYTOPLASM

Matrix

Colloidal, viscous solution of:

- Proteins
- Carbohydrates & lipids
- Enzymes & minerals
- Ions & salts

Organelles

- Living structures
- Permanent
- Essential in all nucleated cells
- Metabolically active
- Have vital functions
- Specialized structures

Inclusions

- Non-living
- Temporary
- Usually not essential
- Not in all cells
- Metabolically inert
- Result from cell activity

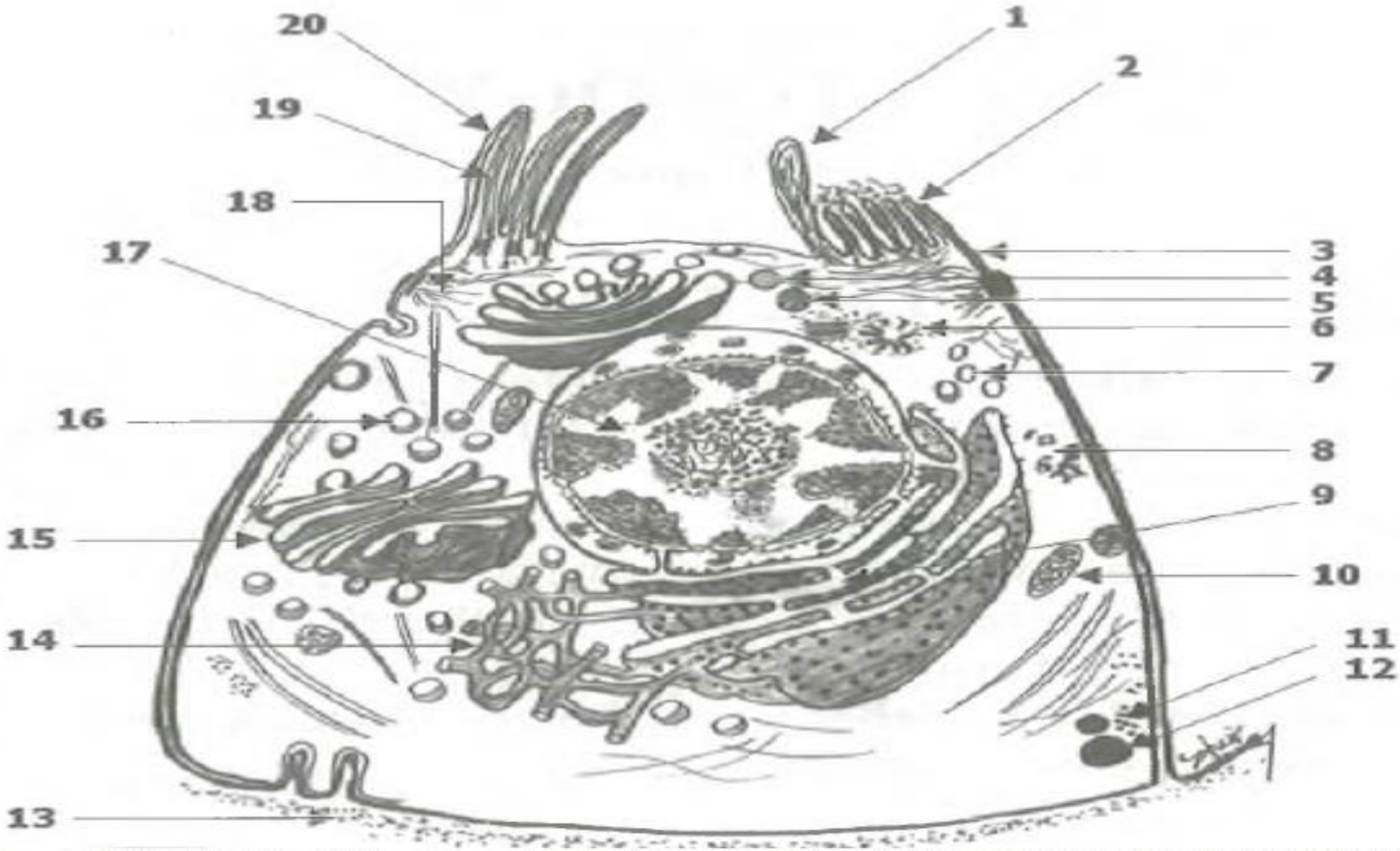


Diagram showing EM structure of the cell. **1** Stereocilia; **2** Microvilli; **3** Cell membrane; **4** Primary lysosome; **5** Secondary lysosome; **6** Centrioles; **7** Transfer vesicles; **8** Free ribosomes; **9** Rough endoplasmic reticulum; **10** Mitochondria; **11** Glycogen granules; **12** Lipid droplet; **13** Basement membrane; **14** Smooth endoplasmic reticulum; **15** Golgi apparatus; **16** Secretory vesicles; **17** Nucleus; **18** filaments; **19** Microtubules; **20** Cilia.

Cytoplasmic Organelles

- **Definition:** specialized intracellular structures that, like body organs, perform specific functions.
- They are divided according to whether they have membranes or not into:

Organelles

Membranous

- Covered by membrane
- Contain enzymes

1. **Cell Membrane**
2. **Mitochondria**
3. **Endoplasmic reticulum (rough & smooth)**
4. **Golgi apparatus**
5. **Lysosomes**
6. **Peroxisomes**
7. **Coated vesicles**

Non-Membranous

- Not covered by membrane
- Do not have enzymes

1. **Ribosomes**
2. **Cytoskeleton:**

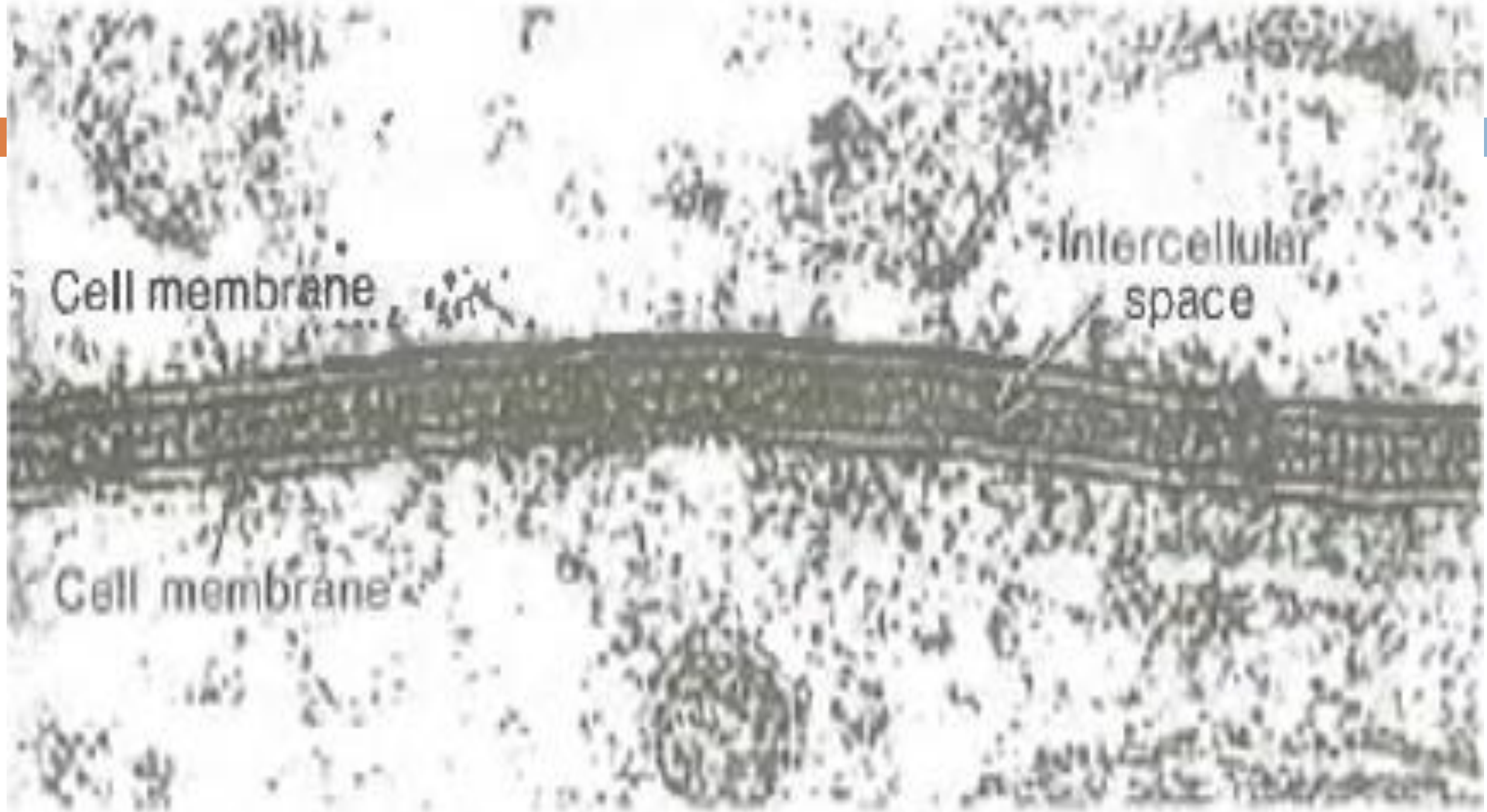
a) **Microtubules:**
- **Centrioles**
- **Cilia & flagella**

b) **Filaments:**
- **Thin**
- **Thick**
- **Intermediate**

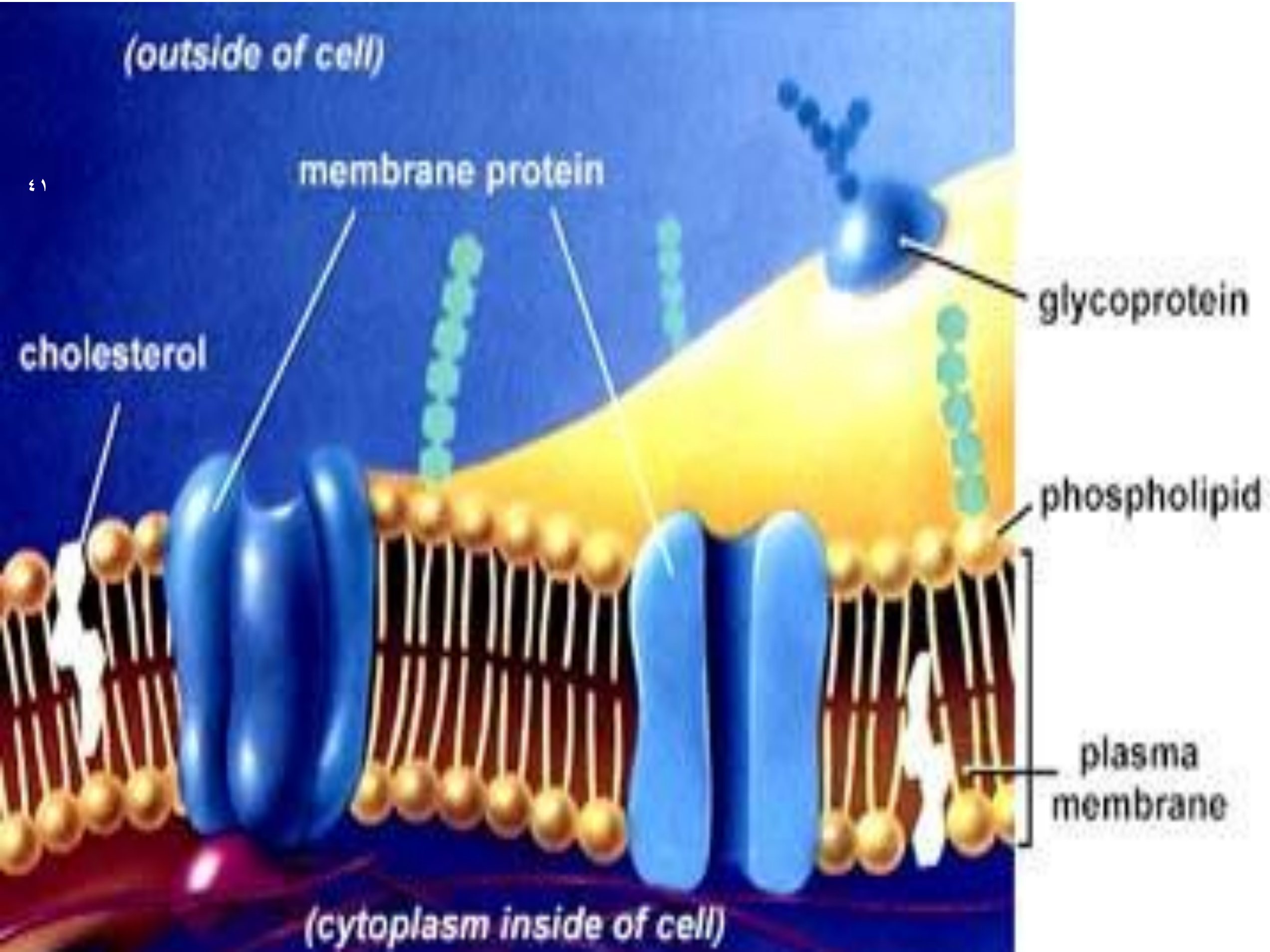
- **A- Membranous Organelles**

- **1- The Cell Membrane (Plasmalemma)**
- **Definition:** a living membrane forming the outermost cover of the cytoplasm.
- **LM:**
- Difficult to be seen because it is very thin (8-10 nm).
- Can be demonstrated when stained with **silver (Ag)** or **PAS** stains.

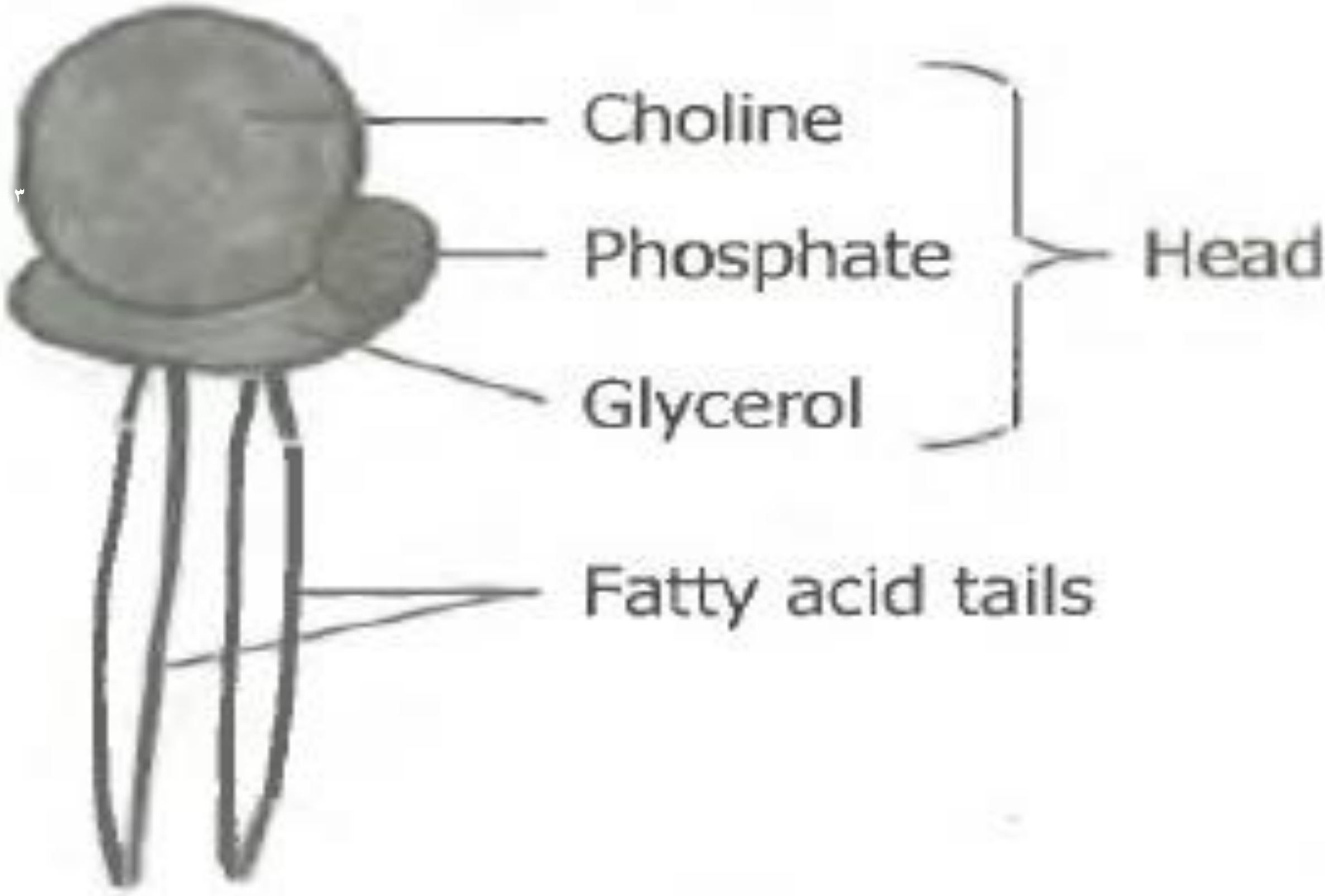
- **EM:**
- Two dark (electron-dense) lines separated by a light one, i.e. 3 parallel lines, so called **‘trilamellar membrane** or **“unit membrane”.**
- There is a fuzzy layer found on the outer surface only, which represents the **cell coat** or **(glycocalyx).**



EM Picture of the cell membrane of two adjacent cells.


(outside of cell)
membrane protein
cholesterol
glycoprotein
phospholipid
plasma membrane
(cytoplasm inside of cell)

- **Molecular Structure:**
- It consists of different components: lipids 30 %, proteins 60% and CHO 10%.
- **1- Lipid component:** consists of phospholipids and cholesterol.
- **a) Phospholipid molecules:** arranged into 2 layers **(lipid bilayer).**



The phospholipid molecule.

- **b) Cholesterol molecules:** present mainly at the cytoplasmic aspect incorporated with the hydrophobic region of phospholipids.

- **2- Protein component:** present in two forms:
- **a) Extrinsic (peripheral) proteins:** small molecules forming a non-continuous layer outside the lipid bilayer; loosely attached to both surfaces of the cell membrane.

- **b) intrinsic (integral) proteins:**
- **Small molecules:** embedded in the hydrophilic region of the lipid bilayer.
- **Large globules:** extend through the full thickness of the lipid bilayer and protrude from both sides **(transmembrane proteins).** They act as channels for water-soluble substances to pass through the membrane**(trans-membrane transport).**

- **3- Carbohydrate (CHO) component:** formed of:
- **a) Glycoproteins:** polysaccharide chains attached to protein molecules.
- **b) Glycolipids:** polysaccharides linked to lipid molecules. They form a carbohydrate-rich coat **(cell coat)** on the outer surface of the cell membrane.

- **Cell coat (glycocalyx):**

- • Formed of molecules of glycoproteins & glycolipids.
- • Present on the external surface of cell membrane.
- • It includes special molecules **(receptors),** which control the entrance of drugs, hormones, bacteria & viruses into the cells.

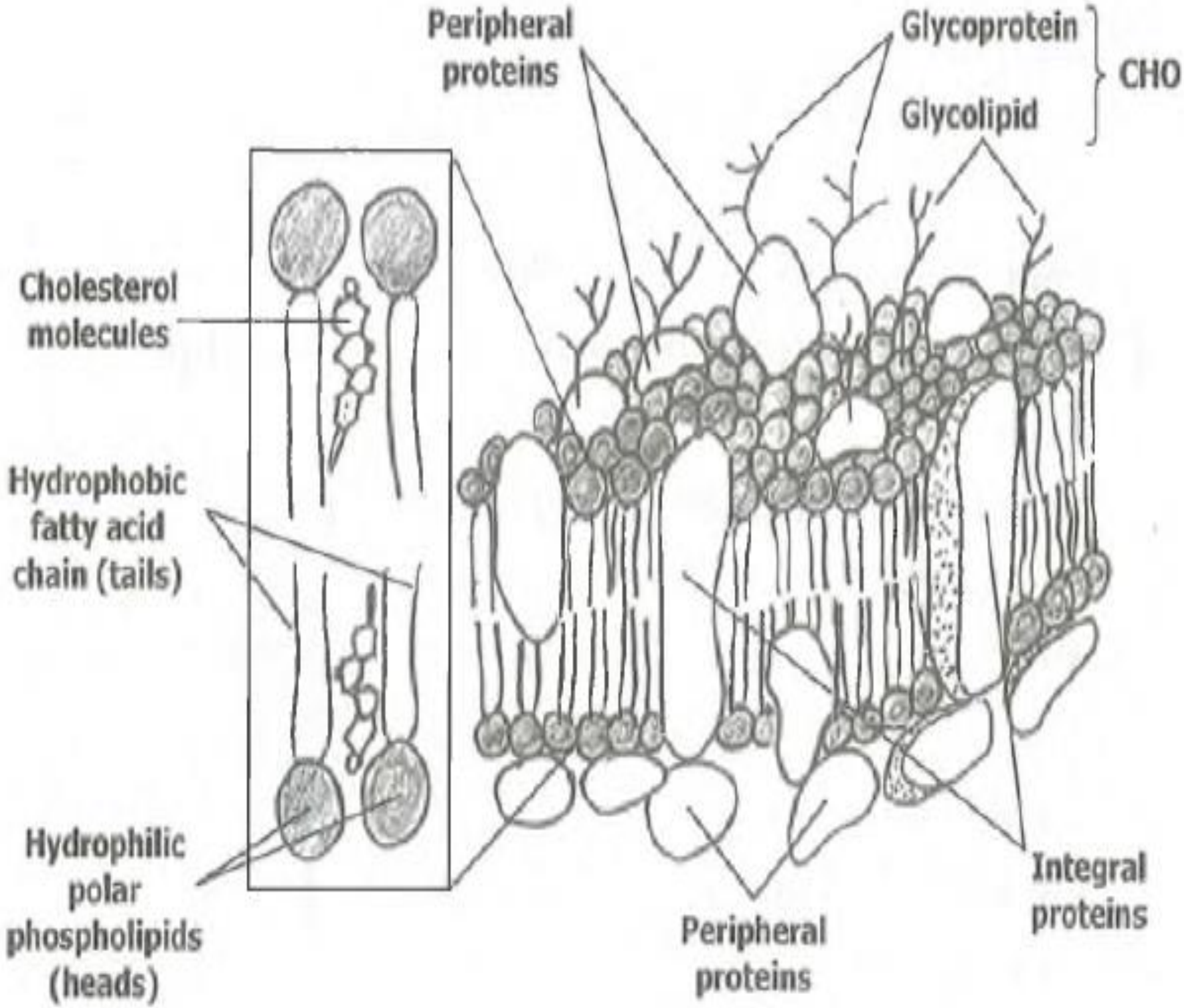


Diagram showing the molecular structure of the cell membrane.

- **Functions of Cell Membrane:**
- It encloses the cell. It is the site that controls the exchange of materials between the cell and its surroundings by several ways:

Simple transport:

- **Passive diffusion:** water, ions and dissolved gasses enter the cell passively across the membrane controlled by a **concentration gradient.**

- **Facilitated diffusion:** substances which do not dissolve in fat need a **carrier,** e.g. glucose & amino acids use integral membrane proteins to enter the cell.

- **Active transport:**
- Against a concentration gradient.
- Needs energy, e.g. Na+/K+ pump.
- **Bulk transport:** by which the cell can engulf or extrude large substances:

- **a)Endocytosis:** This is the active process of the cell membrane, by which large molecules and particles are taken up by the cell in membrane invaginations that close at their necks forming vesicles. Ingestion of solid particles is called **phagocytosis,** while ingestion of fluids is called **pinocytosis.**

- **Phagocytosis (cell eating):** the process by which the membrane can engulf solid particles forming a **phagosome,** e.g. food, bacteria, dead cells & foreign bodies as in white blood cells (WBCs). When particles or bacteria bind to the receptors on the cell surface, the cell membrane extends **pseudopodia** that encircle them. The distal ends of these pseudopodia fuse forming a vacuole containing the ingested particle, which will separate from the cell membrane and sink into the cytoplasm as a phagosome.

- **Pinocytosis (cell drinking):** the process by which the membrane can engulf fluid droplets When liquid droplets touch the membrane, invaginations are formed and encircle the droplets, their edges then fuse forming small **pinocytic vesicles** that separate from the membrane and sink into the cytoplasm

- **b) Exocytosis:** the process by which the cell releases its products or extrudes residual bodies The secretory vesicles approach the cell membrane; their limiting membranes fuse with it, and discharge their contents into the extracellular space. The limiting membranes of the vesicles will be added to the cell membrane.

- **Functions of cell coat:** They are important in:
- Cell immunity.
- Cell recognition (cell identity).
- Cell adhesion.
- Cell protection.
-

- **Cell membrane modifications:** it forms **microvilli** (increase surface area for absorption as in absorptive cells of the small intestine), **cilia** (move particles above the cell membrane in one direction), **flagella** (form the tails of spermatozoa), and **stereocilia** (non-motile long microvilli).
- 6- **Conduction of excitation waves** in nerve cells and muscles.

2- Mitochondria (**Mitos** = thread, **chondros** = granules)

- **Definition:** membranous organelles, containing enzymes responsible for **cell respiration** and **energy production.** They are considered the **power-house** or the **battery** of the cell.
- **Sites:** accumulate in the cytoplasm at sites of most activity.

- **Number:** More numerous in highly active cells
- • Varies from few to hundreds according to the metabolic activity of the cell, e.g. a liver cell has about 1000 mitochondria, while a lymphocyte has few.
- • Mitochondria can divide by simple division as they have their own genetic apparatus (DNA and RNA).

- **LM:** appear as granules or rods when stained with special stains. With **iron hematoxylin** mitochondria appear dark blue. With **Janus green** mitochondria stain green.
- **EM:** appear as rounded or oval membranous vesicles of 0.5-1 μ.m in diameter and 5-10 μm in length, surrounded with two unit membranes:

- • **The outer membrane:** is smooth, thicker (7 nm), and permeable to small molecules and ions.
- • **The inner membrane:** is thinner, and less permeable (selective). It projects into the cavity forming incomplete alternating, shelf-like thin folds called **cristae.**
- • The two membranes are separated by an **inter-membranous space.**

- • **Mitochondrial Matrix:** fills the interior cavity, more electron-dense than the surrounding cytoplasm, and full of granular materials which are:

- **1- Elementary particles:** ‘globular structures with heads attached to the cristae with slender stalk. They contain **respiratory enzymes** (enzymes of Krebs cycle & fatty acids oxidation).

- **2- Matrix granules:** electron-dense granules containing **phospho-lipoproteins** bounded to Ca^{++} & Mg^{++}.
- **3- DNA & RNA:** so mitochondria have its own genome (genetic content).

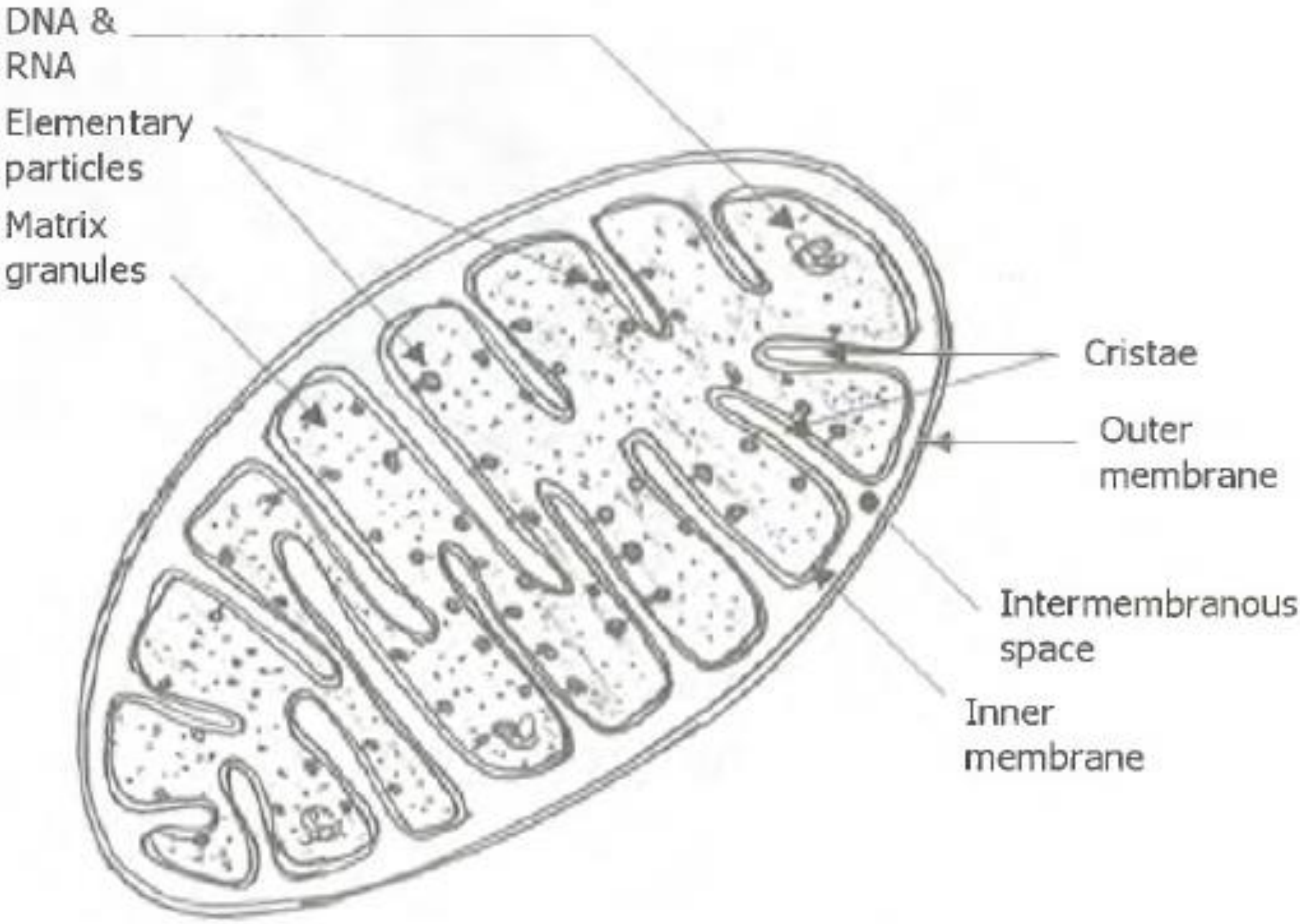


Diagram of EM structure of a mitochondrion.

- **Functions of Mitochondria:**
- **1- Cell respiration:** supply energy for cellular activity as they contain enzymes important for oxidative phosphorylation for glucose and fatty acids oxidation.
- **2- Adenosine triphosphate (ATP)** production as a store of energy, which passes to the cytoplasm. On demand, it splits to release energy and converts to ADP, which returns to mitochondria.
- **3-** Their **Ca^{++}& Mg^{++}** act as catalysts for enzymatic reactions.

- **3- Endoplasmic Reticulum(Endo** = in, **Rete** = net)

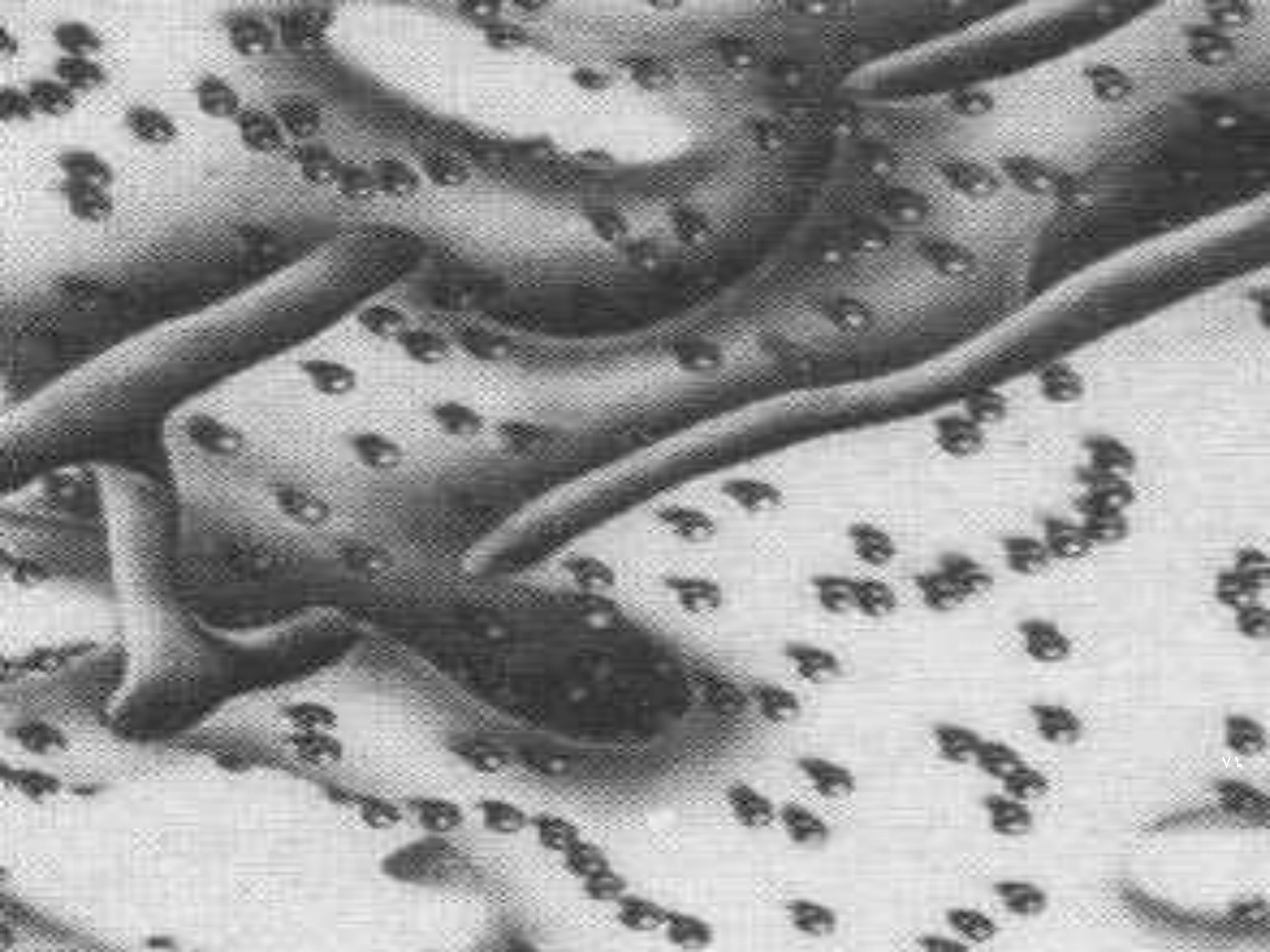
- **Definition:**

- • they are membranous cell organelles Formed of communicating wide and narrow tubules. They are synthesize protein , carbohydrate ,lipid and regulate mineral metabolism

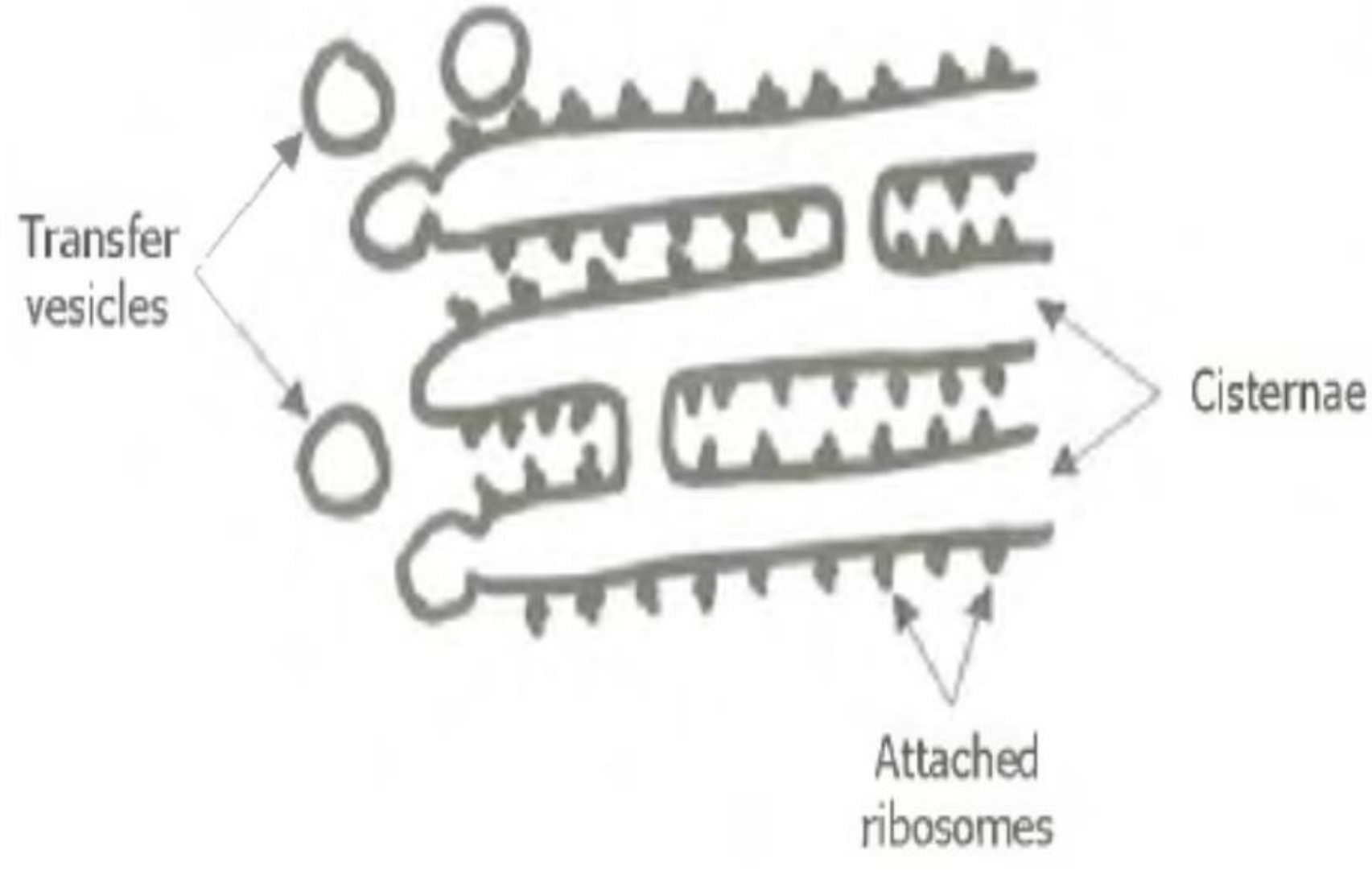
- • There are two types: a) Rough b) Smooth

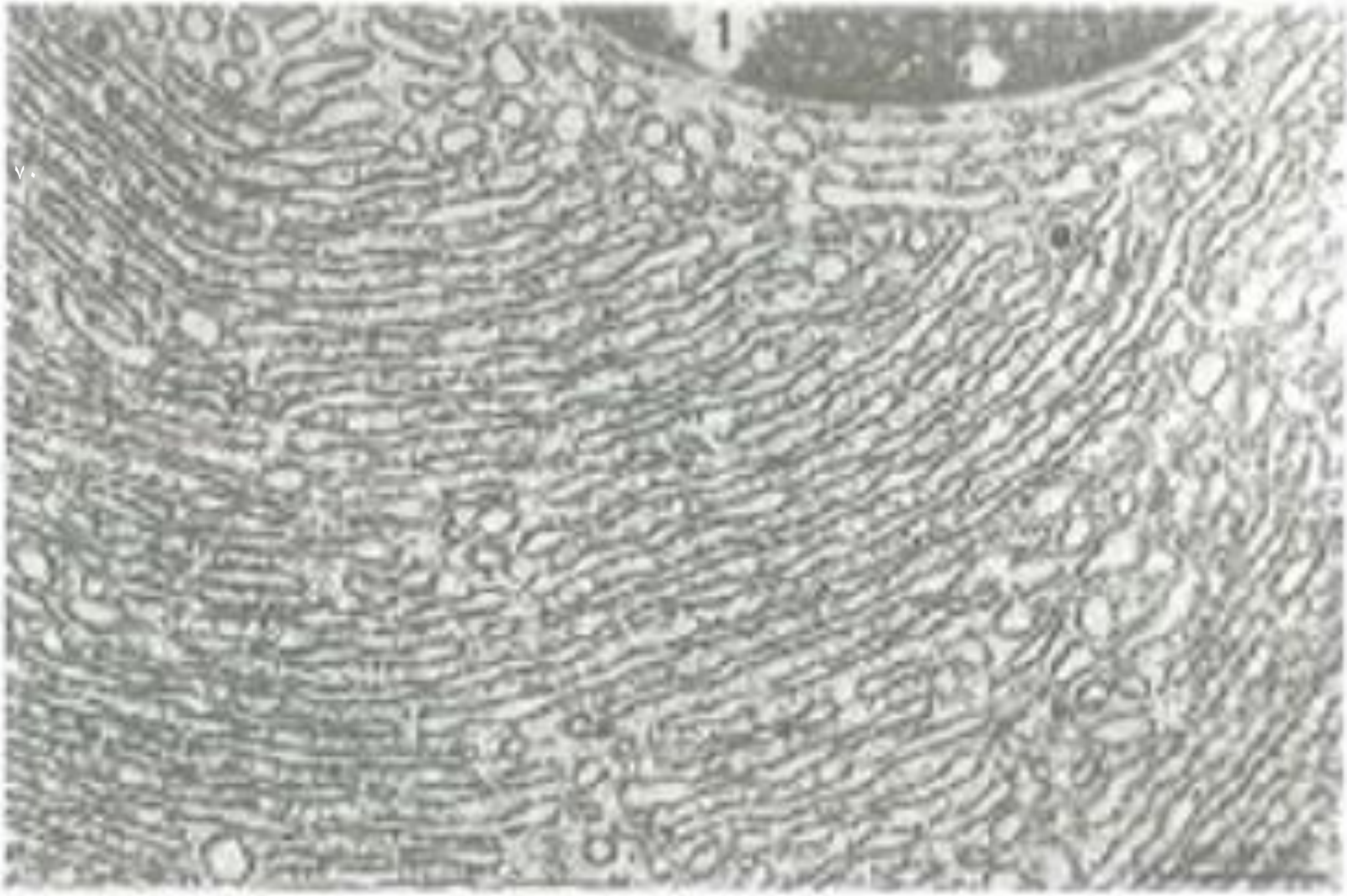
- **a) Rough (Granular) Endoplasmic Reticulum (rER):**
- **Definition:** It is formed of communicating flat tubules (cisternae) with rough surface .Its surface covered by ribosomes its synthesizes protein.

- **Position:** it extends between nuclear membrane and cytoplasm. It is present in great amounts in protein forming cells, e.g. plasma cells, fibroblast, chondroblasts, osteoblasts and pancreatic cells.
- **LM:** its sites cause localized or diffuse **basophilia** in H & E stained sections.



- **EM:** they are formed of communicating flat tubules called **cisternae covered by ribosomes and ribophorins** receptors





EM picture of rER.

Functions of rough endoplasmic reticulum:

- 1. The rough endoplasmic reticulum and the attached ribosomes form protein
- 2. They store the formed protein.
- 3. They package the formed protein . The package protein travel through the cytoplasm as transfer vesicles to fuse with golgi apparatus
- 4. they form the protein needed for the formation of lysosomal enzymes.

- **b) Smooth (non-granular) Endoplasmic Reticulum (sER):**

- **Definition**: they are membrance organelles, formed of narrow anastomosing tubules with smooth wall.
- • **Position:** it extends between nuclear membrane and cytoplasm. It is present in great amounts in cells which synthesize lipid & carbohydrate e.g. liver cells & endocrine cells.
- • **LM:** cannot be demonstrated, but when abundant causes cytoplasmic acidophilia.

- •**EM:** branching and anastomosing tubules with smooth membranes (no ribosomes), which anastomose in an irregular manner forming a network.

- **Functions of smooth endoplasmic reticulum:**
- 1. Lipid synthesis and storage.
- 2. Cholesterol & steroid hormones synthesis as cortisone & testosterone
- 3. Glycogen storage & breakdown as in liver and muscle cells.

- 4. Drug detoxification: excess drugs are removed as in liver cells.
- 5. Mineral metabolism: calcium ions (Ca++) release (pump) in muscle contraction.
- 6. HC1 formation in stomach.
- 7. Acts as an intracellular pathway.

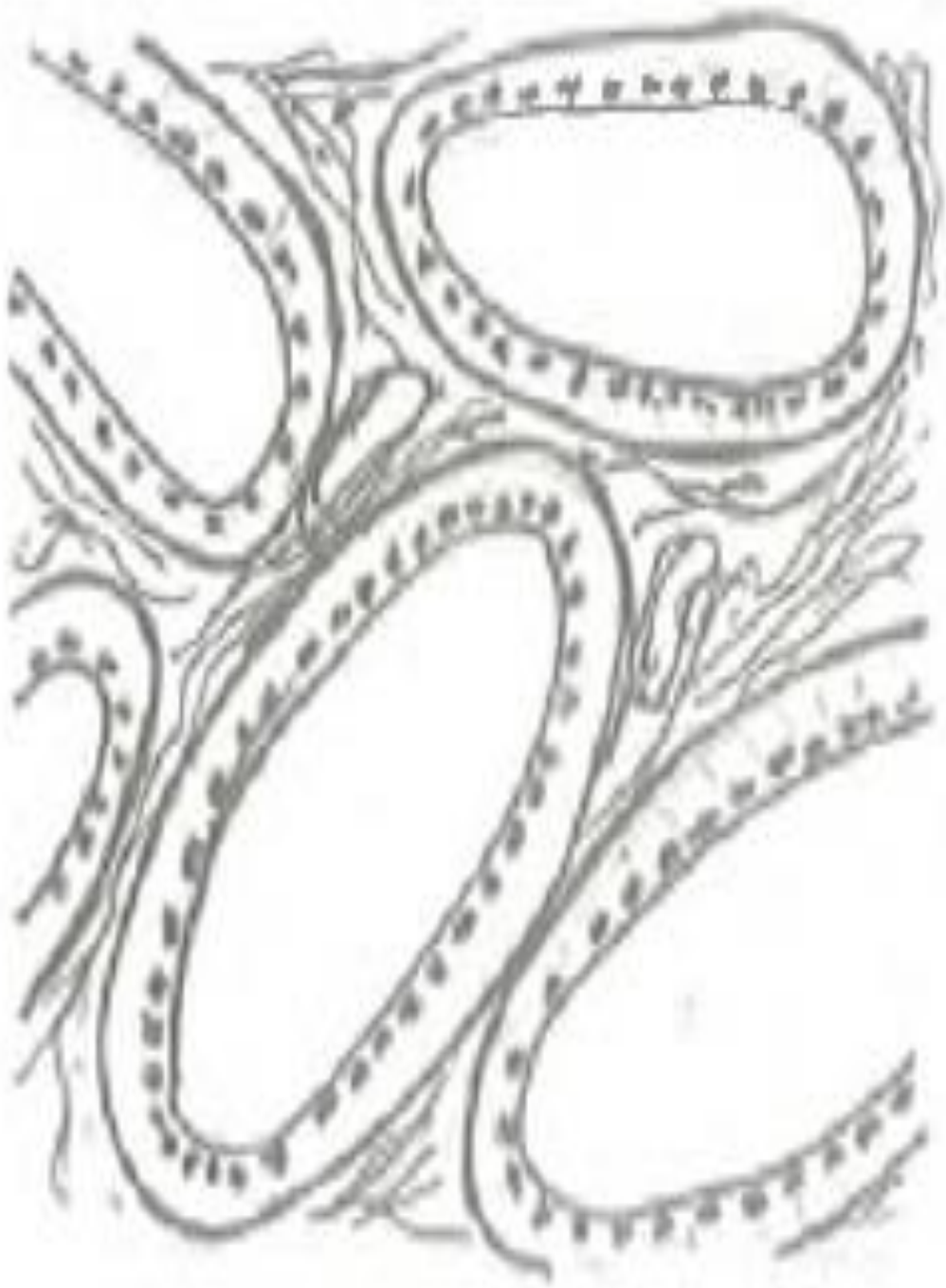
4- Golgi Apparatus

- **LM:**
- Golgi bodies are named after the Italian biologist Camillo Golgi.
- They are major membranous organelles, concerned with secretion.
- They vary in shape, size & site in different cells according to cell activity.

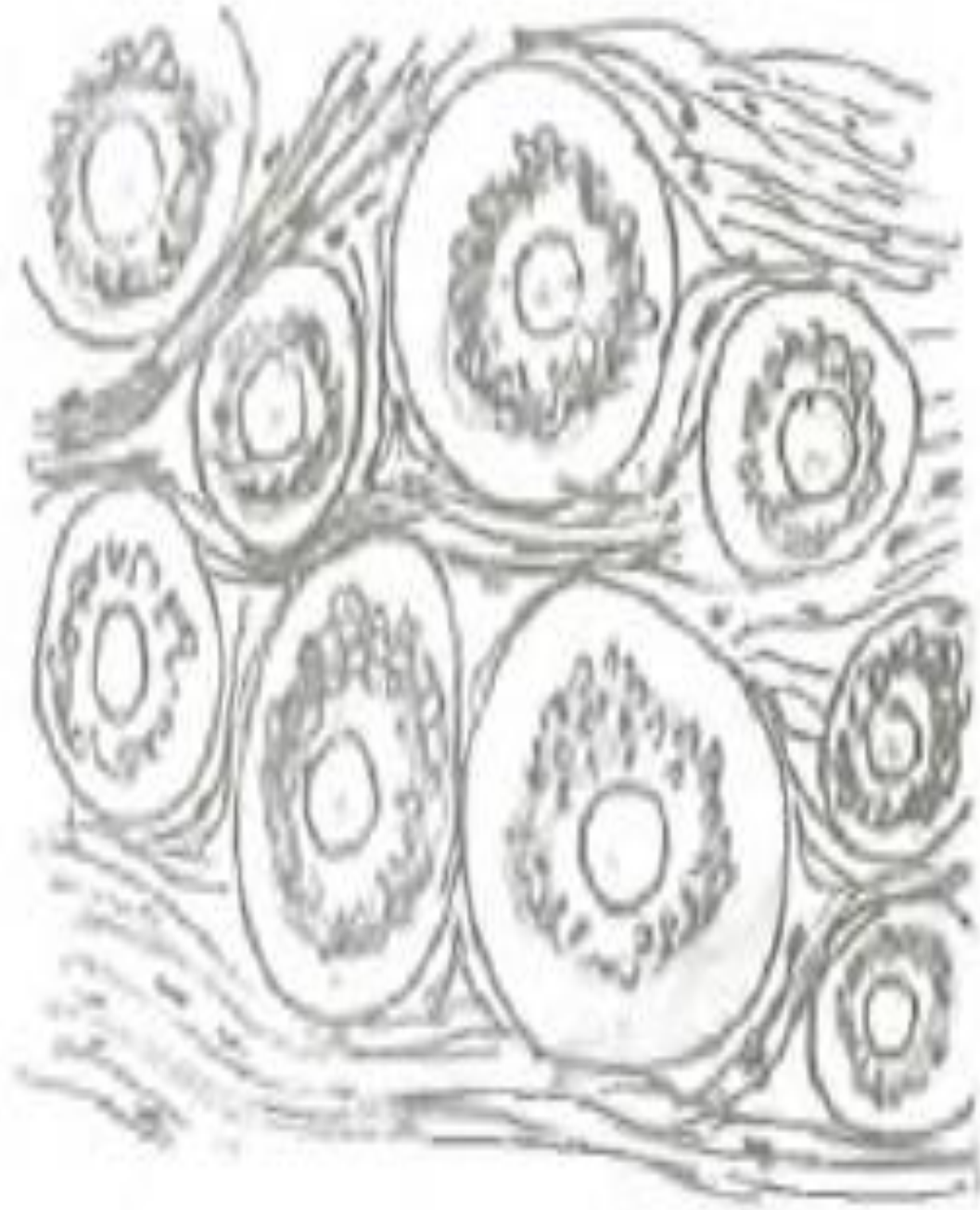
- They are well developed in secretory cells.
- In H & E stained sections, it doesn't appear, but in deeply basophilic cytoplasm (as in protein synthesizing cells), it can be seen as a pale unstained area near the nucleus called **"negative Golgi image",** e.g. in plasma cells.
- With **Silver stain (Ag),** it can be demonstrated as a network of brown granules & fibrils.

Negative Golgi image in plasma cells.

- **Sites:**
- Apical: between the nucleus and the secretory pole of the cell, e.g. in **secretory cells.**
- Perinuclear**:** completely surrounds the nucleus, e.g. in **nerve cells.**



Apical Golgi body in epididymal cells (silver stain).



Perinuclear Golgi body in nerve cells of spinal ganglion (silver stain).

EM:

1-It consists of saccules or cisternae called the golgi stacks.

2-Each stack consists of 4- 6 cisternae (flattened, curved, membrane-bounded, slightly expanded at the ends).

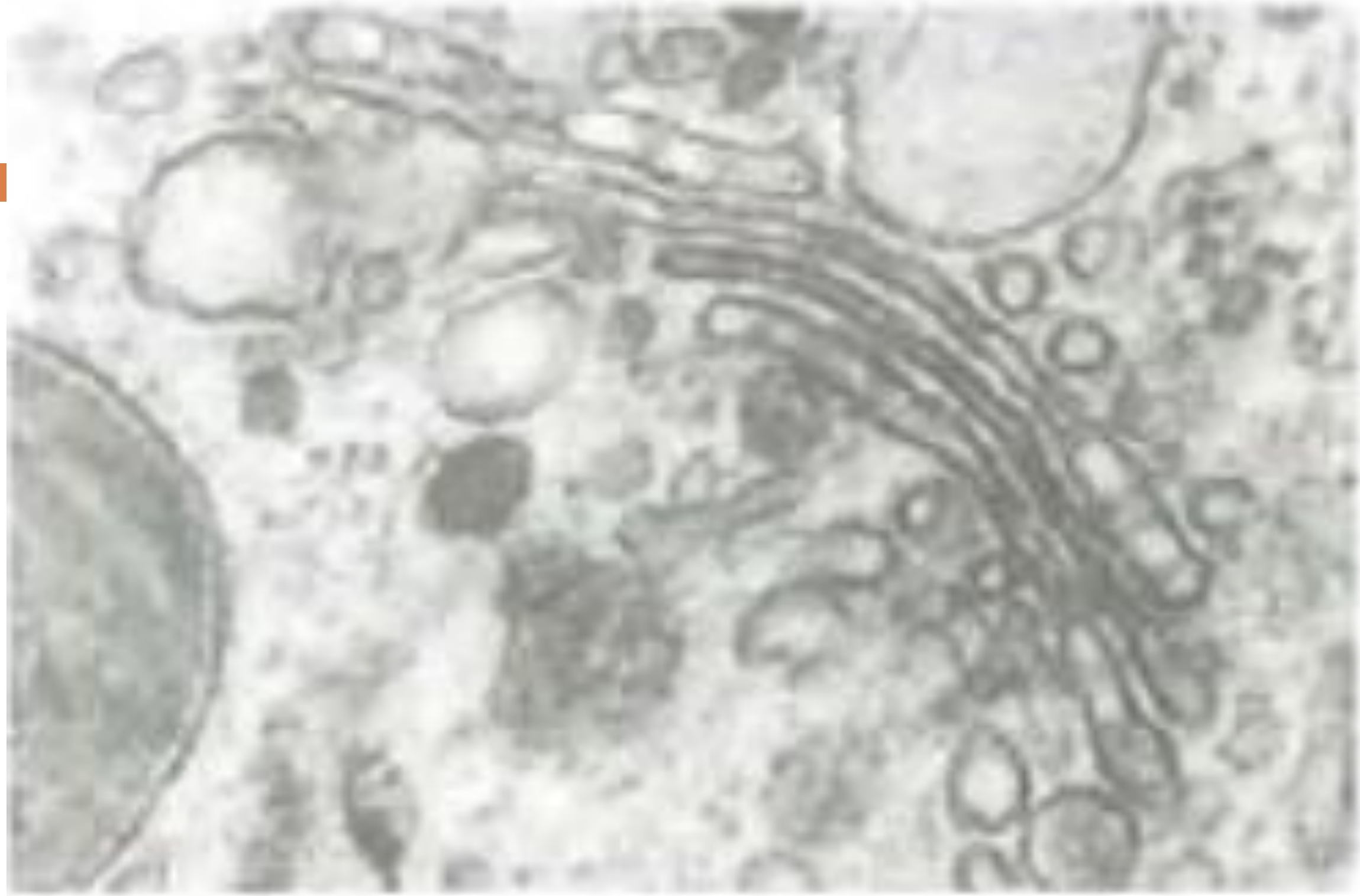
3-The Golgi stack is cup-shaped with a convex surface and a concave surface:

A. The cis face (forming face; immature face):is convex in shape and lies near to the rER.

B. The trans face (secretory face; mature face):is concave in shape.

C. The medial compartment .

- **EM: appear in the following three forms:**
- **1- Flat vesicles or golgi saccules**: they are formed of flat sacs , the sacs are arranged above each other
- **2- Transfer or micro vesicles**: they are small rounded sacs filled with protein.
- **3- Secretory or macro vesicles**



EM picture of Golgi apparatus.

- **Functions of Golgi Apparatus:**
- **1- Concentration & accumulation** of secretory products received from rER to form secretory vesicles and enzymes.
- **2- Chemical modification of proteins** by addition of carbohydrate and sulfates (glycosylation, sulfation).

- **3- Discharge of secretory products** as hormones and enzymes in secretory vesicles by exocytosis.
- **4- Renewal and maintenance of cell membrane** and providing it with integral proteins.
- **5- Formation of secretory vesicles, coated vesicles and lysosomes.**

- **Secretory Vesicles (Secretory Granules):**
- Secretions are modified in the Golgi saccules, condensed and packed into small vesicles that bud off the mature face. They move to the cell membrane where their walls fuse with it and discharge their contents by exocytosis.

Endosome

Definition: are system of vesicles and tubules involved in the endocytotic pathway.

Types:

A-Early endosomes:

Site: at the periphery of the cell as a part in the pathway of the receptor-mediated endocytosis.

Content: the receptors-ligands complex.

The membrane of the endosomes pumps H+ ions into its interior ⟶ lowers the pH of endosomes to less than 6 ⟶ uncoupling of the receptors and the ligands.

The receptors recycle to the cell membrane and the ligands move to the late endosomes.

B-Late endosomes:

- Site: deep within the cytoplasm near the Golgi complex.

They receive:

1- the ligands from early endosomes.
2- clathrin- coated vesicles containing lysosomal enzymes from Golgi complex.
PH: 5.5. (the enzymes become active at the acidic pH of the late endosome).

- The lysosomal enzymes in the late endosome begin to degrade the ligands açcompanied by further decrease in the internal pH which then "mature" to form lysosomes.

- **5- Lysosomes**

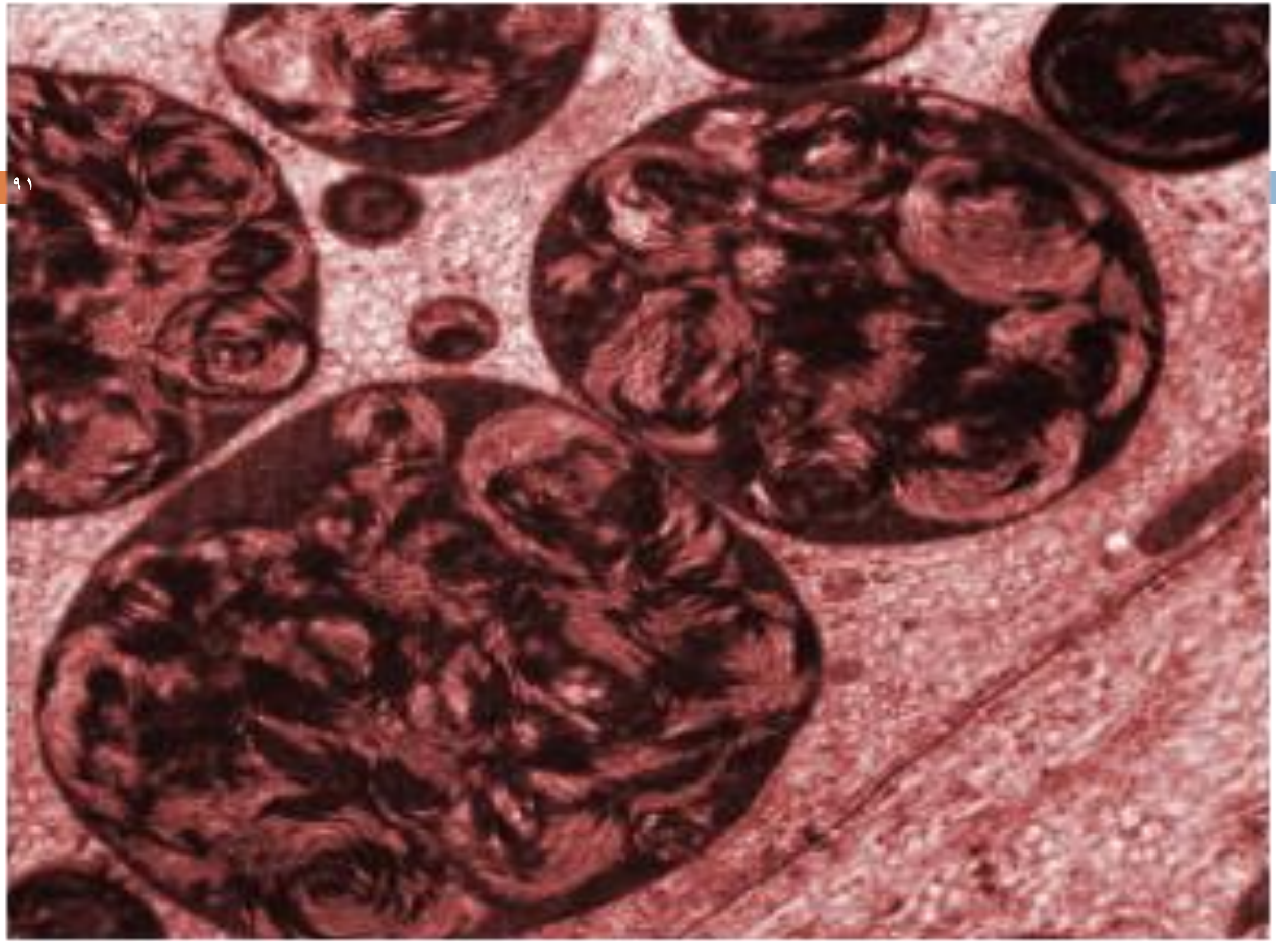
- **Definition:**

- Spherical membranous organelles.

- Concerned with intracytoplasmic **digestion.**

- Contain hydrolytic enzymes (lipase, protease, acid phosphatase, nuclease, etc...).

- **Origin**
- The proteins forming its enzymes are formed in rER, transferred to the immature face of Golgi apparatus as transfer vesicles, processed in Golgi saccules, till they reach the top saccules.
- Arise from the mature surface of Golgi apparatus by budding.



- **Number:** their numbers vary according to the phagocytic activity of the cell, so their numbers are more in white blood cells and phagocytic cells.

- **LM:** they can be demonstrated by histochemical tests that detect the enzymes inside them as acid phosphatase enzyme

- **EM:**
- **Primary lysosomes:**
- • They are newly released vesicles from Golgi apparatus that have not entered into digestive events.
- • Each is a spherical membranous vesicle, surrounded with a single unit membrane.
- • Contain moderately electron-dense **homogeneous** content.

- **Secondary lysosomes:**
- • When a primary lysosome fuses with any intracytoplasmic content, which may enter the cytoplasm from outside or from inside, it becomes a secondary lysosome.
- • They have a **heterogeneous** appearance in EM because of the various materials resulting from digesting.

- • Secondary lysosomes are of many types according to the type of vesicle that primary lysosome fuses with.

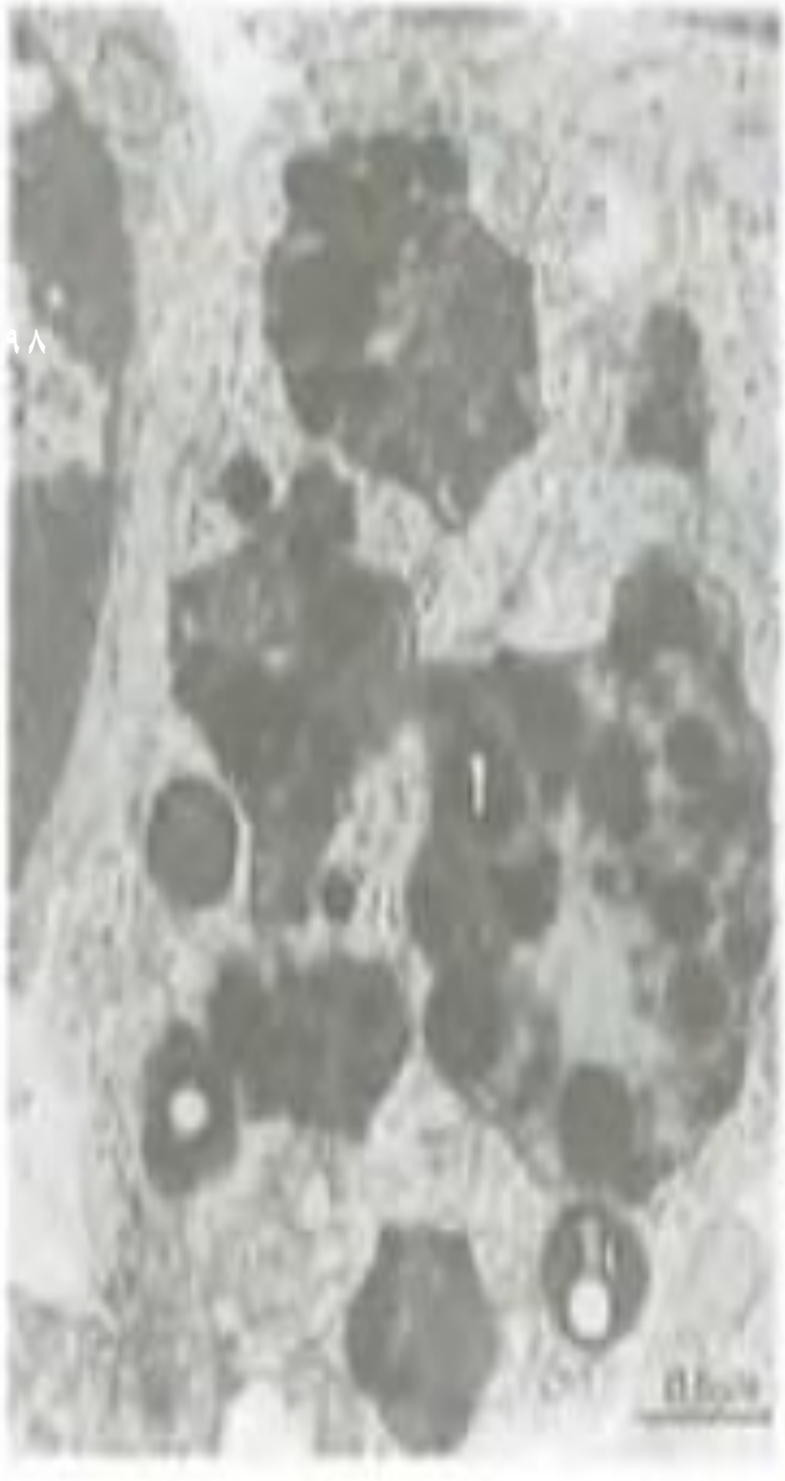
- **• Types of secondary lysosomes:**

- **1- Heterolysosomes:** when a primary lysosome fuses with a **phagosome.** It fuses with its membrane and empties its hydrolytic enzymes into the vacuole to digest its content (bacteria, viruses, etc...).

- **2- Multivesicular bodies:** when a primary lysosome fuses with a **pinocytic vesicle** (liquids taken by the cells by pinocytosis).

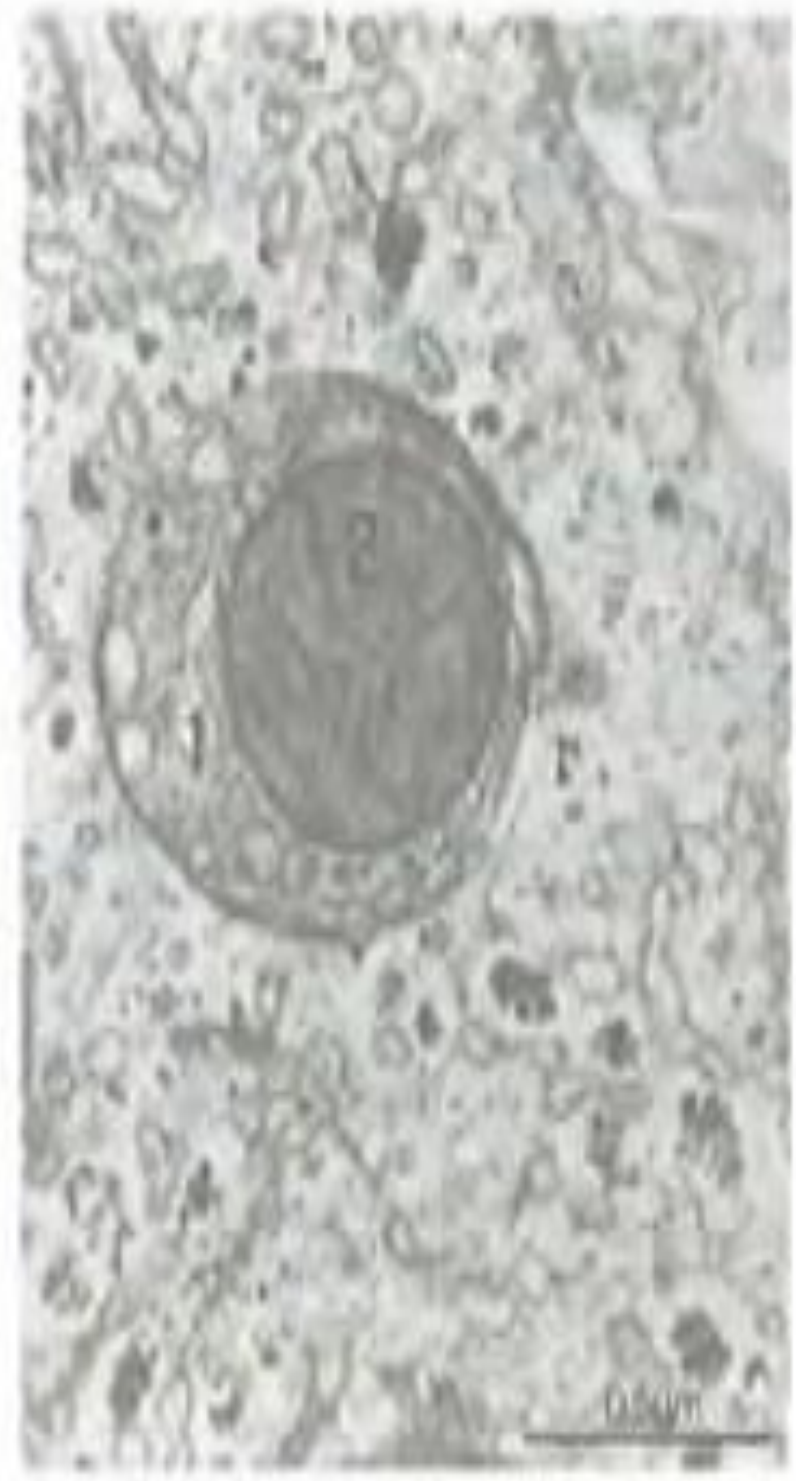
- **3- Autolysosomes:** when a primary lysosome fuses with an **autophagic vacuole** (vacuole containing old or broken down organelles as old mitochondria, i.e. endogenous component from inside the cell). The process is called **autophagy.**

- **4- Residual bodies:** after digestion of the contents of a secondary lysosome, digested nutrients diffuse back to the cytoplasm through lysosomal membrane, while indigestible compounds are retained within vesicles and called residual bodies.



EM picture of heterolysosomes.

