



Универзитет у Крагујевцу  
Факултет медицинских наука  
Интегрисане академске студије медицине  
Катедра за Хистологију и ембриологију

# Intro and CYTOLOGY

**Week 1**

# HISTOLOGY AND EMBRYOLOGY

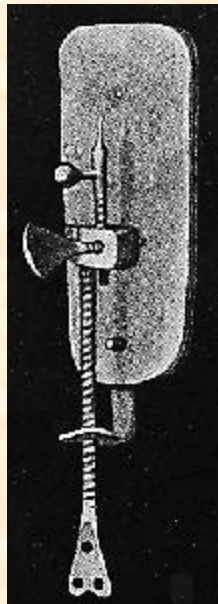
	Textbook	Authors	Publisher	In the library
1	<u>Junqueira's Basic Histology Text and Atlas, 16e</u>	Anthony L Mesher	McGraw Hill, 2021	Yes
2	Gartner & Hiatt's Atlas and Text of Histology Eighth edition, International Edition	Leslie Gartner, Lisa Lee	LWW Lippincott Williams and Wilkins 2022	
3	Histology: A Text and Atlas: With Correlated Cell and Molecular Biology, 6th edition	Michael Ross, Wojciech Pawlina	Lippincott Williams & Wilkins, 2006	Yes
4	<u>The Developing Human : Clinically Oriented Embryology, 11th edition</u>	Keith Moore, TVN Persaud, Mark Torchia	Elsevier Health Sciences, 2018	Yes
5	<u>Workbook for LABS in histology and embryology for medical students</u>	Goran Ranković	Unigraf X copy, Niš, 2019	Yes

# A little bit of history

## Who discovered the cell?

In 1665, Robert Hook (1635-1703), an English microscopist and physicist, while looking at a piece of cork with his microscope saw many small, empty compartments. He named this with the Latin name "cell", that is a small compartment, bearing in mind the comparison with a honeycomb.

In 1648, Van Leeuwenhoek and his first simple microscope in Amsterdam. Allowed him to magnify up to 275 times. It is believed that around 1665 he read Robert Hooke's book, "Micrographia", and that it was this that prompted him to think that his invention could be used for more important things than making textiles.





Finally, who wrote the first book on Histology?

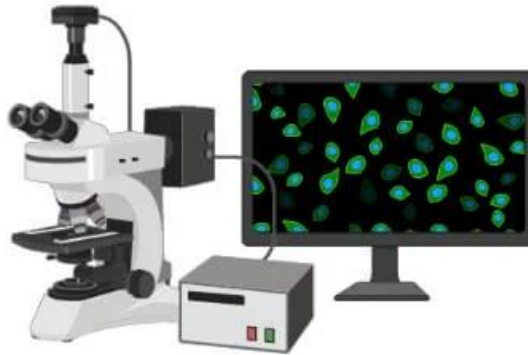
Rudolph von Kölliker (1817-1905), a Swiss professor wrote—“Handbuch der Gewebelehre” (i.e. Book for the Study of Tissues), which was published in 1852, so some authors consider him the **founder of Histology**.



# Microscopes



**Light Microscope**



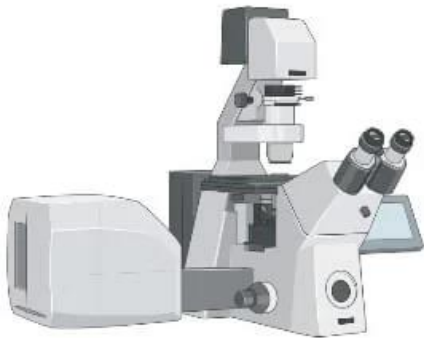
**Fluorescence Microscope**



**Electron Microscope**



**Stereo Microscope**



**Confocal Microscope**



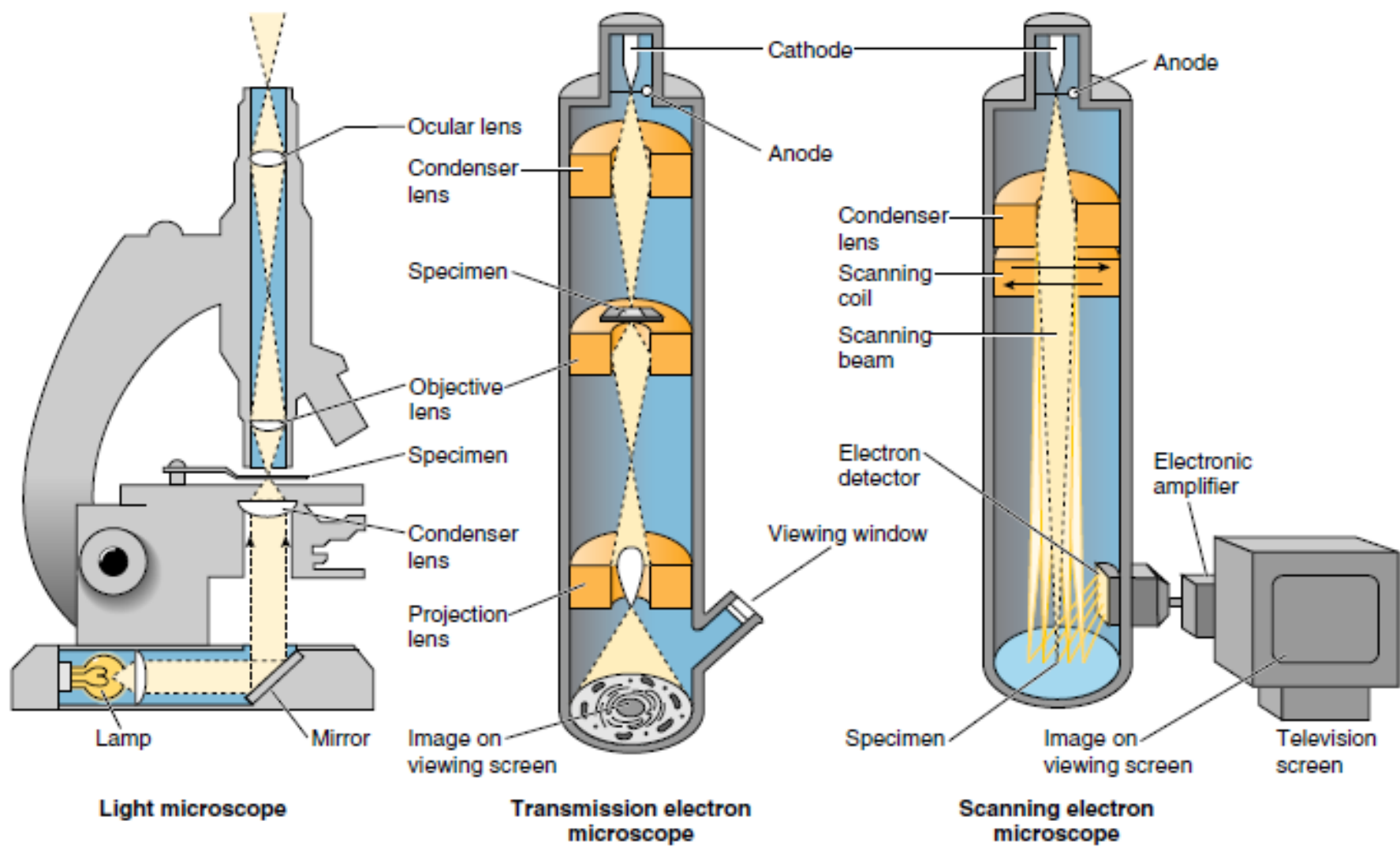
**Atomic Force Microscope**



**Inverted Microscope**



**Retinal Imaging Microscope**





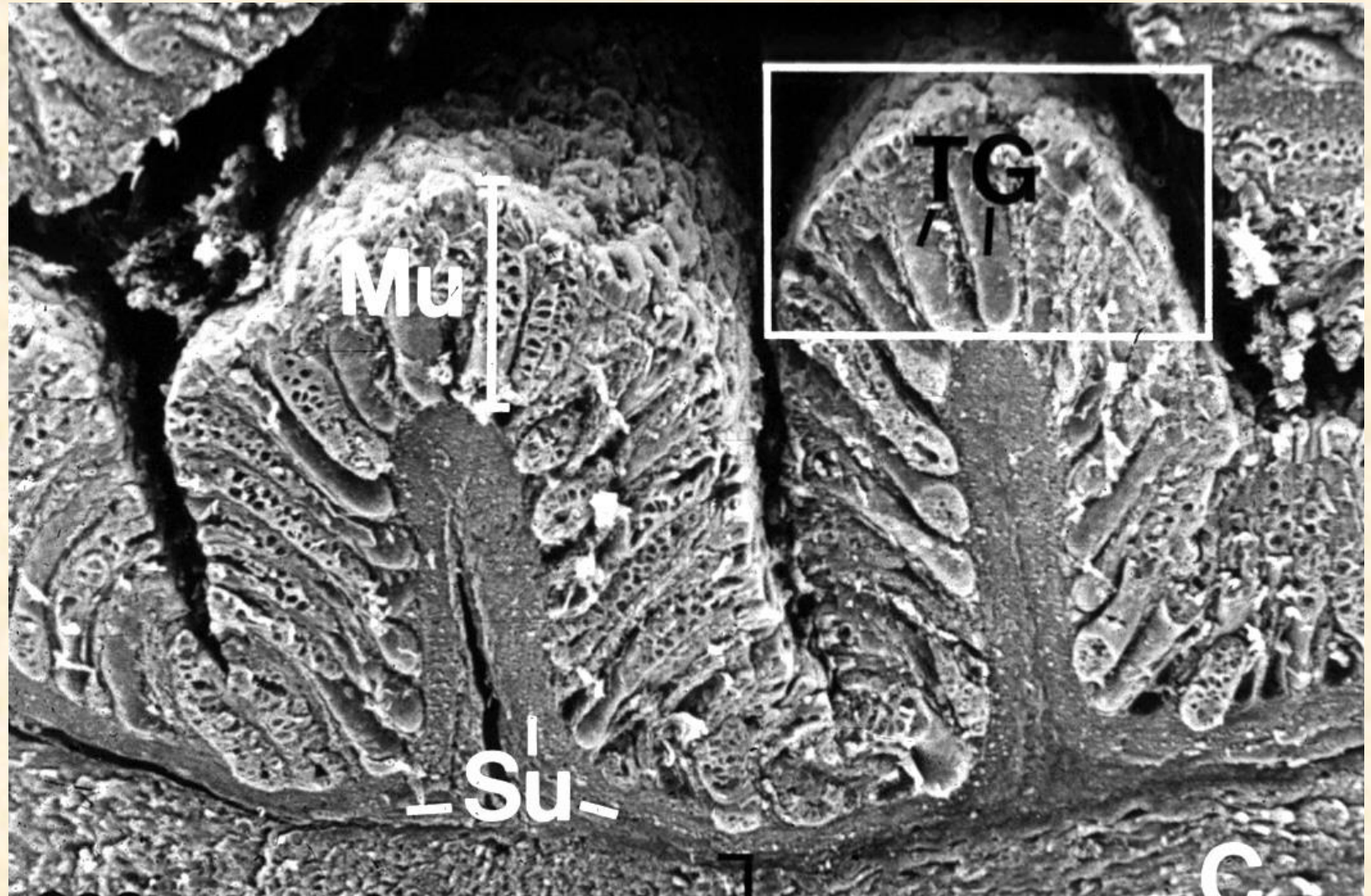
# Light







# SEM

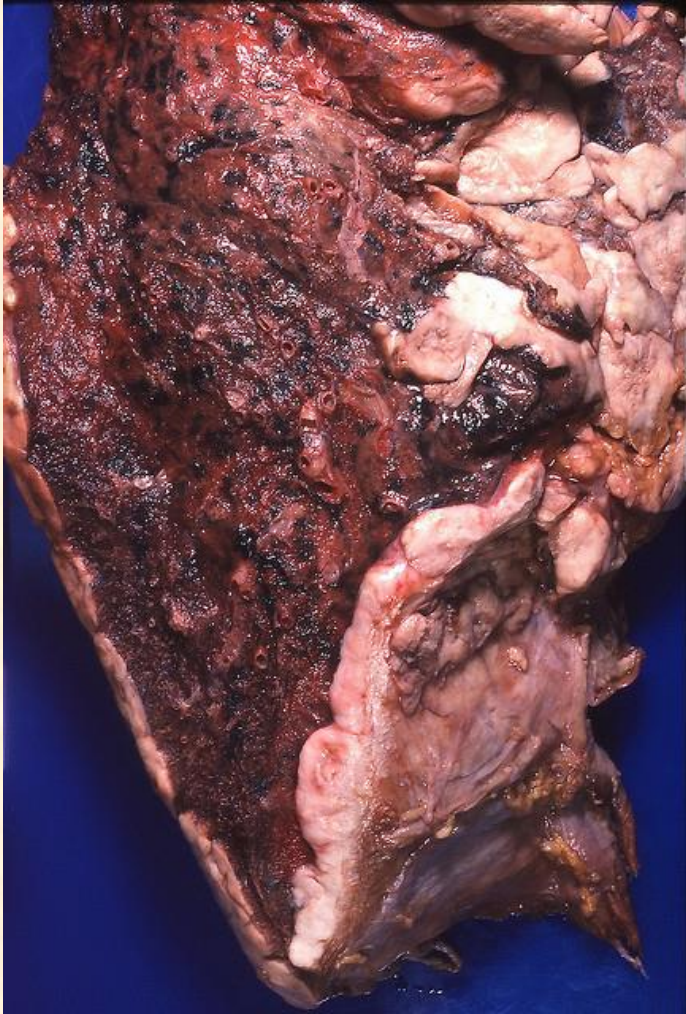




Tissue processing



# Specimens come to the lab fresh or in formalin.



Fresh lung specimen

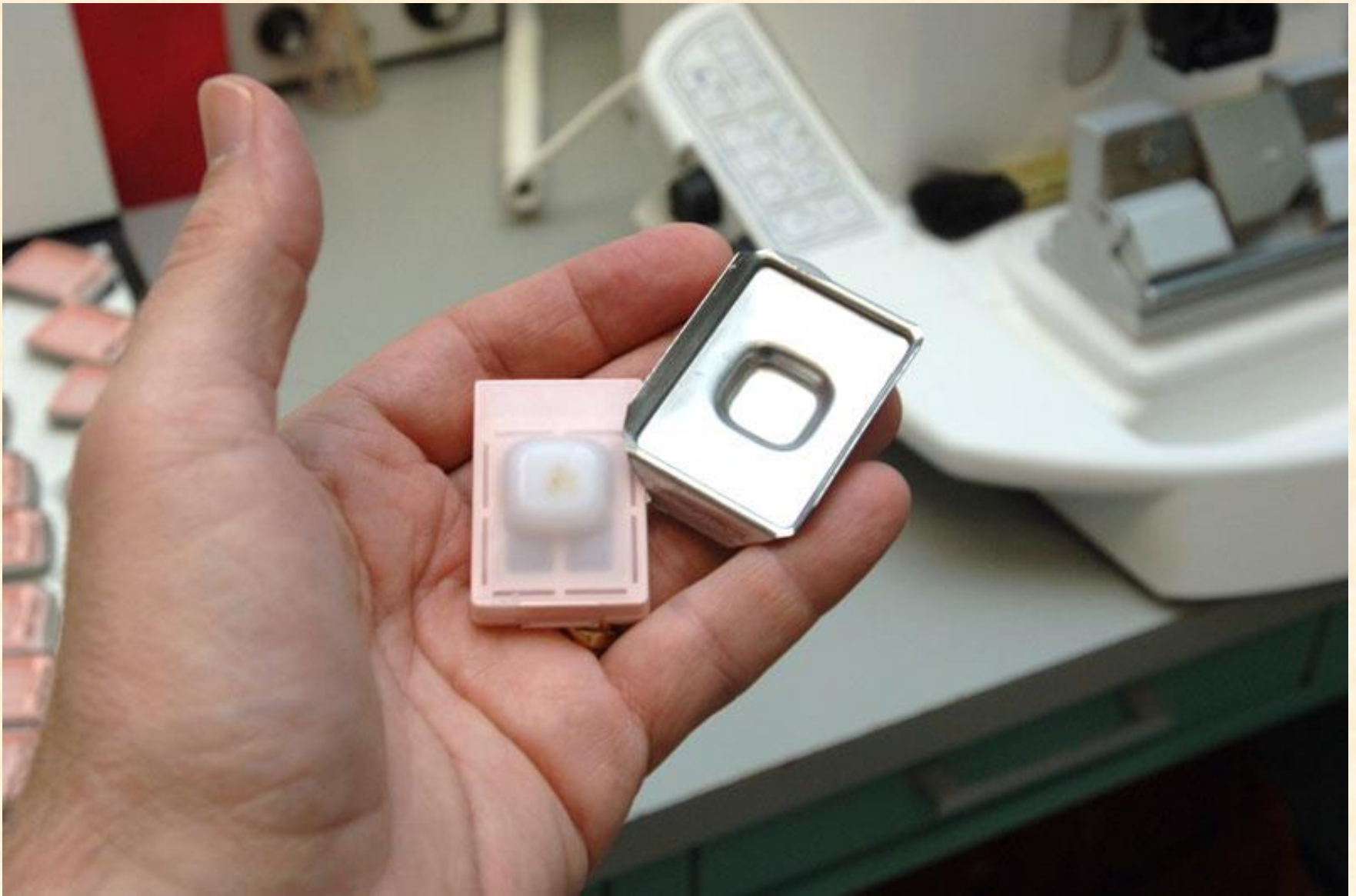


Formalin container for specimen



**The specimen is placed in a “cassette” that goes into an automated processor for tissue fixation.**





**The specimen is embedded in paraffin...**



**...and then cut into super thin slices.**



**The tissue is transferred onto a slide...**





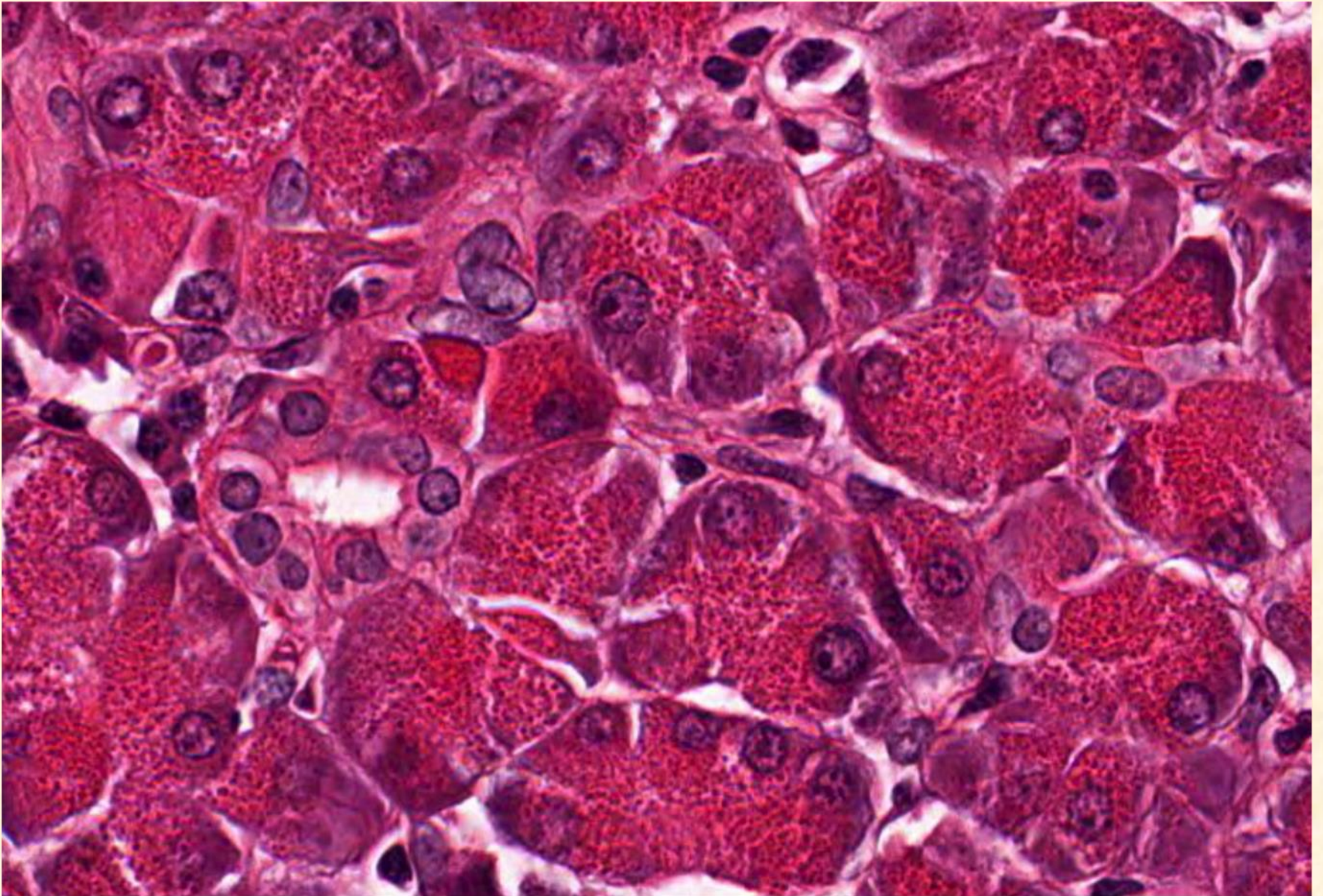
**...and then stained and coverslipped.**

Most tissue specimens are stained with hematoxylin and eosin.

Most blood smears are stained with the Wright-Giemsa stain.

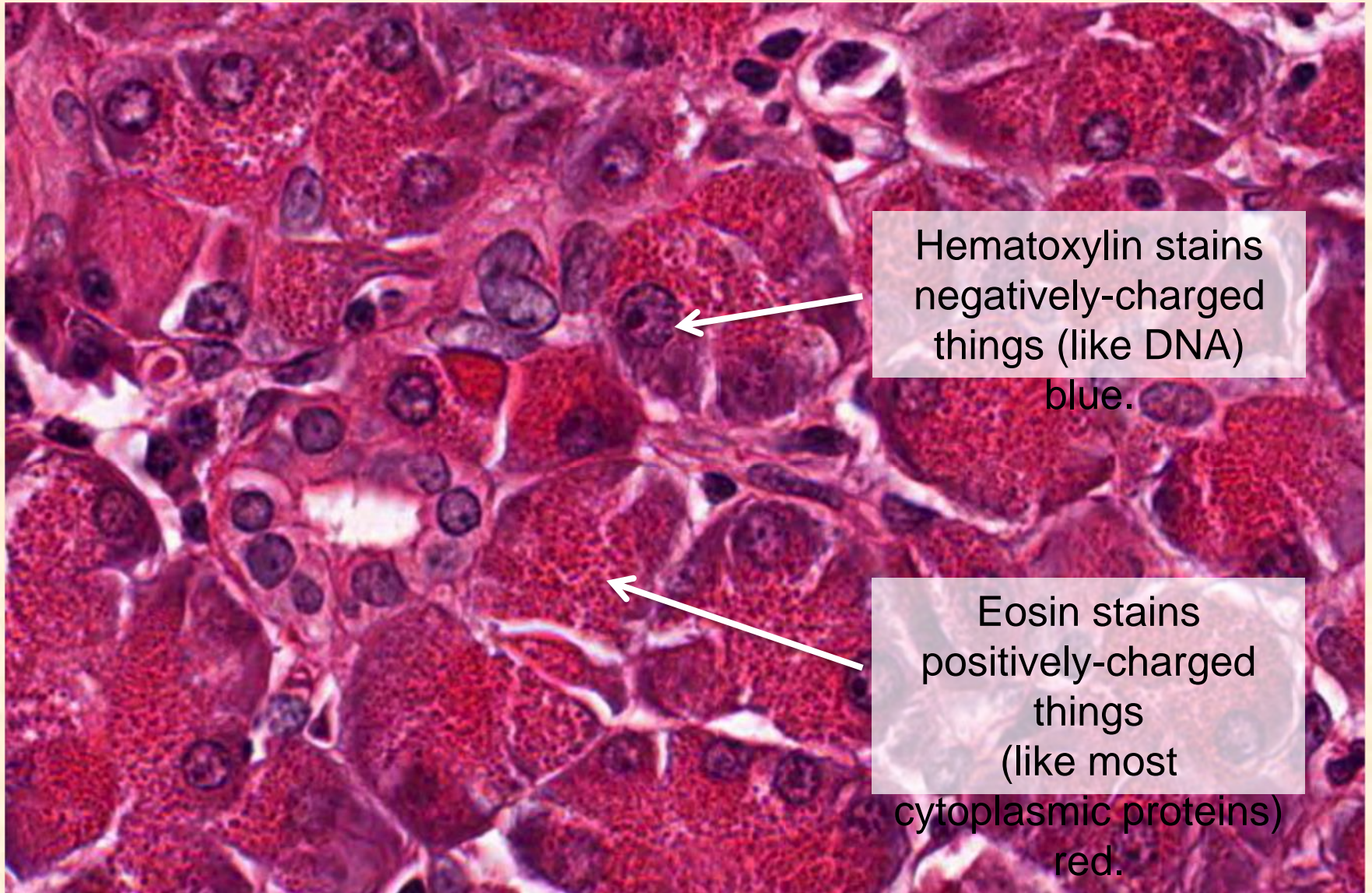
Other stains (like silver, PAS, and acid-fast stains) can be used to highlight special cells or features.





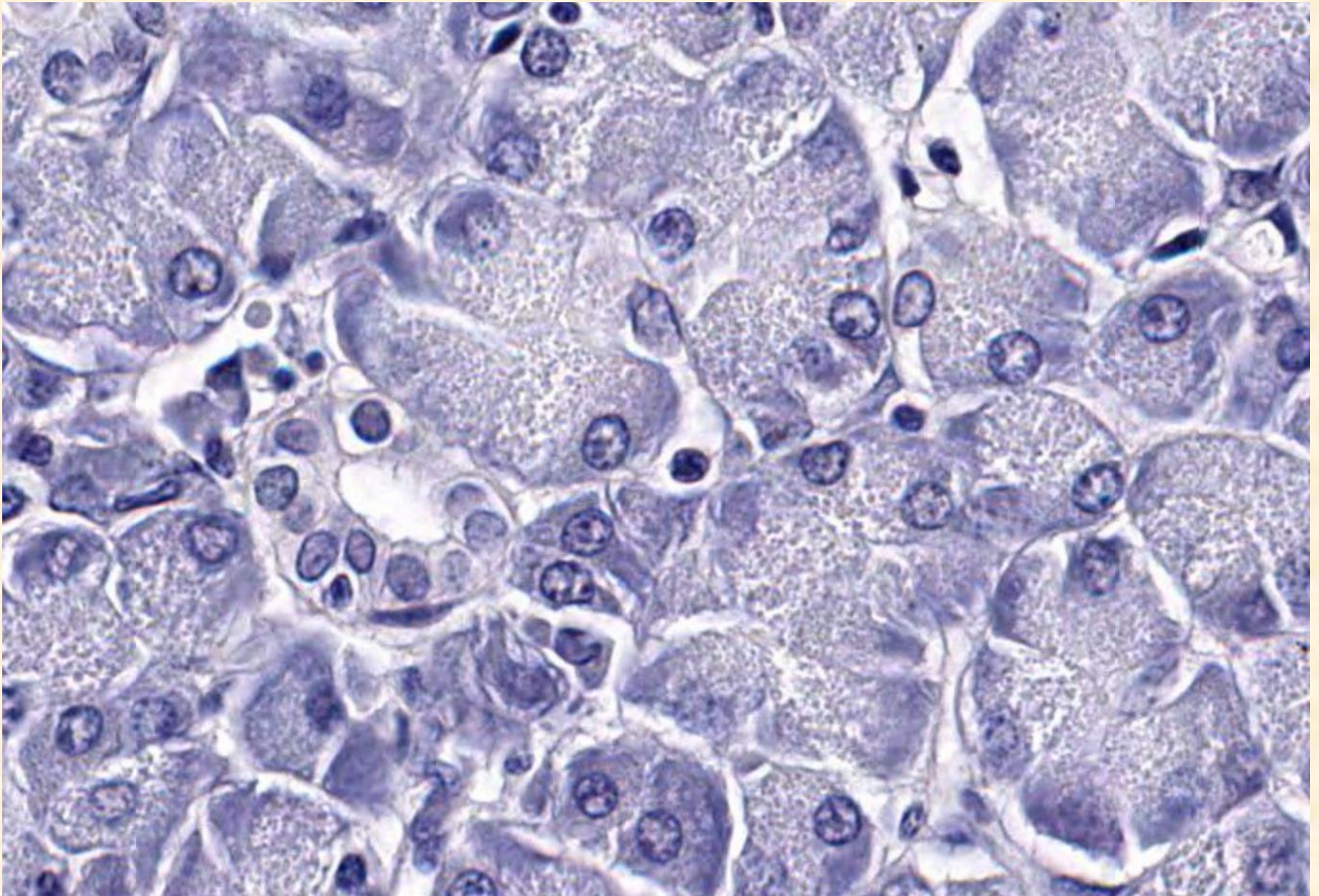
Section of pancreas stained with hematoxylin and eosin





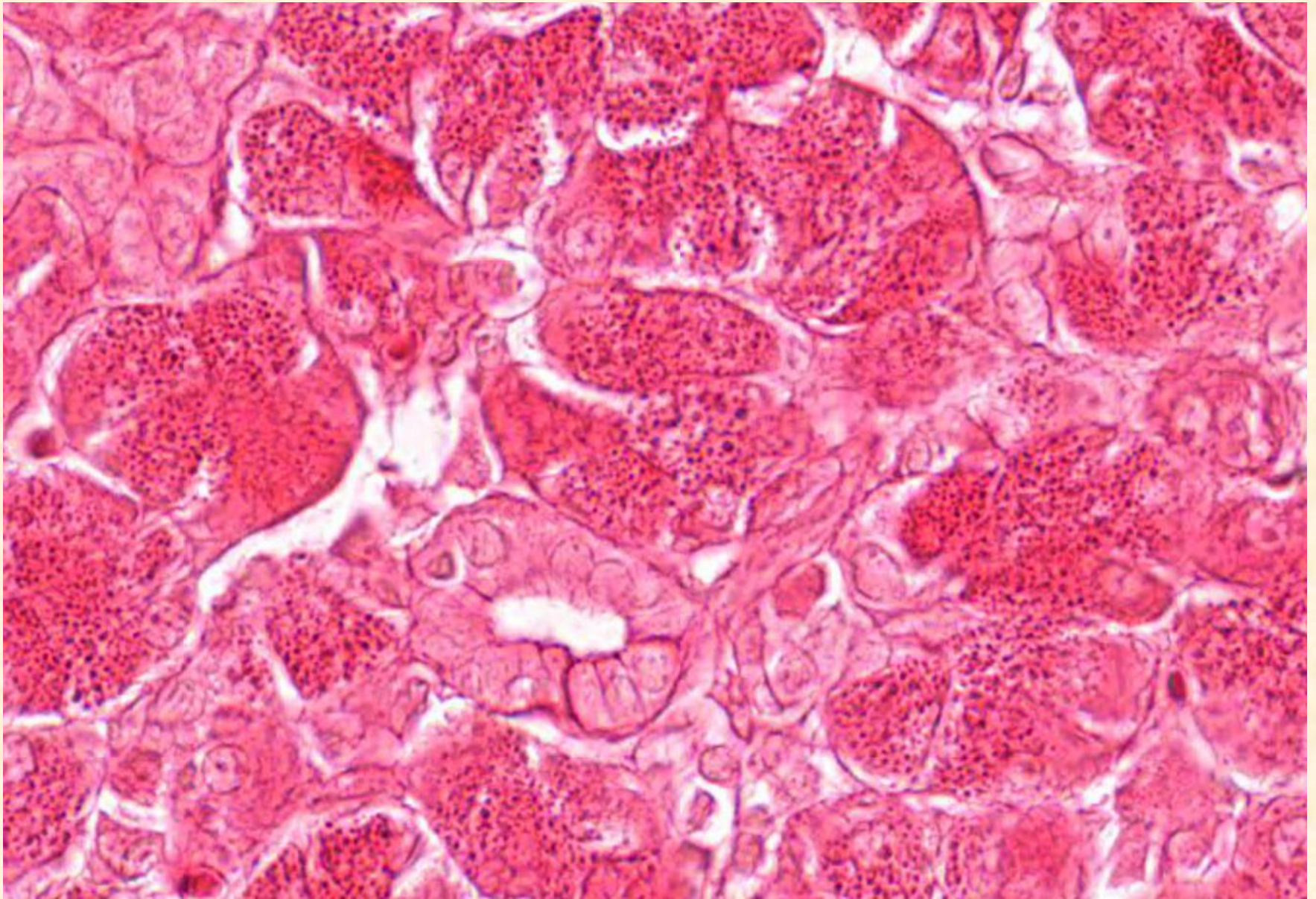
Hematoxylin (blue) is positively charged.  
Eosin (red) is negatively charged.





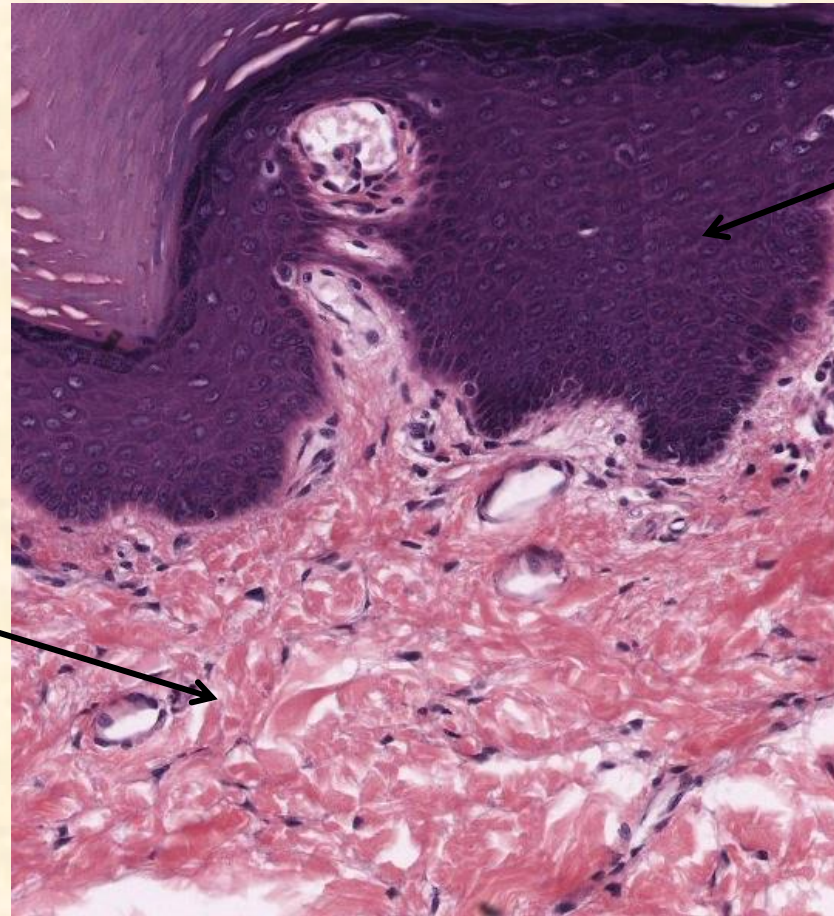
Section of pancreas stained with hematoxylin only





Section of pancreas stained with eosin only

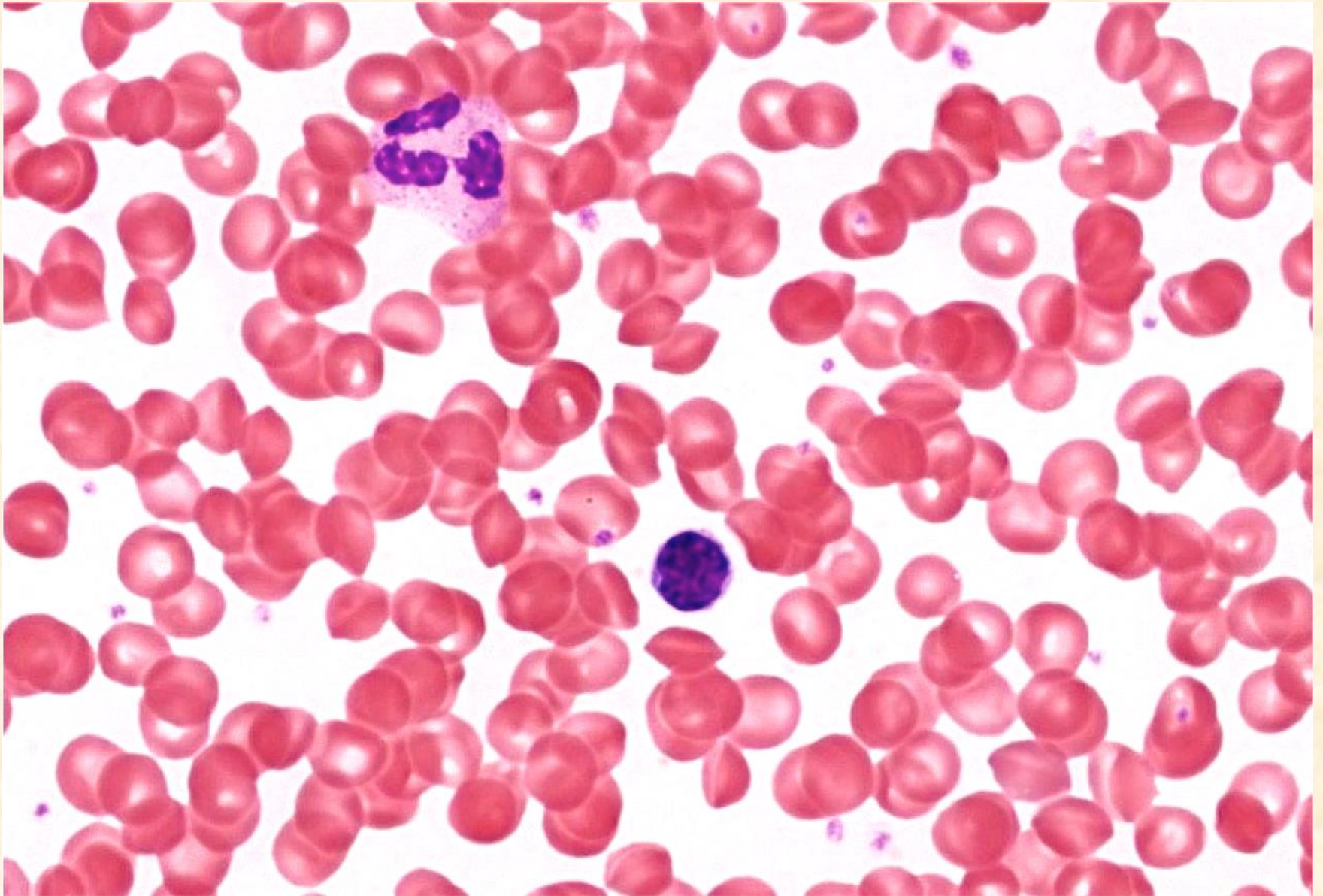




Cell nuclei  
stain purple  
with  
hematoxylin.

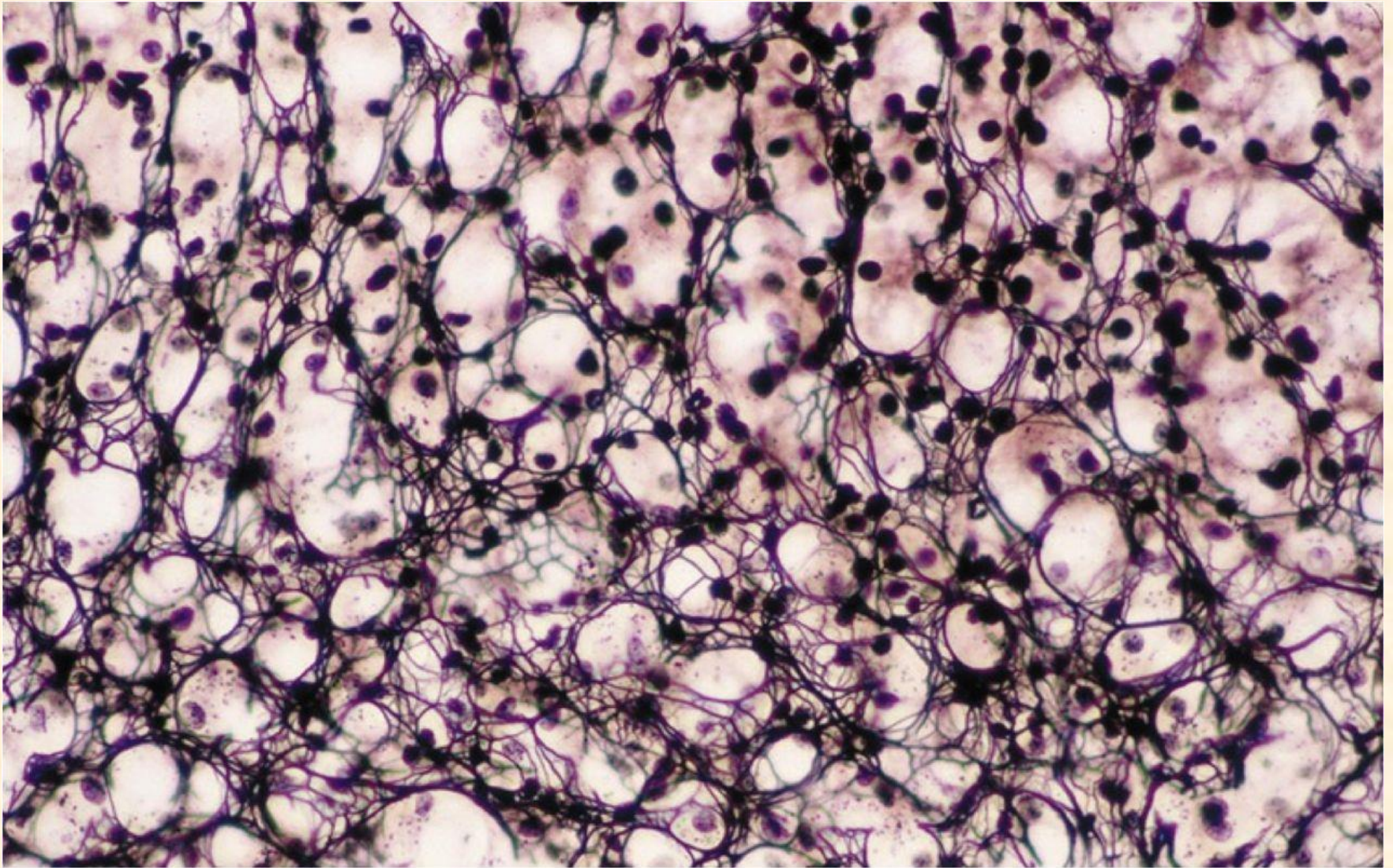
Collagen  
stains pink-red  
with eosin.

Section of skin stained with hematoxylin and  
eosin



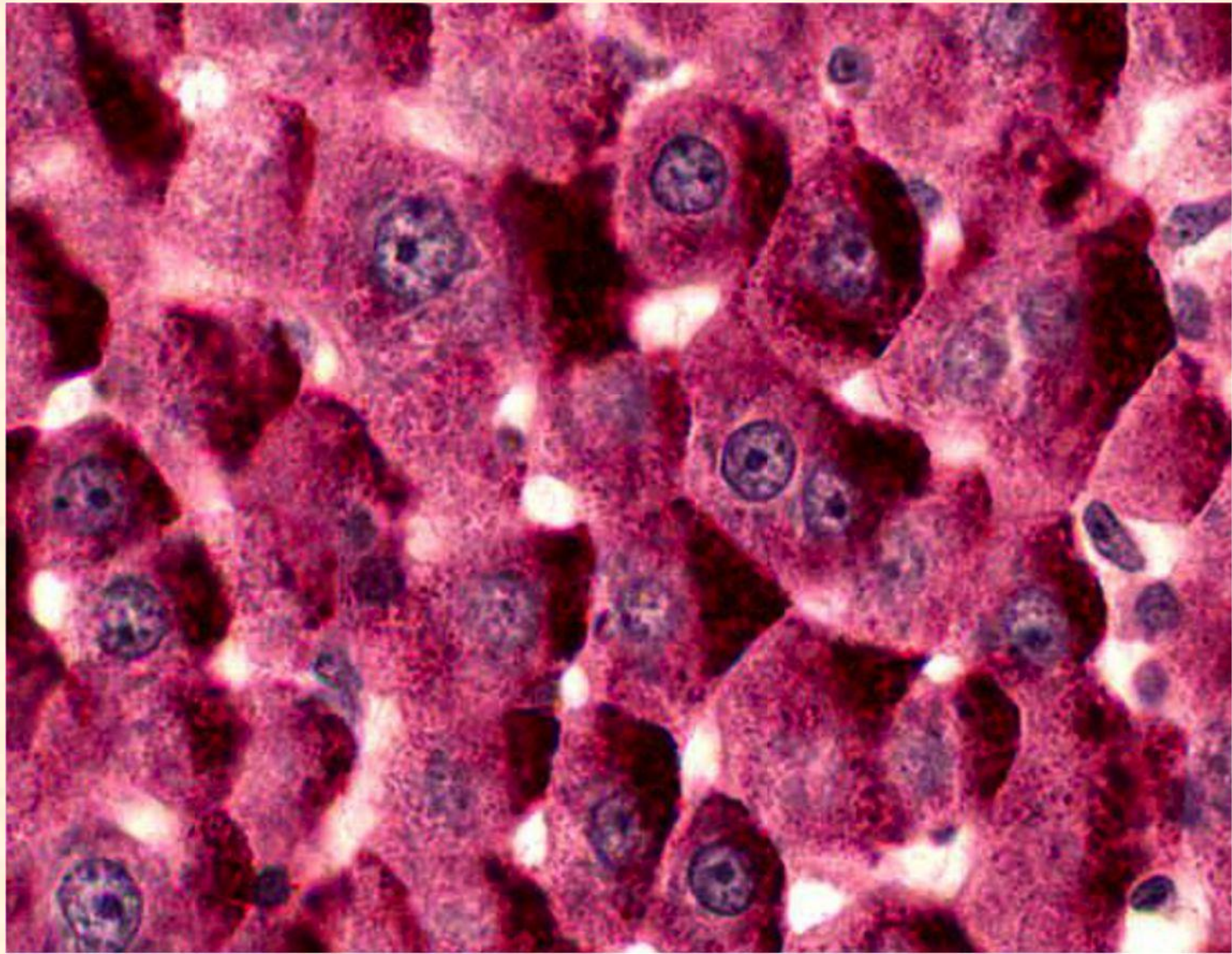
Blood smear stained with Wright-Giemsa stain





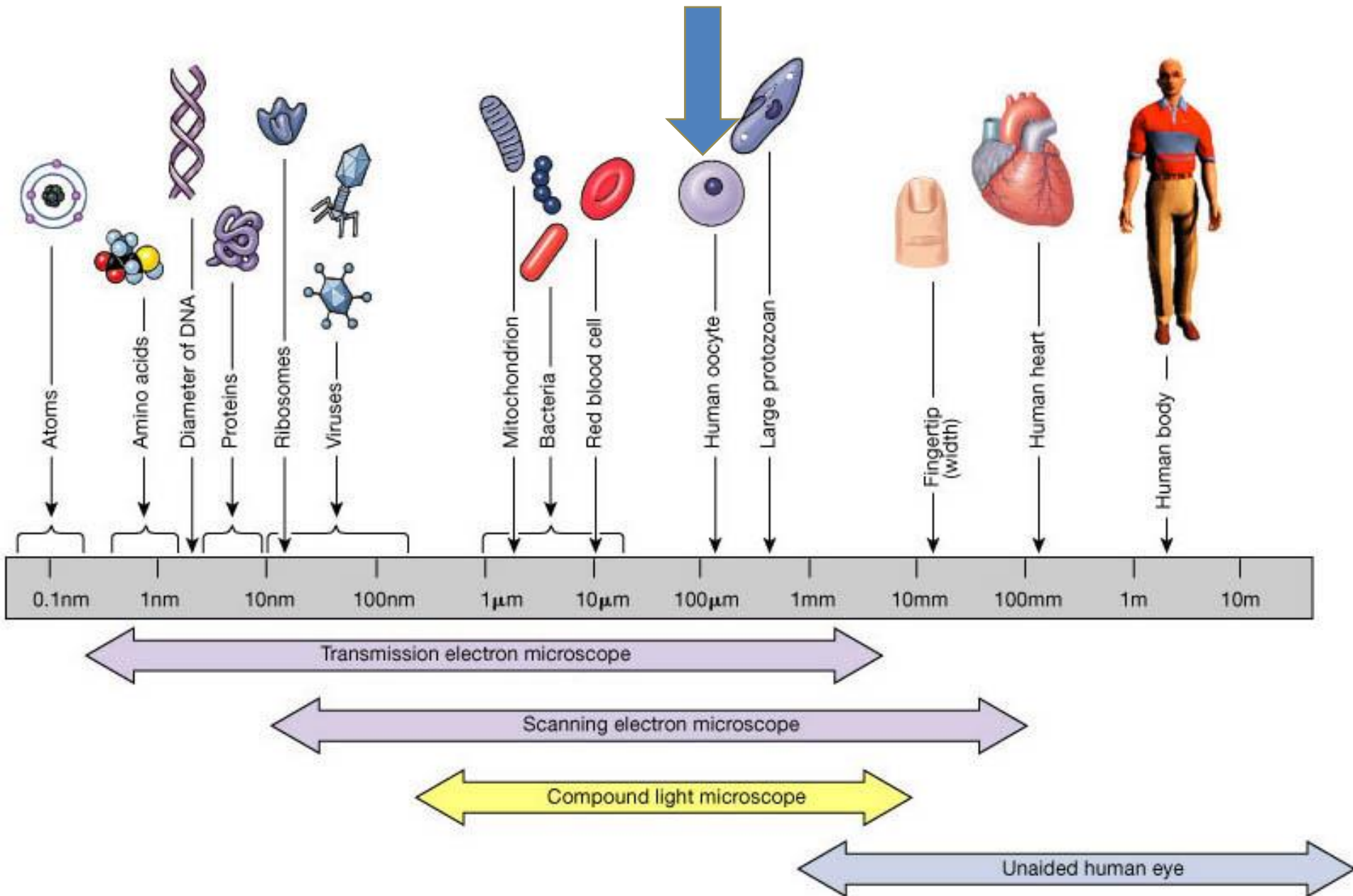
**The silver stain also stains reticulin fibers  
(this is a lymph node).**





The periodic acid-Schiff (PAS) stain highlights sugars (this is a liver section with a lot of glycogen).

# What human eye can see



# So many cell sizes and shapes!

The biggest cells are motor neurons, which can be up to 80  $\mu\text{m}$  in diameter.

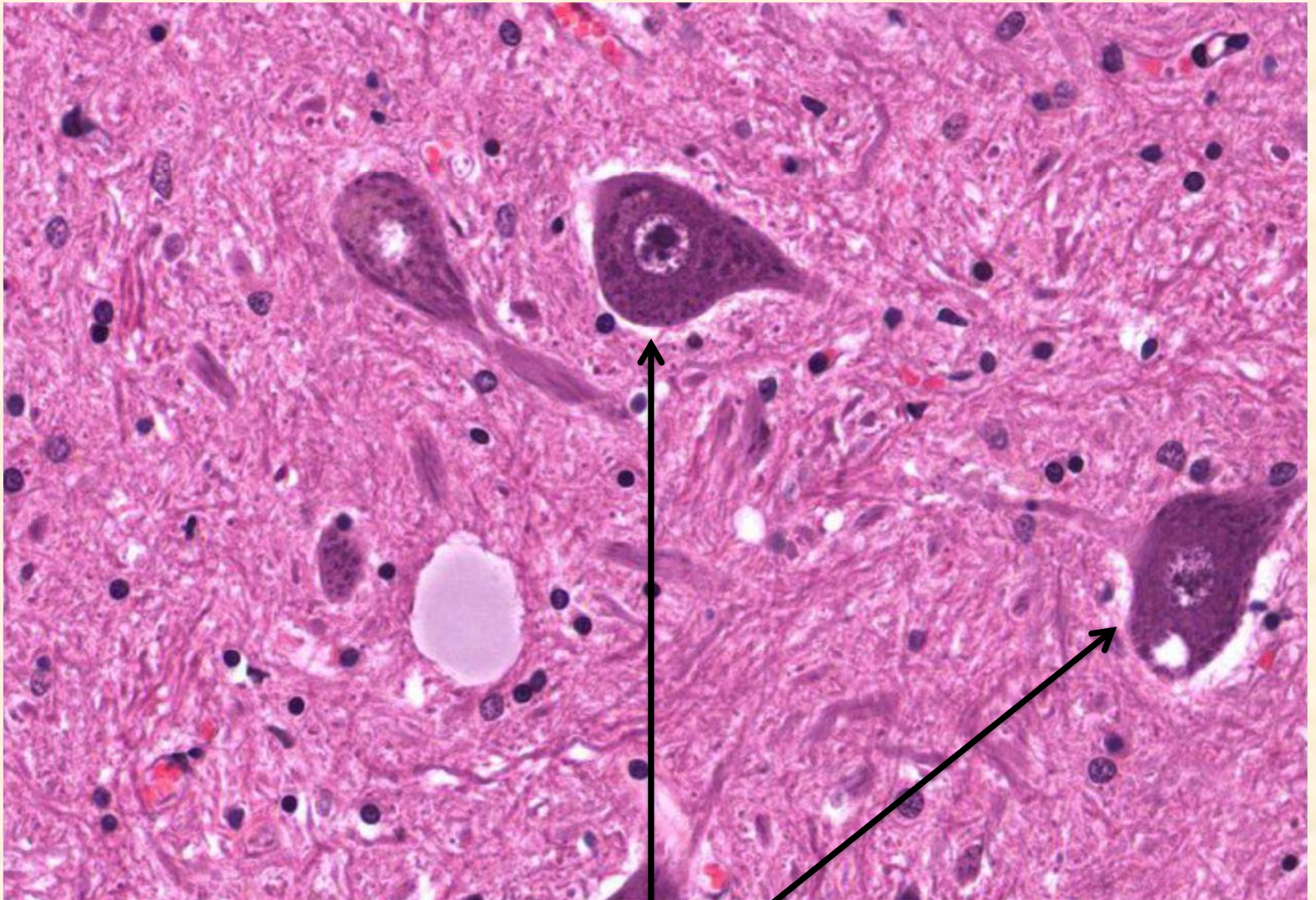
Most cells are somewhere between 10 and 30  $\mu\text{m}$ .

Some cells are quite small (red cells are about 7  $\mu\text{m}$ ).

Cell shapes vary from round to hexagonal to flat

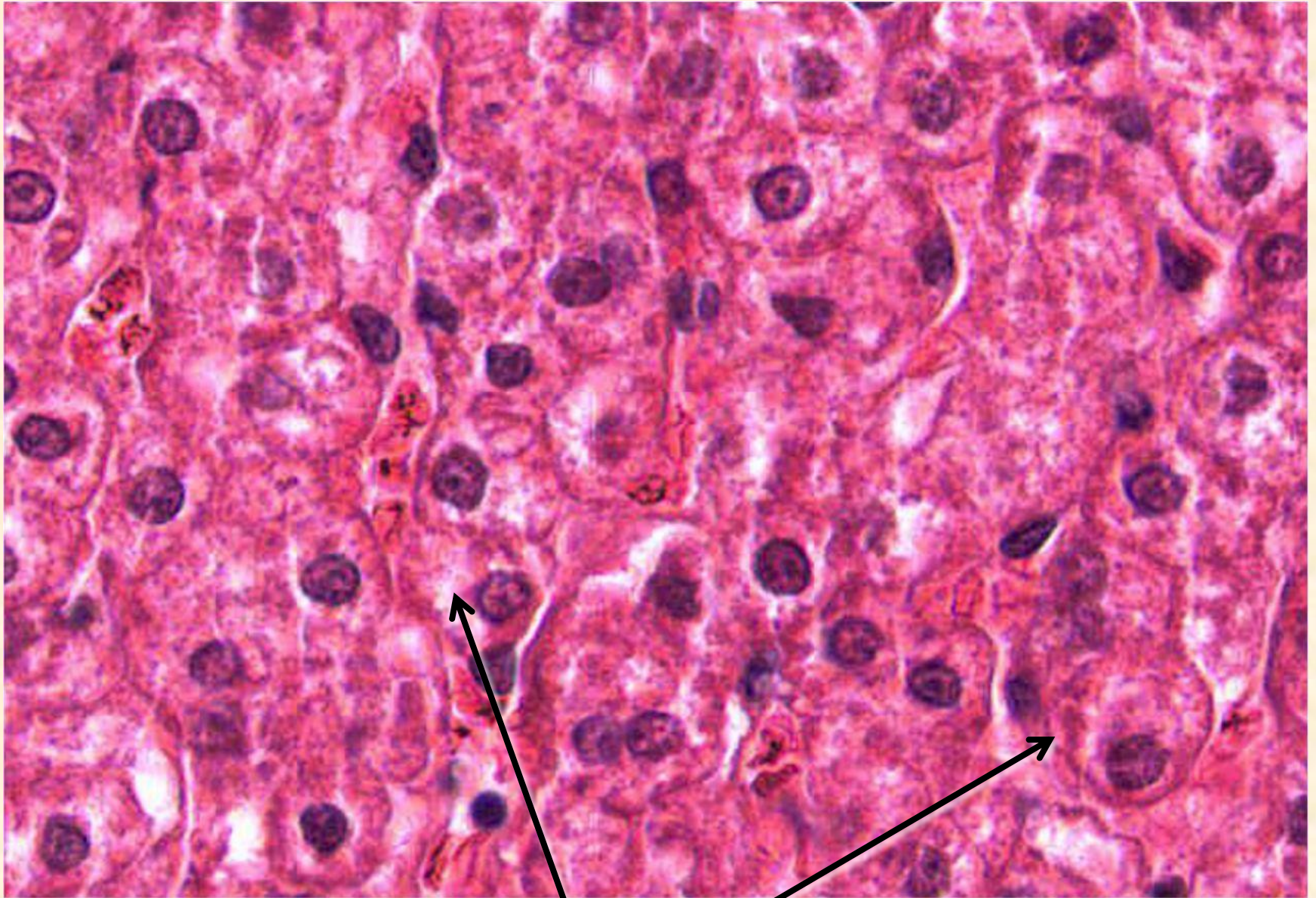
But REALLY...the largest cell, visible by the naked eye is 200 $\mu\text{m}$  in diameter ..... fertilized egg cell





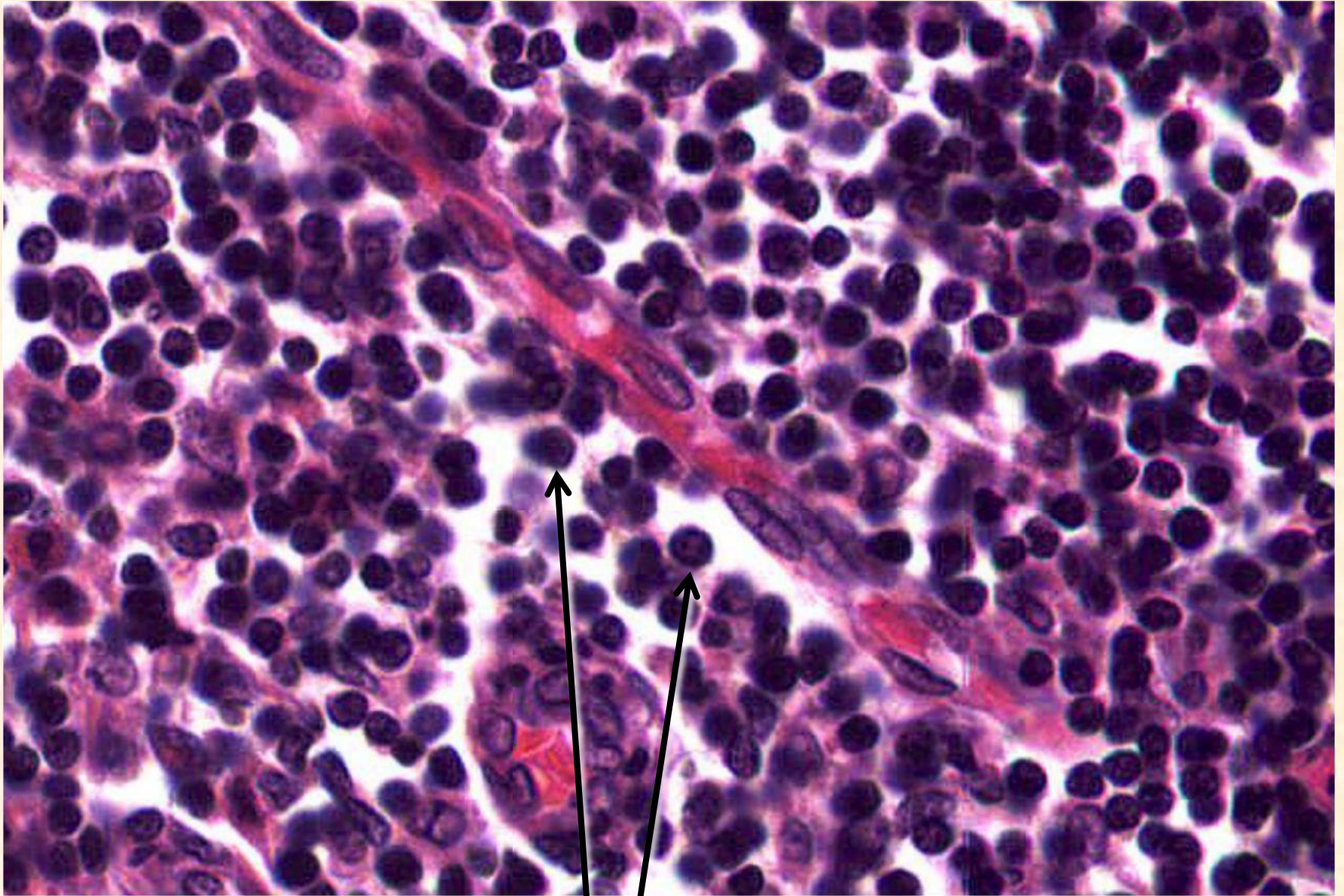
Motor neurons (about 50-80  $\mu\text{m}$ )





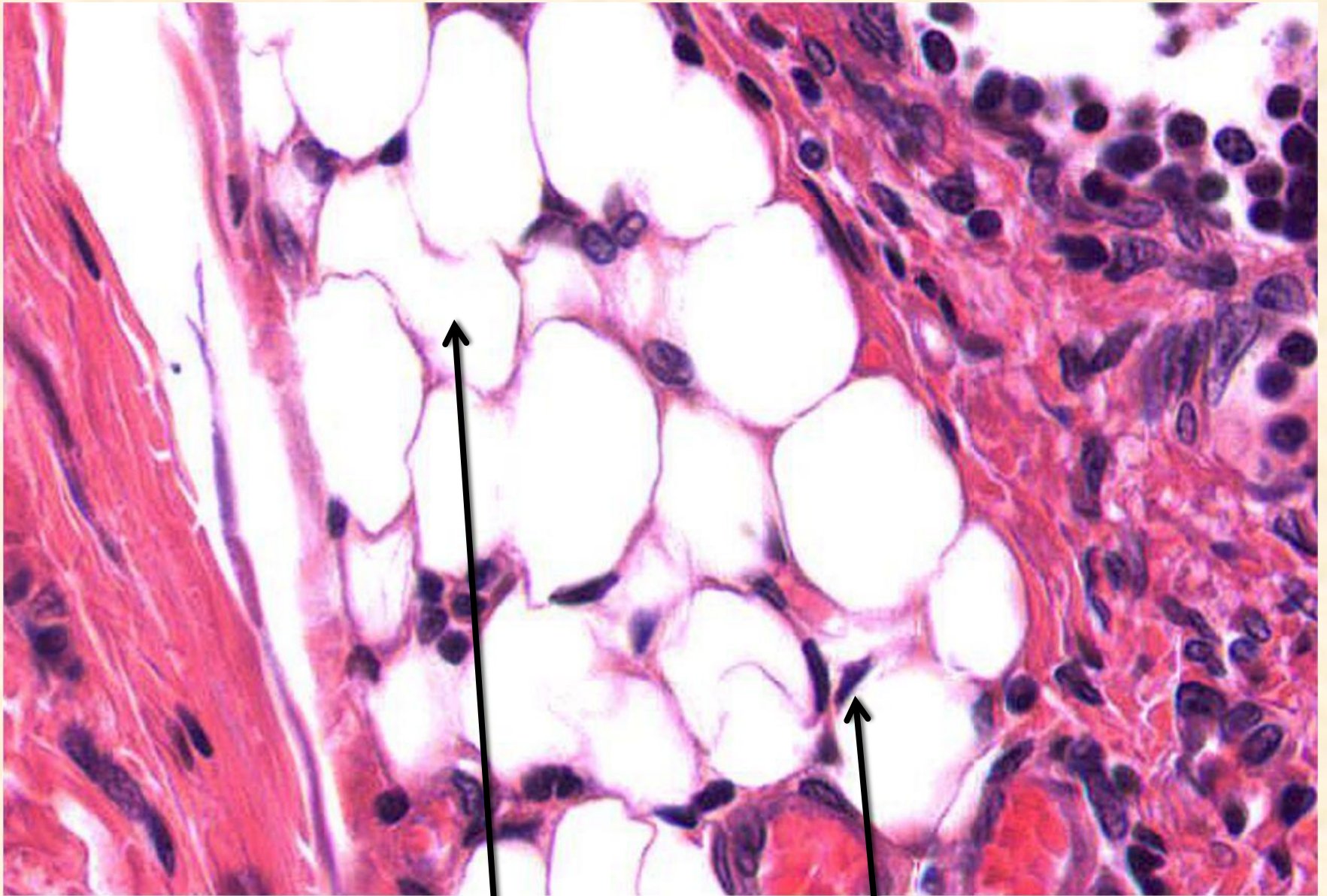
Hepatocytes (about 25  $\mu\text{m}$ )





Lymphocytes (about 9  $\mu\text{m}$ )

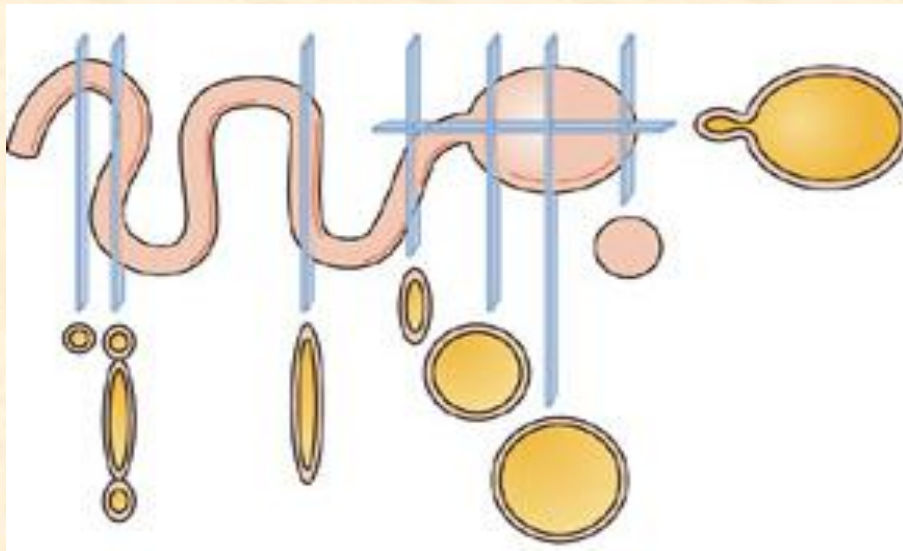




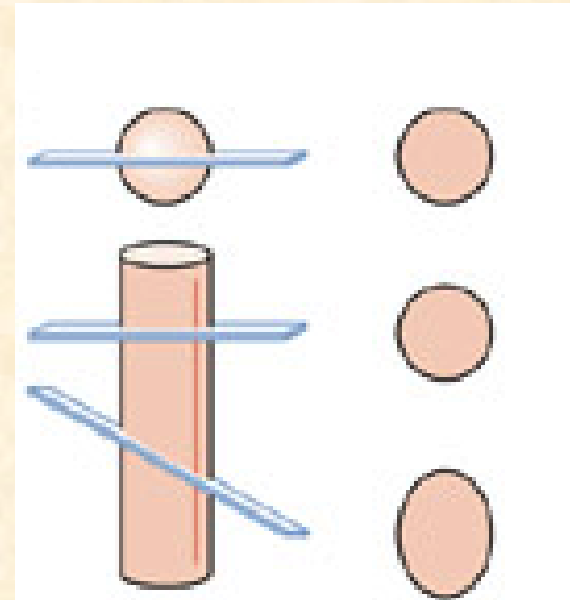
Adipocytes and their nuclei



**Something else to remember:  
slides show 2D images of 3D structures**

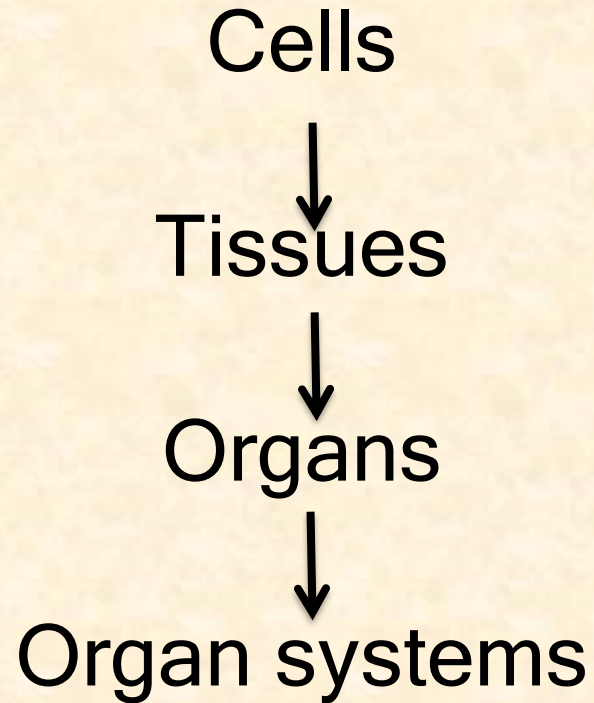


Hollow ball and tube



Solid ball and cylinder

# Histologic Hierarchy



# Cell

- Cells are the basic morphofunctional units of all living organisms.
- There are two fundamentally different types of cells, but they show so many biochemical similarities that some researchers hypothesize that one type evolved from the other:

## Prokaryotic cells

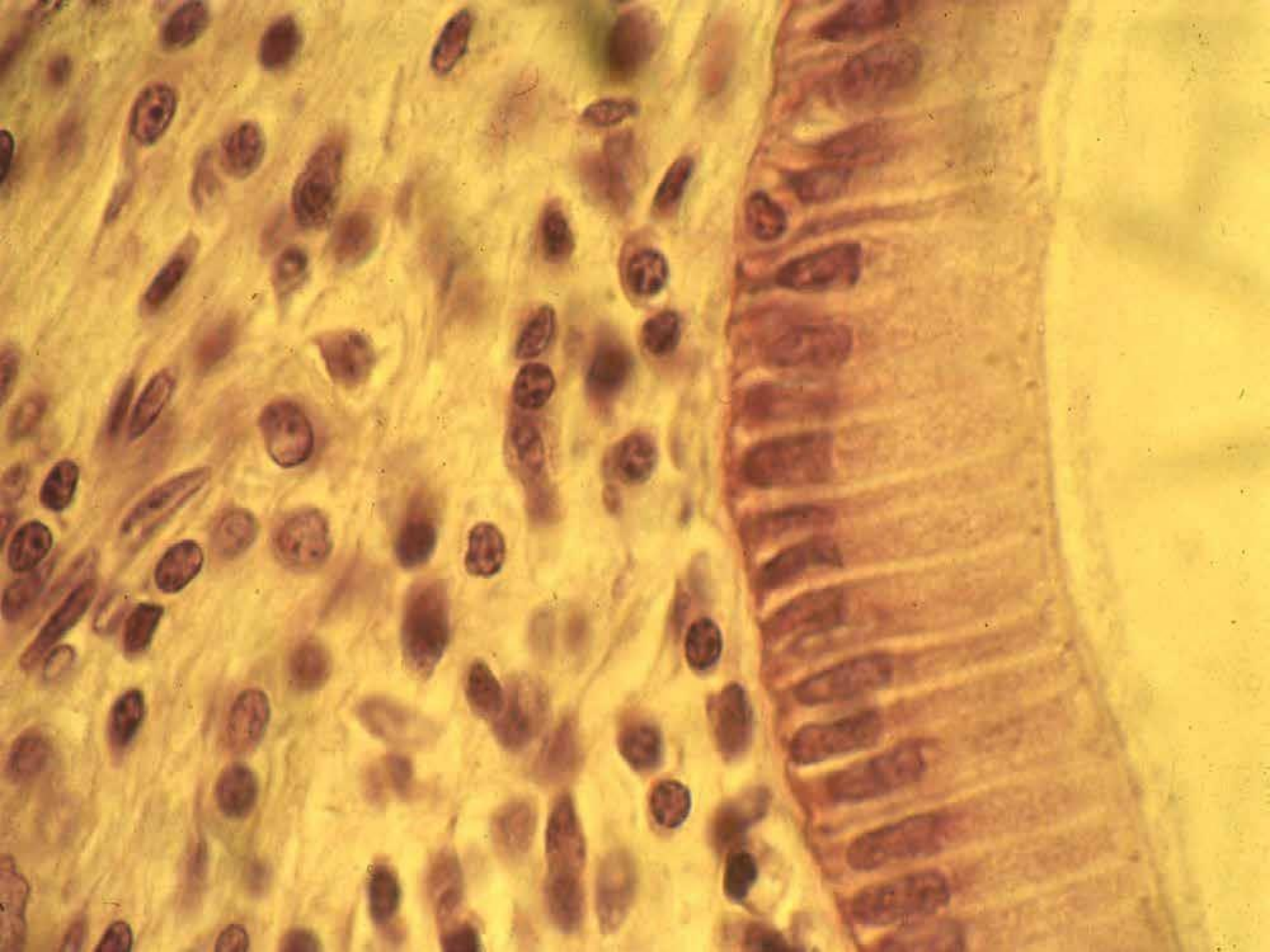
- They do not contain a nucleus; have one intracellular compartment surrounded by plasmalemma (bacteria)