EM picture of multivesicular bodies.



EM picture of an autolysosome.

- **Functions of Lysosomes:**

- **1- Digest** nutrients and solid particles in phagosomes, and fluid droplets in pinocytic vesicles.

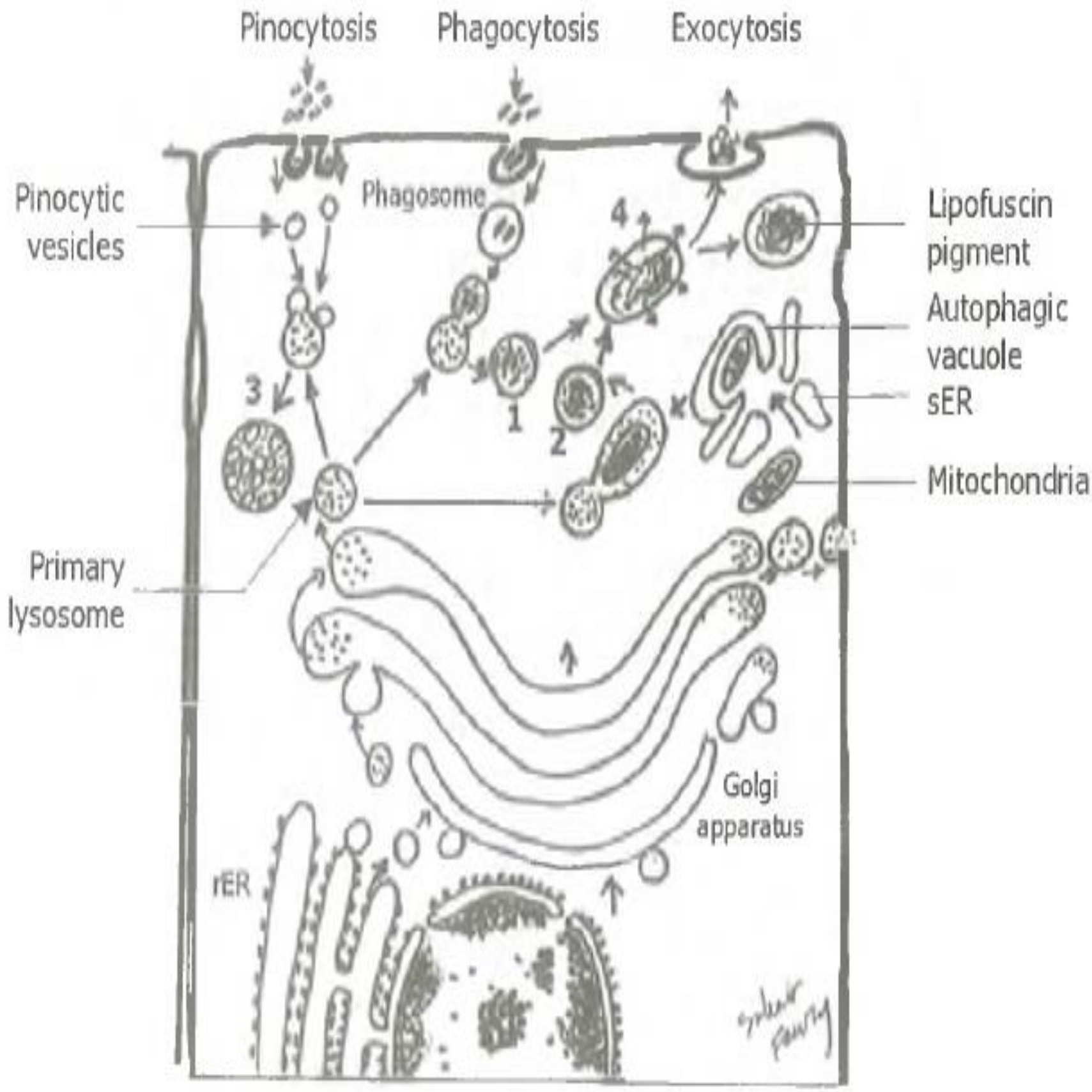
- **2- Defend** the body against foreign invaders, as bacteria and viruses.

- **3- Maintain cell health** by elimination of old or worn out organelles.

- **4-** Digestion of the whole cell after death: as the hydrolytic enzymes escape from the lysosomal membrane after cell death to digest and lyse all its contents **(postmortem autolysis).**
- **5-** Help the sperm to penetrate the ovum **(fertilization).**
- **6-** In thyroid glands: it **breaks the inactive hormone** into the active form.

Diagram showing the formation, types, and functions of lysosomes. Types of secondary lysosomes include:

1. Heterolysosome,
2. Autolysosome,
3. Multivesicular body, and
4. Residual body.



Peroxisomes (Microbodies)

Peroxisomes are single membrane–bounded organelles containing oxidative enzymes.

Peroxisomes (microbodies) are small , membrane-limited spherical organelles that contain oxidative enzymes, particularly catalase and other peroxidases.

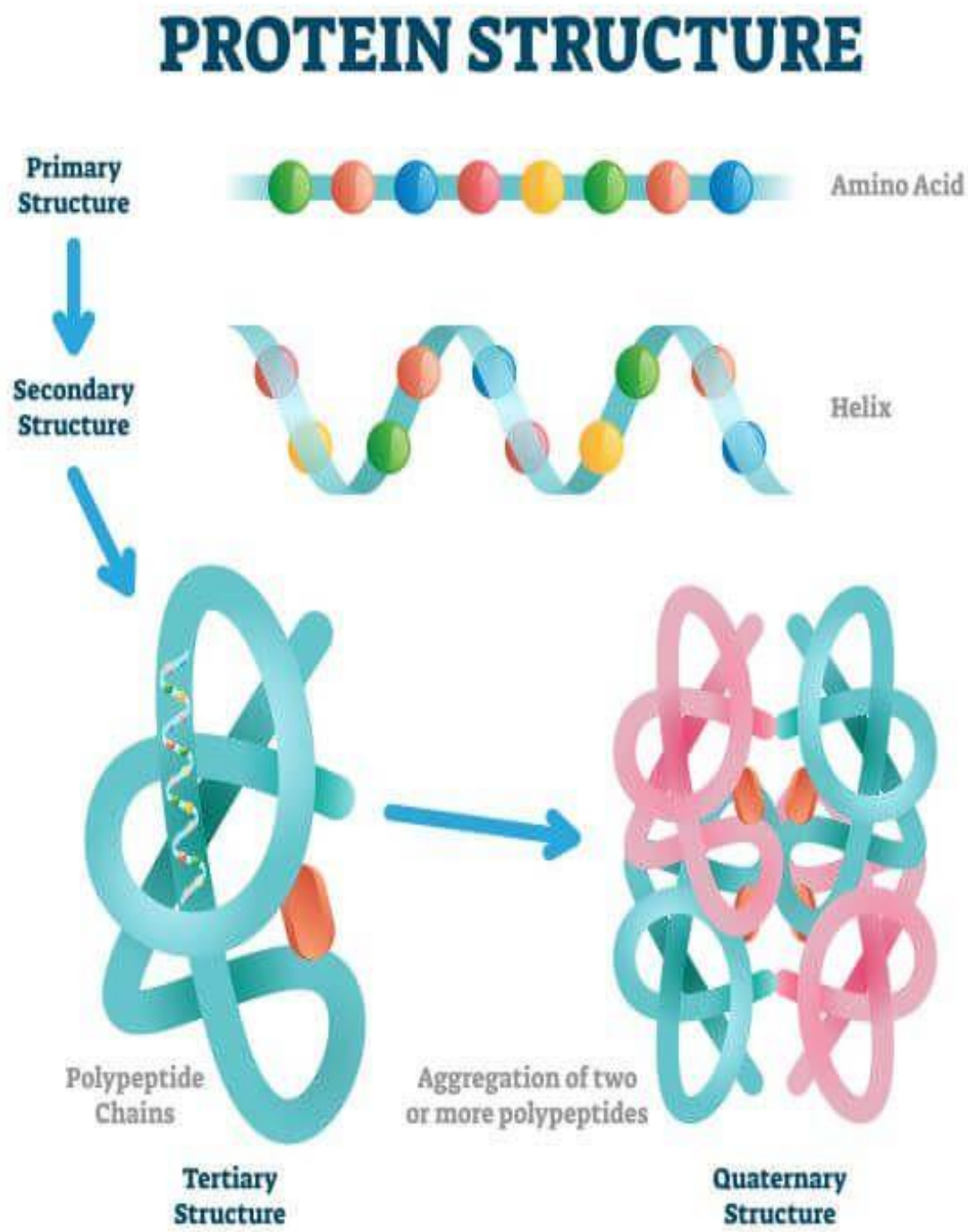
Virtually all oxidative enzymes produce hydrogen peroxide (H_2O_2) as a product of the oxidation reaction. Hydrogen peroxide is a toxic substance. The catalase present in peroxisomes carefully regulates the cellular hydrogen peroxide content by breaking down hydrogen peroxide, thus protecting the cell. In addition, peroxisomes contain D-amino acid oxidases, -oxidation enzymes, and numerous other enzymes.

Cytoplasmic organells that participate in process of protein synthesis

1- Ribosomes (factories)

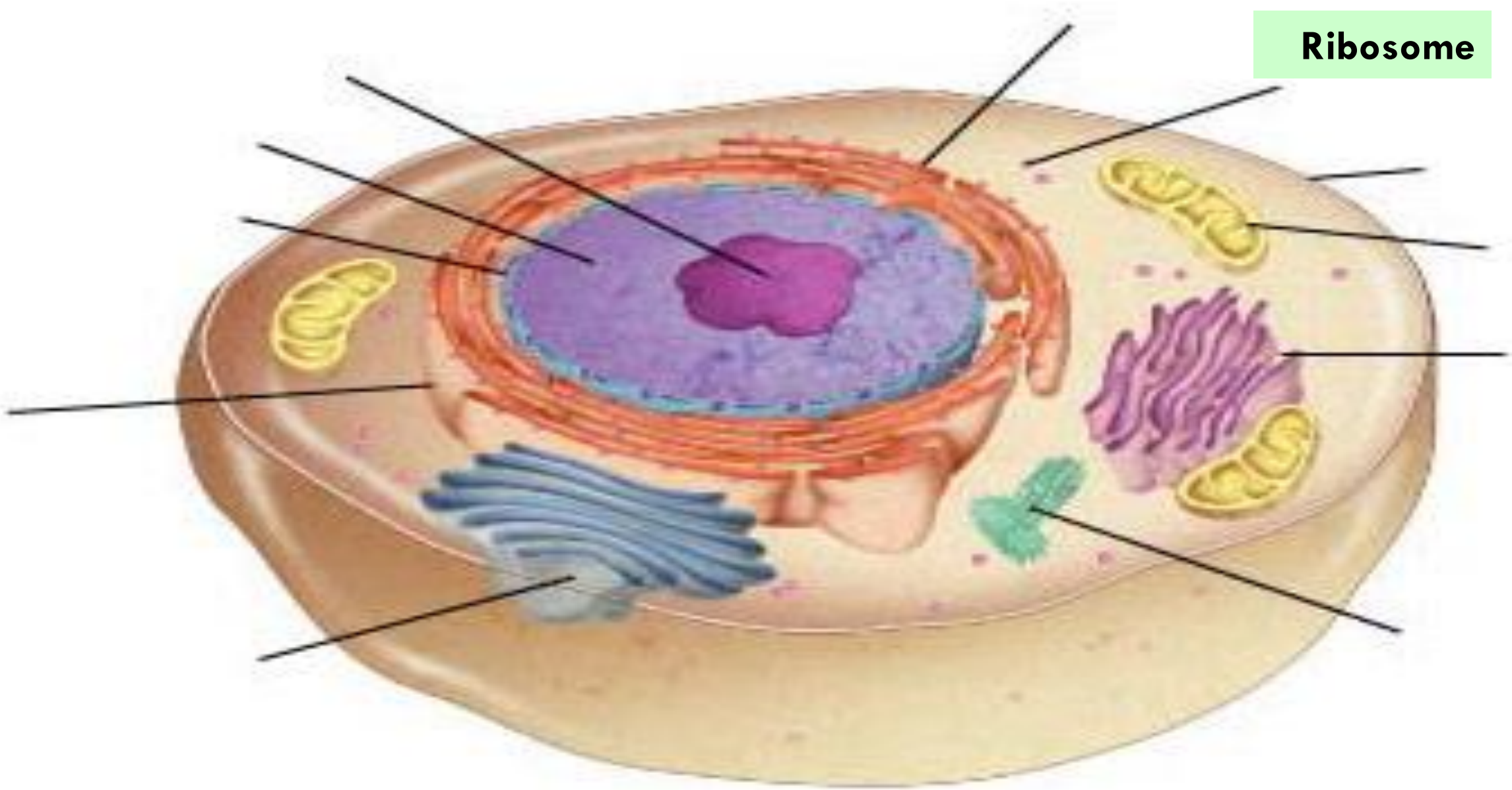
2- Rough endoplasmic reticulum (modification& transport)

3- Golgi apparatus (chemical modification,package,sorting& targeting)



Non-Membranous Organelles

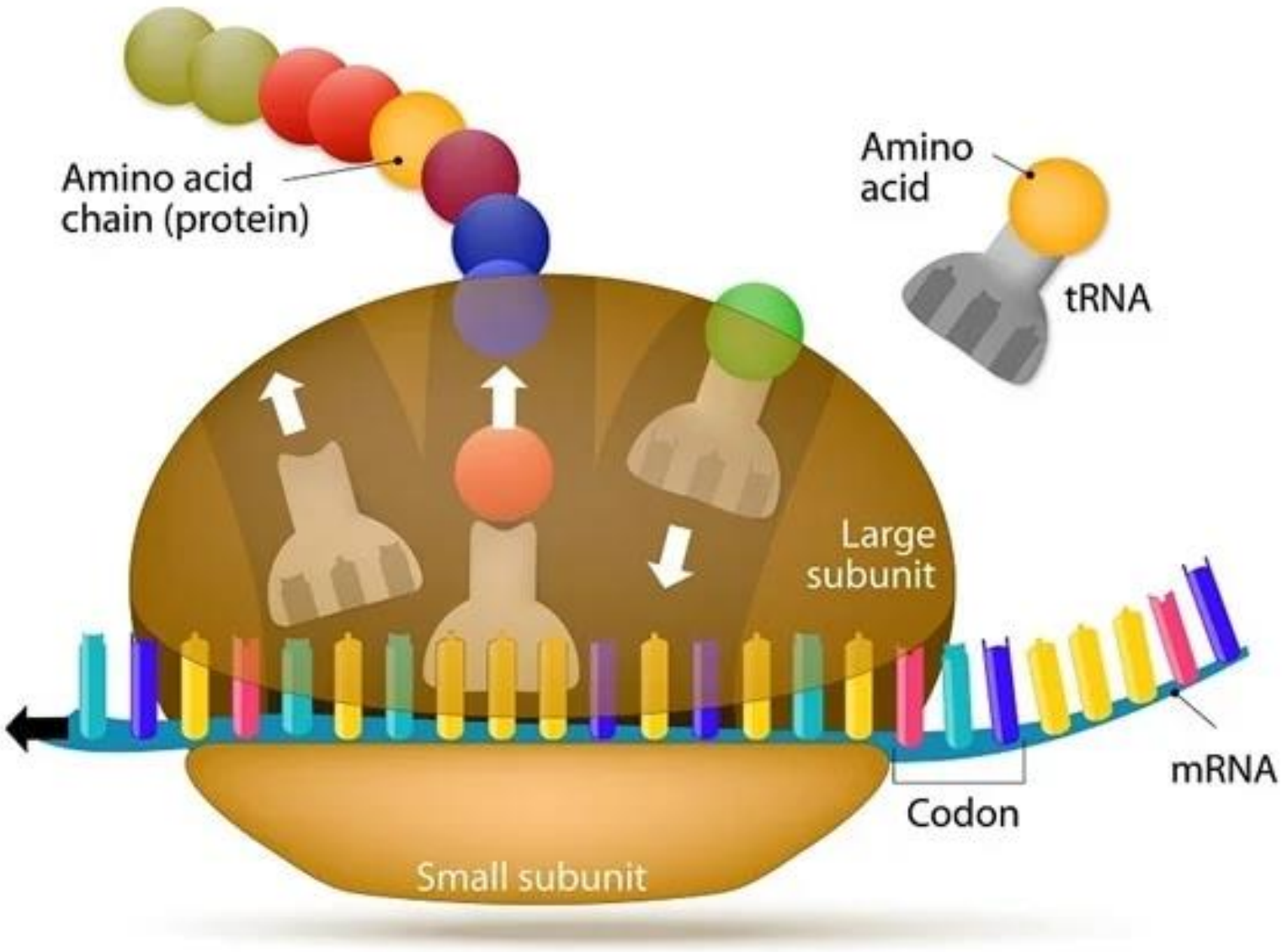
- **Ribosomes**
- **Definition:**
- • Non-membranous rounded or oval bodies (12 x 25 nm).
- • Formed of strands of rRNA and associated proteins.
- • Formed in the **nucleolus** and pass to the cytoplasm through the pores in the nuclear membrane. Ribosomes are the site of protein synthesis.

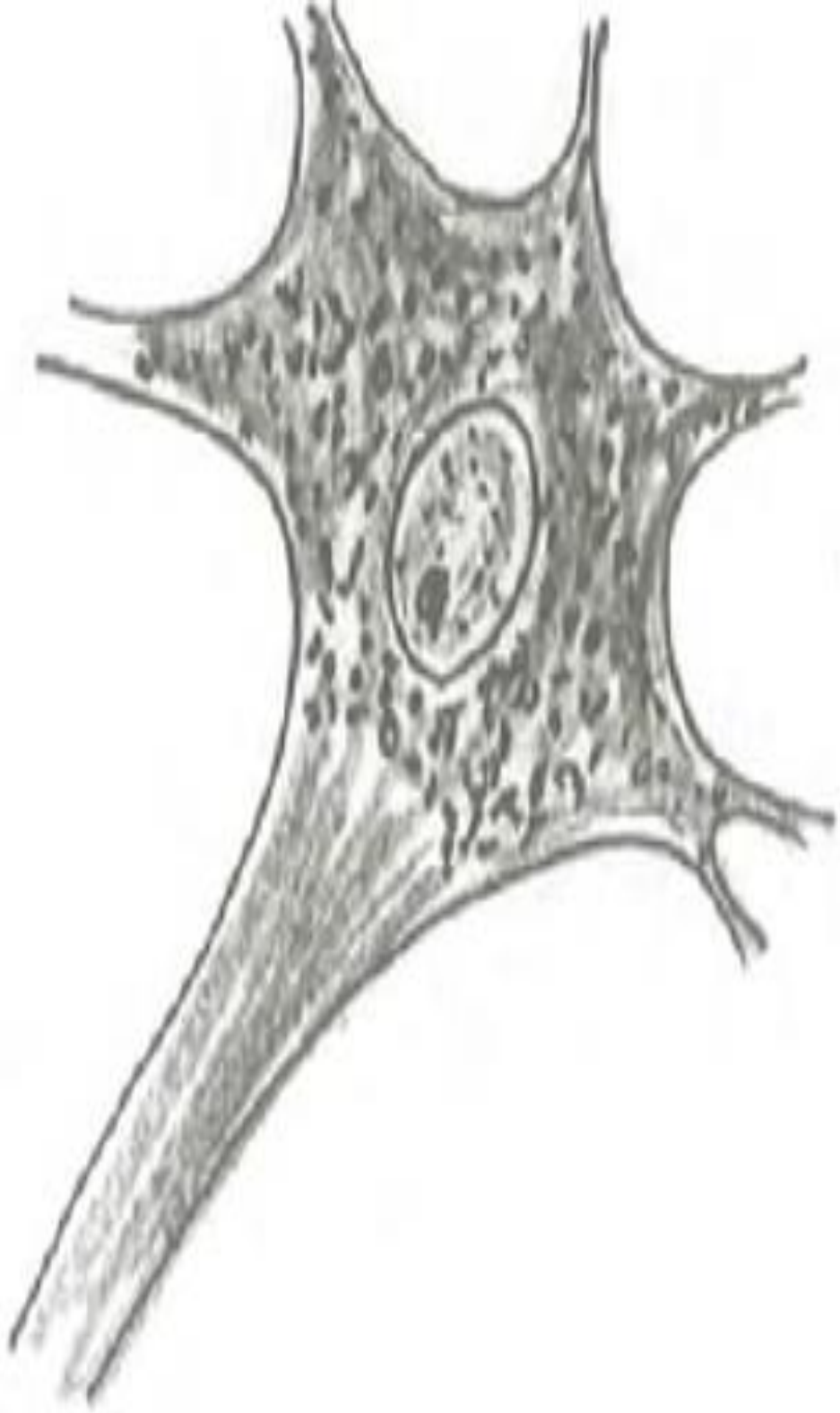

Ribosome

- **Number:** cells that have high rates of protein synthesis have great numbers of ribosomes, and also have prominent nucleoli.

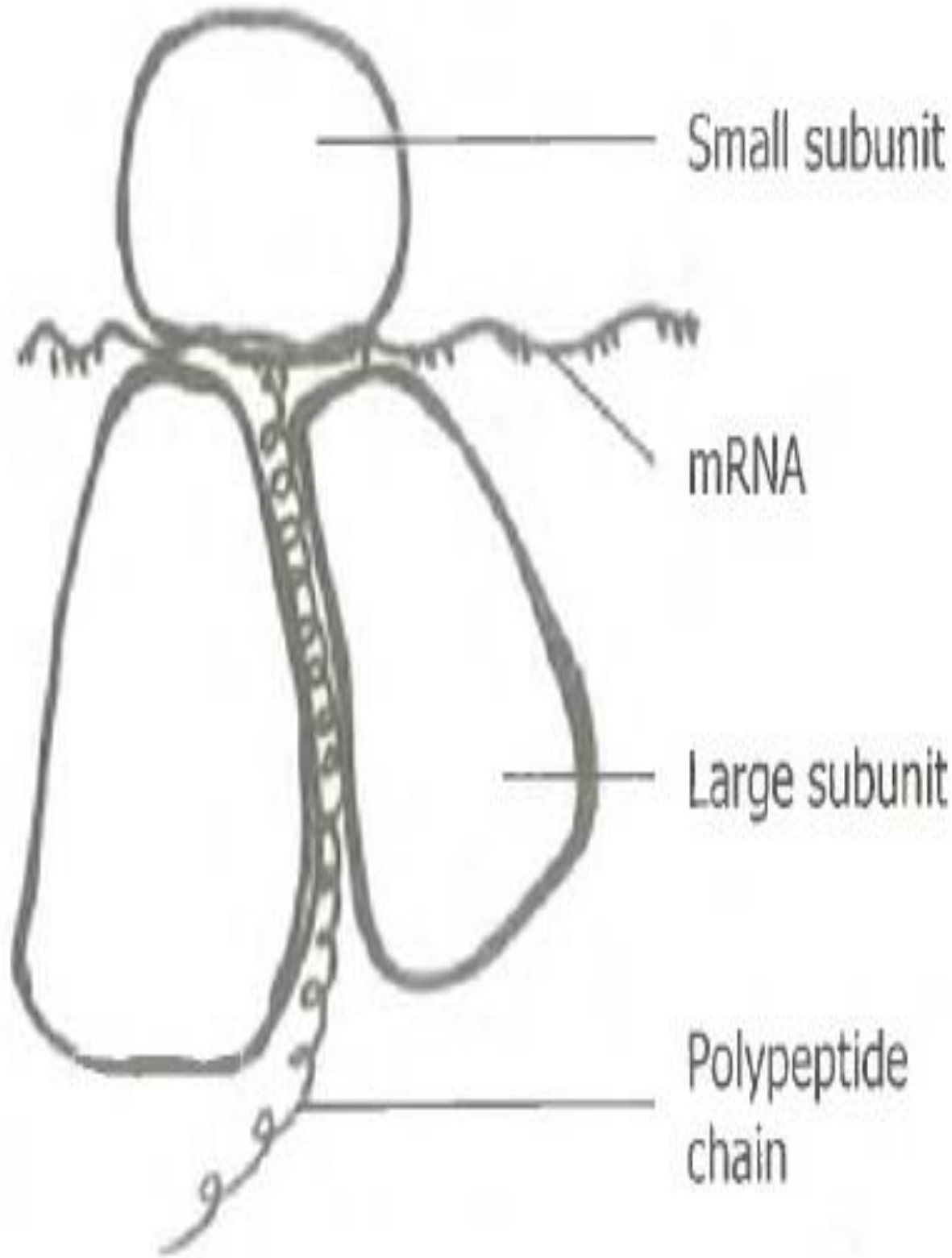
- **LM:** too small to be seen but when abundant they cause cytoplasmic basophilia due to their acidic content (RNA). Cytoplasmic basophilia may be:

- • **Diffuse:** as in embryonic cells.
- • **Localized:** as in pancreatic cells (at the base of the cell).
- • **Spotty:** as in nerve cells (Nissl's granules).


Amino acid
chain (protein)
Amino
acid
tRNA
Large
subunit
mRNA
Codon
Small subunit



Spotty basophilia in nerve cells (Nissl's granules).



Structure of a ribosome.

- **EM:** small electron-dense granules, each is formed of 2 subunits of unequal size, small & large ones. These 2 subunits unite together when the ribosome attaches to mRNA. The large subunit contains a ribosomal groove in its center which contains the polypeptide chain. Ribosomes are found in 3 forms:

- **1- Free:** scattered diffusely & singly.
- **2- Polysomes (polyribosomes):** linked together by a strand of mRNA.
- **3- Attached:** bind to the outer surface of rER

- **Function of ribosomes:**
- **Protein synthesis** (the factory that builds proteins).
- Free ribosomes form proteins that build up the cytoplasm (for growth &regeneration of organelles), i.e. proteins for local use.
- Attached ribosomes form proteins secreted by the cells as enzymes and hormones, i.e. proteins for export.

2- Cytoskeleton

- The gel-like consistency of the cytoplasm is due to a network of filamentous protein structures.

- Its principle components are **microtubules** and **filaments,** together with some proteins to link them to each other and to cell membrane forming a framework (**microtrabecular lattice**).

a) Microtubules

Definition:

Straight hollow tubular structures.

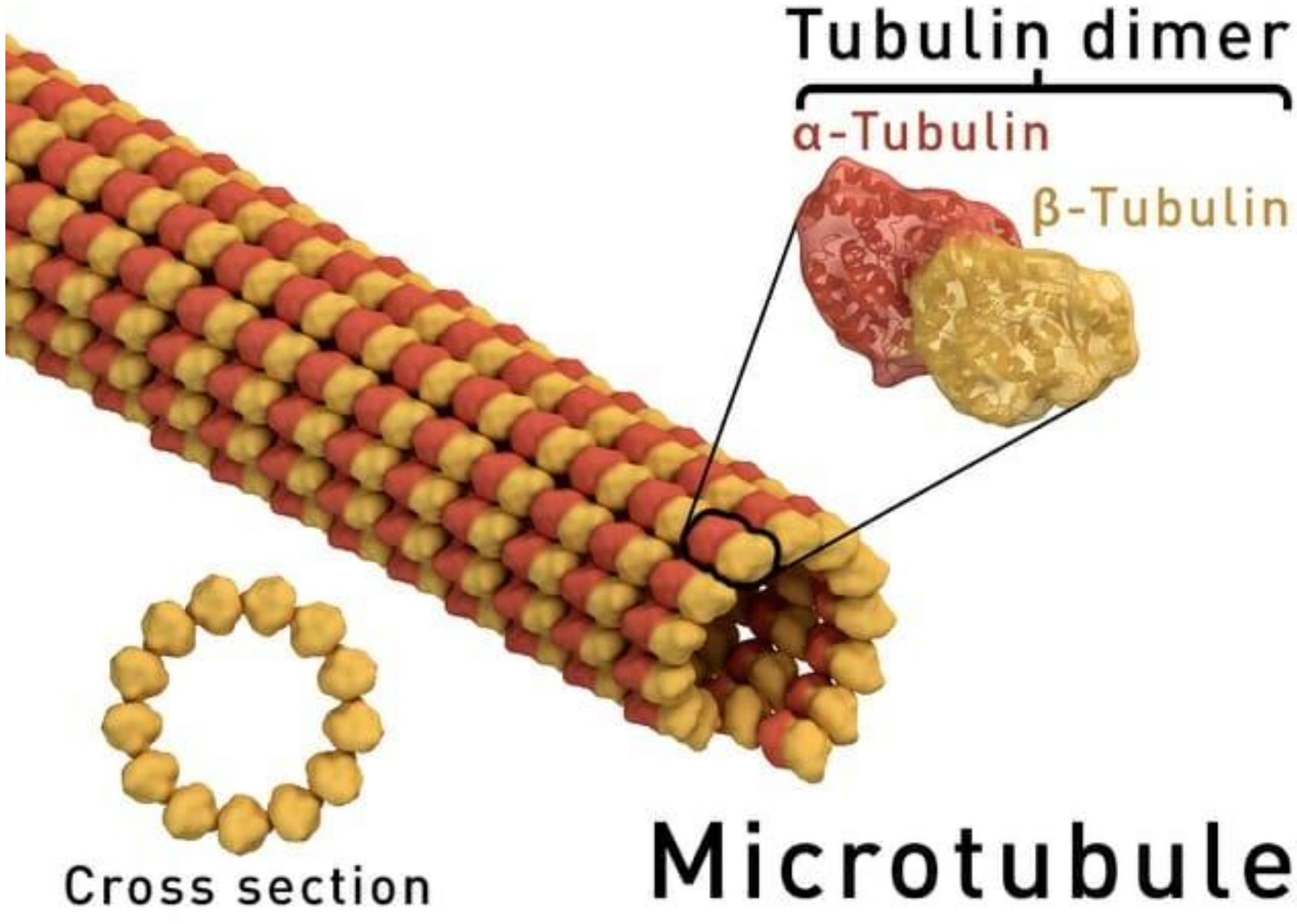
Have constant diameter (25 nm), but unfixed length.

Rigid but flexible.

Structure:

- Built by the assembly (polymerization) of subunits of a globular protein called tubulin.
- There are two forms of microtubules:

Dynamic form: with continuous assembly and disassembly causing rapid reshaping of the cell.
Stable form: another form of microtubules in which polymerized tubulin form solid stable structures that form the walls of centrioles and axonemes of cilia & flagella.
LM: not seen except when present in bundles or if stained with immunofluorescent technique.
EM: long, thin cylindrical tubes, tiny circles in cross sections.


Tubulin dimer
α-Tubulin
β-Tubulin
Cross section
Microtubule

Functions:

1- Form the skeleton of the cell, and preserve its shape.

2- Help intracellular transport of macromolecules.

3- Form the mitotic spindle during cell division.

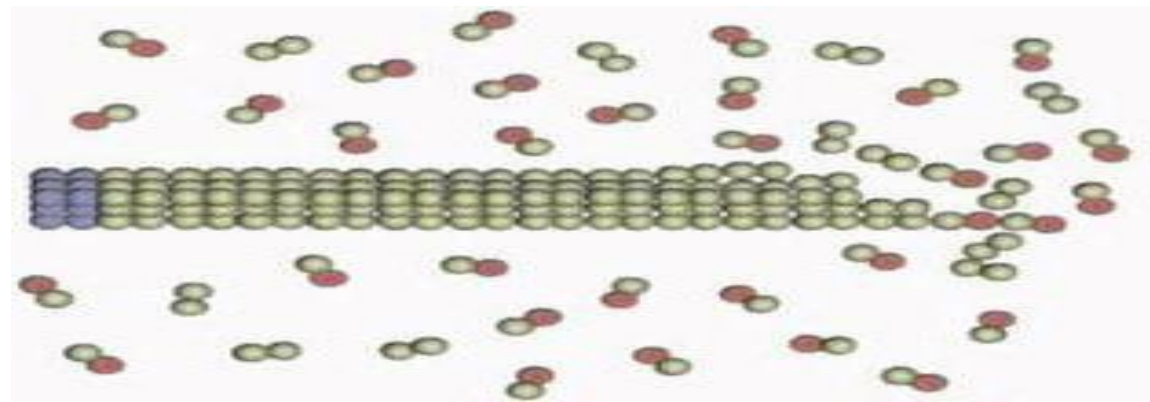
4- Formation of centrioles, cilia & flagella.

Structures Formed by Stable Microtubules

1) Centrioles

Definition: non-membranous organelles, present near the nucleus in an area in the cytoplasm called centrosome.

LM: can be demonstrated with iron hematoxylin stain as two small dark bodies in the middle of a paler area in the cytoplasm (centrosome). This area is very rich in tubulin molecules, from which new microtubules are formed and is known as Organizing Center Microtubule (MTOC).



EM:

2 cylindrical structures, oriented at right angles to each other (0.2 um diameter and 0.5 pm in length).

In cross section, each centriole appears as a hollow cylinder and its peripheral wall formed of 9 bundles of microtubules, each bundle is formed of 3 microtubules (triplets), i.e. the wall of the centriole is formed of 9 X 3 = 27 microtubules. The inner microtubule (A) (near the center) is a Complete cylinder, made of 13 protofilaments, while B or C microtubules are made of less than 13, usually 10 sharing the wall of adjacent one.

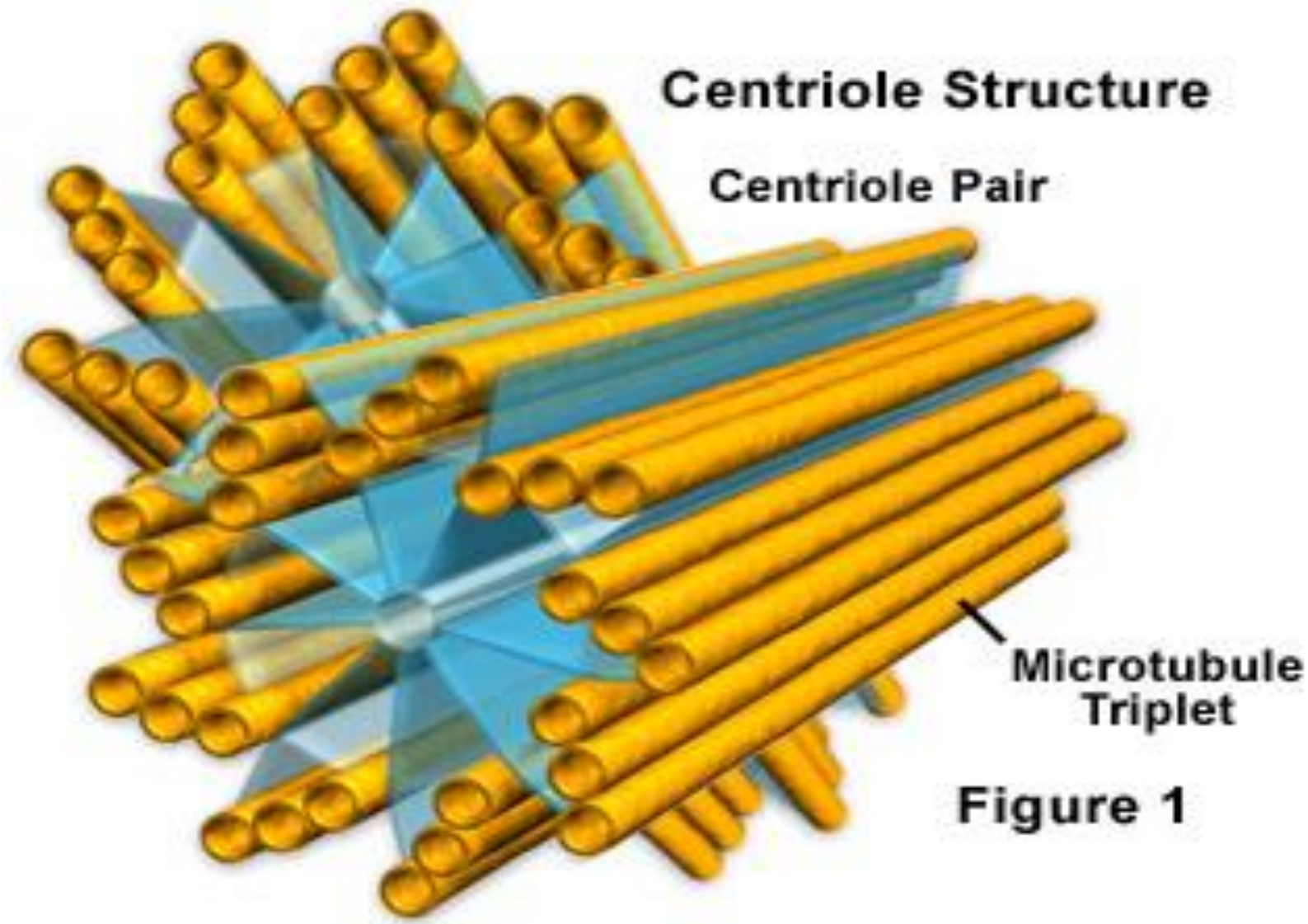


Figure 1

Functions of Centrioles:

1· Play an important role in cell division: the two centrioles duplicate, each pair of centrioles migrates to one side of the cell. They are surrounded by an area rich in tubulin (MTOC) from which the microtubules of the mitotic spindle are formed.

2- Share in the formation of cilia, flagella.

2) Cilia

Definition:

Motile hair-like processes from the free surface of some epithelial cells.

Cilia are formed of microtubules covered by cell membrane.

Origin: each cilium grows out and remains attached to a basal body, which is embedded in the cytoplasm. Basal bodies are identical to centrioles, and are produced by them through duplication thousands of times during development.

LM: hair-like striations.

EM: a cilium is formed of 3 parts: a basal body, a shaft and rootlets.

1- basal body:

It is formed of a single centriole, which after replication from the original centrioles, migrates to the surface.

It is similar in structure to a centriole, i.e. 27 microtubules in 9 triplets.

It is embedded in the cytoplasm.

2- Shaft (axoneme):

Finger-like cytoplasmic projection from the surface membrane.

From each triplet, the inner 2 microtubules (A & B) grow by polymerization of tubulin dimers from their plus end → doublets pushing the cell membrane in front of them. So. the shaft is formed of 9 bundles of doublets at its periphery in addition to polymerization of 2 microtubules in the center (2 singlets).

So each shaft is formed of (9 x 2) + 2 = 20 microtubules.

3- Rootlets: develop at the lower end to fix the basal body and shaft to the underlying cytoplasm.
They are formed by growth of the third outer microtubule (C) in each triplet in the basal body into the cytoplasm (i.e. 9 microtubules)

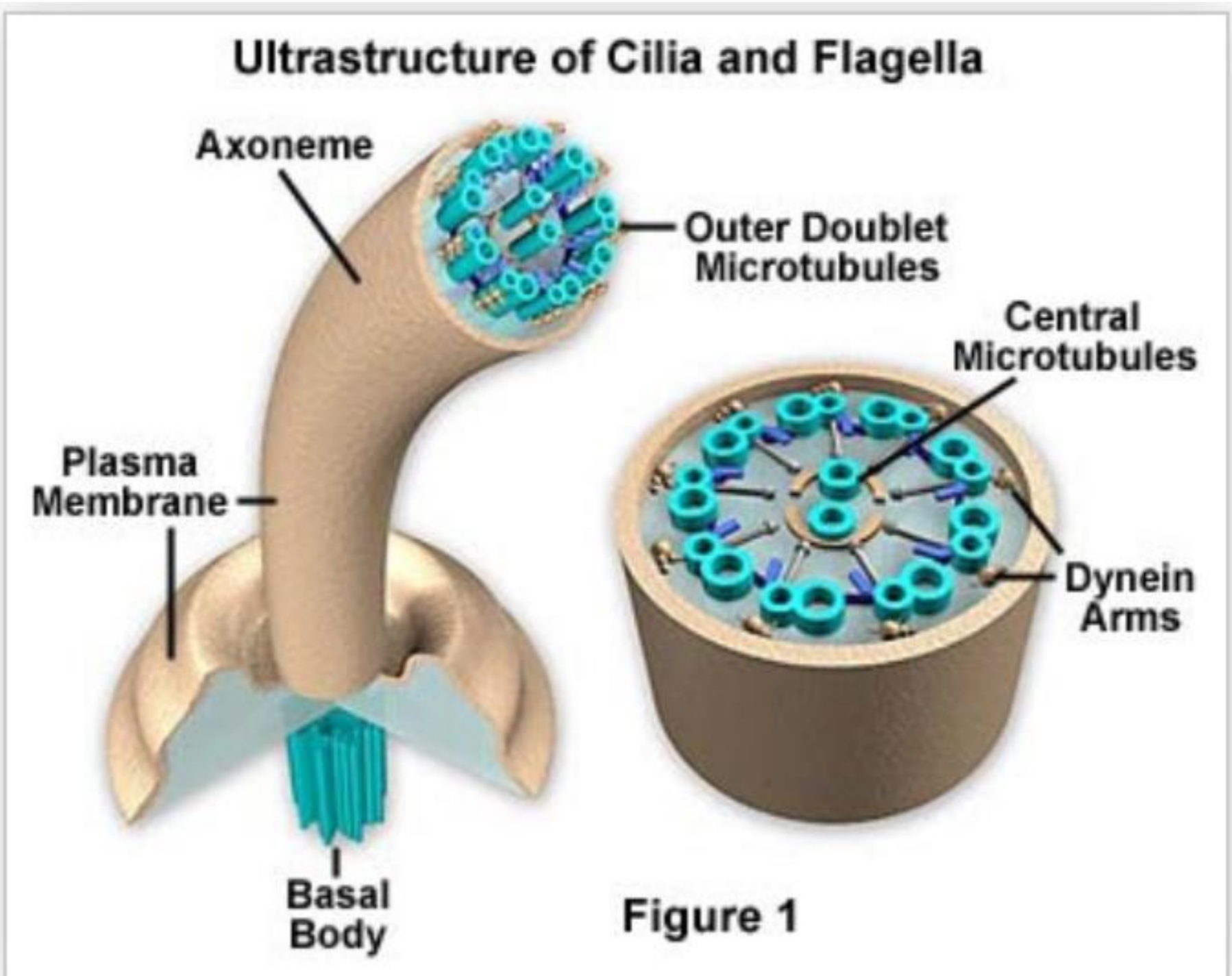

Ultrastructure of Cilia and Flagella
Axoneme
Outer Doublet Microtubules
Central Microtubules
Plasma Membrane
Dynein Arms
Basal Body

Figure 1

Functions of Cilia:

1- Their rhythmic beating helps to move fluids or particles in one direction over the surface of the cell. The best example is the cilia in the respiratory tract that beat upwards and move a film of mucus, on which foreign particles are stuck, to be expelled out by coughing or sneezing.

2- Modified cilia act as receptors in sense organs, e.g. rods & cones in the retina.

3) Flagella

Definition: They are motile projections from the cell, designed to move the cell itself. They have exactly the same basic structure as the axoneme of the cilium (nine peripheral doublets and 2 central singlets), but extremely longer. A flagellum forms the tail of the spermatozoa, which helps its whip-like movements.

b) Filaments

Definition: A group of minute threads or fine strands of molecular proteins responsible for elasticity and contractility of cytoplasm (muscles of the cell). They differ in size and distribution according to cell types.

Types: According to size, 3 forms are present:

1- Thin filaments: 6 nm, e.g. actin (microfilaments).

2- Intermediate filaments: 10 nm, e.g. keratin, vimentin, desmin, neuoro-filaments & glial filaments.

3- Thick filaments: 15 nm, e.g. myosin filaments.

(1) Thin filaments (Microfilaments)
(Actin filaments)

Definition: extremely fine strands of molecular actin (6 nm).

Sites & Functions:

1- Form a band or dense mesh beneath the cell membrane (peripheral ectoplasm).

2- In skeletal muscles, interact with myosin (thick) filaments for contraction.

3- Initiate cell membrane mobility and changes in cell shape (amoeboid movements
pseudopodia, endocytosis) as in WBCS.

4- In microvilli, microfilaments form their core to keep their shape.

(2) Intermediate filaments

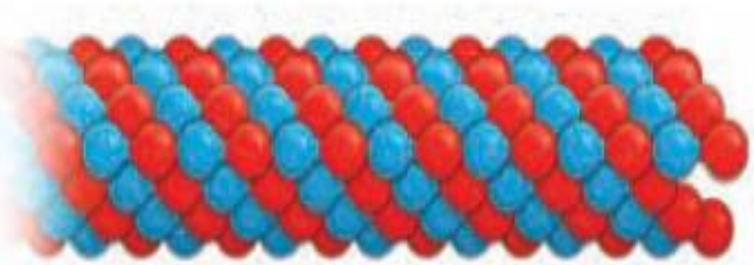
Definition: a family of filaments of similar microscopic structure, but different in chemical nature. They are intermediate in size between microtubules and microfilaments (8-11 nm in diameter).

Types: several types, each is formed of protein molecules characteristic for it:

1- Keratin filaments (tonofilaments): found in epithelial cells and form hairs and nails.
2- Vimentin: found in cells of mesenchymal origin as most CT cells. Also provide mechanical strength to muscle cells and nuclear envelope.
3- Desmin: found in muscle cells to join myofibrils together.
4- Neurofilaments: in neurons to support their long processes.
5- Glial filaments: in glial cells for support.

(3) Thick filaments (Myosin filaments)

Their diameter is about 15 nm. Formed of the protein myosin and are arranged in bundles. Each thick filament is formed of 200-300 myosin molecules. They are present in muscle cells and interact with actin filaments for muscle contraction.

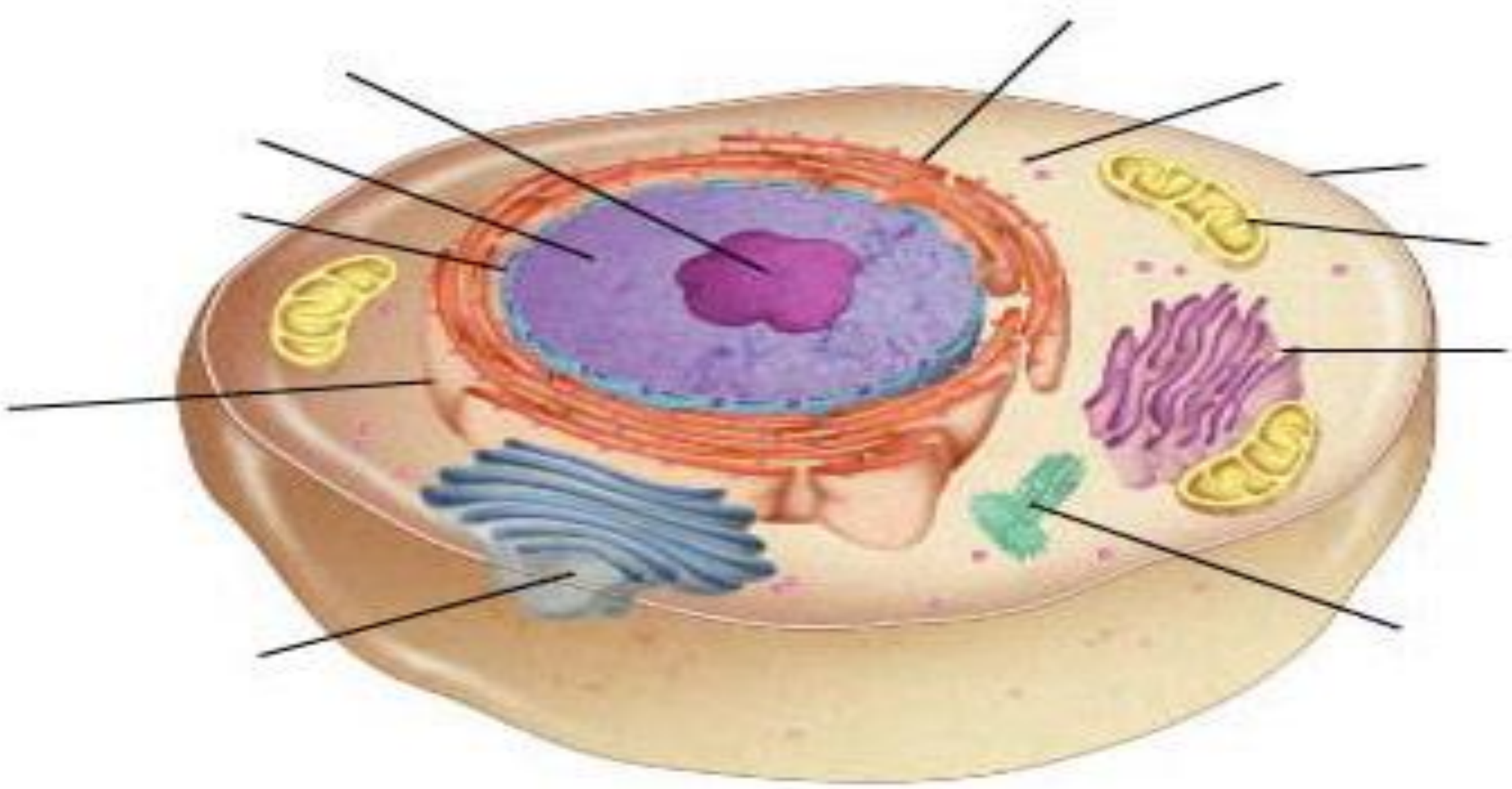
	Actin Filaments (Microfilaments)	Intermediate Filaments	Microtubules
			
Shape	Double-stranded linear helical array	Rope-like fibers	Nonbranching long, hollow cylinders
Diameter (nm)	6–8	8–10	20–25
Basic protein subunit	Monomer of G-actin (MW 42 kDa)	Various intermediate filament proteins (MW ~50 kDa)	Dimers of α- and β-tubulin (MW 54 kDa); γ-tubulin found in MTOC is necessary for nucleation of microtubules; δ-, ϵ-, ζ-, η-tubulins are associated with MTOC and basal bodies

- **Functions of cytoskeleton:**

- • It maintains the shape of the cell & its attachment.
- • It holds and moves the organelles, helps the migration of the vesicles.
- • It has an important role in cellular movement (amoeboid movement).
- • It has an important role in endocytosis, exocytosis and cell division

Nucleus

- **Definition:**
- • The largest and most distinct component of the cell.
- • Present in all cells, except RBCs and platelets (not true cells).
- • Plays an important role in heredity, cell division and controls all cellular functions.



- **Number:** usually one nucleus is present in each cell **(mononucleated),** but some cells have two nuclei **(binucleated)** as liver cells and some have more than two nuclei **(multinucleated)** as osteoclasts & skeletal muscles.
- **Position:** usually central, but may be eccentric, basal or peripheral. Nucleus is
- always present in the wider part of the cell.

- **Shape:** rounded, flattened, oval, kidney-shaped, segmented, lobulated, or
- bilobed (horse shoe).

Flattene

Ova

Oval

Rounded

Bilobed

Segmented

Lobulated

Kidney-shaped

(horse

Centra

Eccentric
(peripheral)

Basa

Various shapes & positions of nuclei.

- **LM:**
- • Dark blue with basic stains as hematoxylin, methylene blue.
- • Its basophilia is due its content of DNA & RNA.
- **Appearance:** according to cell activity it may appear:

- **Lightly stained (vesicular):** pale with fine extended chromatin (open face nucleus) and prominent nucleolus, e.g. nerve cells nucleus.

- **Deeply stained (condensed):** masses of dense condensed chromatin granules (closed face nucleus), e.g. small lymphocytes.

- **Structure of the nucleus:** 4 components:
- 1- Nuclear membrane (Envelope).
- 2- Chromatin material.
- 3- Nucleolus.
- 4- Nuclear sap.

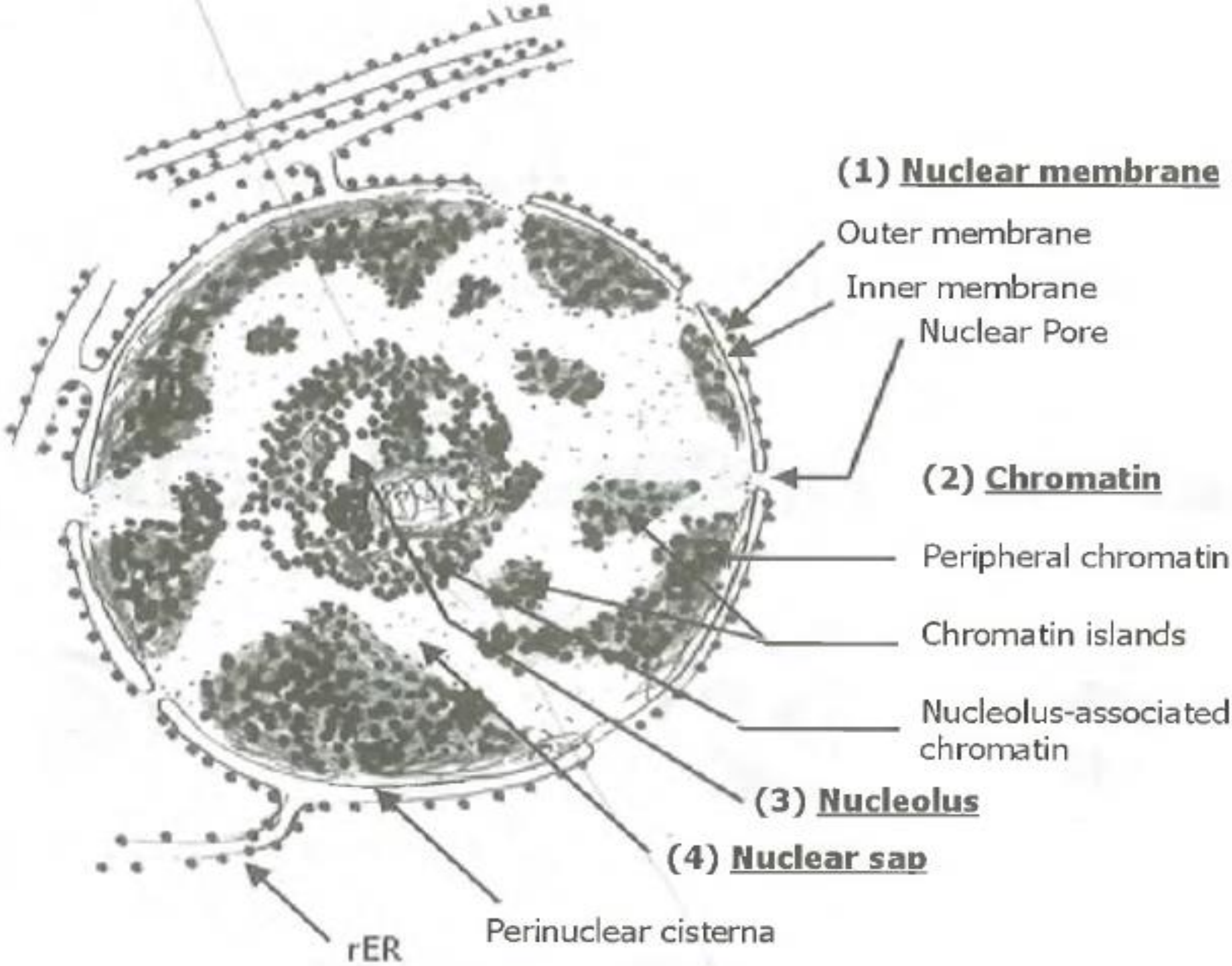


Diagram showing components of the nucleus.

- **Structure of the Nucleus**
- **Nuclear Membrane (Nuclear Envelope)**

- **LM:** blue line (basophilic) due to chromatin on its inner side & ribosomes on its outer side.

- **EM:**

- • **Double walled membrane** formed of two parallel unit membranes separated by a **perinuclear space** (10-30 nm) and interrupted at intervals by **nuclear pores.**

- **- Outer nuclear membrane (facing the cytoplasm):** It is rough & granular due to ribosomes attached to its outer surface, continuous with rER cisternae.

- **- Inner nuclear membrane (facing the nucleus):** It is fibrillar due to attached chromatin threads on its inner side (peripheral chromatin).

- **-Nuclear pores:** circular openings at intervals where the outer & inner membranes fuse to be continuous as diaphragms.

EM of nuclear membrane.

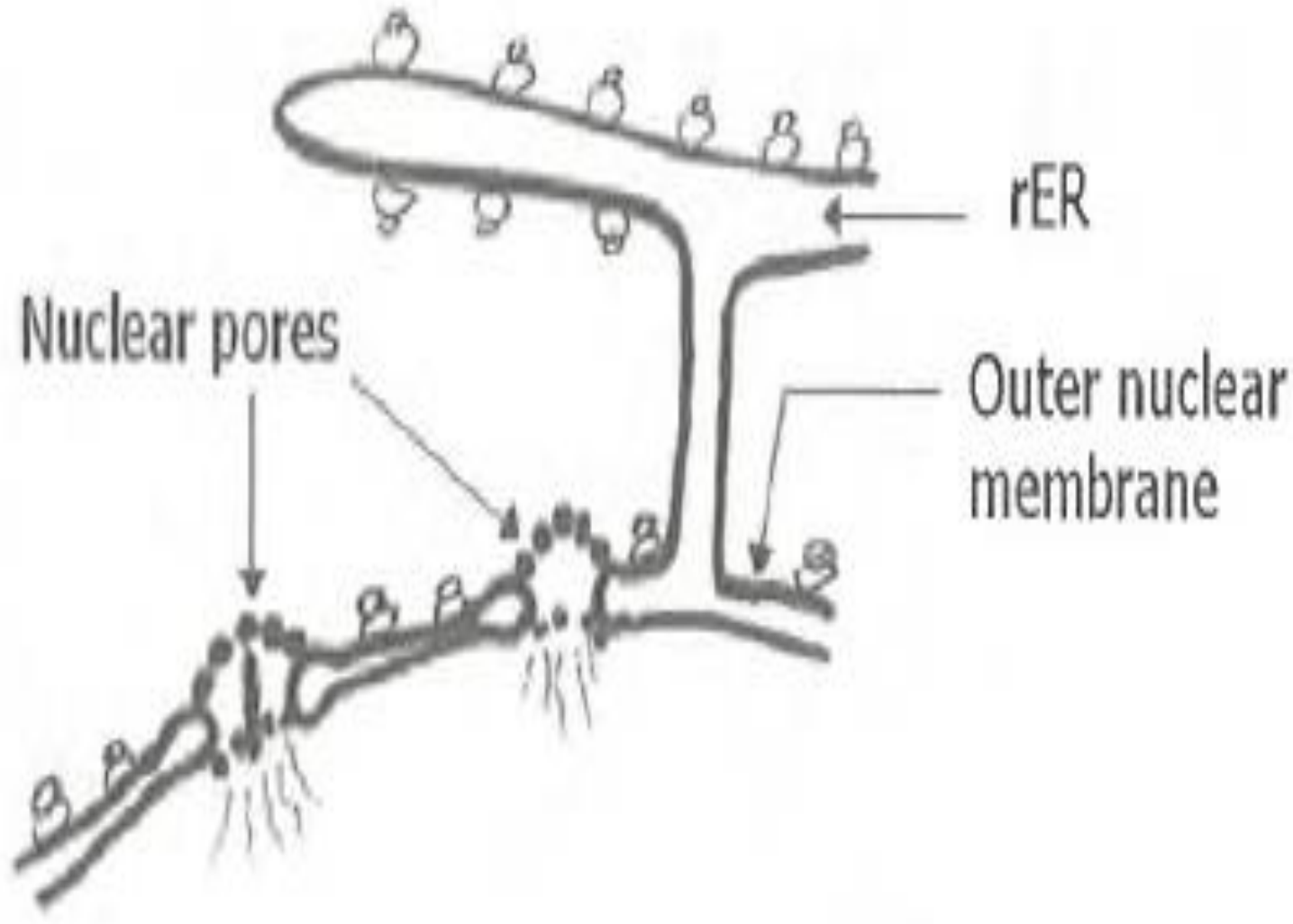
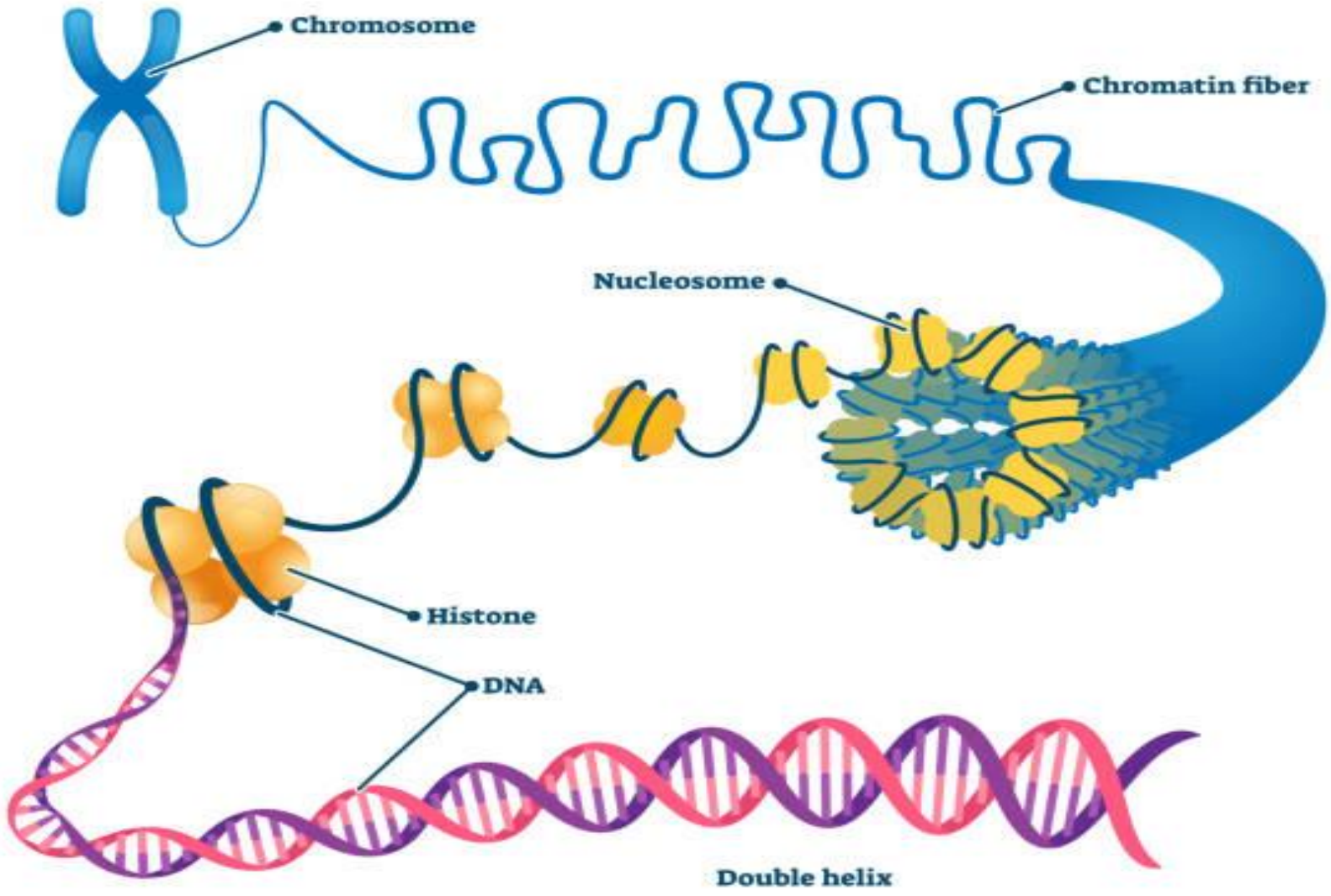


Diagram of nuclear pores.

- **Chromatin**
- **Definition:**
- Particles and threads in the interphase nucleus that change into chromosomes during cell division.
- It represents the genetic material, formed of nucleoproteins (DNA + Histone).
-

CHROMATIN


Chromosome
Chromatin fiber
Nucleosome
Histone
DNA
Double helix

- **LM:**

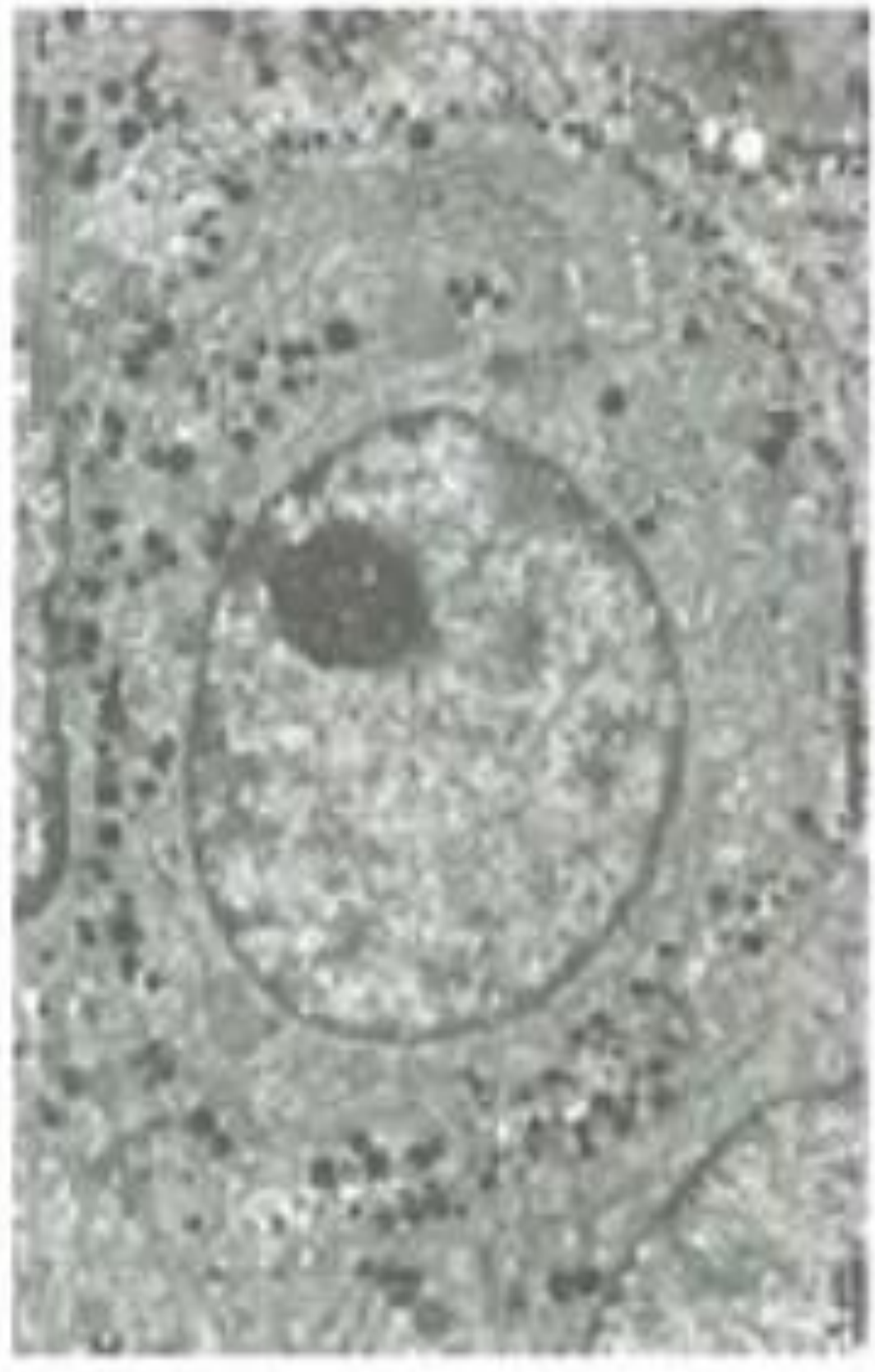
- • Basophilic masses or threads.
- • Have two forms according to cell activity:
- **1- Euchromation (extended or active):**
- - Not visible in LM which makes the nucleus pale in staining (vesicular nucleus).
- - It represents extended (uncoiled) chromosomes.
- - Contains the active genes that direct protein synthesis in protein-forming cells.

- **2- Hetrochromatin (condensed or inactive):** Appears dark in LM (condensednucleus) as it represents coiled chromosomes, and contains the inactive genes.
- **EM:**
- **1- Euchromatin:** appears as fine granules only at high magnifications.
- **2-Heterochromatin:** appears as masses of nucleoproteins condensed at different sites in the nucleus

- **Sites of heterochromatin:**
- **a) Peripheral chromatin:** attached to the inner surface of nuclear membrane.
- **b) Chromatin islands:** aggregated clumps scattered in the nuclear sap.
- **c) Nucleolus-associated chromatin:** condensed around the nucleolus.

- **Functions of Chromatin:**
- Carries genetic information.
- Directs & controls protein synthesis.
- Formation of RNA (mRNA, rRNA & tRNA).

Heterochromatic nucleus.



Euchromatic nucleus.

- **3- Nucleolus**
- **LM:**
- • Rounded, deeply basophilic mass, as it is rich in nucleic acids and surrounded with chromatin.
- • Usually one or two in each nucleus.

- **EM:** It has **dark and light areas.**

- • **The dark area is called (nucleolar organizer)** it is surrounded with granular and fibrillar parts. The Granular part formed of granules of mature ribosomal RNA. The fibrillar part is formed of filaments of newly formed ribosomal RNA.
- • **The light areas:** formed of the nucleolar sap.

- **Functions of the Nucleolus:** formation of ribosomal RNA & ribosomes, which pass through nuclear pores to the cytoplasm and start protein synthesis.

- N.B.: Protein-forming cells have large prominent one or more nucleoli.

- **4- Nuclear Sap**
- **Definition:**
- • A colloidal solution that fills the spaces between chromatin material and nucleolus.
- • Formed of: nucleoproteins, enzymes, sugars, calcium, potassium & phosphorous ions.

- **Functions of Nuclear Sap:** Provides a medium for transport of RNA through nuclear pores to cytoplasm for protein synthesis.

- **Functions of the Nucleus**
- 1- It carries all the genetic information and hereditary factors.
- 2- It controls all the cell functions including protein synthesis.
- 3- It is responsible for the formation of RNA.
- 4- It directs cell division.