## Eukaryotic cells

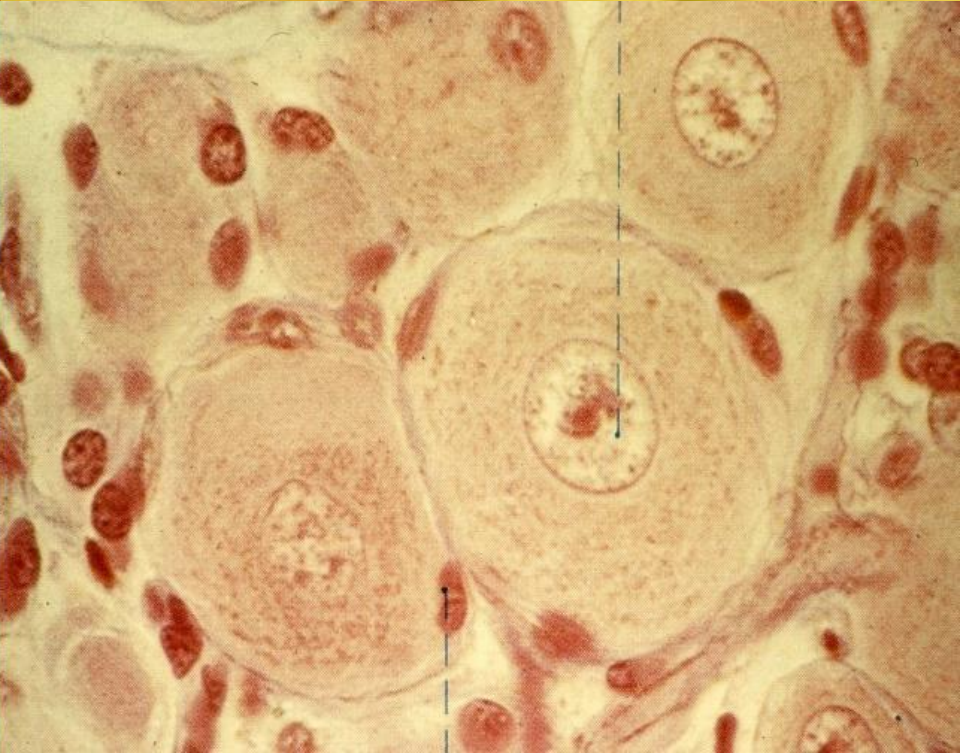
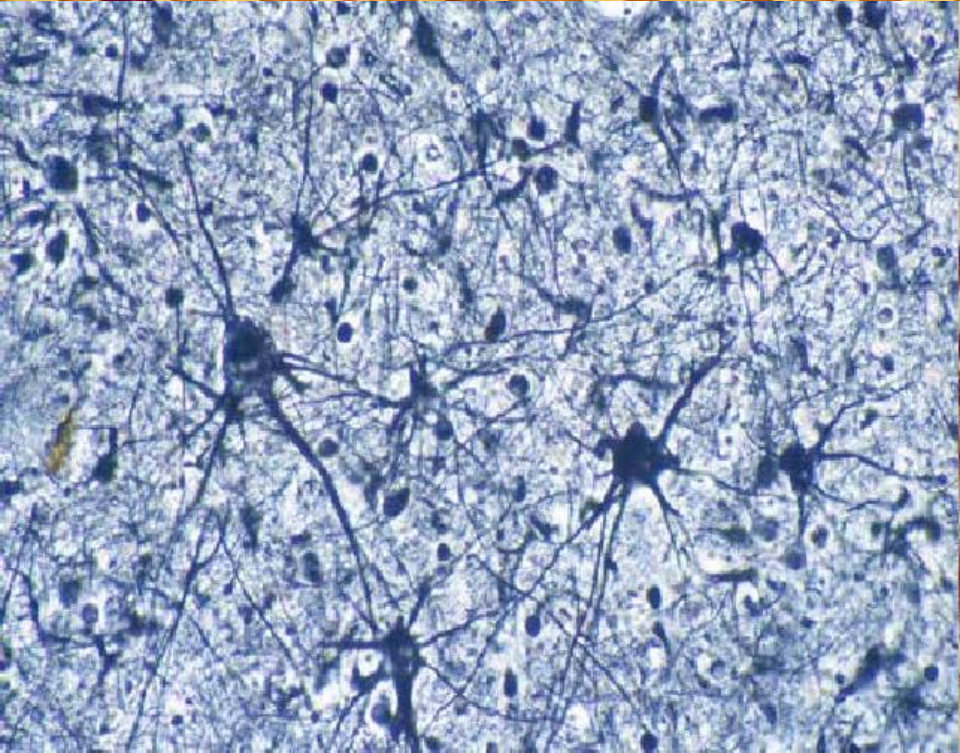
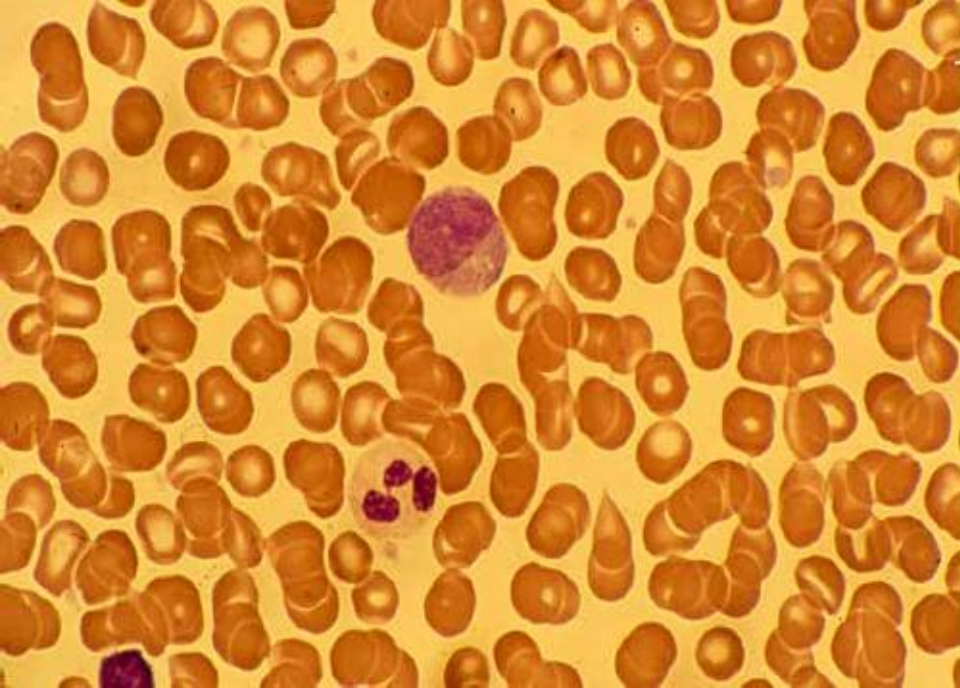
- They contain a nucleus and numerous membrane-encased compartments, organelles (fungi, plants, animals, humans).

In humans 200+ types of cells of various shape and sizes

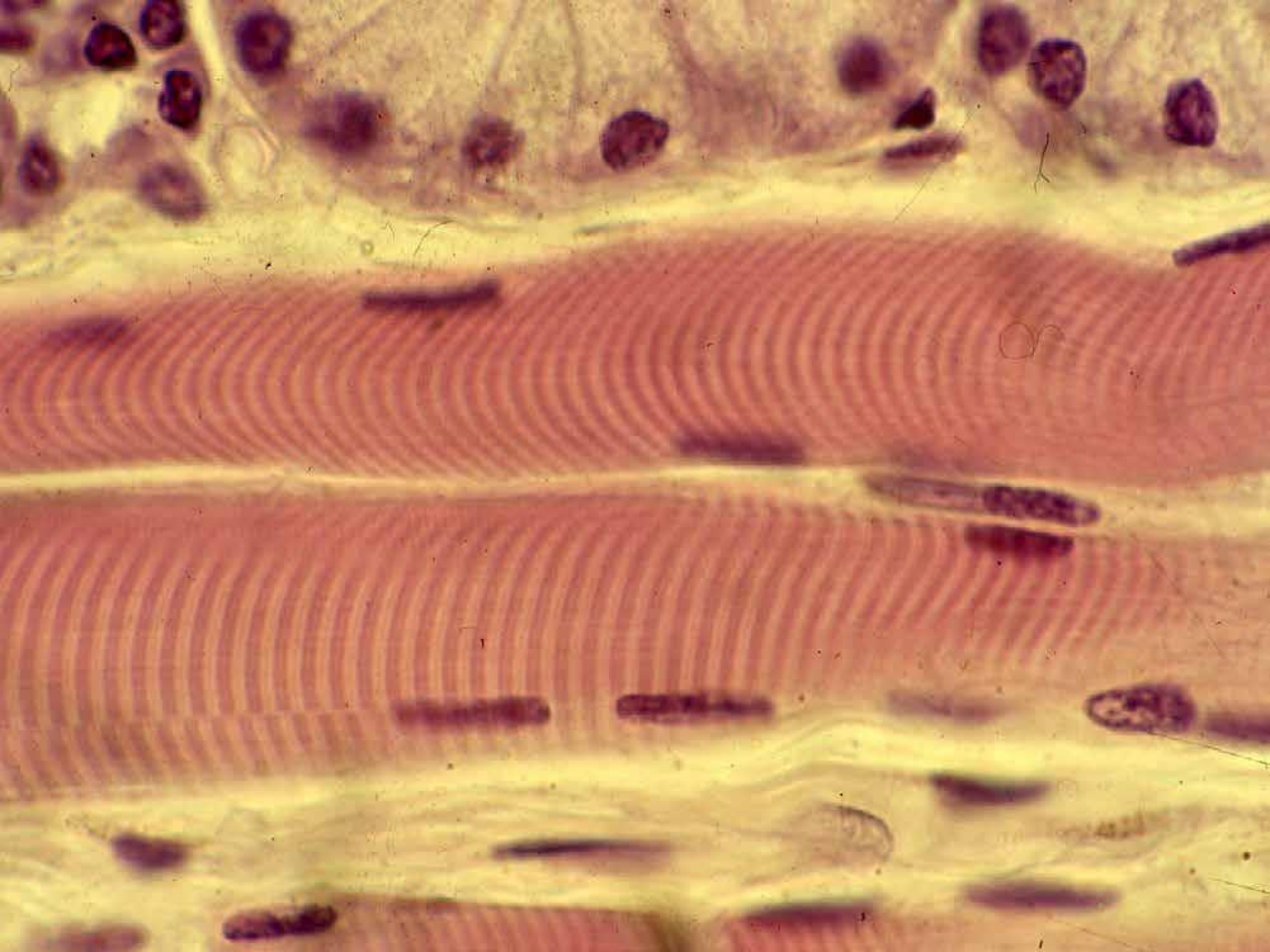




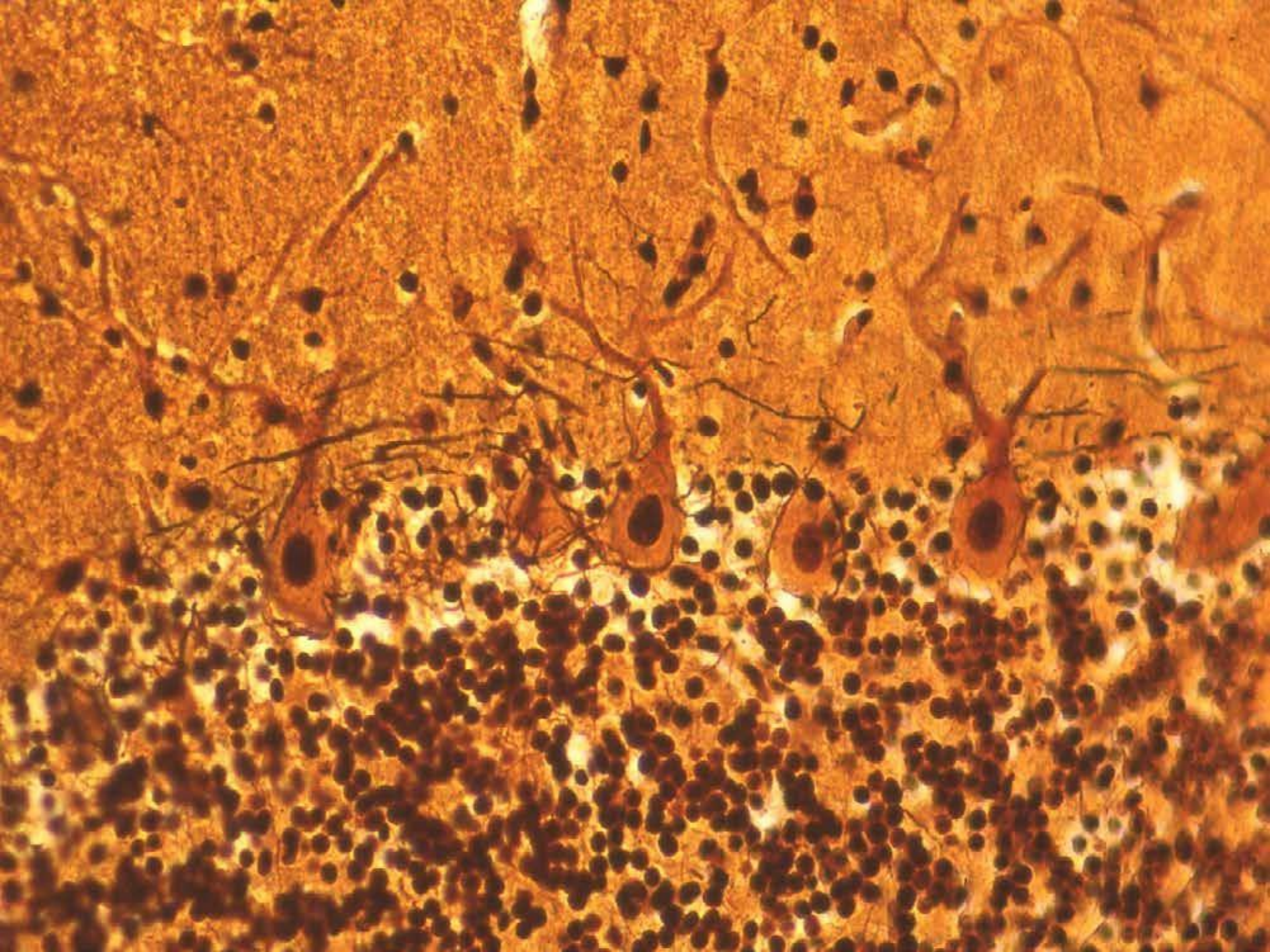








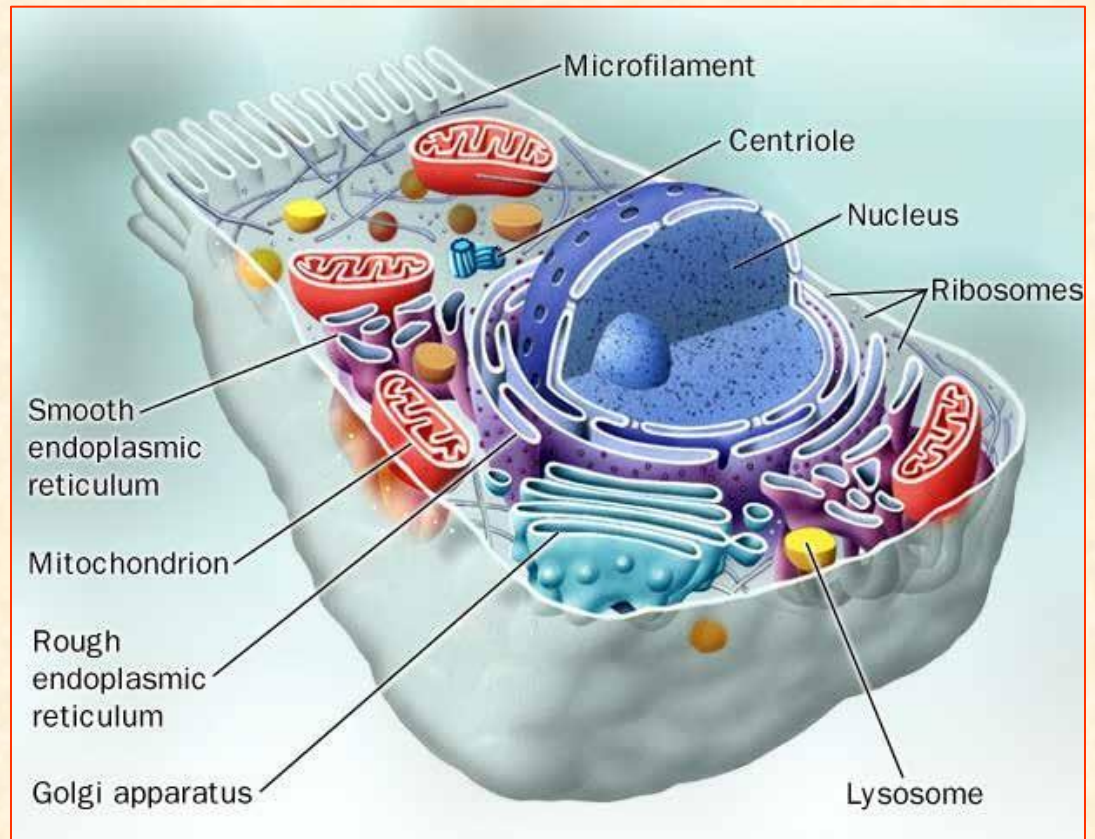




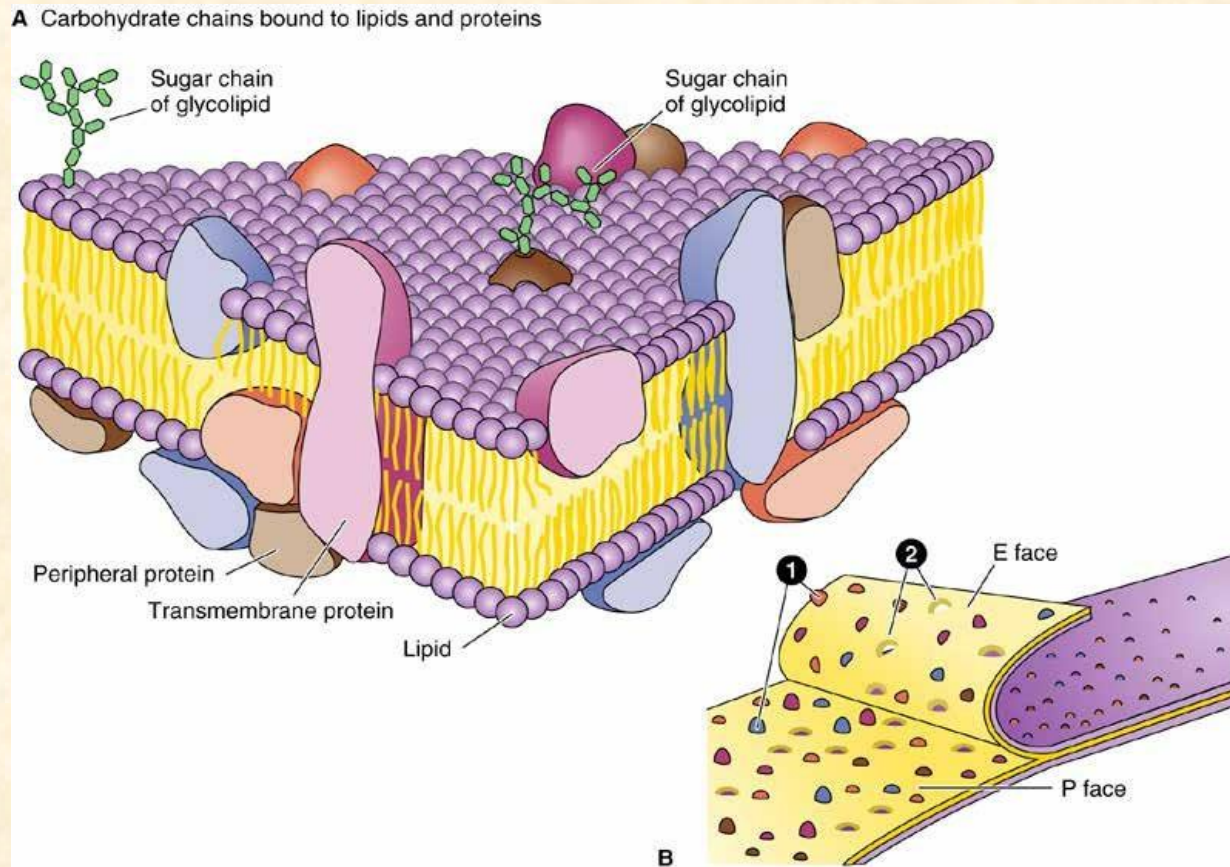


# Basic cell structure

- **Plasmalemma or cell membrane**
- **Cytoplasm**
  - ❖ Cytosol
  - ❖ Cytoskeleton
  - ❖ Organelles
  - ❖ Inclusions
- **Nucleus**

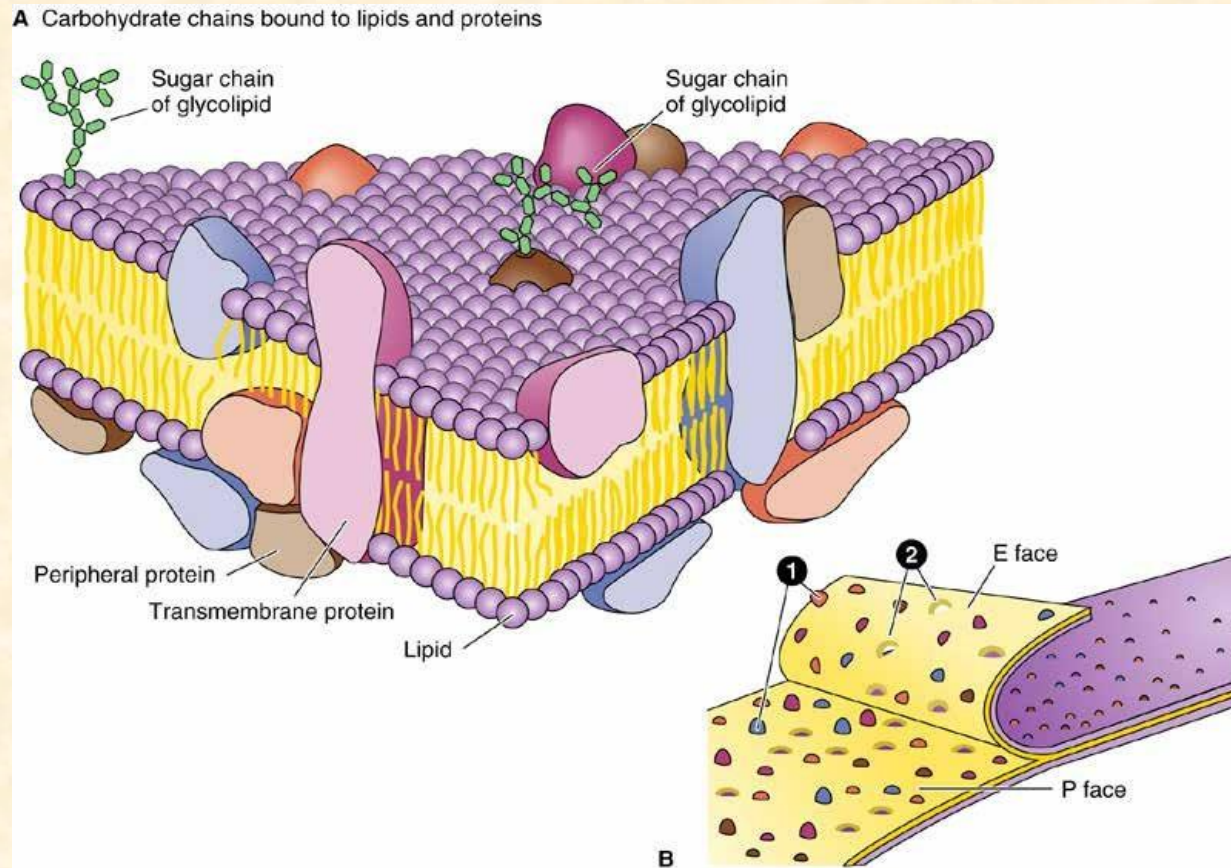


# Cell membrane



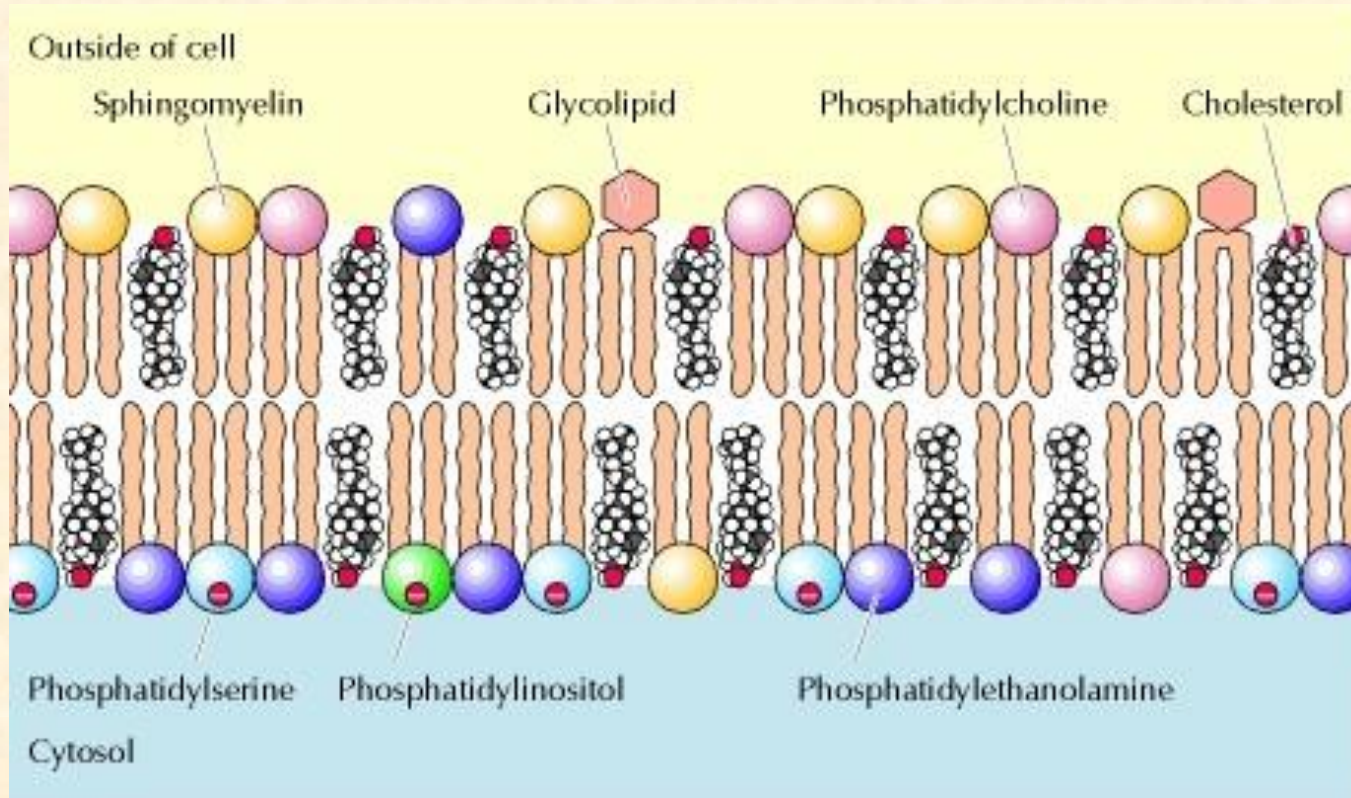
- The thickness of the plasmalemma is 7.5-10  $\mu\text{m}$ . At TEM, its three-layer construction is noticeable.
- A fluid lipid bilayer in which proteins are scattered like a mosaic (Fluid mosaic model, 1972. Singer and Nicholson)





- **Lipids** - phospholipids (glycerophospholipids and sphingophospholipids), glycolipids and cholesterol.
- **Proteins**
  - integral (can pass through the entire membrane - transmembrane) and
  - peripheral (are bound by weak electrostatic forces to the hydrophilic heads of phospholipids)
- **Glycocalyx** (carbohydrates of the plasmalemma on the surface of the plasmalemma)

# Lipids



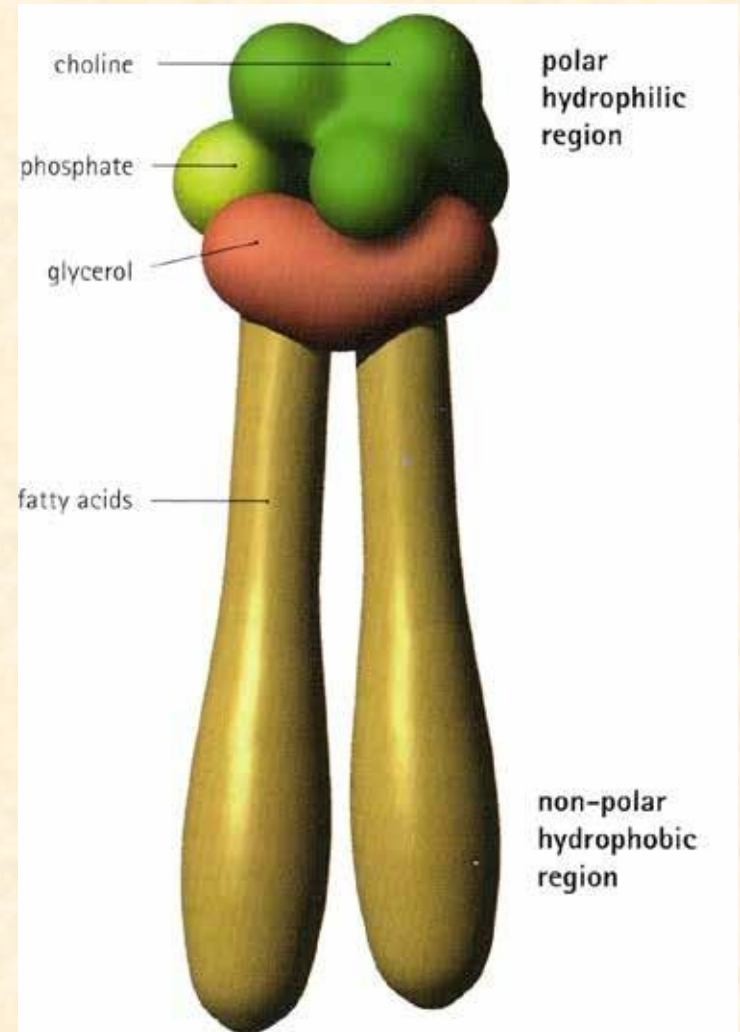
Lipids include

- **phospholipids** (glycerophospholipids and sphingophospholipids),
- **glycolipids** and
- **cholesterol**.

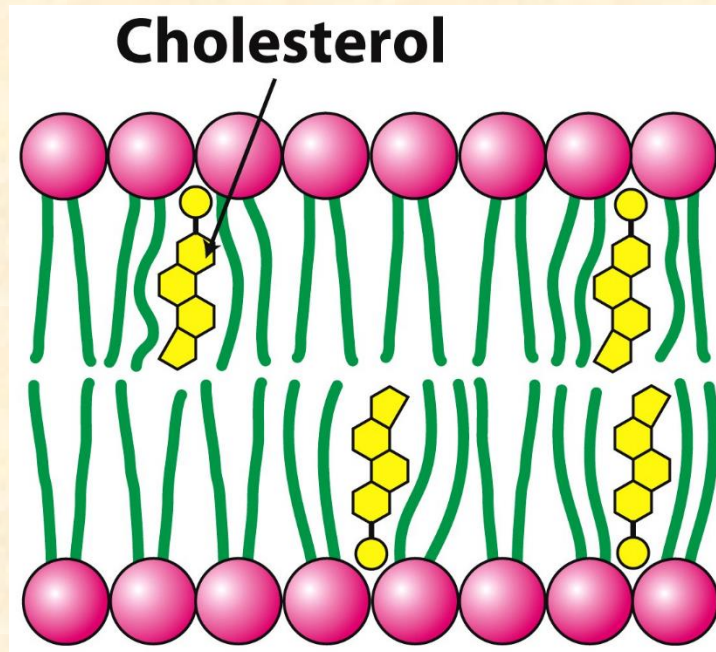


# Phospholipids

- **Phospholipids** consist of a polar, **hydrophilic head** and **nonpolar hydrophobic tails** (unsaturated fatty acids that increase membrane fluidity).
- They are usually divided into glycerol- and sphingo-phospholipids.
- They include: **sphingomyelin**, **phosphatidylcholine**, **phosphatidylinositol**, **phosphatidylserine**, **phosphatidylethanolamine**...



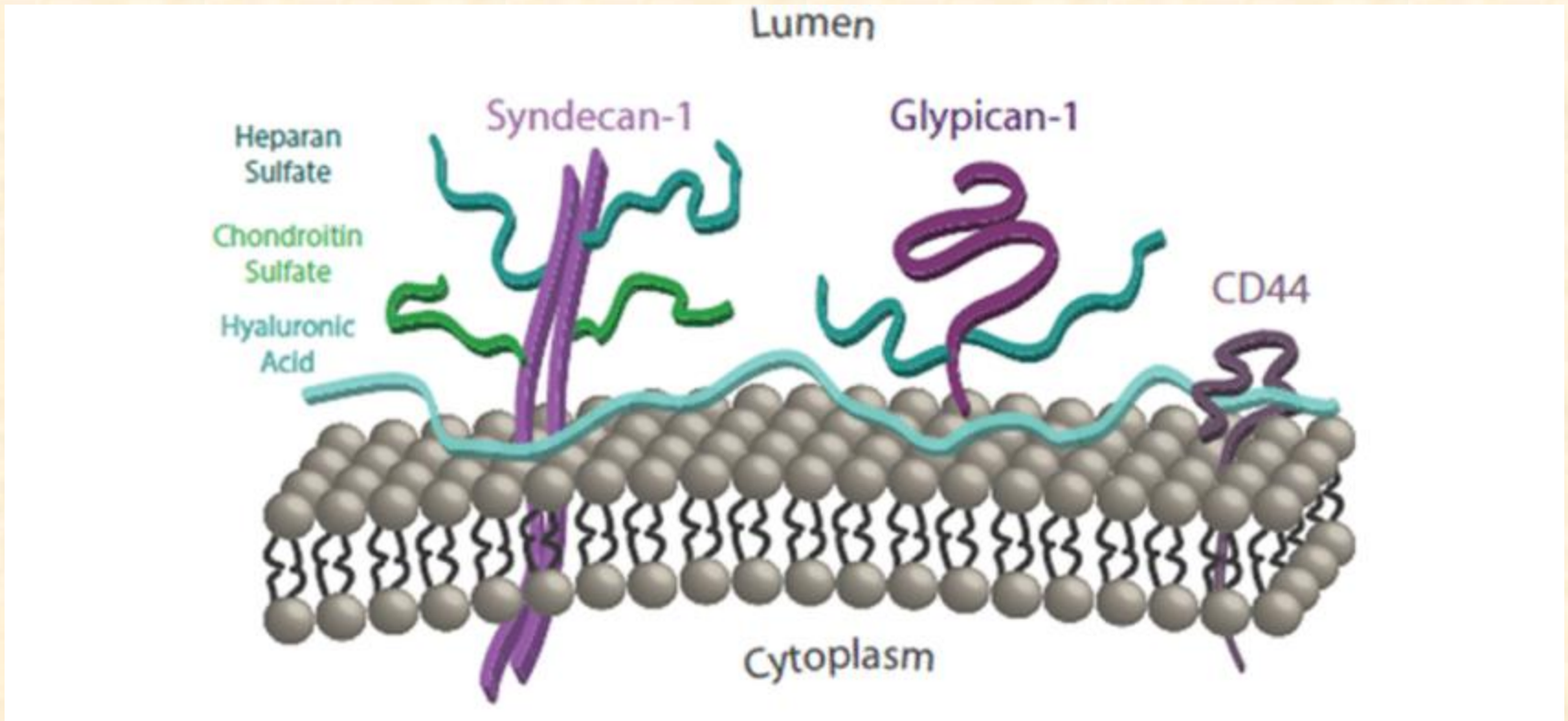
# Cholesterol



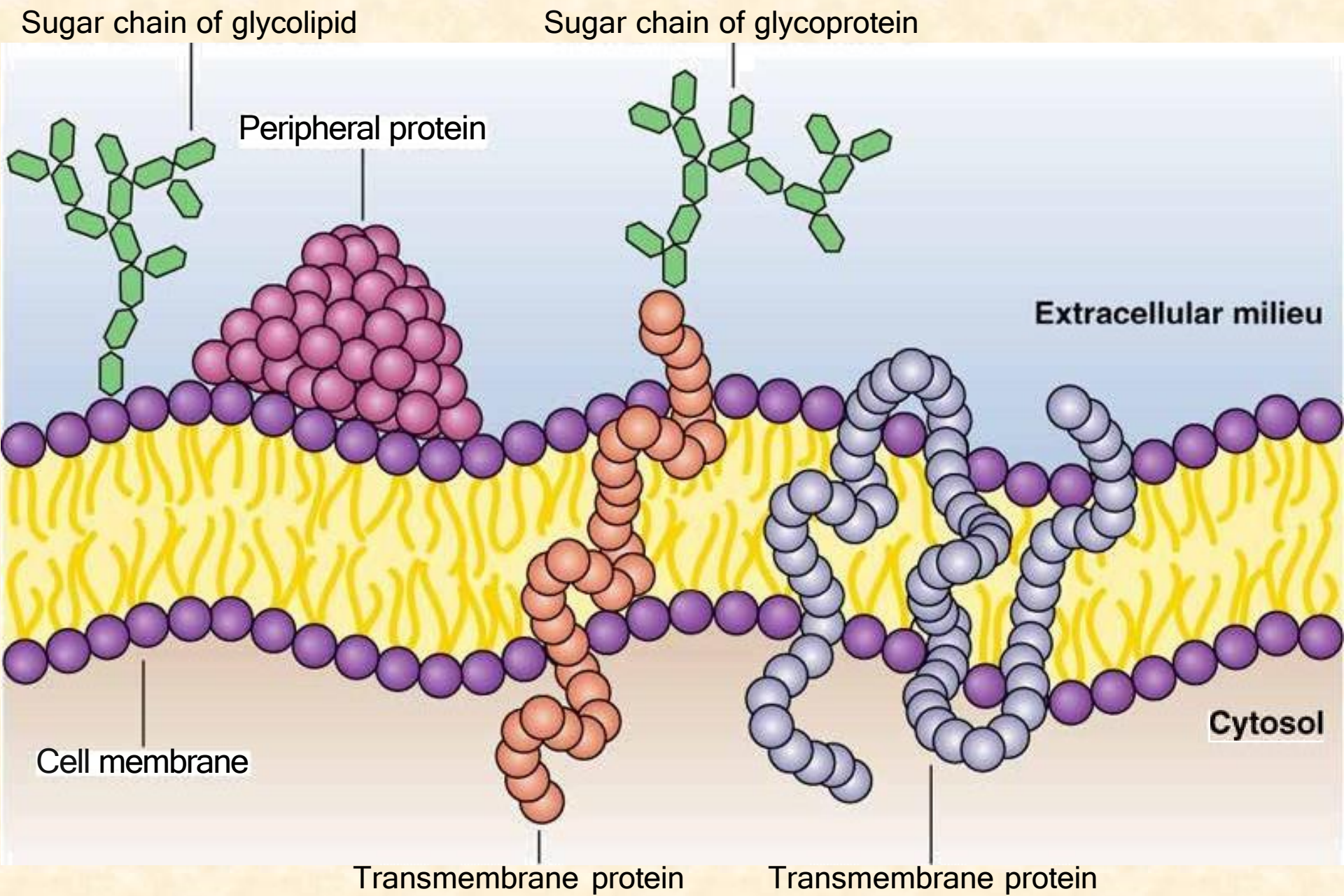
- **Cholesterol** within the hydrophobic part **increases viscosity** (as opposed to unsaturated fatty acids that increase fluidity) and **stabilizes the membrane**.



# Glycolipids

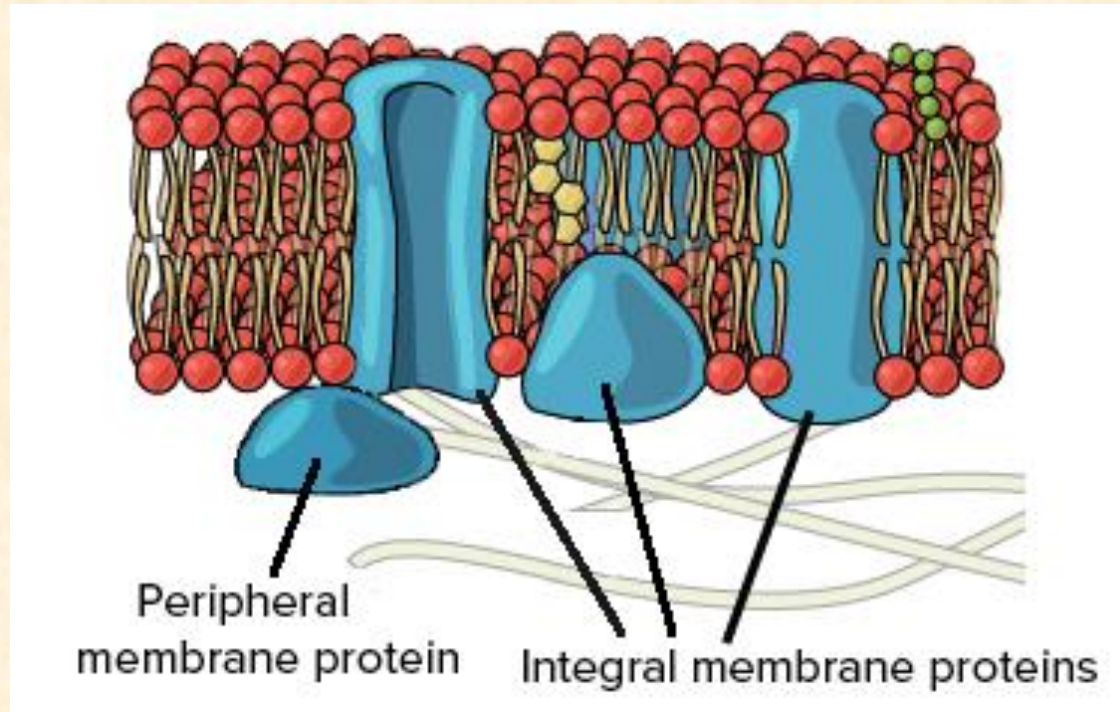


**Glycolipids** have oligosaccharide chains that extend outward from the surface of the cell membrane and thus contribute to lipid asymmetry.





# Proteins



- **Integral proteins** can be immersed in the membrane or pass completely through the new transmembrane proteins (one-pass, multi-pass).
- **Peripheral proteins** are bound to the hydrophilic heads of phospholipids by weak electrostatic forces.

# Plasmalemma protein functions

**Transport proteins** – integral proteins involved in the transport of substances through the plasmalemma. They include:

- **Channel proteins** (protein channels) that form narrow channels through which ions and water pass:
- **Ion channels** (can be sensitive and insensitive)
- **Water channels** (aquaporins)
- **Carrier proteins** – transmembrane proteins that have binding sites for ions and corresponding molecules (when they are involved in active transport, they are called pumps)

**Receptors** - integral proteins or glycoproteins that have a site for binding signal molecules (ligands) and the ability to transmit signals to the interior of the cell.

**Enzymes** – integral proteins that carry out a catalytic action on the appropriate substrate

**Structural proteins** - participate in intercellular junctions and attach cells to the extracellular matrix.



# Transport proteins

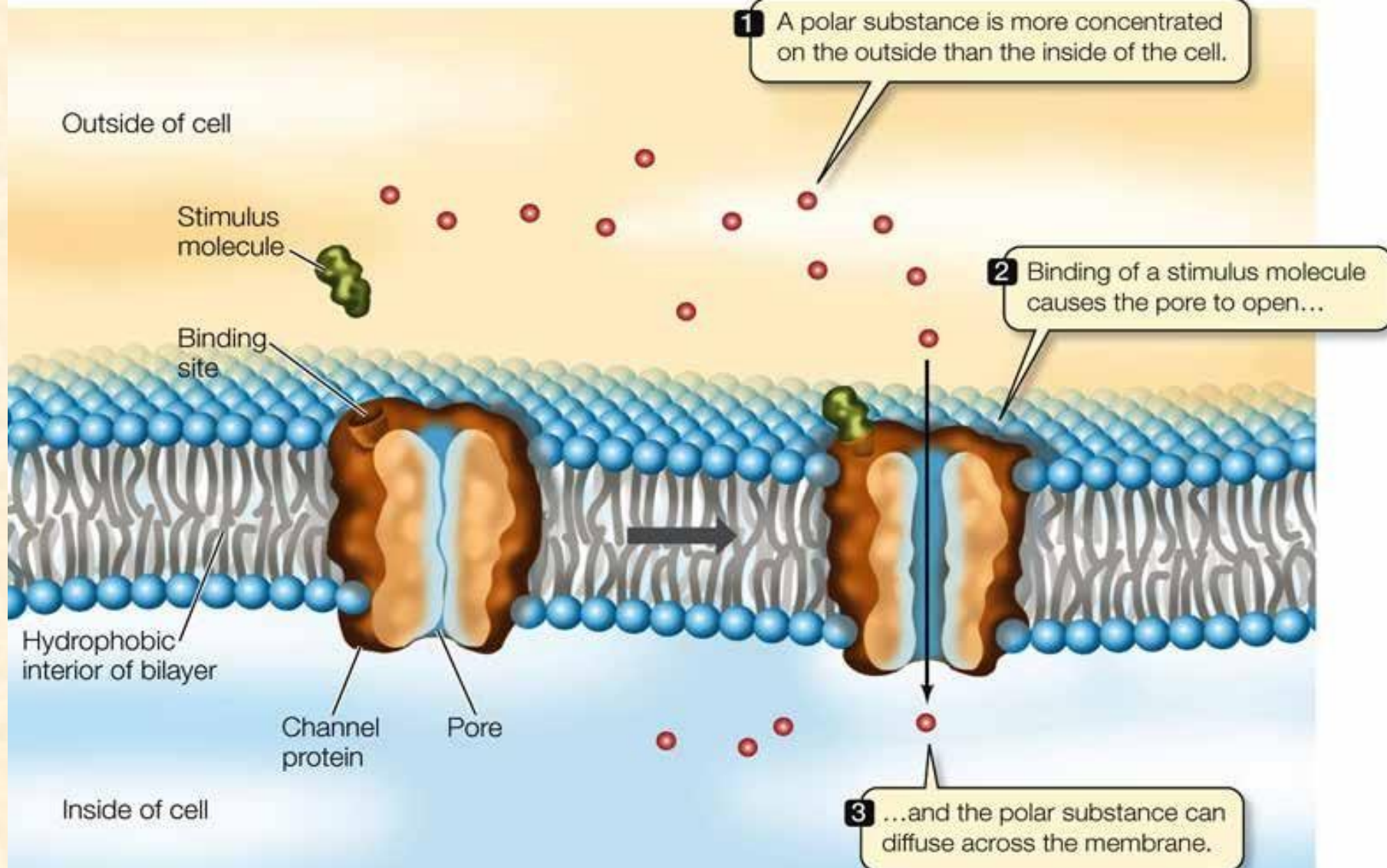
A type of integral protein involved in the transport of substances through the plasmalemma.

They are divided into **channel proteins and carrier proteins**

- **Channel proteins** form ion channels (sensitive (voltage-, ligand- and mechano-sensitive) and insensitive (which are controlled by an electrochemical gradient))
- **Carrier proteins** have binding sites for ions and molecules that need to enter the cell.

After their binding, a conformational change of the carrier occurs, which enables the entry of the given molecule/ion into the cell ("pumps").

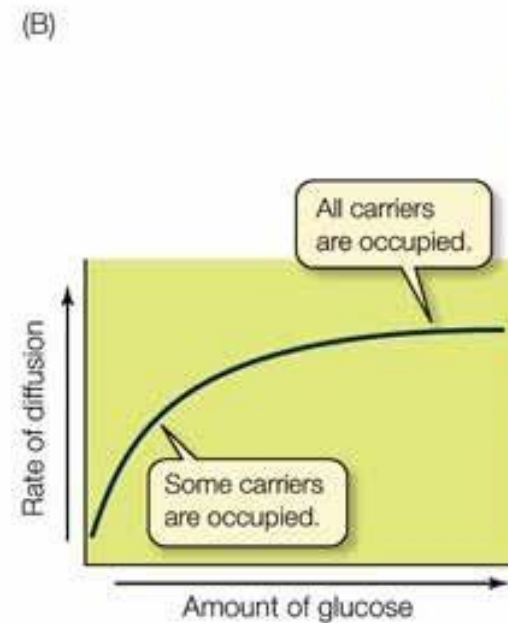
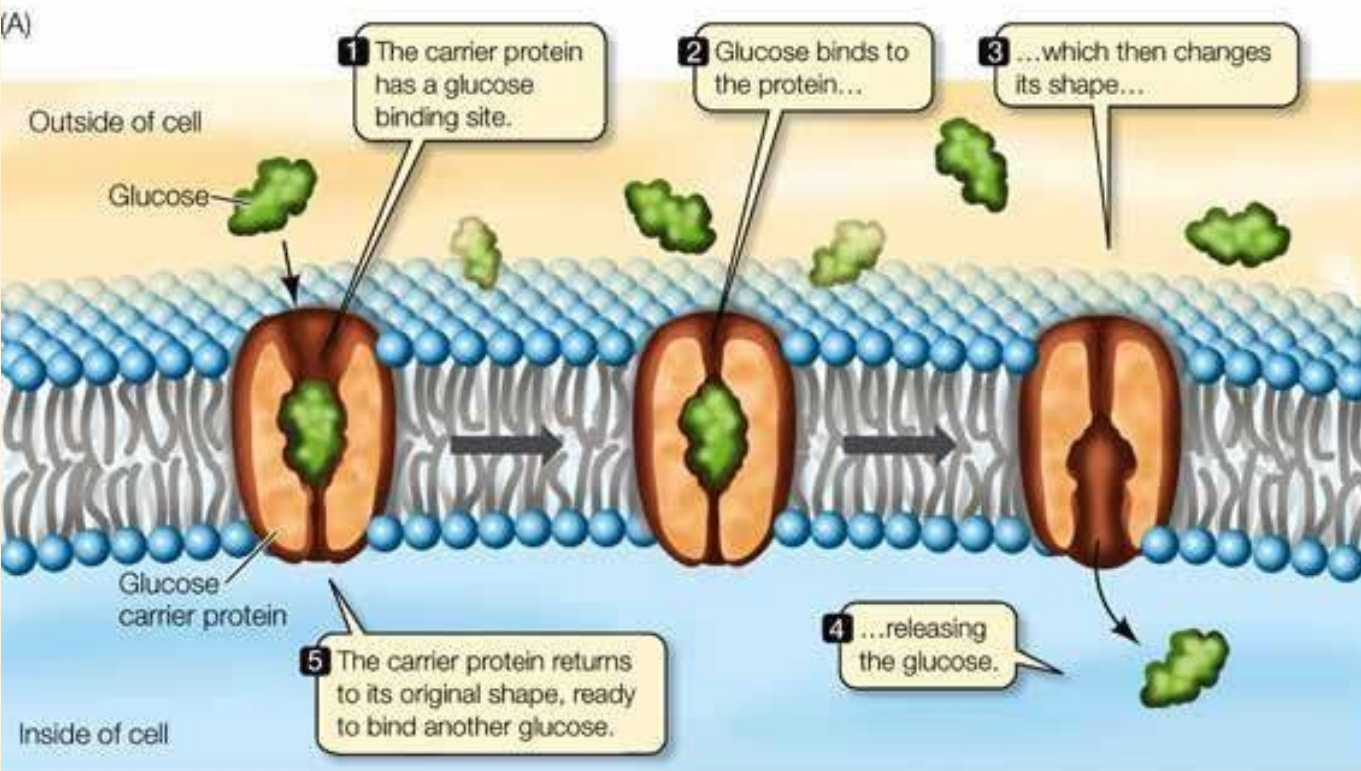
- operate passively,
  - allowing movement of substances across membranes
  - down a concentration gradient due to its kinetic energy



Channel proteins form ion channels

- sensitive (voltage-, ligand- and mechano-sensitive) and
- insensitive (which are controlled by an electrochemical gradient))





**Carrier proteins** have binding sites for ions and molecules that need to enter the cell.

# Receptors

Allow recognition and localized **binding of ligands** (molecules that bind to the extracellular surface of the plasma membrane) in processes such as *hormonal stimulation, coated-vesicle endocytosis, and antibody reactions*.

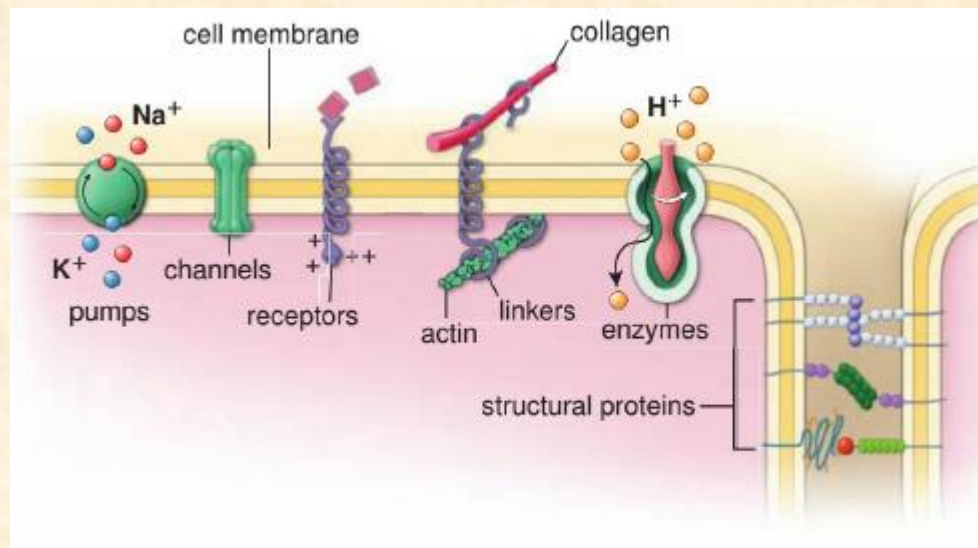
Receptors that bind to signaling molecules **transmit the signal** through a sequence of molecular switches (i.e., second messengers) to the cell's internal signaling pathways, thereby initiating a physiological Response.

- The signal causes a change in the **metabolic activity** of the cell, triggers its **proliferation** or activates the process of programmed cell death (**apoptosis**) or else.
- Cells bearing receptors for a specific ligand are referred to as **target cells** for that molecule



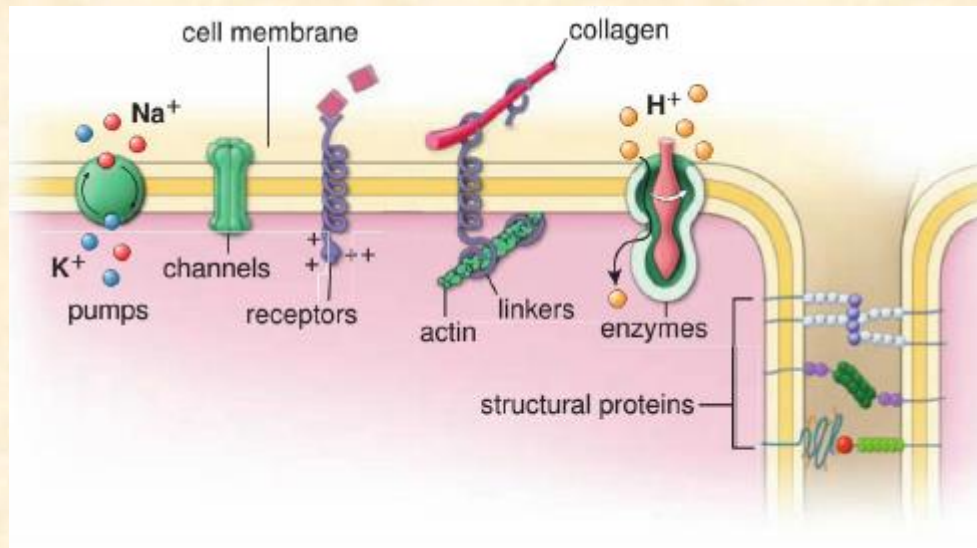
# Enzymes

- Integral proteins that **exert a catalytic effect on the appropriate substrate** in the cell environment (dipeptidases and disaccharidases participate in the terminal digestion of proteins and carbohydrates).
- Lipid rafts (less mobile enzymes)



# Structural proteins

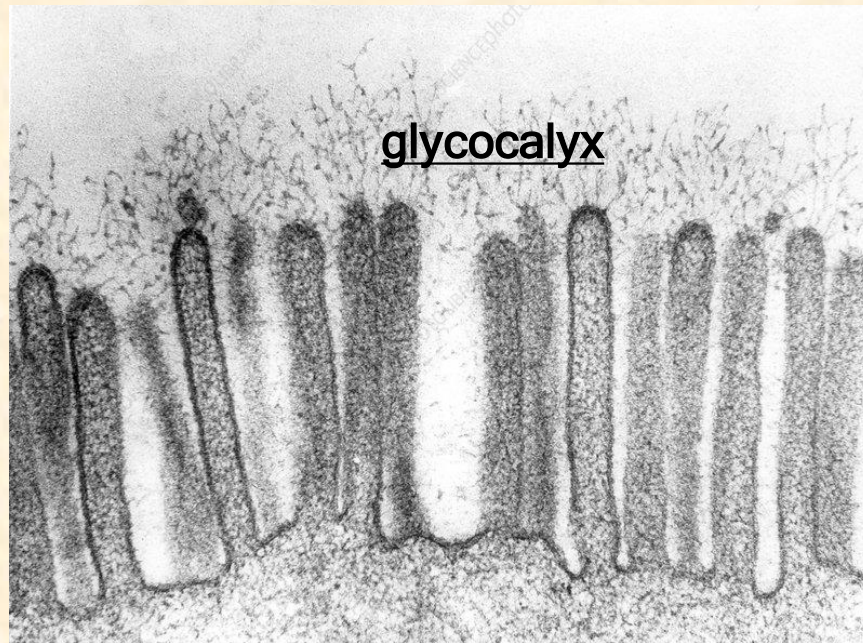
- They participate in intercellular junctions and in the attachment of the cell to the extracellular matrix.
- They are located in specialized regions of the cell.
- They include **integrins, cadherins and connexins**.





# Carbohydrates

- They are located exclusively on the **outside of the plasmalemma**.
- They are **attached to lipids**, building glycolipids, glycoproteins and proteoglycans.
- In this way, a carbohydrate layer called the **glycocalyx** (2-50 nm) is formed on the outside of the plasmalemma, which has a protective function.



# Cell membrane transport

**Passive diffusion** (conditioned by the concentration gradient – ions and small molecules)

**Facilitated diffusion** (through carrier proteins – glucose, amino acids)

**Active transport** (against the gradient with ATP, via pumps, maintaining the difference in conc. ion)

**Vesicular transport** (macromolecules and large particles that cannot pass through the plasmalemma or with the help of its proteins are transported by means of vesicles).

## □ Endocytosis

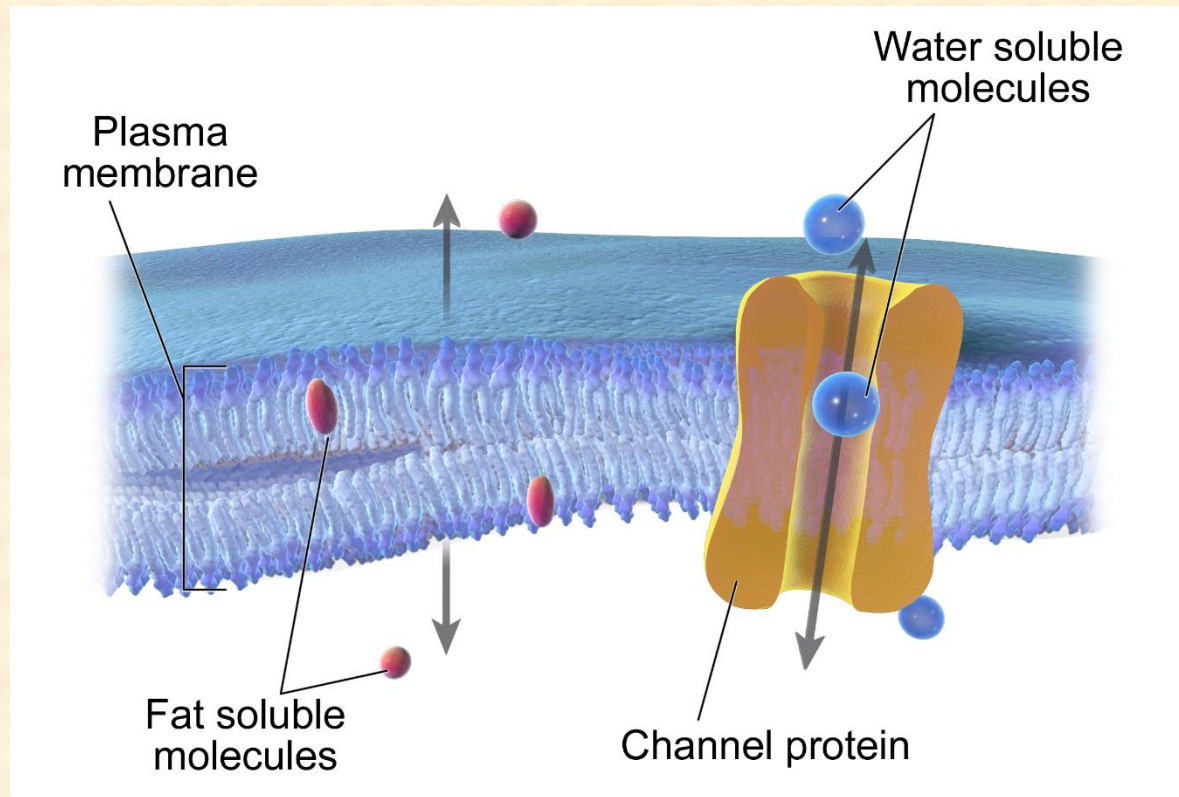
- **Pinocytosis** - a small amount of fluid is taken in via caveolae, which then form a pinocytotic vesicle
- **Receptor-mediated endocytosis** or clathrin-dependent endocytosis - selective uptake of substances from the ECM by means of cargo receptors
- **Phagocytosis** - phagocyte cells internalize bacteria, protozoa, fungi through receptors... They form vesicles (phagosomes) that merge with primary lysosomes into secondary lysosomes where the destruction of the phagocytosed material ends.

□ **Exocytosis** - the process by which non-diffusible substances are released from the cell - enzymes, hormones, neurotransmitters (by constitutive or regulated secretion)



# Diffusion across cell membrane

Some substances (**fat-soluble and small, uncharged molecules**) cross the plasma membrane by simple diffusion down their concentration gradient



# Carrier proteins

- Transfer small, **water-soluble molecules**.
- They are highly selective, often transporting only one type of molecule. After binding to a molecule designated for transport, the carrier protein undergoes a series of conformational changes and releases the molecule on the other side of the membrane.
- Carrier proteins, such as glucose carriers, do not require energy and participate in passive transport.

**Uniport** transports one substance in one direction.

**Symport** transports two different substances in the same direction.

**Antiport** transports two different substances in opposite directions.

- Membrane pumps are enzymes engaged in **active transport**, utilizing energy from the hydrolysis of adenosine triphosphate (ATP) to move ions and other solutes across membranes, against often steep concentration gradients. Because they consume ATP pumps, they are often referred to **as ATPases**.

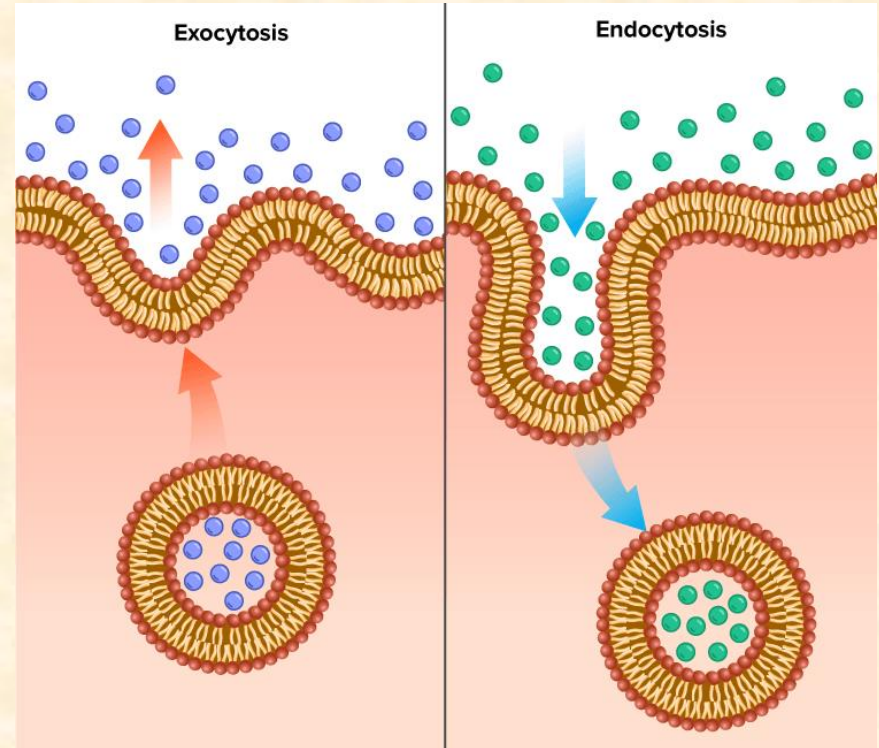


# Vesicular transport

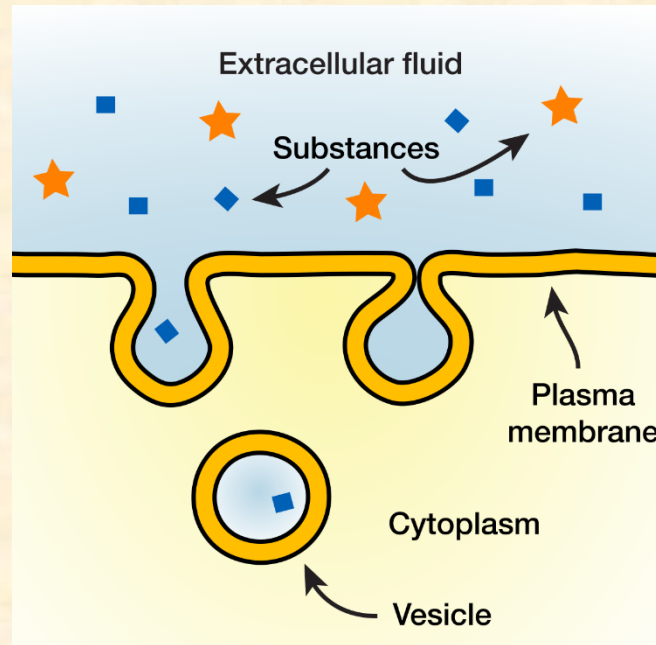
**Endocytosis** is the general term for processes of vesicular transport in which substances enter the cell. It plays key roles in nutrient uptake, cell signaling, and cell shape changes.

- **Pinocytosis** - a small amount of fluid is taken in via caveolae
- **Receptor-mediated endocytosis** or clathrin-dependent endocytosis - selective uptake of substances from the ECM by means of cargo receptors
- **Phagocytosis** - phagocyte cells internalize bacteria, protozoa, fungi through receptors... They form vesicles (phagosomes) that merge with primary lysosomes into secondary lysosomes where the destruction of the phagocytosed material ends.

**Exocytosis** is the general term for processes of vesicular transport in which substances leave the cell.



# Pinocytosis



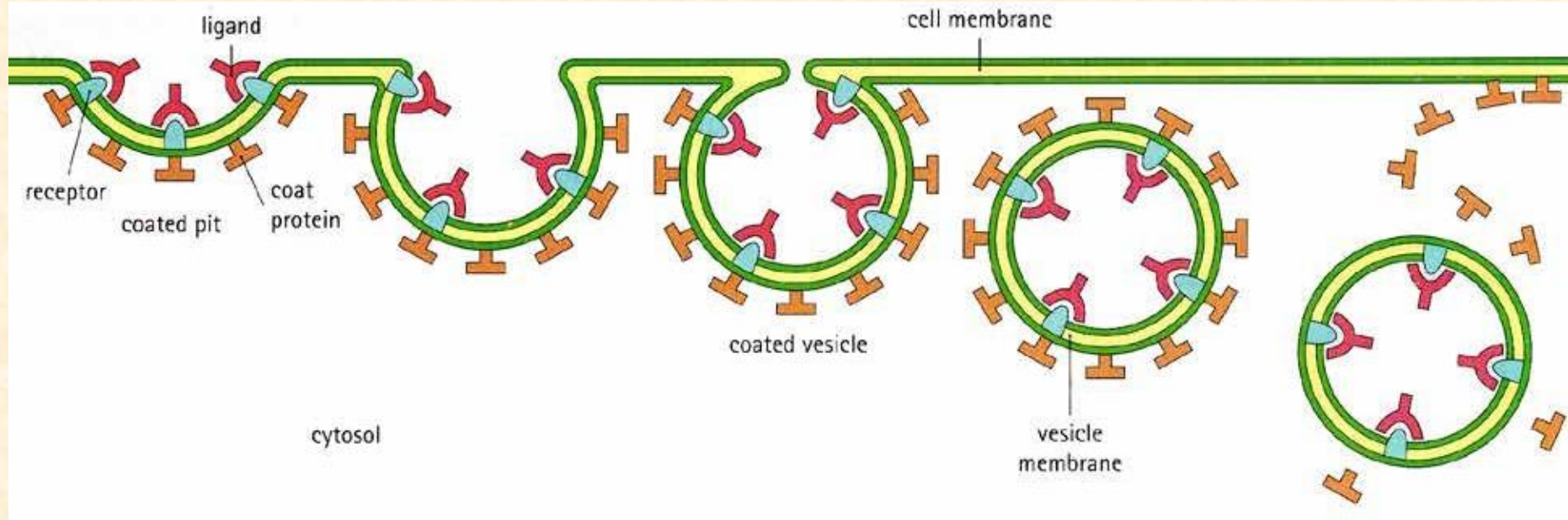
**Pinocytosis** (“cell drinking”) involves smaller invaginations of the cell membrane which fuse and entrap extracellular fluid and its dissolved contents. The resulting pinocytotic vesicles (~80 nm in diameter) then pinch off inwardly from the cell surface.

- **Transcytosis**, accomplishes bulk transfer of dissolved substances across the cell.



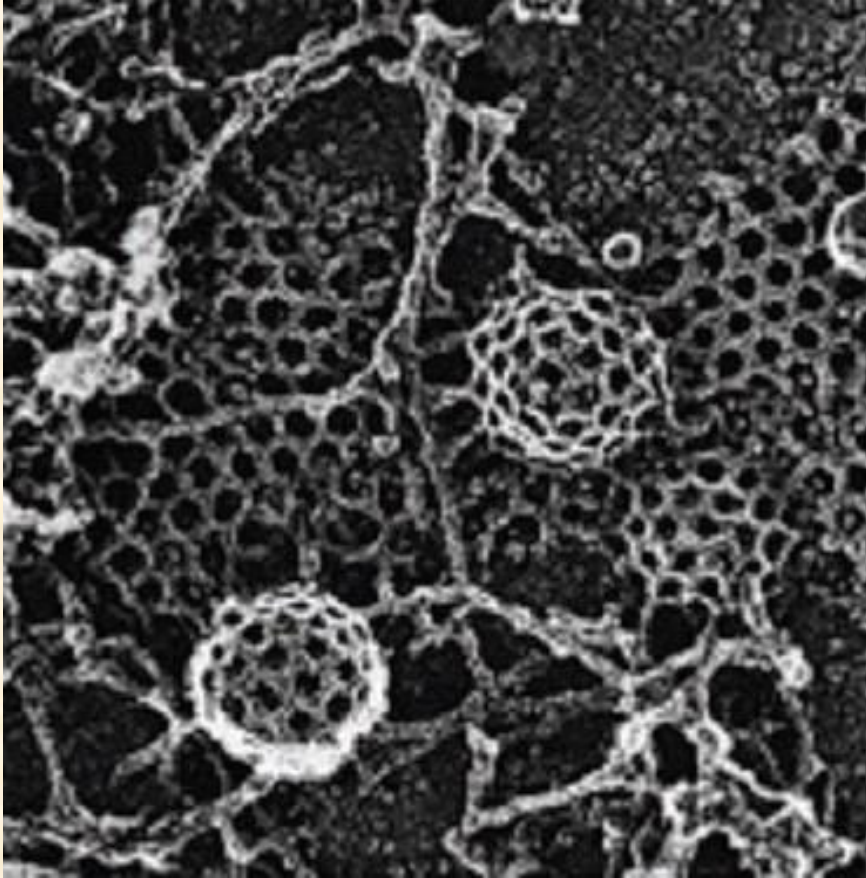
# Receptor-mediated endocytosis

Hormones, growth factors, VLDL, transferrin, viruses and toxins are introduced in this way



Coating on the cytoplasmic surface of endocytic pits contains **clathrin** forming **cage-like** invagination that soon pinches off into the cytoplasm as a **coated vesicle** with the receptor-bound ligands inside. The type of vesicle formed as a result of receptor-mediated endocytosis is referred to as a coated vesicle, and the process itself is known as **clathrin-dependent endocytosis**.