General Histology

Dr:Hani Ahmed Alanqar
Consultant of Histopathology
El shifa hospital

Epithelial Tissue

What is tissue

- A **tissue** is a group of cells
  - Common embryonic origin
  - Function together to carry out specialized activities
- Hard (bone), semisolid (fat), or liquid (blood)
- **Histology** is the science that deals with the study of tissues.
- **Pathologist** specialized in laboratory studies of cells and tissue for diagnoses

Types of tissues :

- **Epithelial**
  - Covers body surfaces and lines hollow organs, body cavities, duct, and forms glands
- **Connective**
  - Protects, supports, and binds organs.
  - Stores energy as fat, provides immunity
- **Muscular**
  - Generates the physical force needed to make body structures move and generate body heat
- **Nervous**
  - Detect changes in body and responds by generating nerve impulses

Development of Tissues

- Tissues of the body develop from three primary germ layers:
    - **Ectoderm, Endoderm, and Mesoderm**
  - Epithelial tissues develop from all three germ layers
  - All connective tissue and most muscle tissues drive from **mesoderm**
  - Nervous tissue develops from **ectoderm**

Epithelial Tissue:

- **Epithelial tissue** consists of cells arranged in continuous sheets, in either single or multiple layers
  - Closely packed and held tightly together
  - Covering and lining of the body
  - Free surface

Essential function of epithelial tissues

– protection - skin
– absorption – small intestine
– secretion – glandular epithelium
– excretion – epithelium of kidney tubule
– sensory – neuro-epithelium
– lubrication – goblet cells and sebaceous gland

Special Characteristics of Epithelia

- **Cellularity**
  - cells are in close contact with each other with little or no intercellular space between them
- **Specialized contacts**
  - may have junctions for both attachment and communication
- **Polarity**
  - epithelial tissues always have an apical and basal surface
- **Support by connective tissue**
  - at the basal surface, both the epithelial tissue and the connective tissue contribute to the basement membrane
- **Avascular**
  - nutrients must diffuse
- **Innervated**
- **Regeneration**
  - epithelial tissues have a high capacity for regeneration

General Features of Epithelial Cells

- **Basement membrane**
  - Thin double extracellular layer that serves as the point of attachment and support for overlying epithelial tissue
  - **Basal lamina**
    - Closer to and secreted by the epithelial cells
    - Contains laminin, collagen, glycoproteins, and proteoglycans
  - **Reticular lamina**
    - Closer to the underlying connective tissue
    - Contains collagen secreted by the connective tissue cells

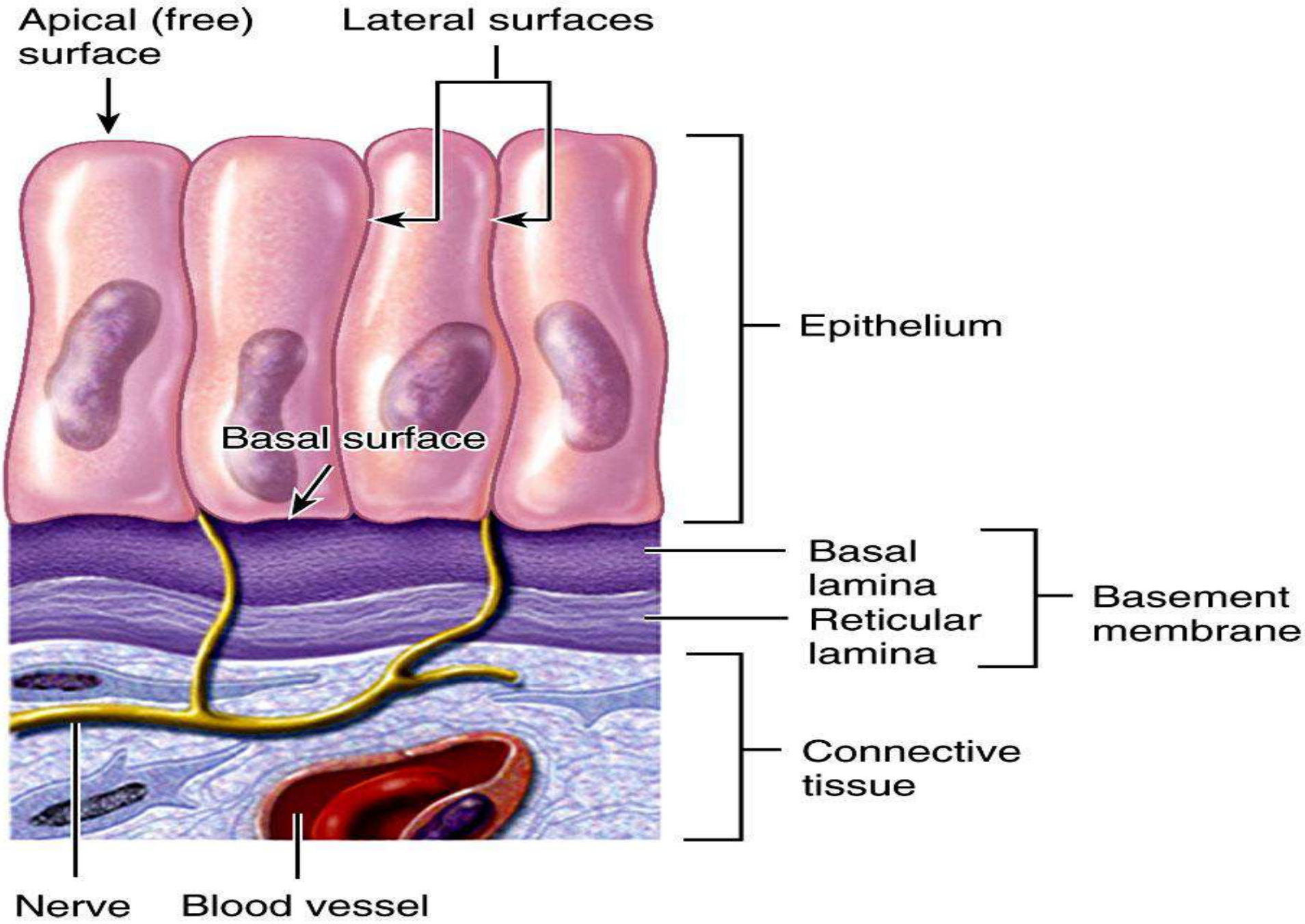


Figure 04.02 Tortora - PAP 12/e

Epithelium is classified according to

A. Number of layers :

1- Simple Epithelium:
made of one layer only.

2- Stratified Epithelium :
made of more than one layer

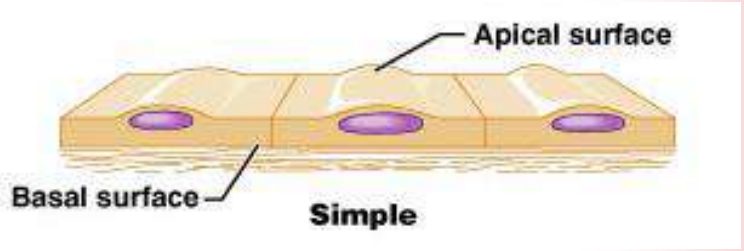
B. Function into :

1- Surface (covering or lining).

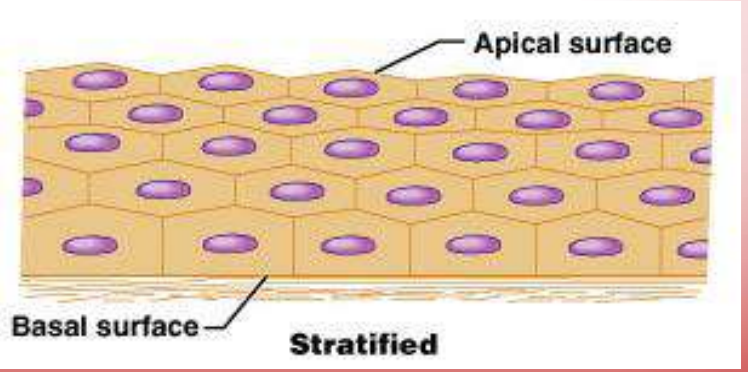
2- **Glandular**

Classifications & Naming of Epithelia

- First name of tissue indicates number of layers
  - Simple – one layer of cells

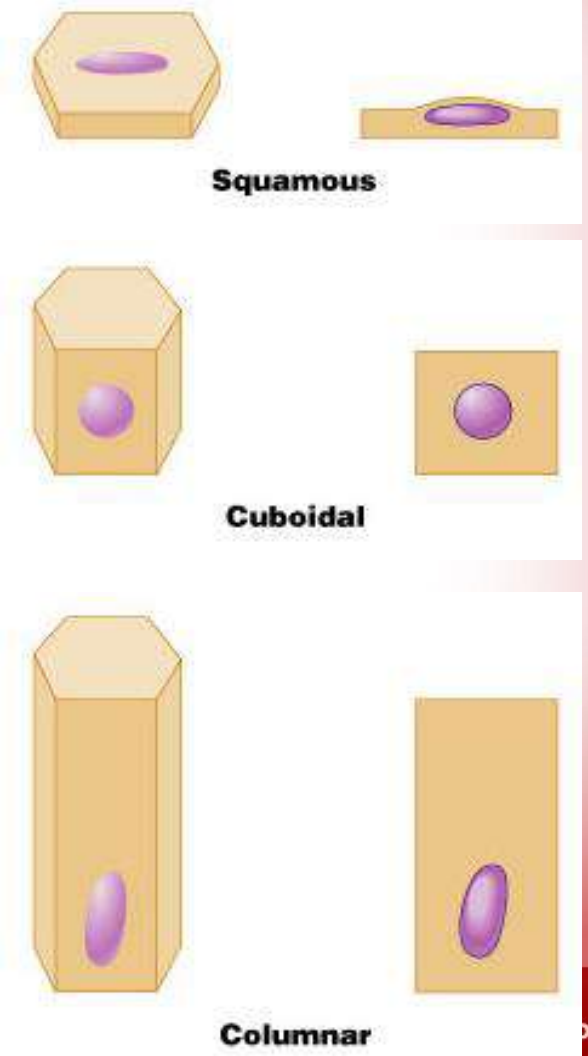


  - Stratified – more than one layer of cells



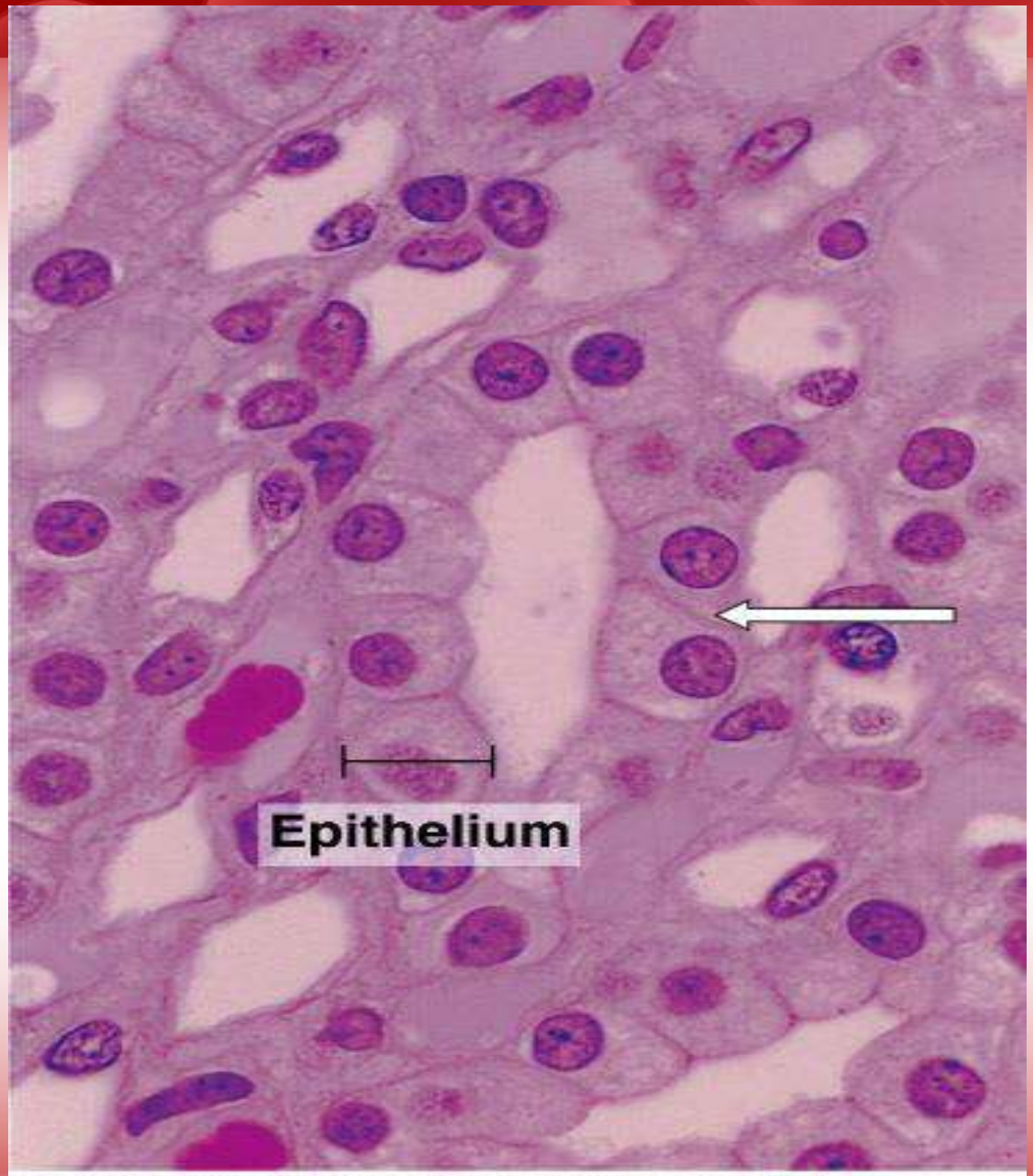
- Last name of tissue describes shape of cells
  - Squamous – cells wider than tall (plate or “scale” like)
  - Cuboidal – cells are as wide as tall, as in cubes

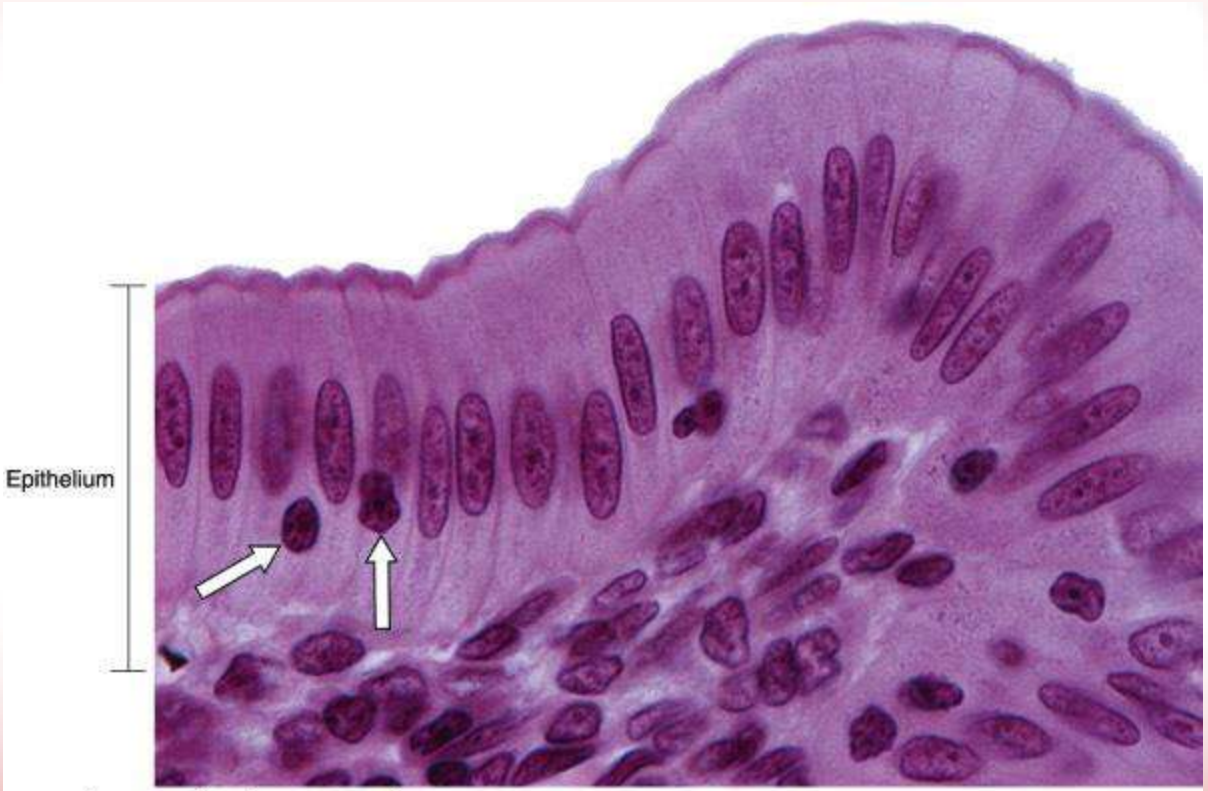
Columnar – cells are taller than they are wide, like columns



Naming Epithelia :

- Naming the epithelia includes both the layers (first) and the shape of the cells (second)
  - i.e. stratified cuboidal epithelium
- The name may also include any **accessory structures**
  - Goblet cells
  - Cilia
  - Keratin
- Special epithelial tissues (don't follow naming convention)
  - Psuedostratified
  - Transitional





Covering & lining epithelial

- Normally classified according to:
  - Arrangement of cells into layers
  - Shapes of cells

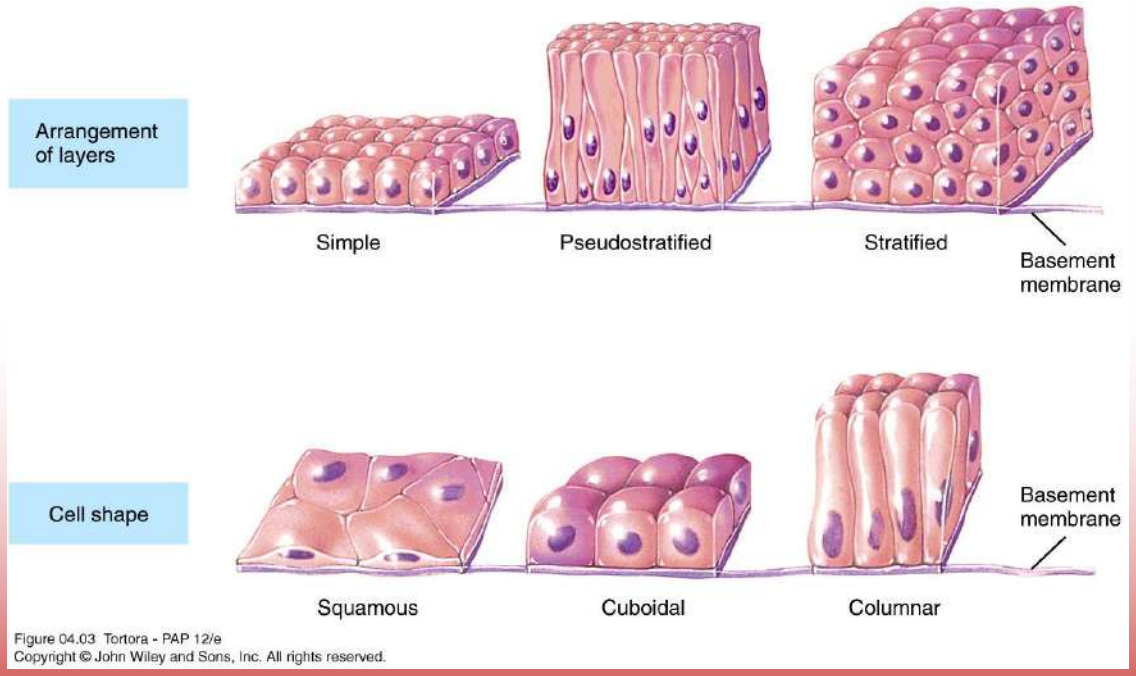


Figure 04.03 Tortora - PAP 12/e

Arrangement of cells in layers

- Consist of one or more layers depending on function
- **Simple epithelium**
  - Single layer of cells that function in diffusion, osmosis, filtration, secretion, or absorption
- **Pseudostratified epithelium**
  - Appear to have multiple layers because cell nuclei at different levels
  - All cells do not reach the apical surface
- **Stratified epithelium**
  - Two or more layers of cells that protect underlying tissues in areas of wear and tear

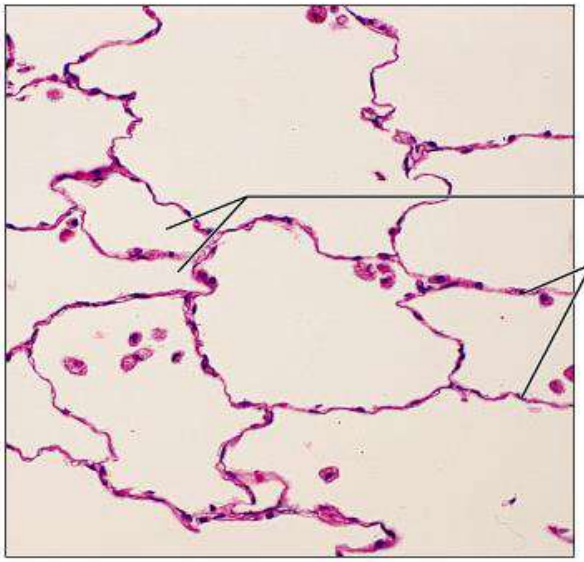
Simple Epithelium

- Simple squamous epithelium
- Simple cuboidal epithelium
- Simple columnar epithelium (nonciliated and ciliated)

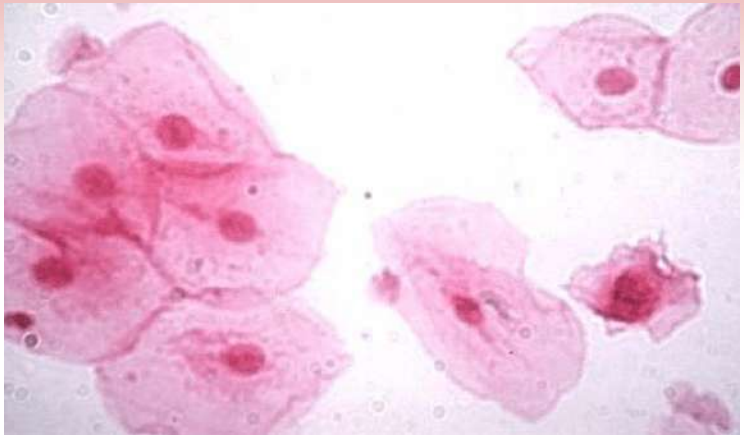
Simple Squamous Epithelium

- Description
  - single layer of flat cells with disc-shaped nuclei
- Special types
  - Endothelium (inner covering)
    - slick lining of hollow organs
  - Mesothelium (middle covering)
    - Lines peritoneal, pleural, and pericardial cavities
    - Covers visceral organs of those cavities

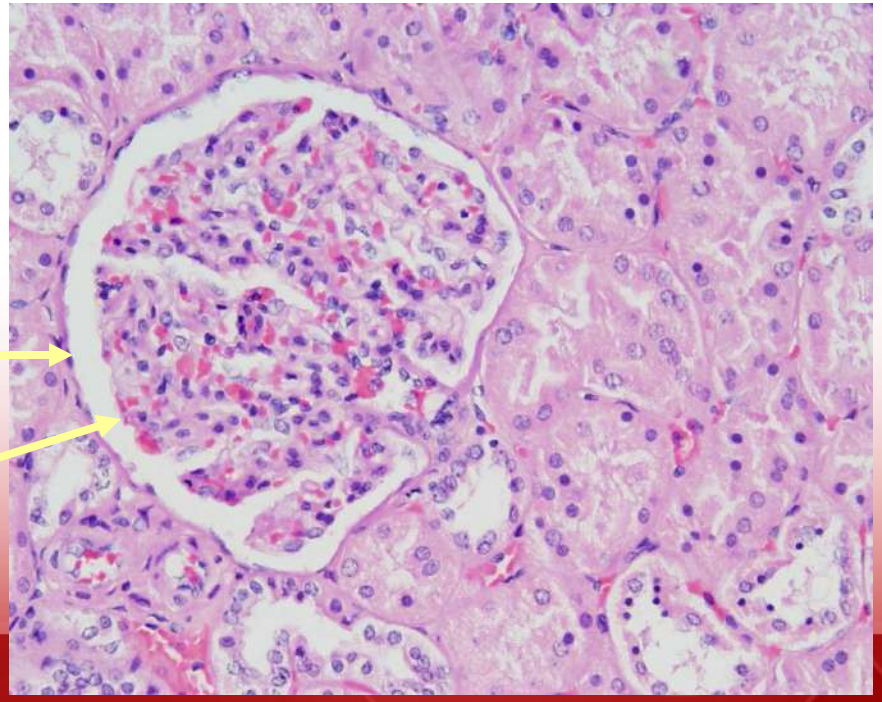
- Function
  - Passage of materials by passive diffusion and filtration
  - Secretes lubricating substances in serosae
- Location
  - Renal corpuscles
  - Alveoli of lungs
  - Lining of heart, blood and lymphatic vessels
  - Lining of ventral body cavity (serosae)



Photomicrograph: Simple squamous epithelium forming part of the alveolar (air sac) walls (400×).

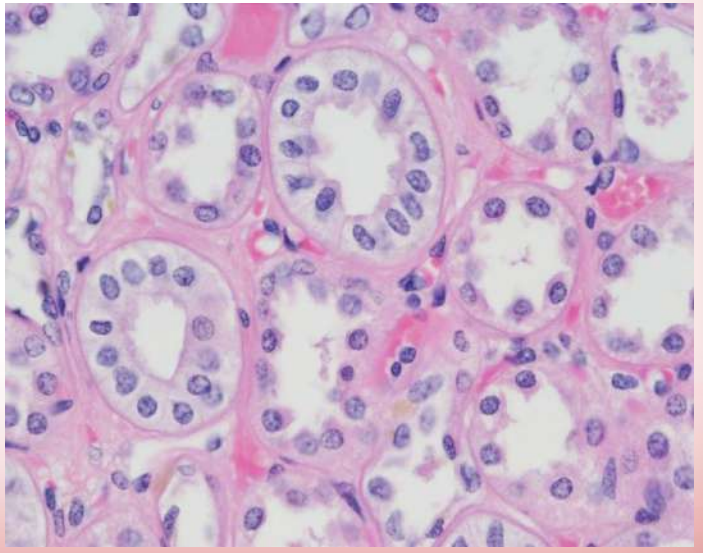


Simple squamous lining the walls of the capillary



Simple Cuboidal Epithelium

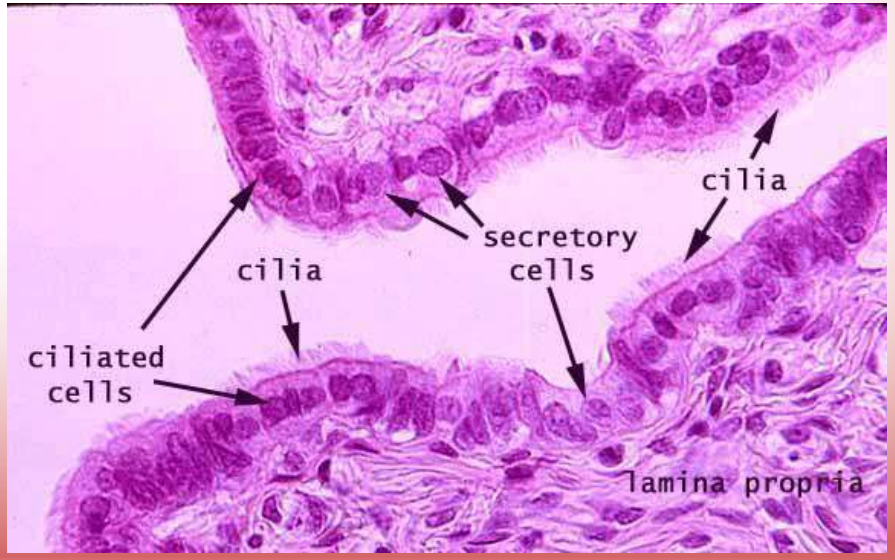
- Description
  - single layer of cube-like cells with large, spherical central nuclei
- Function
  - secretion and absorption
- Location
  - kidney tubules, secretory portions of small glands, ovary & thyroid follicles



Simple Columnar Epithelium

- Description
  - single layer of column-shaped (rectangular) cells with oval nuclei
    - Some bear cilia at their apical surface
    - May contain goblet cells
- Function
  - Absorption; secretion of mucus, enzymes, and other substances
  - Ciliated type propels mucus or reproductive cells by ciliary action

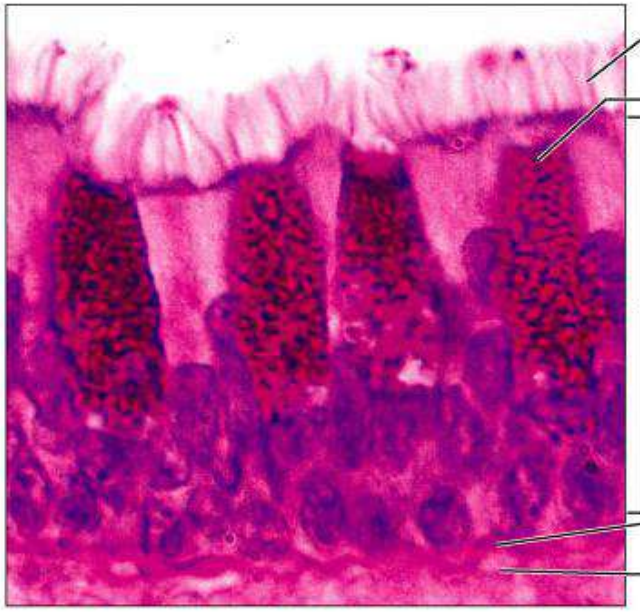
- Location
  - Non-ciliated form
    - Lines digestive tract, gallbladder, ducts of some glands
  - Ciliated form
    - Lines small bronchi, uterine tubes, uterus



Pseudostratified Columnar Epithelium

- Description
  - All cells originate at basement membrane
  - Only tall cells reach the apical surface
  - May contain goblet cells and bear cilia
  - Nuclei lie at varying heights within cells
    - Gives false impression of stratification
- Function
  - secretion of mucus; propulsion of mucus by cilia

- Locations
  - Non-ciliated type
    - Ducts of male reproductive tubes
    - Ducts of large glands
  - Ciliated variety
    - Lines trachea and most of upper respiratory tract



Photomicrograph: Pseudostratified ciliated columnar epithelium lining the human trachea (400×).

Stratified Epithelia

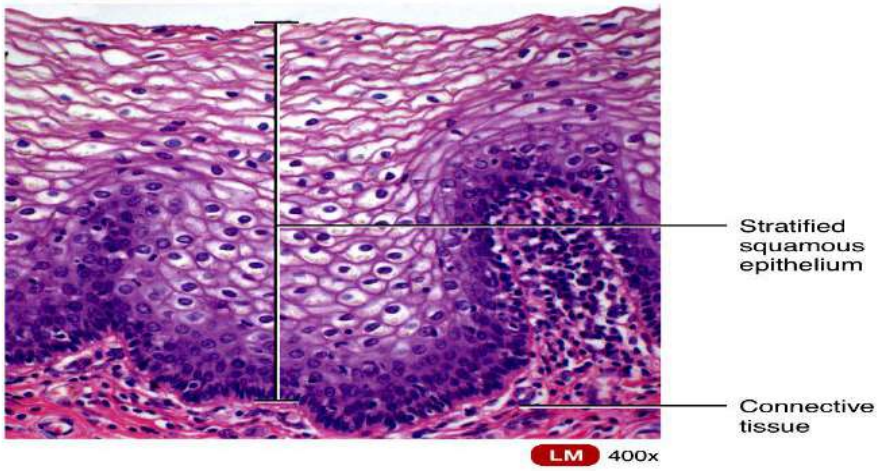
- Contain two or more layers of cells
- Regenerate from below
- Major role is protection
- Are named according to the shape of cells at apical layer

Stratified Epithelia

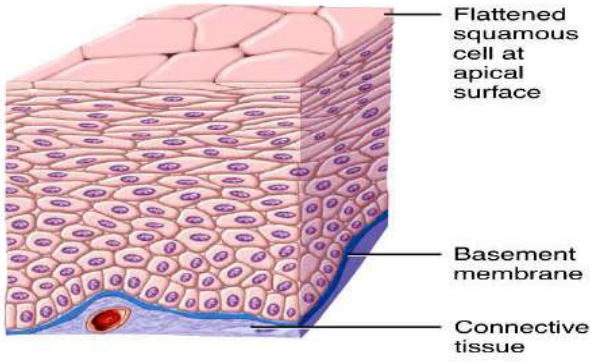
- Stratified squamous epithelium
- Stratified cuboidal epithelium
- Stratified columnar epithelium
- Transitional epithelium

Stratified Squamous Epithelium

- Several layers of cells that are flat in the apical layer
  - New cells are pushed up toward apical layer
  - As cells move further from the blood supply they dehydrate, harden, and die
- **Keratinized form** contain the fibrous protein keratin
  - Found in superficial layers of the skin
- **Nonkeratinized form** does not contain keratin
  - Found in mouth and esophagus



Sectional view of stratified squamous epithelium of vagina

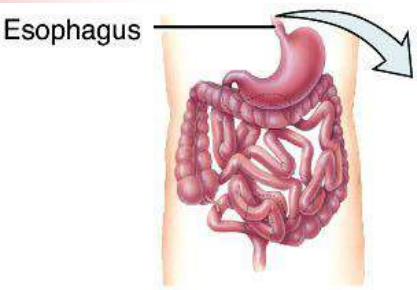


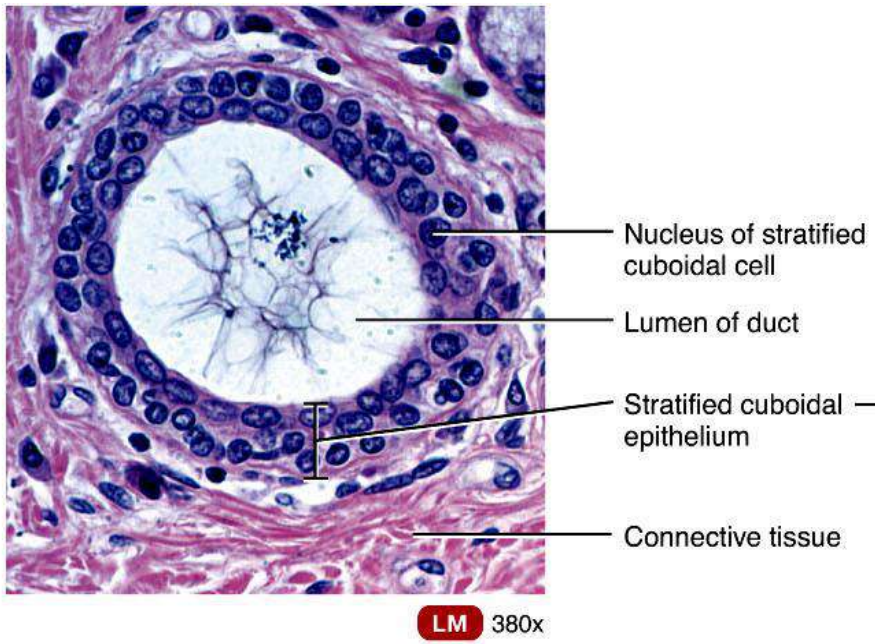
Stratified squamous epithelium

Table 04.table 01f Tortora - PAP 12/e

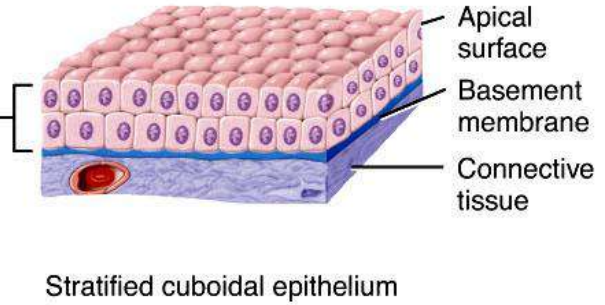
Stratified Cuboidal Epithelium

- Fairly rare type of epithelium
- Apical layers are cuboidal
- Functions in protection





Sectional view of stratified cuboidal epithelium of the duct of an esophageal gland

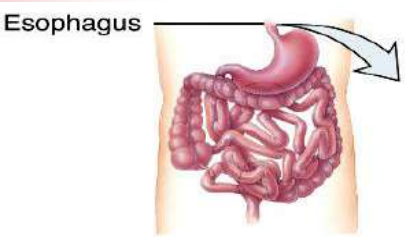


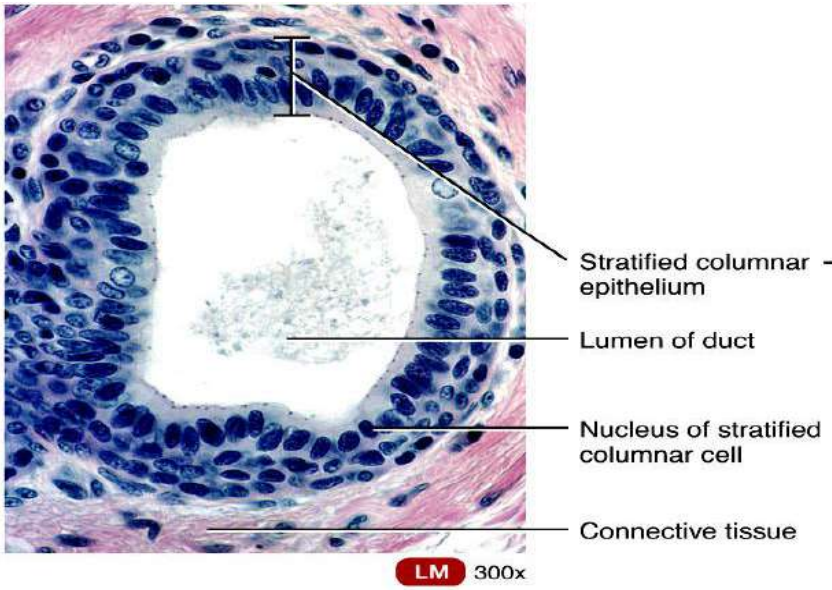
Stratified cuboidal epithelium

Table 04.table 01g Tortora - PAP 12/e

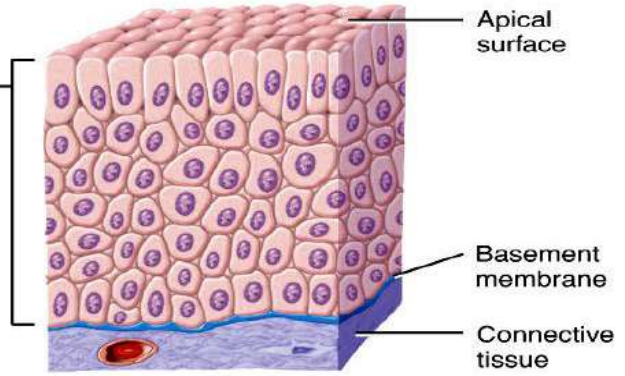
Stratified columnar epithelium

- Also very uncommon
- Columnar cells in apical layer only
- Basal layers has shorten, irregular shaped cells
- Functions in protection and secretion





Sectional view of stratified columnar epithelium of the duct of an esophageal gland

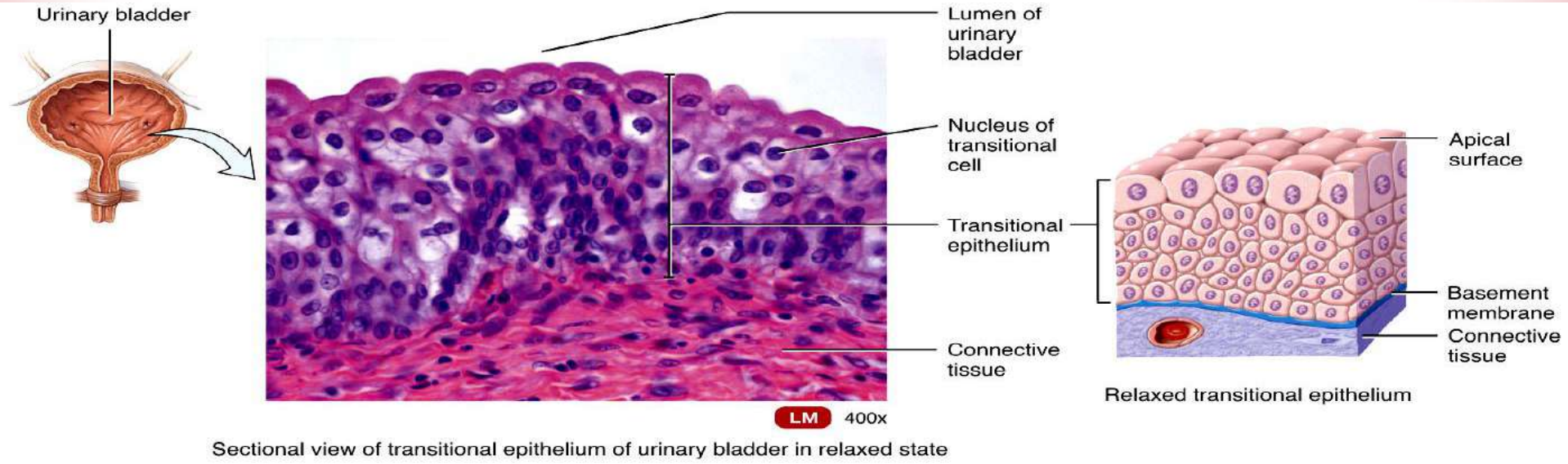


Stratified columnar epithelium

Table 04.table 01h Tortora - PAP 12/e

Transitional Epithelium

- Found only in the urinary system
- Variable appearance
- In relaxed state, cells appear cuboidal
- Upon stretching, cells become flattened and appear squamous
- Ideal for hollow structure subjected to expansion



Sectional view of transitional epithelium of urinary bladder in relaxed state

Table 04.table 01i Tortora - PAP 12/e

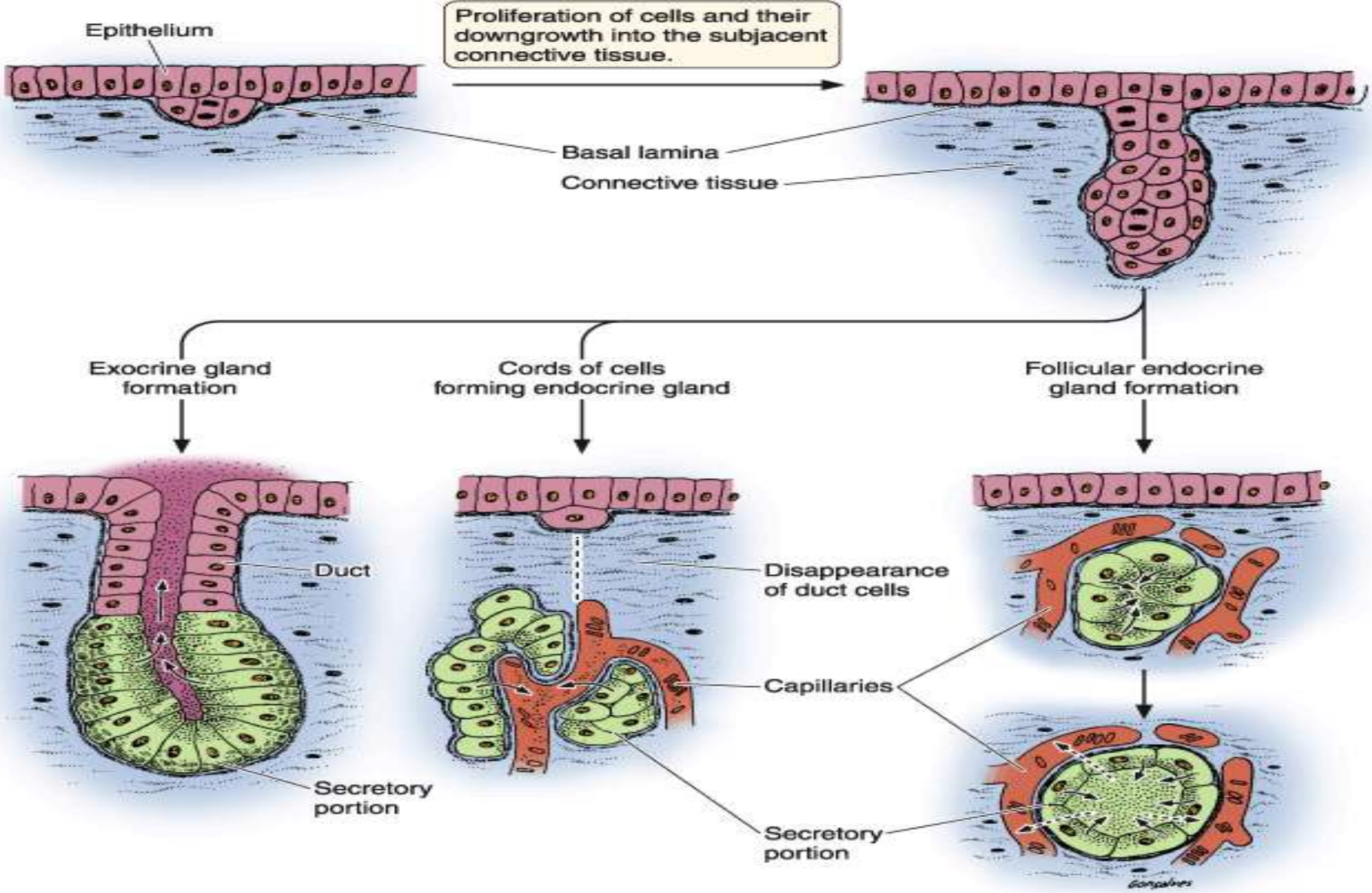
Glandular Epithelium

This one type of epithelial tissue specialized to produce secretion.

It is classified into

A. Endocrine Glands.
B. Exocrine Glands.

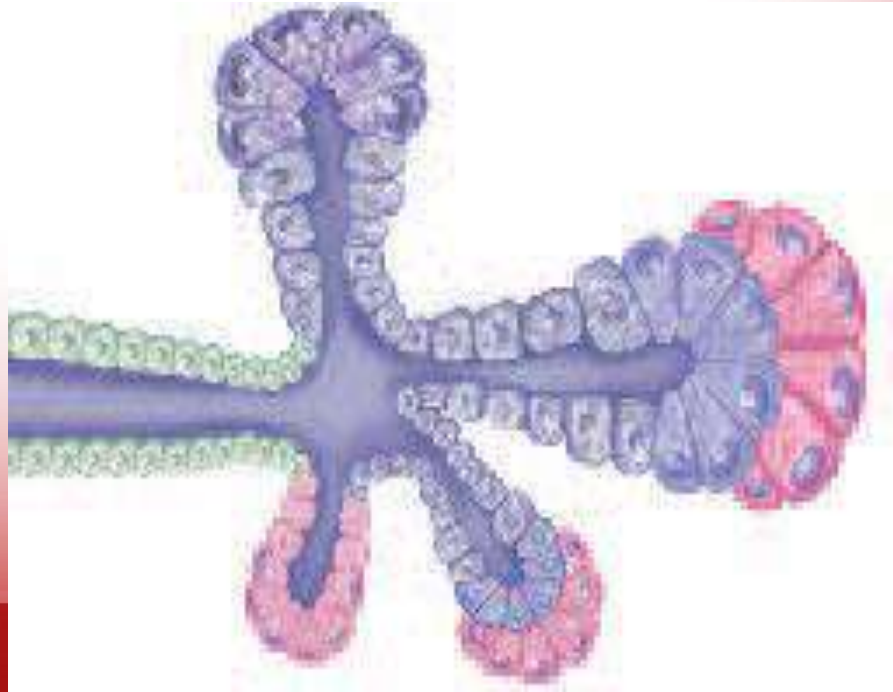
according to the presence or absence of duct systems



Exocrine Glands

- These glands are composed of secretory units and duct systems.

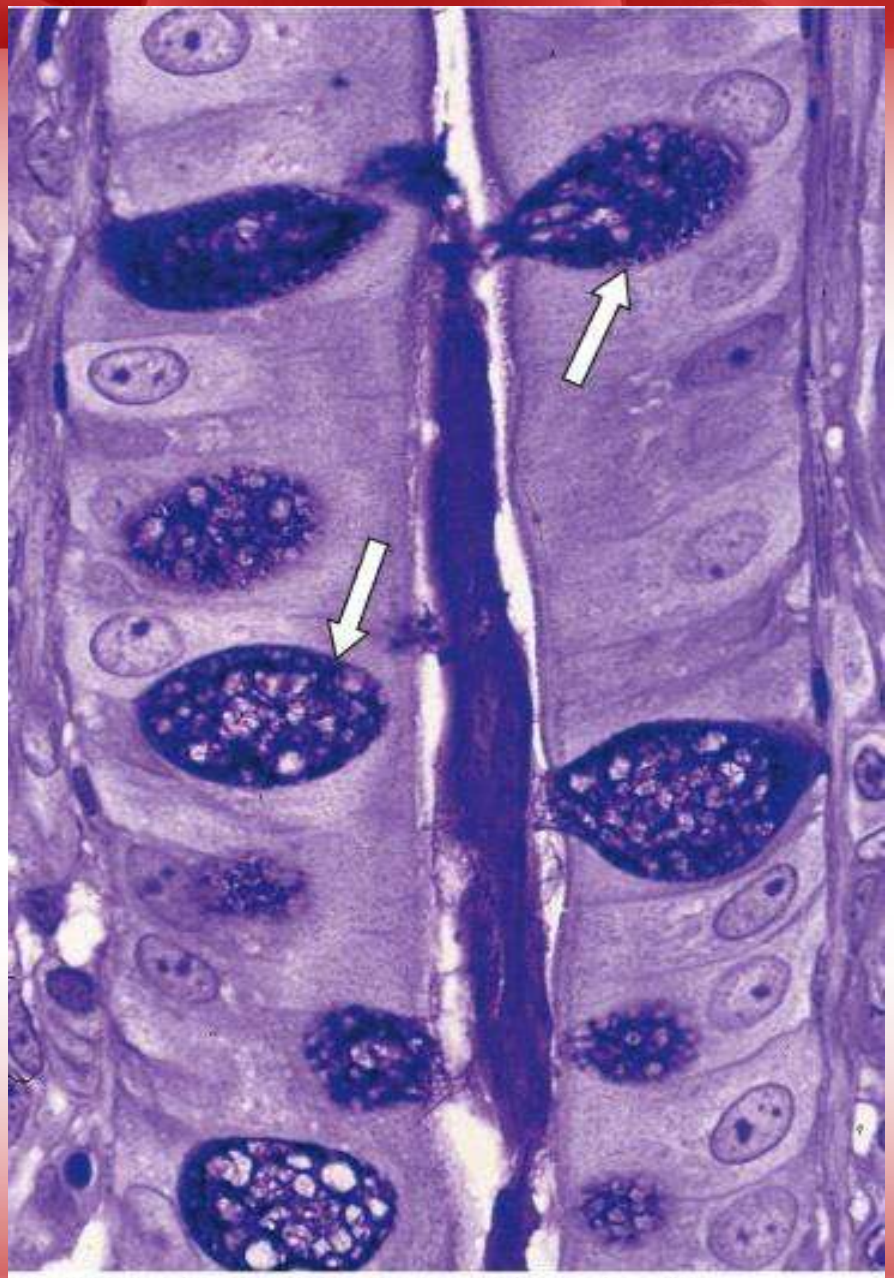
- classified in different ways.



According to Number of Cells :

A. **Unicellular glands** like **goblet cells**, they are present in large number in the lining epithelia of **large intestine** and the **respiratory tract**.

B. Multicellular glands which form most of the glands of the body example **salivary glands**.

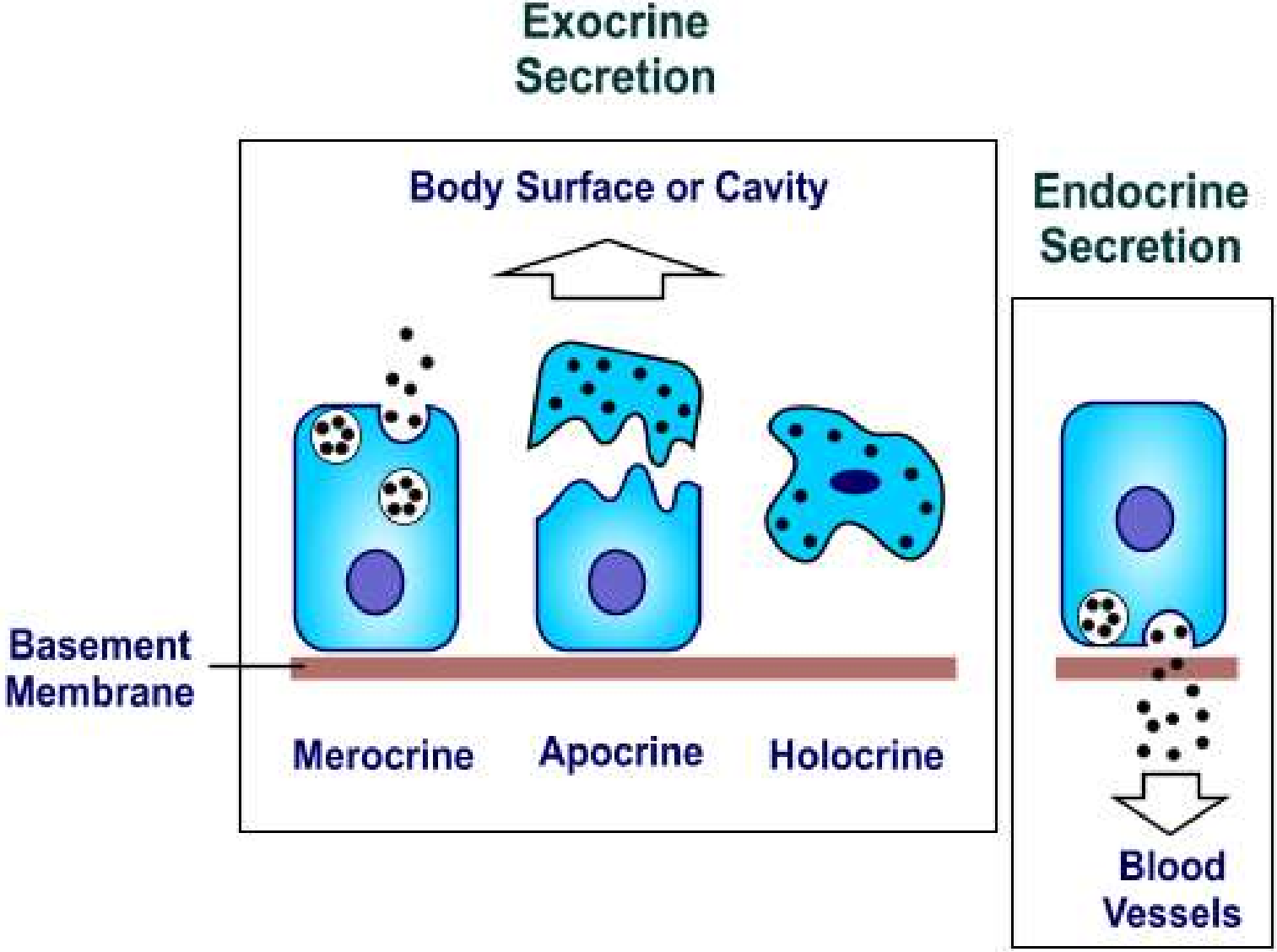


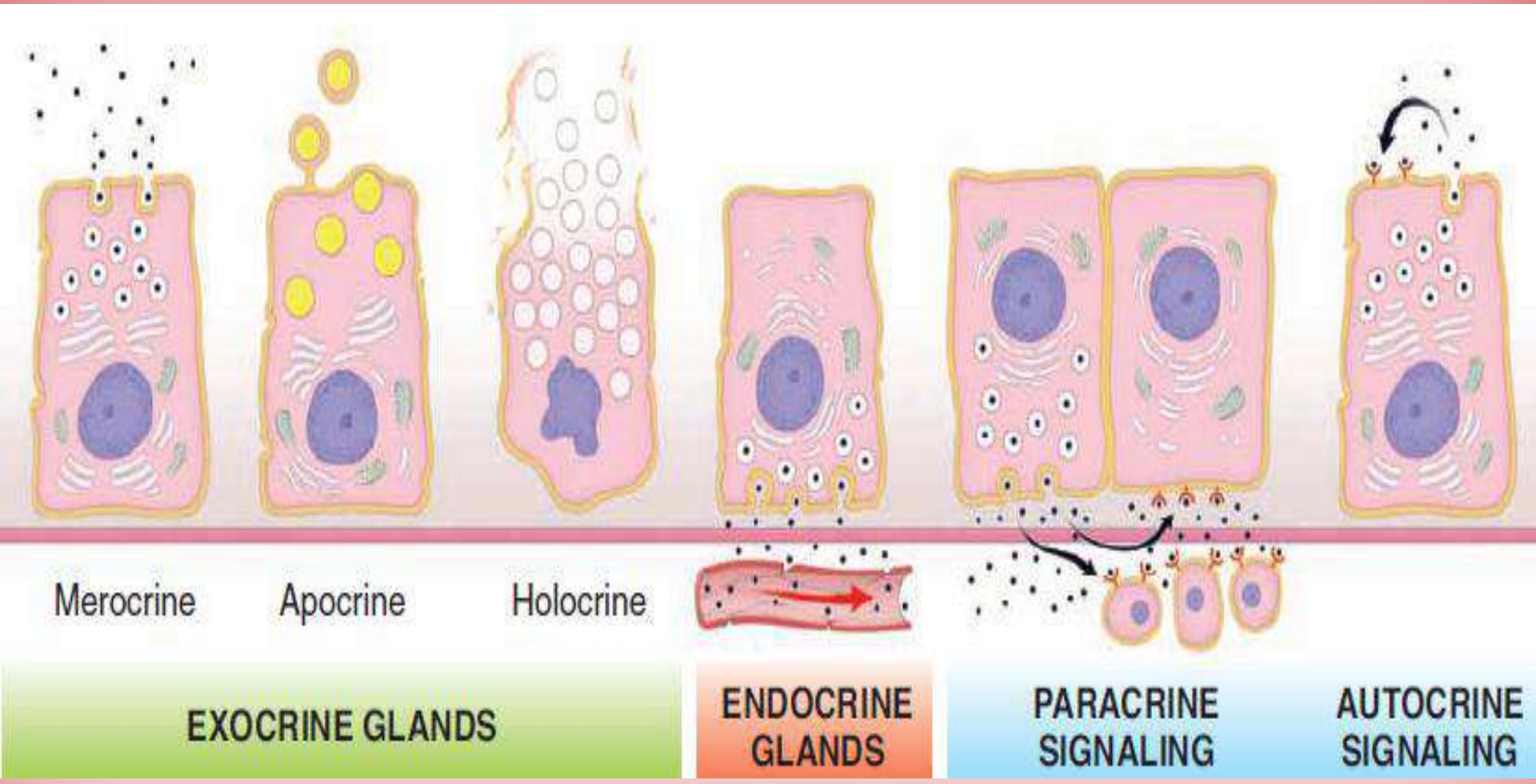
According to the Mode of Secretion :

A. Merocrine Glands (exocrine part of pancreas) :
where the secretory products leave the cells by exocytosis with no loss of cellular material.

B. Holocrine Glands (sebaceous glands) :
in which the secretory product is shed with the whole cell, a process which involves destruction of the secretary cell.

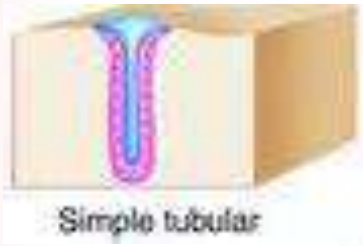
C. Apocrine Glands (Mammary and Axillary's sweat glands) :
in which the secretory product is discharged with parts of the apical cytoplasm.


Exocrine Secretion
Body Surface or Cavity
Endocrine Secretion
Basement Membrane
Merocrine
Apocrine
Holocrine
Blood Vessels


Merocrine
Apocrine
Holocrine
EXOCRINE GLANDS
ENDOCRINE GLANDS
PARACRINE SIGNALING
AUTOCRINE SIGNALING

Simple

- Simple tubular



Large intestine

Sweat gland

stomach

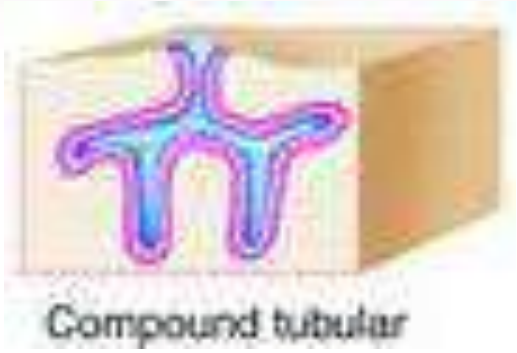
- Simple acinar/alveolar

Penile urethral gland

Sebaceous gland

Compound gland

- Tubular



Brunner's gland (Duodenum)

- Acinar/alveola

Pancreas

Submandibular gland

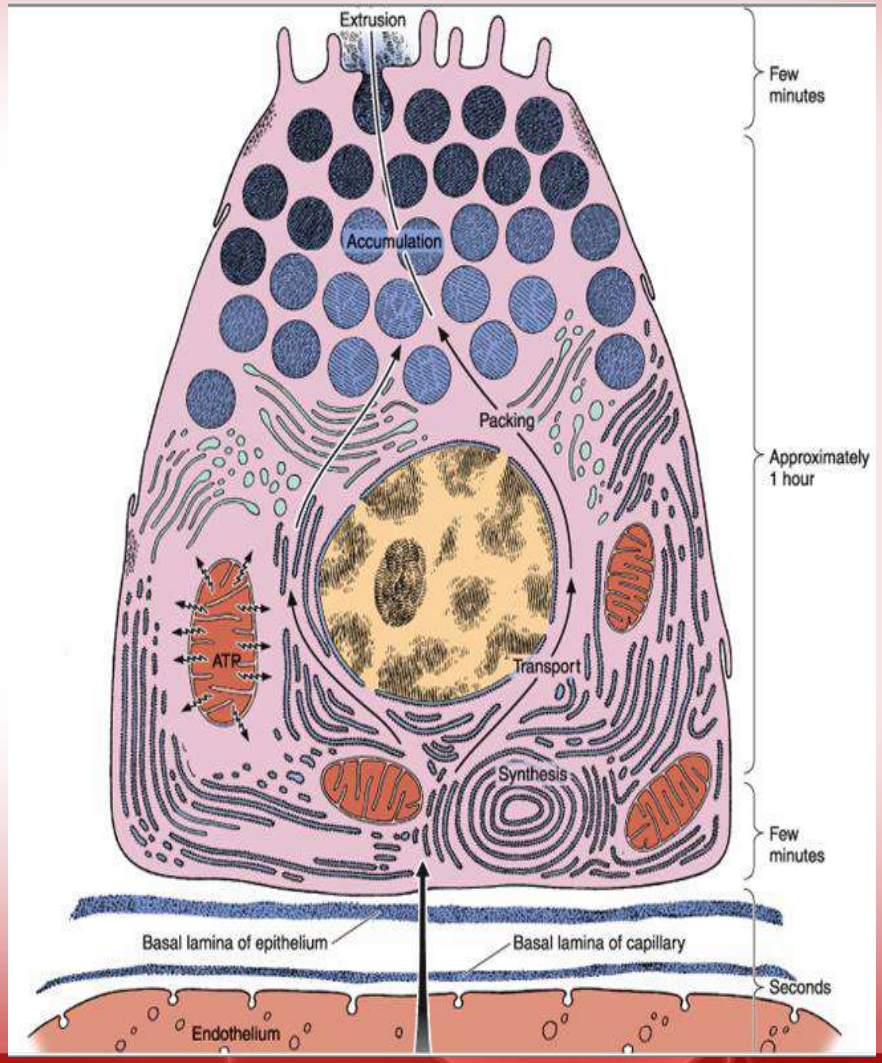
According type of secretion

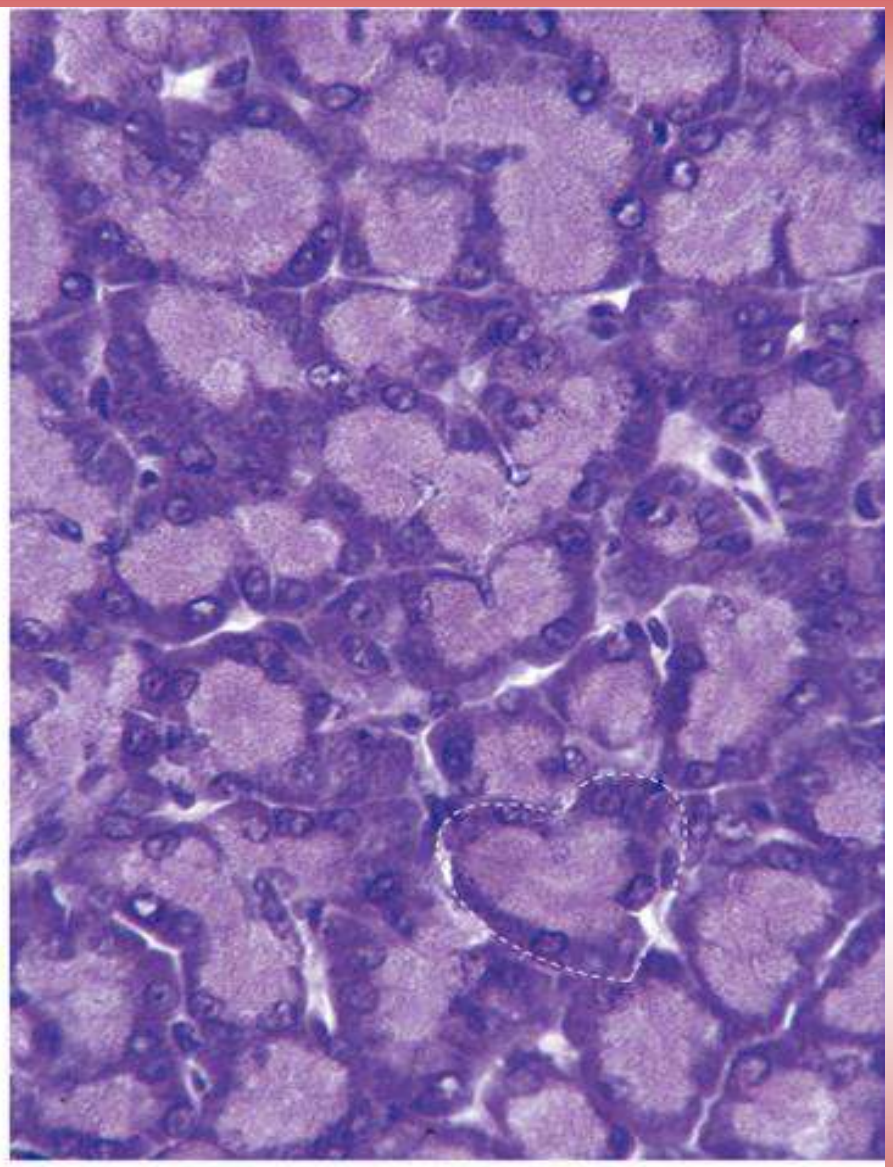
Serous secreting glands

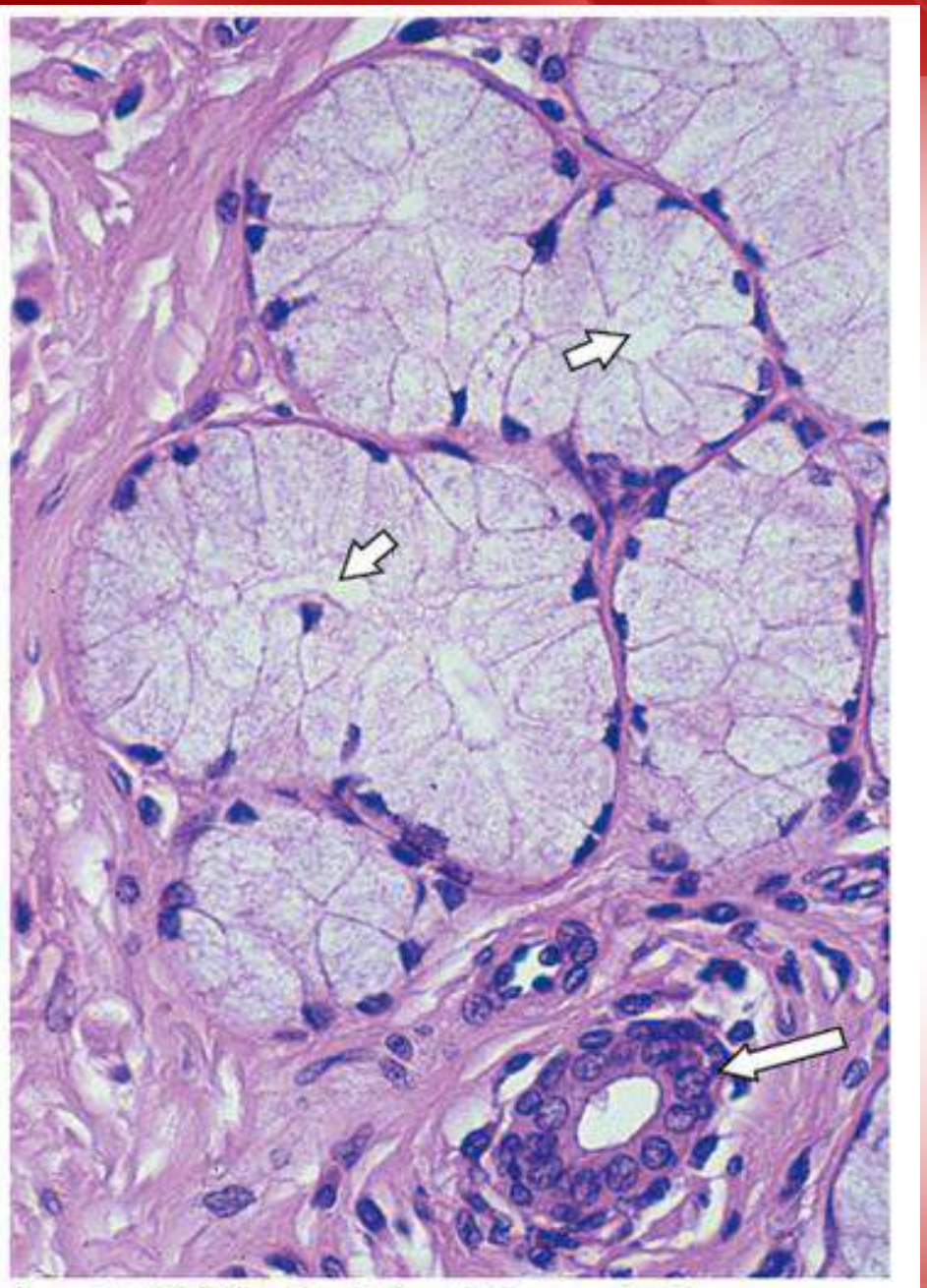
- Secrete watery secretion containing some enzymes e.g parotid salivary glands

Mucous glands

Secrete viscous glycoprotein secretion(mucus) e .g goblet cells





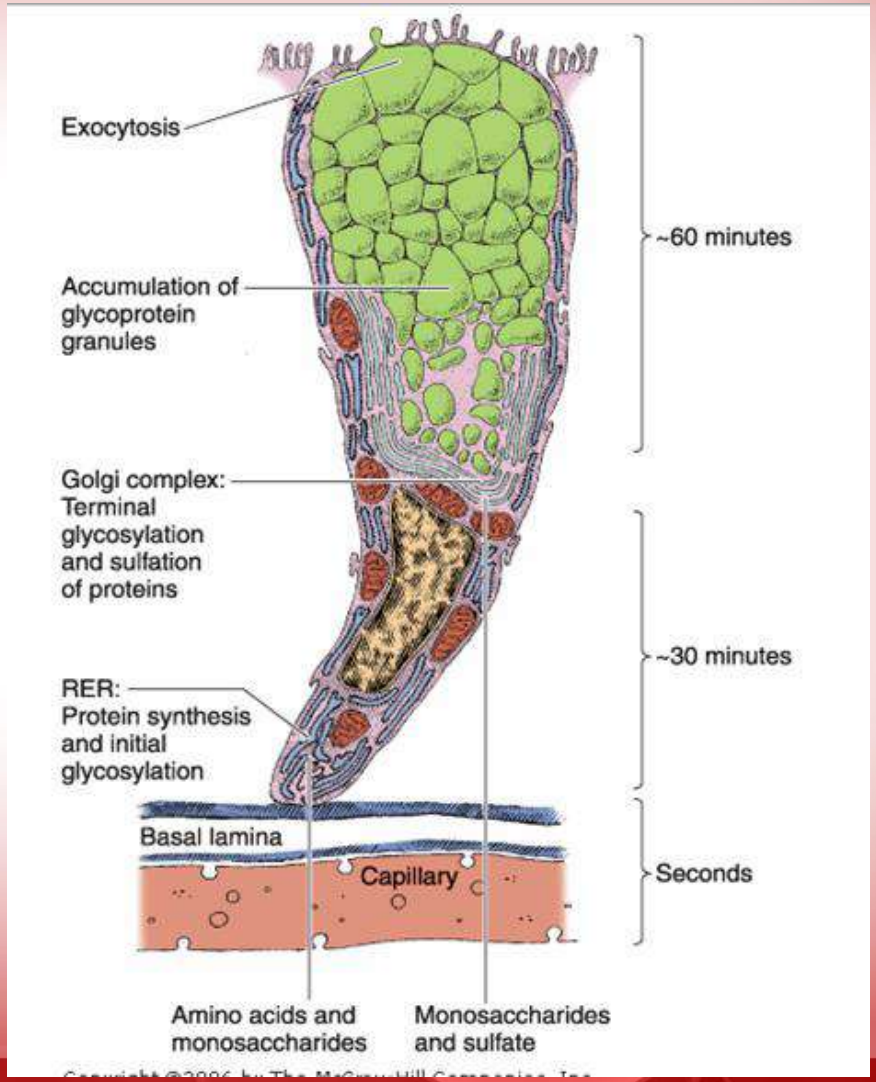


Muco-serous glands

- Secrete mucous and serous acini e.g submandibular and sublingual glands

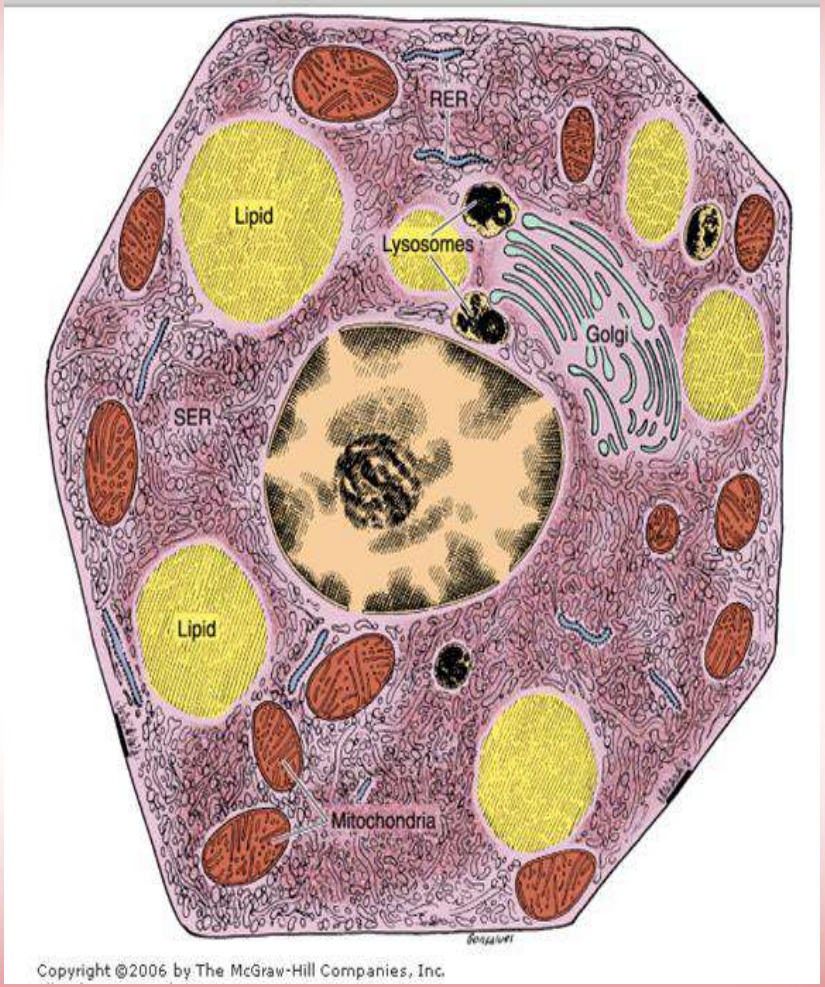
Waxy glands

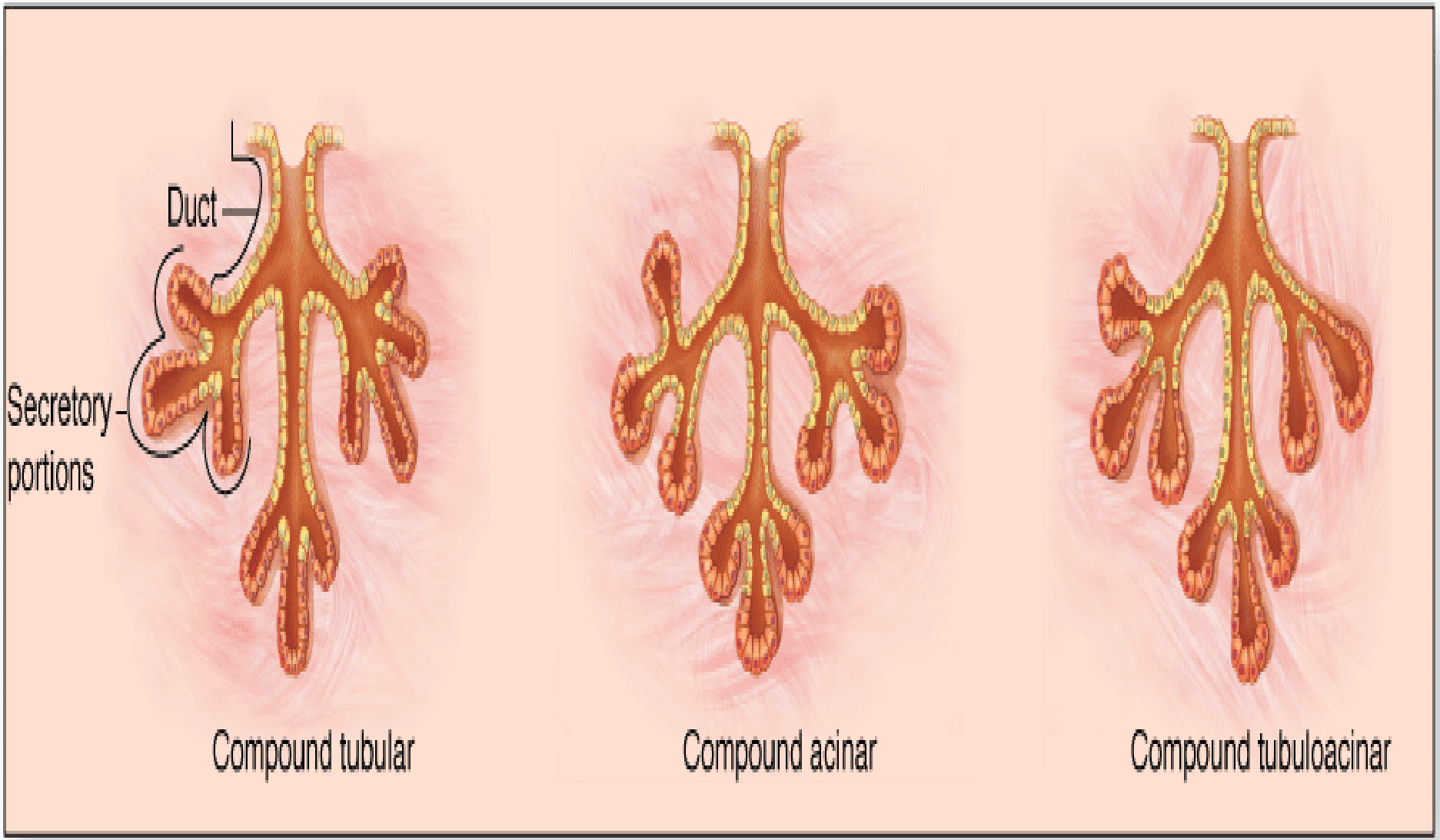
Secrete waxy secretion e.g ceruminous glands in the external ear



Cellular glands

- Secrete cells as sperms and ova e.g testis and ovary





b Compound glands

Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: http://www.accessmedicine.com

Neuro-epithelium

- Epithelial cells are modified and specialized to receive particular stimuli.
- Epithelial cells act as sensory receptors for special sensation.

Sites:

Tongue: taste buds.

Eye: rods and cones in retina (for vision).

Internal ear: for hearing and equilibrium.

Myoepithelium

- Special type of epithelial cells that are modified to contract .
- Branched cells with multiple processes , which contain contractile filaments .
- Located around the basal parts of some epithelial secretory cells . They surround the glandular acini and help in squeezing them to discharge their secretion into the ducts , e.g in sweat , salivary and mammary glands ..

Specializations of the Cell Surface

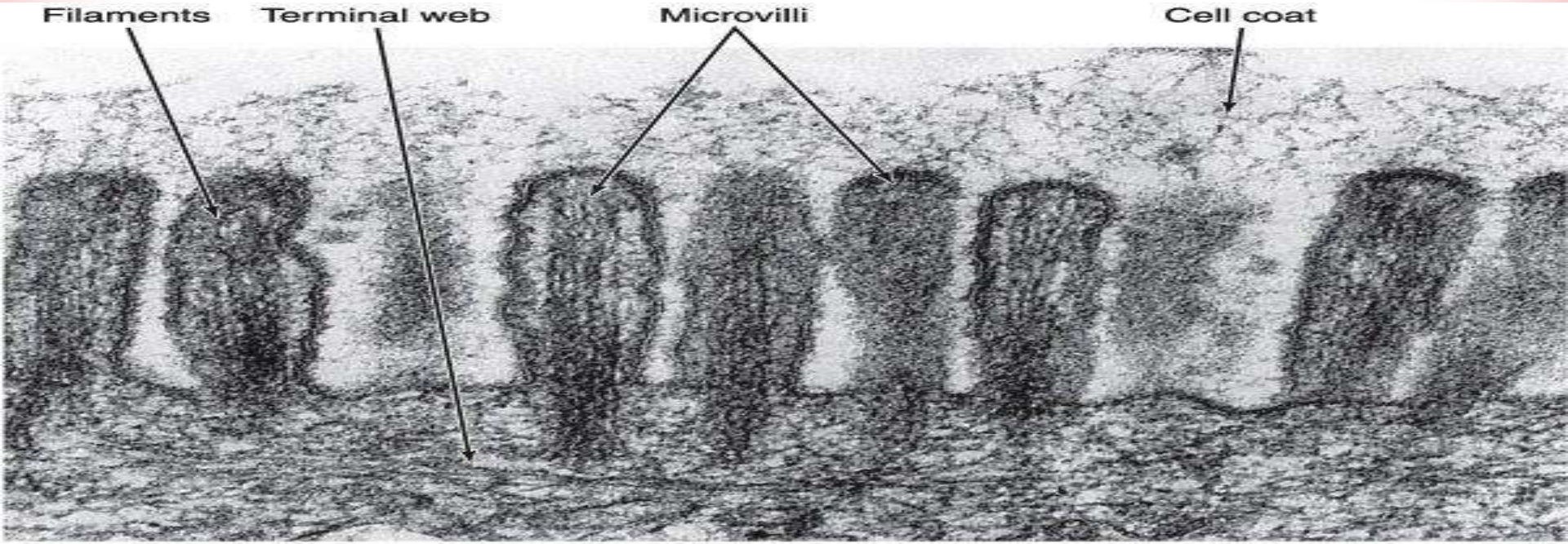
- Microvilli
- Stereocilia
- Cilia & Flagella

Microvilli

– irregular, fingerlike projections with an extracellular glycoprotein layer (cell coat).

Can be seen under LM

Contain a core of Actin filaments

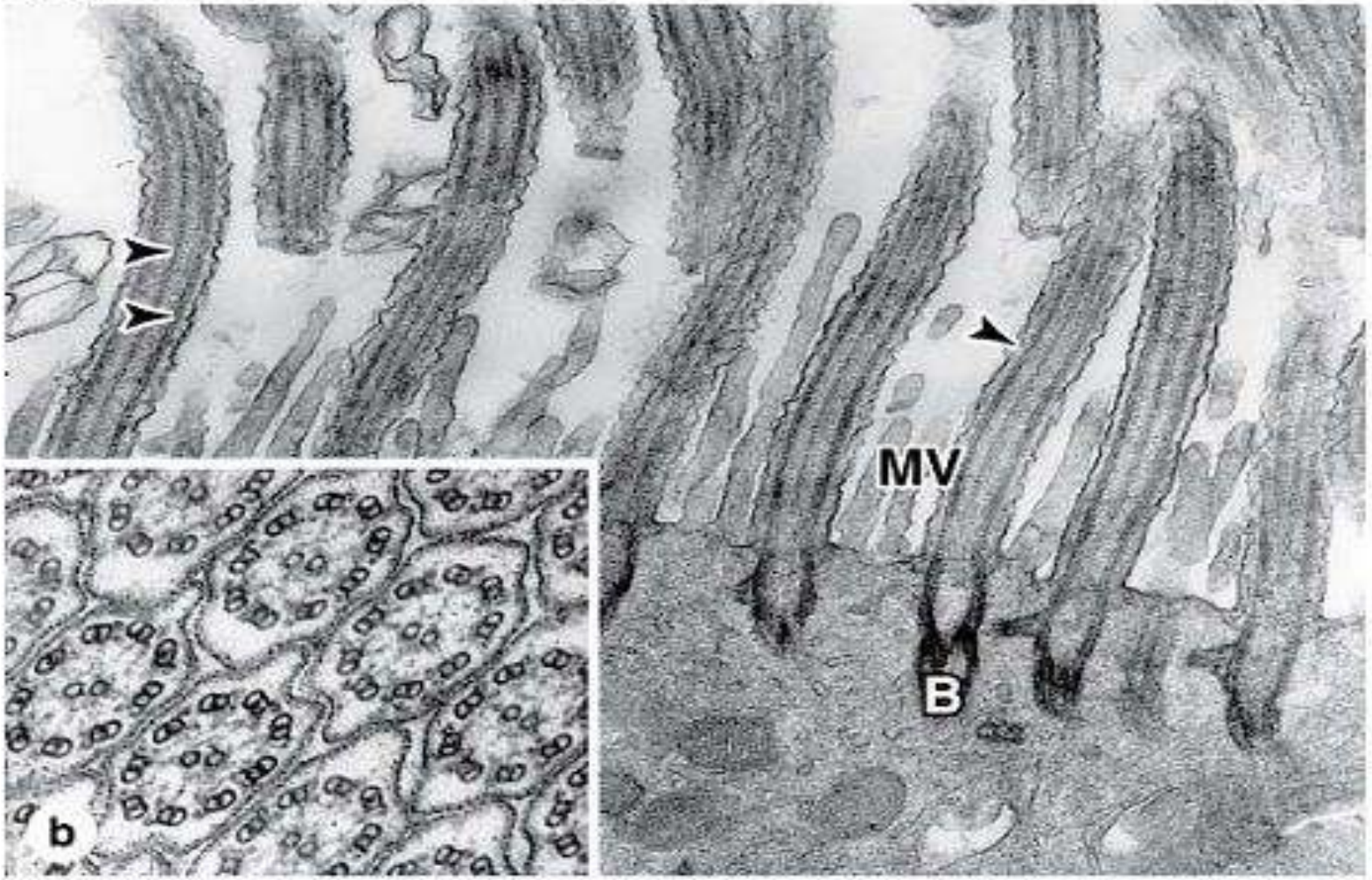


Sterocilia

– long, non-motile microvilli
  - epididymis and ductus deferens

Cilia & Flagella

- **Cilia**
- Cilia are elongated, highly motile structures on the surface of some epithelial cells, which is much longer and two times wider than a typical microvillus.
- A ciliated cell of the trachea lining is estimated to have about 250 cilia
- **Flagella**
- present in the human body only in spermatozoa are similar in structure to cilia but are much longer and are normally limited to one flagellum per cell



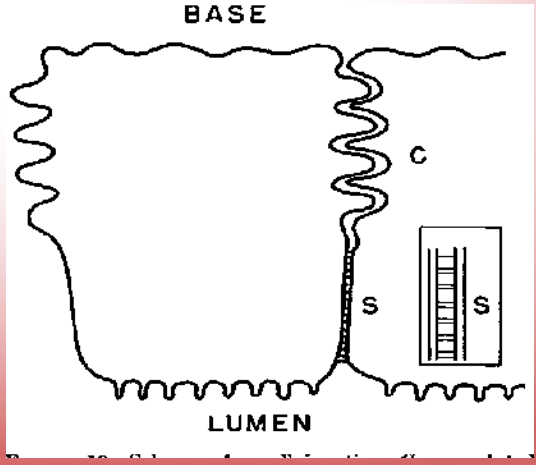
Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition:* http://www.accessmedicine.com

. Inset: Cilia seen in cross section clearly show the 9 + 2 array of the axoneme microtubules in each cilium

Lateral specialization & intercellular junctions

1.cellular interdigitation: increase the surface area for transport e.g. cells of intestine and kidney tubules

2.intercellular junctions: link the neighboring cells together by cell adhesion molecules



Types of cell junctions

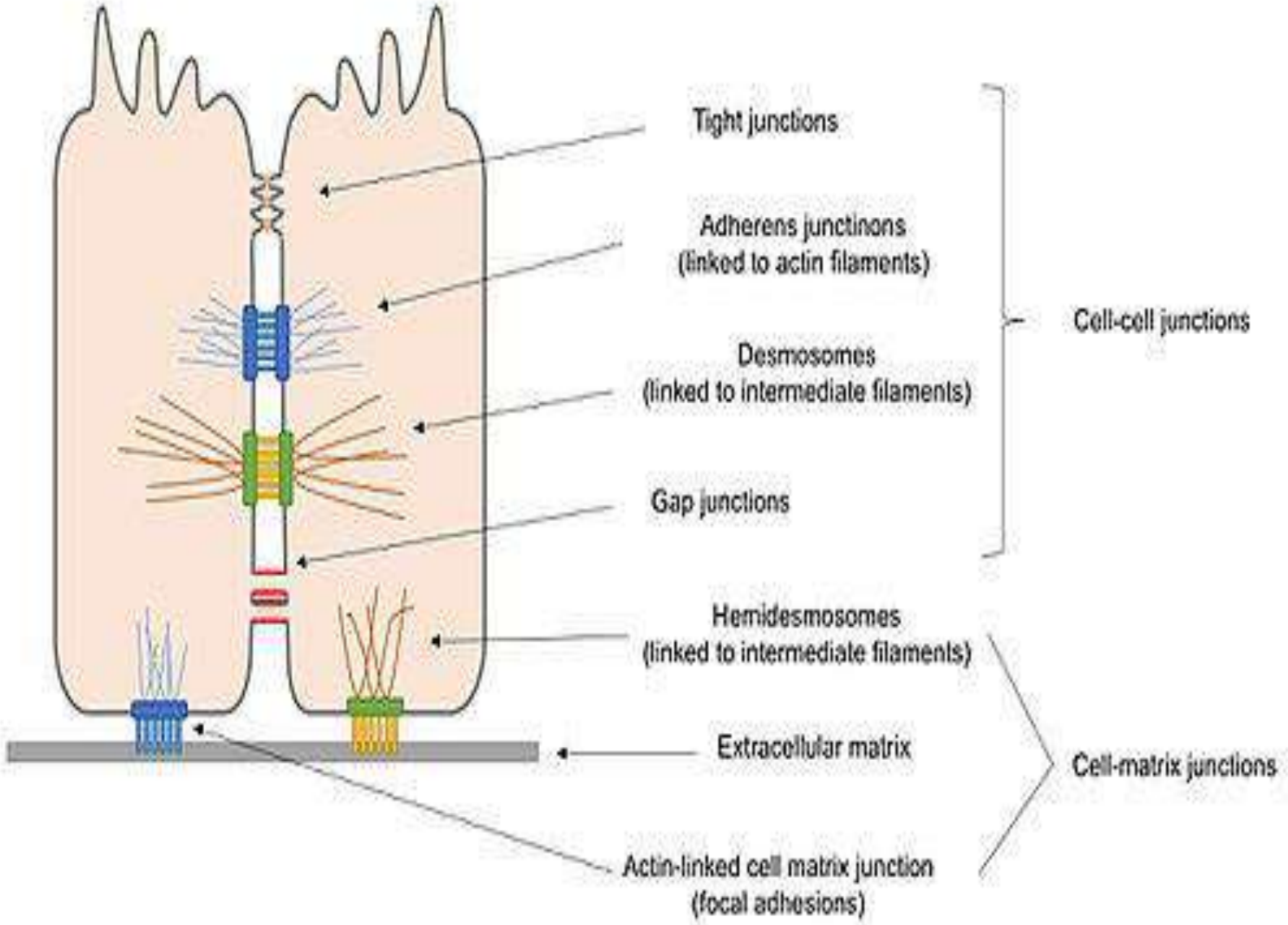
1.Cell to cell adhesions

2.Cell to matrix adhesions

The cell adhesion is adynamic e.g

Non adhesive cell become adhesive

Adhesive cells become non adhesive


Tight junctions
Adherens junctinons
(linked to actin filaments)
Desmosomes
(linked to intermediate filaments)
Gap junctions
Cell-cell junctions
Hemidesmosomes
(linked to intermediate filaments)
Extracellular matrix
Cell-matrix junctions
Actin-linked cell matrix junction
(focal adhesions)

Cell adhesion molecules (CAM)

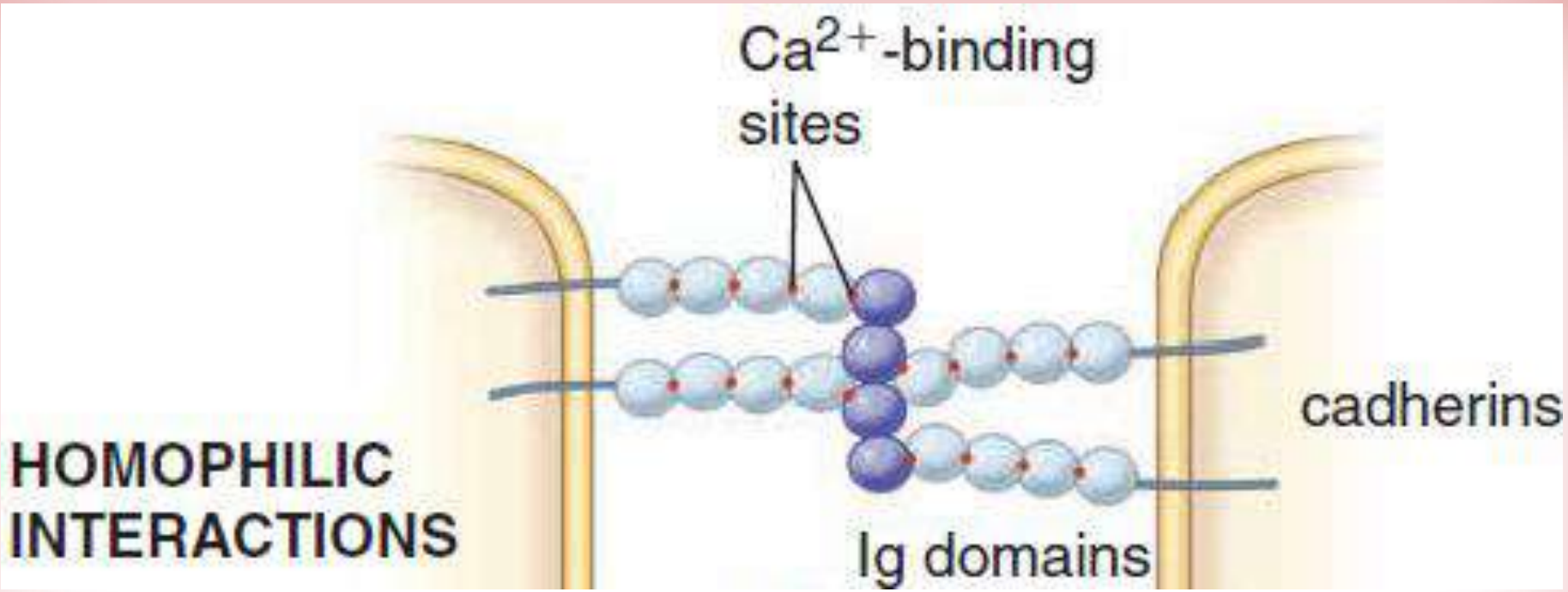
• **Definition:** transmembrane glycoproteins, consists of three parts (domains):

1- Extracellular part: binds with the other CAMS of adjacent cells or extracellular matrix proteins.

2- Intramembranous (transmembrane)part.

3- Cytoplasmic part: attached to cytoskeleton of the cell through linker proteins.

CAMS may be calcium-dependent, affected by the extracellular ca ions concentration while others are calcium- independent.



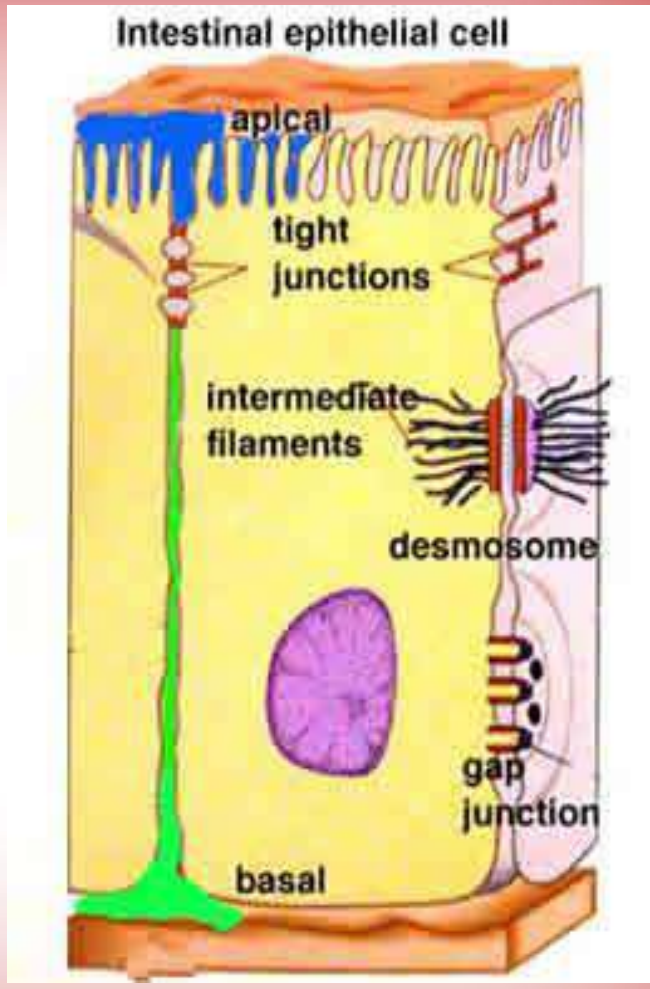
HOMOPHILIC INTERACTIONS

Cell to cell junction

I-Tight junction(occluding junction, Zonula occludens)

Site: at the apical of the cells.

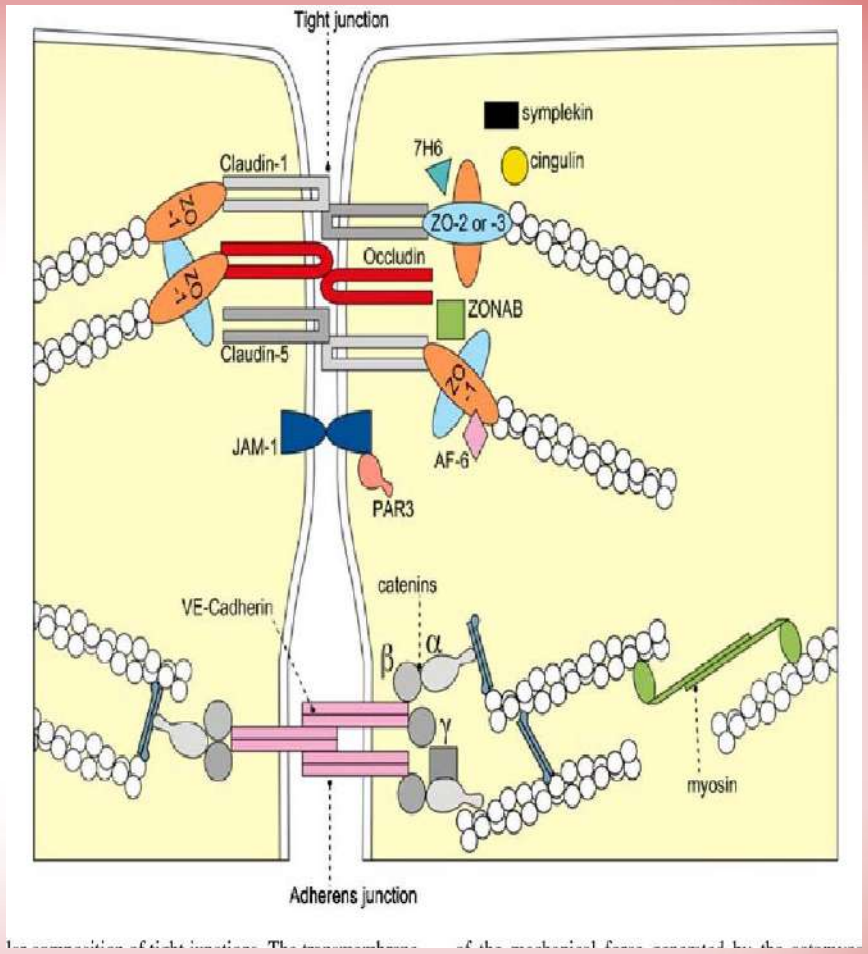
Function: restrict the passage of molecules between epithelium (barrier) e.g. epithelial cells of intestine



- Structure:

A- The outer leaflets of adjacent cell membranes are fused together, forming a belt-like junction.

B- 2 transmembrane proteins (occluding and claudin) join together to seal (occlude) the intercellular space → no intercellular space between the adjacent cells.



II-Anchoring junctions

Site: in cells that are subjected to severe mechanical stress e.g. cardiac muscles and epidermis of skin.

Function: provide strong attachment and act as a link between the cytoskeleton of adjacent cells.

• Histological structure: 2 types:

•**Zonula adherens :**

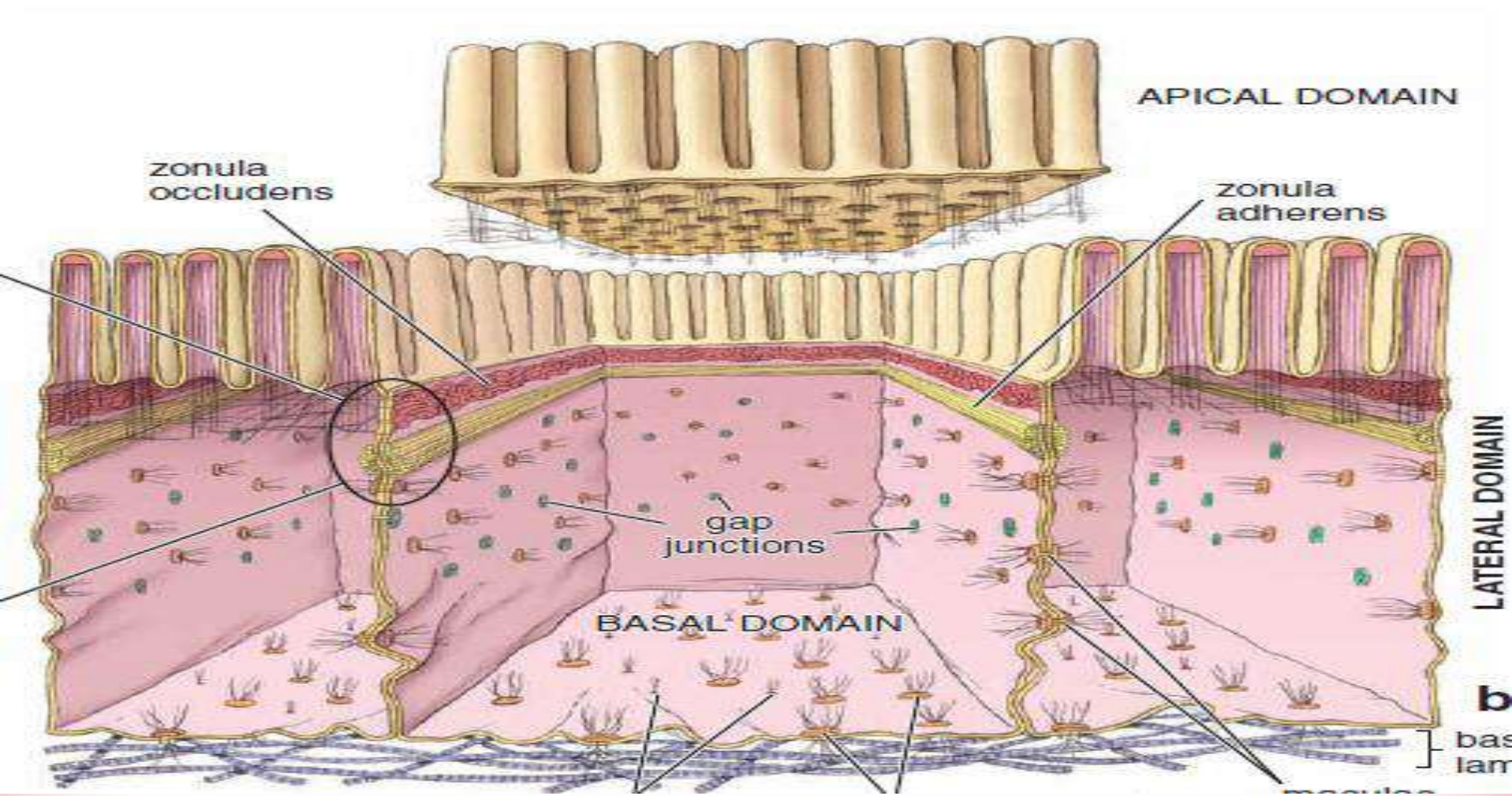
1- Belt-like specialization that encircles the apical part of the 2 adjoining cells.

2- The intercellular space between the adjacent cell membranes is 20 nm (the usual intercellular space).

3- Cadherins adhere the two cells together (ca-dependant).

4- The cytoplasmic part of cadherins are attached to actin filaments inside the cells.

Removal of Ca leads to disruption of the junction.


APICAL DOMAIN
zonula occludens
zonula adherens
gap junctions
BASAL DOMAIN
LATERAL DOMAIN

Macula adherens (desmosomes) :

1-A spot-like specialization of the cell membrane

2-Two plaques located opposite each other on the cytoplasmic aspects of the adjacent cell membranes, to which cytokeratin filaments are inserted.

3-Cadherins connect the two cells together (Ca dependent)

4- the intercellular space between the opposing cell membranes is 30 nm.

5-Dense vertical filamentous material is present in the intercellular space that represents the extracellular domains of cadherins.

In the presence of a calcium chelating agent, the desmosome breaks into 2 halves and the cells separate

Inter cellular junction complex:
in several epithelia the zonula occludens , the zonula adherens and macula adherens are present in a definite order from the apex toward the base of cell

III-Communicating junction (gap junction, nexus)

Sites: in epithelial cells, cardiac and smooth muscle cells.

Function:

They permit the exchange of molecules e.g. ions, amino acids allowing passage of signals involved in contraction and communication from one cell to another.

- Histological structure:

1-A spot-like junction, formed of protein channels.
2- The channel is called connexons which are formed of 6 transmembrane proteins called connexins.
3-When two connexons of opposing cell membranes are in register, they form a channel connecting the cytoplasm of adjacent cells.
4- The intercellular space is 3nm.

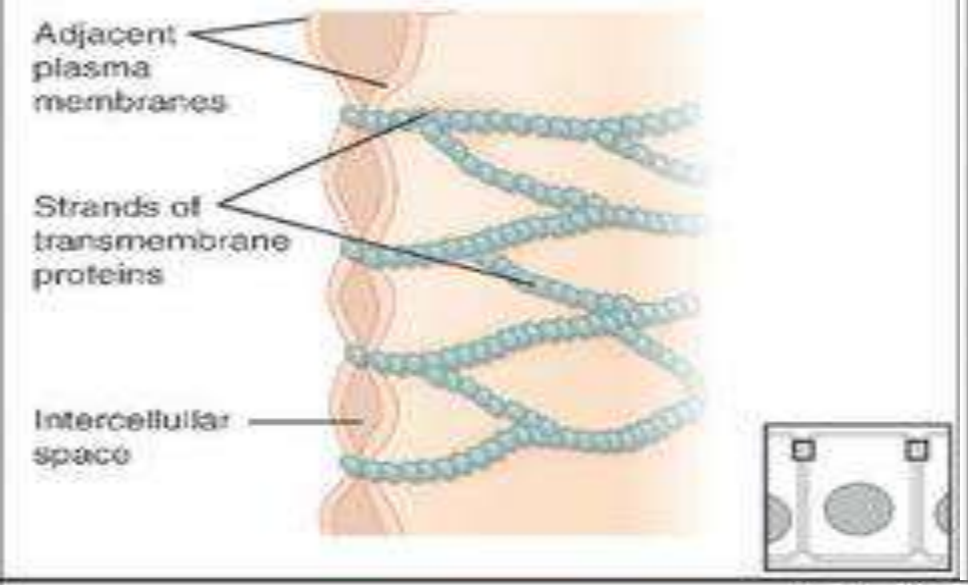
Hemidesmosomes

•**Definition:** half of desmosome but are different functionally and in their content.

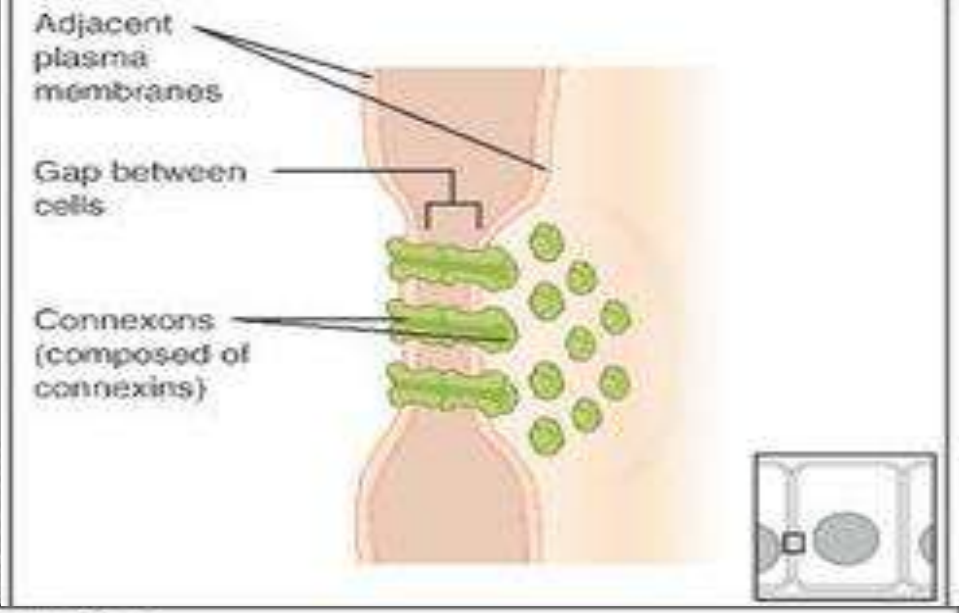
• **Site:** at the base of the epithelial cells to connect them with the basement membrane.

Structure: The CAMS are integrins, their extracellular parts bind to proteins of the basal lamina while the intracellular parts bind to keratin filaments.

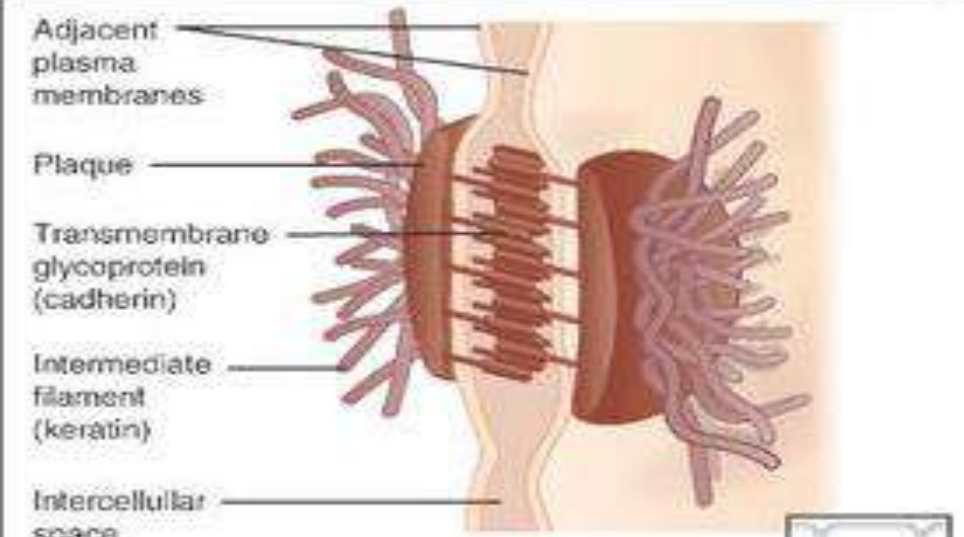
Tight junction



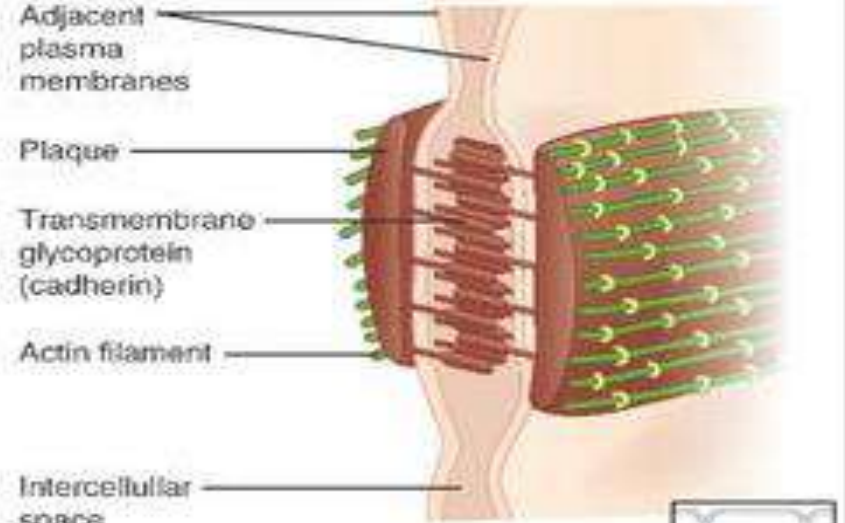
Gap junction



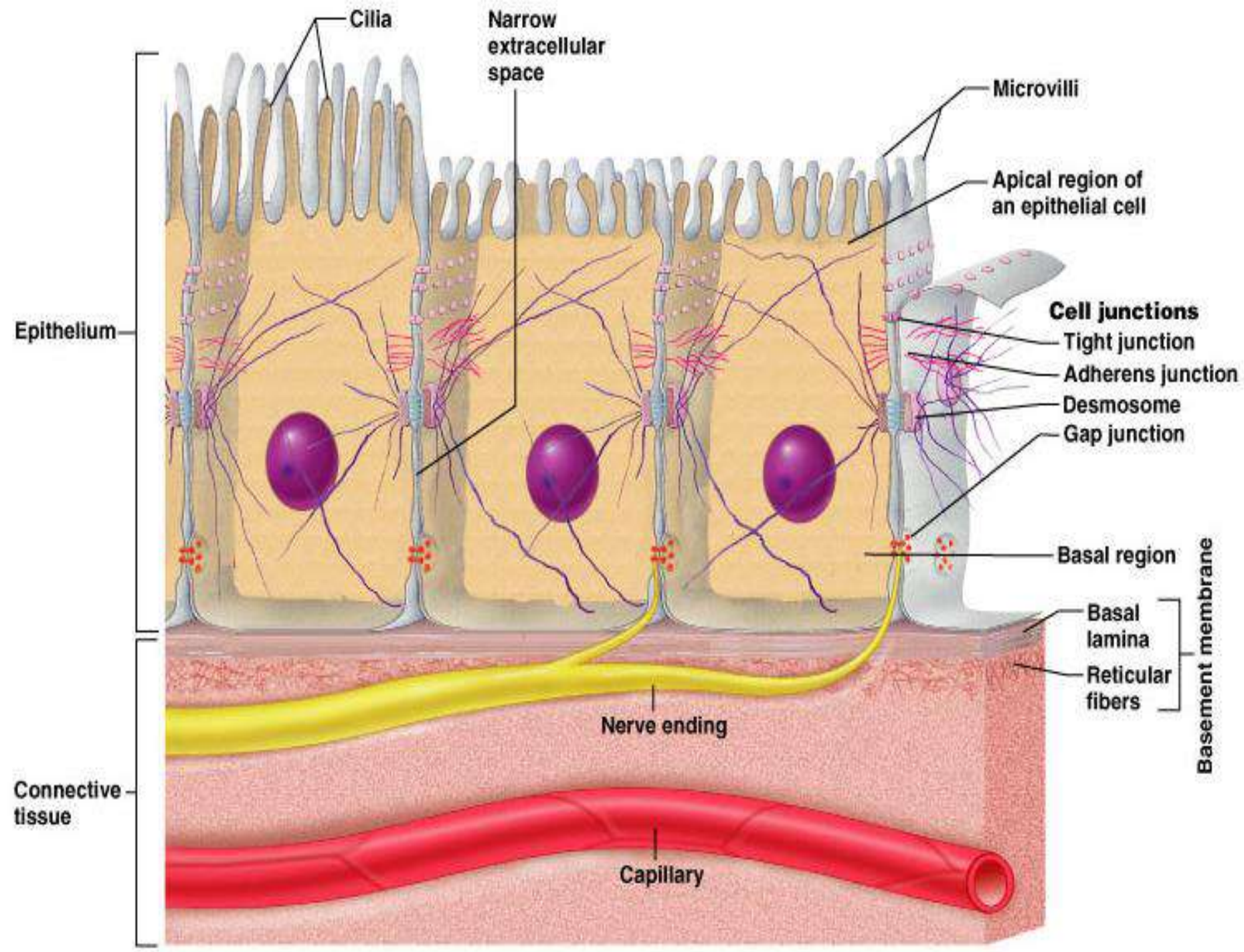
Anchoring junctions



Desmosome



Adherens


Cilia
Narrow extracellular space
Microvilli
Apical region of an epithelial cell
Epithelium
Cell junctions
Tight junction
Adherens junction
Desmosome
Gap junction
Basal region
Basal lamina
Reticular fibers
Basement membrane
Nerve ending
Connective tissue
Capillary

“Intelligence plus character-that is the goal of true education”

Martin Luther King Jr.

General Histology

Dr/hani ahmed alanqar
consultant of pathology

Connective Tissue

Connective Tissue

Origin

- Mesoderm , Mesenchymal Tissue [undifferentiated mesenchymal cells (UMCs) + Matrix].

Characteristics:

1- It is mesodermal in origin.

2- It is formed of widely separated cells with large amount of ground intercellular substances (matrix).

Characteristics:

3- It is penetrated by blood vessels, lymphatics and nerves.

4- It connects, supports and protects other tissues & organs.

Functions

- **1)Structural support**
- C.T. serve several functions, of which the most prominent function is structural support to enable maintenance of anatomical form of organs and organ systems.

- -Examples include the C.T. capsules surrounding organs (such as the kidney, lymph nodes).
- loose C.T. acts to fill the spaces between organs.

- Tendons (connecting muscles to bone) and the elastic ligaments (connecting bones to bones) are examples of specialized orderly forms of C.T..
- -Skeletal tissues (cartilage and bone) are special forms of C.T.

- **2)Metabolic functions**
- -C.T. serve a **nutritive role**.
- -All the metabolites from the blood pass from capillary beds and diffuse through the adjacent C.T. to cells and tissues.

- -Similarly **waste metabolites** from the cells and tissues diffuse through the loose C.T. before returning to the blood capillaries.

- -**Adipose tissue** (especially that of the hypodermis) serves as an **energy store** and also provides **thermal insulation**. Surplus calories can be converted into lipid and stored in adipocytes.

- **3) Blood components and blood vessels**
- -Hematopoietic tissues (blood-forming tissues) are a further specialized form of C.T..

- -These include the myeloid tissue (bone marrow) and the lymphoid (lymphatic) tissue.
- -The lining of the blood and lymphatic vessels (endothelial cells) as well as the peripheral blood, are also specialized forms of C.T.

- **4) Defensive functions**
- -Various components of the C.T. play roles in the **defense or protection of the body** including many of the components of the vascular and immune systems (plasma cells, lymphocytes, neutrophils, eosinophils, basophils, mast cells).

- The various macrophages of the body are also categorized as C.T. cells. These all develop from monocytes and are grouped as part of the **Mononuclear Phagocyte System** of the body.

- -Macrophages are important in tissue repair as well as defense against bacterial invasion.
- -The fibroblasts of C.T. proliferate in response to injury of organs and migrate to and deposit abundant new collagen fibers, resulting in the formation of **fibrous scar tissue**.

Components

- C.T. consists of **3 main components**:

1- **Cells**

2- **Fibers**

3- **Matrix**

A- soft.....C.T. proper

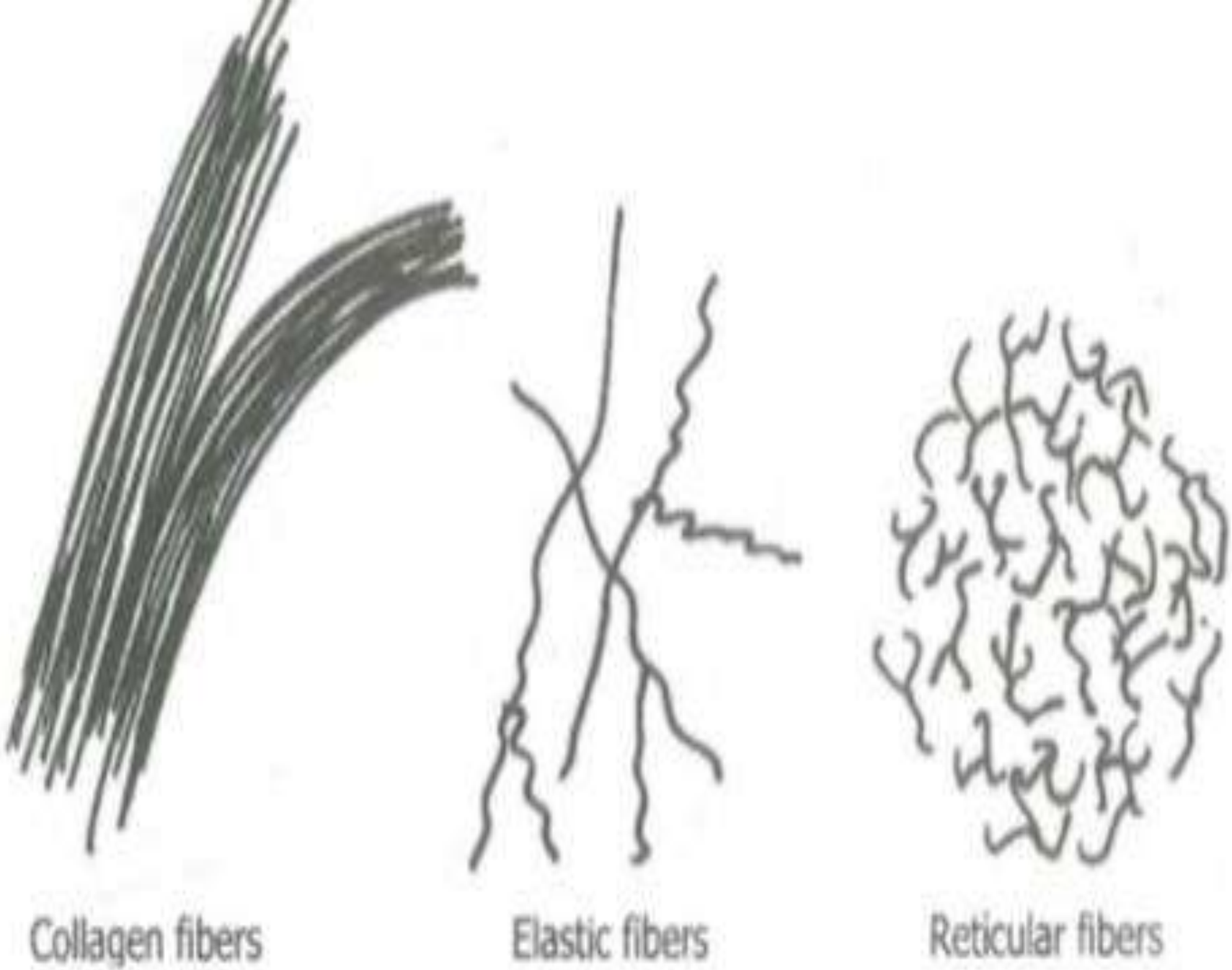
B- Fixed rubbery.....cartilage

C- solid…… bone

D-fluid……. blood

Connective Tissue Fibers

- The C.T. fibers are formed of protein molecules that polymerize to form thin threads.
- **There are three types of C.T. fibers:**
- **1- White Collagenous fibers.**
- **2- Yellow Elastic fibers.**
- **3- Reticular fibers.**


Collagen fibers
Elastic fibers
Reticular fibers

1- White Collagenous Fibers :

- **Structure:**

They are formed by aggregation of tropocollagen molecules secreted mainly by **fibroblasts** and other cells as chondroblasts, osteoblasts and odontoblasts.

- **N.B.**: collagen protein is the most abundant protein in the human body.
- **L.M**
- • In histological preparations, they appear as wavy branching bundles, formed of **non-branching** fibers.

- They are colorless when single, white when condensed, so they are called white fibers, e.g. in tendons &aponeuroses.
- Fibers are parallel to each other in the bundles.
- They are **acidophilic** in staining.

- H & E pink.
- Mallory's stain blue.
- Van Gieson's stain red.

Characters:

- They are soft, strong, flexible but not elastic.
- Collagen protein can be digested by pepsin &trypsin enzymes, and is affected by boiling (transforming into gelatin), acids and alkalies(stanning).

- **Functions:**

give strength and rigidity to tissues, and resist pulling forces.

2 - Yellow Elastic Fibers:

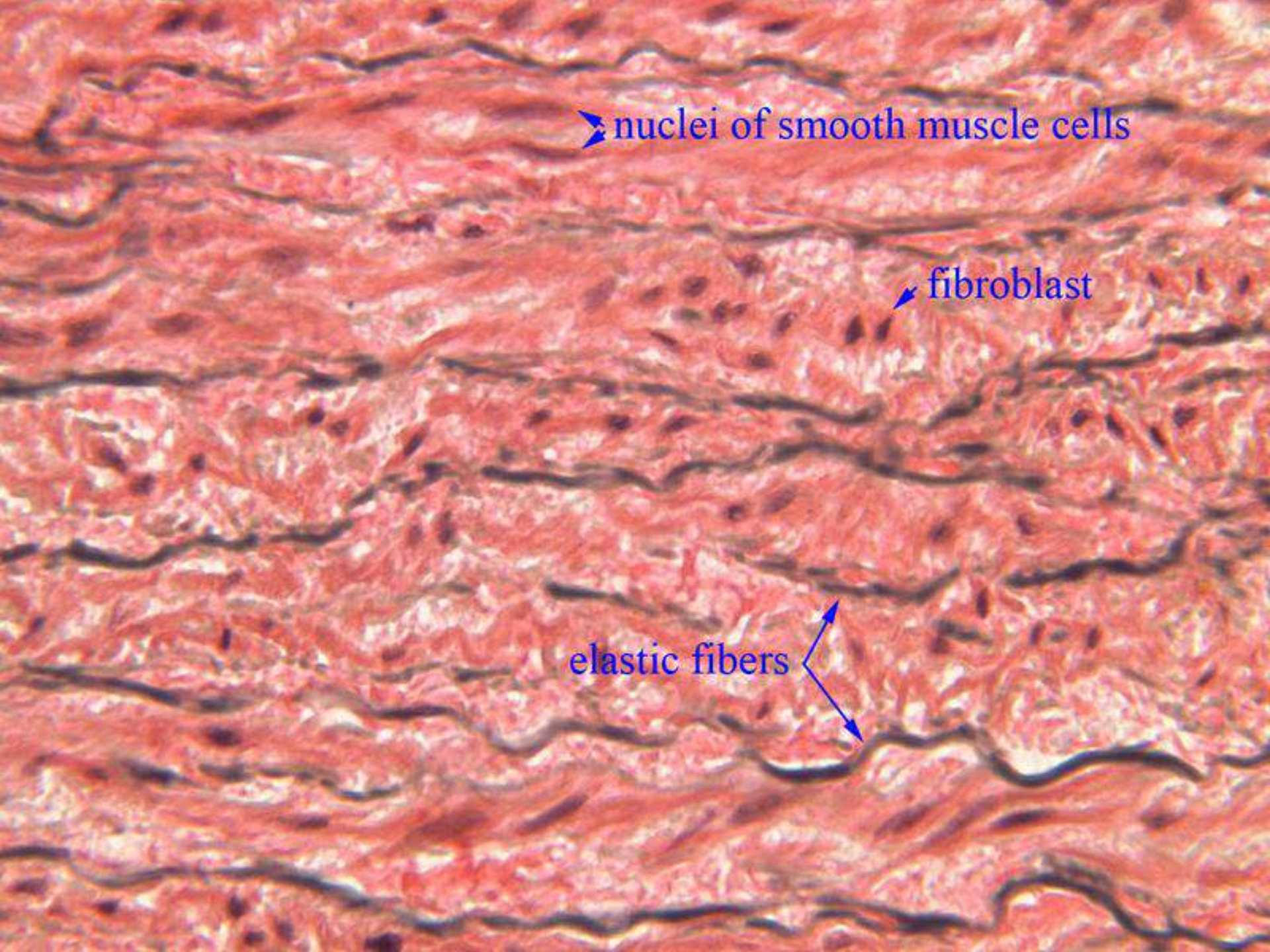
Shape:

- They are formed of thin, long, branching fibers.
- Fibers are stretchable and yellow in color in fresh state.
- They run singly not in bundles, but branch and anastomose to form networks.

- **Structure:**
- Elastic fiber formed of a core of elastin protein synthesized in
- fibroblasts, surrounded with a sheath of microfibirils of a glycoprotein called fibrillin.

- **Characters:**
- Resistant to boiling & chemicals, resist digestion by enzymes as
- usual proteases, but can be digested by pancreatic elastase.

- **Staining:**
- H & E pink.
- Orcein stain brown.
- Van Gieson's stain yellow.
- **Functions:**
- They provide the tissue with the power of stretch and elasticity.


nuclei of smooth muscle cells
fibroblast
elastic fibers

3 - Reticular Fibers:

- **Shape:**
- They are formed of delicate (very thin) fibers that branch & anastomose to form network or reticulum.

- **Structure:**
- They are formed of a thin type of collagen (type III).
- **Staining:**
- Silver stain → brown to black (argyrophilic).

- **Functions:**

- Form the stroma of organs (the background).

Ground Substance (Matrix)

Definition:

- It is the inter-cellular substance in which cells & fibers are embedded. It is amorphous, jelly like, and translucent.

Structure:

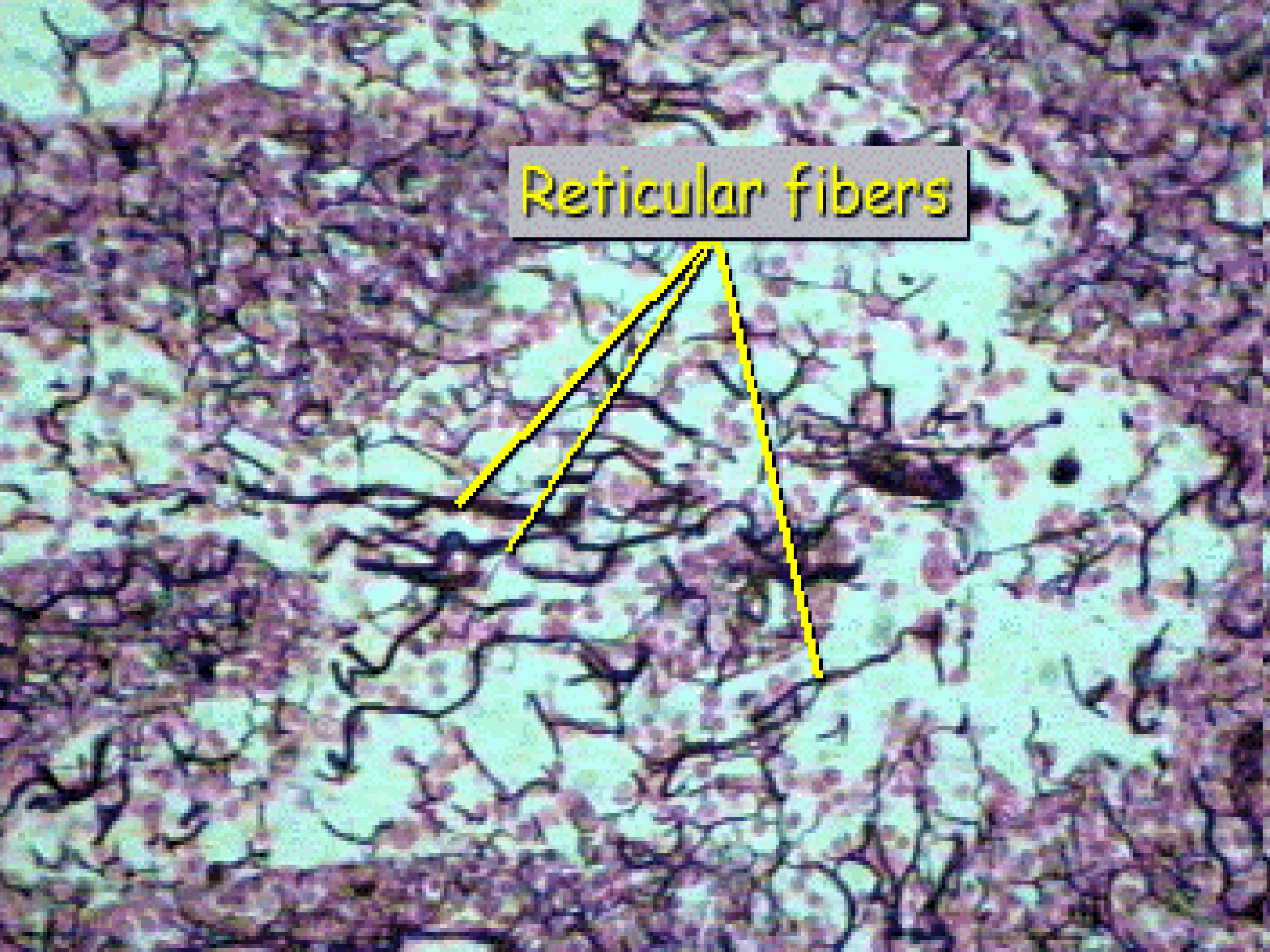
- it is formed of a viscid substance which has a complex mixture of:
- **1- Proteoglycans (glycosaminoglucans, GAGs):**
- **2- Glycoproteins:**
- **3- Tissue fluid:** salvation water surrounding these structures, similar to plasma.

Staining:

- - Ground substance can be stained metachromatically with basic stains as toluidine blue giving purple color.
- - PAS red color
- - Silver brown color.

Functions:

- 1 - Through its aqueous phase all nutrients & gasses and wastes pass between blood and cells.
- 2- Cells & fibers are bounded.
- 3- It acts as a physical barrier for spread of bacteria and microorganisms.


Reticular fibers

Connective Tissue Cells

- **<u>The fixed cells</u>** are the intrinsic component of the C.T., they are the stable

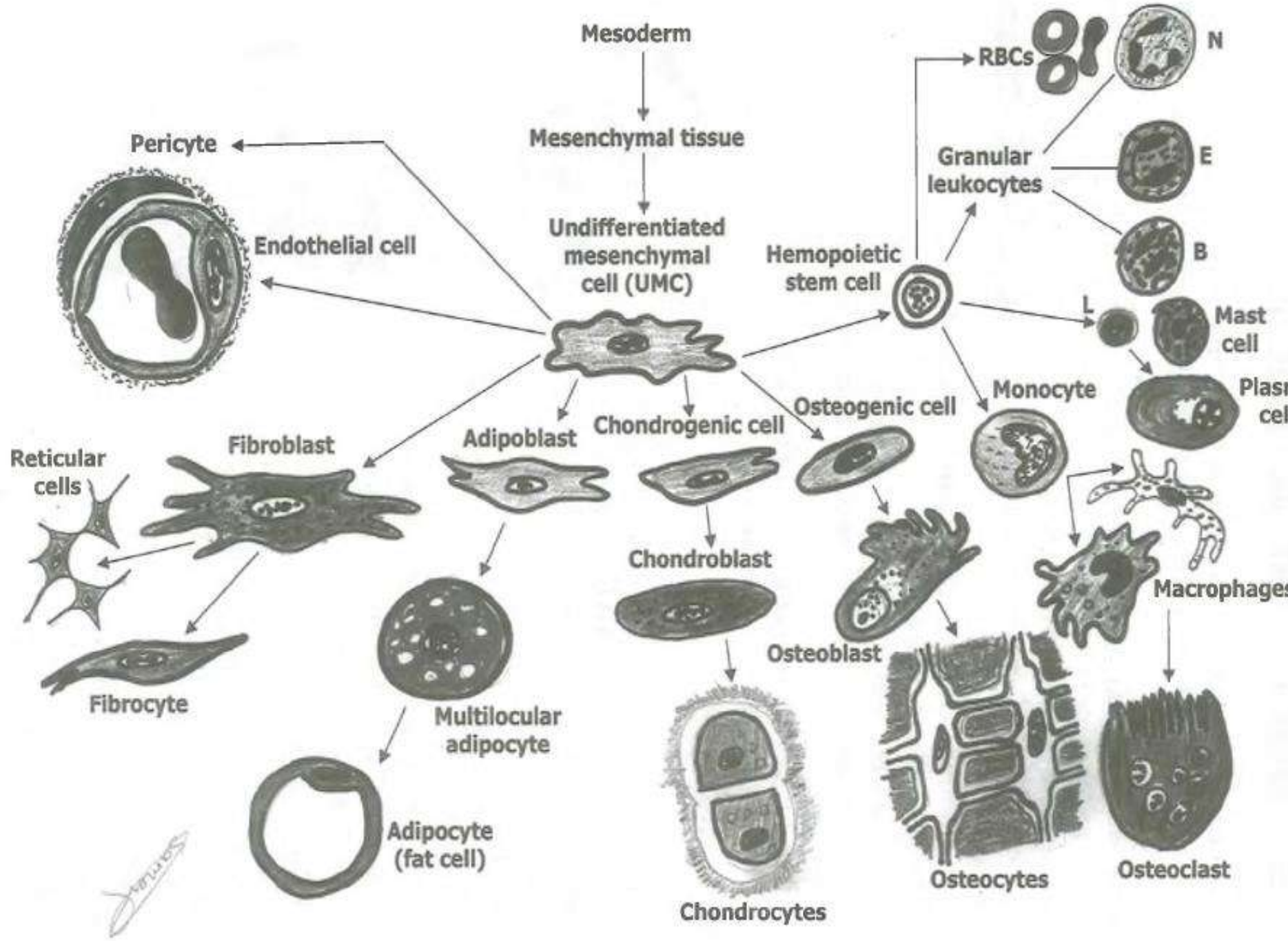
population of long lived cells.

They are produced in the C.T. & remain in it.

- **The free cells** changeable population, they are motile cells, belonging

to the immune system, entering the C.T. from the blood, and wander through it.

They are short lived, and continuously replaced from the blood.


Mesoderm
Mesenchymal tissue
Undifferentiated mesenchymal cell (UMC)
Pericyte
Endothelial cell
Hemopoietic stem cell
RBCs
N
Granular leukocytes
E
B
L
Mast cell
Plasm cel
Monocyte
Osteogenic cell
Chondrogenic cell
Adipoblast
Fibroblast
Reticular cells
Fibrocyte
Chondroblast
Osteoblast
Macrophages
Multilocular adipocyte
Adipocyte (fat cell)
Chondrocytes
Osteocytes
Osteoclast

A- Fixed C.T. Cells

1- Undifferentiated Mesenchymal Cells (UMCs):

- **Sites:**
- Unspecialized stem cells present in the embryo.
- They have the ability to differentiate into other types of C.T. cells (mother cells).

- In adult, they remain undifferentiated in certain areas to act as a life-longsource for some cells e.g.:

- in bone marrow —≫blood cells.

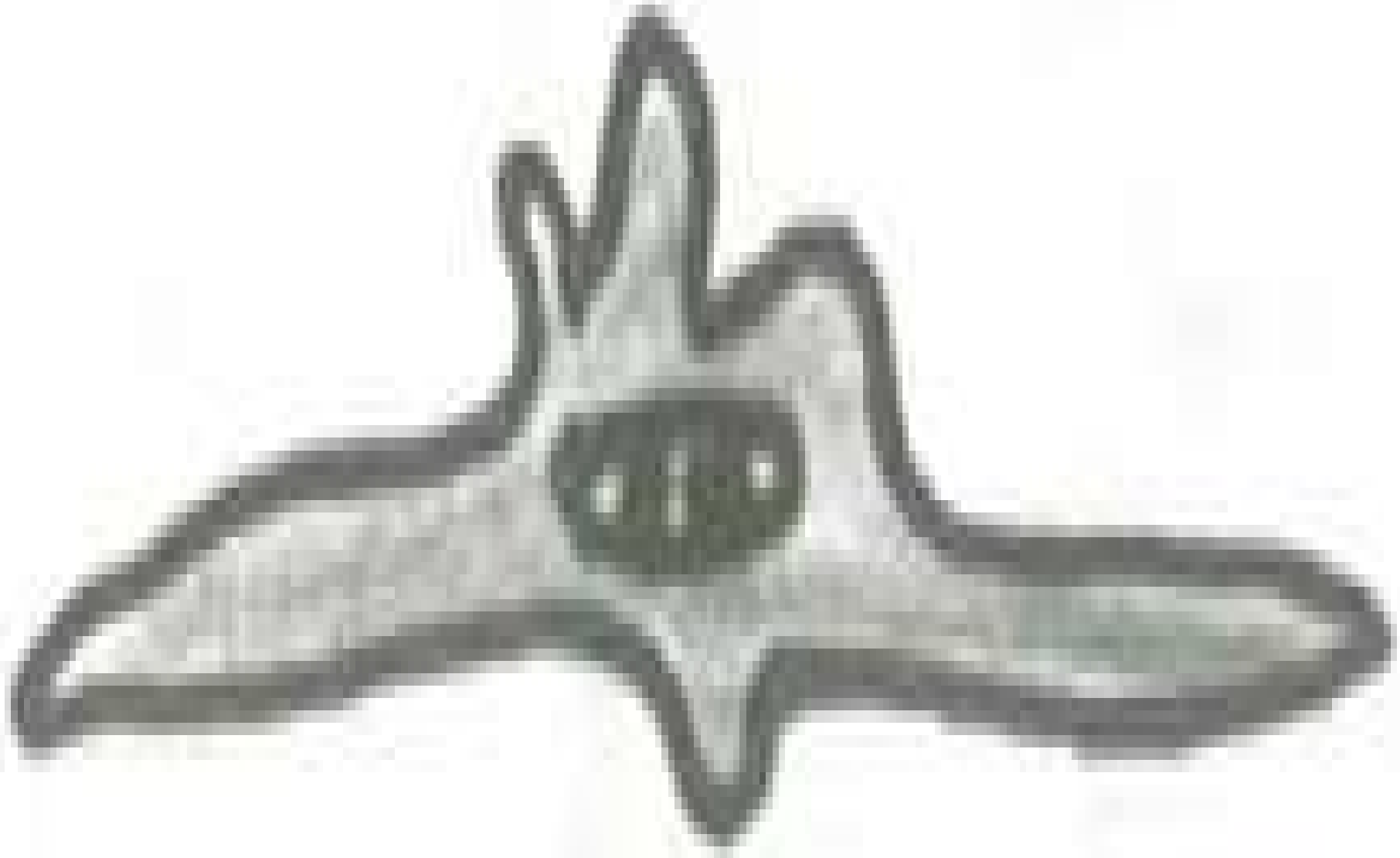
- around blood vessels —≫pericytes.

- Small, irregular, branched star (stellate) shaped cells.
- Pale basophilic cytoplasm.
- Central large oval nucleus with visible nucleoli.

- **EM:** few organelles, many ribosomes & fine chromatin in the nucleus.
- **Functions:**

1- Can differentiate into other types of C.T. cells.

2- In bone marrow, it gives blood cells.



2- Pericytes:

Sites:

- Present around blood capillaries.
- They are considered UMCs of the adults.