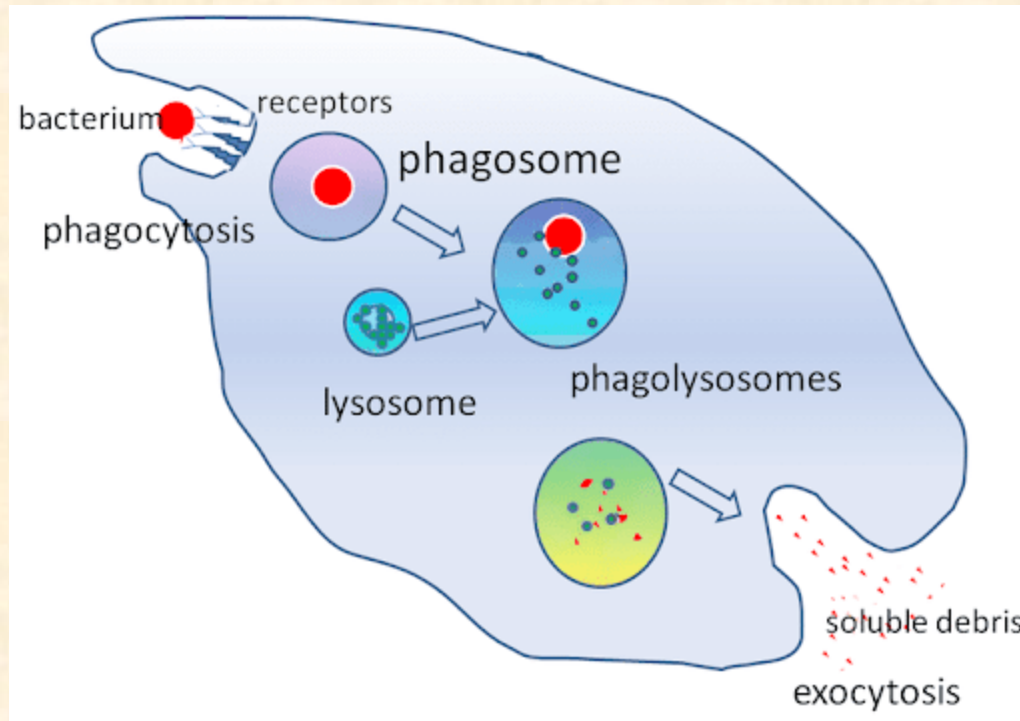
Another type of receptor-mediated endocytosis prominent in very thin cells produces invaginations called caveolae.



Receptor-mediated endocytosis  
Clathrin coated vesicles

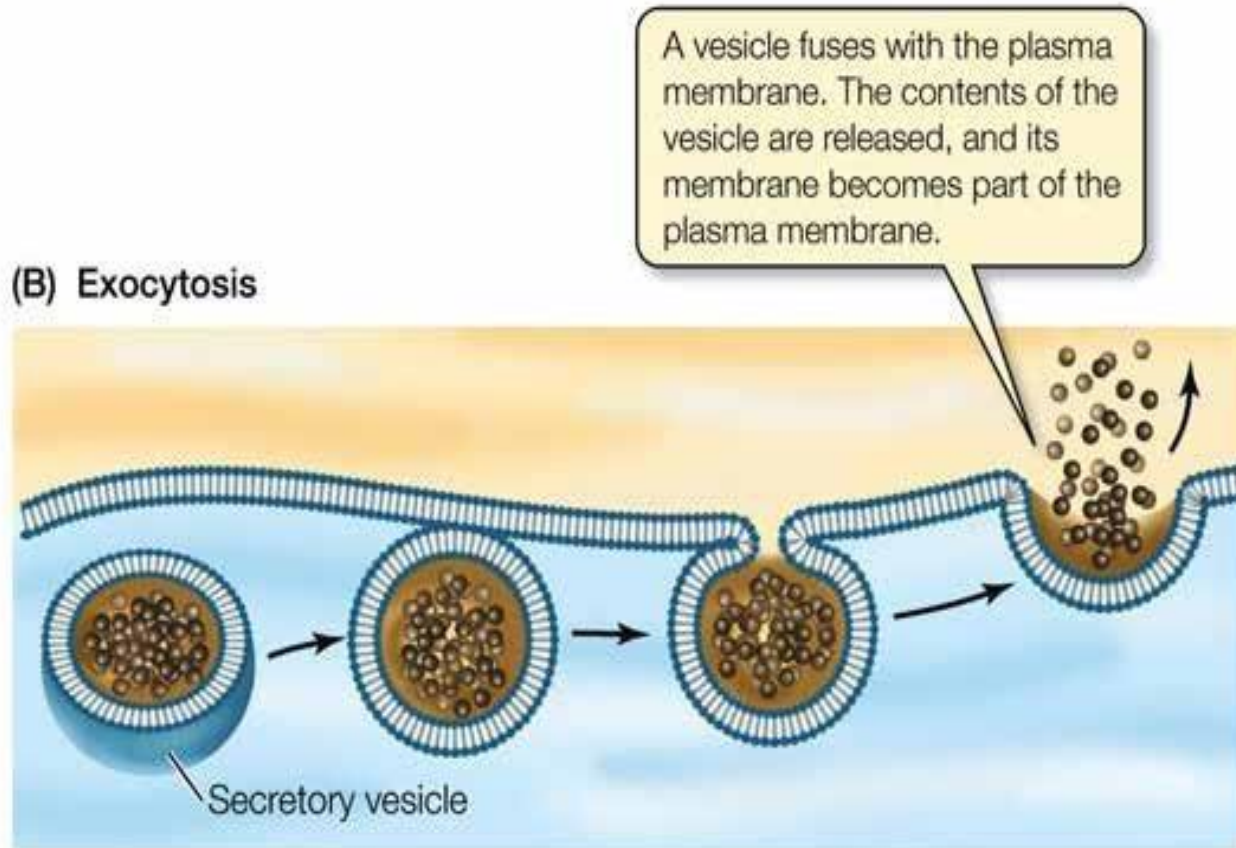


# Phagocytosis



- **Phagocytosis** [Gr., cell eating] is the ingestion of large particles such as cell debris, bacteria, and other foreign materials.
- Pseudopodia engulf phagocytosed particles into large vesicles (larger than approximately 250 nm in diameter) called phagosomes which then merges with a lysosome for degradation of its contents
- Phagocytosis is performed mainly by a specialized group of cells belonging to the mononuclear phagocytotic system (Macrophages and neutrophils)

# Exocytosis



- Exocytosis is the process by which a vesicle moves from the cytoplasm to the plasma membrane, where it discharges its contents to the extracellular space.



**Cytoplasm**

**Cytoplasm** is the entire contents of the cell that fills the space between the nuclear envelope and the plasmalemma.

The components of the cytoplasm are:

- **Cytoskeleton** – a system of filaments and tubules involved in maintaining cell shape, cell movement, as well as the movement of organelles and macromolecules within the cell.
- **Organelles** – metabolically active subcellular structures responsible for various cell activities.
- **Inclusions** – metabolically inert products of cellular metabolism or depots of nutrients.
- **Cytosol** - fluid matrix composed of water, nucleotides, amino acids, proteins, enzymes, metabolic products, RNA, glucose, ATP and inorganic ions.



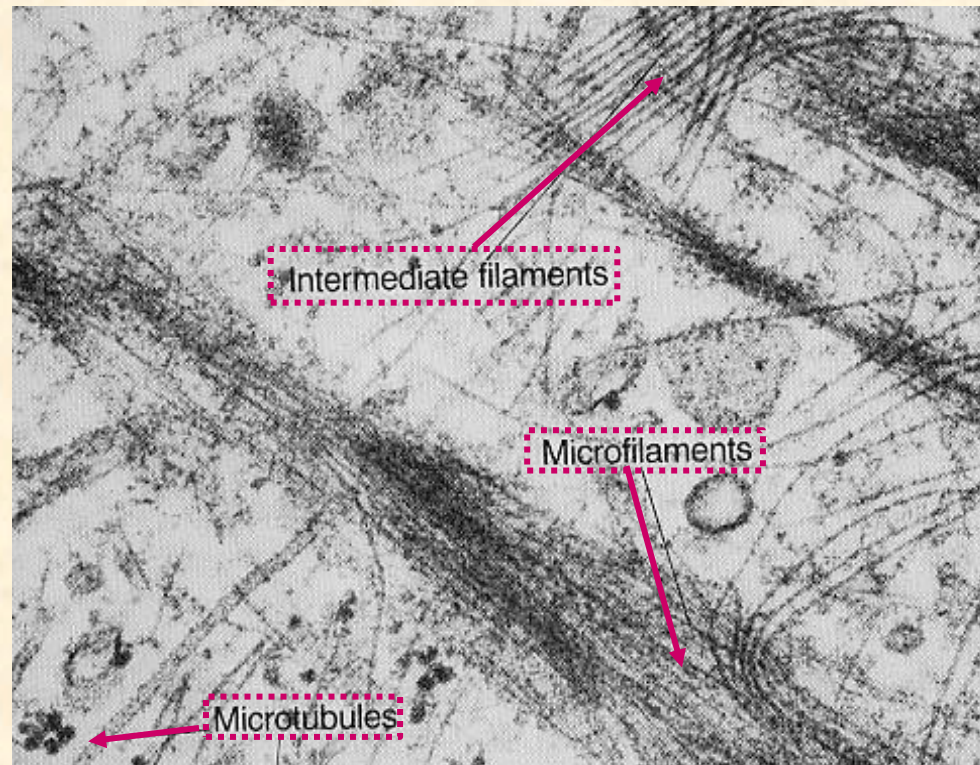
# **Cytoskeleton**

# Cytoskeleton

The cytoskeleton consists of a three-dimensional network of protein filaments and tubes that supports the cell, determines its shape and the position of organelles in the cytoplasm.

The components of the cytoskeleton are:

- **Microfilaments**
- **Intermediate filaments**
- **Microtubules**

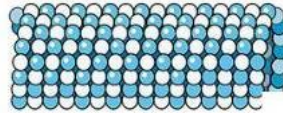




# Cytoskeleton



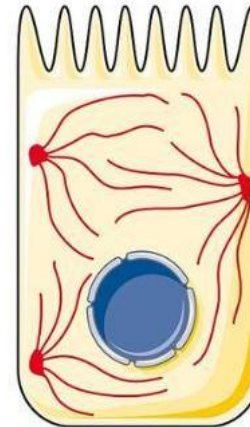
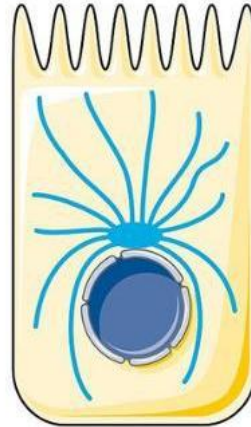
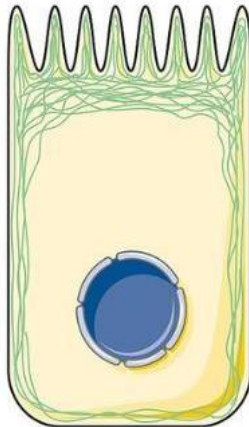
**MICROFILAMENTS**



**MICROTUBULES**

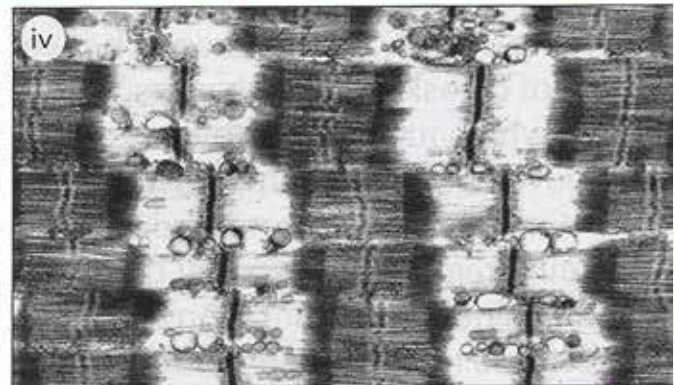
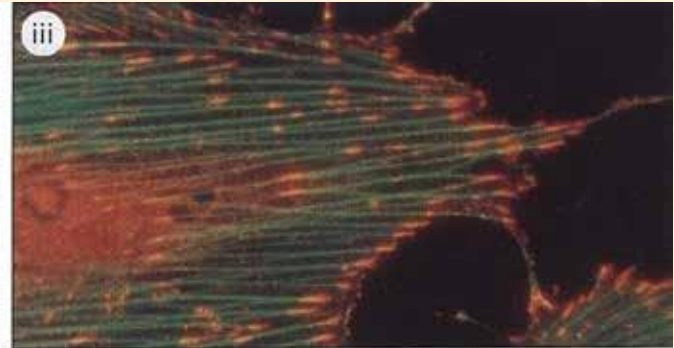
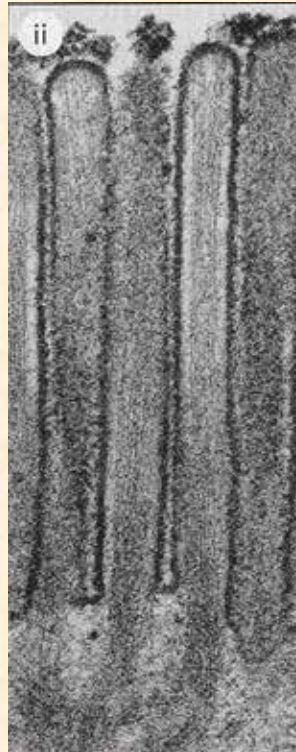
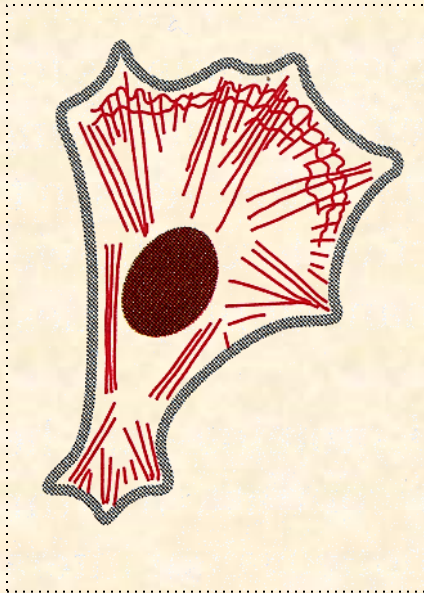


**INTERMEDIATE  
FILAMENTS**



Actin filaments and microtubules change their length by constant polarization and depolarization, while intermediate filaments are much more stable structures

# Actin filaments (microfilaments)



The thinnest filaments of the cytoskeleton (also called microfilaments) have an average diameter of **6nm**. They are formed by the polymerization of globular subunits of **G actin**. They form bundles in the cell, they provide support to the cell as well as **microvilli and stereocilia** (fimbrin and villin).

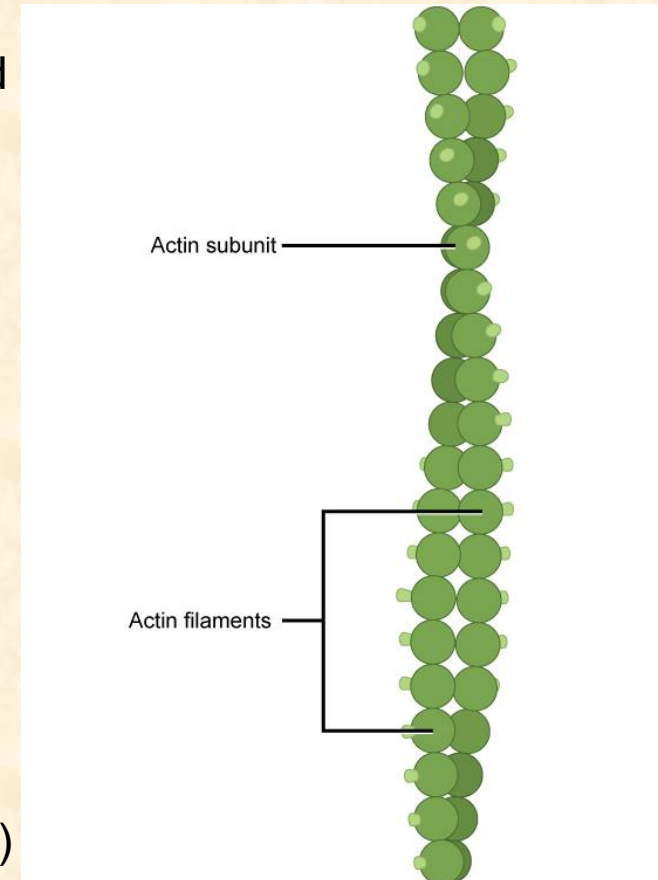


They consist of two chains of polymerized **G actin monomer** chains (43kDa) arranged in the form of a helix, with a fast-growing (+) (cap) and a slow-growing (-) pole, thus building **F actin or an actin filament** (microfilament) (alpha, beta and gamma).

❑ It occurs in 6 isoforms

$\alpha$  - skeletal,  $\alpha$  - cardiac  $\alpha$  - vascular  $\alpha$  - enteric

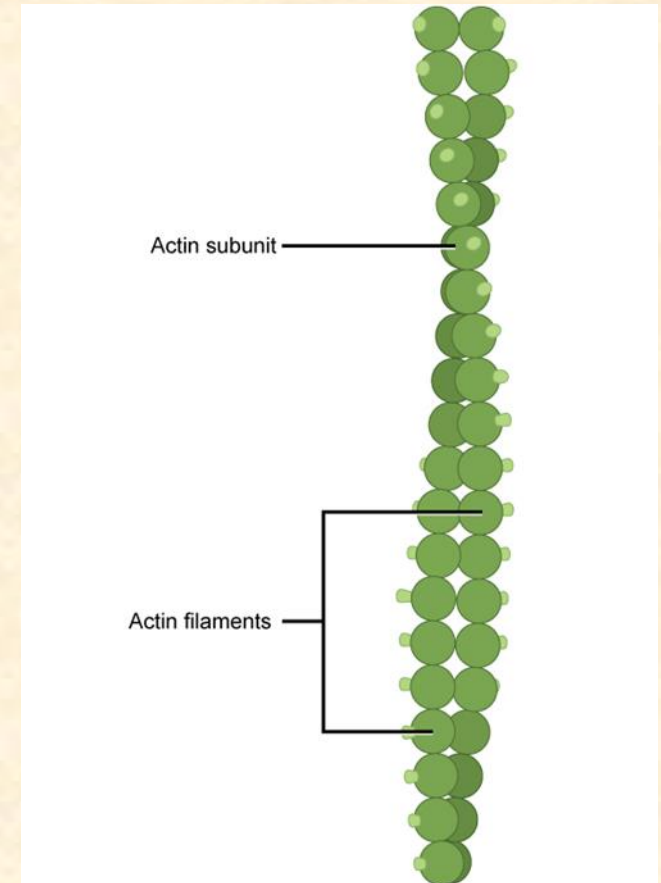
$\beta$  and  $\gamma$  - cytoplasmic (in non-muscle cells)



F actin performs its numerous functions in interaction with a large number actin binding proteins

- **tropomyosin** - stabilization of F actin
- **myosin II** - sliding of F actin in muscle cells (contraction)
- **myosin I** - movement of vesicles along F actin
- **fimbrin, fascin and  $\alpha$  - actinin** - assembly of actin filaments
- **filamin** - cross-linking of F actin
- **spectrin I/II** - cross-linking of F actin in the cell membrane skeleton

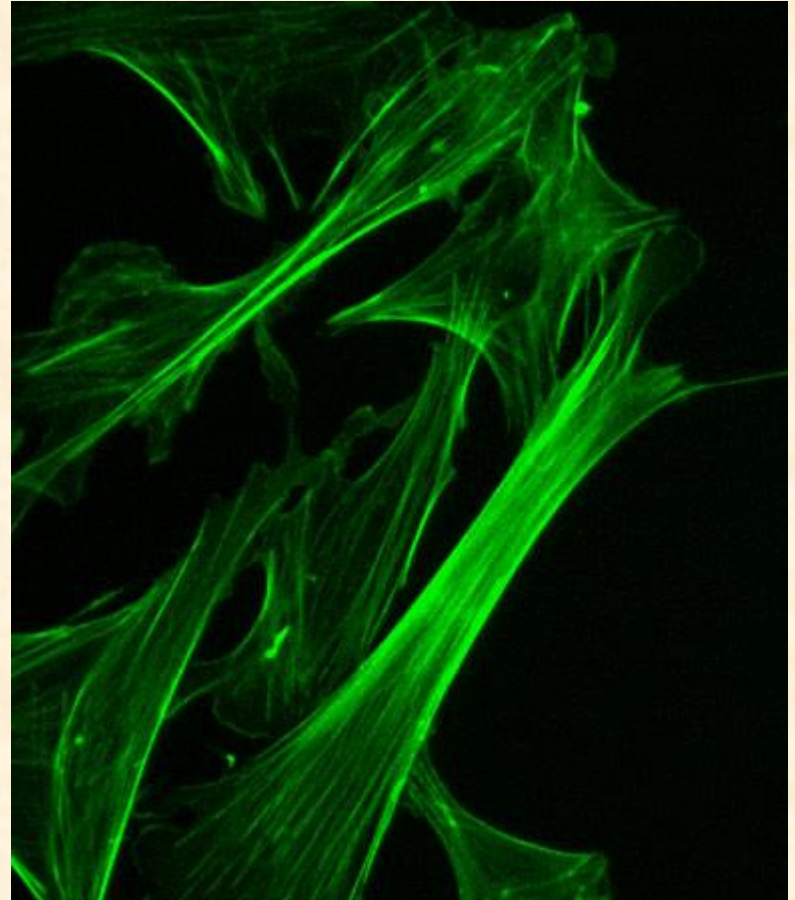
Half of G actin is free in the cytosol, half is polymerized. Depolymerization changes the viscosity and contractility of the cytoplasm



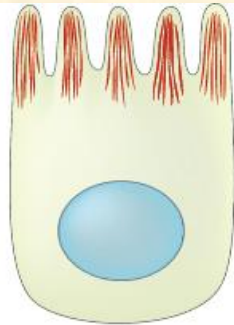


# F actin functions

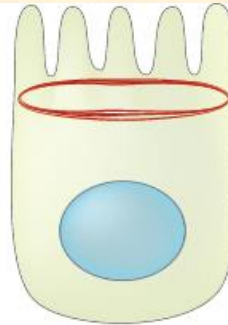
- Cell movement ("creeping") stress fibers
- They give cells contractile strength
- Together with the motor protein myosin responsible for muscle contraction
- They participate in connecting the cell with neighboring cells and the extracellular matrix
- They form the skeleton of numerous specializations of free cell surfaces (microvilli, stereocilia, filopodia, lamellopodia)



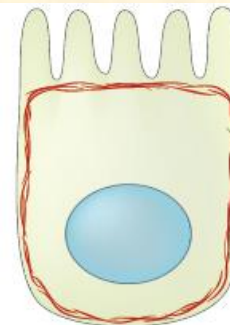
## Distribution in various cells



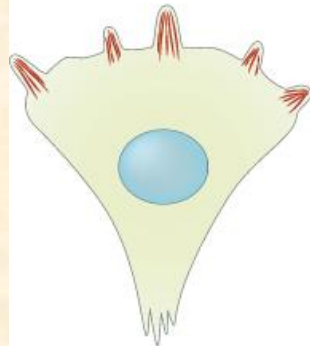
Microvilli



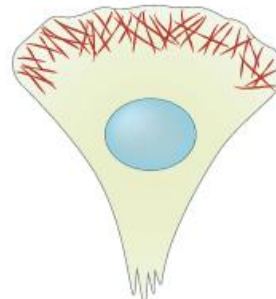
Adhesion belt



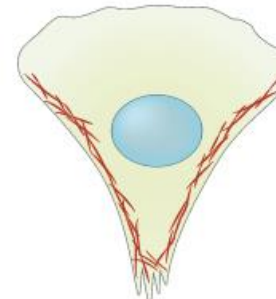
Cell cortex



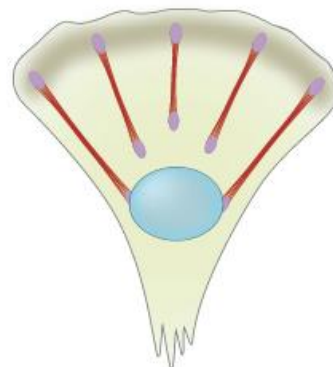
Filopodia



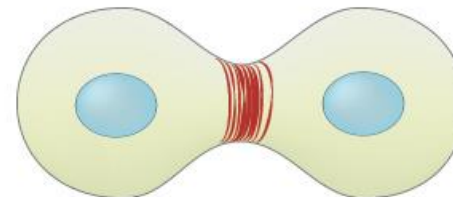
Lamellapodium



Cell cortex

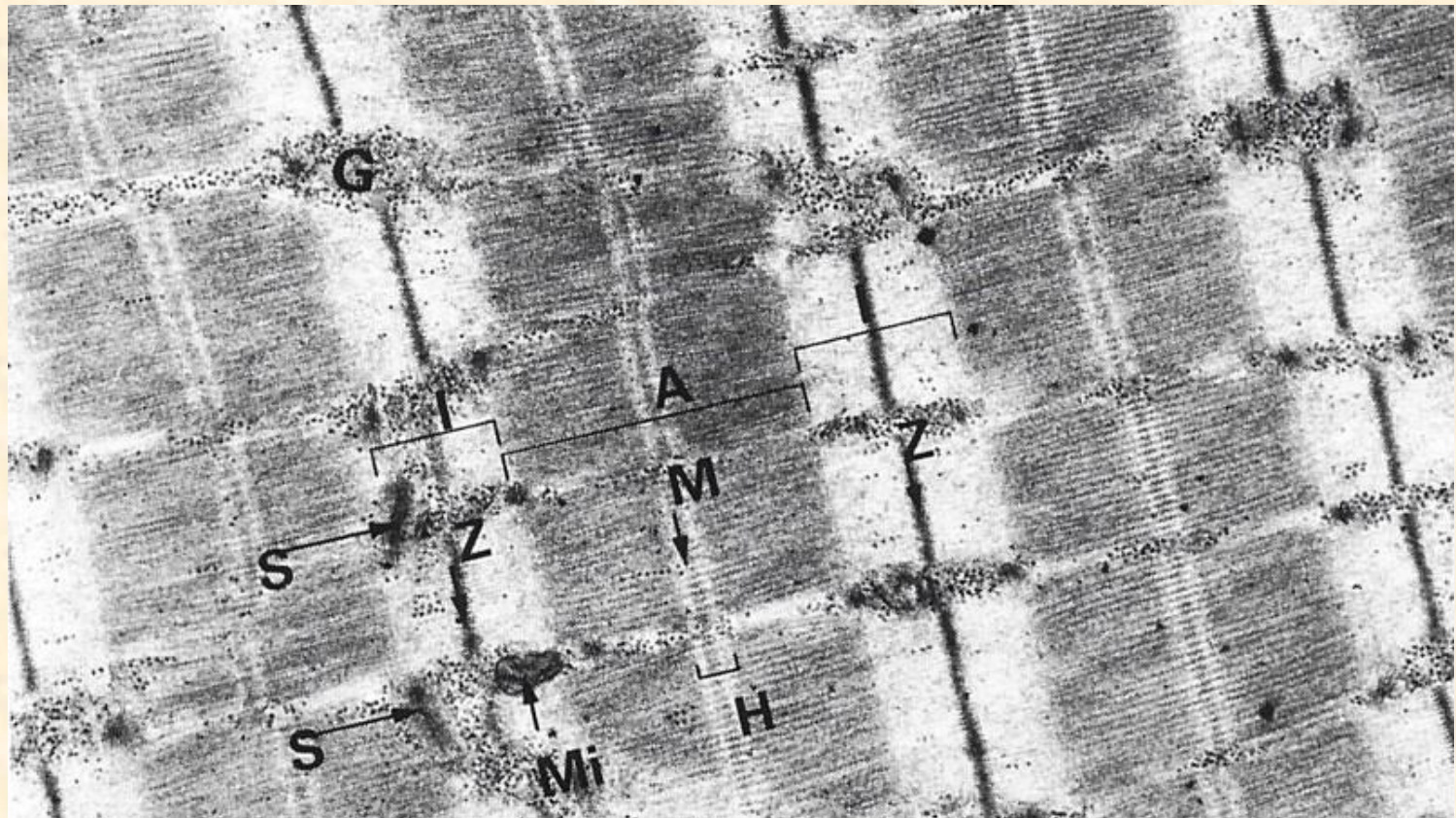


Stress fibers



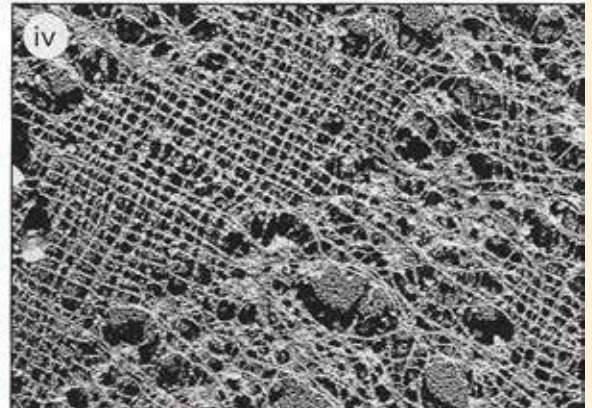
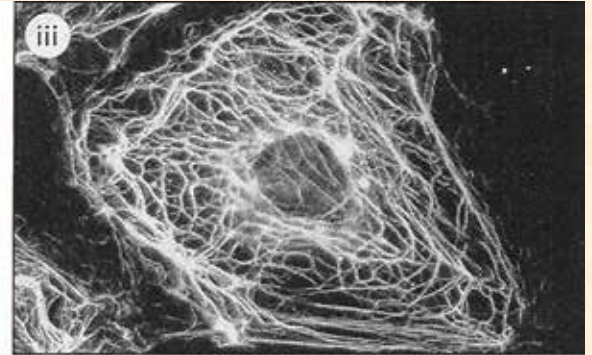
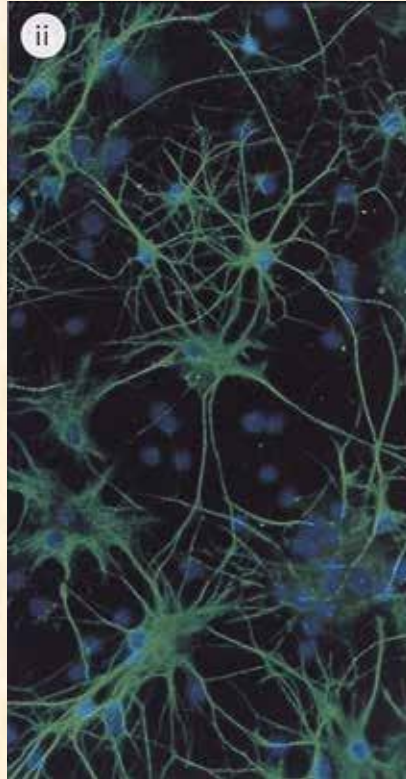
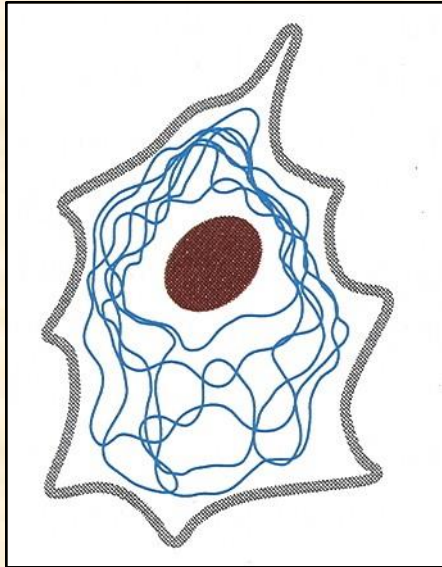
Contractile ring





In striated muscle cells together with thick myosin filaments, they form the **sarcomere** - the basic contractile unit of skeletal and cardiac muscle cells.

# Intermediate filaments

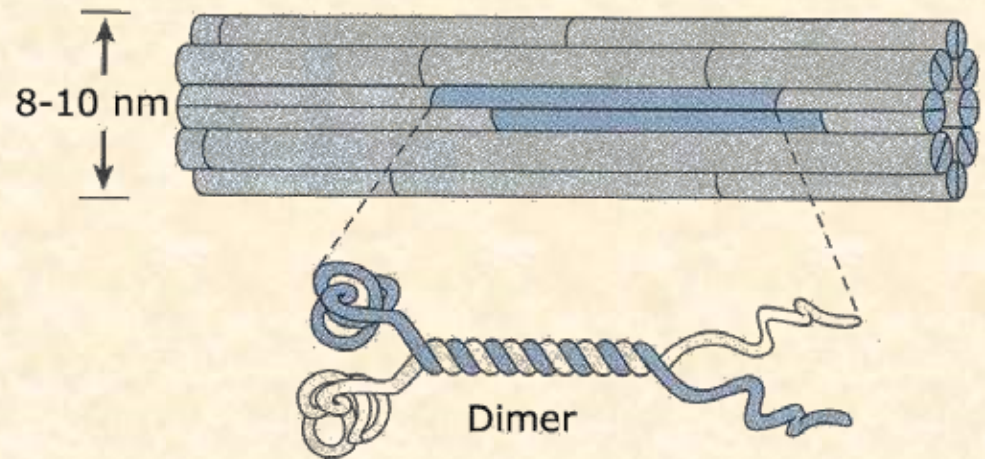


Filaments intermediate in size between the other two, with a diameter averaging 10 nm

Intermediate filaments are stable, confer increased mechanical stability to cell structure, and are made up of different protein subunits in different cell types.



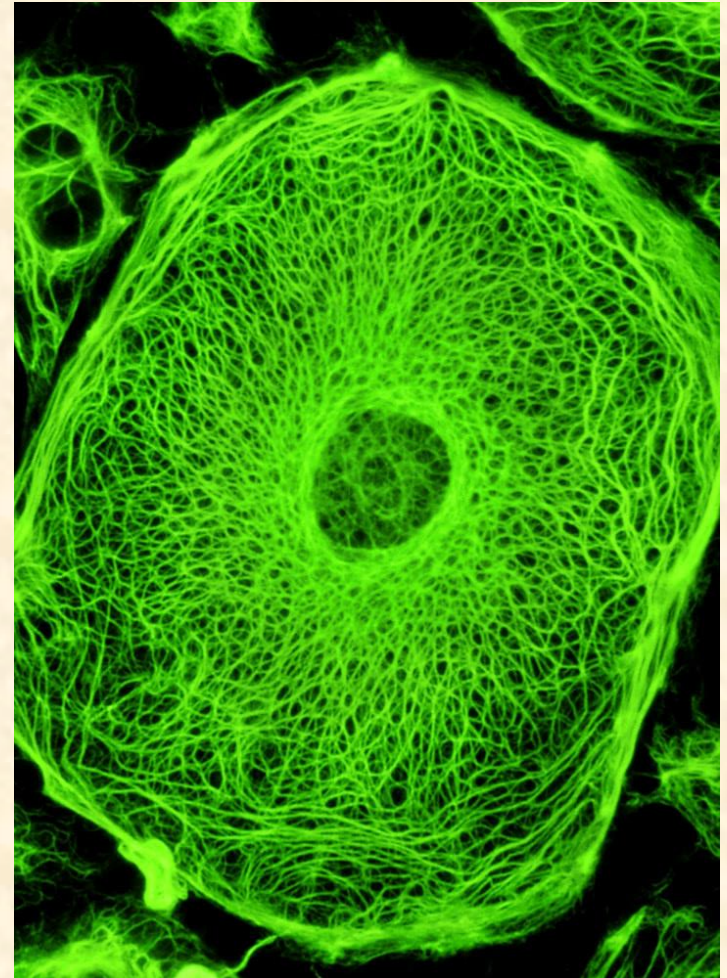
- Intermediate filaments provide mechanical support to the cell.
- They determine the position of the nucleus and organelles.
- They participate in the formation of desmosomes and hemidesmosomes.