LM:

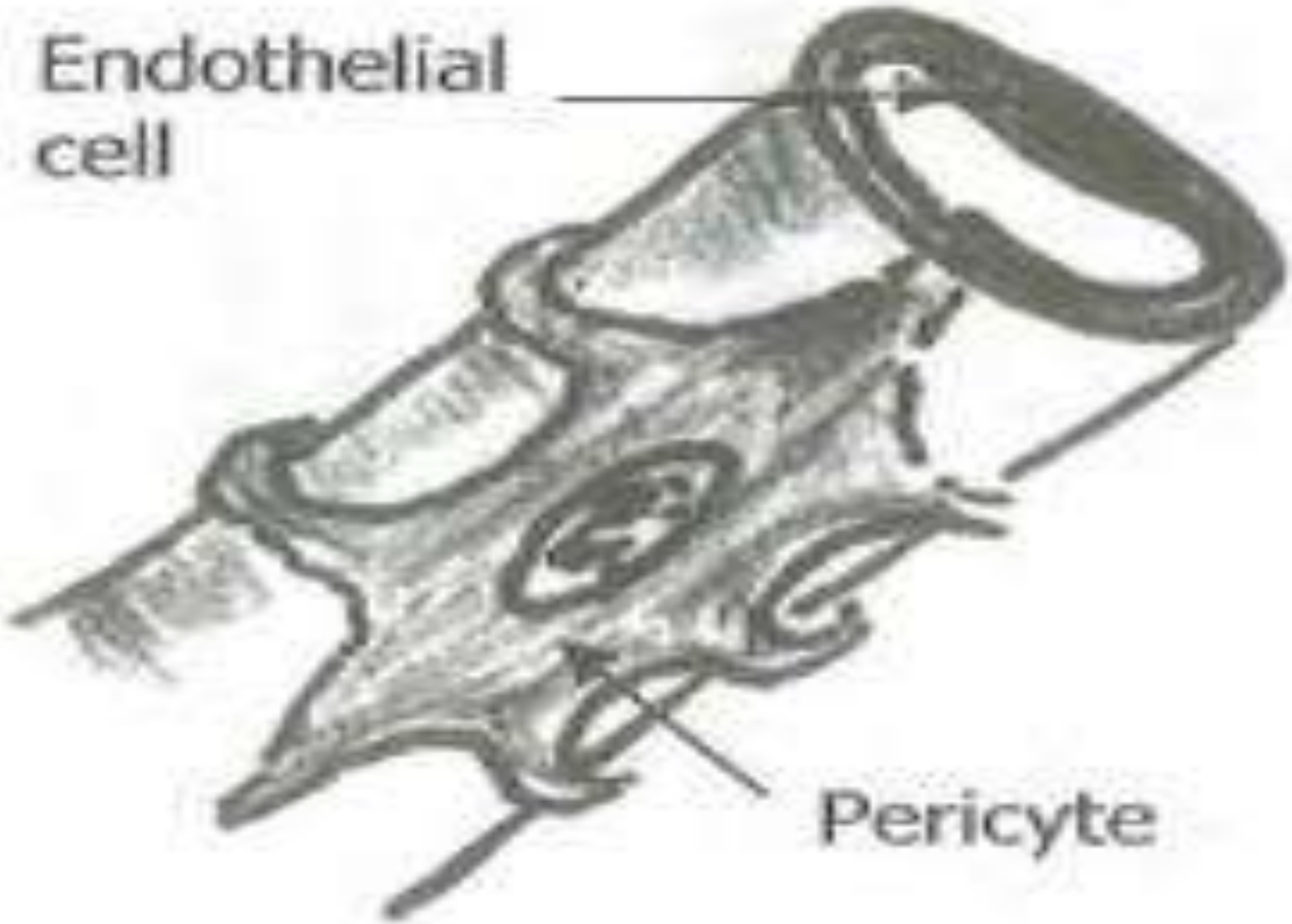
- branched, with oval nucleus & pale cytoplasm.

Functions:

1- The same functions of UMCs but after birth.

2- After birth they give rise to fibroblasts & smooth muscles (duringhealing).

3- Their contraction leads to vaso-constriction.



Pericyte around blood capillary.

3 - Endothelial Cells:

Sites:

- Lining all blood vessels & heart.

LM:

- Thin flat squamous epithelium with flat nucleus, little cytoplasm & few organelles around the nucleus.

Functions:

1- Can divide to produce new endothelial cellsin injury.

2- Can produce their own basement membrane.

3- Can produce antithrombotic factors, which prevent platelet aggregation.

4- Can produce collagen (type 4).

EM picture of Pericyte.

4 - Fibroblasts:

Origin:

- UMCs &Pericytes.

Sites:

- **The most common type;** found nearly in all types of C.T. proper.

LM:

- Flat, elongated, branched cells, with thin processes, (fusiform in side view).
- Large, pale, oval nucleus & prominent nucleolus.
- Deeply basophilic cytoplasm.

EM:

- Euchromatic nucleus & large nucleolus.
- Large amount of rER& free ribosomes.
- Well developed Golgi apparatus.
- Mitochondria.

N.B.: these are the EM picture of cells active in protein synthesis. EM picture of fibroblast

Functions:

1) Formation of C.T. fibers by secretion of their proteins (procollagen and elastin, that form collagenous, elastic & reticular fibers).

2) Secretion of the ground substances of the matrix, i.e. the cells that build the C.T.

3) Healing & repair of C.T. after injury.

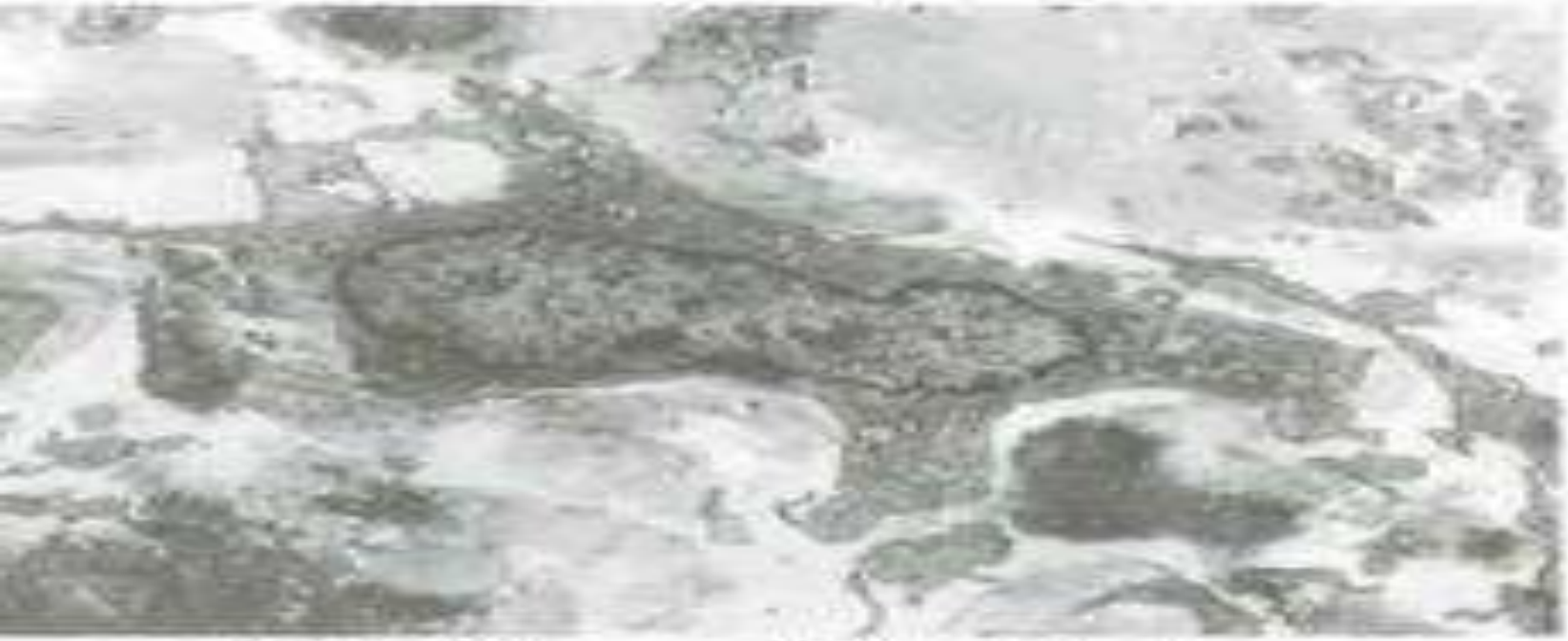
N.B: Fibrocytes:

- They are old inactive fibroblasts.
- They are smaller, spindle shaped with fewer processes.
- Less basophilic cytoplasm with darker nuclei.
- Smaller amount of rER.

Functions of fibrocytes:

Continuous slow turnover of extracellular components, for maintenance of C.T.

LM picture of fibroblasts.



EM picture of fibroblast.

Fibrocytes in C.T.

5- Fat Cells (Adipocytes):

Origin: UMC ⟶ Lipoblast:

- Unilocular adipocyte (white fat cells)
- Multilocular adipocyte (brown fat cells)

- **A- Unilocular fat cell:**

Sites: most abundant in white adipose C.T.

LM:

- Rounded or oval, large (120 um) cells.
- Nucleus is peripheral & flattened.
- Cytoplasm is reduced into a very thin film around a large fat globule.

- In H & E —>fat dissolves, so cells appear as large vacuoles **(signet-ring** appearance).
- In frozen sections, it stains orange with **Sudan III.**

EM:

fat droplets appear as electron-dense (black) inclusion that occupies most of cytoplasm.

- **Functions:**
- 1- Storage of fat.
- 2- Support some organs e.g. kidney.
- 3- Heat insulator.
- **N.B.:** fat cells do not divide, but have a long life span.

B- Multilocular fat cell:

Sites: present in brown adipose C.T.

LM: small rounded cells with central rounded nucleus and many small fat droplets. They have many mitochondria, so appear brown due to the cytochrome pigments present in the mitochondria.

Functions: heat generation.

6 - Fixed Macrophages (Histiocytes):

Origin: from mesenchyme derived **monocytes** (a type of white blood cells).

They are described as fixed or resident when they are attached to fibers.

Sites: scattered in the C.T. along the collagen fibers.

LM:

- Large branched cell with irregular cell boundary pseudopodia), so they have variable shapes, may be fusiform or stellate.
- It has darkly stained, small, oval or kidney shaped nucleus.
- Its cytoplasm is acidophilic, granular and vacuolated.
- Can be demonstrated with vital stains as **trypan blue** or **Indian ink,** where the stain granules are phagocytosed by the cells.

EM:

- The surface of macrophage is irregular due to pseudopodia, surface folds and microvilli.
- The cytoplasm contains large particles that these cells have phagocytosed.

- **Lysosmes,** primary & different types of secondary lysosomes, which attack and degrade the ingested materials, also residual bodies that contain indigestible remains.

- Few cisterns of rER, **prominent Golgi** (from which arise the lysosomes).
- In certain conditions macrophages fuse and form large multinucleated cells called foreign body giant cells that are able to phagocytose large foreign materials.

- N.B.: Giant cells are common in **tuberculosis,** and in chronic nodular lesions called **granulomas.**

Functions:

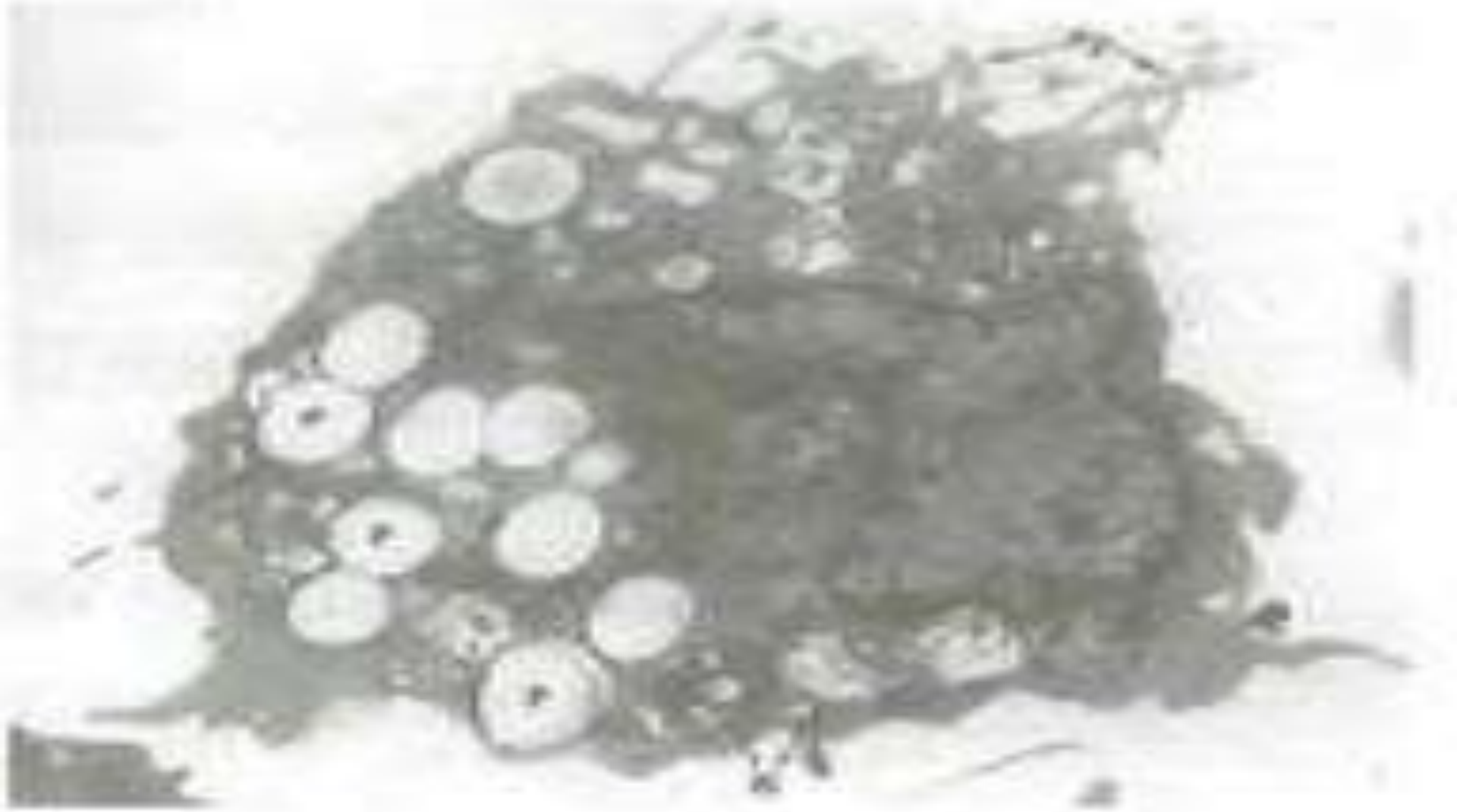
1. **Phagocytic** cell (engulf & digest micro-organisms).

2. Clean wounds from any dead cells (debris).

3. Interaction with lymphocytes & neutrophils by producing interleukins which stimulate attraction of neutrophils and mitosis of lymphocytes.

- 4. Act as **antigen presenting cells;** they can trap, transport & partially digest antigens to present them to lymphocytes.
- 5.Histiocytes can fuse with each other to form large multinucleated cell**(Foreign Body Giant cell)** to engulf large foreign bodies.
- 6.Destruction of old RBCs in liver & spleen.

Macrophage.



EM of a macrophage.

7 - Reticular Cells:

Site: in stroma of different organs.

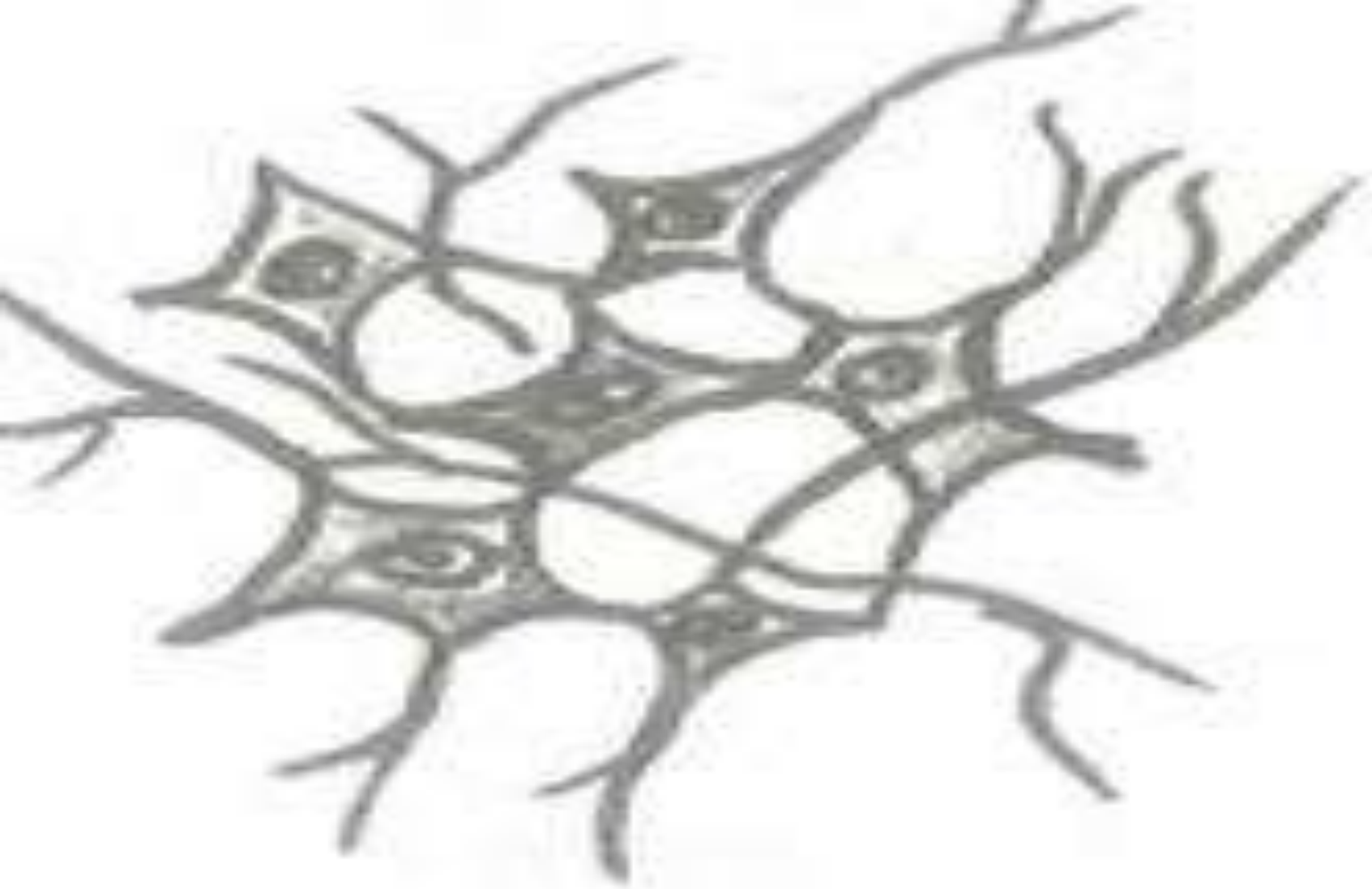
LM: small stellate shaped branched cells with long processes. They form a reticulum together with reticular fibers which stain with silver stain.

Functions:

1.Supportive function.

2.Can act as phagocytic cells on need, i.e. when stimulated with an antigen.

3.Can differentiate into blood cells in bone marrow.



Reticular cells.

B- Free C.T. Cells

8 - Plasma Cells

Origin: from further differentiation of B-lymphocytes

(B-lymphocyte → plasmablast → plasma cell).

Sites: abundant in lymphoid tissue.

LM:

- Oval cell with rounded eccentric nucleus & a clear nucleolus.
- The nucleus has a **cart-wheel** or **clock-face** appearance due to regular radiating chromatin masses under the nuclear membrane.
- The cytoplasm is deeply basophilic with a pale(unstained area) **“negative Golgi image”** near the nucleus.

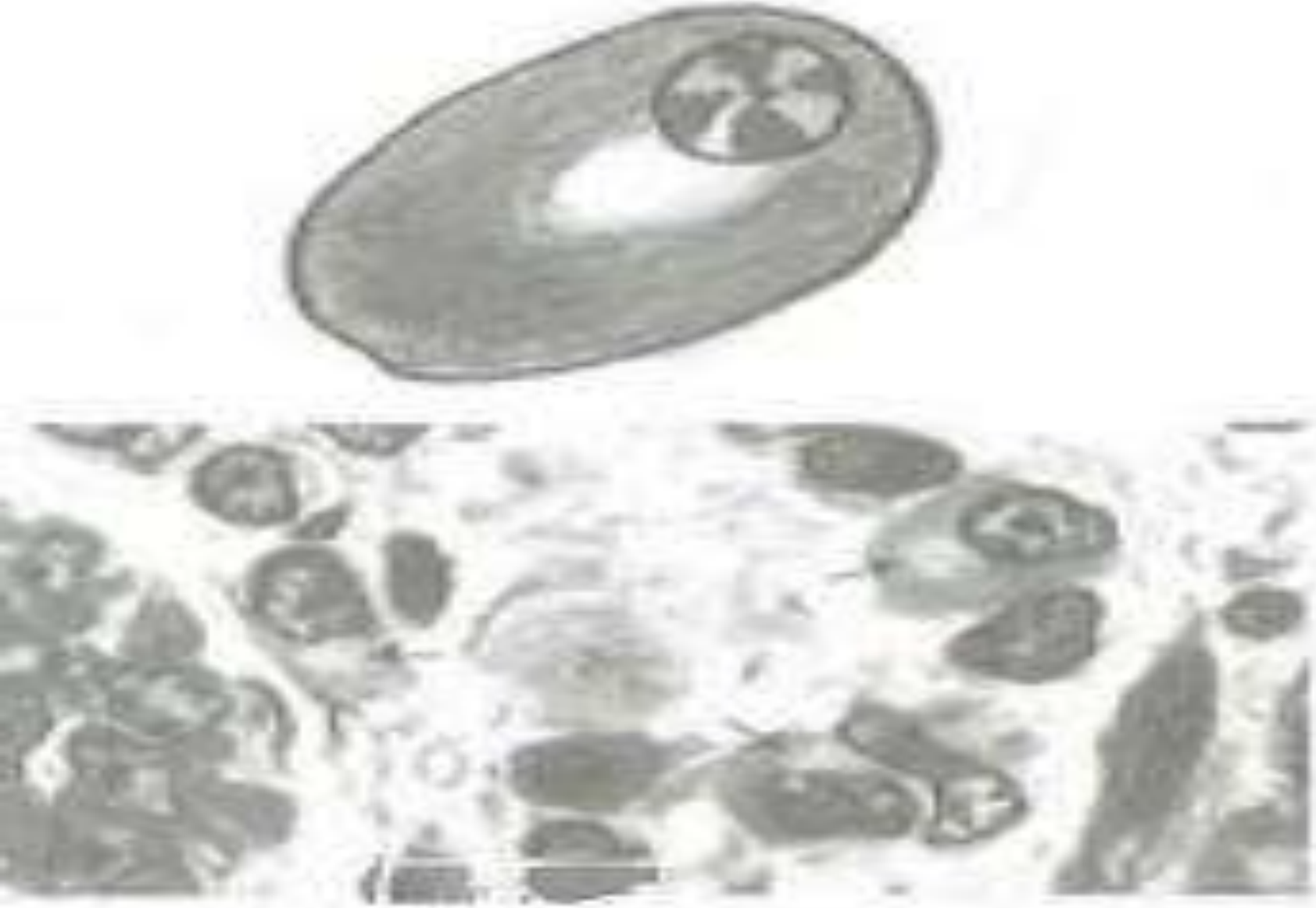
EM:

Features of cells active in protein synthesis:

- Well developed Golgi apparatus.
- Closely spaced numerous cisternae of rER filling the cytoplasm.
- Mitochondria.

Functions:

formation& secretion of **antibodies.**



LM picture of plasma cells.
Arrows point to –ve Golgi image.

9 - Mast Cells:

Origin: UMCs in bone marrow.

Sites:

- In loose C.T. around blood vessels.
- Under epithelium of respiratory & digestive tracts.

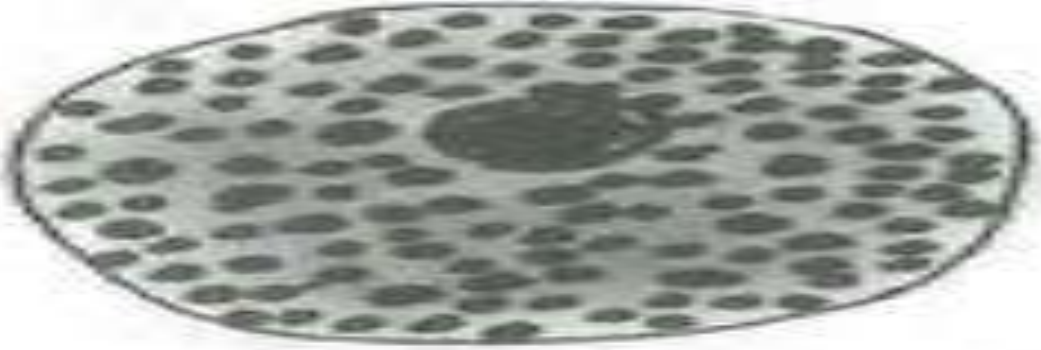
LM:

- Oval cells with eccentric nuclei.
- Cytoplasm is full of basophilic granules which stain metachromatically with basic stains (e.g. toluidine blue), so they stain purple or red instead of blue.

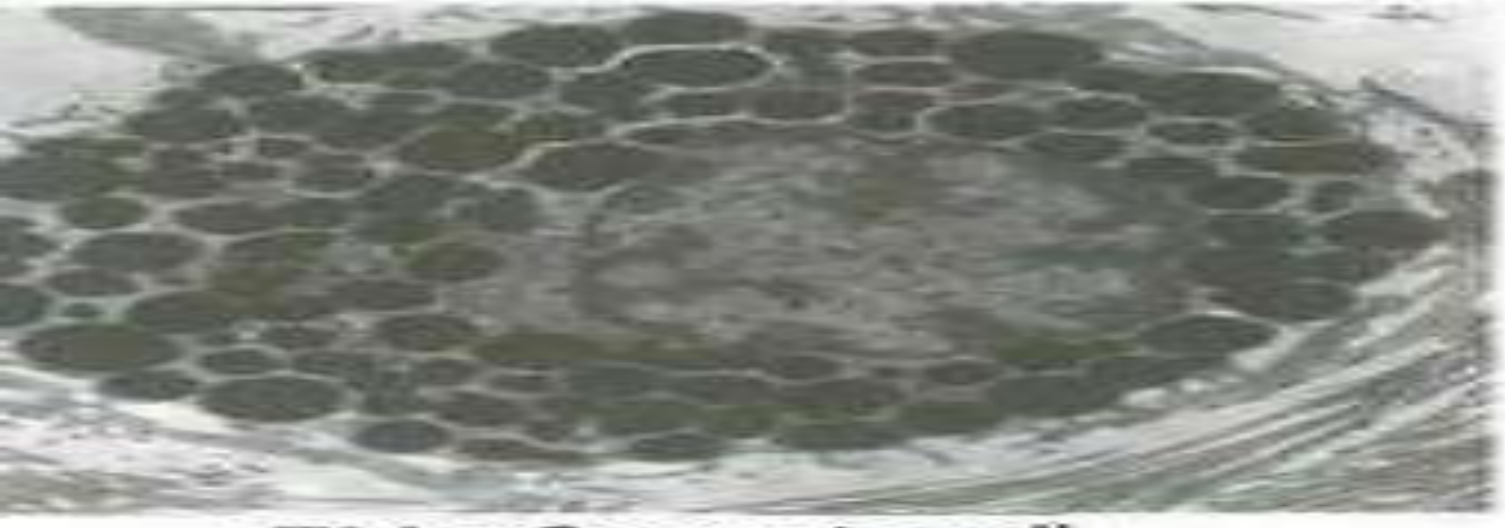
<u>EM:</u> Features of secretory cells:

- Well developed Golgi.
- Mitochondria, rER.
- Electron-dense membrane-bounded granules filling the cytoplasm.

- **Functions:**
- 1- Secretion of **histamine** which causes vasodilation that initiates allergic reactions.
- 2- Secretion of **heparin** (anticoagulant) which prevents blood clotting.
- 3- Secretion of **eosinophil chemotactic factor,** which attracts eosinophils to site of allergy.



LM of mast cell.



EM of mast cell.

10 - Free Macrophages:

- **Origin:** Arise from blood monocytes, which circulate in the blood stream
- then migrate to the loose C.T. where they undergo direct transformation into macrophages. They are described free as they are wandering in the C.T.

- **LM:** They have the same features of fixed macrophages as they are the same cell, but at different sites.
- **Functions:** They have the same function as fixed macrophages.

11 - Blood Leukocytes:

Origin:

- All blood leukocytes migrate from blood stream to C.T. to perform their defensive functions (wandering cells) (see blood for their structures &functions).

12 - Pigment Cells (Melanophores):

Origin: C.T. macrophages which phagocytose melanin pigments produced in melanocytes.

Sites: C.T. dermis of skin & eye.

LM:

Small branched cells with small dark nuclei, cytoplasm is granular.

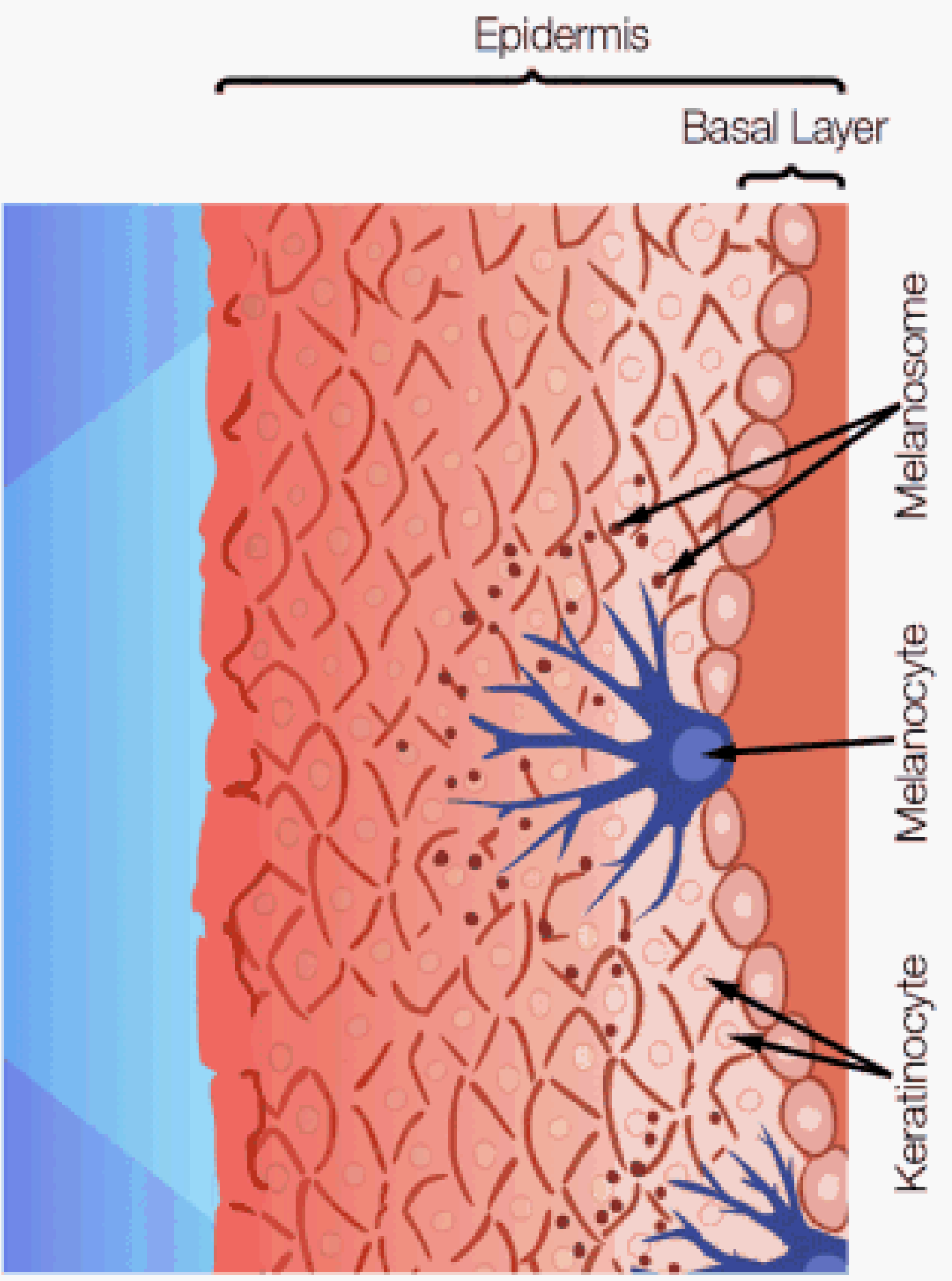
EM:

Cytoplasm is full of melanin granules(melanosomes).

Functions:

1- Carry melanin pigments which gives the color of the skin

2- Absorb ultraviolet rays and protect the skin from the injurious effect of the sun


Epidermis
Basal Layer
Melanosome
Melanocyte
Keratinocyte

Types of Connective Tissue Proper

- According to the relative abundance of the basic components of C.T. (cells, fibers & matrix) C.T. is classified into two classes:

Loose:

1)Loose alveolar CT

2)Adipose CT

3)Reticular CT

4)Mucoid CT

Dense:

1)White fibrous CT

2)Yellow elastic CT

A- Loose Types of Connective Tissue Proper

1- Loose (Areolar) Connective Tissue:

- It is the typical and the most common type of C.T. proper.
- **Structure:**
- Cells: It contains all types of C.T. cells (most common cells are fibroblasts, macrophages, fat cells and mast cells).

- Fibers: All types, white collagenous is the most common.
- Matrix: most abundant matrix.

Characters:

- It is a loose type that contains potential cavities (areolae) which can accommodate large amounts of fluids or gasses.
- Delicate consistency, flexible, well vascularized & nonresistant to stress.

Sites:

Found everywhere in the body (except the brain):

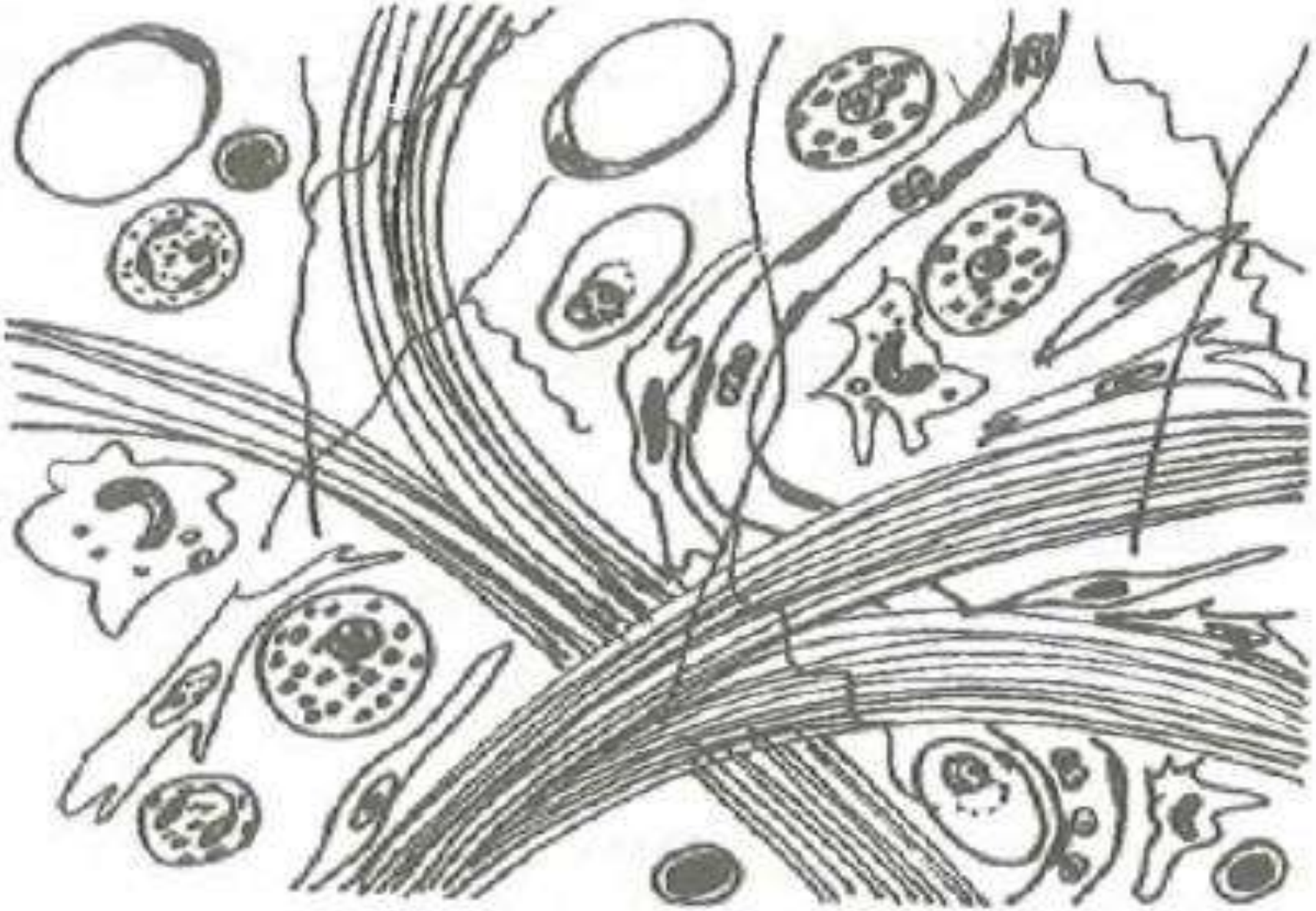
- Packing and filling the spaces between cells & fibers and organs, e.g. subcutaneous tissue (dermis of skin) .
- Submucosa, serous membranes.
- Around blood vessels & nerves along their course.

Functions:

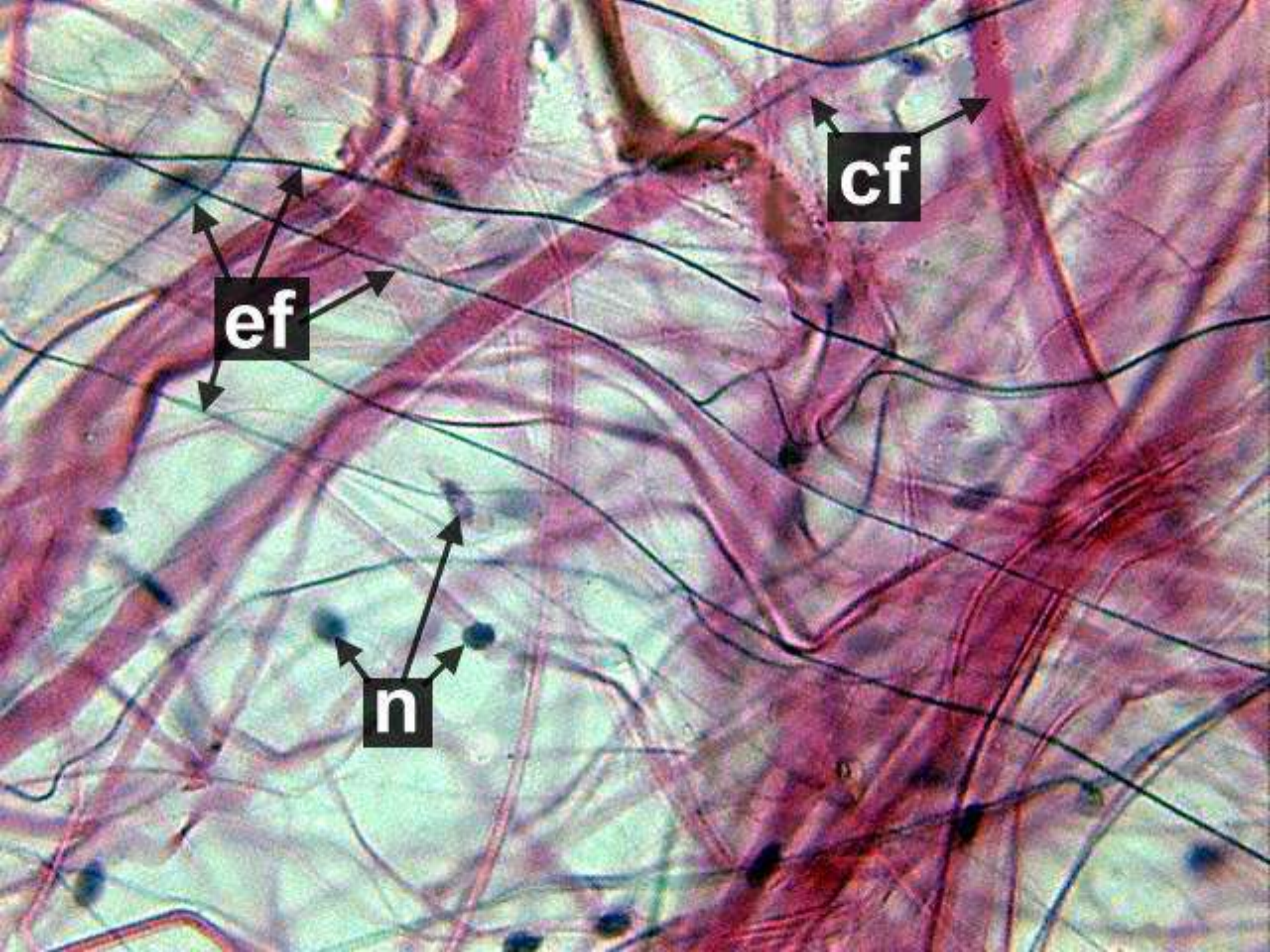
1- Exchange of nutrients to & from blood vessels.

2- It binds structures together.

3- It limits the spread of infection.



Loose (areolar) CT.


cf
ef
n

2- Adipose Connective Tissue:

- Similar to areolar C.T. but fat cells predominate over other components.
- **Structure:**
- Cells: It is formed of a large number of fat cells, closely packed few fibroblasts, Mast cells & lymphocytes are scattered in the narrow spaces between fat cells.

- Fibers: reticular fibers form a network around the fat cells, Collagen and elastic fibers are present in between the lobules of fat cells.
- **Types of adipose tissue:**

There are two types according to vascularity & functions:

- **a) White adipose connective tissue:**
- **Sites:**
- Widely distributed in the body.
- Under the skin especially in females, e.g. mammary gland, gluteal region, abdominal wall & mesentery.
- Around the kidney & blood vessels.

- **Characters:**
- • Fat cells are large (up to 150 um), filled with one globule of nonpigmented yellowish fat.
- • It is poor in blood supply and is affected by diet & hormones.

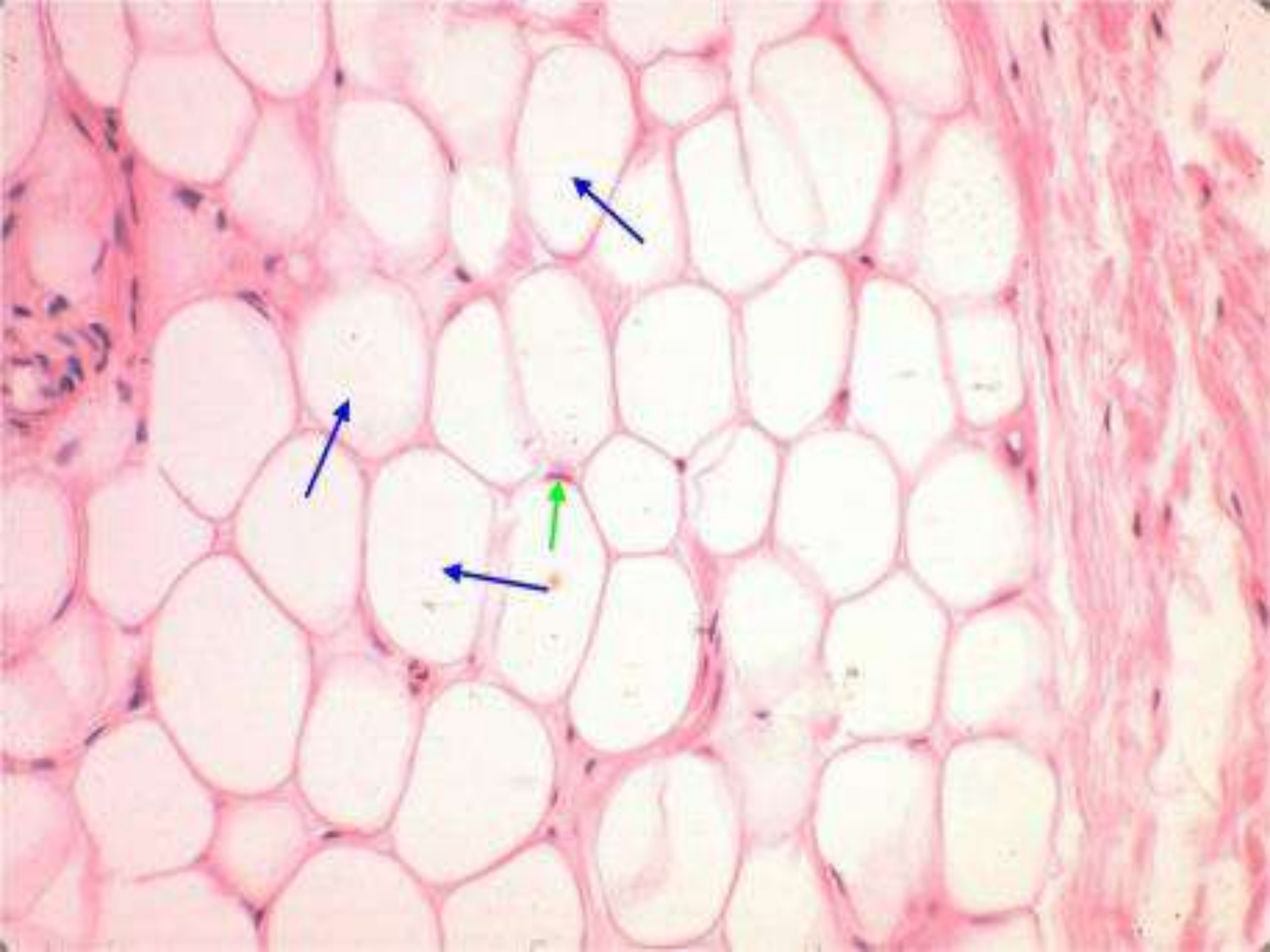
- **Functions:**

1- Storage of fat.

2- Heat insulation.

3- Support the kidney and other organs.

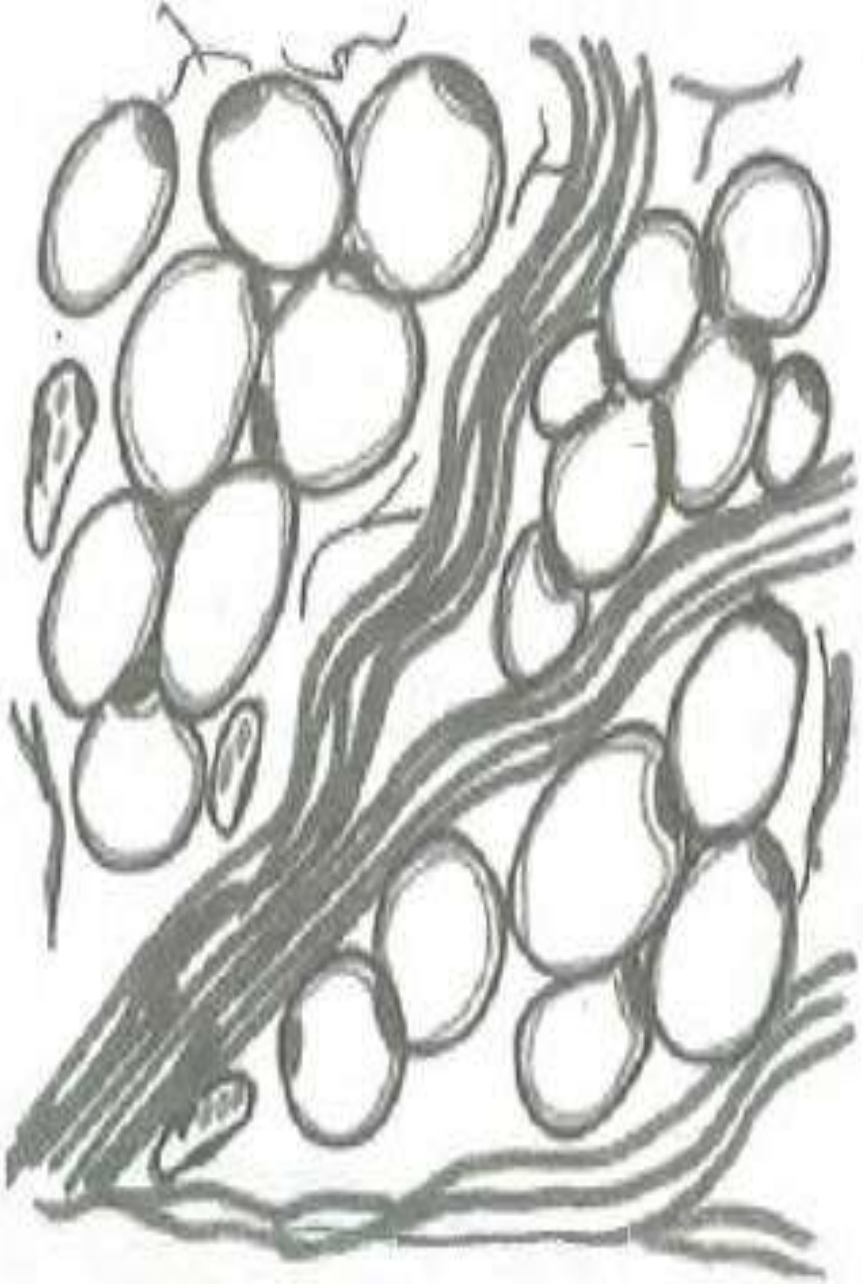
4- Gives the skin its shape and contour,



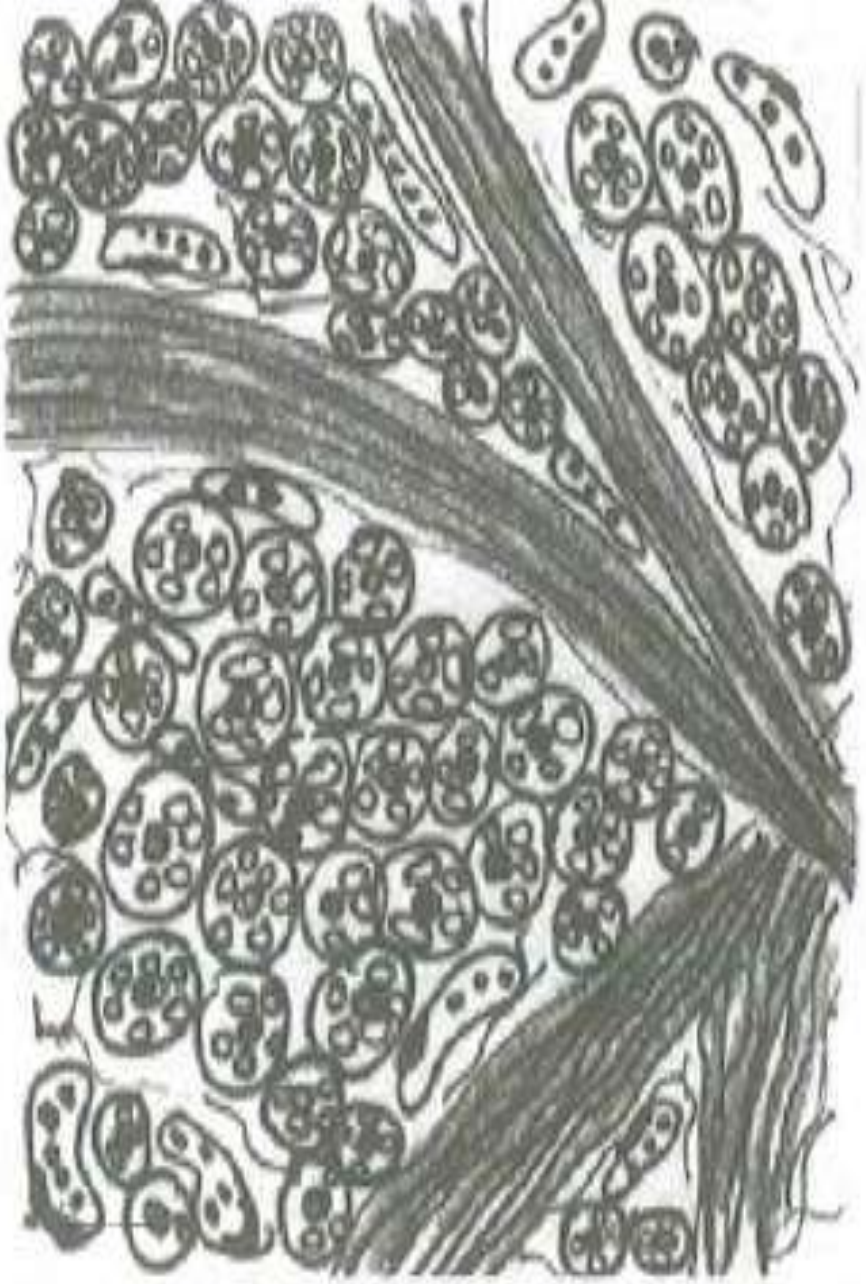
- **b) Brown adipose connective tissue:**
- **Sites:**
- present in limited areas: interscapular region, mediastinal region & axillary region in embryo and newborn & is gradually replaced by white fat.

- **Character:**
- Fat cells are small in size with central nucleus, filled with multiple small droplets of pigmented fat.
- Its brown color is due to cytochrome pigments in mitochondria &high vascularity. It is not affected by starvation.

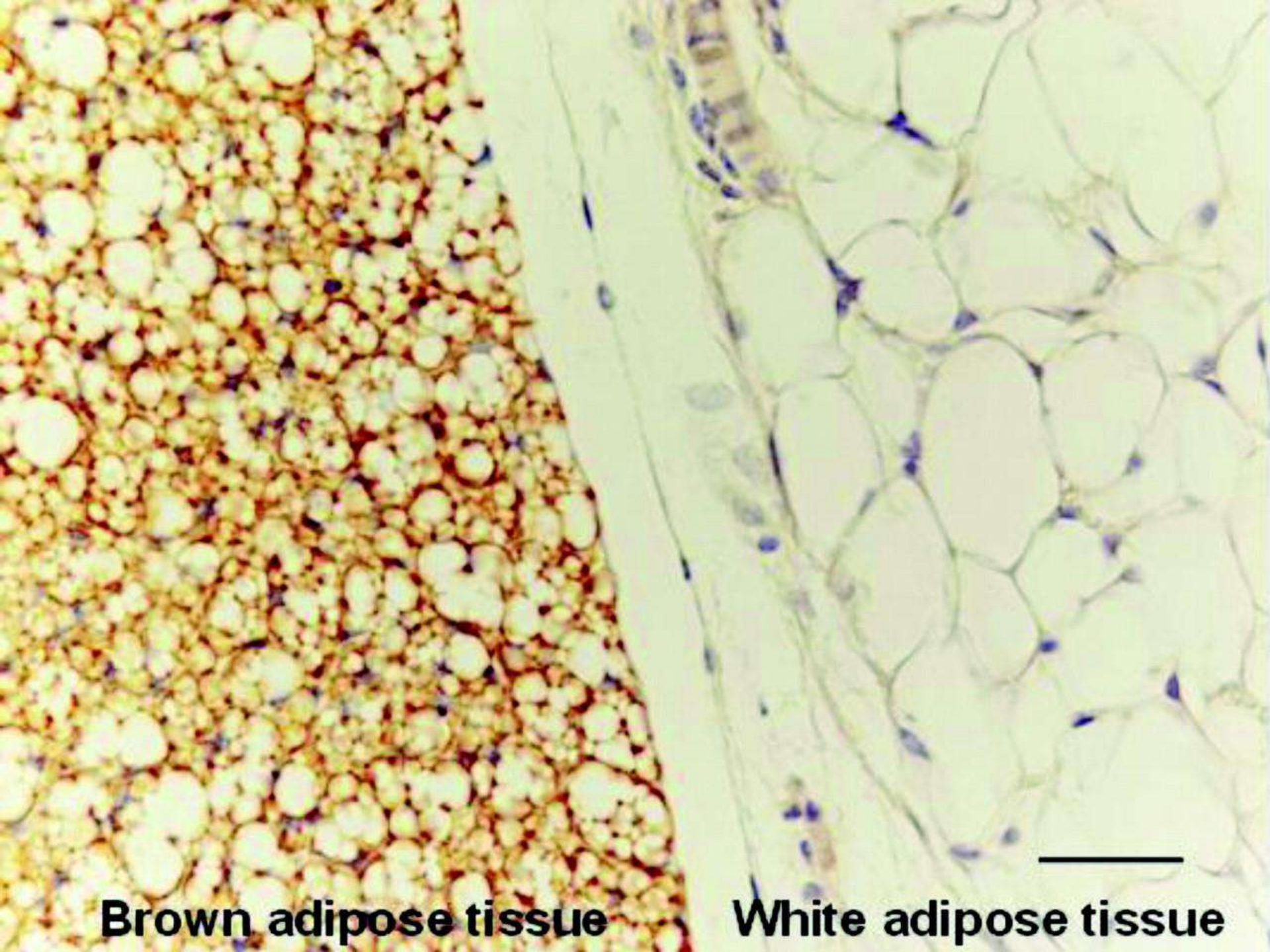
- **Functions:**
- Heat generation in newly born infants.
- Release of heat on exposure to cold to warm the body.



White adipose CT



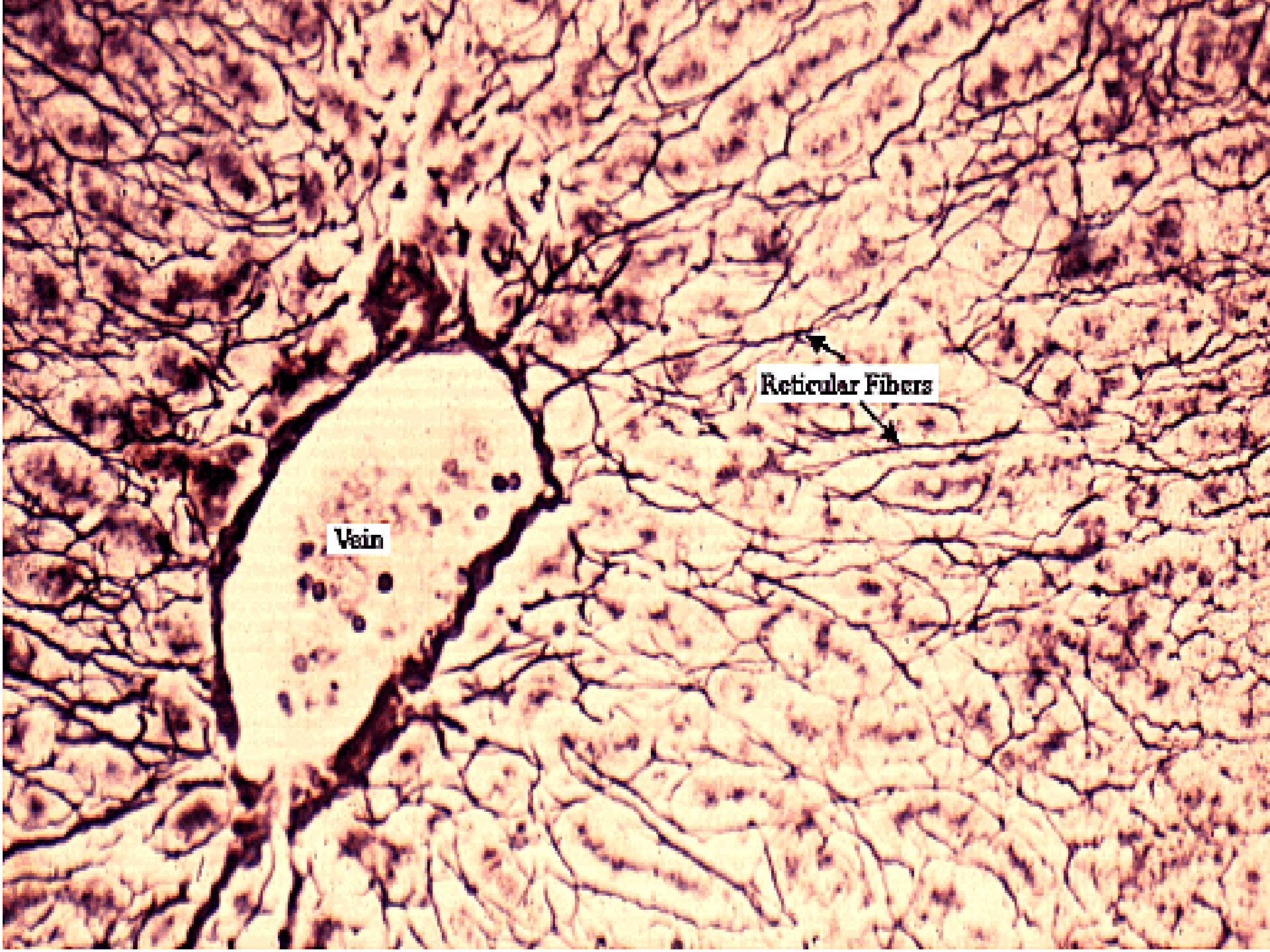
Brown adipose CT


Brown adipose tissue
White adipose tissue

3- Reticular Connective Tissue:

- Delicate type.
- **Structure:**
- **Cells:** primitive fibroblasts, called reticular cells. They are stellate with long processes which are joined with cell junctions.
- **Fibers:** fine network of branching reticular fibers, forming) network with the cells.

- **Sites:**
- It forms the framework (stroma) for cellular solid organs (to support the functioning cells, e.g. lymphatic organs (spleen & lymph node), kidney, liver, bone marrow, lung and all endocrine glands.
- **Staining:**
- Silver stain (Ag) ⟶ brown black.


Reticular Fibers
Vein

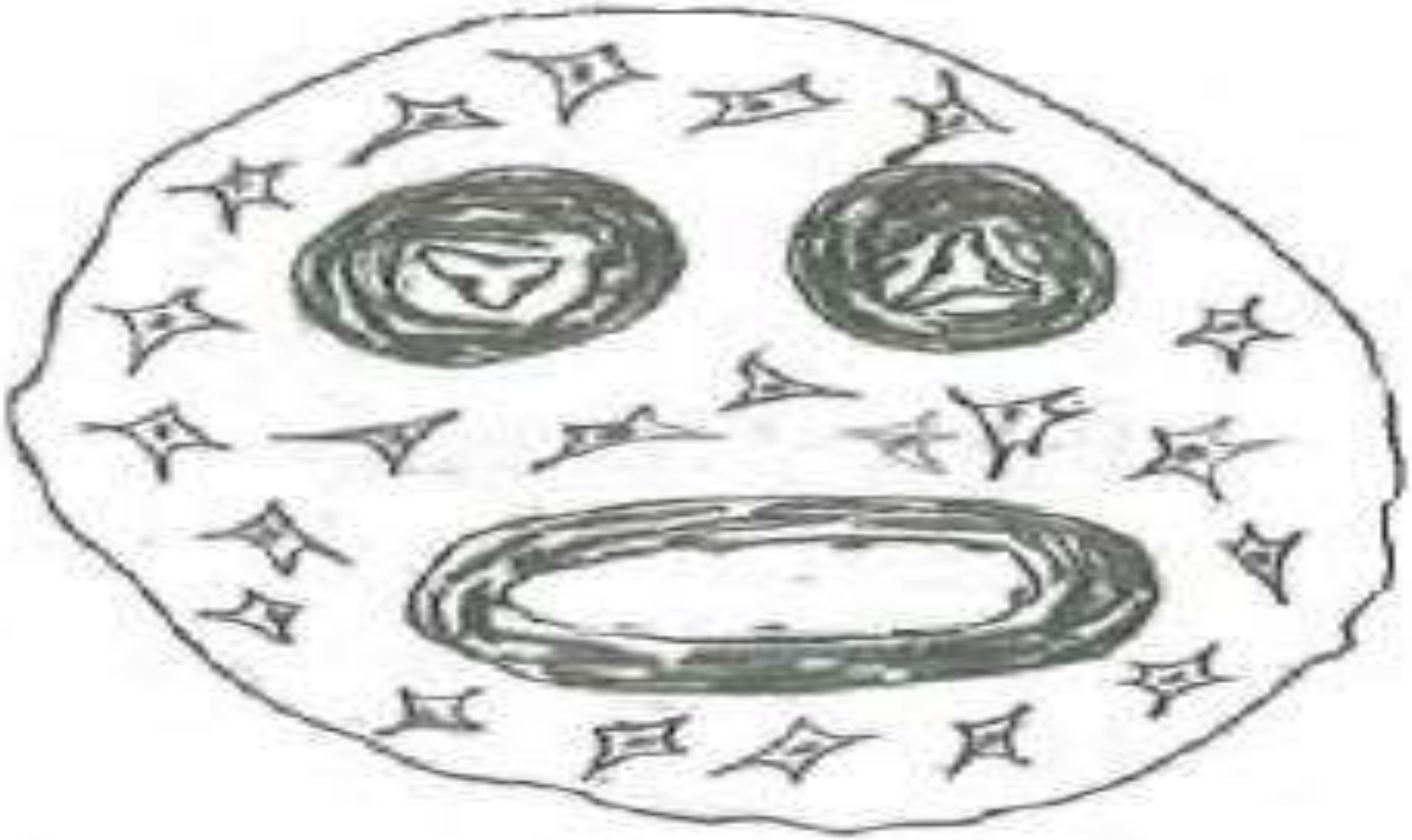
4- Mucoid Connective Tissue:

- It is an embryonic, jelly-like C.T., in which the ground substance predominates.
- **Structure:**
- **Cells:** mesenchymal cells or young fibroblasts which are stellate, branched with multiple connected processes.
- **Fibers:** very fine collagen and reticular fibers.

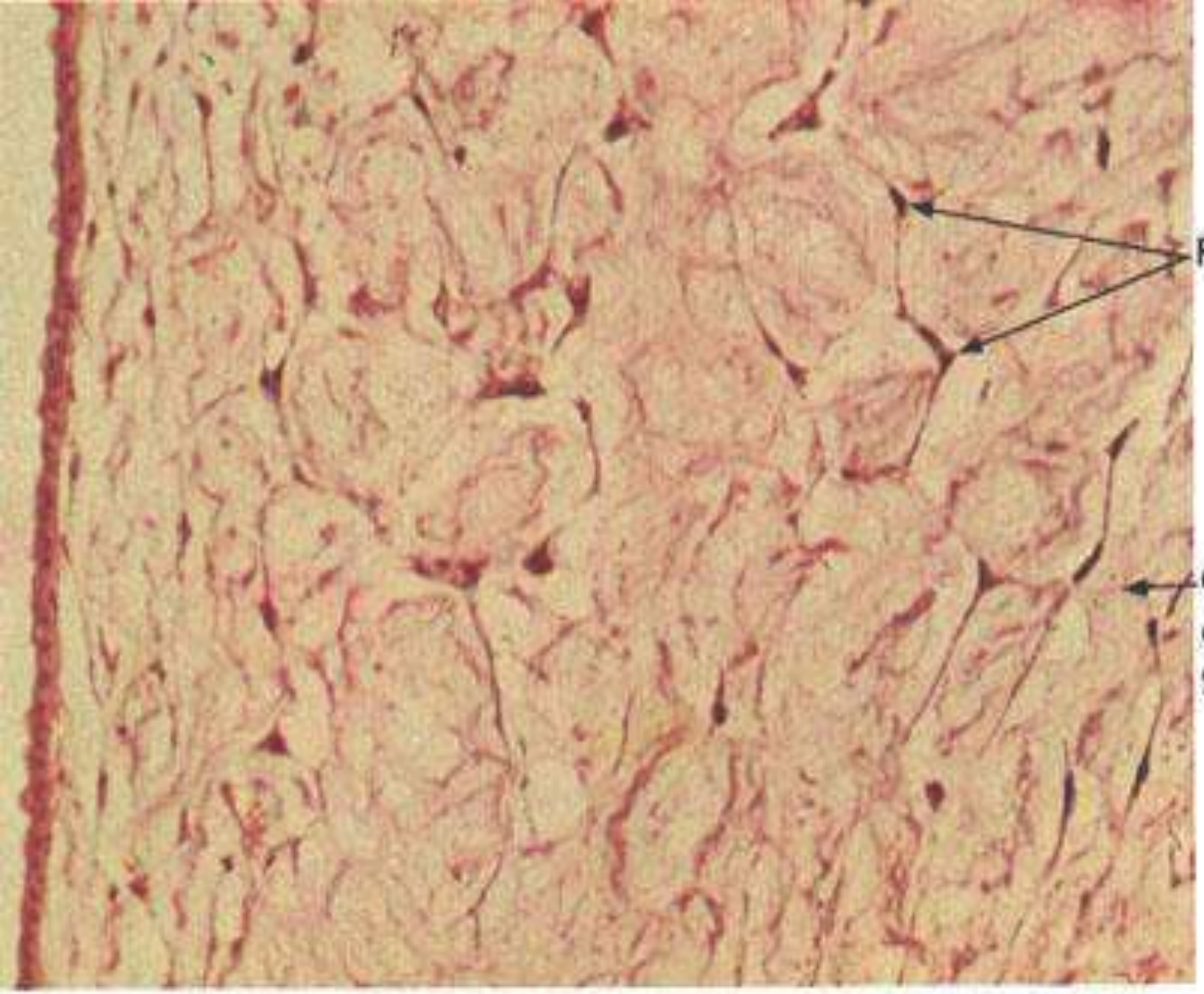
- **Matrix:** large amount of soft, jelly-like ground substance, rich in mucus and

hyaluronic acid.

- **<u>Sites:</u>**
- Umbilical cord, where it forms the main component and is called Wharton's Jelly.



Mucoid C.T. (in umbilical cord)


Fibroblasts
Collagenous connective tissue
100 μm

B- Dense Types of Connective Tissue Proper

5- White Fibrous Connective Tissue:

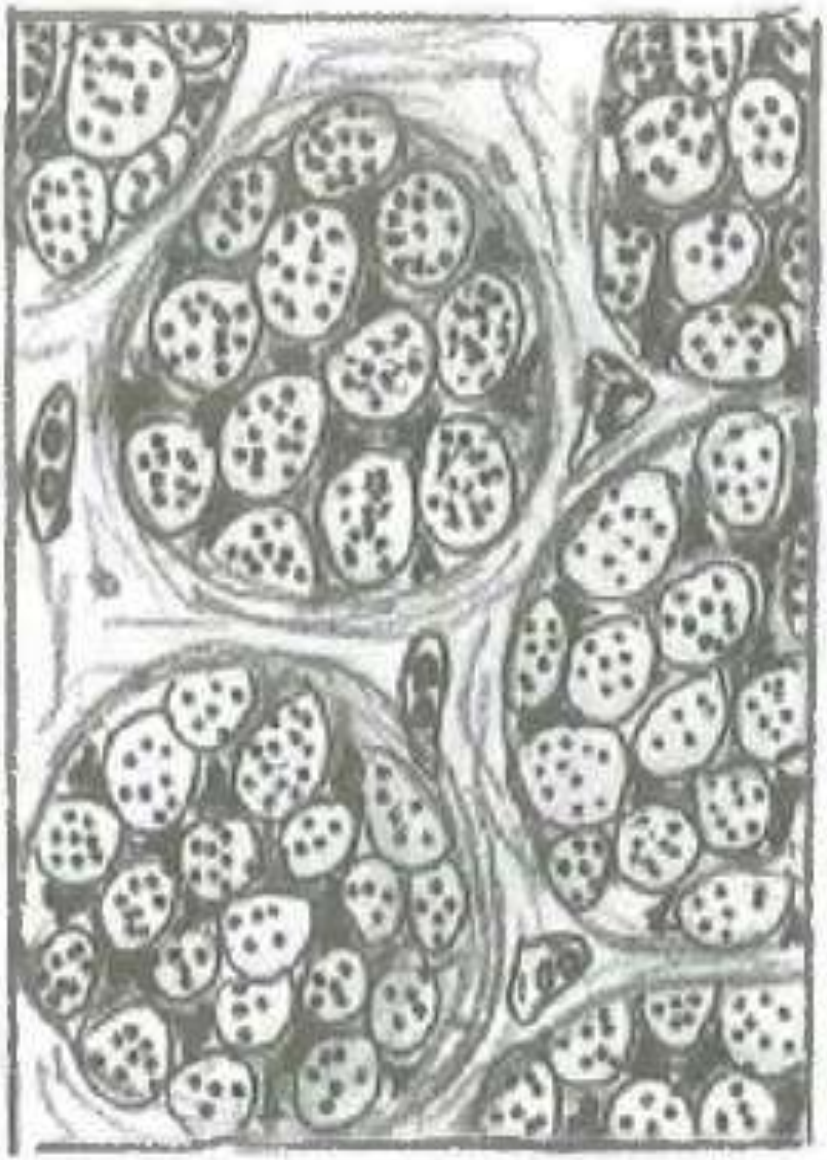
- Very dense due to great predominance of collagen fibers with few cells.
- **Structure:**
- **Fibers:** packed collagen fibers in bundles.
- **Matrix:** minimal amount (poor in blood supply).

- **Cells:** fibroblasts (tendon cells). Fibroblasts are enclosed between the packed collagen fibers.
- **Characters:**
- appears white in fresh state, less flexible & more resistant.
- **Types:**
- according to the arrangement of bundles of collagen fibers, there are two types:

- **a) Regular white fibrous connective tissue:**
- The bundles of collagen are arranged regularly & parallel. Fibroblasts are arranged in rows in between the collagen bundles (tendon cells), with very little amount of matrix.

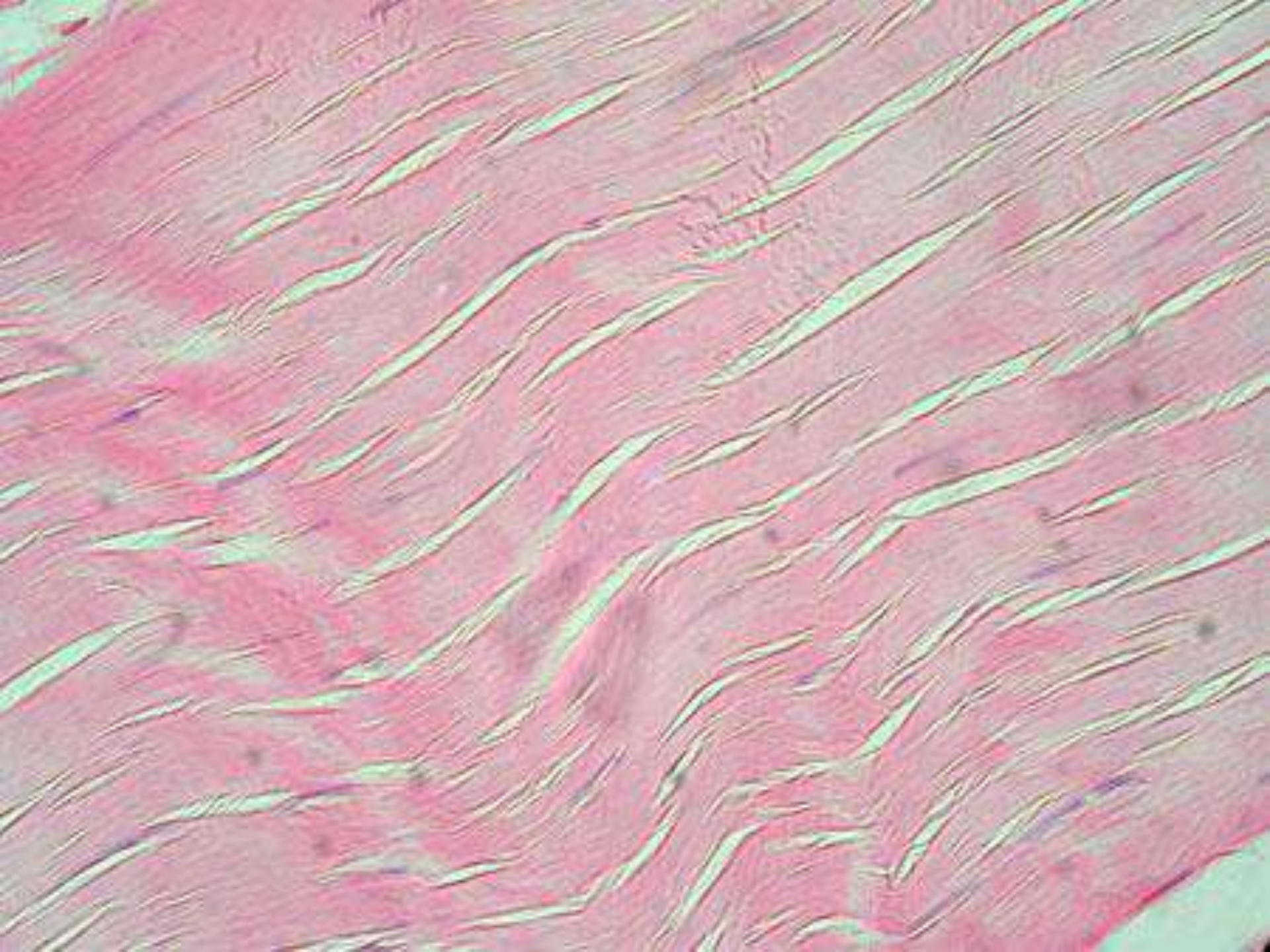
- **Sites:**
- **Tendons** of skeletal muscles.
- **Cornea:** under the epithelium.
- **Function:** to withstand stretch in one direction.

LS

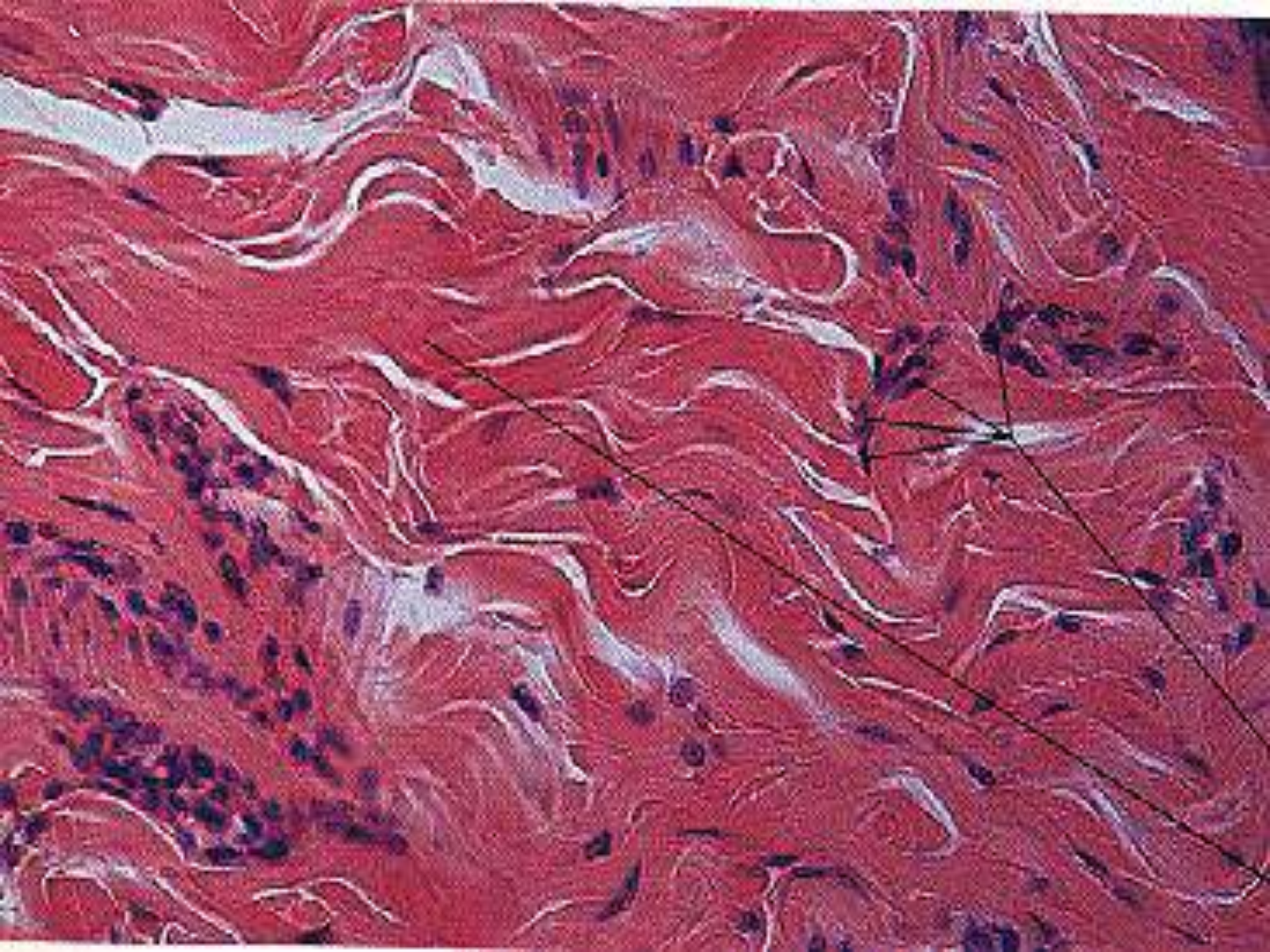


TS

Regular white fibrous CT (in a tendon).



- **b) Irregular white fibrous connective tissue:**
- The bundles of collagen fibers are interwoven & irregularly arranged.
- **Sites:**
- Dermis of the skin.
- Capsule of organs & ligaments.
- Periosteum&perichonderium.
- Sclera of the eye.
- **Function:**to withstand stretch in different directions.

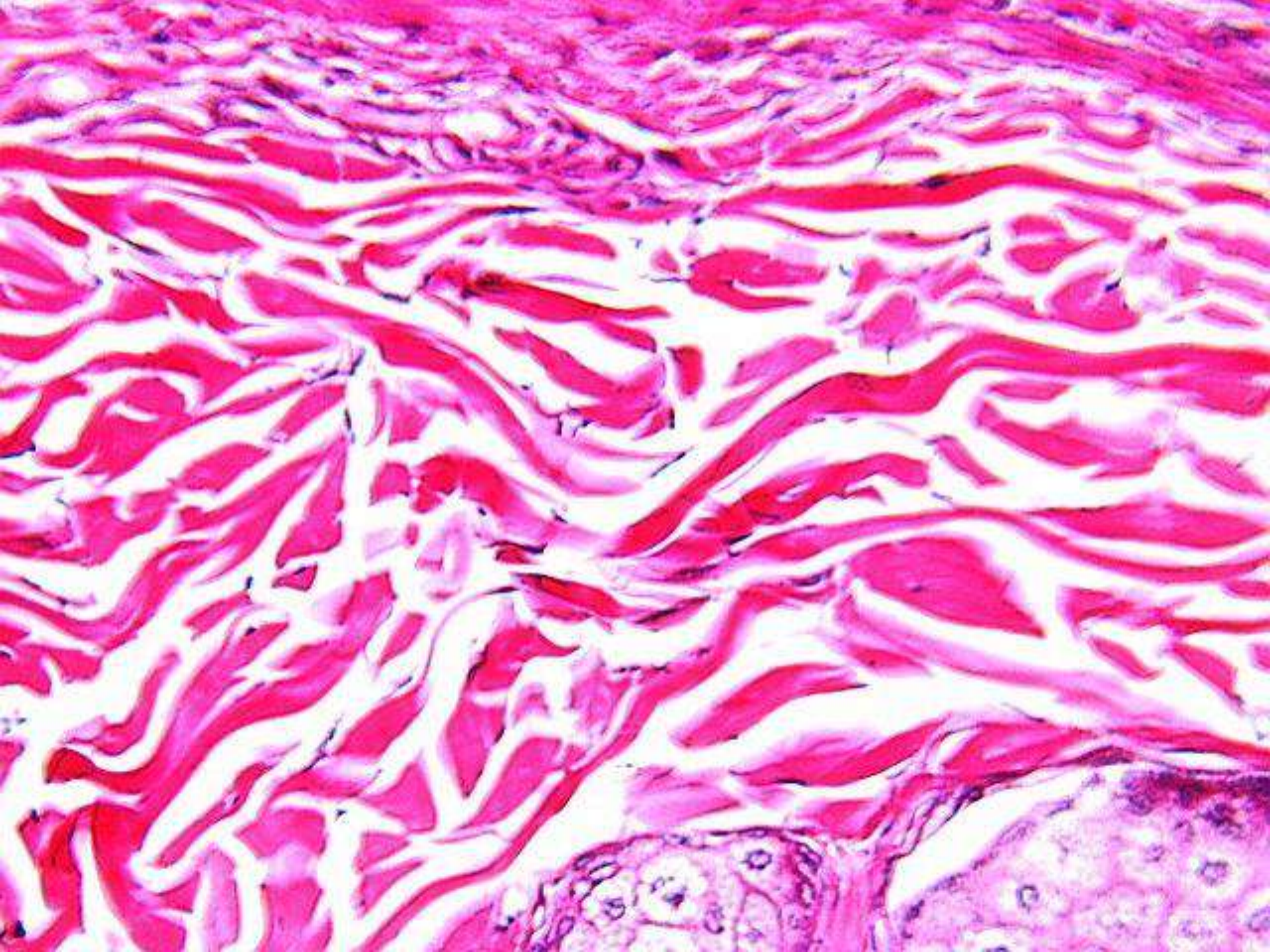


6- Yellow Elastic Connective Tissue:

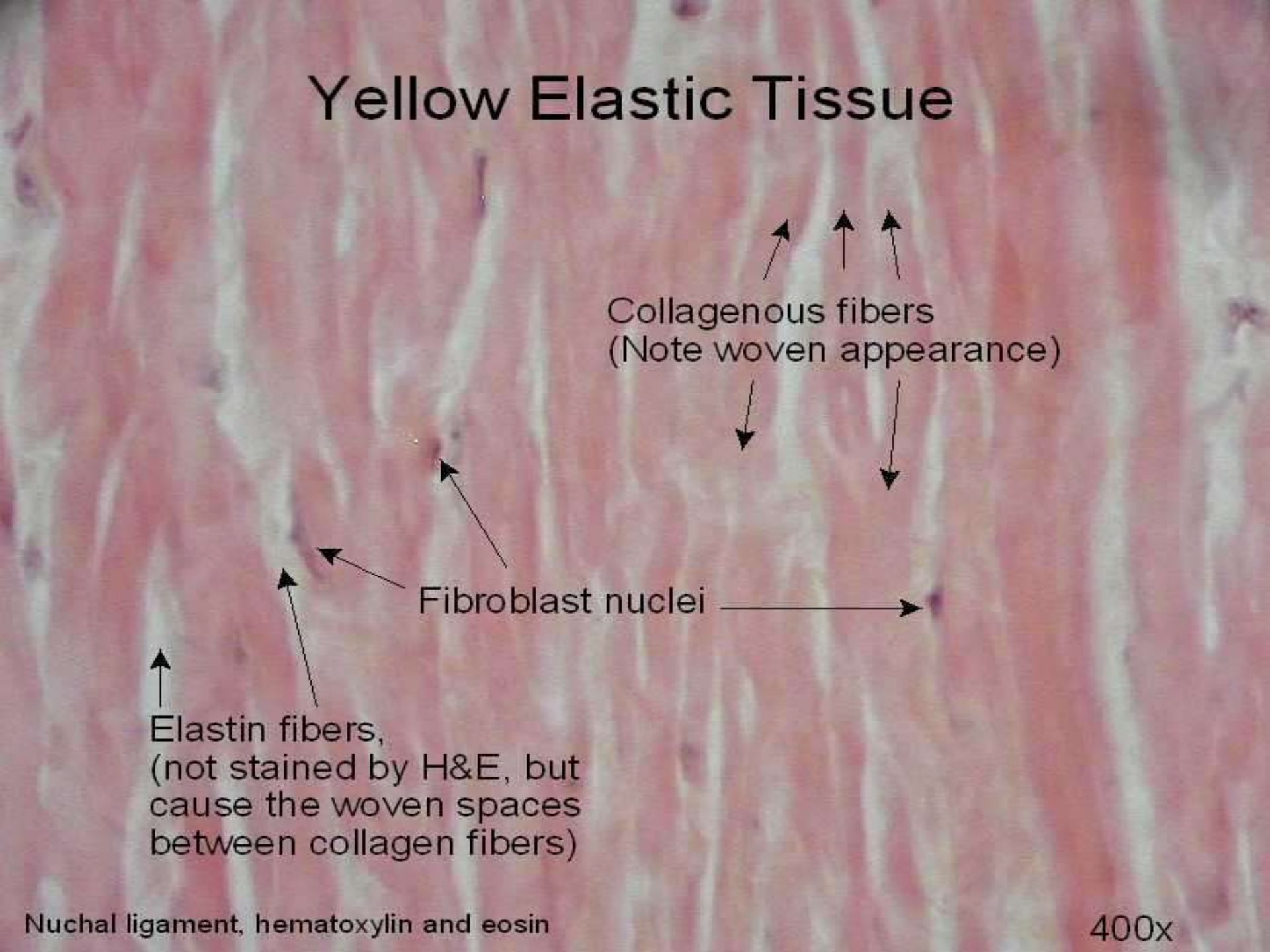
- Dense type, with great predominance of elastic fibers, so it appears yellow in fresh state.
- **Structure:**
- **Fibers:** regular parallel elastic fibers.
- **Cells**: few fibtroblasts & fibrocytes.

- **Sites:**
- Aorta & large arteries.
- Bronchi, bronchioles & around alveoli.
- Ligaments (e.g. ligamentum flavum: joining the vertebrae, ligamentum nuchae: at the back of the neck especially animals & suspensory ligaments of the penis).
- Vocal cords.

- **Staining:**
- Orcein stain ⟶ brown black.
- **Function:**
- It forms elastic membranes.
- It has a great elastic power (recoil when stretched, i.e. rubber like).



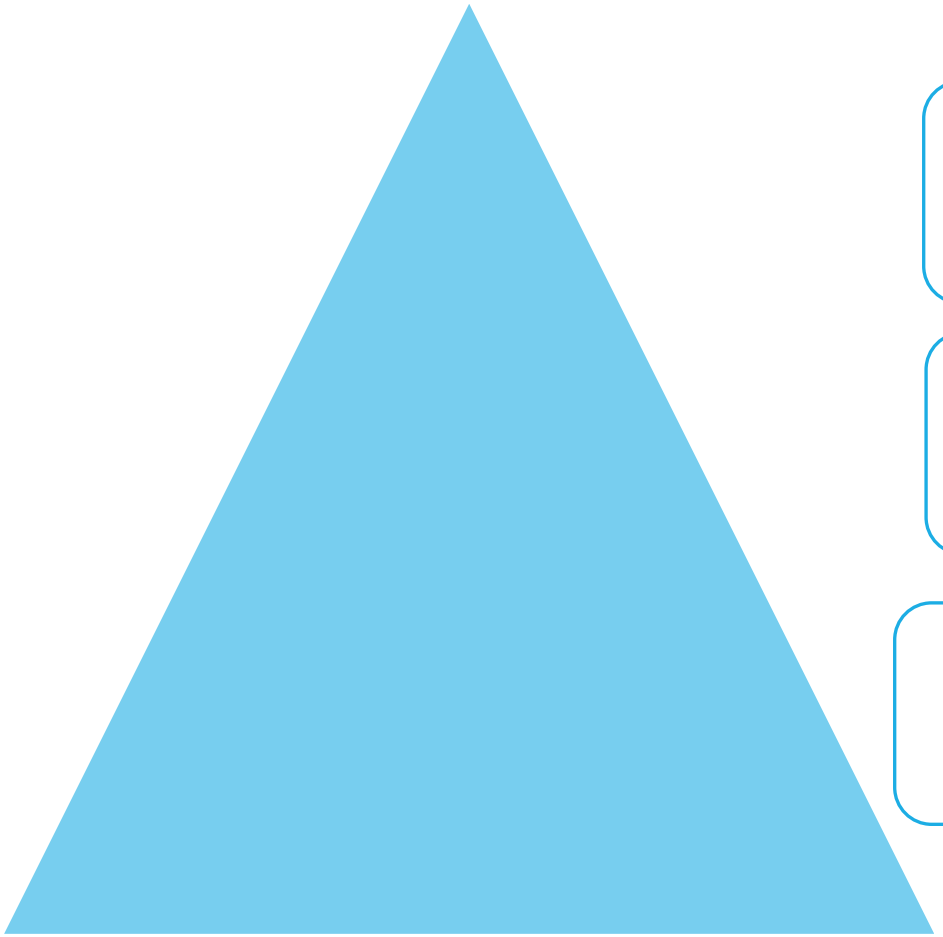
Yellow Elastic Tissue



Nuchal ligament, hematoxylin and eosin

400x

General histology



Blood

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Consultant of pathology

Definition

Blood is a viscous fluid which flows inside blood vessels, and is continuously circulating throughout every tissue by the pumping action of the heart.

• It is considered a type of C.T. as it is formed inside the bone marrow from mesenchymal tissue, and its cellular elements on entering the blood stream become suspended in considerable amount of intercellular liquid substance(plasma).

Volume: about 3-5 liters in an adult which constitutes about 5-7% of the body weight.

Color: a thick drop appears red (due to overlapping of RBCs), while a thin film appears **greenish yellow.**

Composition

is composed of **formed elements** suspended in a liquid phase called the **plasma.**

1- Formed elements (45%):

- Red Blood Corpuscles (RBCs) or **Erythrocytes.**
- White Blood Cells (WBCs) or **Leukocytes.**
- Blood Platelets.

2- **Plasma (55%):** which is formed of 90% water, 7% plasma proteins (albumin, globulin and fibrinogen), 2% organic substances as amino acids, glucose, lipids, hormones, enzymes & nitrogenous compounds and 1% inorganic salts.

N.B.: The fluid component of the blood leaves the small vessels and enters the C.T. as tissue fluid

Examination of Blood

For histological examination of the blood cells, a drop of blood is smeared on a glass slide, air dried, this is called **blood film.**

The blood film is then **stained** with a mixture of acidic and basic stains(neutral stain).

The most common stain used in routine work is **Leishman's stain,** which is formed of a mixture of red acidic stain **eosin** and blue basic stain **methylene blue** dissolved in methyl alcohol (for fixation and preservation of the cells).

RBCs are acidophilic due to hemoglobin which is a basic protein,

While leukocytes nuclei are basophilic, their cytoplasm differs in staining according to the type.

As the number of RBCs (5 million/mm^3) is by far larger than leukocytes (4-11 thousands/mm^3), with ratio (1:500), so in blood film, the RBCs look like a red sea in which blue dots are scattered.

Functions of the Blood

1- Transport as blood carries **O_2,** nutritive materials, hormones and enzymes to different tissues and cells and it removes C02 and waste products from them.

2- Protection of the body from bacteria, foreign bodies, antigens and viruses.

3- Control of body temperature and acid-base balance.

Red Blood Corpuscles (RBCs) (Erythrocytes)

Minute corpuscles that give the blood its red color.

They develop in the bone marrow as true cells, but before entering the blood stream they extrude their nuclei and their organelles are also lost.

They are reduced to be a bag or corpuscle, bounded by a plasma membrane and contain a colored iron binding protein called hemoglobin.

This transformation helps the RBCs to perform their specialized function of transporting O_2 from the lungs to the tissues, and CO_2 from tissues to lungs.

Shape:

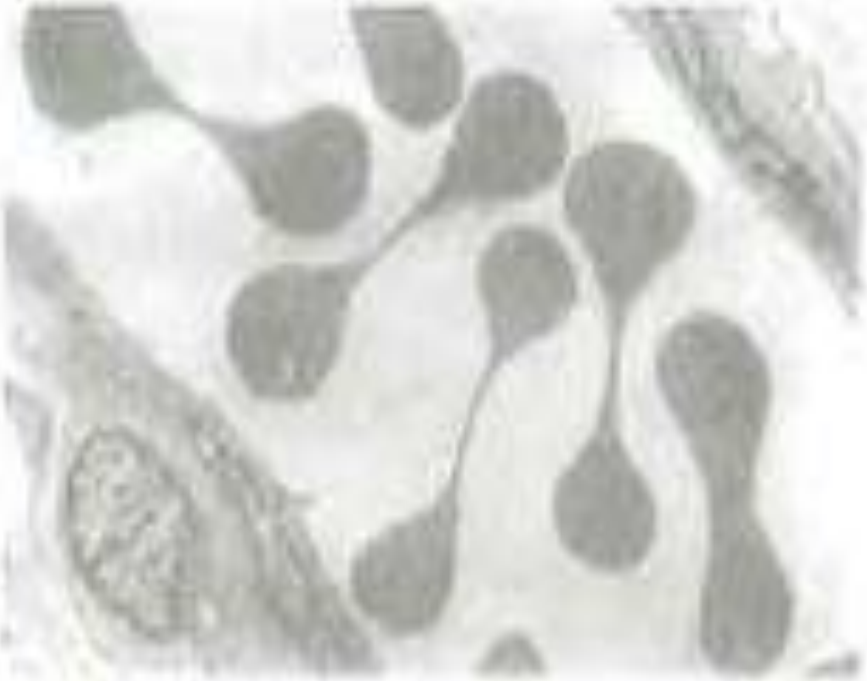
LM:

In surface view: they appear **rounded, non-nucleated, acidophilic** with pale centers and dark periphery, **uniform in size** having a diameter of about 6-9 μm (average diameter 7.5 μm).

In side view: they are **biconcave,** thick at periphery, thin at the center.

This shape provides the cell with larger surface area relative to its volume , thus increasing its capacity for gas exchange.

N.B.: RBCs may be seen adhering one above the other, stacked in columns resembling piles of coins **(Rouleaux appearance).**



EM:

RBCs are nothing but membranous bags that carry hemoglobin (Hb).

The only cell organelle is the cell membrane (plasma membrane).

They have **no nuclei, no organelles,** so they are **not true cells.**

The only cell inclusion is Hb, which appears as an electron-dense mass filling the whole corpuscle due to its iron content, in addition to some enzymes as carbonic anhydrase, reductase and enzymes of glycolysis.

Size of RBCs:

RBCs have a uniform diameter of 6-9 μm. The average diameter of RBCs is**7.5** μm .

Their thickness is 2 μm at the periphery & 1.1 μm or less at the center.

Life Span of RBCs:

RBCs live about **120 day (4 months), ±7 days.**

Senile RBCs are destroyed in liver & spleen by phagocytic cells.

Their iron is stored and recirculates while the pigments are excreted as bile pigments.

Color of RBCs:

The color of RBCs is due to their Hb content.

A fresh single RBC appears greenish yellow, with a pale central area.

A drop of blood appears red (due to overlapping of RBCs).

RBCs are acidophilic in staining as Hb is a basic protein..

Number of RBCs:

The average number is **5 millions/mm3.**

The normal number of RBCs in adult **males** is **4.5-5.5 millions/mm3** of blood.

This number is less by 1/2million in adult **females** to be **4-5 millions/mm3** .This decrease is due to the monthly loss of blood in menstruation & the effect of female hormones.

Abnormalities in number:

A- Polycythemia:

It is the increase in number of RBCs **to more than** 6 **million/mm3.**

Causes of polycythemia:

Physiological:

1- Hemoconcentration: due to decreased plasma volume (vomiting &diarrhea).

2- Excessive production of RBCs due to physiological hypoxia as: in high altitudes, after muscular exercises and in newly born infants.

Pathological: Chronic heart and lung diseases that cause pathological hypoxia, which in turn stimulates the bone marrow to produce new RBCs.

B- Oligocythemia (anemia):

- It is the decrease in number of RBCs **to less than 4 million/mm3.**

Causes of anemia:

1-Deficiency anemia: due to deficiency of substances essential for erythropoiesis as iron, vitamin B12, or proteins in diet.

2-Hemolytic anemia: results from hemolysis, which is the destruction of RBCs due to abnormal shape or content caused by congenital diseases as spherocytosis and favism (glucose-6-phosphatedehydrogenase deficiency).

3-Hemorrhagic anemia: due to acute or chronic blood loss as repeated bleeding from any cause (e.g. excessive menstrual bleeding, bleeding piles, bleeding nose).

4 -Aplastic anemia: due to damage of bone marrow resulting from exposure to x-ray irradiation or prolonged use of antibiotics.

Functions of RBCs:

1- Transport oxygen from blood to the tissues. Hb combines rapidly with O_2 in the lung to form **oxyhemoglobin,** which is a reversible compound that carries O_2 inside RBCs to the tissues, where it is released easily.

2- Remove carbon dioxide from tissues to the lungs. Hb combines with $CO_2$from the tissues to form **carbaminohemoglobin,** which is also a reversible compound, which releases CO_2 in the lung.

Adaptation of RBCs to Function:

1- Cell membrane structure:

It is plastic and flexible, so RBCs can change their shape and squeeze themselves in narrow capillaries.

It is also a lipoprotein, which is highly selective to help gas exchange.

2- Shape:

Their biconcave shape increases the surface area exposed for gas exchange.

The rounded edges help their easy passage in small branched vessels.

3- Content:

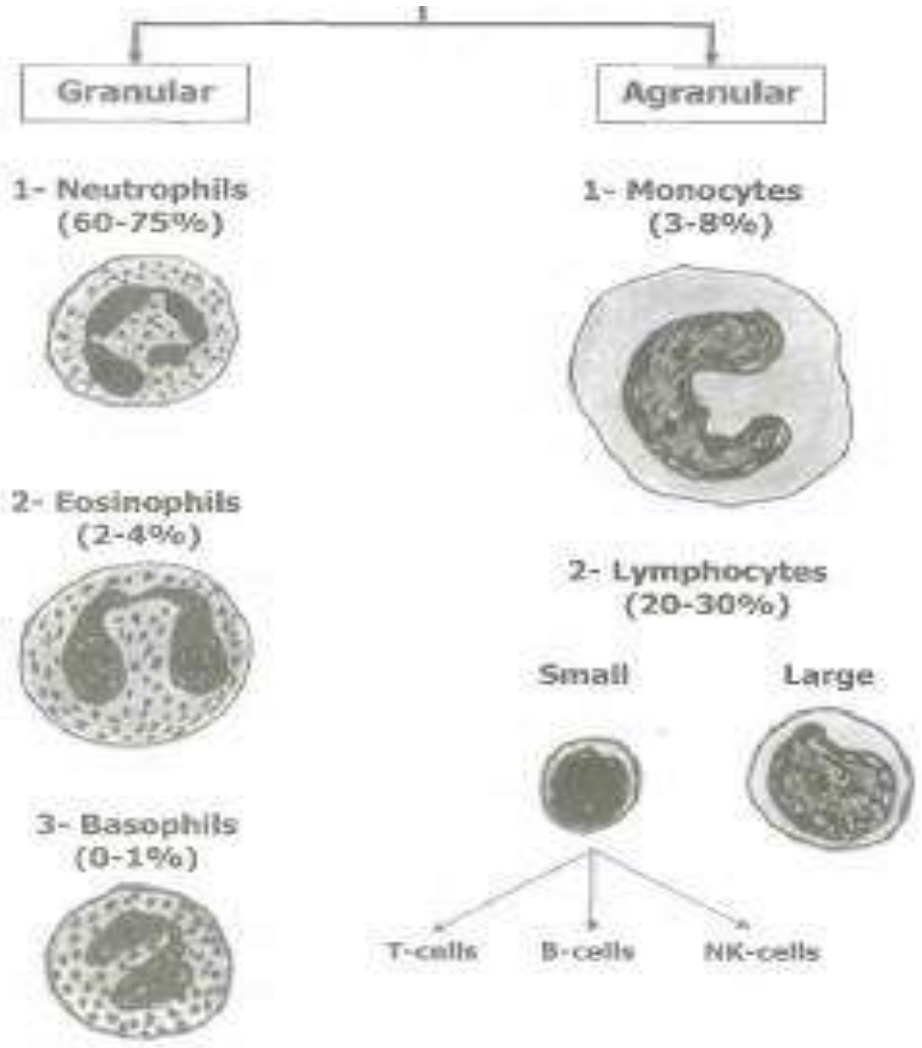
RBCs contain no nuclei or organelles, leaving more space for Hb.

They also contain carbonic anhydrase enzyme needed for carriage of CO_2,and Hb reductase which helps Hb to combine with O_2rapidly.

Differences between RBCs & leukocytes

	RBCs	Leukocytes
Number	4.5-5.5 millions/mm^3	4000-11000/mm^3
Types	One type	5 types
Content	Not true cells, contain only Hb No nuclei, no organelles	True cells, as they contain nuclei & organelles
Shape	Biconcave discs	Spherical in blood stream and irregular (ameboid movement) in C.T.
Appearance	Rouleaux	No rouleaux
Color	Greenish yellow	Colorless
Movement	No movement	Ameboid movement
Size	7.5 µm	Variable: 6-18 µm
Osmotic pressure	Fragile, affected by changes in osmotic pressure	More resistant
Function	Carry O_2 & CO_2 (inside BVs)	Phagocytosis (outside BVs) and immune response
Development	Red bone marrow	Bone marrow & lymphatic tissue
Life span	4 months (120 days)	Few days to years
Site	Blood	Blood, lymph and C.T.

White Blood Cells (WBCs) (Leukocytes)



General Characters:

Color: white when condensed, but in the fresh state they are colorless.

Size: different sizes, 6-20 μm.

Shape: spherical in blood stream, irregular in C.T. (highly mobile with different shapes due to their amoeboid movement).

Content (True cells): They contain nuclei, organelles & inclusions. They have no Hb, but they have different granules.

Origin: from myeloid tissue (bone marrow), but some differentiate in the lymphatic tissue.

Osmotic fragility: they are more resistant to changes in osmotic pressure than RBCs.

Life span: different according to the type, varying from few days up to years.

Number: the total number of leukocytes in normal adult is **4000-11000/mm3**

Abnormal number:

Leukocytosis:

the increase in number of WBCs above the upper limit(11000/mm3). It may be:

- **Physiological:** in infants, during pregnancy, labor & lactation, after meals, cold bath, muscular exercises and exposure to sun.

- **Pathological:** due to infections especially acute pyogenic infections (accompanied with pus formation) and chronic infections.

Leukopenia: the decrease in number below the lower range (4000/mm^3),it occurs in:

- Bleeding.

- Starvation.

- X ray irradiation.

- Excessive use of some antibiotics (e.g. chloramphenicol).

- Typhoid fever and some influenza viruses.

Functions:

The main function of WBCs is **protection.** WBCs are the main line of body **defense** against foreign invaders. Their defensive functions are performed by:

Non-specific defense mechanism.... phagocytosis.

Specific mechanism ⟶ immune response.

WBCs functions are performed outside the blood stream, in the C.T., in association with other C.T. cells as mast cells.

Types:

There are **five types** of leukocytes, classified according to:

1) Presence or absence of granules in their cytoplasm into: granular & non granular.

2) The staining characteristics of these granules.

3) The shape & lobulation of their nuclei.

Leukocytic count:

- Total leukocytic count : is the total number of leukocytes/mm^3 of blood.

- It is done by the hemocytometer, using a special pipette .

- Differential leukocytic count : is the percentage of each type of leukocyte to the total number of leukocytes.

- It is done by preparing a blood film, stained with Leishman's stain ,counting each type in each 100 leukocytes counted and calculating its percentage.

I/ Granular Leukocytes (Granulocytes)

Common characters of granular leukocytes:

Have **specific stainable granules**, and according to the staining of these granules granulocytes are further subdivided into: eosinophils, basophils and neutrophils.

They have a single but **segmented nucleus** .

They have **few organelles**, condensed nucleus (heterochromatic, with no obvious nucleolus).

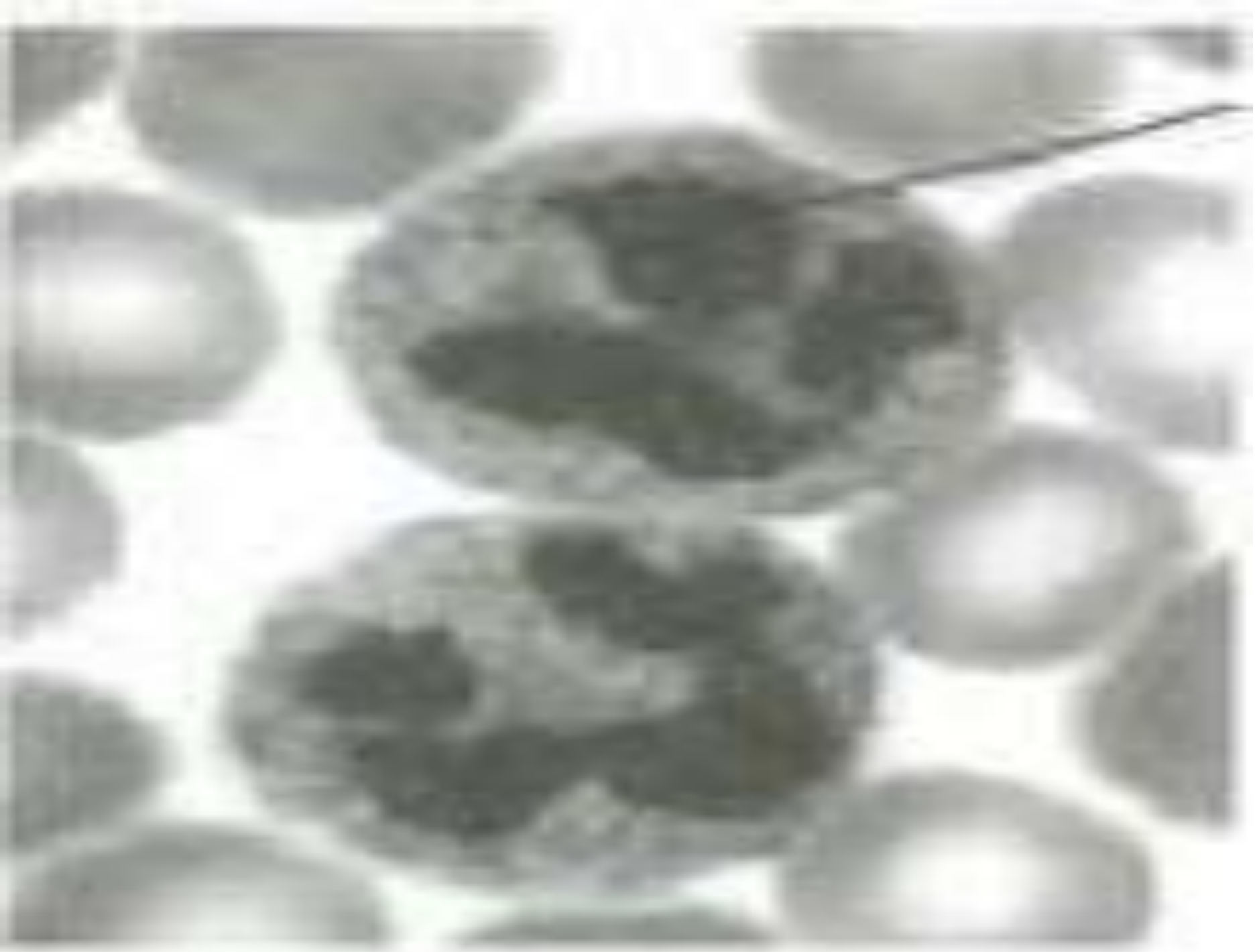
They are end cells that will not divide or regenerate their lost organelles so they have short life span and are compensated by new cells from the bloodstem cells.

1- Neutrophils (Polymorphonuclear leukocytes, PMNs, Microphages)

Neutrophil is a highly motile cell, with great phagocytic activity.

It is considered the first line of defense against any foreign invader.

Percentage: 60-75 % (the most common type of leukocytes).



Life span : very short, about 3-4 days.

Diameter: 10-12μm.

LM :

Nucleus : single, segmented , formed of 2-5lobes , which are connected with thin chromatin threads, darkly stained, very variable in shape and number of segments (so called polymorphonuclear leukocytes).

Cytoplasm: stippled, stains light pink (neither acidophilic nor basophilic), due to:

1 specific granules : numerous, fine neutrophilic granules.

2- Azurophilic granules: few, larger granules that stain purple with azurestain.

EM:

•Nucleus: may appear as many segments, with condensed heterochromatin and no nucleolus.

• Cytoplasm:

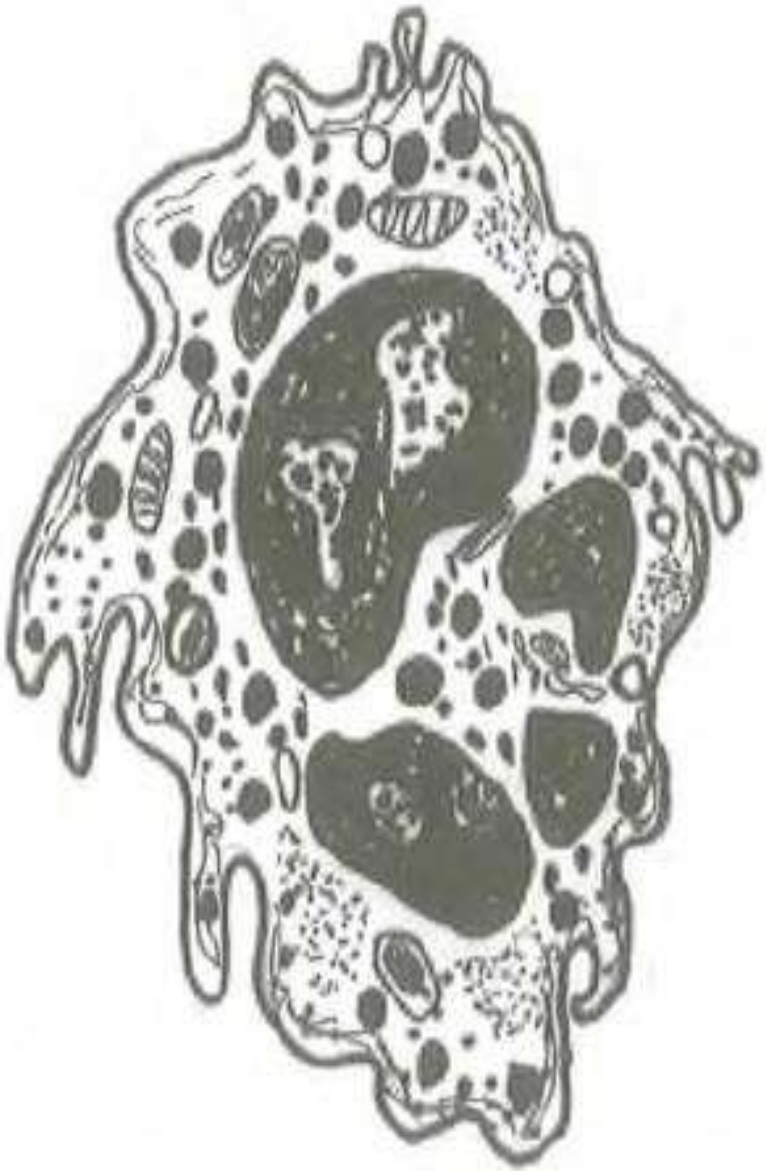
- Highly irregular cell boundaries (due to pseudopodia).

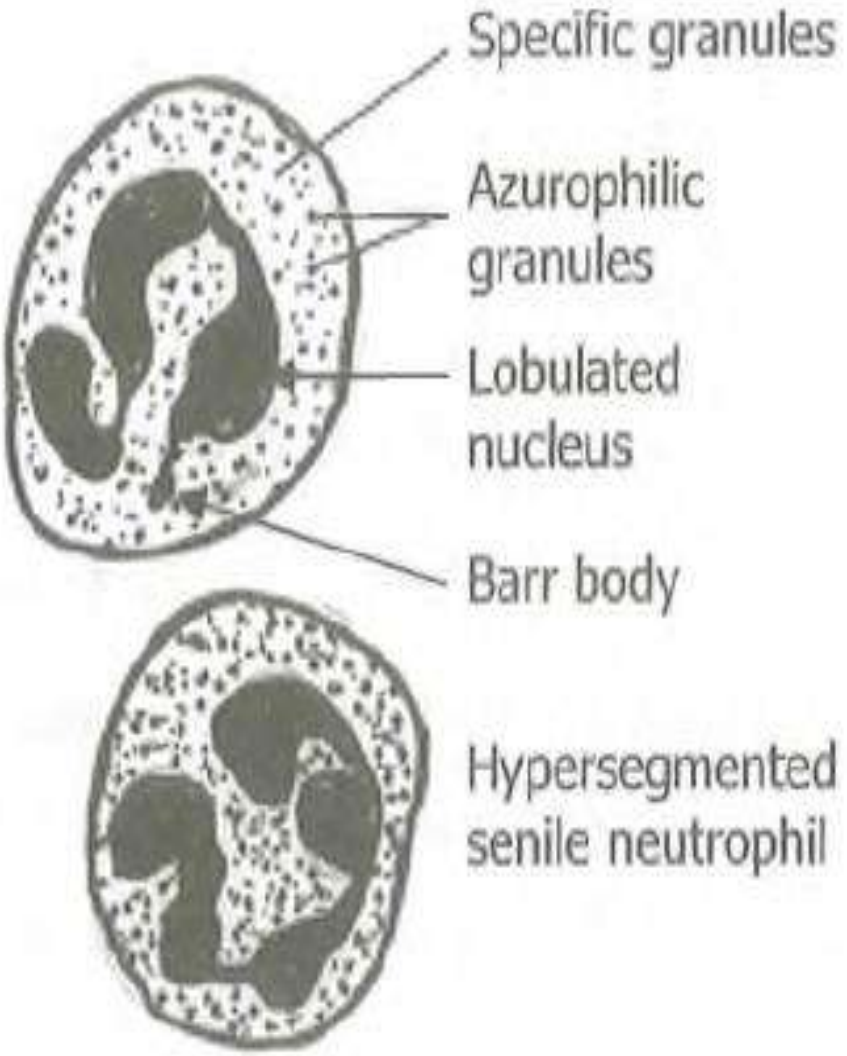
- Peripheral microfilaments & microtubules, to help amoeboid movement.

- Few organelles (few mitochondria, rER& small Golgi), abundant **glycogen** granules (which provides energy to the cells after they leave the blood stream).

EM

LM





LM and EM picture of neutrophil.

Contains two types of granules:

1- Primary (azurophilic) granules:

Large, oval or rounded, dark and less numerous (20%), they contain **hydrolytic enzymes** as **acid hydrolase** and **myeloperoxidase.**

They correspond to **primary lysosomes.**

2- Secondary (specific) granules:

Smaller, elongated, pale, but with variable densities.

They are more numerous (80 %).

They contain **phagocytin**(a bactericidal protein), **lactoferrin** (abacteriostatic iron-binding protein) &**collagenase.**

Functions

1- Phagocytosis of any foreign invader:

Bacterial toxins attract neutrophils from blood to C.T.

They squeeze between the capillary endothelium, to become active phagocytic cells in C.T. (microphages), which engulf bacteria forming phagosomes.

2- Secretion of proteolytic enzymes:

First, specific granules, then the azurophilic granules fuse with the phagosome, discharging their enzymes to destroy it by their bacteriostatic effect (which attract iron from bacteria to stop their growth) and their bactericidal effect (which kills bacteria).

Second, collagenase and hydrolytic enzymes cause lysis of the bacteria and surrounding tissues.

3- Production of pus & pyrogens:

The toxins liberated from the bacteria cause death of neutrophils forming **pus cells.**

Dead neutrophils (pus cells) and destroyed C.T. debris, micro-organisms and tissue fluid form a yellowish semi-liquid called **pus.**

Toxins & pus produce substances called pyrogens that elevate the body temperature (fever) and inhibit the growth of bacteria. These pyrogens stimulate the heat regulating centers in the brain.

4- Attraction of monocytes to the site of infection: to clean the area byphagocytosis of cellular debris & pus, as the monocytes have longer lifespan.

5-Secretion of trephone substances which help healing of wounds.

6- Stimulation of bone marrow: to produce more neutrophils during infection.

Neutrophilia:

It is the **increase** in the number of neutrophils above **75%.**

It occurs in cases of **acute inflammation:** e.g. abscesses, acute tonsillitis and acute appendicitis.

Neutropenia:

It is the **decrease** in number of neutrophils below 60%.

It occurs in cases of damage or depression of bone marrow, where the blood cells are formed, e.g. exposure to irradiation, toxins, some viral infections as influenza, prolonged use of some antibiotics and **typhoid fever**

2- Eosinophils

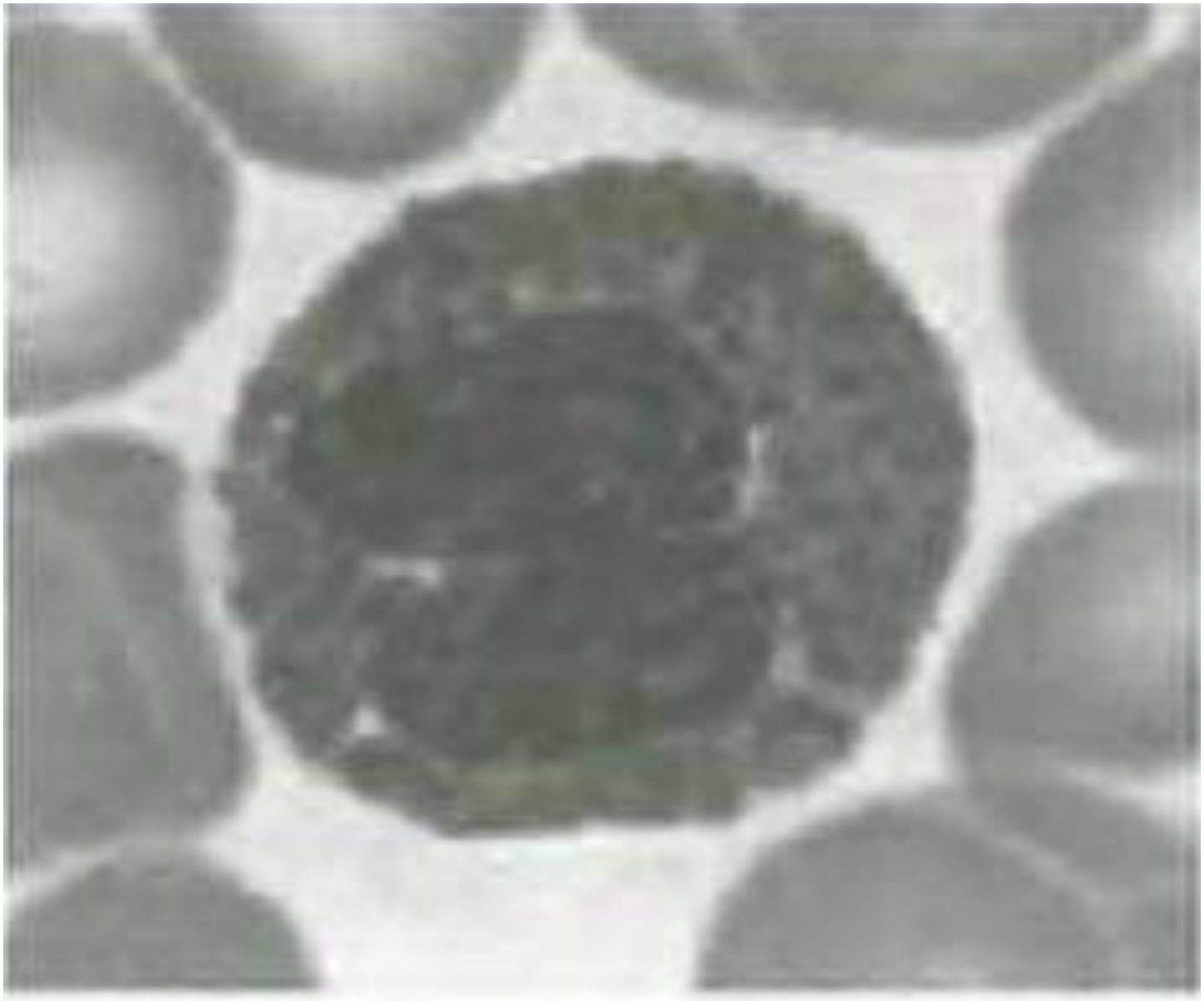
- They are the regulatory cells in **allergic inflammation.**
- They have functional correlation with basophils and mast cells.
- They are motile, but with a **limited phagocytic activity.**

Percentage: 2-4 %.

Life span: 8-10 days.

Diameter: 12 -14 μm.

Distribution: in C.T. under the mucous membranes of GIT & respiratory tract.



LM:

Nucleus: dark and bilobed (horse-shoe shaped),connected with a thick chromatin thread, with no obvious nucleolus.

Cytoplasm:deeply acidophilic as it is packed with **specific granules** which are prominent, coarse ,**acidophilic, shiny red** (refractile) granules.

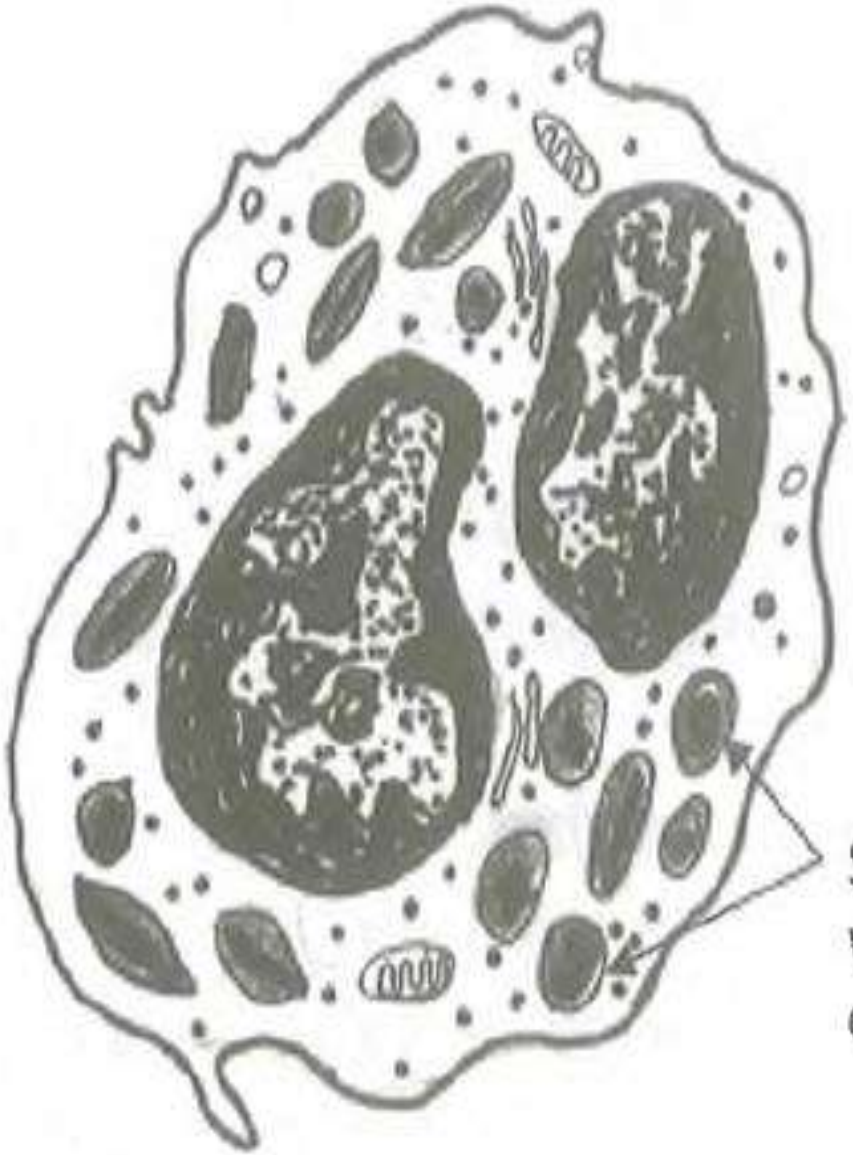
EM:

Nucleus: appears as 2 segments, which are heterochromatic, but less condensed than neutrophils with no obvious nucleolus.

Cytoplasm: contains few organelles and two types of granules:

EM

LM



Bilobed nucleus

Pink acidophilic shiny granules

Specific granules with a crystalloid electron-dense core

LM and EM picture of eosinophil.

1- Specific granules: They are large, oval (ellipsoid), membrane-bound with electron-dense crystalloid core **(internum),** formed of basic protein which his the cause of acidophilia in LM, embedded in granular matrix**(externum)** of hydrolytic_enzymes (histaminase and sulphatase).

2- Azurophilic granules: They are small, rounded with moderate density.

They contain non-specific lysosomal enzymes.

Functions:

Eosinophils are attracted (chemotactically) to the site of allergy by **eosinophil chemotactic factor,** secreted with histamine and heparin by mast cells & basophils (when stimulated with an antigen). They try to reduce and localize the allergic reaction by the following ways:

Destroy histamine & heparin by histaminase and sulphatase (i.e. terminates the allergic reaction).

Engulf antigen-antibody complexes.

Killing & inactivation of parasitic larvae: by discharging the basic protein in the crystalloid core of their granules on their surface. This protein is cytotoxic for the parasites.

Eosinophilia:

Increase in number more than 5%. It occurs in:

1- Allergic diseases: bronchial asthma, eczema, urticaria.

2- Parasitic diseases: Ascariasis, Bilharziasis.

Eosinopenia:

Decrease in numberless than **1%.** It occurs with cortisone treatment (inhibits their formation in bone marrow).

3- Basophils

Percentage: The least type (the rarest), **0-1%.** They are difficult to be seen in blood film.

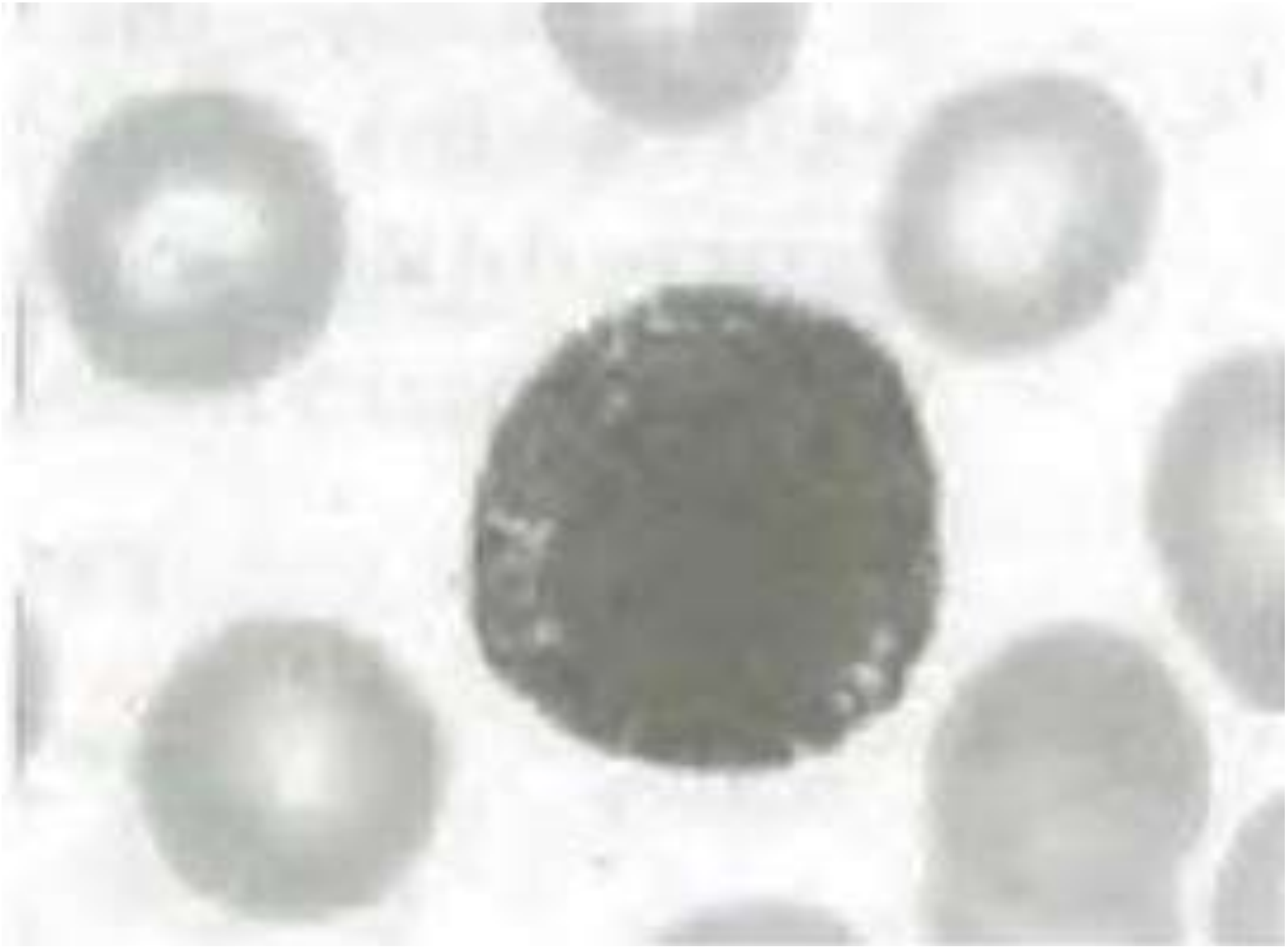
Life span: 10-15 days.

Diameter: nearly the same as neutrophils , **10-12 μm.**

LM:

Nucleus: is divided into two irregular segments(bilobed), twisted to form an **S-shape.**

Cytoplasm:is filled with basophilic specific granules, which are large (obscuring the nucleus),irregular in size and few in number.



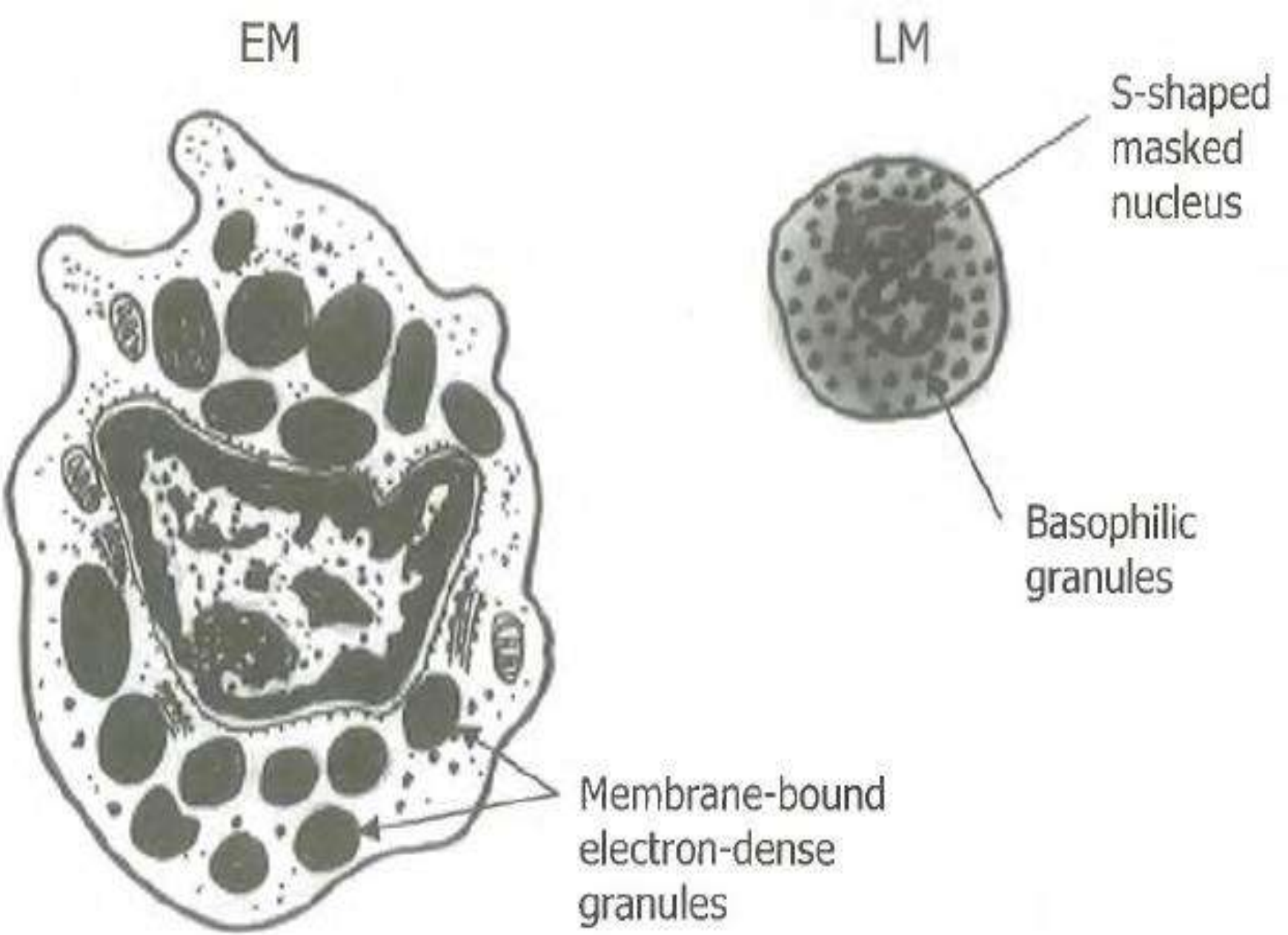
EM:

Nucleus: heterochromatic, but less condensed than neutrophils.

Cytoplasm: few rER, small Golgi, few mitochondria and some glycogen. It is full of granules, which are two types:

1- Specific granules: are large and membrane-bound. They contain histamine, heparin and eosinophil chemotactic factor.

2-Azurophilic granules: small, represent lysosomes and contain hydrolytic enzymes.



LM and EM picture of basophil.

Functions:

Basophils play little role in some allergic reactions (due to their few number).They supplement the function of mast cells, i.e. activated in immediate hypersensitivity reactions (anaphylaxis), through their surface IgE receptors, similar to those of mast cells. On migrating to the C.T., their functions are accomplished by:

- Secretion of histamine (causes vasodilatation increasing capillary permeability) and heparin (anticoagulant).
- Release of eosinophil chemotactic factor (attracts eosinophils to site of allergy).
- Slightly motile with very limited phagocytic activity.

Basophilia:

(increase in their percentage) occurs in some viral diseases as chicken pox and liver cirrhosis. Also in cases of allergic and parasitic diseases.

II/ Non-Granular Leukocytes

- They are two types of leukocytes, namely lymphocytes & monocytes, which contain no obvious stainable specific granules, but still contain small azruophilic granules which contain hydrolytic enzymes.
- Their nuclei are not lobulated.

1- Lymphocytes

- They are the functioning units of the specific immune response (the 2nd line of the body defense against foreign invaders).
- They are immune competent cells as they have **surface receptors,** which enable them to distinguish the self from the non-self so can identify different antigens.

Percentage: 20-30% (2nd common type).

Life span: Varies from days to few years, according to type & function.

Diameter: Two size ranges can be found in peripheral blood:

Small lymphocytes:

- They represent most of the lymphocytes in circulating blood, i.e. 15-20%.

- Their diameter is small (6-8 **μm),** nearly the same size of RBCs.

Large lymphocytes:

- Few lymphocytes 5-10%. They appear **medium sized or large (12-15 µm).**

- They may represent different stages of activated lymphocytes.

LM:

- **Nucleus:** large and rounded (not lobulated).

- In small lymphocytes it is deeply stained, condensed with no obvious nucleolus. It is very large, occupying most of the cell volume, leaving only a thin rim of cytoplasm difficult to be seen under LM.

- In large lymphocytes, it is slightly paler in staining, less condensed, with aprominent nucleolus.

Cytoplasm:

In small lymphocytes, it is very scanty (thin rim around the nucleus), not apparent in LM.

In large lymphocytes, cytoplasm is more abundant and basophilic, with no specific stainable granules, but few azurophilic granules.

EM:

- **Nucleus:**

- In small lymphocytes, it has highly condensed heterochromatin.

- In large lymphocytes, nuclei are large, indented and have fewer condensed chromatin with prominent nucleolus.

- **Cytoplasm:**

- The cell membrane shows few short microvilli.

- In small lymphocytes, cytoplasm shows few organelles, many ribosomes and few scattered mitochondria.

- In large lymphocytes, cytoplasm is more abundant, with more ribosomes & polysomes, more developed organelles as mitochondria, rER, Golgi and few azurophilic granules.

Distribution & mobility:

- Lymphocytes are actively motile, continuously moving between lymphatic tissue, where they settle, and blood. They take the *"tennis-racquet"* appearance while moving, as they have a head (containing the nucleus) & a tail (containing the rest of cytoplasm).

- Lymphocytes are the only leukocyte which when leave the blood stream, can return back to the circulation.

- They are found in blood, lymph & lymphatic tissue (lymph node, spleen ,thymus, tonsils).
- They are also found under the wet epithelial membranes of the gut ,respiratory & genital tracts. They are also seen in saliva, where they can attack microbes.

Functions of lymphocytes:

They perform two types of immunity:

1- Cell mediated immunity.

2- Humoral immunity.

N.B:

1 - **Immunity** means being safe from re-infection.

2- Lymphocytes are not phagocytic cells.

Differences between small and large lymphocytes.

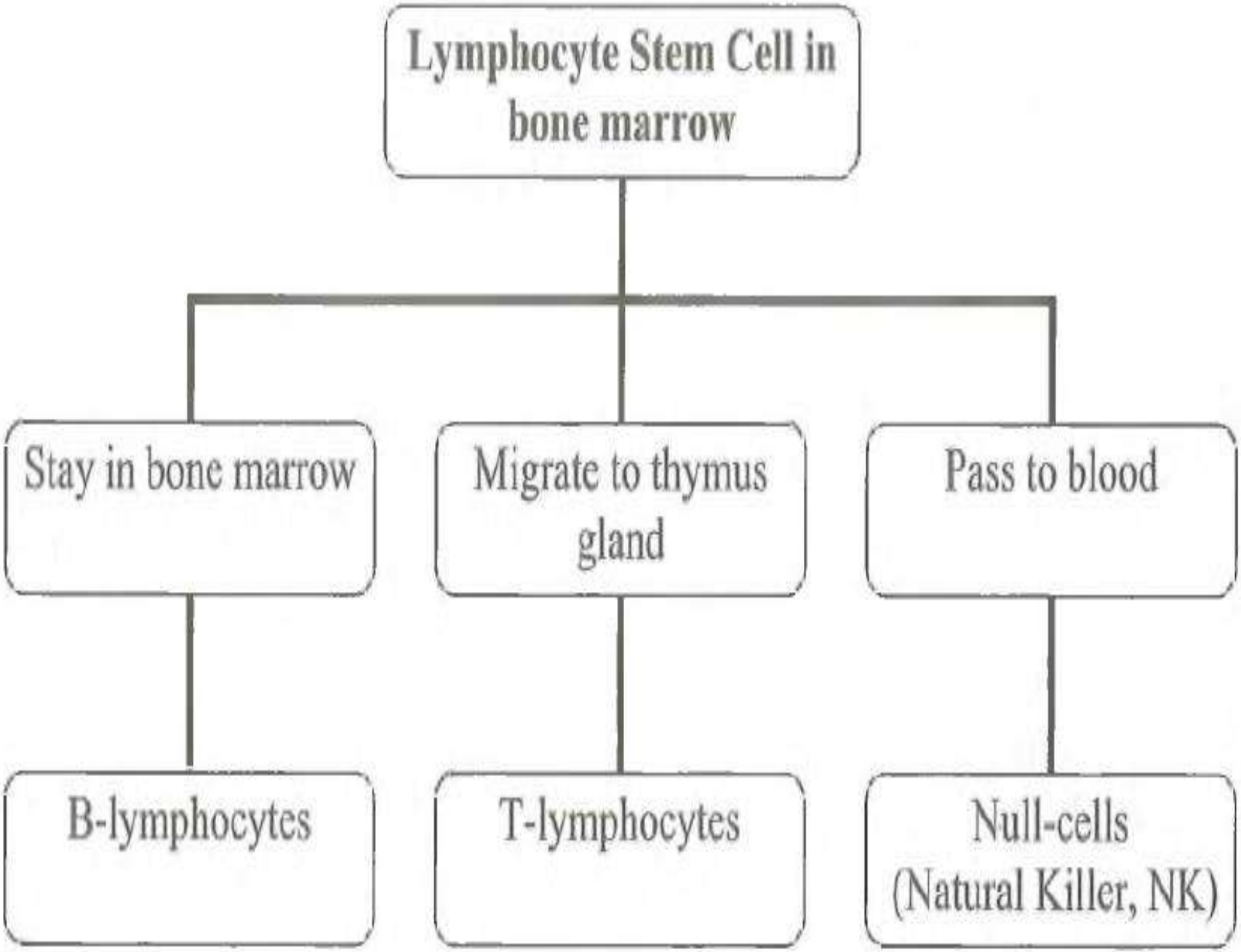
	Small lymphocytes	Large lymphocytes
Diameter	6-8 µm	10-15 µm
Nucleus	Small rounded deeply stained with no obvious nucleolus.	Large, indented, lightly stained with a prominent nucleolus.
Cytoplasm	Just a slightly basophilic non-granular rim.	More abundant cytoplasm, more basophilic.
EM	• Condensed chromatin. • Few mitochondria, ribosomes, small Golgi & centrioles. • Covered with microvilli.	• Less condensed chromatin, prominent nucleolus. • More abundant cytoplasm, more mitochondria, ribosomes, golgi & rER.
Mobility	Highly mobile cells that pass through capillary wall (tennis racquet).	
Distribution	• In blood, lymph & lymphatic tissues (lymph nodes, spleen, thymus, tonsils & gut associated lymphocytic aggregations. • Present in and under wet epithelial membranes & in saliva.	