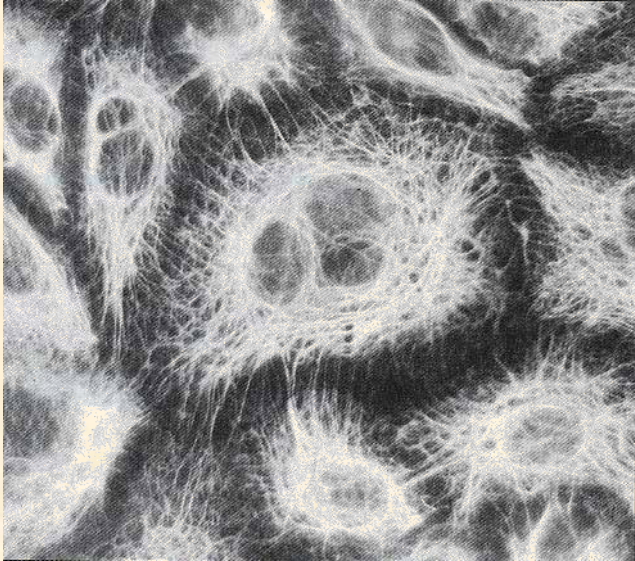


Intermediate filaments include:

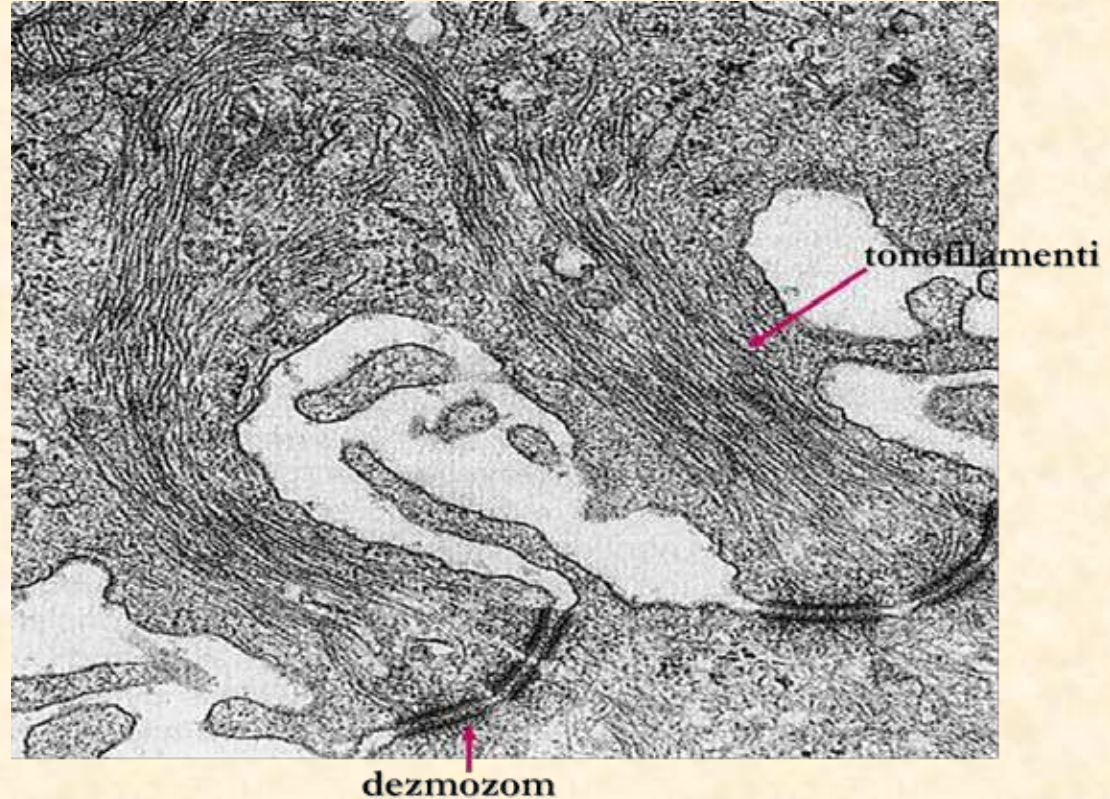
- **Keratin filaments** - cytokeratins or tonofilaments (present in epithelial cells, especially expressed in keratinocytes)
- **Vimentin filaments** (present in cells of mesenchymal origin)
- ❖ **Desmin filaments** (present in muscle cells)
- ❖ **Glial fibrillar acidic protein** (GFAP) (found in astrocytes, oligodendrocytes, microglia)
- **Neurofilaments** (found in neurons, there are three types: NF-L, NF-M and NF-H)
- **Nuclear lamins** (A, B, C) (localized around the nucleus, there are three types: **filaggrin**, **cinnamin** and **plectin**)



# Keratin filaments



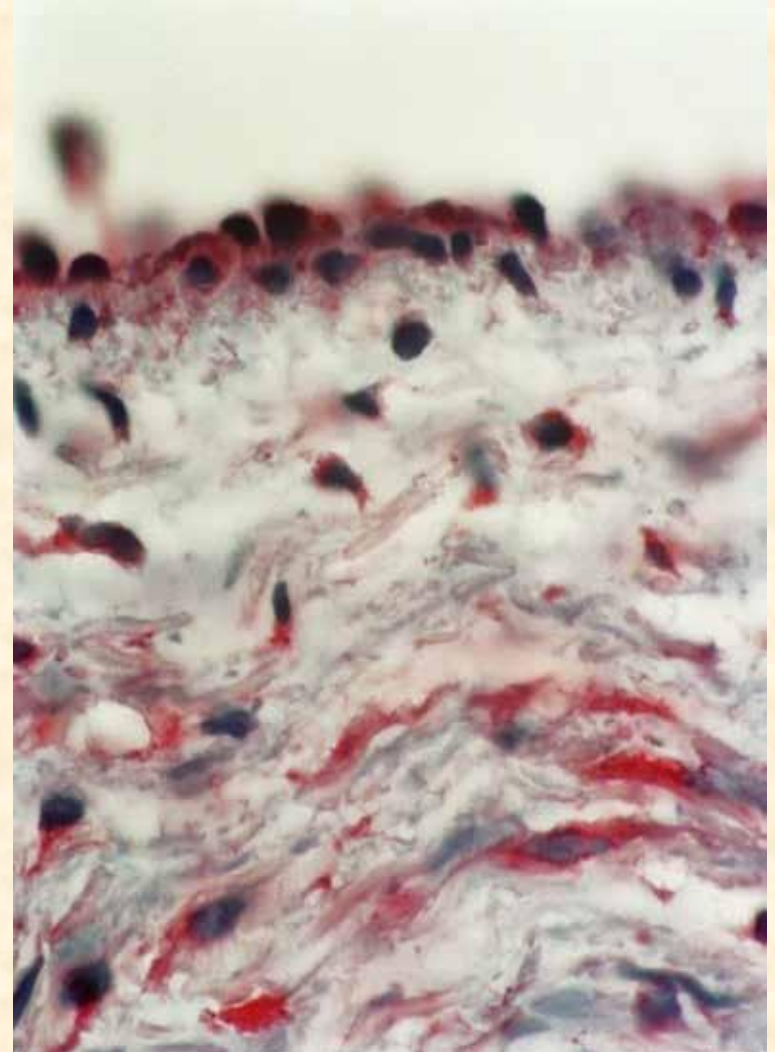
- Typical IF of epithelial cells
- There are over 20 species
- In epidermis causes keratinization
- Tonofilaments bundles around the nucleus and in most of the cytoplasm





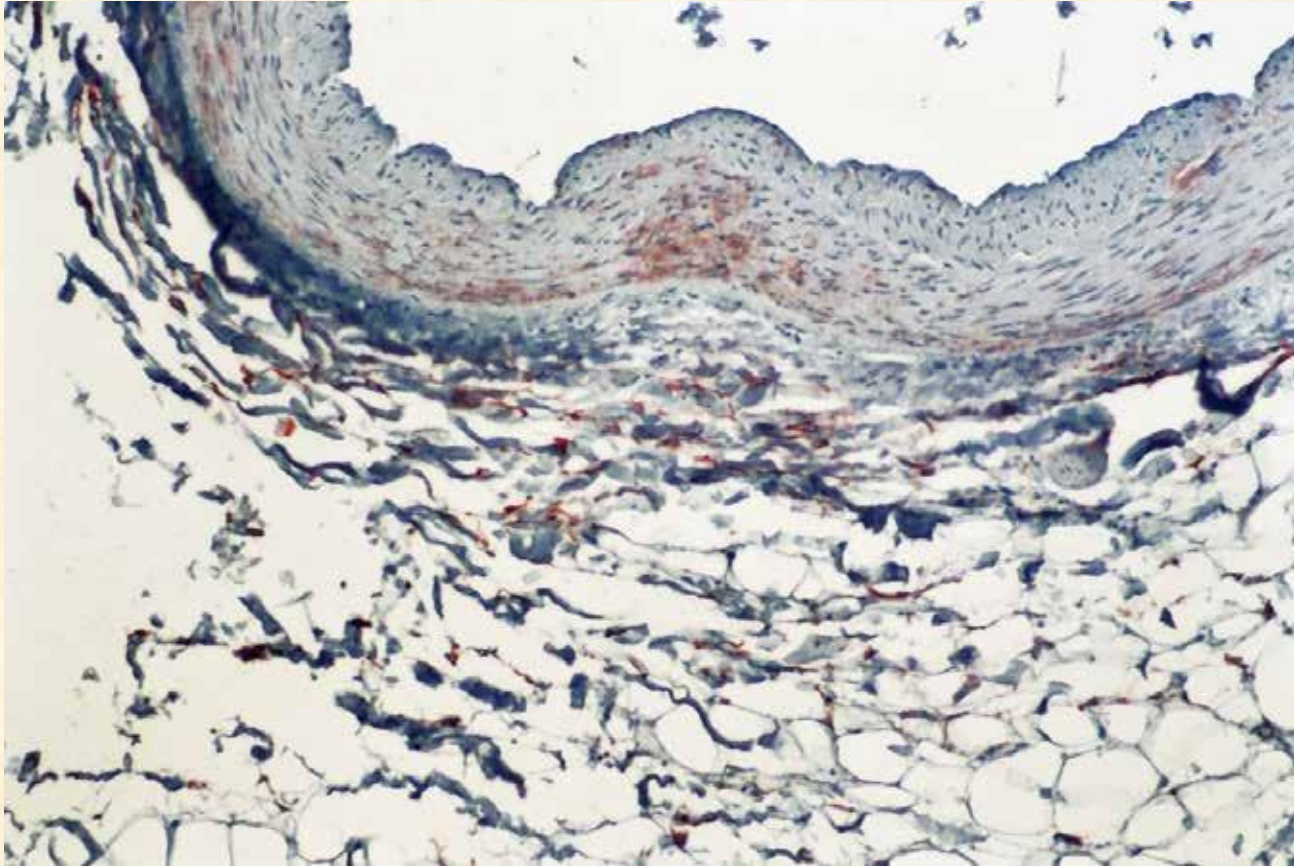
# Vimentin filaments

- Vimentin filaments are made of **vimentin protein**.
- They are found in the cytoplasm of cells of mesenchymal origin: **fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, lymphocytes and blood cells**.
- Some of the mentioned cell populations contain other types of intermediate filaments in addition to vimentin ones.
- Vascular smooth muscle cells **co-express vimentin and desmin**, and glial cells co-express **vimentin and glial acidic protein (GFAP)**.



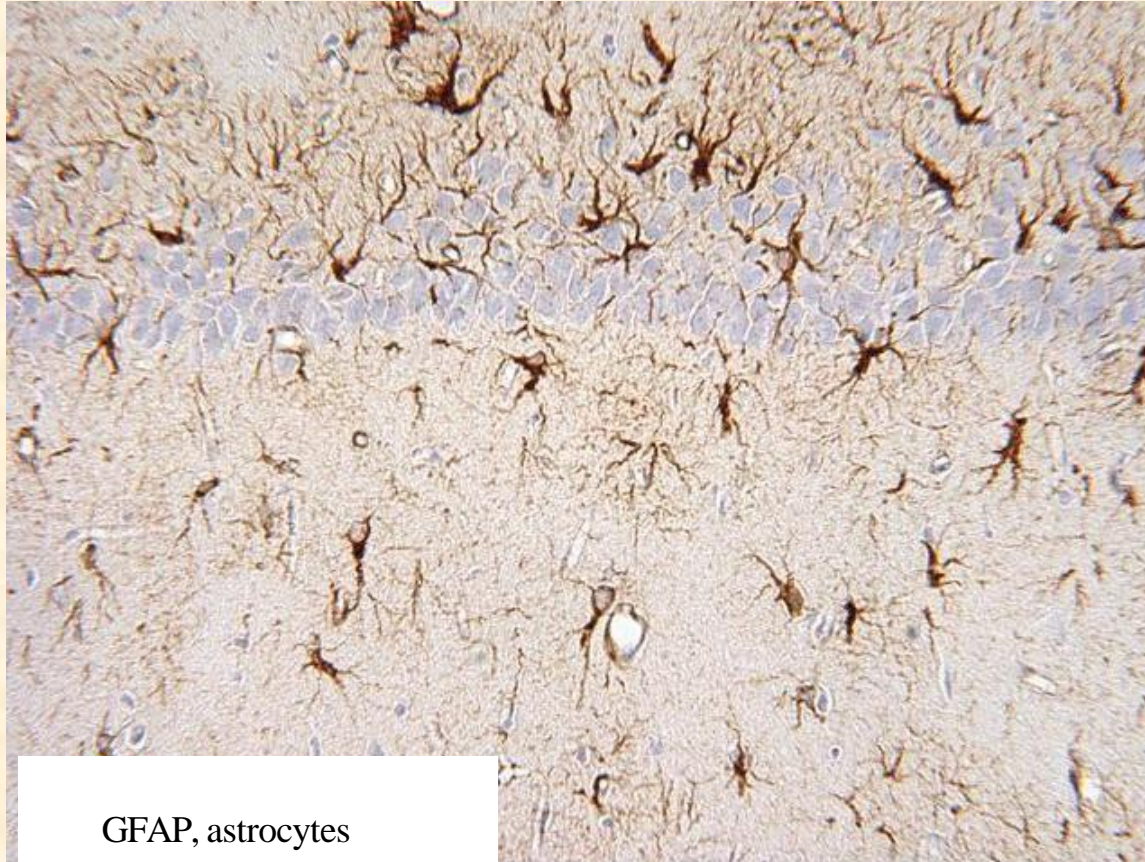


# Desmin filaments



- Desmin filaments are built from the protein desmin. They are represented in the cytoplasm of differentiated smooth, skeletal and cardiac muscle cells.

# Glial fibrillar acidic protein



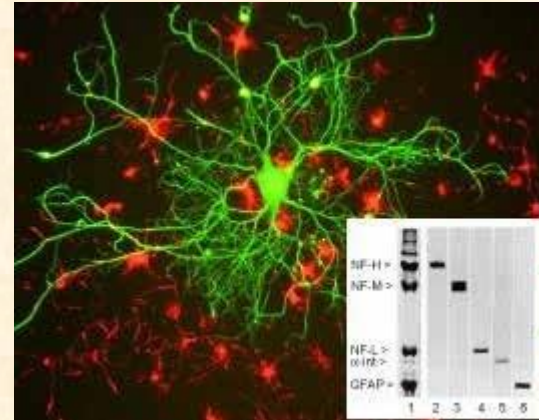
GFAP, astrocytes

- Глијафиламенти (ГФАП) су интермедијарни филаменти астроцита, олигодендроцита, микроглије, Шванових и сателитских ћелија, као и ћелија које су пореклом од неуроглије (питуцити)

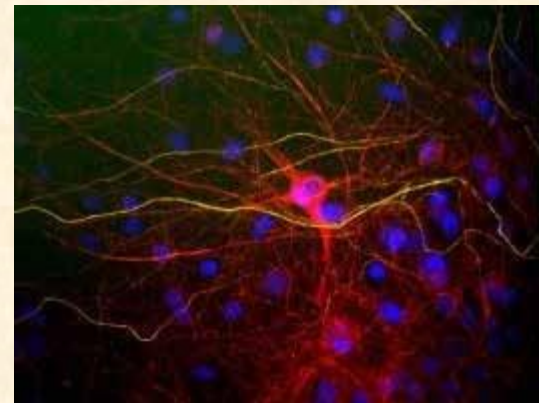


# Neurofilaments

- They contain intermediate filament proteins that are expressed mostly in axons of nerve cells
- The three types of neurofilament proteins are of different molecular weights: NF-L (a low-weight protein), NF-M (a medium weight protein), and NF-H (a high-weight protein)

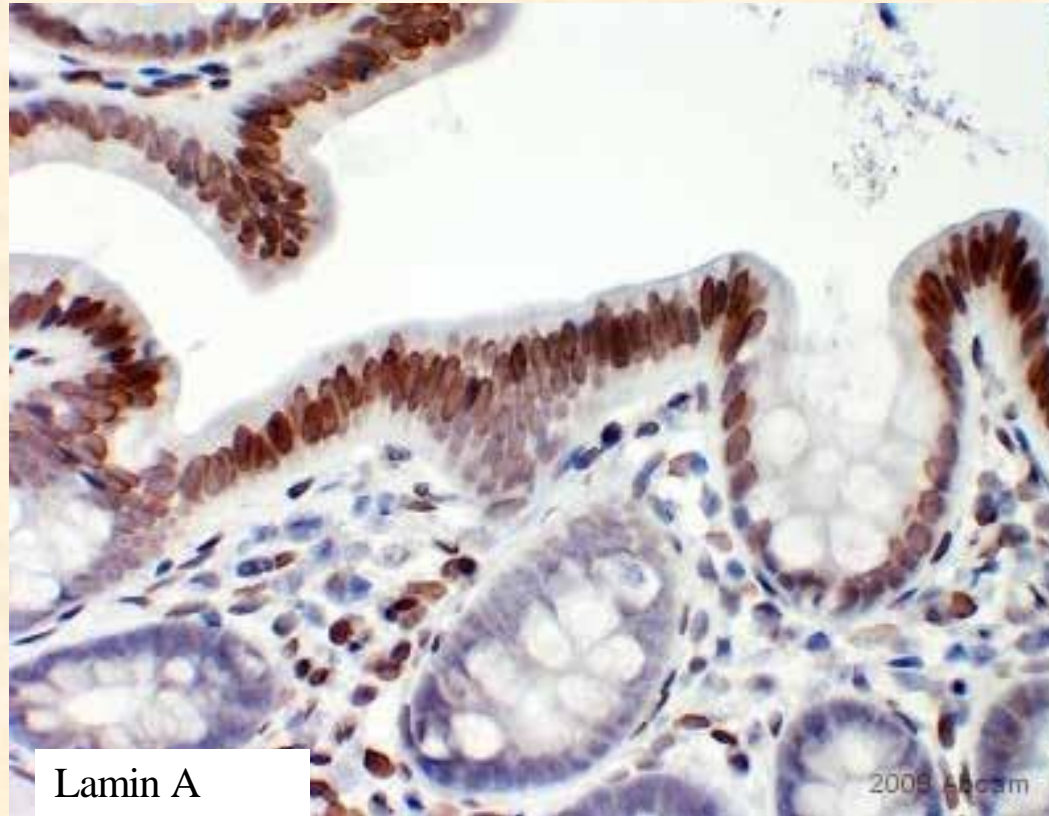


NF-L и  $\alpha$ -interneksin



NF-H и NF-L

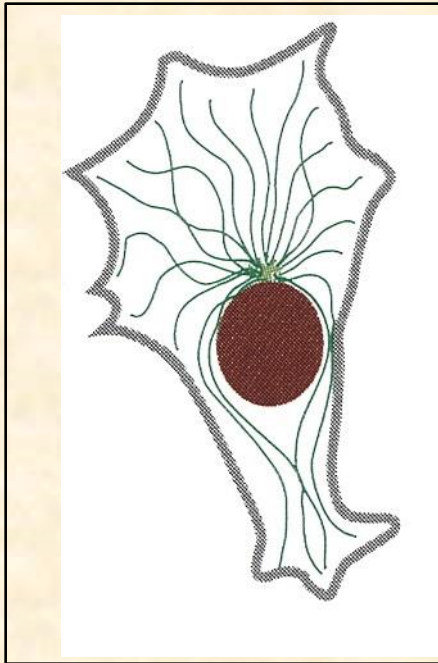
# Lamins



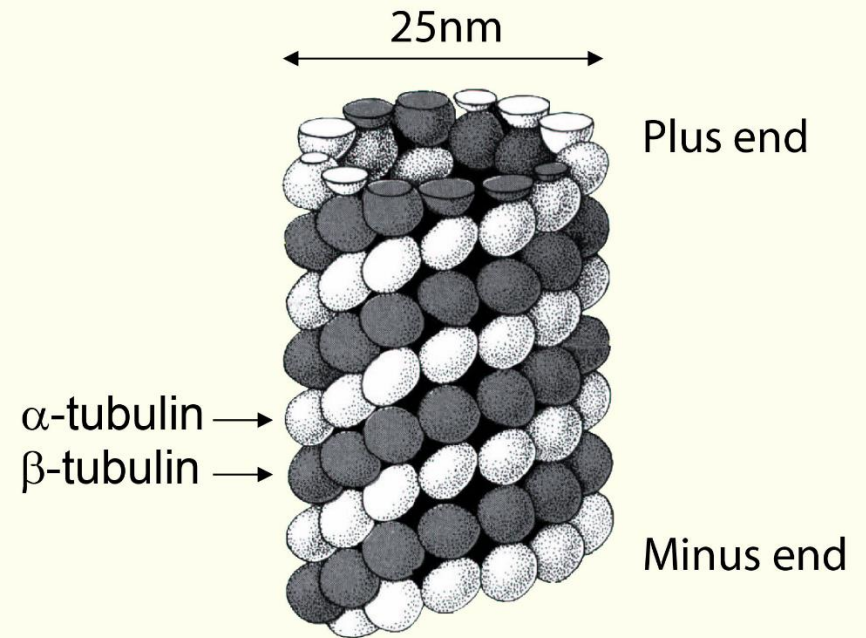
- **Lamins (A, B and C type) are** present in the cell nucleus, **where they form a structural framework called the nuclear lamina just inside the nuclear envelope.**



# Microtubules

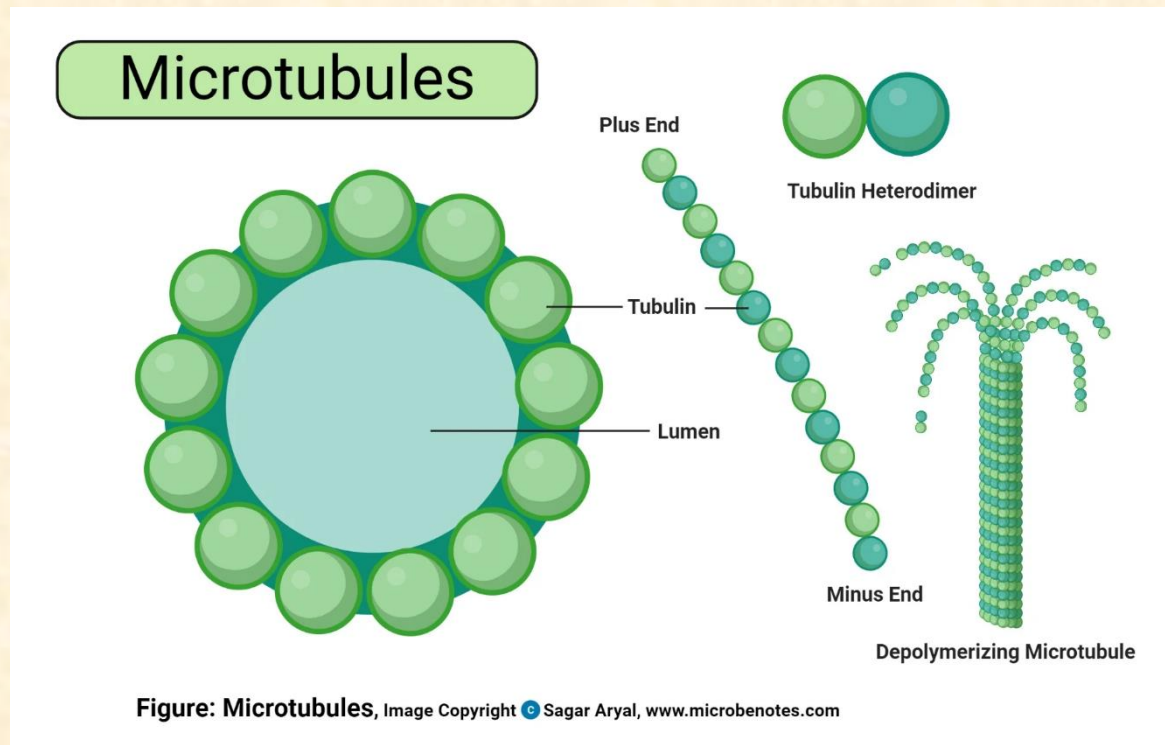


Microtubule Schematic



Each microtubule is hollow, with an outer diameter of **25 nm** and a wall 5-nm thick, a structure that confers significant rigidity to help maintain cell shape.

- They represent the **strongest elements** of the cytoskeleton.
- They are formed by the polymerization of monomeric units, heterodimers  $\alpha$  and  $\beta$  tubulin into protofilaments.
- The microtubule wall contains **13 protofilaments**
- They play an important role in intracellular transport.
- They build the **centriole and the division spindle**.
- They make up the skeleton of **kinocilia and flagella**.

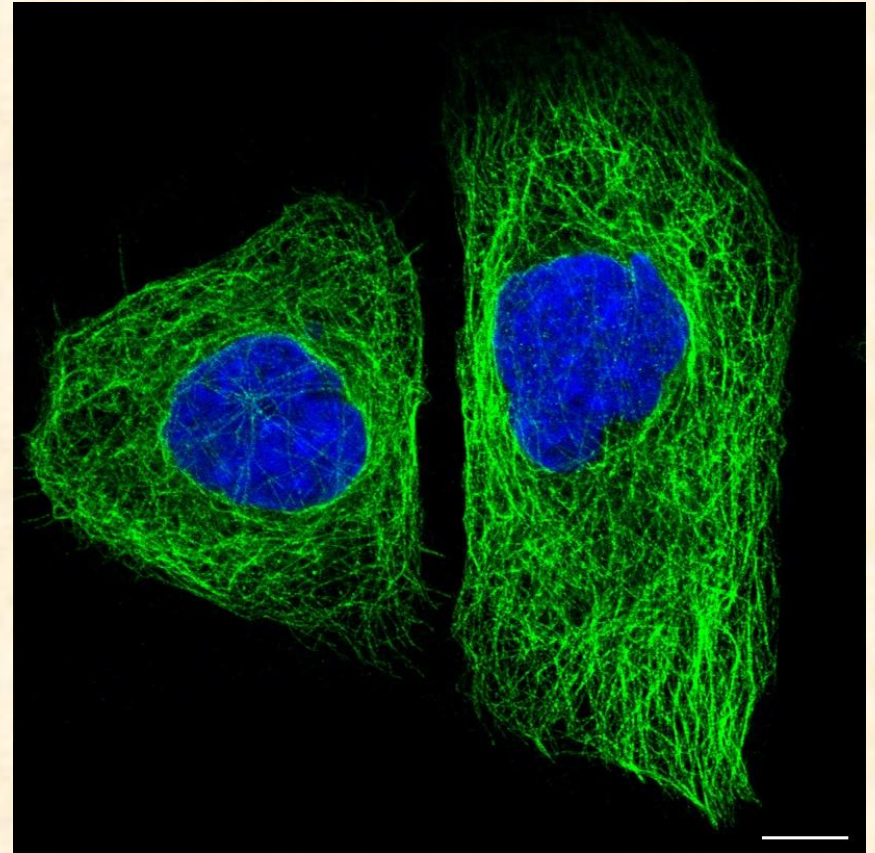


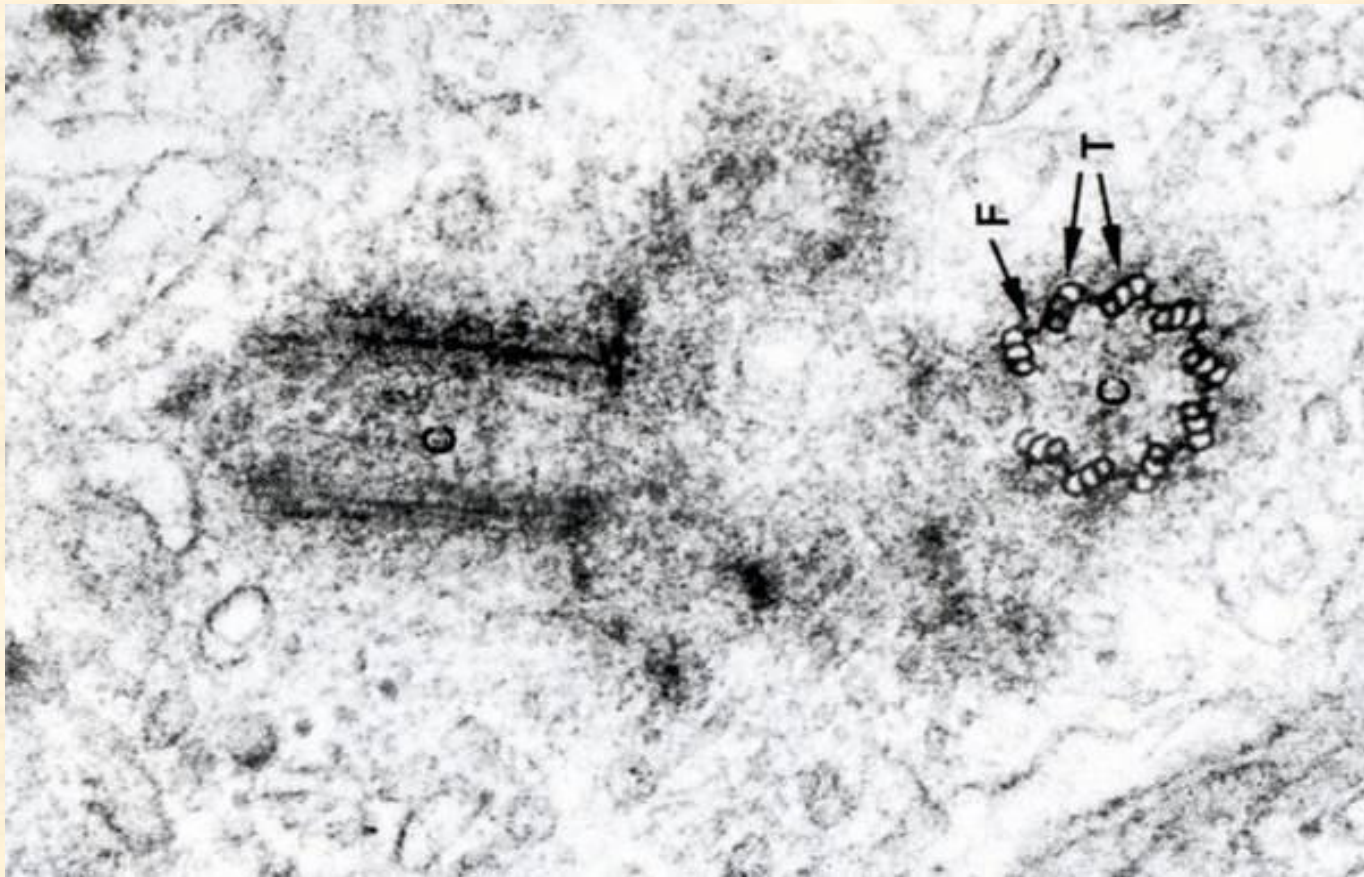


The arrangement of microtubules differs depending on whether it is the centriole and the basal body, the microtubules of the dividing spindle, or the skeleton of kinocilia and flagella.

Microtubules are **stabilized by MAP proteins (dynein and kinesin)**, motor proteins that have ATPase activity.

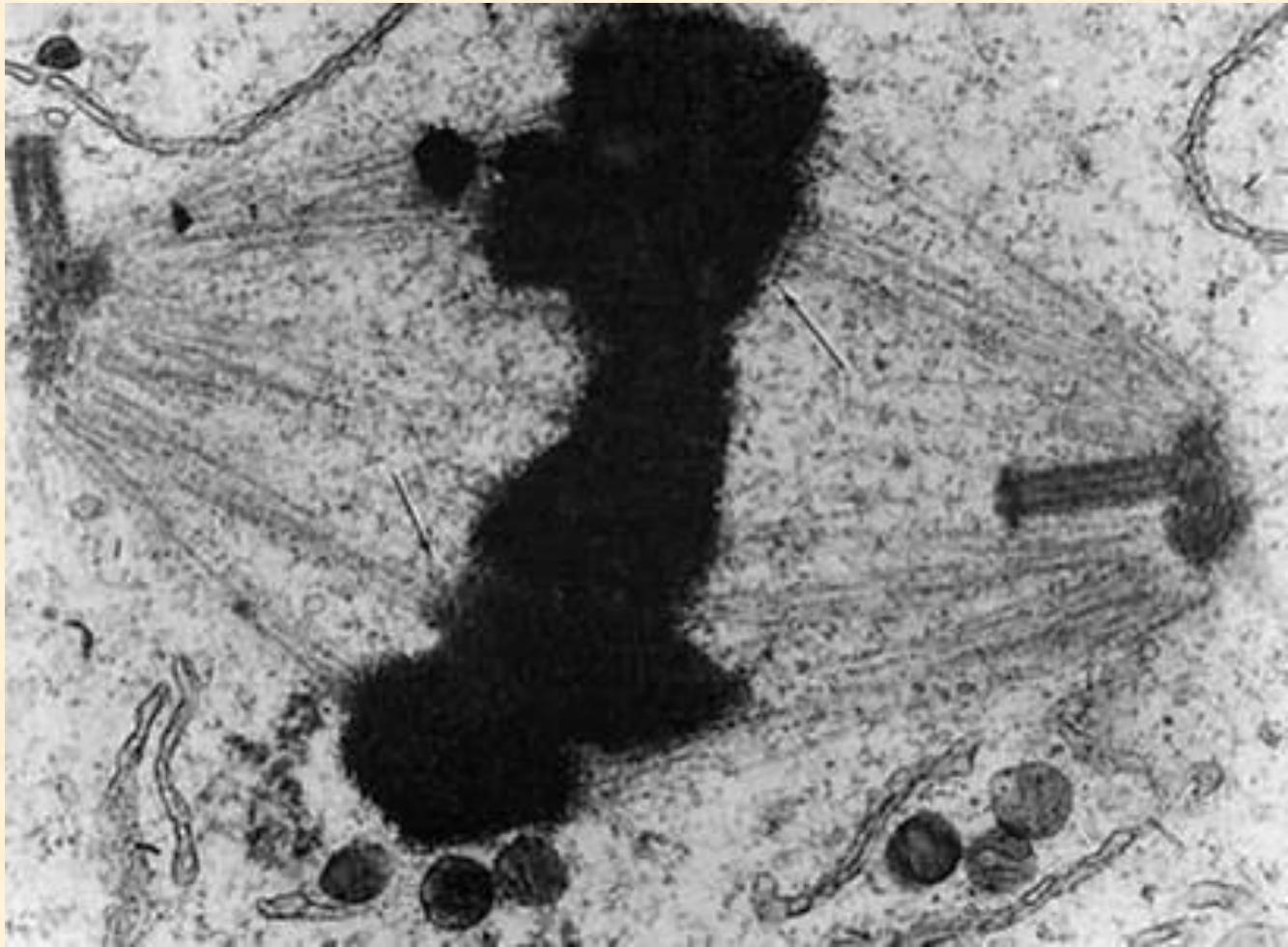
**Kinesins** carry material away from the MTOC near the nucleus toward the plus end of microtubules (anterograde transport);  
**dyneins** carry material along microtubules in the opposite direction





Centriole and MTOC





Mitotic spindle

# **Cytoplasmic organelles**



Organelles are components, or. compartments of the cytoplasm that are distinguished by the appropriate shape, size, structure and function.

Organelles can be divided into two groups:

### **Non-membranous**

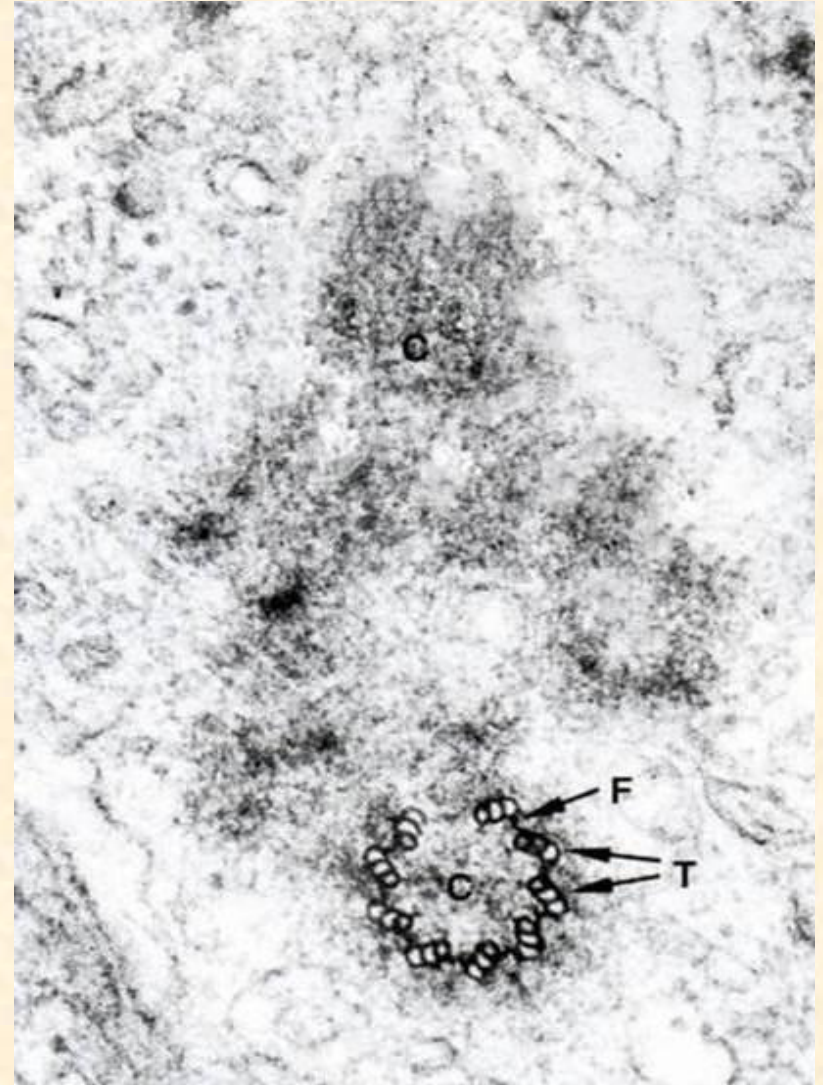
- Centrioles
- Ribosomes

### **Membranous**

- Endoplasmic reticulum (rough and smooth)
- Golgi apparatus
- Lysosomes
- Peroxisomes
- Mitochondria

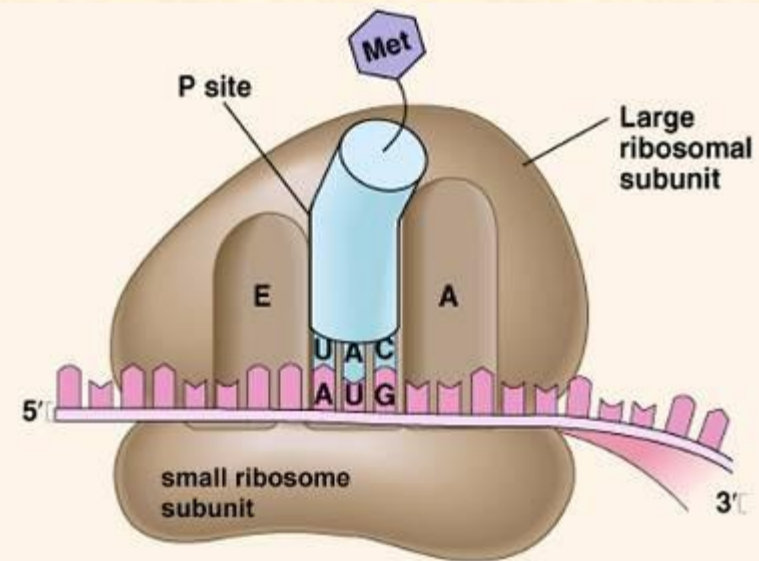
# Centrioles

- The wall of the centriole is composed of 9 triplets of microtubules, arranged in a circle.
- The triplet consists of three partially fused microtubules (A - closest to the central axis, B and C).
- Microtubule A is connected to microtubule C of the neighboring triplet
- Microtubule A is complete, or contains 13 protofilaments.
- Non-dividing cells, as well as proliferating cells in the interphase of the cell cycle, contain two centrioles.
- Pair of centrioles and the pericentriolar material is called the centrosome (MOC).
- Centriole replication begins in the S phase of the cell cycle.