Classification of small lymphocytes:

Small lymphocytes are classified according to their maturation sites &

functions into **T and B** lymphocytes and **null** cells:

- They arise from the same stem cell in the bone marrow.
- They differ in maturation & differentiation sites.
- There are no histological or morphological differences between them, so they can't be differentiated by light or electron microscope. They can only be differentiated by immunohistochemical staining as they differ only in their surface receptors (markers).


Lymphocyte Stem Cell in bone marrow
Stay in bone marrow
Migrate to thymus gland
Pass to blood
B-lymphocytes
T-lymphocytes
Null-cells (Natural Killer, NK)

1/ T-Lymphocytes:

- They represent about 75% of circulating lymphocytes.
- Their life span is long (1-2 years).
- The immature cells that will be T-lymphocytes, migrate from the bone marrow to the **thymus** where they mature and become programmed to recognize a particular antigen (thymic education) by addition of certain **polypeptide** molecules on their surfaces **(receptors).**

- The T-lymphocytes then leave the thymus back to the circulation, taking the family name **(T),** to settle in lymphoid organs as lymph nodes and spleen.
- They are continuously re-circulating from these sites seeking for antigens.

Functions of T-lymphocytes:

- They provide cell mediated immunity **(cellular immunity).**
- T-cells attack foreign cells such as fungi, viruses, parasites and tumor cells **directly,** i.e. cell to cell contact.
- When T-cells come in contact with the antigen to which they respond, they are activated into large lymphocytes, which undergo a series of divisions that gives a clone of identically programmed T-cells.

- As regards the way of response to antigens, they form **subtypes.** Some subtypes regulate antigen destruction indirectly by helping other cells in immune reactions **(Regulators),** while others kill target cells directly **(Cytotoxic).**

Subtypes of T-lvmphocvtes (Regulators and Cytotoxic):

A) Regulators:

1- T-Helper cells:

- They are activated by interleukin-I secreted by macrophages.
- They secrete interleukin-II, which **helps activation of B-lymphocytes.**

2- T-Memory cells:

- They develop when an antigen attacks the body for the first time, but they do not react to it. They keep the memory of this antigen and remain in lymphoid tissue for rapid response on second exposure**(secondary immune response).**

3- T-Suppressor cells:

- They switch off the immune response when the stimulus is removed.
- They suppress the immune response to self-antigens (i.e. prevent autoimmune reactions)
- They suppress T-helper cells, and subsequently control the B lymphocytes response.

B) Cytotoxic:

1- T-Cytotoxic: They kill foreign or abnormal cells by **direct contact** and lysis, as virus infected cells, cancer cells and fungi.

2- T-Killer cells:

When expose to antigen, they kill the antigenic cells by releasing hormone-like factors called **lymphokines** such as interferon(which inhibits viral replication), interleukin II (which stimulates T-lymphocytes),macrophage activating factors (which activate the phagocytic power of macrophages) and colony stimulating factors (which stimulate granulopoiesis). These cells contribute to graft rejection.

2/ B-Lymphocytes:

- They represent about 25% of circulating lymphocytes.
- Their life span is short (few weeks up to 3 months).
- They were named B lymphocytes as they were discovered to develop in amass of lymphoid tissue called **Bursa of Fabricius** present in **birds.** But in man, their maturation was found to occur in the bone marrow.

- In the bone marrow they acquire surface receptors in the form of immunoglobulin M (IgM), which are programmed for a particular antigen.
- They settle in lymphoid tissue, and re-circulate between blood and lymphoid tissue.

Functions of B-Lymphocytes:

1- Humoral Immunity:

- They are activated when exposed to their specific antigen, with the help of interleukin II secreted by T-helper cells.

- The activated B-lymphocyte differentiates, enlarges, changes to plasmablast, then plasma cell which secretes the specific **antibody** for the original antigen **(primary immune response).**

- These antibodies circulate in the blood allover the body to meet the antigen.

2- B-Memory cells: Some activated B-cells remain in lymphatic tissue, to respond rapidly to the second exposure for the same antigen, by transformation into plasma cells, which secrete antibody **(secondary immune response).**

Null cells:

- They are Non-T, Non-B cells that arise from the bone marrow as undifferentiated lymphocytes, having neither T nor B cell specific receptors.
- They can develop in the absence of the thymus.
- **Percentage:** They constitute about **5%** of all circulating lymphocytes.
- **Size:** They are larger than small lymphocytes **(12-15** μm).

- **Functions:**

1- They act as cytotoxic **Natural killer (NK),** as they can destroy foreign

cells directly (as viral infected cells, cancer cells, parasites and fungi).

2- They may act as a reserve circulating undifferentiated stem cells for lymphocytes.

Lymphcytosis:

it is the increase in the number of lymphocytes above 40%which occurs in:

1- Physiological in children.

2-Chronic infections: as Whooping cough, T.B. (Tuberculosis), and Syphilis.

3- Leukemia.

Differences between T- and B-lymphocytes.

	T-lymphocyte	B-lymphocyte
% of lymphocytes	about 75%	25 %
Site of maturation	Thymus gland	Bone marrow in mammals, bursa of Fabricius in birds
Functions	Cellular immunity	Humoral immunity
Subtypes	1- T-helper cells 2- T-suppressor cells 3- T- killer cells 4- T-cytotoxic cells 5- T-memory cells	1- Active B lymphocytes → transform to plasma cells 2- B-memory lymphocytes

2- Monocytes (Macrophages in C.T.)

- The second type of non-granular leukocytes which lack specific granules.
- They are **highly phagocytic** cells. They have an important role in both the non-specific & specific immune responses.
- They leave the blood stream and migrate by amoeboid motion to C.T., where they are called **macrophages.**

Percentage: 3-8%.

Life span: three days in blood stream and up to three months in C.T.

Diameter: 14-18 µm (the largest leukocyte). It may reach 30 µm in C.T.

LM:

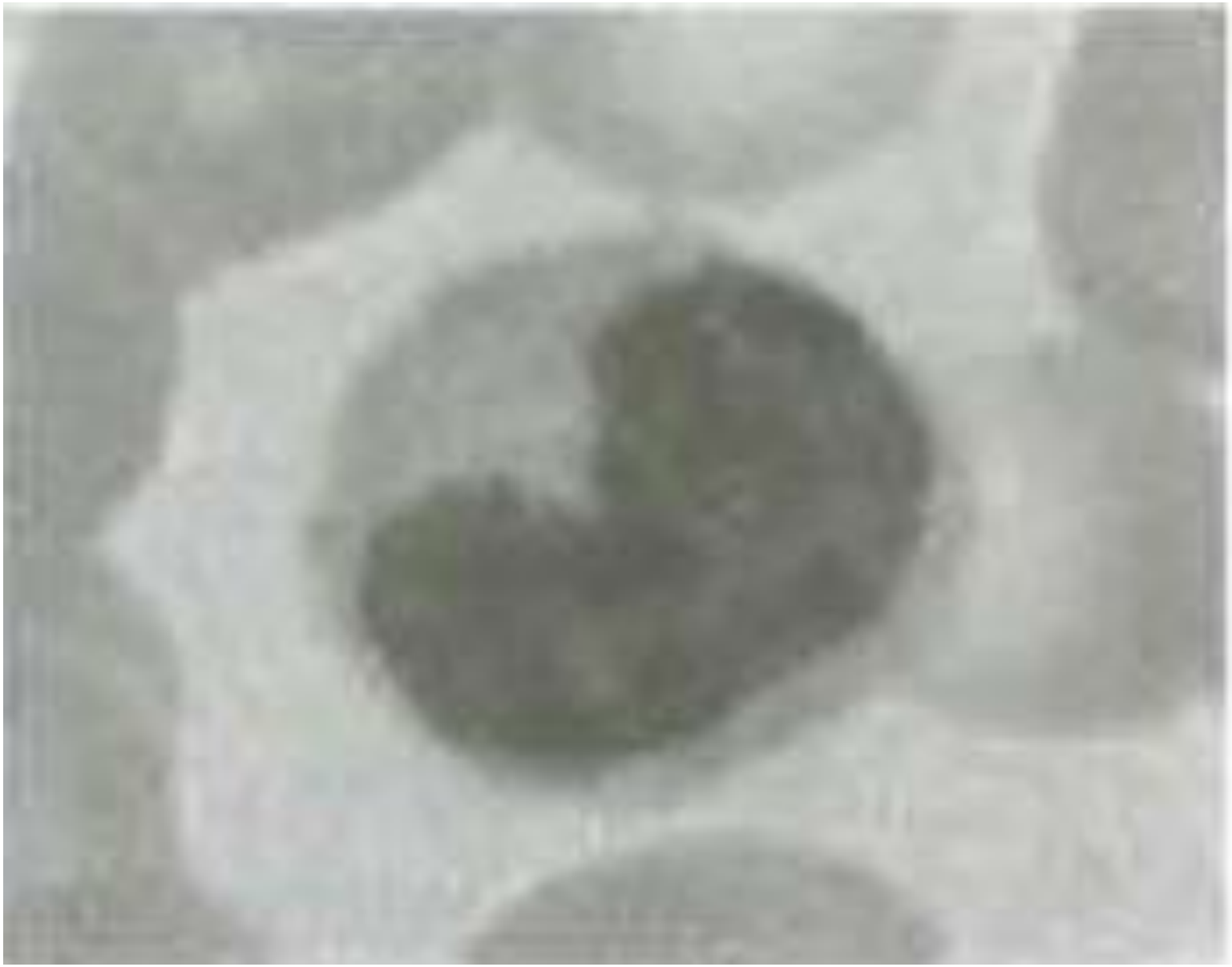
- **Nucleus:**

- Large characteristic indented **kidney-shaped,** and eccentrically placed.

- Pale, less intensely stained than other leukocytes,with one or two obvious nucleoli.

- **Cytoplasm:**

Abundant, pale basophilic, grayish-blue in staining, which gives the unclear **frosted-glass appearance,** due to fine purple azurophilic granules (lysosomes), but no specific granules.



EM:

- **Nucleus:**

- Less condensed peripheral chromatin, and extended central chromatin.

- One or two nucleoli.

- **Cytoplasm:**

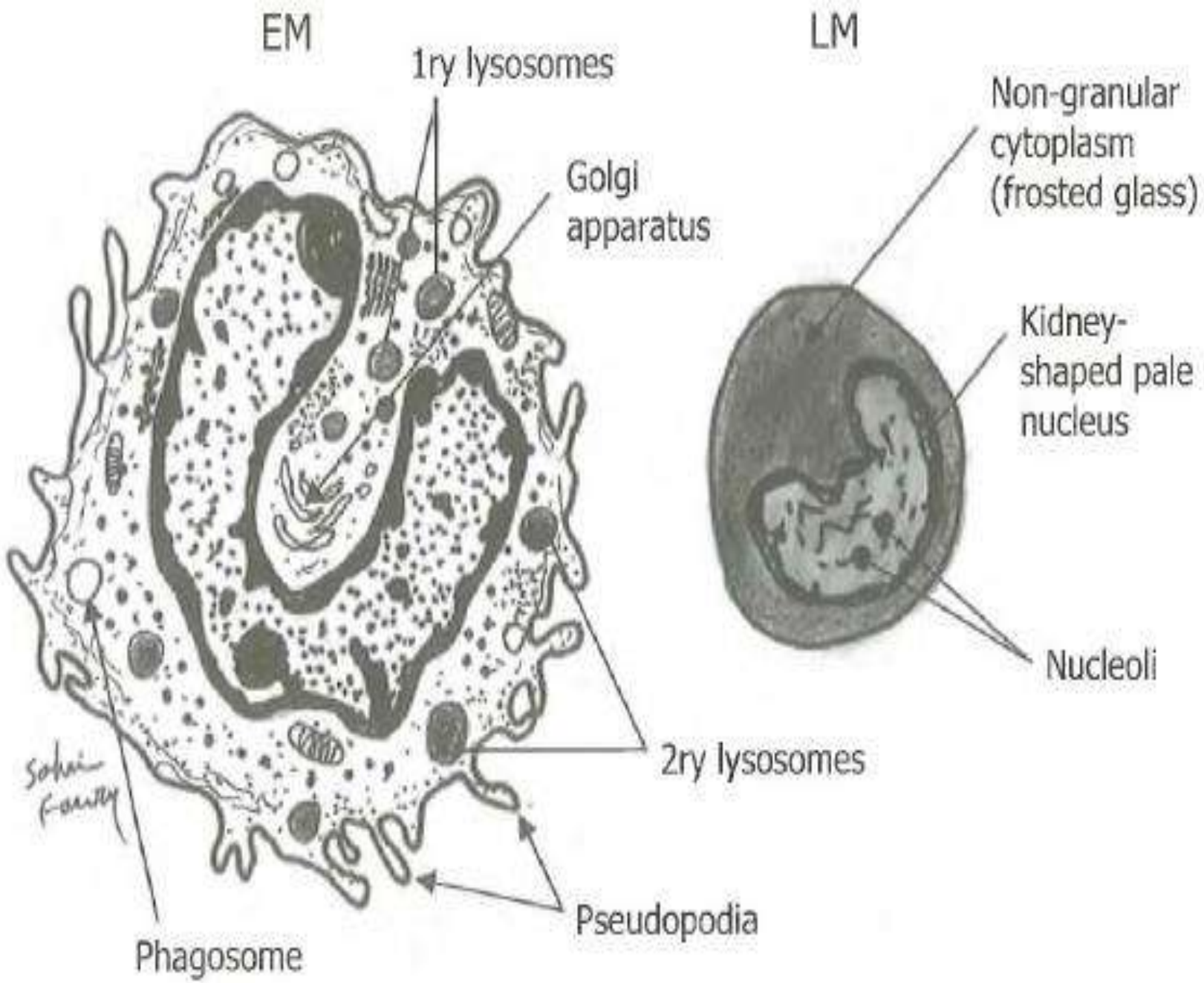
- The cell surface is irregular due to pseudopodia.

- Many vesicles are also seen on the surface (reflecting the phagocytic activity).

- Small amount of rER and mitochondria.

- Prominent large **Golgi** apparatus (the origin of lysosomes).

- Large number of **lysosomes** appearing as dense granules, which correspond to azurophilic granules in LM.



LM & EM picture of monocyte.

Sites & Distribution:

Monocytes are found in the peripheral blood for 3 days, then leave to C.T. They are the phagocytic cells of the blood.

- In C.T. they differentiate into macrophage system (reticulo-endothelial system).

• They are given different names depending on the tissue they reside in, e.g.

- in C.T..... **histiocytes,**

- in lung.....**dust cells,**

- in liver.....**von-kupffer cells,**

- in brain.....**microglia.**

N.B: In these tissues, macrophages remain stationary, like watchful soldiers ever ready to attack and phagocytose invaders.

Functions of monocytes:

- Monocytes are the precursors of macrophages which phagocytose microorganisms, dead cells, and dead neutrophils.
- The phagocytic cell of the blood: for bacteria, viruses & antigen-antibody complexes.
- They can fuse to form giant cells to phagocytose large foreign bodies.
- Antigen presenting cell to lymphocytes, and secretion of interleukins.

Monocytosis:

Is the increased percentage above 8% which occurs in:

1- Chronic infections: as glandular fever, typhus, syphilis & T.B.

2- Protozoal diseases as Malaria.

3- Lymphomas and monocytic leukemia.

Blood Platelets

Small non-nucleated, disc-like, cytoplasmic cell fragments.

- In lower vertebrates, they are called **thrombocytes** as they are nucleated.

Origin:

- Platelets arise in the bone marrow by fragmentation & detachment (budding)from large cell in bone marrow (mega karyocytes).

- Mega karyocytes, in the bone marrow, protrude thin processes that cross the wall of the sinusoids and fragment at their tips liberating the platelet.

Number: 200,000-400,000/mm . They are found in aggregates & clumps, which accounts for the wide range of their number.

Life span: 5-10 days, after which they are phagocytosed by macrophages in the spleen. The spleen also acts as a store for about 1/3 of the platelets.

Size: 2-4 μm in diameter.

LM:

They appear as non-nucleated, rounded or oval fragments that can be differentiated into two concentric zones:

- Outer pale basophilic clear transparent zone called **hyalomere**(hyalo = glass, mere = piece).

- Central dark granular zone called granulomere.

EM:

- Platelets are covered with a thick glycoprotein cell coat, which cause the

aggregation of platelets into clumps.

The surface is irregular due to protrusion of pseudopodia.

Functions:

They play a vital role in controlling hemorrhage due to blood vessel injuries via the following steps:

1- Platelet aggregation: to form a platelet plug as a first step to stop bleeding.

2- Secretion of serotonin which is a vasoconstrictor.

3- Blood coagulation: where platelets, together with factors from the blood plasma, deposit fibrin that forms a network trapping RBCs, leukocytes and platelets to form a **blood clot,** or **thrombus.**

4- Clot retraction: the clot retracts due to contraction of platelet microfilaments and ATP.

5- Clot removal: when blood vessel is restored by new tissue formation, the clot is removed by interaction between the proteolytic enzyme plasmin and the platelet lambda granules.

Platelet Disorders:

Thrombocytopenia (purpura):

It results from severe reduction in the number of platelets. It is characterized by prolonged bleeding time, and excessive bleeding after minor traumas due to failure of platelets to seal injuries in blood vessels.


Thank You

General Histology

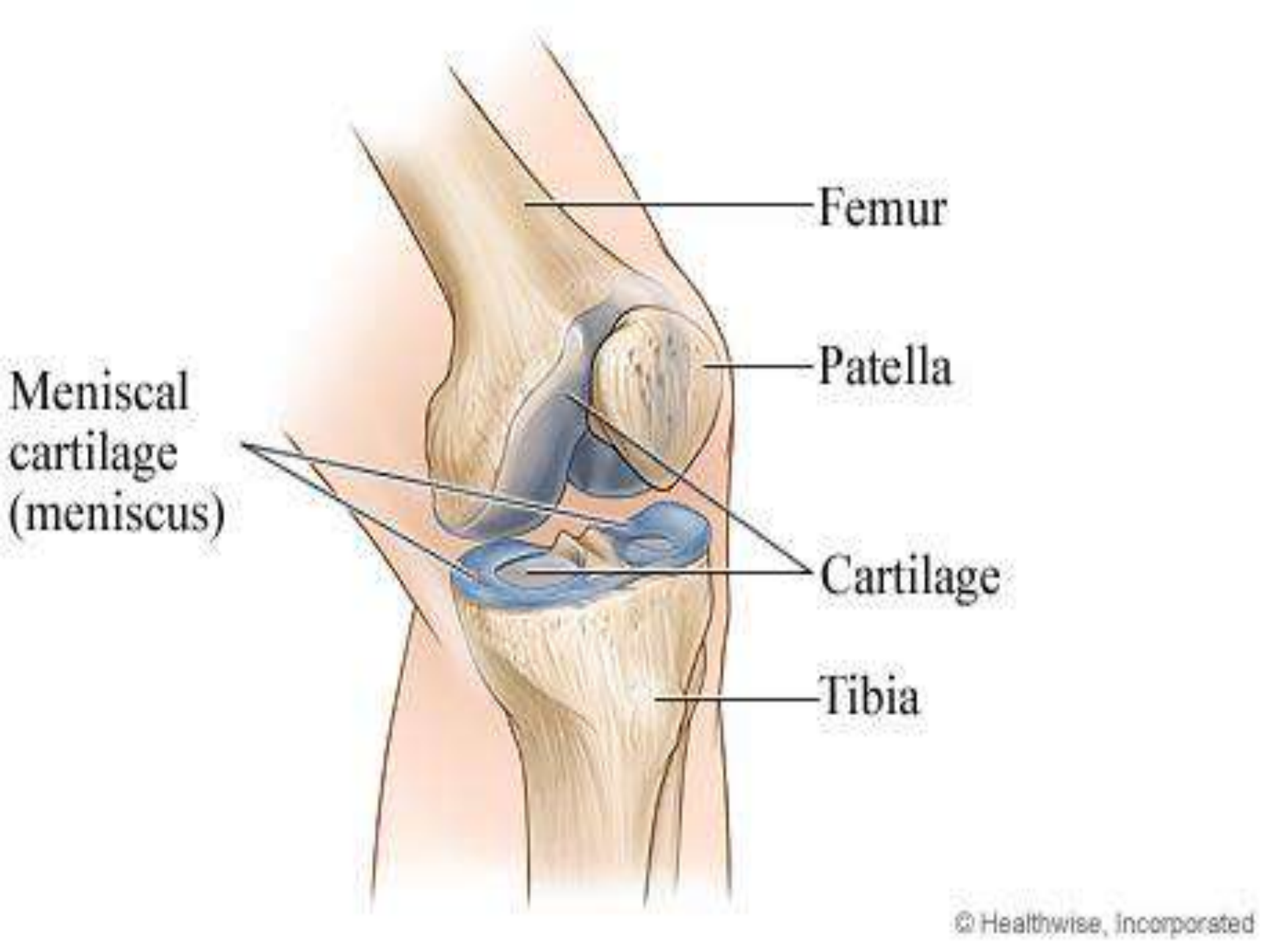
Dr/hani ahmed alanqar
consultant of pathology

Cartilage

Cartilage

Definition

- Cartilage are modified type of connective tissue in which the extracellular matrix is hardened to provide rigidity , support and attachment for soft tissue.



Characters of cartilage:

- It resembles C.T. proper in that the cells are widely separated by a considerable amount of intercellular material (matrix).
- Being a type of C.T., it arises from primitive mesenchymal cells (UMCs).

- The matrix is rigid (firm) but flexible. It is formed of ground substance in which the cells & fibers are embedded.
- It is avascular (non-vascular), nourished by **diffusion** of **O_2** & nutrients from the surrounding C.T. or synovial fluid in the joint cavities.
- It has no lymph vessels or nerves.

- Cells are isolated in small cavities in the ground substance called lacunae

Functions of cartilage:

- Support soft tissues with some flexibility, tissue attachment & weight bearing.
- Keeps airway patent.
- Shock-absorbing, as it is resilient.
- Smooth sliding surface for joints (for easy movement of bones).
- Development and growth of bones, before and after birth.

Composition of cartilage:

like other C.T. it is formed of cells, fibers and ground substance:

- The **Cells** are called chondroblasts and chondrocytes.

- The **Fibers** are collagen and elastic fibers, which are embedded, in varying proportions, in the ground substance to form the matrix.
- The **Matrix,** which is abundant, firm & compact.

Types of cartilage:

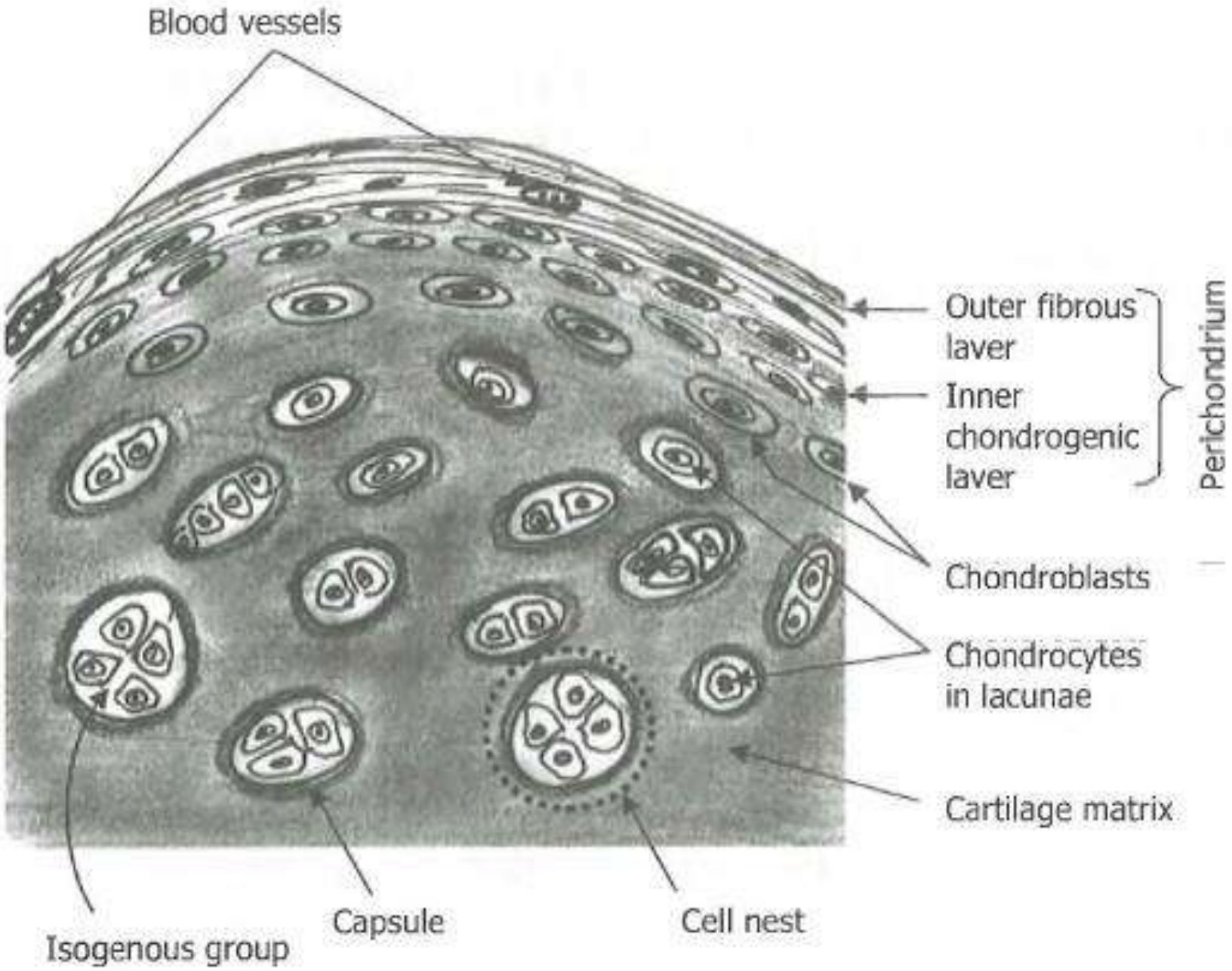
- There are **three types of cartilage** based on the amount of ground substance, relative abundance and type of fibers embedded in it, which makes the different types of cartilage vary in appearance and mechanical properties.
- These types are **hyaline,** yellow **elastic** and white **flbrocartilage.**

Hyaline cartilage

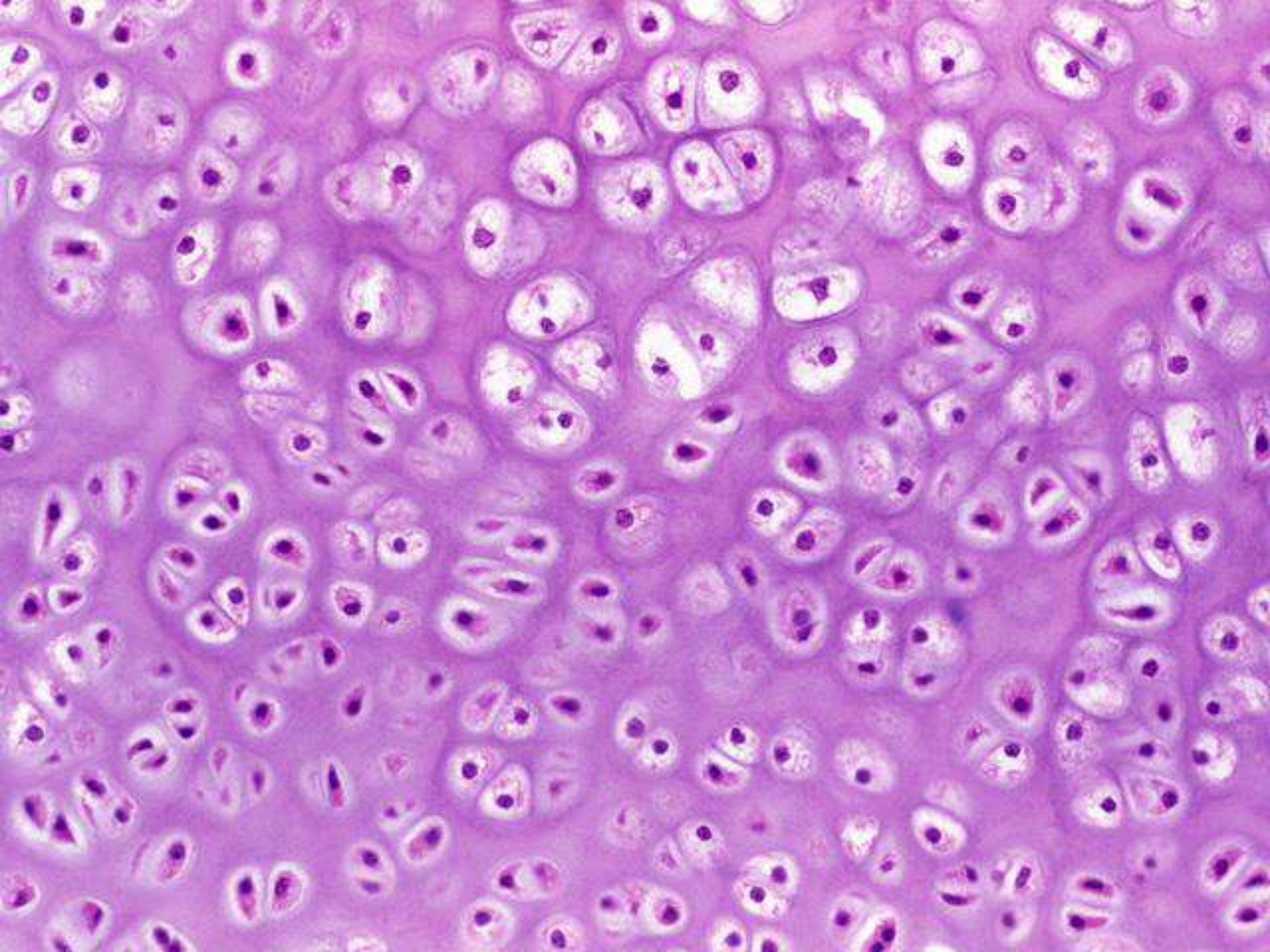
- has the typical structure of cartilage. The other types are considered as variants of its basic structure.
- The most common type.
- It appears translucent, with glassy appearance, (Hyalo = glass).

- **Sites:**

1. Fetal skeleton
2. Epiphyseal plate
3. Costal cartilage
4. Articular surface of bones
5. Respiratory passages (nose, larynx, trachea & bronchi).



Hyaline cartilage.



Structure

- **A) Perichondrium:** it is a capsule-like structure formed of dense fibrous C.T.
- that surrounds the hyaline cartilage **except** at the articular surface of joints.
- It is formed of two layers:

- **1- Outer fibrous layer:** white fibrous C.T., formed of fibroblasts, which secrete the collagen fibers (Type I), it is rich in blood vessels & nerves.

- **2- Inner chondrogenic (cellular):** rich in chondrogenic cells that differentiate into **chondroblasts.** They are flat or oval cells with dark basophilic cytoplasm, which secrete the matrix of cartilage & collagen(type II).

Functions of perichondrium:

- Nutrition of non-vascular cartilage (by diffusion).
- Formation of new cartilage cells during growth.
- Provides attachment for muscles.

- **B) Cartilage Cells:**
- **1- Chondroblasts :**
- **Origin:** arise from mesenchyme (UMCs), which withdraw their processes, proliferate and become crowded. They are transformed into chondrogenic cells that enlarge and differentiate into chondroblasts which start to secrete the matrix.

Site: always on the surface of cartilage, at the inner aspect of perichondrium.

LM:

- Flat to oval or spindle in shape, with deep basophilic cytoplasm
- Flat oval pale stained nucleus, with prominent nucleolus.
- It can divide.

EM:

features of protein forming cells (euchromatic nucleus, abundant ribosomes, rER, large Golgi apparatus & many mitochondria).

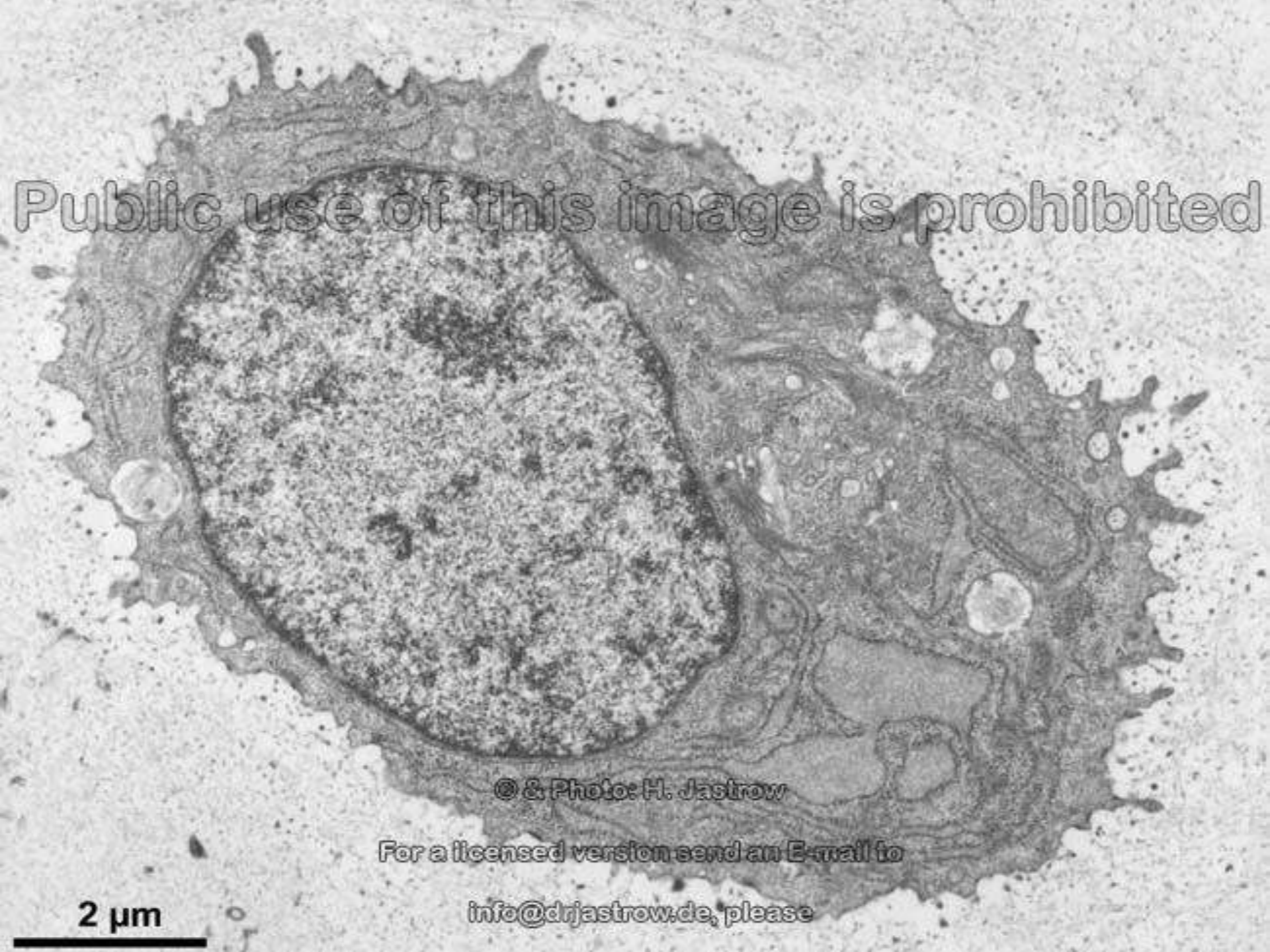
Functions:

1.Formation of cartilage matrix.

2.Formation of cartilage collagen (type II).

3. Cartilage growth from outside (appositional growth).

4. They secrete matrix and when surrounded with it they become called chondrocytes.


Public use of this image is prohibited
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info@drjastrow.de, please
2 µm

2- Chondrocytes: They are the mature cartilage cells.

- **Origin:** they develop from chondroblasts. When chondroblasts mature, they secrete enough matrix to be completely surrounded by it and imprisoned in lacunae, they are now called chondrocytes.

- **LM.:**
- Rounded with pale basophilic cytoplasm and central rounded dark nucleus.
- Rounded darkly stained central nucleus.
- Embedded in the matrix inside spaces called lacunae, either single forming primary lacunae or **divide** once or twice giving clusters of cells formed of 2 or 4, up to 8 cells in secondary lacunae surrounded by **capsule** of condensed matrix.

- The superficial cells are small, flat, oval and single in their lacunae & parallel to the surface.(young chondrocyte)
- The older cells (mature chondrocytes) become deeper in the matrix. They are rounded or triangular, with pale cytoplasm (rich in fat & glycogen which dissolve during preparation). They are found in groups (2, 4 & 8) surrounded with darkly stained **capsule** of matrix, which is due to more condensation of matrix ground substance (GAGs), forming a more basophilic line. This group of cells is called **cell nest.**

- **NB:**
- During life, chondrocytes completely fill the lacunae, but during tissue preparation, the cells frequently shrink, so the lacunae appear as empty spaces.
- **EM.:**
- Features of protein forming cells, in addition mature chondrocytes show large lipid droplets and glycogen granules

- **Functions:**
- Maintain the cartilage matrix, by continuous turnover, like the chondroblasts. They are responsible for the formation and secretion of matrix components.


chondrocyte
lacuna
10 µm

- **C) Fibers:**
- **Collagen** fibers **(type** II), which are very fine type of collagen, embedded in the matrix. However they can't be seen by LM and the ground substance appears homogenous, and transparent. This is because:
- - They are very thin, less than the resolution power of LM.

- - They have the same refractive index as the surrounding matrix.
- - **N.B**.: They can be seen after digestion of the matrix by enzymes.

- **D) Ground substance (Matrix):**
- It is produced by chondroblasts and chondrocytes.
- It is rubbery, homogenous, transparent and **markedly basophilic.** It also stains metachromatically with some basic stains.
- The matrix consists of: proteoglycans, glycoproteins and water(60%).

Yellow Elastic Cartilage

It is yellow in fresh state, more opaque & flexible.

Structure:

- Always covered by perichondrium.

- Has the same structure of hyaline cartilage, but with large number of branching **elastic fibers** embedded in the matrix forming a network that gives this type the yellow color, with few collagen fibers (type II).

- Cells are chondrocytes in lacunae that form small cell nests (mostly 2 cell isogenous groups).

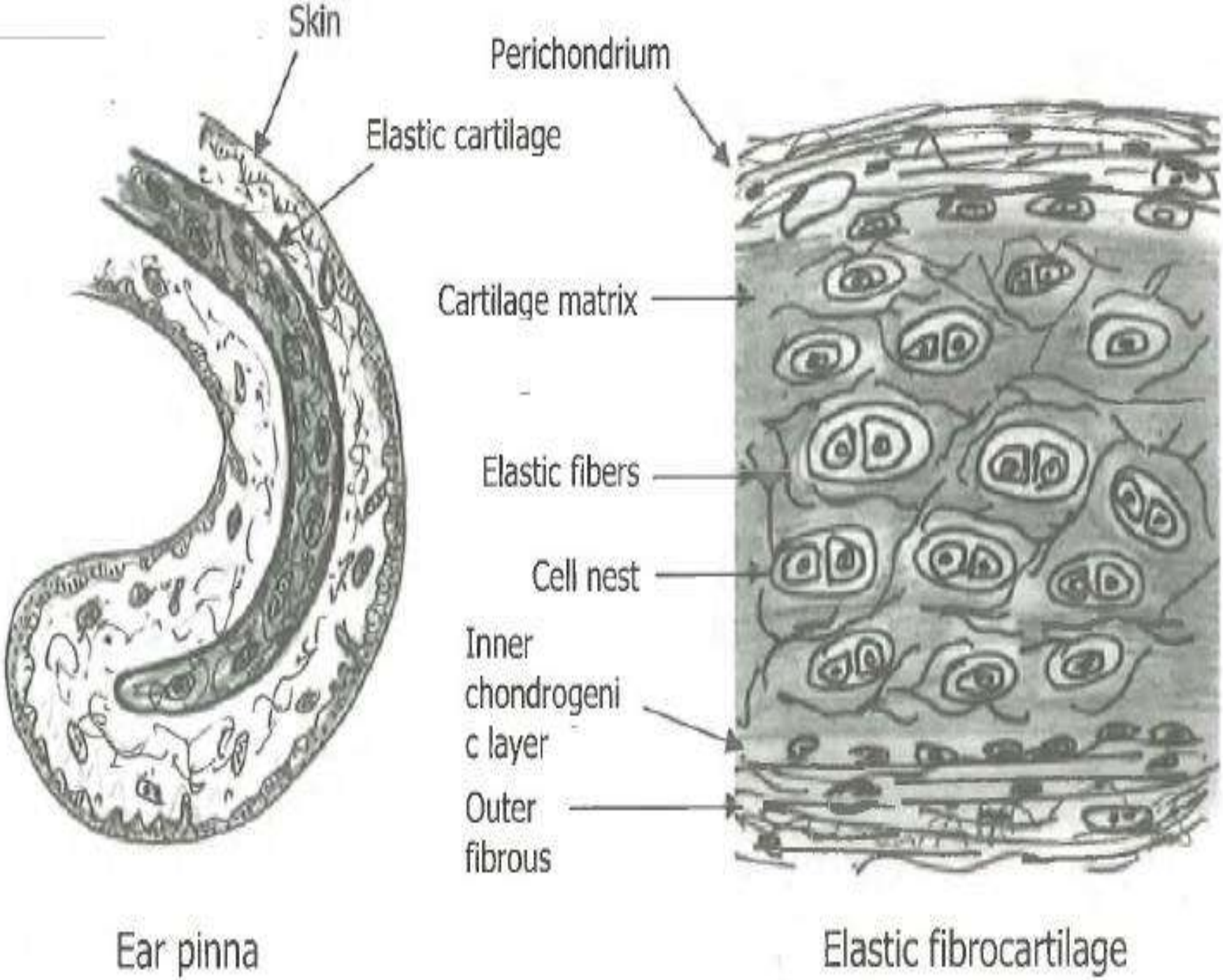
Sites:

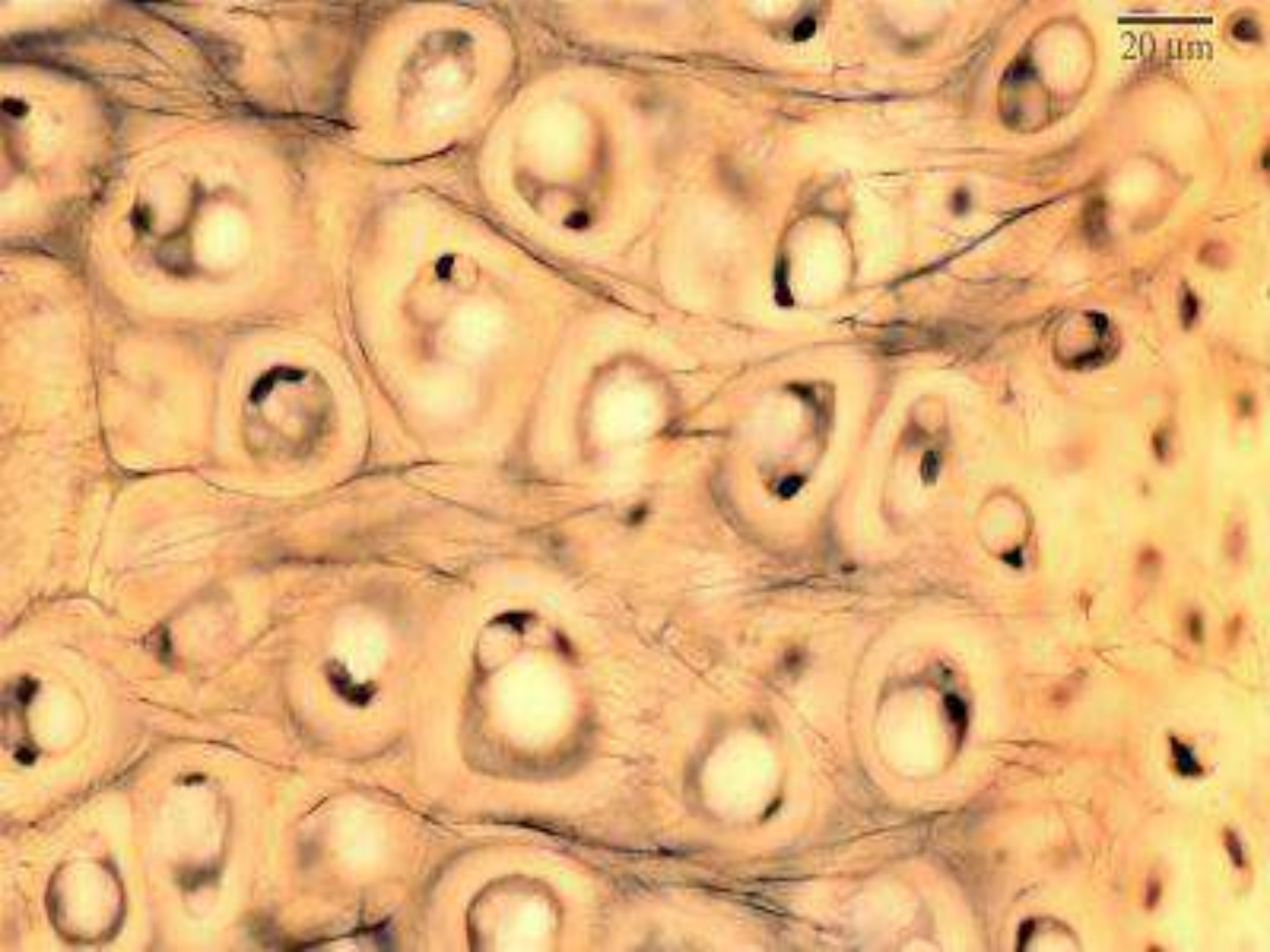
it is found where support with flexibility is required:

- Ear pinna.
- Eustachian tube.
- Epiglottis and some laryngeal cartilages
- External auditoiy canal.

- **Functions:**

- it is very flexible, recovers its shape after being deformed.


Skin
Elastic cartilage
Perichondrium
Cartilage matrix
Elastic fibers
Cell nest
Inner chondrogenic layer
Outer fibrous
Ear pinna
Elastic fibrocartilage


20 µm

White Fibro Cartilage

- It is never surrounded with perichondrium.
- It is a tough type of cartilage which is important in bone to bone attachment.
- It has intermediate character between hyaline cartilage and Elastic Cartilage .

- **Dense regular white fibrous C.T.**
- **Structure:**
- • It is formed of dense collagen fibers (type I), present in parallel thick bundles. •Cartilage cells inside lacunae are present in rows between collagen bundles, embedded in very scanty matrix around them

Sites:

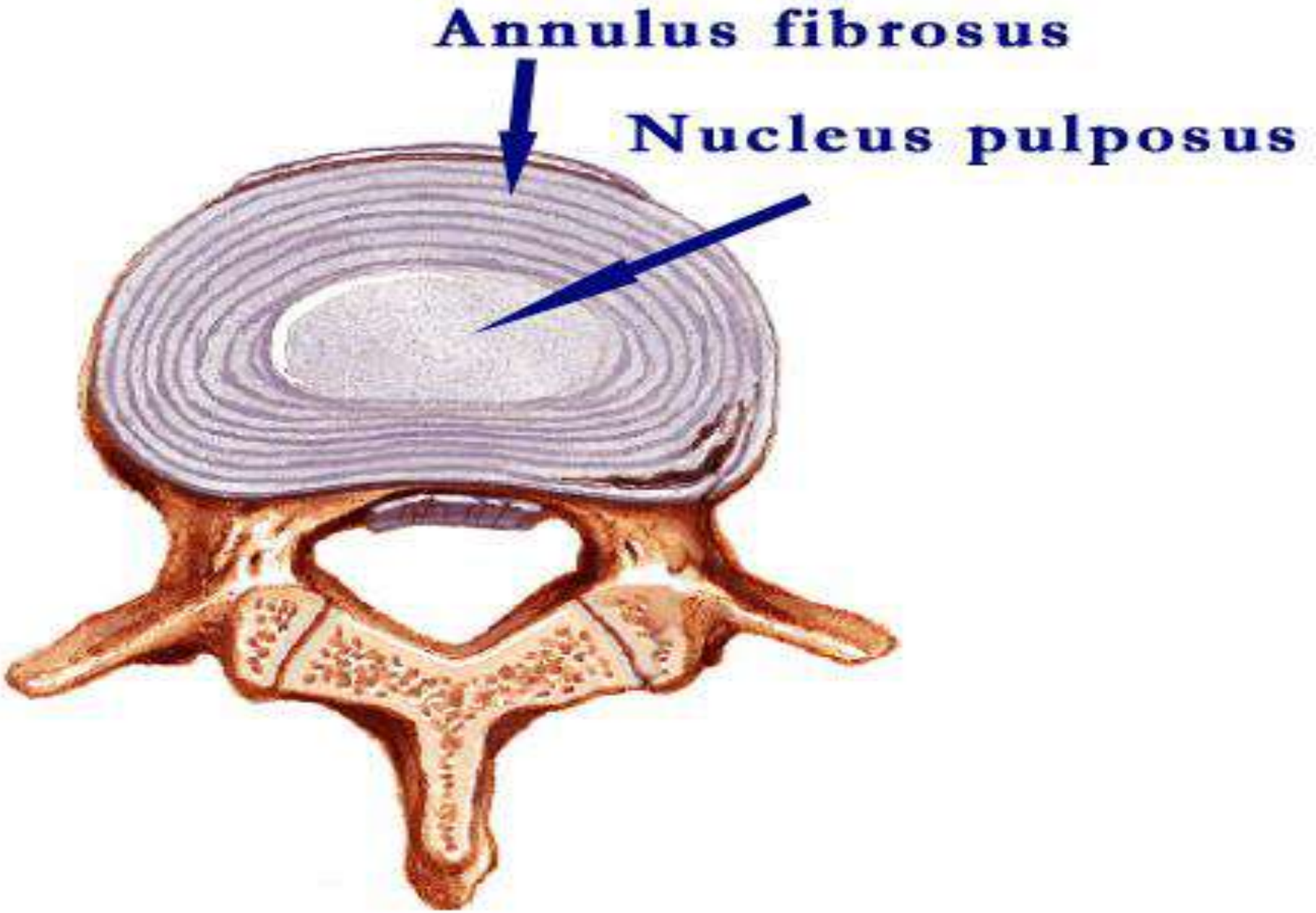
- Mandibular joint.
- Sternoclavicular joint.
- Intervertebral disc.
- Symphysis pubis.
- Cartilage around the hip (acetabulum) and shoulder (glenoid cavity) joints.
- Semilunar cartilages of knee joints.

Functions:

- It is a strong and tough type that can resist great tensile stretch.
- It attaches bone to bone with limited mobility.

Structure of the intervertebral disc:

- It is present between the bodies of two adjacent vertebrae.
- It is formed of an outer fibrous ring formed of white fibrocartilage (collagen type I), called annulus fibrosus, and an inner soft jelly-like inner mass containing collagen type II, called nucleus pulposus.


Annulus fibrosus
Nucleus pulposus

Growth of Cartilage

1- Appositional growth (Exogenous): It is the growth resulting from the addition of new layers of cartilage to the surface by the activity of the inner cellular layers of perichondrium (chondroblasts).

2- Interstitial growth: It is the growth of cartilage from inside by division of young chondrocytes which proliferate and produce matrix around them.

Healing of cartilage

cartilage has a limited ability to repair, if extensively damage the damage tear will be repaired with fibrous tissue


THANK YOU

General Histology

Dr: Hani Ahmed Alanqar
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BONE

Bone

Definition:

1-It is a hard ,strong and vascular type of connective tissue.

2-It is formed as any C.T of 3 main components , bone cells & fibers (collagen type I) embedded in solid matrix

3- The matrix is solid due to deposition of mineral salt (mainly calcium and phosphorus) in osteoid tissue

4-Bone is covered from external surface by a specialized fibrous C.T membrance (the periosteum)and lined from the internal surface by loose cellular C.T layer(the endosteum) in most areas

Function of bone:

- Support
- Protection (protect internal organs)
- Movement (provide leverage system for skeletal muscles, tendons, ligaments and joints)
- Mineral homeostasis (bones act as reserves of minerals important for the body like calcium or phosphorus)
- Hematopoiesis: blood cell formation
- Storage of adipose tissue: yellow marrow

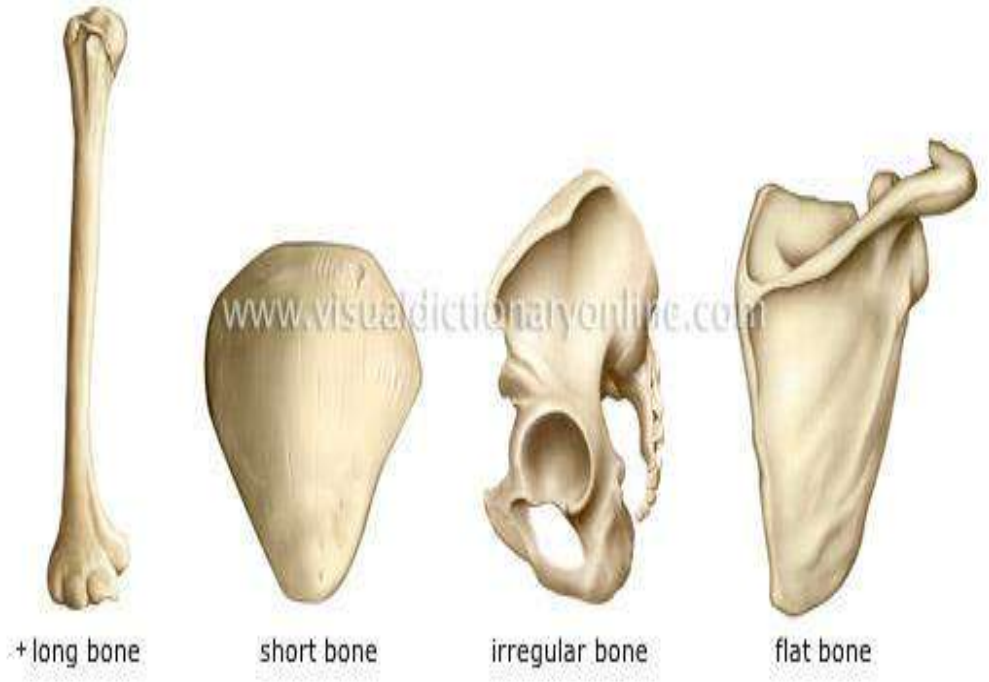
Shape:(anatomical classification)

1- long bone e.g bone of limbs

2- short bones e.g bones of hand & feet

3-flat bones e.g scapula , ribs , sternum

4- irregular bones e.g vertebrae



Type of bone (histological classification)

1- compact (regular) or ivory bone

2- cancellous (irregular) or spongy bone

Structure of bone:

A- Bone matrix

B- Bone cells (osteogenic cells ,osteoblast , osteocytes & osteoclast)

C- Periosteum & Endosteum

A – Bone matrix:

It consists of closely packed layers (lamellae) of calcified collagen bundles, embedded in amorphous intercellular substance.

1- Organic component (osteoid):35% of bone weight, consists of :-

-Fibers: collagen fibers mostly type I which form 90% of the organic portion that's is why bone matrix is highly acidophilic in decalcified H&E

-Ground substance : rich in carbohydrate &protein(proteoglycans and glycoprotein)

The organic part of matrix is secreted by the bone cells

2- Inorganic component: 45% of bone weight formed mainly of ca phosphate , carbonate , citrate and hydroxide. It also contain magnesium , potassium and sodium . The crystals of ca phosphate and hydroxide appear as plates or needles like crystals called **hydroxyapatite crystals** ,that lie alongside collagen fibers and contribute for the lamellar appearance of bone

3-water: 20%

B- Bone cells

There are 4 types of bone cells:

1- Osteogenic cells (osteoprogenitor cells):

Origin : UMCs & pericytes . It is the stem cell derived from embryonic mesenchyme .

Site : at the inner layer of periosteum and in the endosteum .

LM : flat cells with flat dark nuclei & pale basophilic cytoplasm .

EM :FEW ORGANELLES (ribosome & centrioles) , i.e. features of mitotically active cells

Functions:

- It is the main stem cell that gives origin to other types of bone cells . They are active in bone growth during embryonic life . In adults , they are active during bone fractures and repair .
- In low O2 tension (avascular medium) they may change into chondrogenic cell .

2- Osteoblasts (bone forming cells):

origin : osteogenic cells .

Site : at the inner cellular layer of the periosteum and in the endosteum (always at the growing surfaces of bone).

LM :

- Oval branched cells with few processes , rounded eccentric pale nuclei & prominent nucleoli . The cytoplasm is deeply basophilic .it may show an unstained area called negative Golgi image .
- Its cytoplasm is very rich in alkaline phosphatase enzyme .
- When these cells are active in bone formation , they increase in height and are seen arranged on the bony surfaces giving an epithelial –like appearance as a layer of oval , cubical or columnar cells , but with gaps between cells .

EM: All features of actively secreting cells : extensive rER , large Golgi ,many free ribosome ,plenty of mitochondria and many secretory vesicles .

Function :

They are the bone building cells as they are responsible for :

1. Synthesis of organic matrix of bone **(osteoid)**
2. Secretion of alkaline phosphatase which attracts the Ca salts from blood to be deposited in the matrix around osteoblasts and their processes .
3. When osteoblasts are surrounded with the newly formed matrix , imprisoned in spaces called lacunae .they mature and transform into osteocytes .

3- osteocytes (mature bone cells):

Origin : osteoblasts .

Sites : present **singly** inside bony lacunae , between bone lamellae . Cells are communicated with each other through the canaliculi , which connect lacunae .

LM :

- Smaller than osteoblasts .
- Oval ,flat ,branched cells with small oval dark nuclei , faint basophilic cytoplasm .
- Each cell lies inside a small cavity (lacuna) and lacaunae are connected by canaliculi through which osteocyte processes are connected by gap junctions .

EM: Features of active cells ,but they contain less rER and smaller Golgi than osteoblasts . The cells have thin processes that extend inside the cylindrical bony canaliculi in which the adjacent cell processes of the neighboring osteocytes are connected by gap junctions , so the cells are intercommunicated . They also contain alkaline phosphatase .

Functions: it is the bone maintaining cell (bone preserver)

1. Maintains bone matrix by formation of collagen fibers & glycoproteins .
2. Maintains the hardness of the matrix by continuous deposition of Ca salts (continuous exchange between bone & blood).

4-Osteoclasts (bone destroying cells):

It is a multinucleated giant cell .

Origin :formed by fusion of many blood monocytes .

Sites :at the bony surfaces undergoing resorption ,in a shallow depression near the bone marrow cavity produced by their erosive action called **howship's lacuna .**

LM :very large cell (up to 150 **µm**),with foamy **acidophilic** cytoplasm and multiple nuclei (up to 50) . The cell

surface facing the bone is ruffled or striated (brush border) .

E M :

- The surface facing the bone (ruffled border) , consists of finger-like processes projecting from the cell membrane to the bone surface (bone resorption occurs here)
- The cytoplasm contains many mitochondria , Golgi , ribosomes characteristic vesicles , vacuoles & many **lysosomes .**

Functions: bone eating cell . It is responsible for bone remodeling during growth or after fractures . The function is produced by :

- Release of hydrogen ions (H+) by the action of its carbonic anhydrase enzyme , which produce an acidic medium that dissolves ca salts
  __>decalcification and absorption of minerals .
- Their lysosomal enzymes (acid phosphatase , collagenase & proteolytic enzyme) lead to lysis of organic matrix (bone resorption or erosion).

C. Periosteum & Endosteum

All bones except at joint surfaces are covered both at the external and internal surfaces with a layer formed of vascular C.T. and bone forming cells.

a) The periosteum

It is the vascular non –calcified C.T. sheath covering the bone outer surfaces . It consists of :

1. Outer fibrous layer :a layer of dense collagenous C.T. , of collagen fibers & fibroblasts . It is very rich in blood vessels , which will penetrate to inside the bone .
2. Inner osteogenic (cellular) layer : It is more cellular ,formed of closely arranged osteogenic cells , which can divide and differentiate into osteoblasts , which are also seen in this layer .

Function of periosteum :

1. Nutrition (supplies bone with blood)
2. Muscle attachment .
3. Repair of fractures.
4. Apposional growth .

b- The Endosteum:

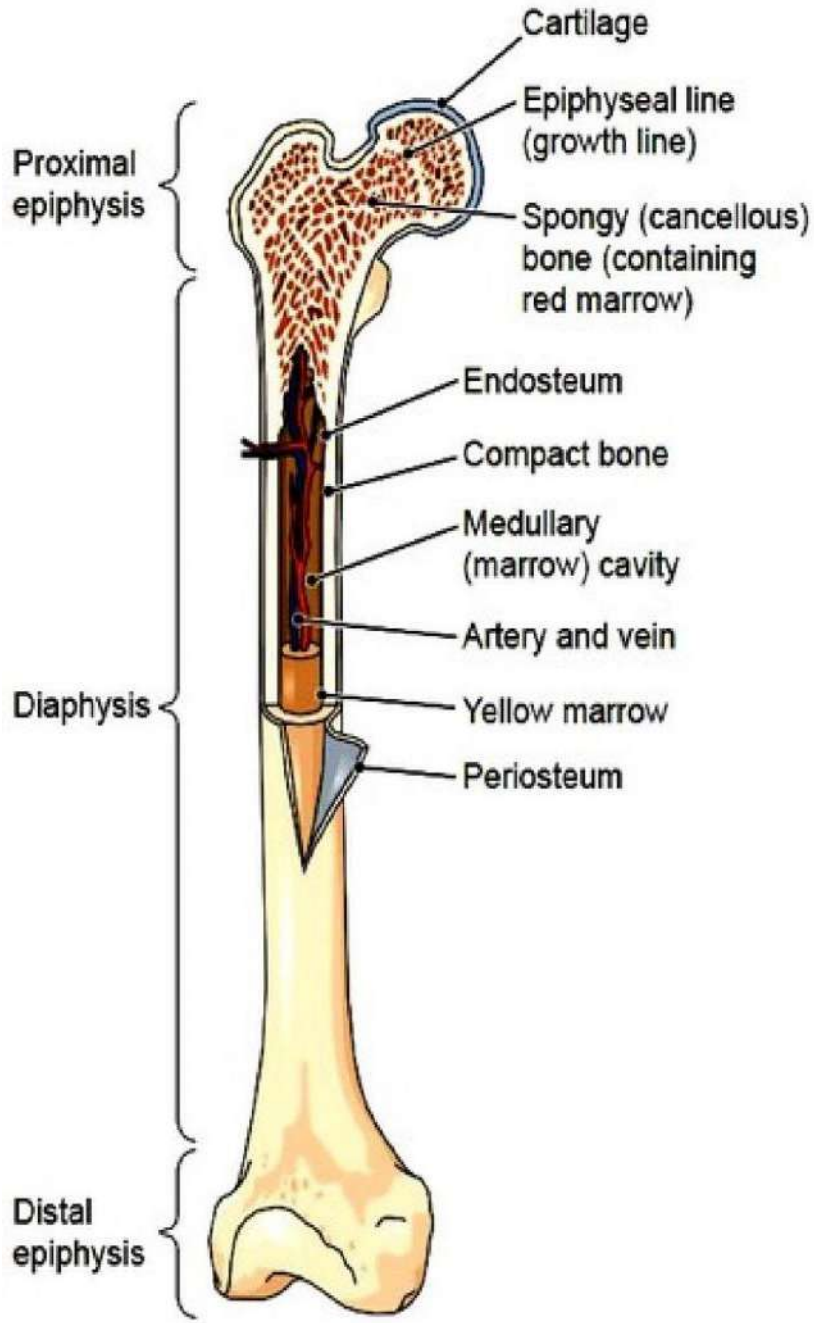
- It lines all internal surfaces and cavities within the bone(marrow cavities).
- It is formed of single layer of osteogenic cells and osteoblasts with little amount of loose C.T. it supplies osteogenic cells and osteoblasts for growth and repair of bone.

Histological study of bone

A- compact bone

- It is highly organized hard C.T , dense and mature
- It is present in the shafts of long bones , inner and outer table of flat bones and outer covering of short irregular bones.
- It is the solid or ivory type of bone (mature bone), in which the bone is formed of layers called lamellae.
- A bone lamella is a thin plate of bone formed of calcified collagen bundles and mineral salts deposited in ground substance around it
- Between the lamellae are small spaces or lacunae in which osteocytes are present
- Lamellae are arranged parallel to each other or concentrically regularly arranged around blood vessels. They are organized on long bone into sets of concentric rings ,**called osteon or haversian systems**

- Each osteon includes a central channel , the haversian canal , which contain a blood vessels in its center . The long axis of the osteons is ususally parallel to the long axis of bone.
- Note: Long bones formed of a shaft called **diaphysis** , and two ends called **epiphysis** . In between the diaphysis and epiphysis in growing bones , there is a cartilage plate called **epiphyseal plate**
- **Methods of preparation of bone section:**
- 1- Decalcified bone section: ca salts are dissolved by treating the bone with mineral acid as nitric acid 10%
- 2-Ground bone section: bone is left to dry in air then grinding or sawing it into thin transparent slices. Thin section are mounted on the slides , examin without stain


Cartilage
Epiphyseal line (growth line)
Spongy (cancellous) bone (containing red marrow)
Endosteum
Compact bone
Medullary (marrow) cavity
Artery and vein
Yellow marrow
Periosteum
Proximal epiphysis
Diaphysis
Distal epiphysis

1- decalcified compact bone

Transverse section of long bone , which is decalcified and stained with H&E , it consists of :

Periosteum: covers the bone outside , formed of 2 layers:

Outer fibrous layer: formed of collagen fibers , fibroblasts , fibrocytes and blood vessels.

Inner cellular layer of osteogenic cells and osteoblasts

External circumferential lamellae: bone lamellae present under the periosteum and arranged in layer parallel to the circumference of the bone, enclosing osteocytes in their lacunae in between them

Haversian systems (osteons): the structure units of compact bone.

- They are cylindrical structures running parallel to the long axis of bone
- Each is formed of concentrically arranged bone lamellae (5-20)layers, around a vascular channel (Haversian canal) which contain blood vessels , nerves and loose C.T . Osteocytes inside their lacunae are embedded between bone lamellae , lacunae are connected together by canaliculi through which they get their nutrition

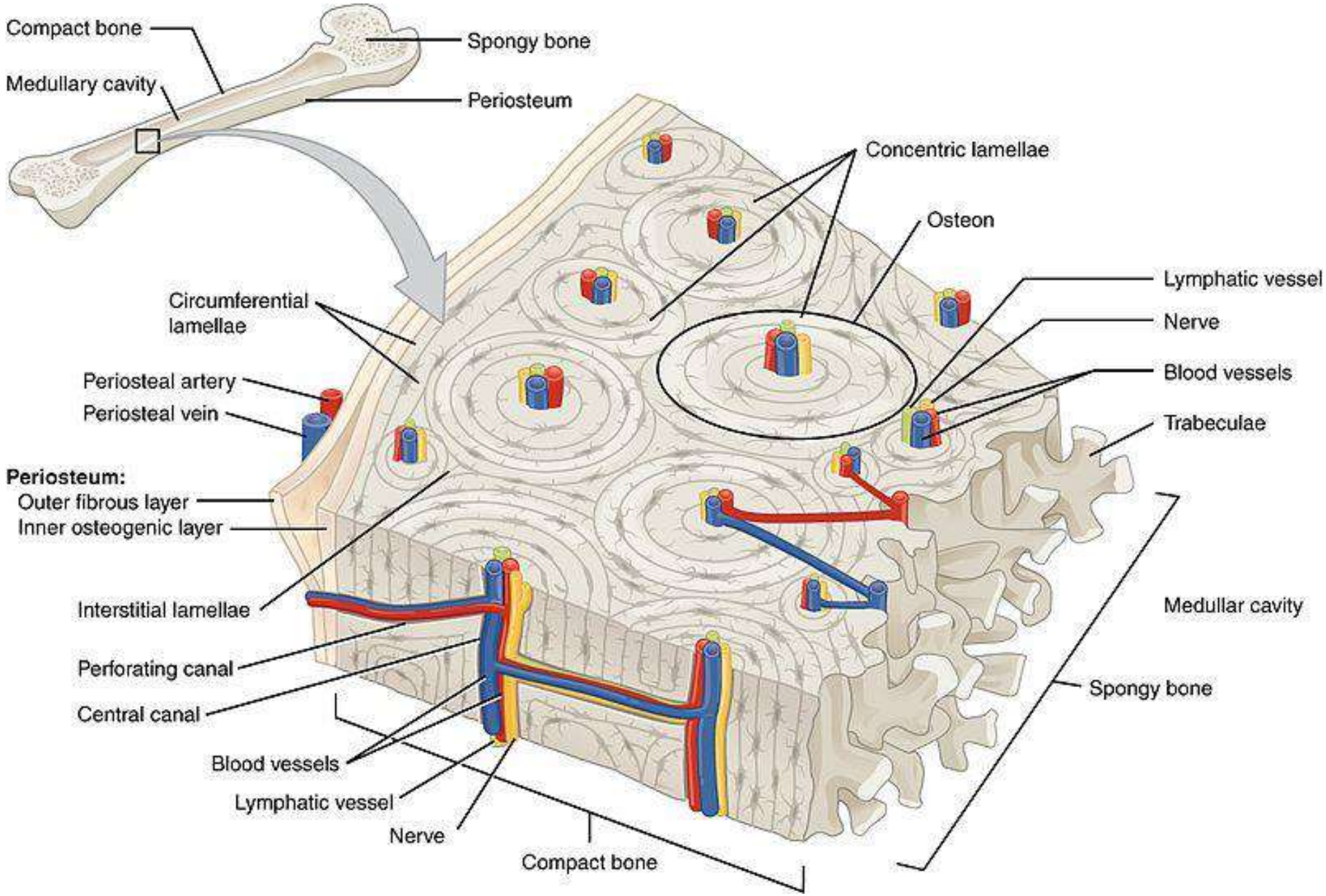
Volkmanns canals: are they communicating transverse canals , linking the Haversian canal in the Haversian systems with one another and with the periosteum and bone marrow cavity to get better nutrition

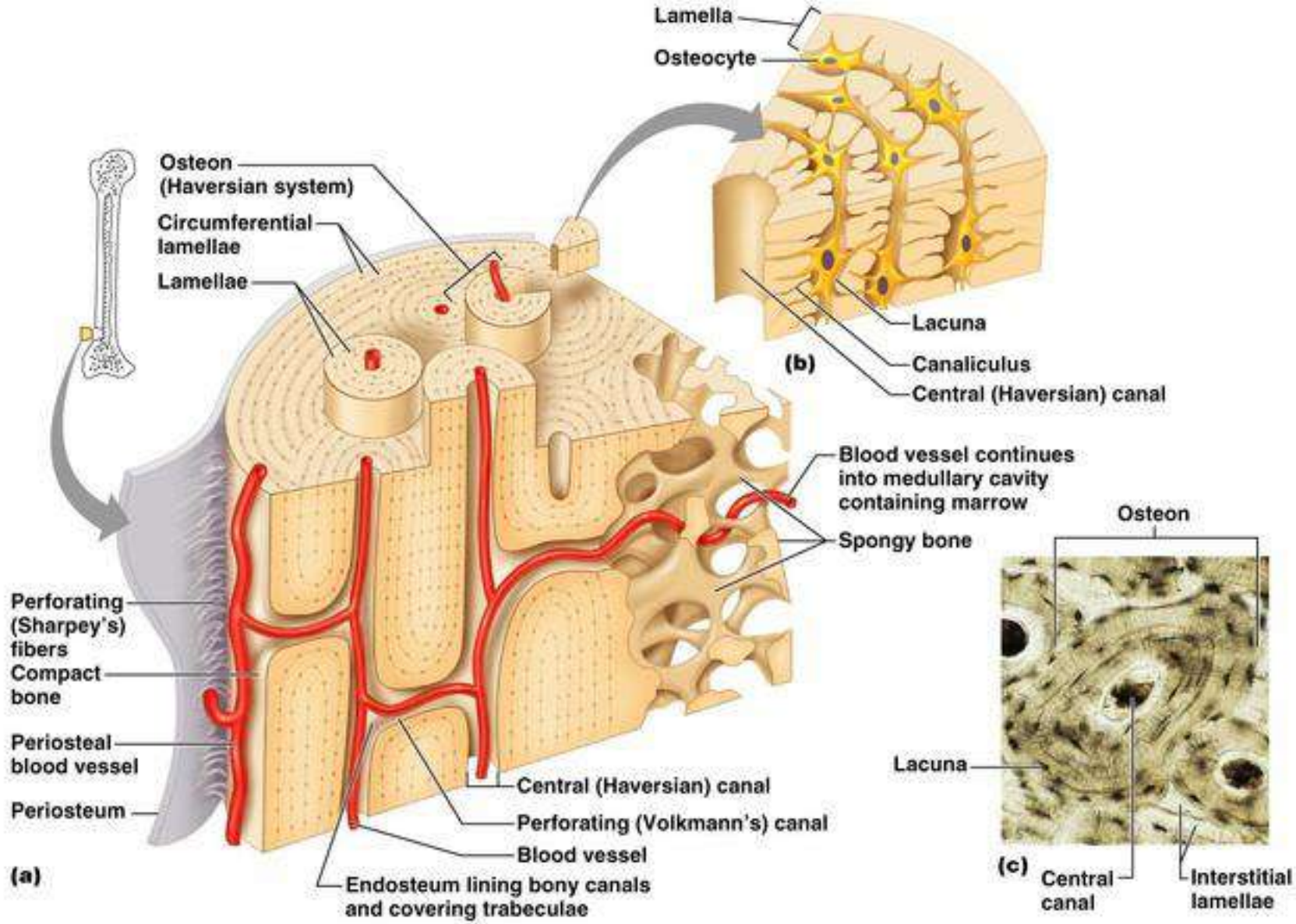
Internal circumferential lamellae: bone lamellae surrounding the bone marrow cavity and parallel to endosteum lining it.

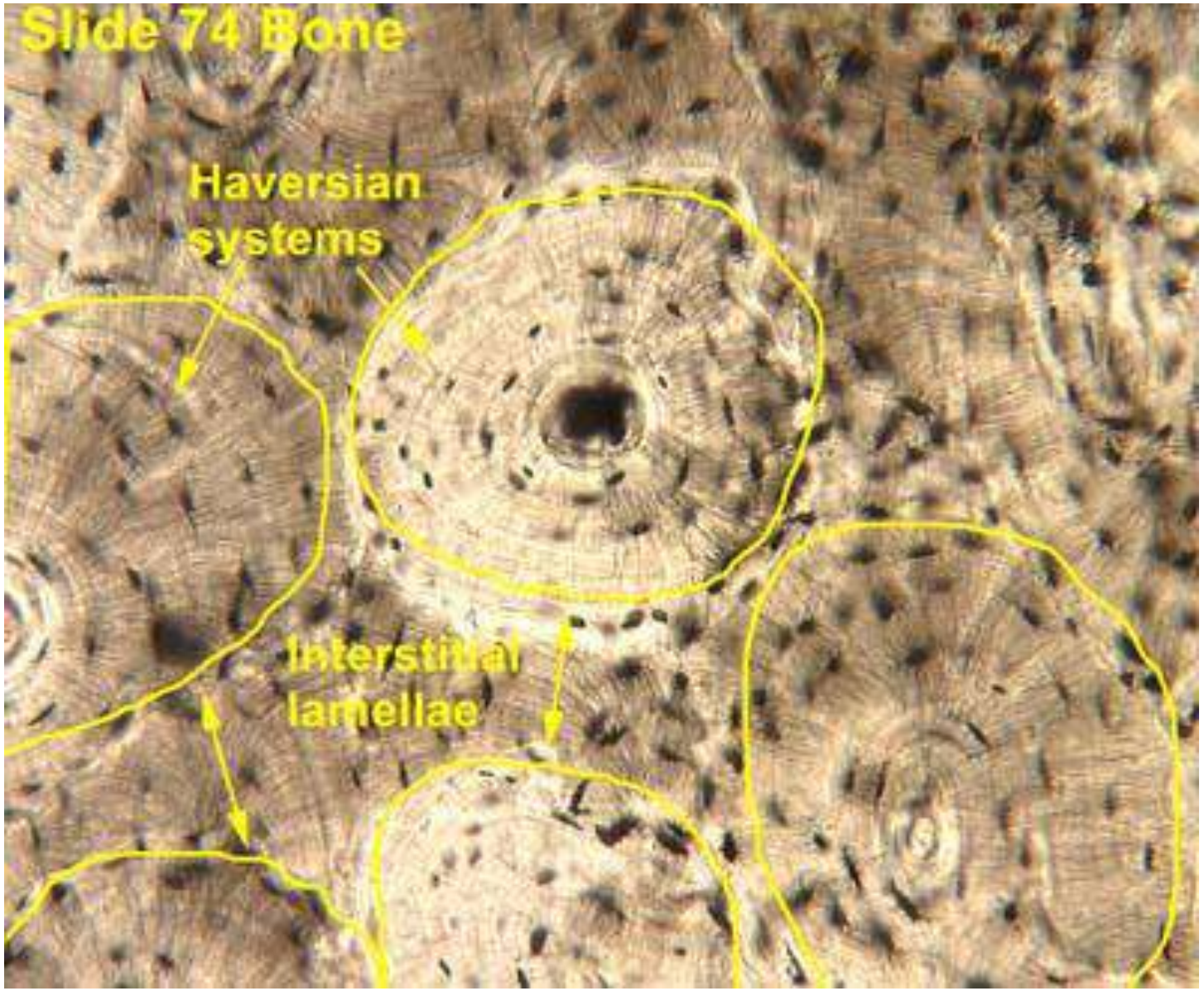
Interstitial lamellae : present in between the haversian system . They consist of irregularly arranged lamellae

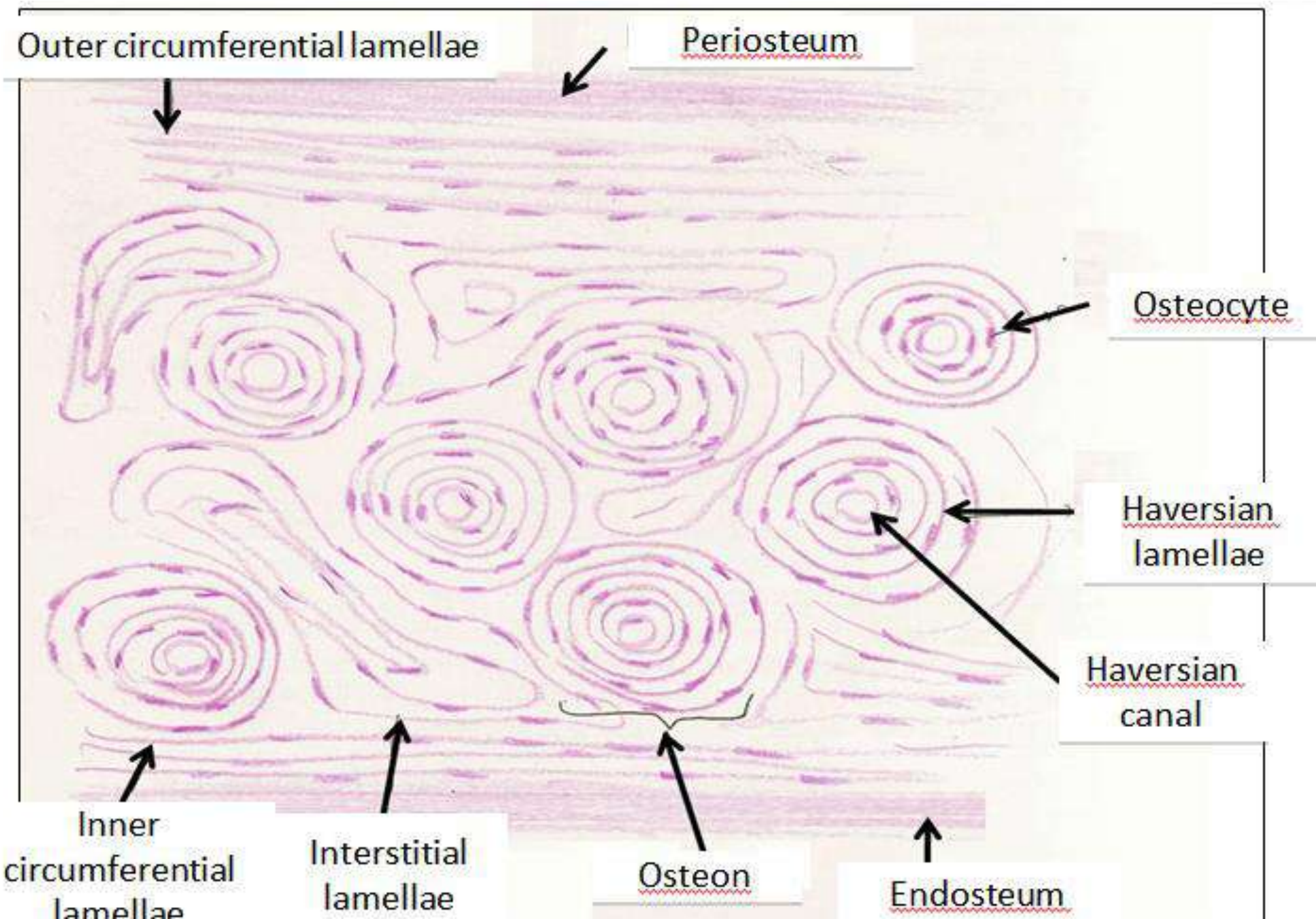
Endosteum: lines the central marrow cavity and consists of one layer of osteogenic cells and osteoblasts with little amount of ct

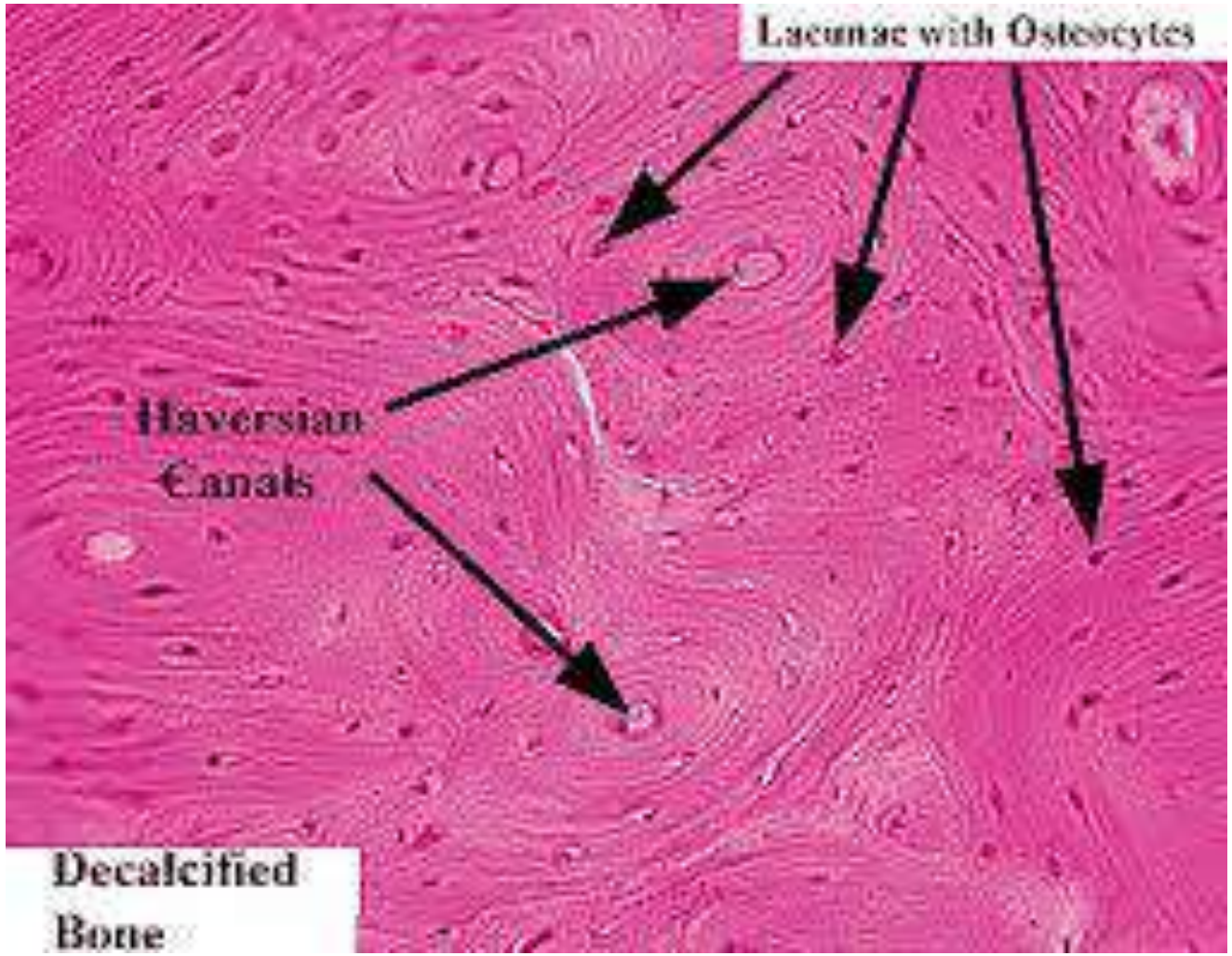
Perforating fibers of sharpey: they are calcified collagen fibers that arise from tendons and ligaments at the site of muscle attachment


Compact bone
Spongy bone
Medullary cavity
Periosteum
Concentric lamellae
Osteon
Lymphatic vessel
Nerve
Blood vessels
Trabeculae
Circumferential lamellae
Periosteal artery
Periosteal vein
Periosteum:
Outer fibrous layer
Inner osteogenic layer
Interstitial lamellae
Perforating canal
Central canal
Blood vessels
Lymphatic vessel
Nerve
Compact bone
Medullar cavity
Spongy bone




Slide 74 Bone
Haversian systems
Interstitial lamellae


Outer circumferential lamellae
Periosteum
Osteocyte
Haversian lamellae
Haversian canal
Inner circumferential lamellae
Interstitial lamellae
Osteon
Endosteum


Lacunae with Osteocytes
Haversian Canals
Decalcified Bone

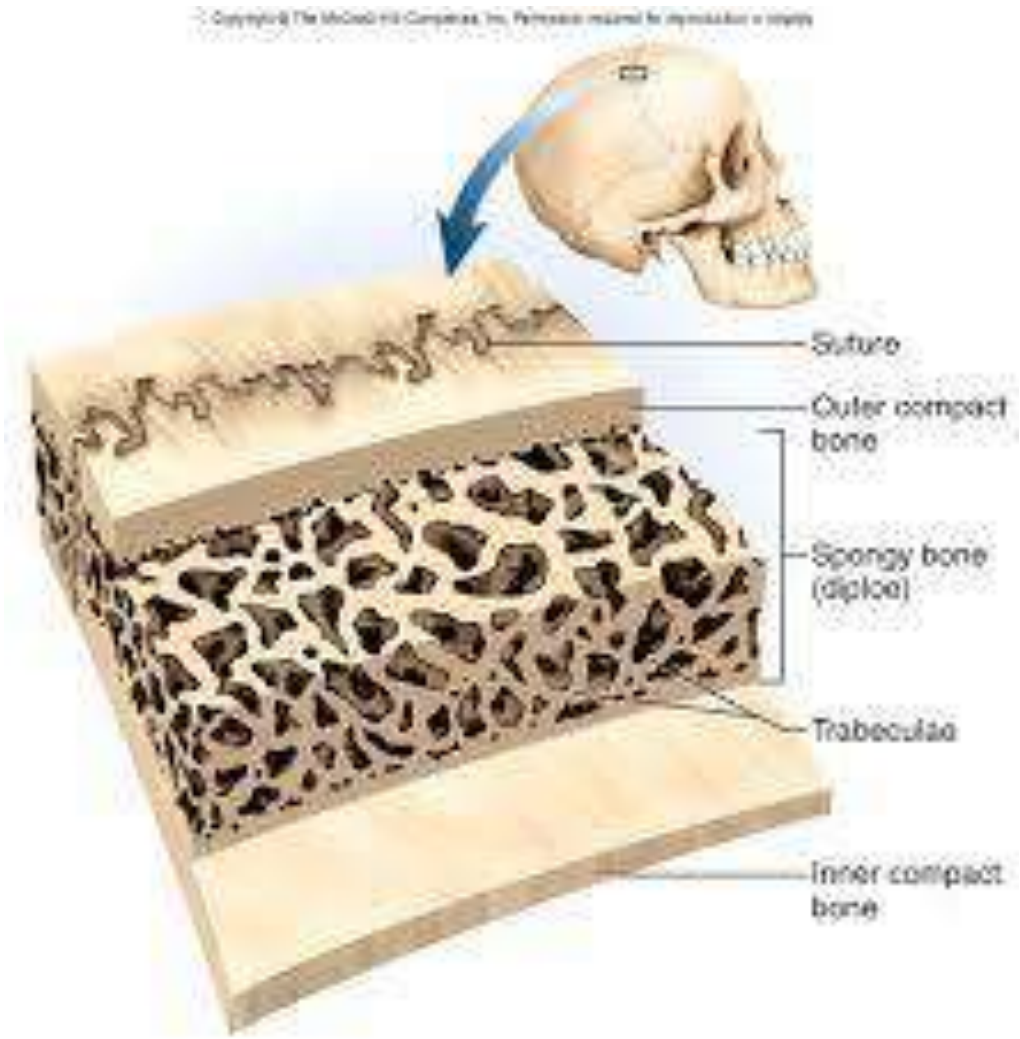
B – Spongy (cancellous) bone

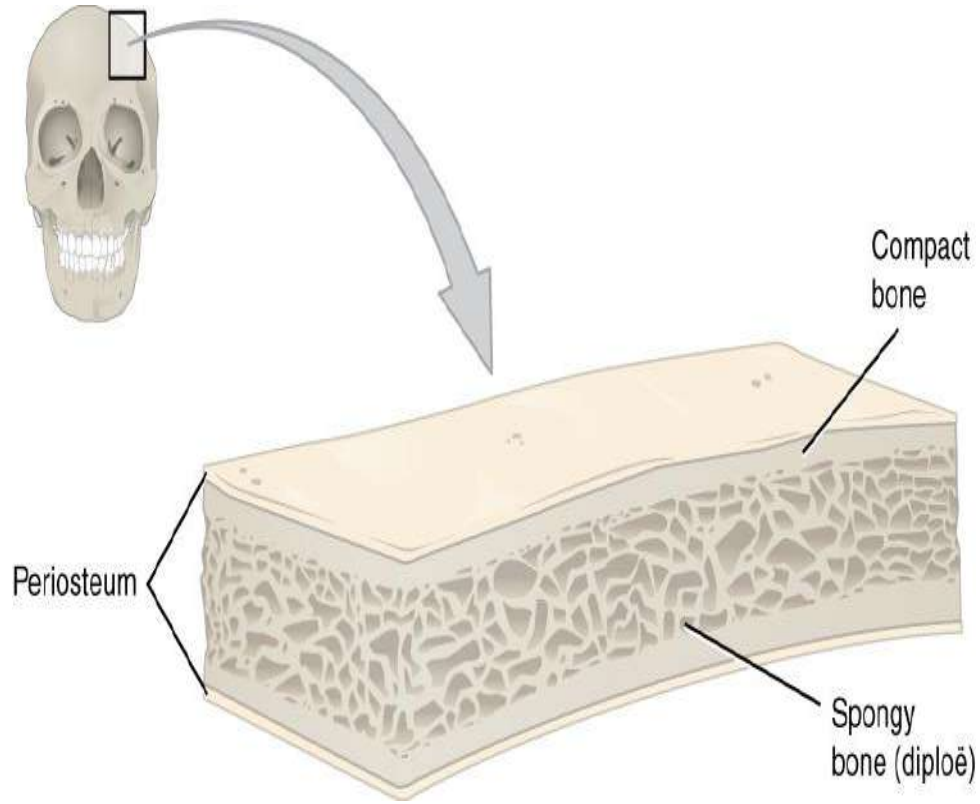
It is an irregular type of bone which bone doesn't have a lamellar structure . It is the first bone to form during fetal life and during bone repair

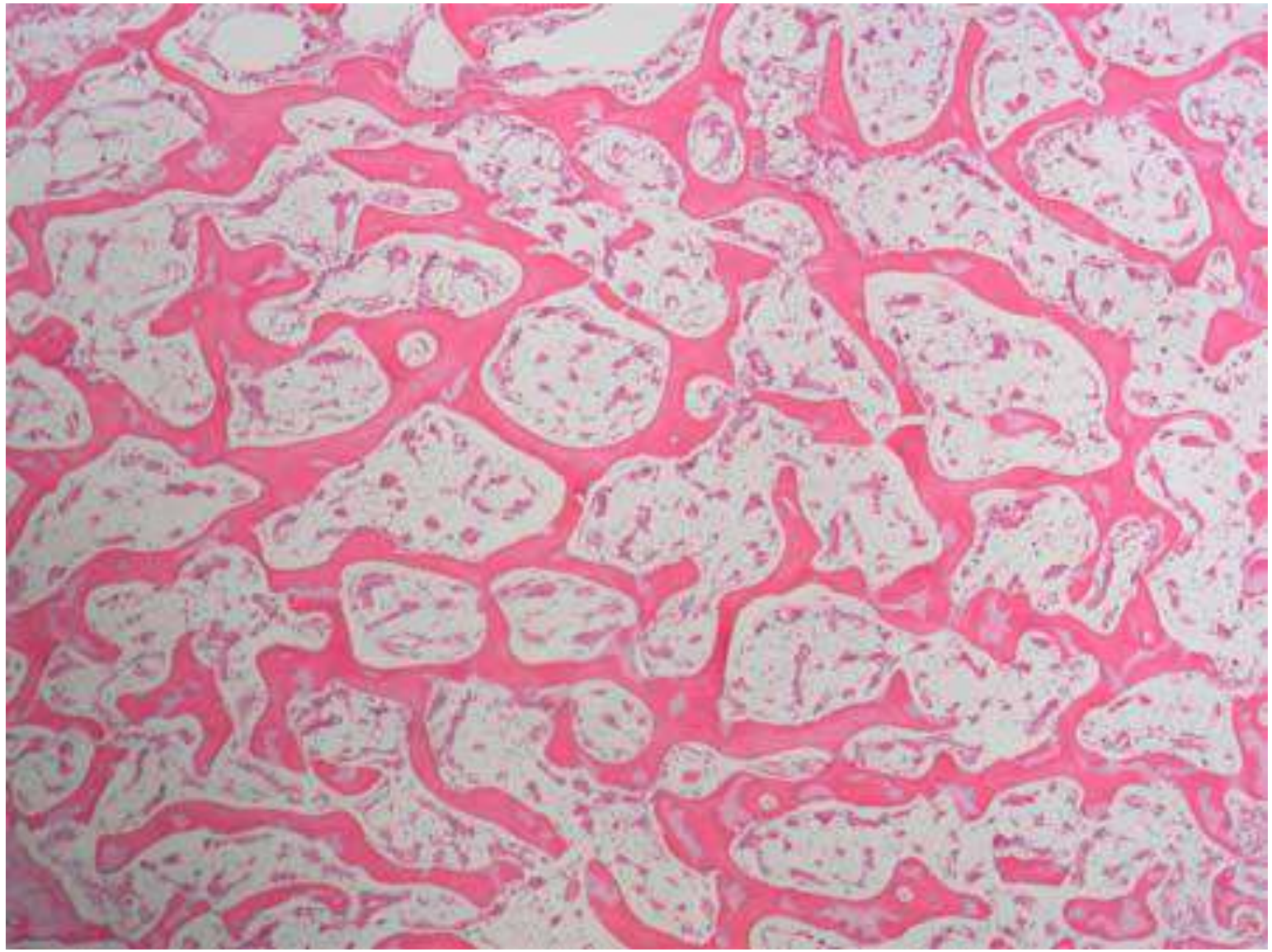
Site: present in the center of epiphysis , flat and irregular bones

Structure:

- It is formed of branching and anastomosing irregular bone trabeculae, which form a meshwork .osteocytes in their lacunae and embedded in these trabeculae.no haversian systems
- The trabeculae enclose multiple irregular bone marrow cavities containing blood forming cells ,fat cells and blood vessels . This arrangement gives the spongy appearance


Suture
Outer compact bone
Spongy bone (diploe)
Trabeculae
Inner compact bone


Compact bone
Periosteum
Spongy bone (diploë)



Formation of bone (Ossification)

Bone is formed from mesenchymal tissue in the embryo. There are two methods for bone development or ossification:

1- Intramembranous ossification: in flat bones as skull , mandible , maxilla & clavicle which develop within a condensed mesenchymal membrane.

2- Intracartilagenous or endochondral ossification: as in long bones , which develop by replacement of pre-existing cartilage model.

N.B: Primary bone is formed first and is later replaced by secondary mature bone.

Intramembranous ossification

This type of ossification occurs in flat bone

1-the site of the future bone is occupied with mesenchymal membrane.

2-the starting point for ossification is the appearance of a highly vascular area in the middle of this membrane called the primary ossification center. The mesenchymal cells (UMCs) condense , proliferate and differentiate into osteogenic cells

3-osteogenic cells increase in size and number and differentiate into osteoblastic cells.

4-osteoblasts divide and synthesize organic matrix (osteoid)(collagen type I ,proteoglycans & glycoproteins), secrete it around themselves. They also secrete alkaline phosphatase which stimulates the deposition of ca salt in the matrix around them & their processes.

5-when osteoblasts are trapped in their own matrix inside lacunae surrounded with calcified matrix , they are called osteocytes.

Intracartilagenous (endochondral) ossification

- This type of bone formation occurs in long bones of limbs and takes place by replacement of cartilage model .
- Development of cartilage model :
- In the embryo , a limb bud of mesoderm outgrows , condenses and takes the shape of the future bone .
- The mesenchymal cells (in the avascular environment) differentiate into chondroblasts which produce cartilage matrix ,resulting in the development of a model hyaline cartilage . Perichondrium , made up of outer fibrous and inner chondrogenic layer also develops at its periphery.
- The cartilage model grows by appostional and interstitial methods .

• **Cartilage is replaced by bone**: through the following steps:

A-Primary center of ossification: which starts to appear at the center of cartilage model (diaphysis) where the following changes occur simultaneously :

1-The chondrocytes at the middle of the cartilage model proliferate , enlarge (hypertrophy) and mature. Calcium salts start to be deposited in the matrix around their lacunae , leading to their deprivation from the nutrition. Chondrocytes then die and leaving empty lacunae , separated by calcified cartilage matrix which basophilic in H&E stain.

2- The perichondrium becomes more vascular & active so mesenchymal cells differentiate into osteoblasts which start to lay down a thin collar of bone matrix in the inner layer of the perichondrium (now it become periosteum)

3- Osteoclast open holes in the bone collar allowing periosteal capillaries and osteogenic cells and UMCS (vascular bud) to invade the cavities inside the model.

4- The osteogenic cells attach themselves to the remnants of the basophilic calcified cartilage matrix and then change to osteoblasts, which start to lay bone matrix (acidophilic) in an irregular manner (spongy bone)

5-**Remodling** : osteoclast start to resorb the irregular trabeculae in the center , lead to formation of a single marrow cavity in which mesenchymal cells gives the bone marrow cells . At the same time , osteoblasts deposit new bone to preserve the general shape of bone during growth

6- **Zone of ossification and Haversian system formation**: concentric bone lamellae produced by osteoblast are deposit around the blood vessels to form Haversian canal

Secondary center of ossification: appear after birth in the two end of the developing bone (epiphyses) , which are still cartilage by increase vascularity.

1-Chondrocytes in the center of the epiphysis proliferate , enlarged and mature then deposit ca salts in the matrix around them. This is followed by death of chondrocytes leaving “empty spaces” in the middle of the epiphysis.

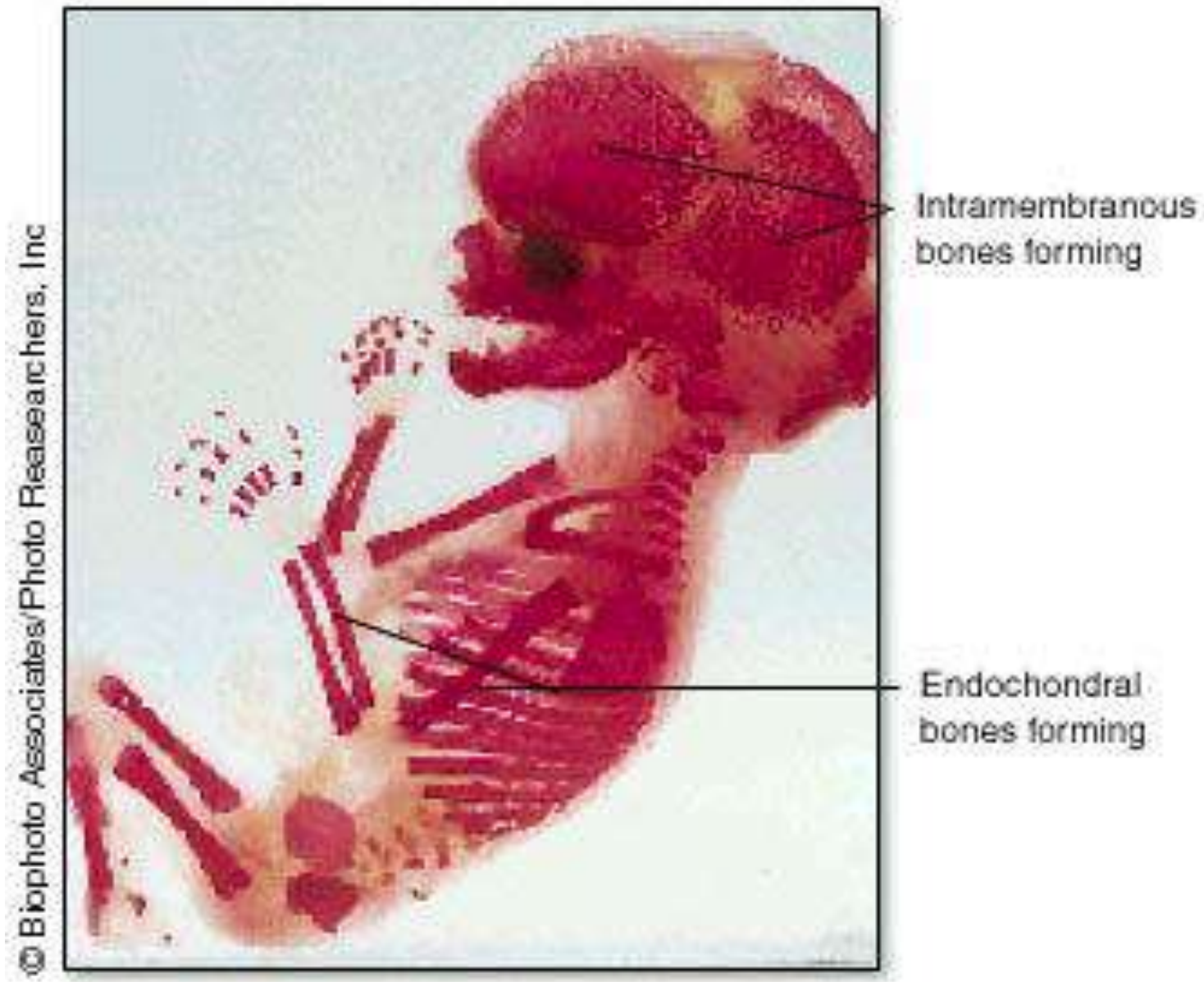
2- Capillaries & osteogenic cells invade the cartilage through the holes produced by osteoclast.

Osteoblast lay down bone matrix forming secondary ossific center.

Thus the cartilage at the middle of the two head is replaced by spongy bone
Except in two regions:

-Epiphyseal plate: between epiphysis & diaphysis which is left for growth till age of 21-23 ys.

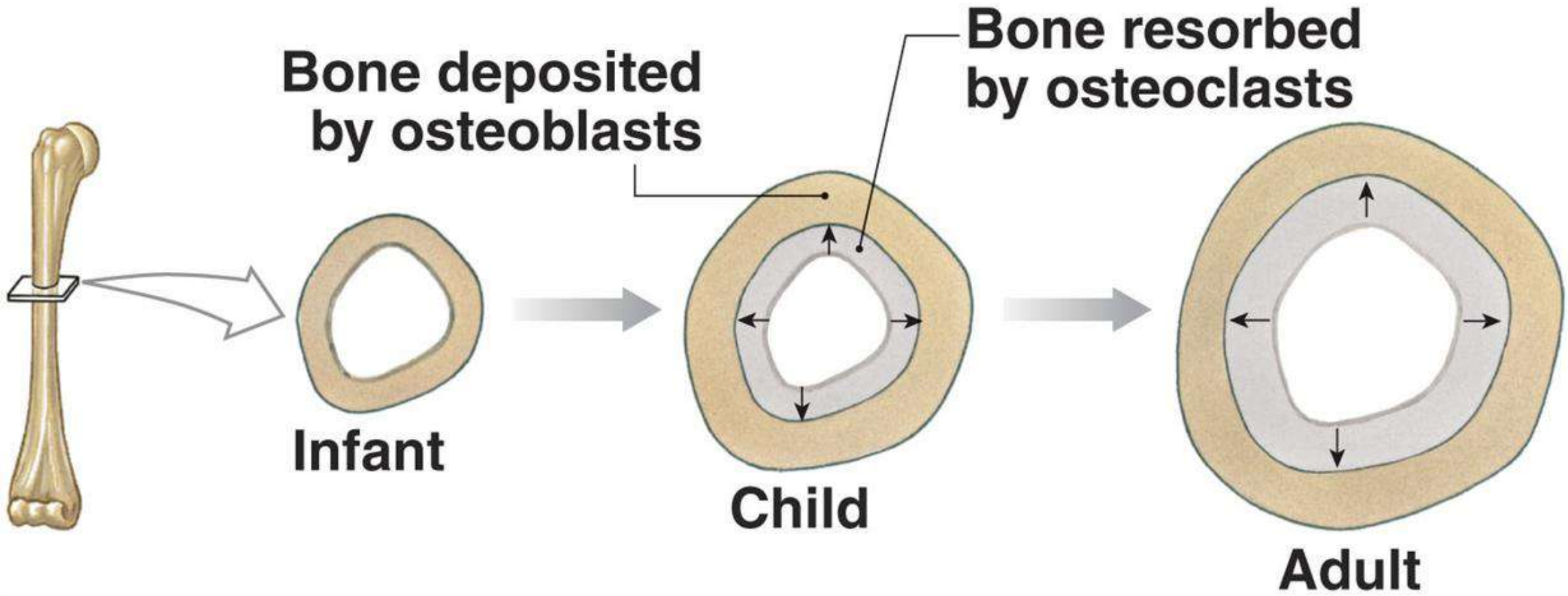
-Articular cartilage : which will never ossify throughout life.


© Biophoto Associates/Photo Reasearchers, Inc
Intramembranous bones forming
Endochondral bones forming

Postnatal growth of bone:

1-growth in width (appositional growth): by continuous deposition of new compact on the outer surface under the periosteum and continuous bone resorption of old bone from inside by osteoclasts this leads to the increase in the diameter of bone and also the bone marrow cavity increase in size.

2- growth in length (interstitial growth): occur in the epiphyseal plates , where proliferation of cartilage take place . At the age of 21-23 ys ossification is complete and growth stops with no further proliferation of cartilage and the epiphyseal disc is completely ossified



The epiphyseal plate (growing end of bone):

During growth of long bones , the epiphyseal plate has a characteristic microscopic appearance (where intracartilagenous ossification occurs). The study of a longitudinal section of growing bone reveals distinct histological zones ,beginning with the epiphyseal side of the plate (the stages of intracartilagenous ossification) in the following zones :

1. Zone of resting cartilage : a reserve zone formed of hyaline cartilage where the cells are small , inactive , and irregularly arranged .
2. Zone of proliferation :(increase in number) ,chondrocytes undergo repeated mitosis , and arrange in parallel rows separated by bars of matrix .

3. Zone of cell maturation & hypertrophy : the chondrocytes are greatly enlarged ,and acquire high glycogen and alkaline phosphatase content . The cartilage matrix between neighboring cells becomes thin .

4.Zone of calcification : mature chondrocytes secrete alkaline phosphatase content m which deposit Ca in the thin cartilage matrix . This will cut the nutrition from chondrocytes .

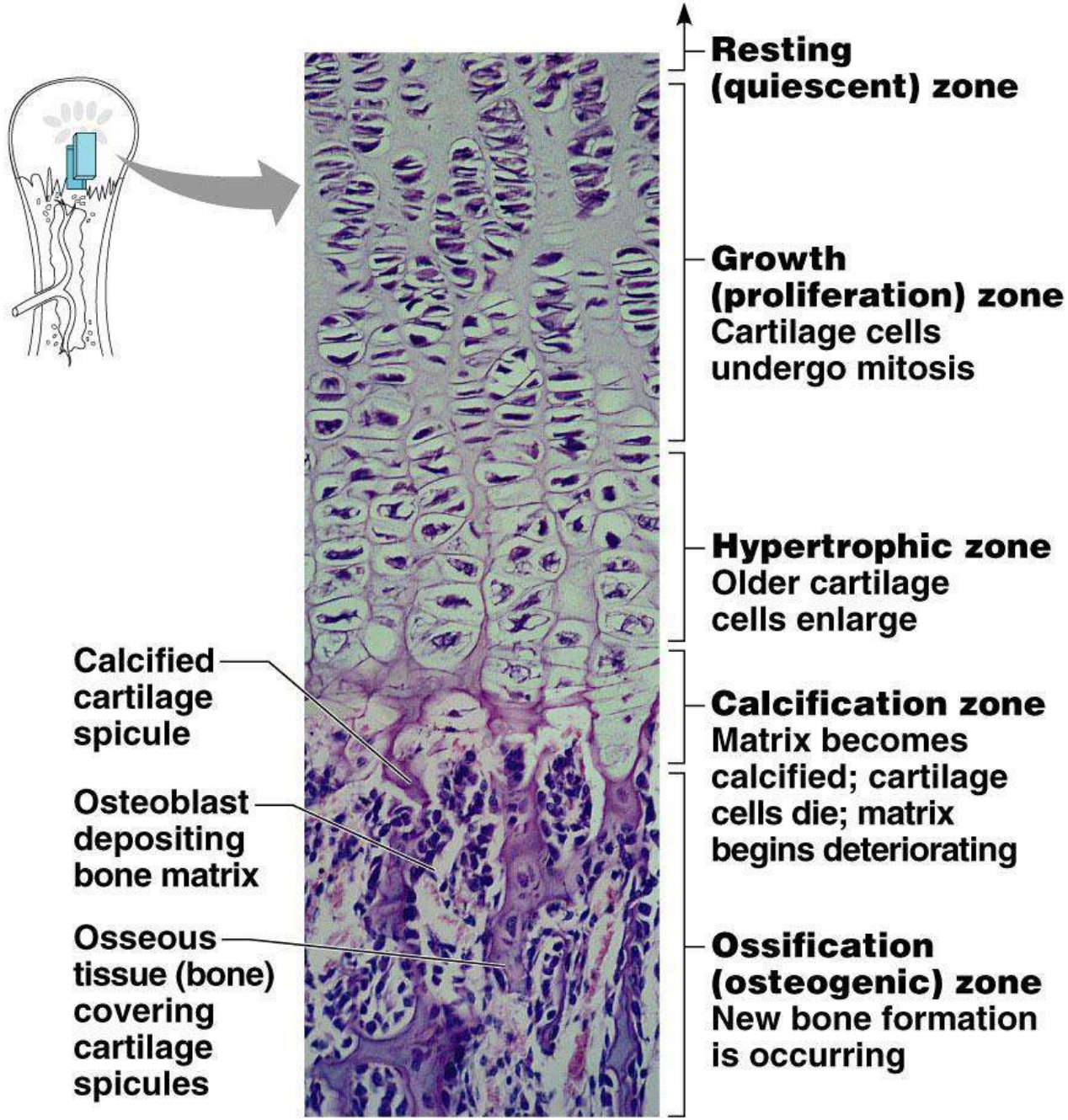
- The trapped chondrocytes will die leaving their lacunae empty .
- Lacunae will break and join leaving large empty spaces.

5.Zone of invasion : vascular bud(capillaries & osteogenic cells) invades the empty spaces in the calcified matrix through holes produced by the action of osteoclasts .

6.Zone of ossification (spongy bone formation) : the osteogenic cells change inti osteoblasts which lay down bone matrix on the calcified cartilage remnants . The resulting bone trabeculae fuse forming spongy bone .

7. Remodeling :This is done by osteoclasts ,which resorb bone trabeculae from certain areas, while osteoblasts deposit new bone , to preserve a single marrow cavity and general shape of bone during growth .

8. Zone of ossification & Haversian system formation : concentric bone lamellae ,produced by osteoblasts ,are deposit from the outer to the inner aspect of a space in the spongy bone containing blood vessels , until the cavities are reduced to be the central canals(Haversian canals).


Resting (quiescent) zone
Growth (proliferation) zone
Cartilage cells undergo mitosis
Hypertrophic zone
Older cartilage cells enlarge
Calcification zone
Matrix becomes calcified; cartilage cells die; matrix begins deteriorating
Ossification (osteogenic) zone
New bone formation is occurring
Calcified cartilage spicule
Osteoblast depositing bone matrix
Osseous tissue (bone) covering cartilage spicules

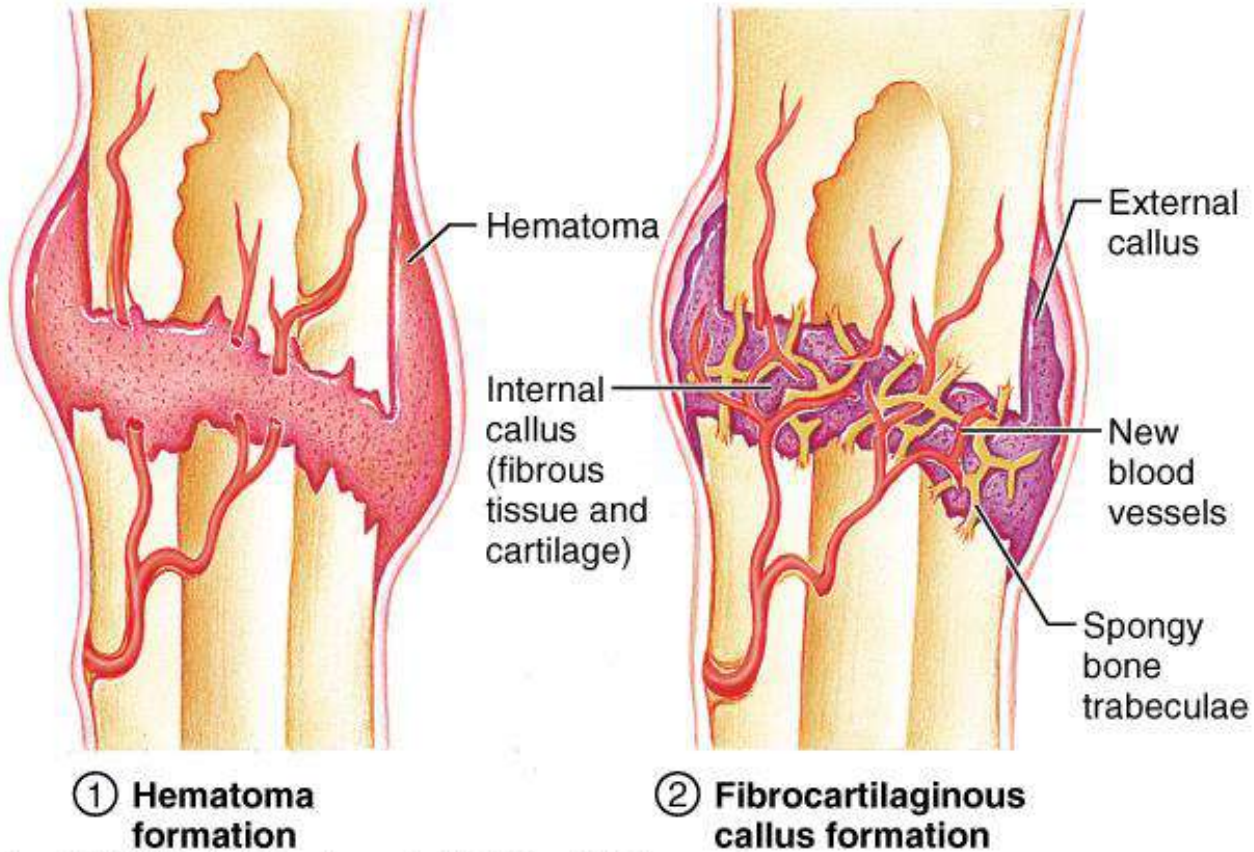
Bone Repair :

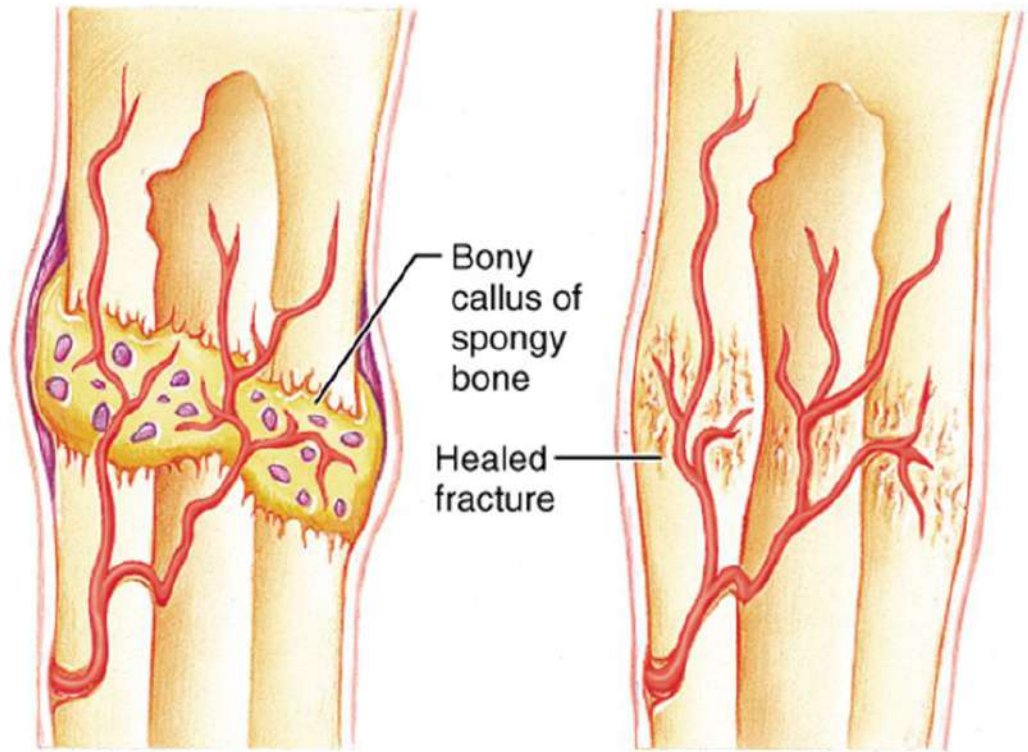
- Bone fracture leads to damage of bone matrix , and bone cell in the region as well as blood vessels supplying the area .
- Hemorrhage is followed by the formation of blood clot . Macrophages remove the damaged tissues .
- Fibroblasts proliferate in the periosteum and endosteum and surround the area externally and internally , forming fibrous tissue between the two broken ends
- Osteogenic cells , from the periosteum and blood capillaries migrate to the area of the clot , starting deposition of bone ,

- Also chondrogenic cells differentiate , starting to lay cartilage matrix . This mass of tissue formed of cartilage and bone at the site of the organized clot is called callus .

The callus :

- It is the organized clot formed of cartilage , which will be replaced by bone ,**the primary bone .**
- Finally excess bone in the callus is resorbed and is replaced by **secondary bone** and union of the two end of the fracture is complete





③ **Bony callus formation**

④ **Bone remodeling**

Joints

- **Joints** are places where bones meet, or articulate, allowing at least the potential for bending or movement in that portion of the skeleton.
- Joints with very limited or no movement are classified collectively as **synarthroses** and freely mobile joints are called **diarthroses**.
- **Intervertebral discs** are synarthroses in the vertebral column which cushion adjacent vertebrae.
- Each intervertebral disc consists of a thick outer layer of fibrocartilage forming a tough **annulus fibrosus** and a shock-absorbing inner, gel-like core, the **nucleus pulposus**.
- Diarthroses have a **joint cavity** filled with lubricant **synovial fluid**, enclosed within a tough, fibrous **articular capsule**; ends of the bones involved are covered with hyaline **articular cartilage**.

■Specialized connective tissue of the **synovial membrane** lines the capsule, with folds extended into some areas of the joint cavity.
■**Macrophage-like synovial cells** of the synovial membrane remove wear-and-tear debris from synovial fluid.
■**Fibroblast-like synovial cells** of the synovial membrane synthesize hyaluronan which moves into the synovial fluid with water from local capillaries to lubricate and nourish the articular cartilage.

General Histology

Nervous Tissue

Dr.Hani Ahmed Alanqar
Consultant of pathology

Contents

- Neurons:
- Classification of Neurons:

According to number of processes:

According to function:

According to length of axon:

- Structure of Neurons:

Cell body (Perikaryon):

Processes:

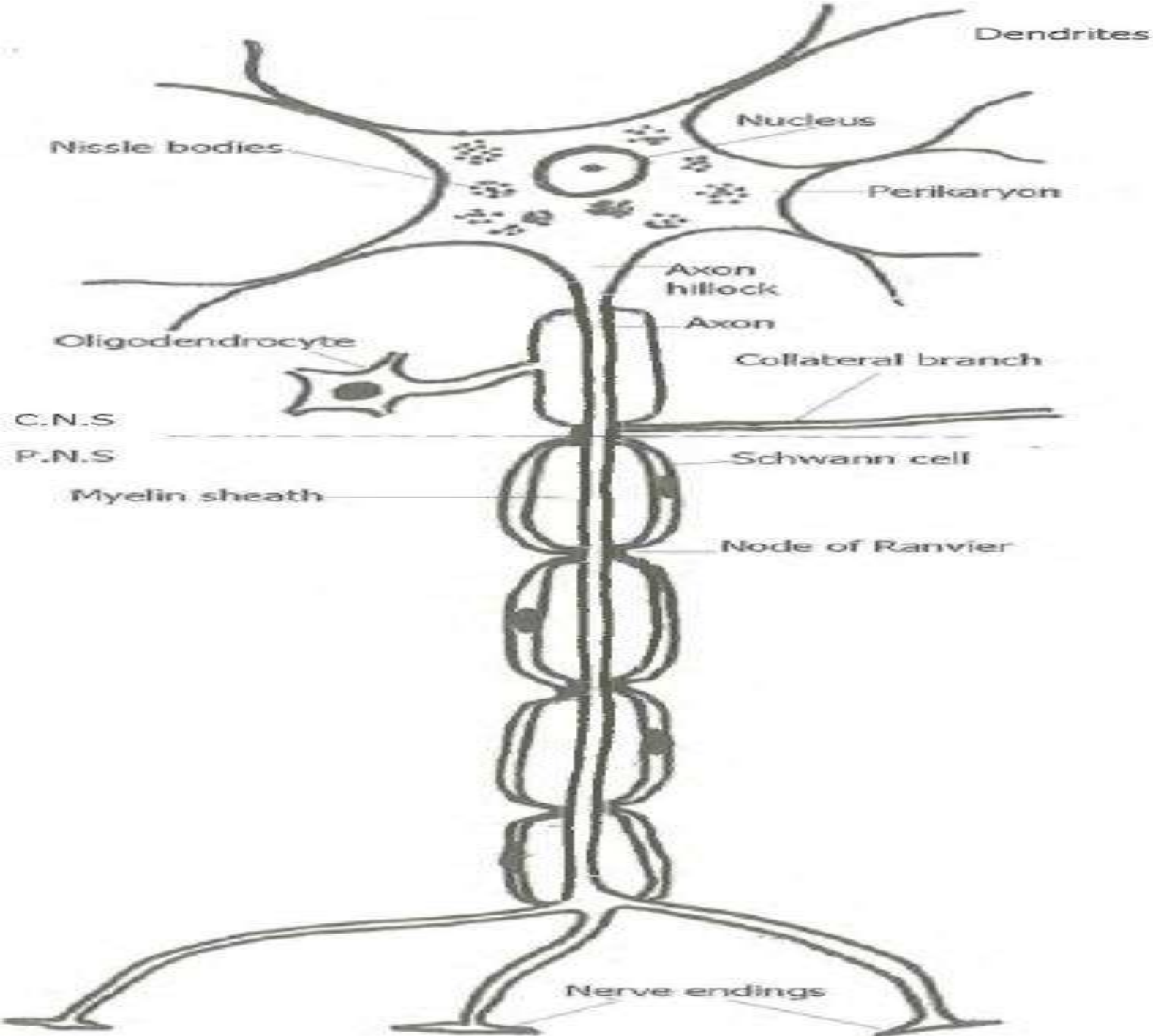
Nerve Fiber:

- Types of nerve fibers:
- Ganglia

- **Nervous tissue** is divided **anatomically** into the **central nervous system** consisting of brain and spinal cord and **peripheral nervous system** composed of nerve fibers, nerve ganglia and nerve endings.
- **Structurally,** nerve tissue consists of two cell types: **nerve cells** (neurons) and **glial cells.**

Neurons

- They are responsible for reception, transmission and processing of stimuli; the release of neurotransmitters; and triggering certain cell activities.
- Most of neurons consist of 3 parts; the **cell body** (perikaryon) which is the trophic center for the whole nerve cell and is also receptive to stimuli, the **dendrites** which are multiple processes and the **axon** which is a single process.


Dendrites
Nucleus
Nissle bodies
Perikaryon
Axon hillock
Axon
Oligodendrocyte
Collateral branch
C.N.S
P.N.S
Schwann cell
Myelin sheath
Node of Ranvier
Nerve endings

Classification of Neurons

- **According to number of processes:**

- **Unipolar:** have a single process that is close to the perikaryon and divides into 2 branches to form a T shape, with one branch extending to a peripheral ending to act functionally as a dendrite but its structure is similar to that of axon and the other towards the central nervous system to represent the axon.

- The stimuli that are picked by the dendrites travel directly to the axon without passing through the Perikaryon. It is found in the spinal ganglia(the sensory ganglia in the dorsal roots of the spinal nerves) and mesencephalic nucleus of trigeminal nerve.

- *NB: This type has been referred to, in the past, as a pseudounipolar neuron as from the functional point of view it is bipolar but this term has now been discarded.*

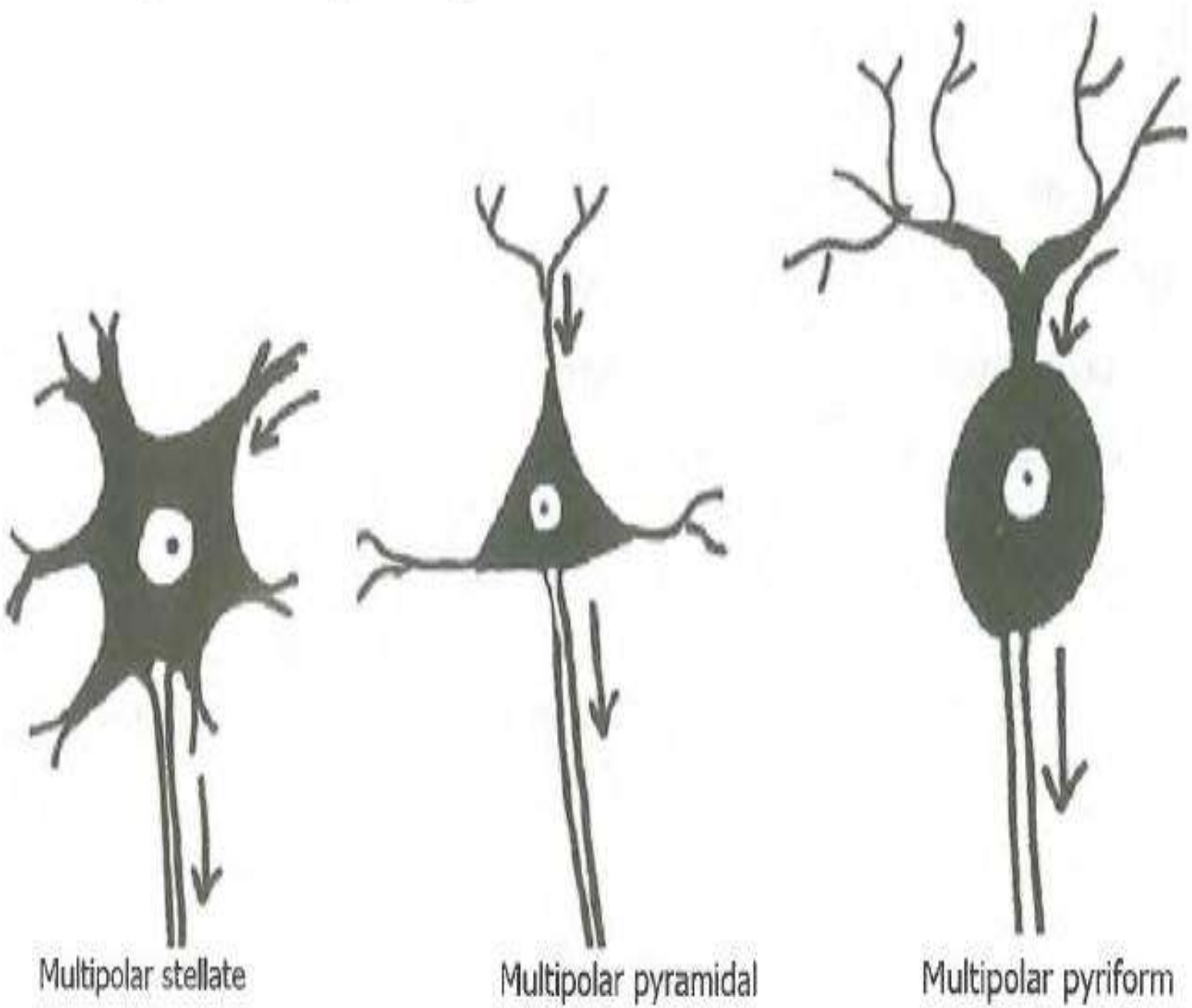
Unipolar

Bipolar: have one dendrite and one axon.

This type is present in cochlear and vestibular ganglia in ear, retina in eye and the olfactory mucosa.

Multipolar: have one axon and many dendrites. They take different forms:

- Stellate as the anterior horn cells in spinal cord.
- Pyramidal as pyramidal cells in cerebral cortex.
- Pyriform as purkinje cells in cerebellar cortex.

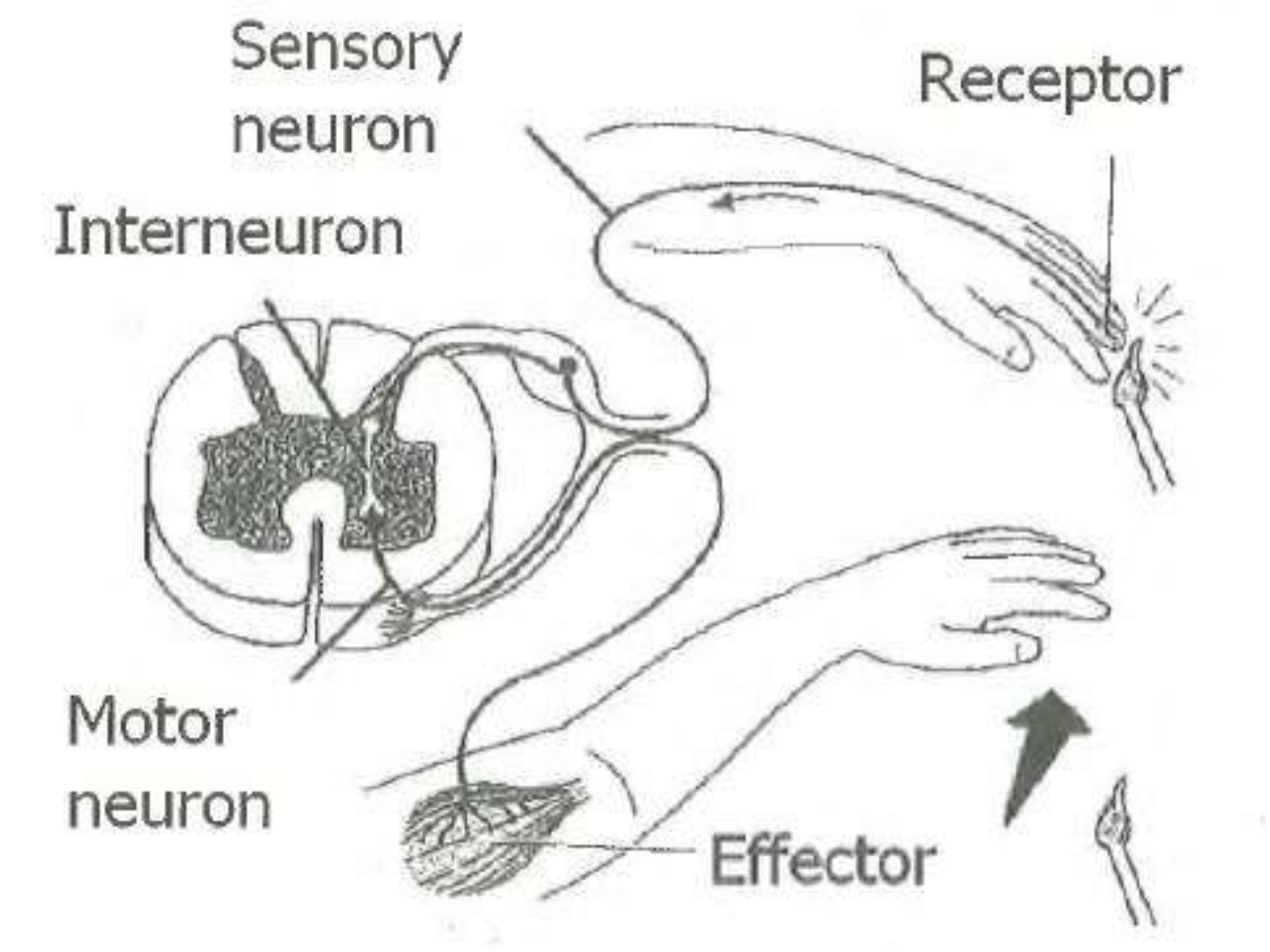

Multipolar stellate
Multipolar pyramidal
Multipolar pyriform

According to function:

Sensory (efferent) neurons receive sensory stimuli as cells of dorsal root ganglion.

Motor (afferent) neurons control effector organs such as muscles and glands as anterior horn cells in spinal cord.

Interneurons connect neurons as in retina and spinal cord.


Sensory neuron
Receptor
Interneuron
Motor neuron
Effector

According to length of axon:

Golgi type 1: neurons have long axon that leaves the grey matter and enters white matter as motor neurons in spinal cord, pyramidal cells in cerebral cortex and purkinje cells in cerebellar cortex.

Golgi type 2: neurons have short axon that does not leave the area of Perikaryon and never enters the white matter as in interneurons in cerebral and cerebellar cortex.

Structure of Neurons

Cell body (Perikaryon):

It is the part of the neuron containing the nucleus and surrounding cytoplasm.

Size: varies from 4 μ*m* as in granular cells in cerebellar cortex to 100 μm as in motor neurons in spinal cord.

Shape: depends on the number of cell processes;

- Pseudounipolr have globular shape.

- Bipolar have fusiform shape.

- Multipolar are stellate, pyramidal or pyriform.

Nucleus:

It is usually large spherical, euchromatic with a prominent nucleolus reflecting the intense synthetic activity of these cells.

Cytoplasm:

1. It contains highly developed **rough endoplasmic** reticulum and numerous **polyribosomes** suggesting that these cells synthesize both structural proteins and proteins for transport. When stained, rough endoplasmic reticulum and free ribosomes and polysomes appear under the light microscope as basophilic granular areas called **Nissl bodies.** Their number varies according to neuronal type and functional state. They are abundant in large nerve cells as motor neurons

2. The **Golgi** complex consists of multiple parallel smooth cistemae around the periphery of the nucleus.

3. Mitochondria are scattered throughout the cytoplasm.

4. Neurofilaments (intermediate filaments with a diameter of 10 nm) are abundant in Perikaryon and processes. They bundle together as a result of the action of fixatives to form neurofibrils (2 ɱm in diameter) that are visible by the light microscope (stained brown by Ag). They provide structural support.

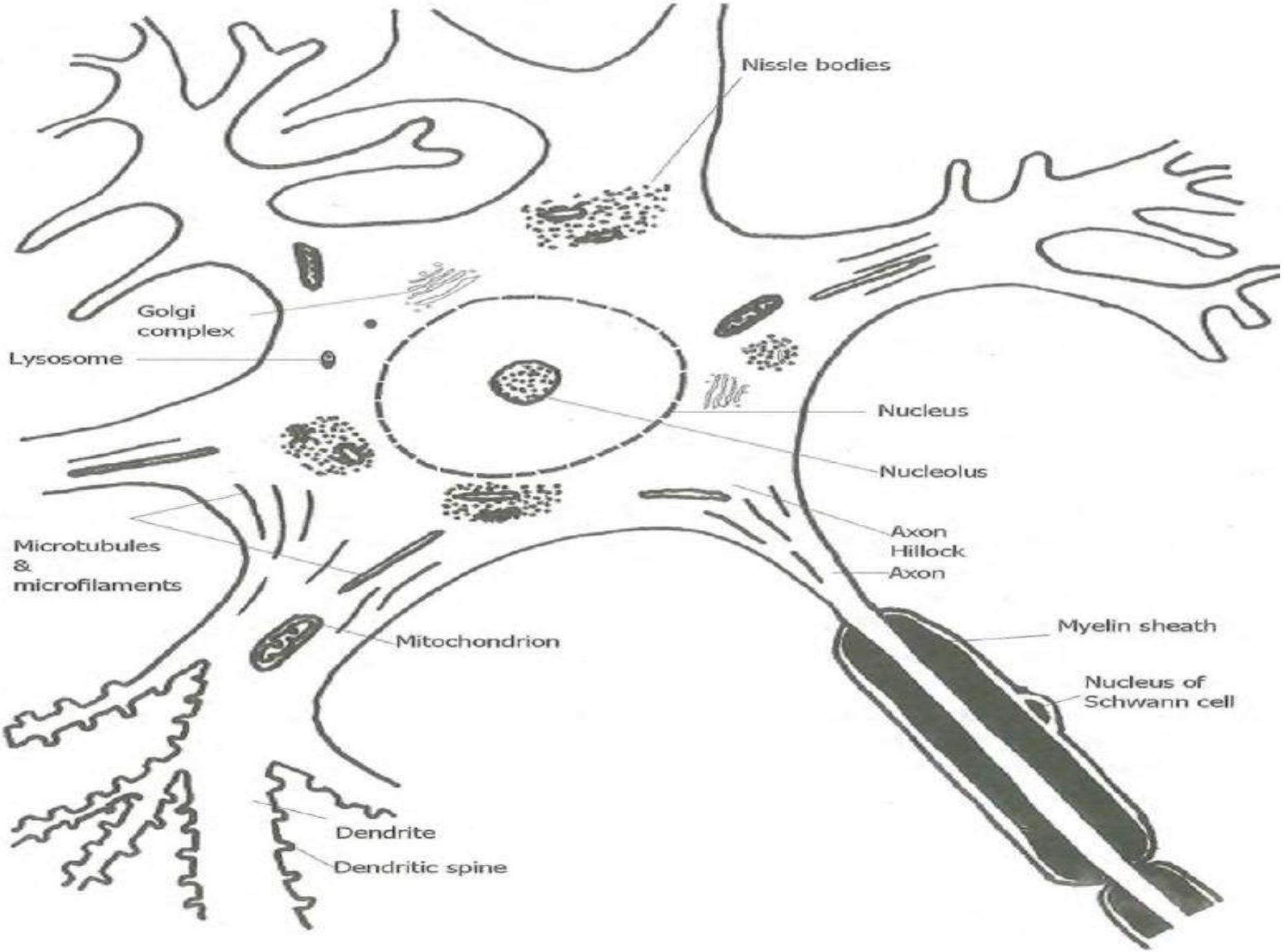
5. **Microtubules** (20-28 nm in diameter) are arranged in parallel bundles in Perikaryon and processes. They are involved in axonal transport of neurotransmitter substances, enzymes and other cellular constituents.

6. **Centrioles** cannot be seen as neurons can not divide.

7. Inclusions in the form of:

a. Lipofuscin pigment which is golden brown. It is a residue of undigested material by lysosomes. Its amount increases with aging.

b. Melanin pigment and **iron** containing pigment are present in cells of substantia nigra in mid brain.


Nissle bodies
Golgi complex
Lysosome
Nucleus
Nucleolus
Microtubules & microfilaments
Axon Hillock
Axon
Myelin sheath
Mitochondrion
Nucleus of Schwann cell
Dendrite
Dendritic spine

Processes:

Dendrites	Axon
1. Usually numerous.	1. Single.
2. Short.	2. Long.
3. Thick.	3. Thin.
4. Branching like a tree. Branches arise at acute angle.	4. Not branching except at the end. It may give collateral branches near the cell body that arise at right angle.
5. Become thinner as they subdivide into branches.	5. Has a constant diameter.
6. Contain Nissl bodies.	6. Does not contain Nissl granules.
7. Covered by spine like processes called dendritic spines that are specialized for synaptic contacts.	7. No spines.
8. Carry nerve impulses to the cell body.	8. Carries nerve impulses away from the cell body.

Both the dendrites and axon have mitochondria, neurofibrils and microtubules.

Nerve Fiber:

A nerve fiber consists of an **axon.** The axon is covered by axolemma and contains cytoplasm called axoplam. It arises from a conical extension of the cell body called axon hillock.

Types of nerve fibers:

Unmyelinated nerve fibers: have no myelin sheath. It is subdivided into;

1. Unmyelinated fibers without sheath of Schwann cells (neurolemma) as in gray matter (naked)
2. Unmyelinated nerve fibers with sheath of Schwann cells as in sympathetic post ganglionic fibers.

Myelinated nerve fibers: have myelin sheath. It is subdivided into;

1. Myelinated nerve fibers without sheath of Schwann cells as in white matter .
2. Myelinated nerve fibers with sheath of Schwann cells as in peripheral nerve fibers .

Ganglia

- They are collections of nerve cells outside the central nervous system.
- They are of 2 types sensory (spinal) and autonomic (sympathetic and parasympathetic).

Differences between spinal and sympathetic ganglia:

Spinal ganglion	Sympathetic
1. Covered by thick connective tissue capsule.	1. Covered by thin connective tissue capsule.
2. Blood vessels are less.	2. Blood vessels are more.
3. Cells are pseudounipolar.	3. Cells are stellate multipolar.
4. Cells have glomeruli formed by coiling of the axon around the cell body before splitting in a T form.	4. No glomeruli.
5. Cells are variable in size.	5. Cells are uniform in size.
6. Cells are larger.	6. Cells are smaller.
7. Cells are surrounded by large number of satellite cells.	7. Few satellite cells.
8. Cells are arranged in groups or rows.	8. Cells are scattered.
9. Cells are separated by myelinated nerve fibers.	9. Cells are separated by umyelinated nerve fibers.
10. No synapse between cells.	10. Synapse is present.