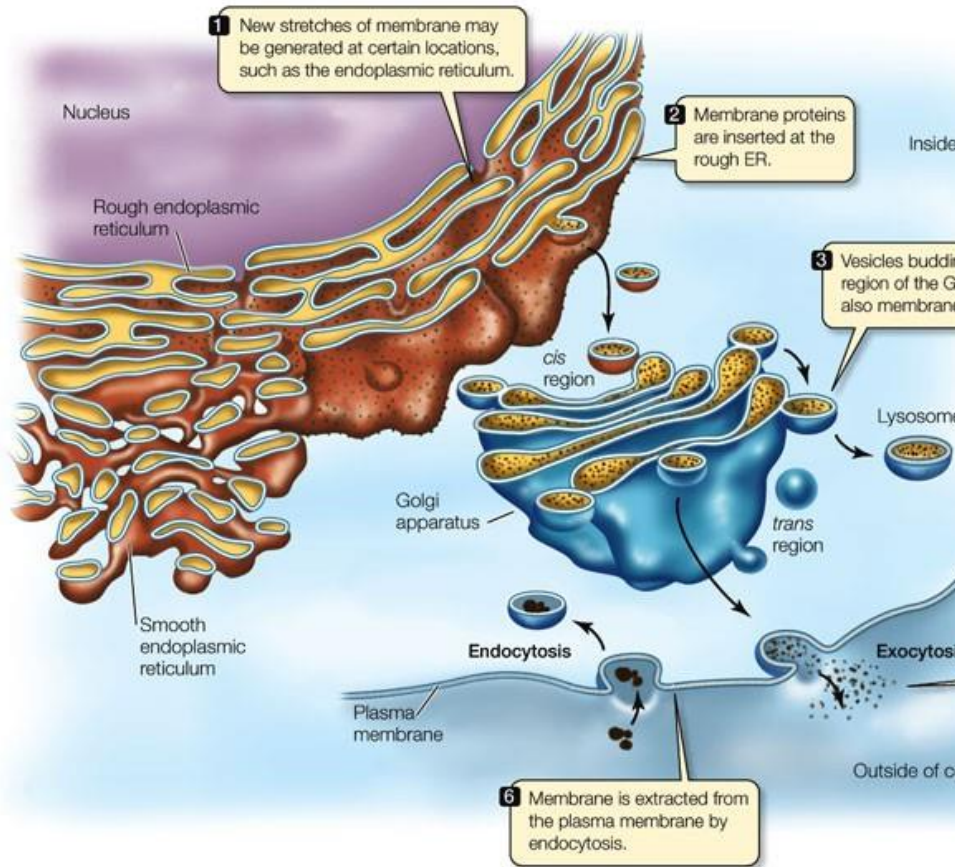
# Ribosomes

- Small electron-dark granules, about 20 nm in diameter.
- Present in all cells and participate in protein synthesis.
- Built from two unequal subunits.
- The smaller subunit contains 1 molecule of rRNA and 33 proteins.
- The larger subunit contains 3 rRNA molecules and 49 proteins.
- Ribosomal RNAs are **produced in the nucleolus**, while protein components are synthesized in the cytoplasm, and then enter the nucleus and nucleoli where they combine with rRNA.
- Subunits leave the nucleus through pores and join together in the cytoplasm.
- (monoribosomes) or (poly(ribo)somes)
- Polysomes can be free in the cytosol or bound to the membrane of the ER and outer nuclear envelope.



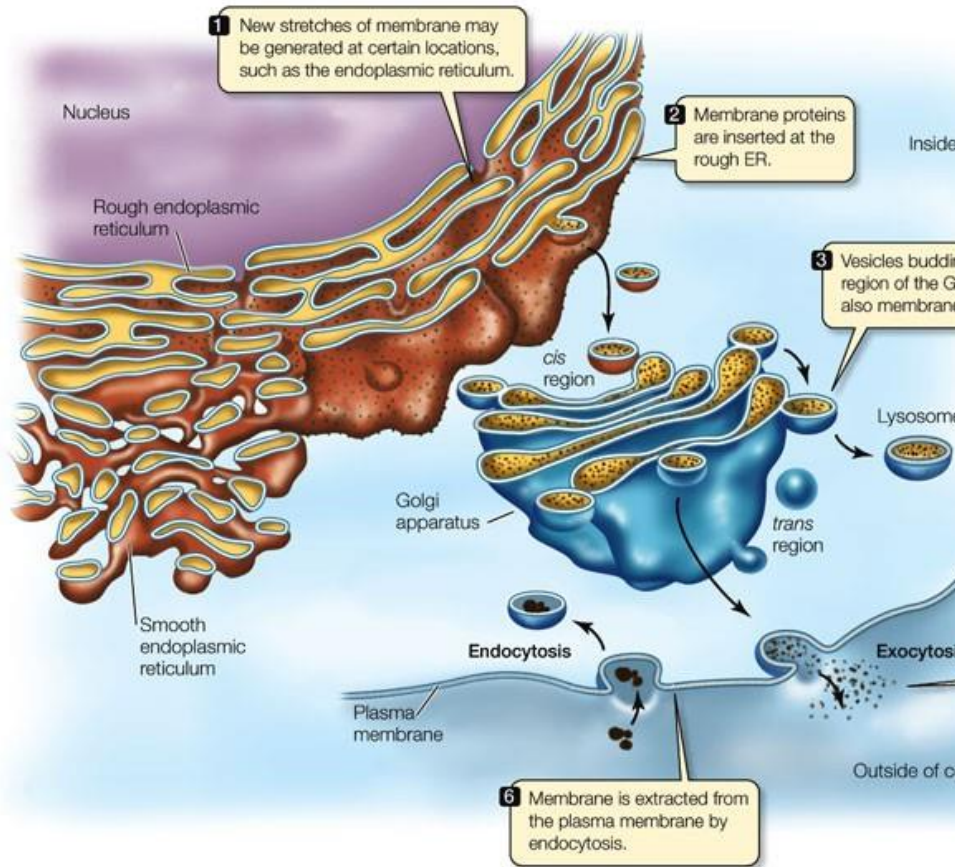


# Endoplasmic reticulum



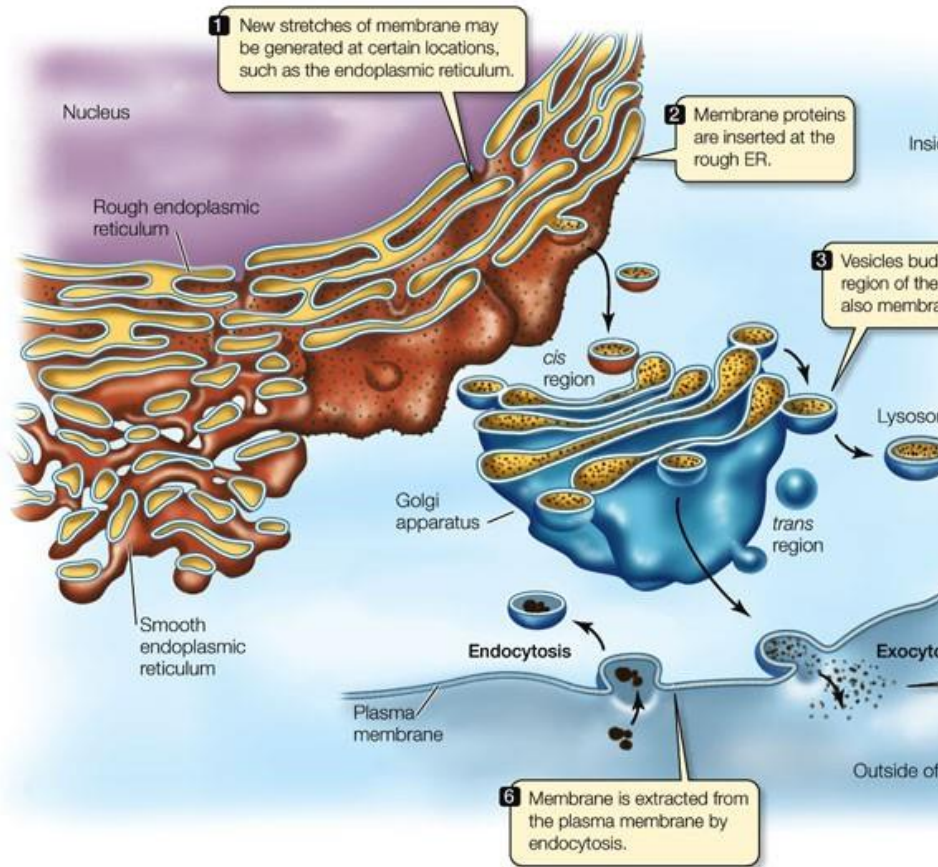
- System of connected intracellular cavities separated by their own membrane.
- ER occurs in two forms that differ in structure and function:
  - **Rough ER (rER) whose membrane is sprinkled with polyribosomes**
  - **Smooth ER (sER) whose membrane is devoid of ribosomes.**
- Only rER has the ability to synthesize proteins.

# rER



- rER consists of parallel cisternae connected by short tubules.
- rER membranes are continuous with the outer nuclear membrane.
- All cells except erythrocytes contain this organelle, and it is especially expressed in cells that synthesize: protein secretion, ECM components, specific proteins (eg immunoglobulins)...

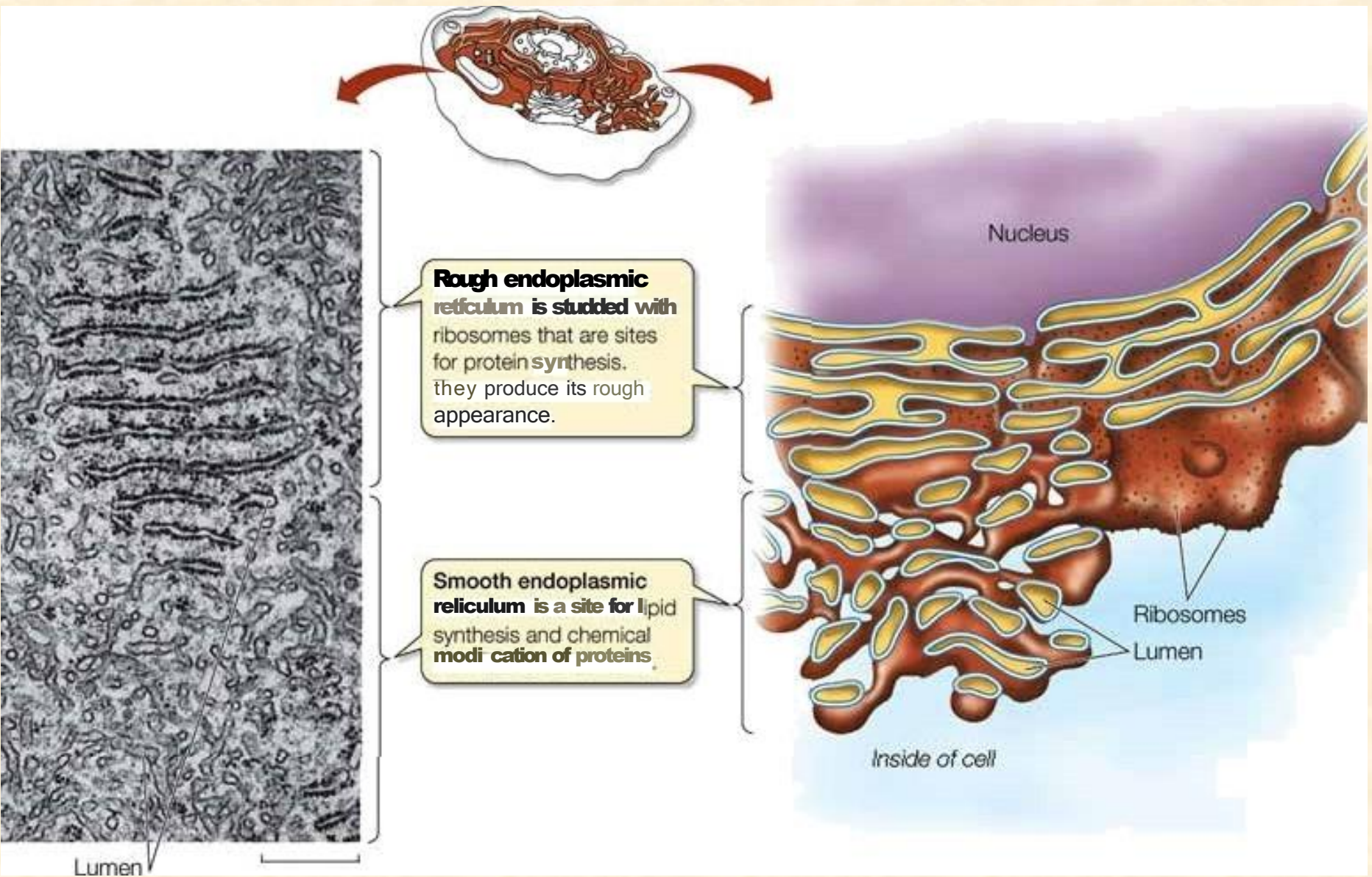
# sER



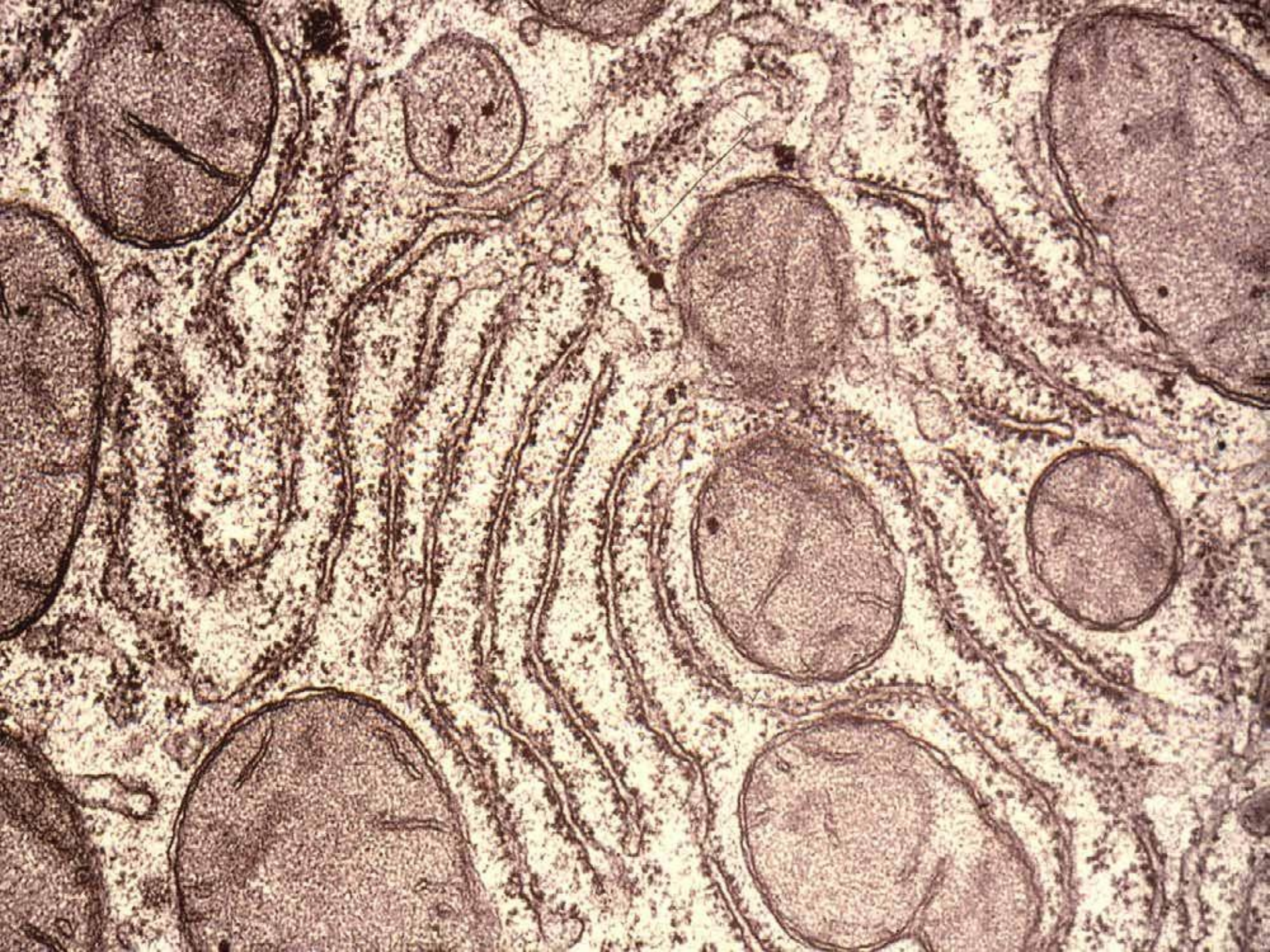
- The membrane system of sER is connected to the membranes of rER/
- Lipid biosynthesis takes place in sER, which takes place in all cells, as well as synthesis of steroid hormones, glycogenolysis, detoxification of metabolites in certain types of cells.
- It is most expressed in hepatocytes.
- Enzymes responsible for lipid synthesis are bound to the sER membrane.

**sER has a specific form and function in skeletal and heart muscle cells - sarcoplasmic reticulum.**





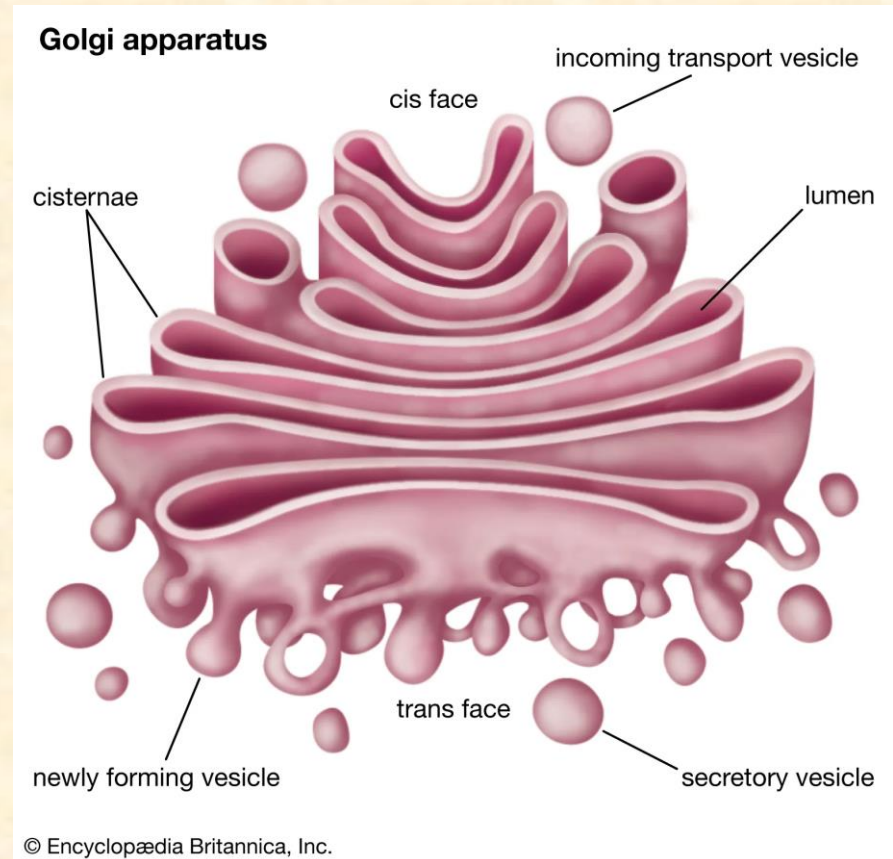




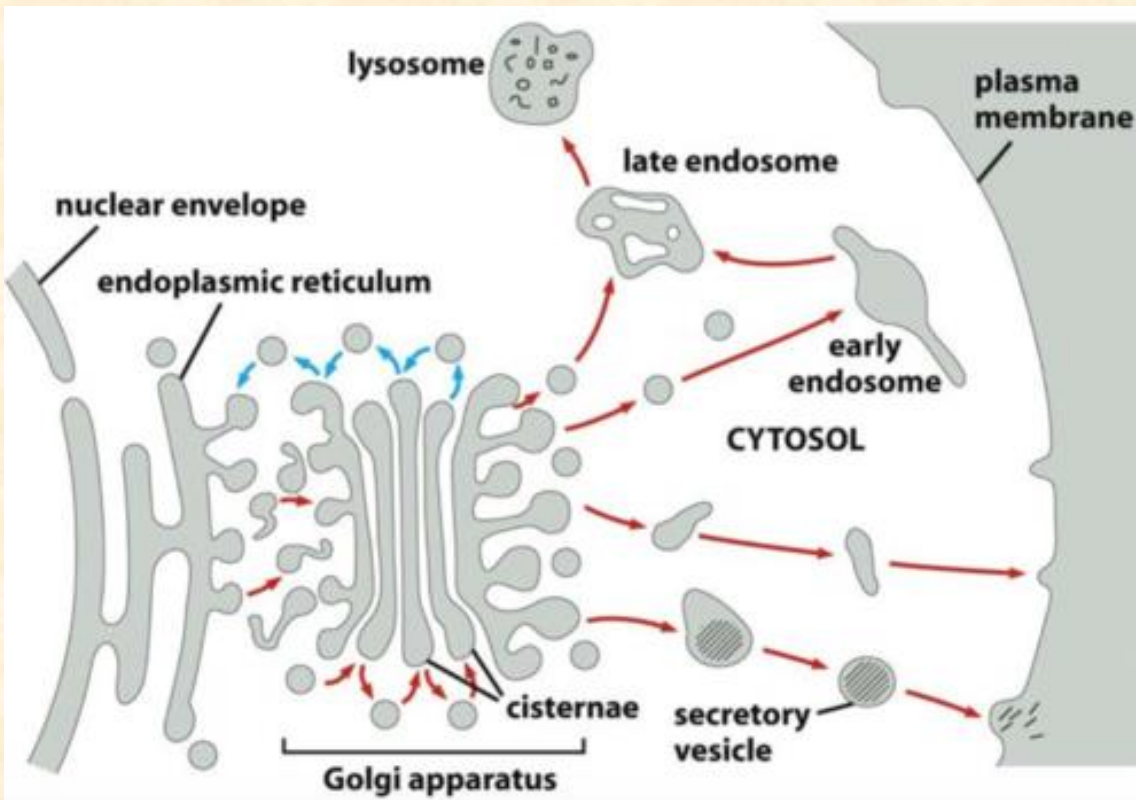


# Golgi apparatus

- Golgi apparatus (complex) involved in the synthesis of carbohydrates, as well as in the modification, sorting, packaging and targeting of proteins and lipids synthesized in the ER.
- It is composed of 3-10 discoid sacs at a distance of 5-30 nm.
- The convex (**cis**, forming) side faces the nucleus or rER, while the concave (**trans**, maturing) side faces the plasmalemma.
- Each cistern of the Golgi apparatus contains an appropriate set of enzymes and is involved in one phase of protein and lipid processing.
- On the trans side, molecules are sorted and packaged into secretory vesicles or primary lysosomes.



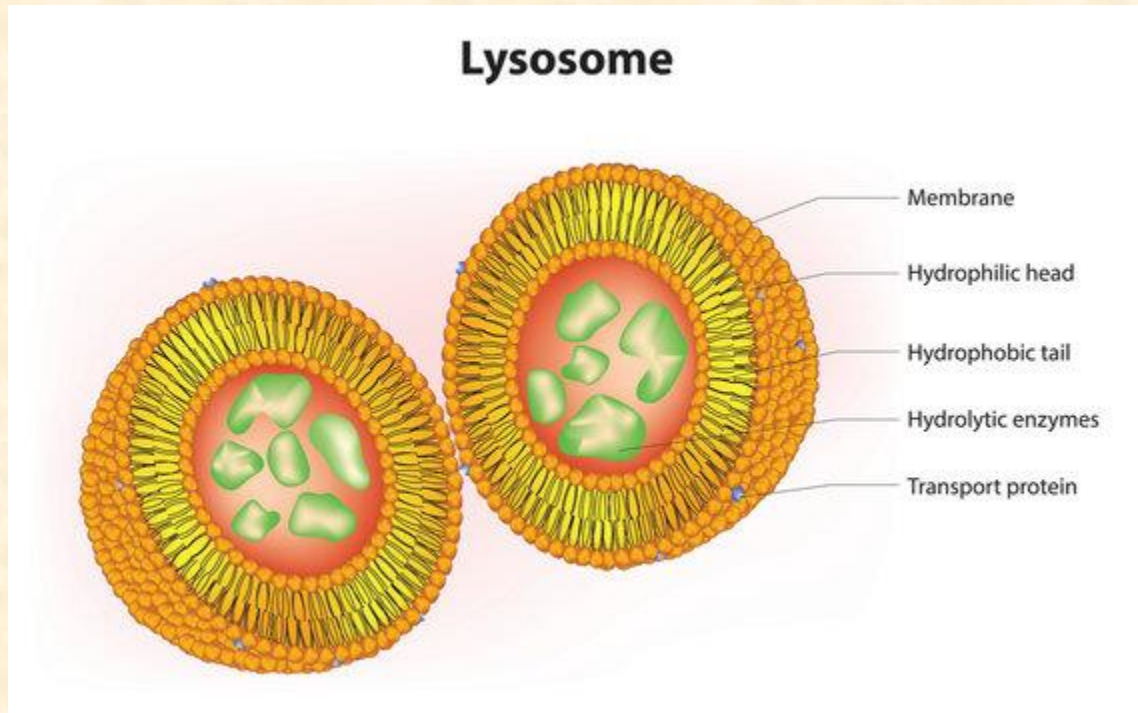




- **Constitutive secretion– exocytotic vesicles** move with the help of microtubules to the plasmalemma and release the contents outside the cell -.
- **Regulated secretion– vesicles** coated with clathrin, accumulate in the cytosol, lose the clathrin coat and form secretory granules that are released by adequate stimulation.
- **Lysosomal vesicles** destined for the lysosome containing many acid hydrolases. The vesicle first fuses with the late endosome, and the contents are then transferred to the lysosome via unknown mechanisms.

# Lysosome

- Lysosomes are places of intracellular digestion and renewal of cellular components.
- Lysosomes (Greek: **lysis, dissolution + soma, body**) are membrane-bound vesicles that contain a large number of different hydrolytic enzymes (more than 40) whose function is intracytoplasmic digestion.





- Lysosomal enzymes are synthesized and secreted in the rER, then transported to the Golgi complex where they are modified and packaged into lysosomes.
- Digestion processes mediated by lysosomes: **specific receptor endocytosis**; **pinocytosis** (non-specific uptake of extracellular fluid); **phagocytosis** (intake of extracellular particles) and **autophagy** (intracellular proteins - microautophagy or organelles - macroautophagy).

#### Lysosomal types

- **primary lysosomes.**
- **secondary lysosome.**
- **residual bodies.**

(in some long-lived cells (eg neurons, cardiac muscle cells), large amounts of residual bodies accumulate and are labeled as **lipofuscin or senescence pigment**).

# Clinical significance

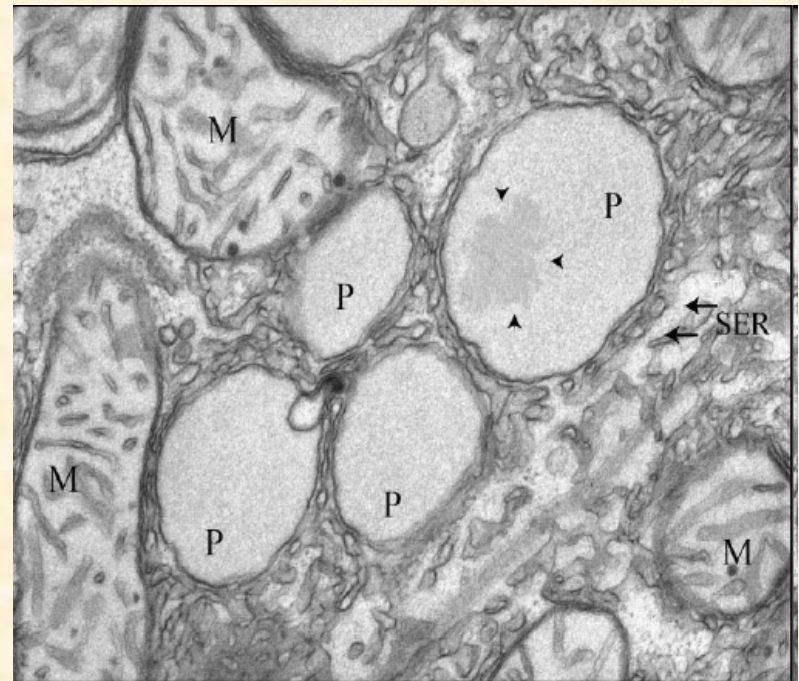
- genetically inherited deficiencies, or mutations called lysosomal storage diseases (LSD),
- inborn errors of metabolism caused by a dysfunction of one of the enzymes. incidence is estimated to be 1 in 5,000 births.
- accumulation of specific macromolecules or monomeric compounds inside the endosomal-autophagic-lysosomal system.
- This results in abnormal signaling pathways, calcium homeostasis, lipid biosynthesis and degradation and intracellular trafficking, ultimately leading to pathogenetic disorders.
- The organs most affected are brain, viscera, bone and cartilage



# Peroxisome

- **Peroxisomes** (peroxide + soma, body) are spherical organelles surrounded by a membrane whose diameter varies from 0.5 to 1.2  $\mu\text{m}$ .
- They oxidize specific organic substrates by removing the  $\text{H}^+$  atom, which is transferred to the oxygen molecule ( $\text{O}_2$ ), creating **hydrogen peroxide** that damages the cell.
- However, peroxisomes contain the enzyme catalase, which breaks down  $\text{H}_2\text{O}_2$  into water and oxygen
- Catalase activity also has **clinical significance**.

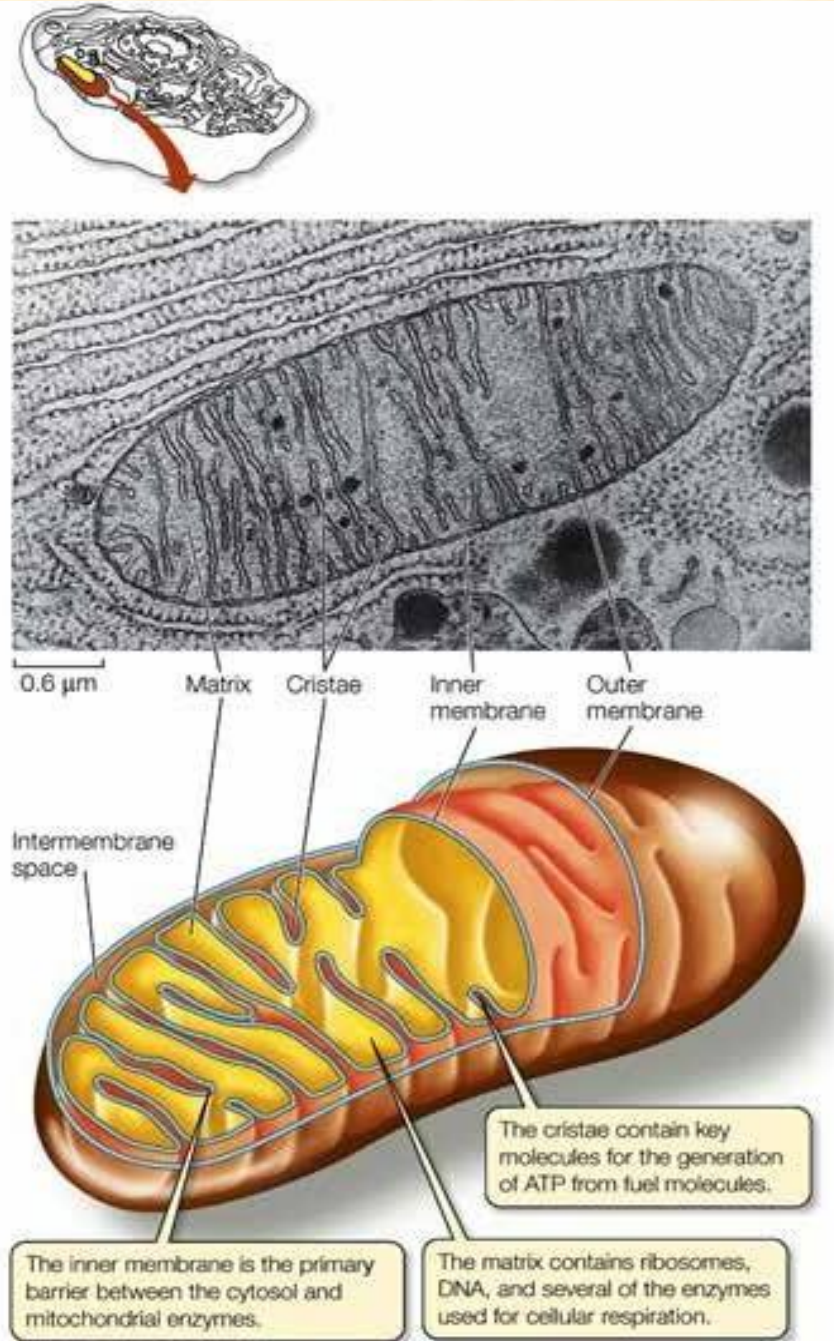
It degrades several toxic molecules and drugs especially in liver and kidney peroxisomes (eg 50% of ingested ethyl alcohol is degraded to acetaldehyde in liver and kidney peroxisomes).



# Mitochondrion

- Synthesis of ATP (0.4-0.8  $\mu\text{m}$ ; 4-8  $\mu\text{m}$ )
- Parathyroidocytes, myocytes, nephrocytes, hepatocytes (up to 2000)
- **Outer membrane** - porous
- **Inner membrane** folds-cristae, oxidative phosphorylation, cardiolipins
- **Intermembrane space**
- **Inner chamber** (matrix)

mitochondrial DNA, RNA, ribosomes and matrix granules, enzymes for pyruvate catabolism,  $\beta$ -oxidation of fatty acids, Krebs cycle, as well as enzymes for mitochondrial genome expression







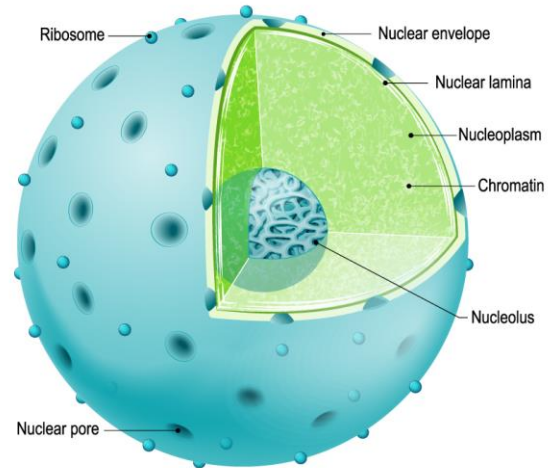
# Inclusions

- Inclusions are products of cellular metabolism that accumulate in the cytoplasm in the form of granules, droplets or crystals.
- Either surrounded by a membrane (pigment granules), or more often immersed in the cytoplasmic matrix.
- They can be important for cell function (glycogen) or completely non-functional.

The most important inclusions:

- **Glycogen** - a polysaccharide created in the GLER from glucose molecules - an important energy source that is stored in cells with high metabolic activity (hepatocytes, myocytes).
- **Lipid inclusions** (mACase droplets) - deposits of triglycerides, as a source of energy or steroid hormones - precursors of steroid hormones; one or more drops.

## Cell Nucleus



# Nucleus

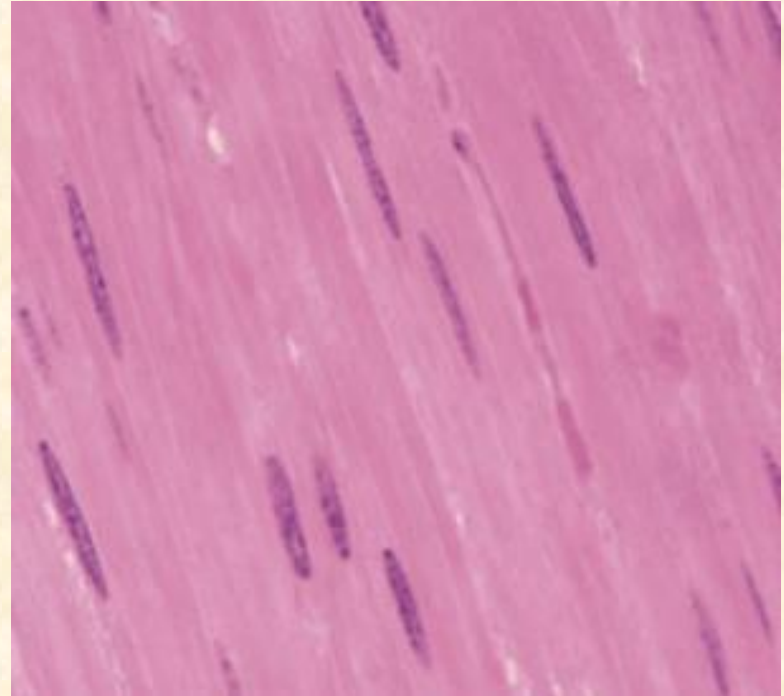
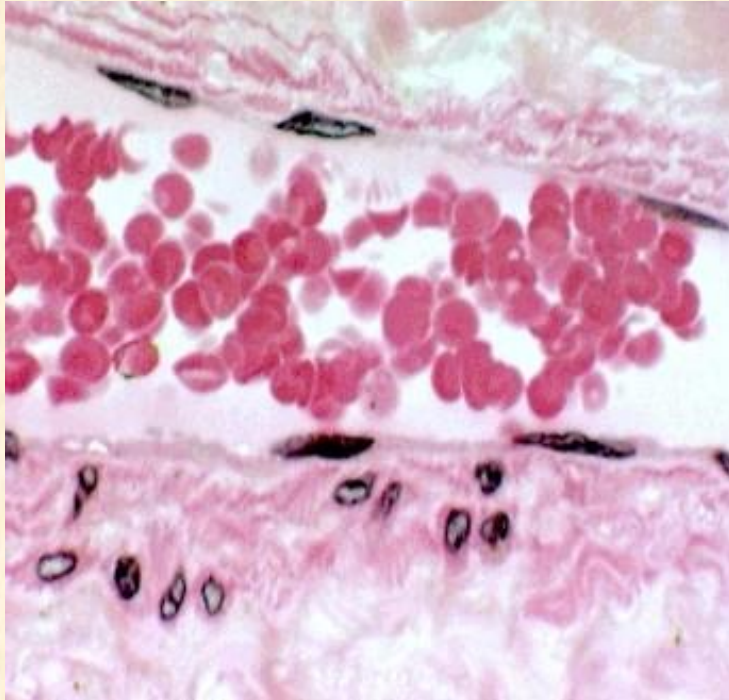


# Nucleus

- The core (lat. nucleus; Greek. karyon) represents the control center from which information about the cellular structure and its function originates.
- It contains almost the entire DNA of the cell, which contains information about protein synthesis **and much more**.
- The nucleus is present in all eukaryotic cells, except in the erythrocytes of mammals and in the keratinocytes of the surface layers of the epidermis.
- Most cells have one nucleus, although some cells have two (hepatocytes, cardiomyocytes) or more nuclei (osteoclasts, skeletal muscle cells).

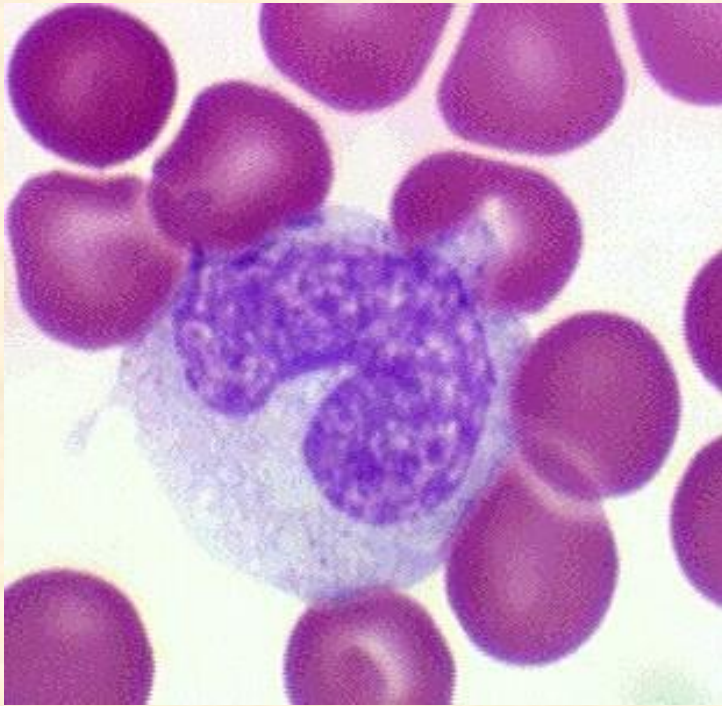
- Cells are designated as **mononuclear**, **binuclear**, and **multinuclear**.
- The number, shape, size, position and dying properties of the nucleus depend on the type and degree of cell activity.
- All nucleated cells possess a nucleus (or more) of standard characteristics, which in physiological conditions may exhibit minor variations in accordance with changes in the functional state of the cell.
- Variations of a larger range indicate a pathological process.

# Shape



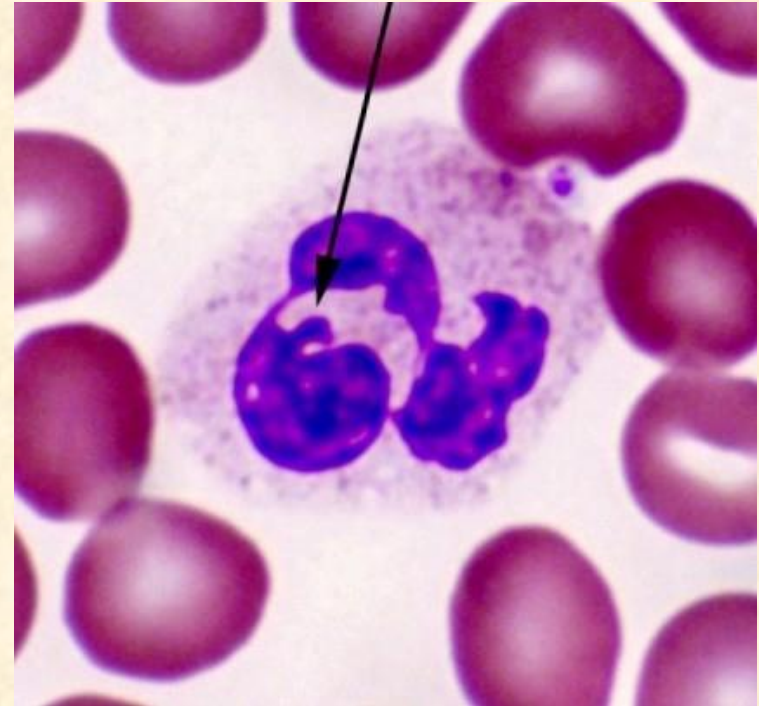
- The shape of the cell is determined by the type, shape and functional state of the cell. It most often has a round or oval shape, although it can be discoid (endothelial cells), elongated (smooth muscle cells)...



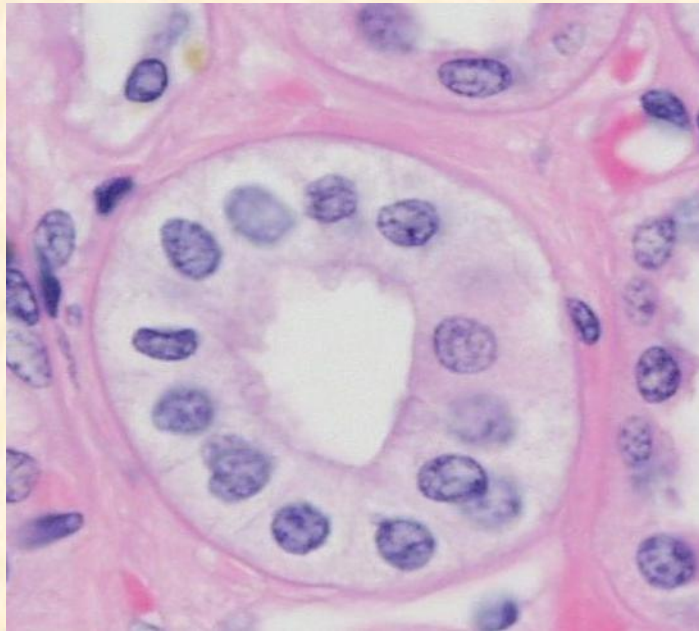


Indented

or



multilobar

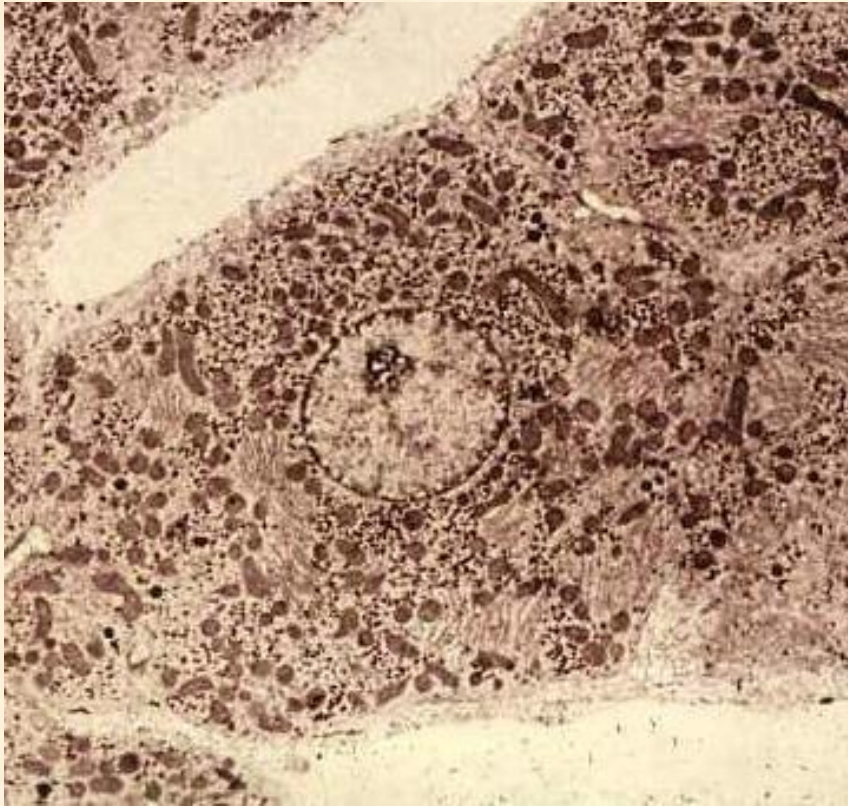


Round, multipolar and cuboidal cells usually have a round nucleus, cylindrical cells have an oval nucleus, and spindle cells have a similarly shaped nucleus.

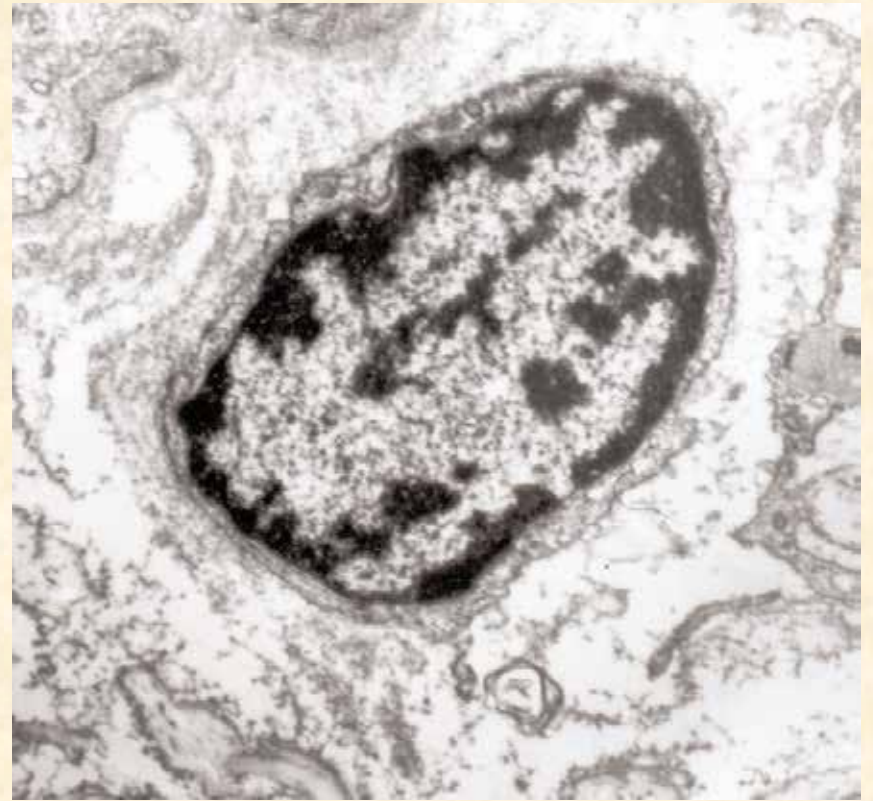
# Size

- The size of the nucleus usually correlates with the size of the cell.
- The diameter is usually in the range of 5-10 $\mu$ m
- The ratio of the volume of the nucleus to the volume of the cytoplasm is constant for a certain cell type and is referred to as the nucleocytoplasmic ratio (N/C).
- The higher the value of this coefficient, the more metabolically active the cell is.
- As a rule, the nucleocytoplasmic ratio is from 1:5 to 1:4, although there is a possibility that relatively large cells contain a small nucleus (hepatocytes) or that small cells have a relatively large nucleus (small lymphocytes).





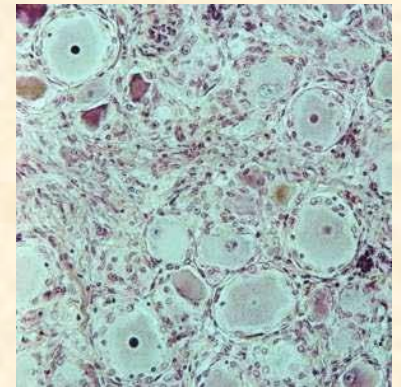
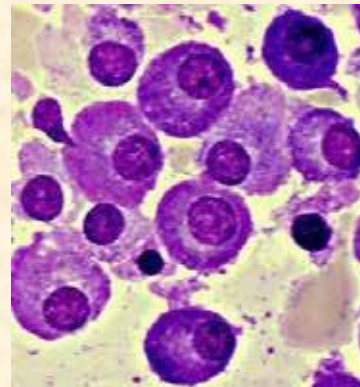
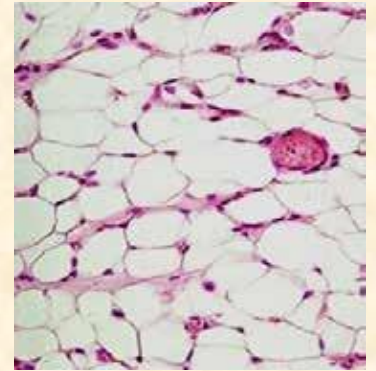
Hepatocyte



Lymphocyte

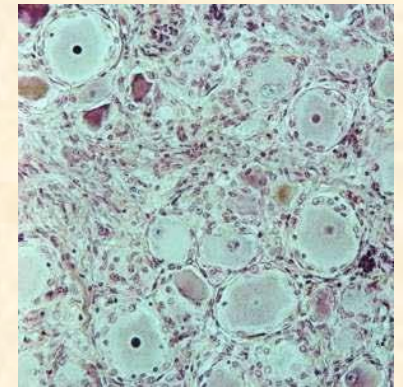
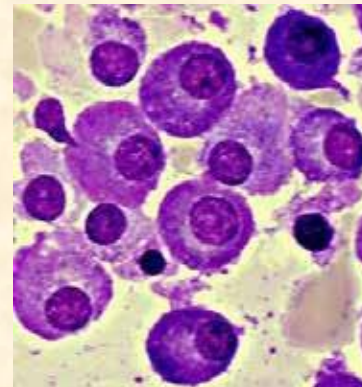
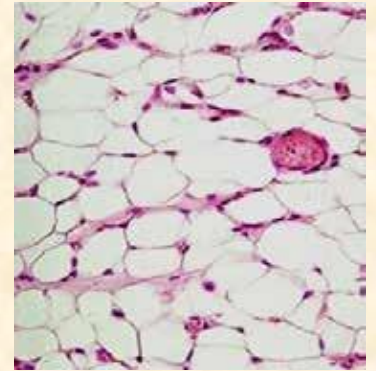
# Position

- The position of the nucleus depends on the shape and type of the cell, and in rare cases also on its functional state.
- In flat, cube, spindle and multipolar cells, it is usually placed in the center.





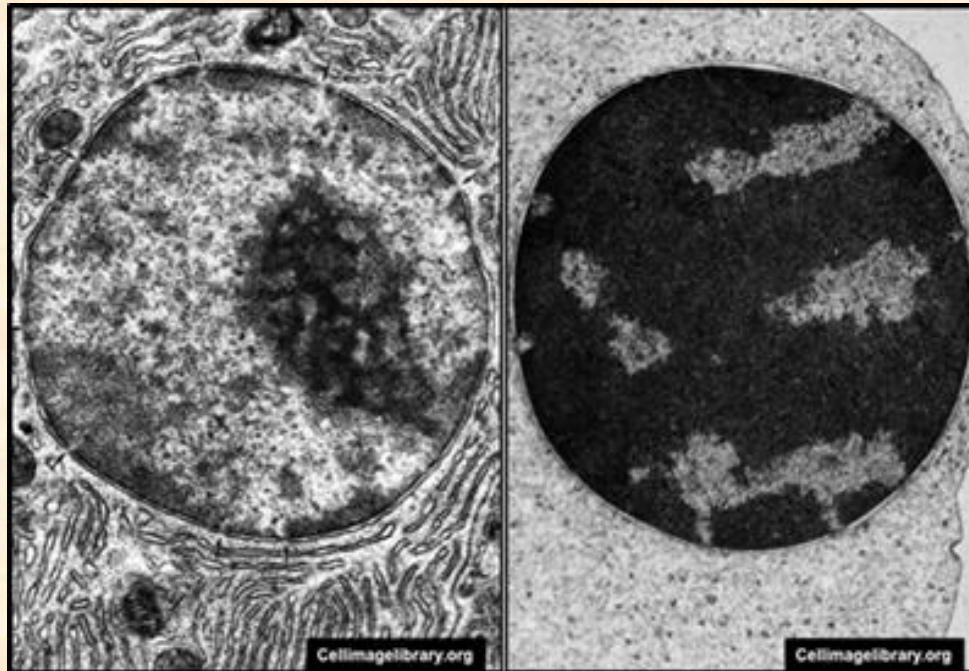
- In the case of round cells, it can have a central, but also an eccentric position (ovum).
- In cylindrical cells, it is most often placed at the base.
- In cells whose cytoplasm is filled with inclusions, the nucleus can be pushed against the plasmalemma (fat cells).





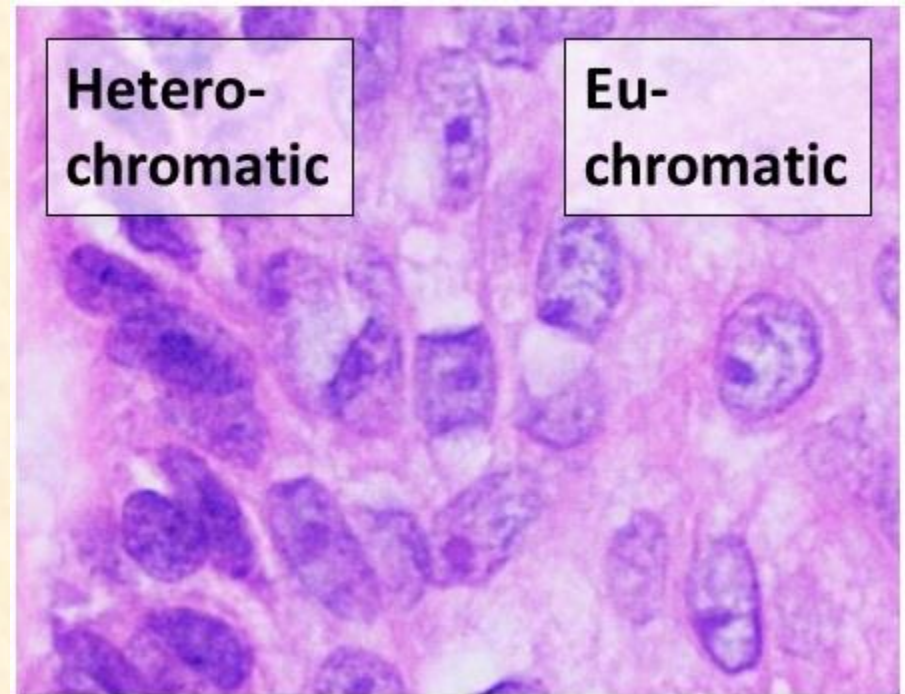
# Dying properties

- Due to the high concentration of DNA and RNA, the nucleus is intensively stained with base dyes (basophilia).
- Synthetically active cells have a light (**euchromatic**).
- Inactive cells have a dark (**heterochromatic**) nucleus.



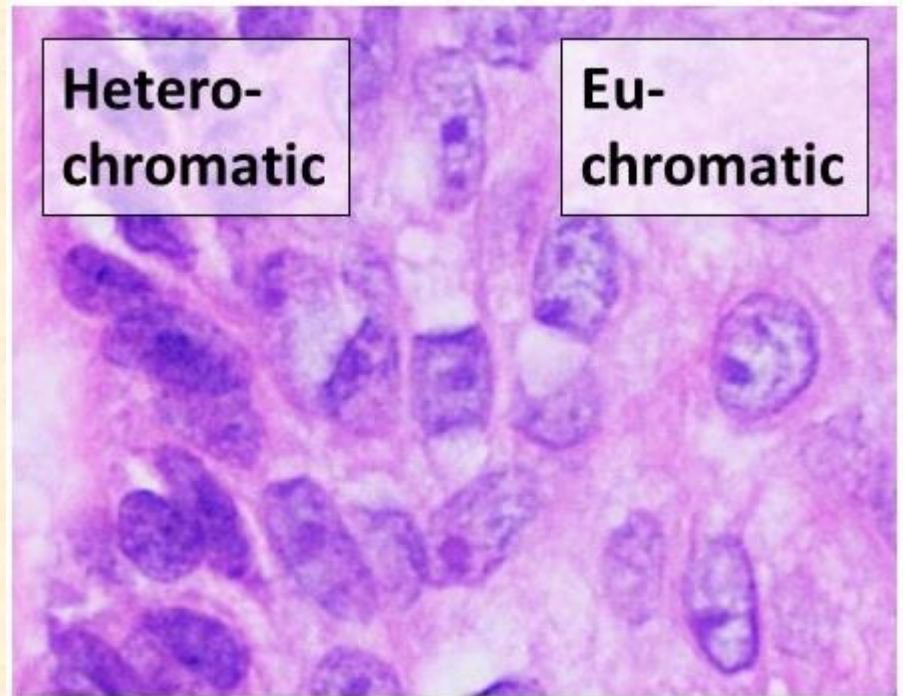
# Euchromatin

- Euchromatin includes regions of the chromosome in which the DNA strands are unwound or less coiled
- Nuclei in which euchromatin predominates are designated as **euchromatic** and are characteristic of metabolically active cells (hepatocytes, neurons).



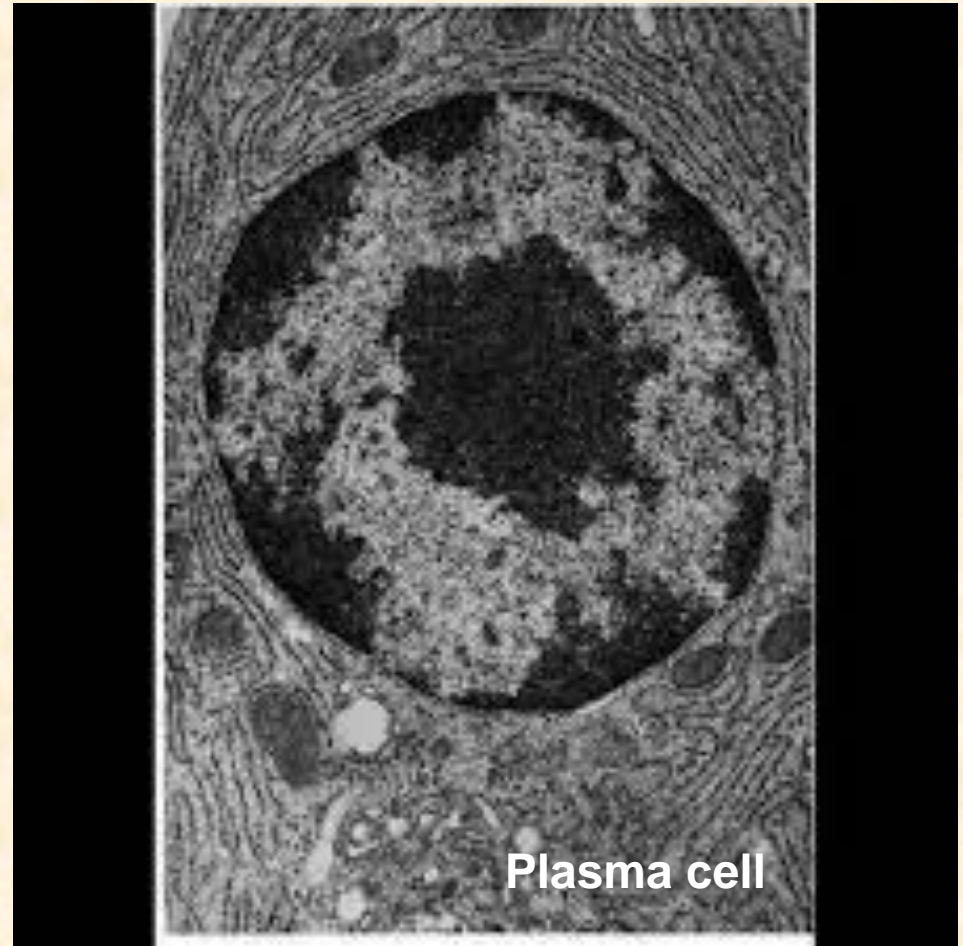
# Heterochromatin

- Heterochromatin is observed in light microscopy in the form of basophilic clumps, and in electron microscopy, in the form of dark granules.
- Heterochromatin dominates in less active and metabolically inactive cells (circulating lymphocytes, spermatozoa).





- The amount of heterochromatin is inversely proportional to the degree of cell activity.
- In certain cells, heterochromatin has a specific arrangement, so it can serve as one of the criteria for their identification (eg, in plasma cells, it is arranged in the form of a spider's wheel).



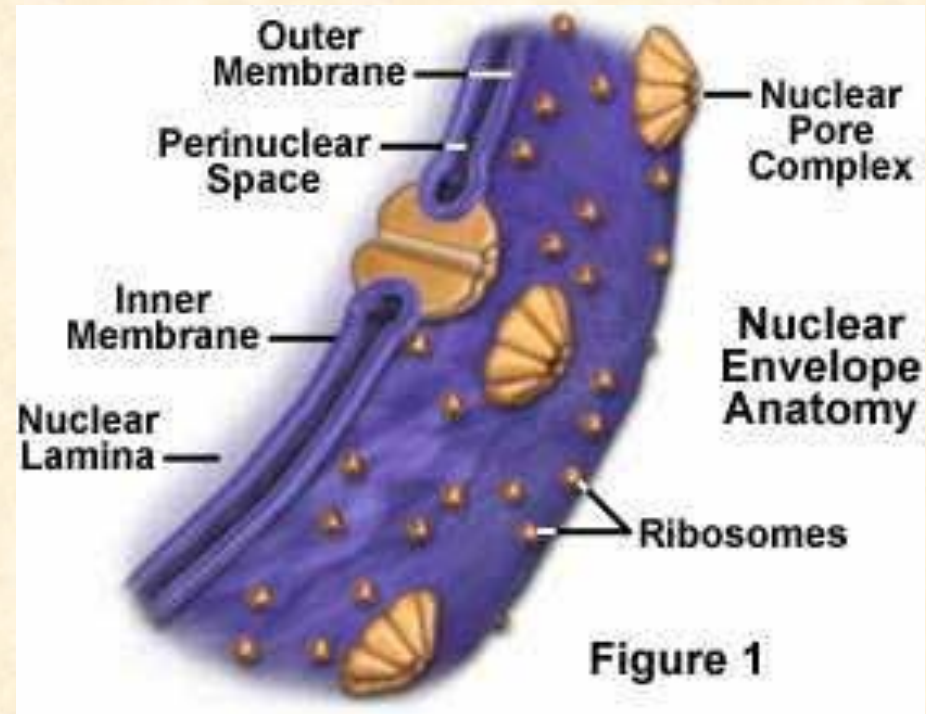
# So.....

Parts of the nucleus

- Envelope
- Nucleoplasm
- Chromatin
- Nucleolus
- Nuclear lamina

# Nuclear envelope

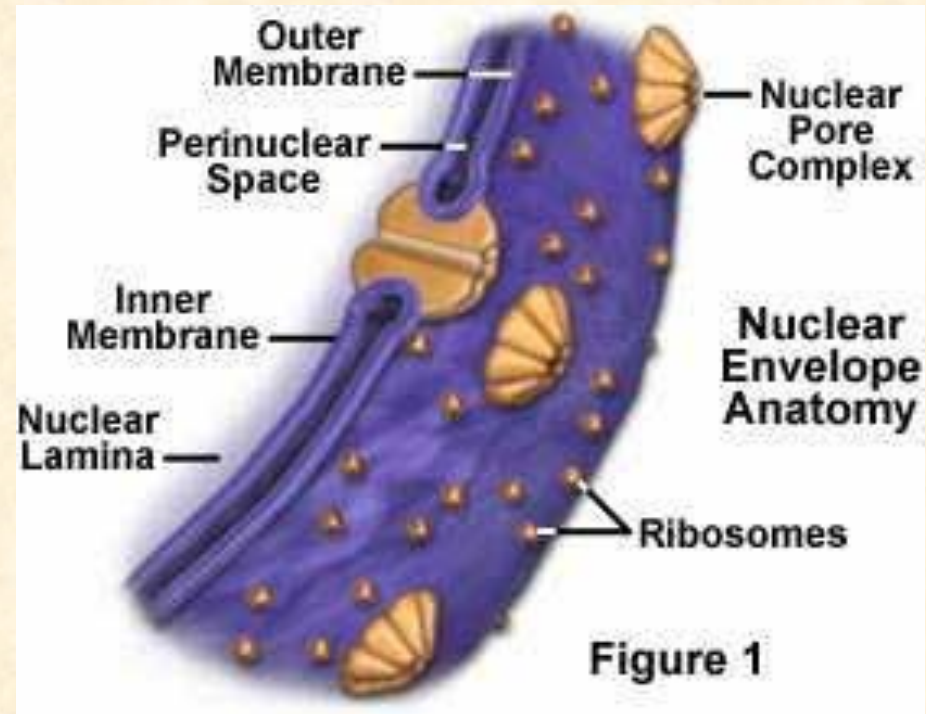
- The nuclear envelope consists of the **inner and outer nuclear membrane**.
- Between them there is an perinuclear (intermembranous) space.





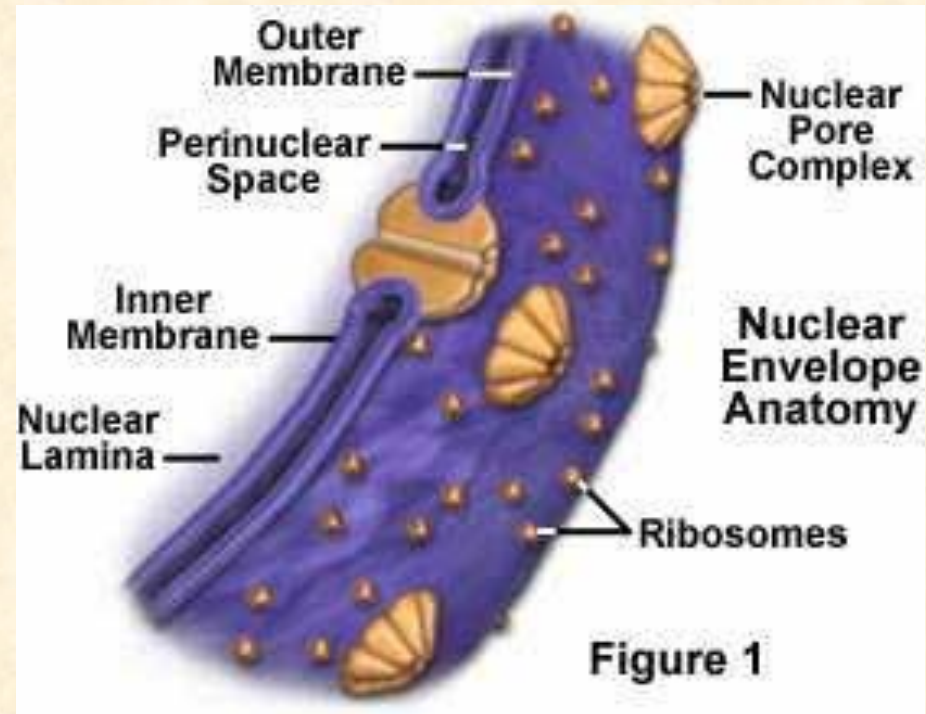
# Outer membrane

- The outer membrane is sprinkled with **ribosomes** on which the transmembrane proteins of the nuclear envelope are synthesized.
- **It is in continuity with the rER** membranes so that the intermembrane space communicates with the cisterns of this organelle.



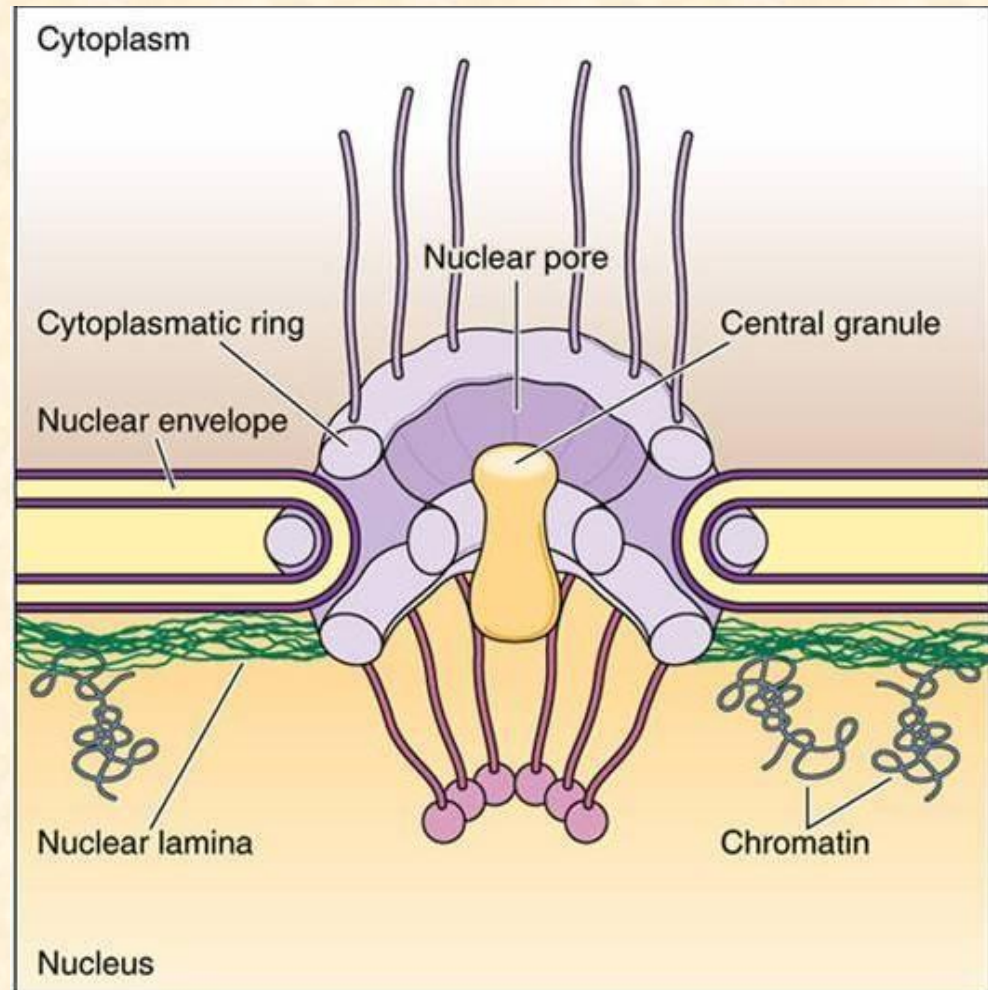
# Inner membrane

- The inner membrane rests on the **nuclear lamina**. Major components of this layer are the **class of intermediate filament proteins called lamins**
- RNA and chromosomes, i.e. peripheral heterochromatin, are attached to its integral proteins.



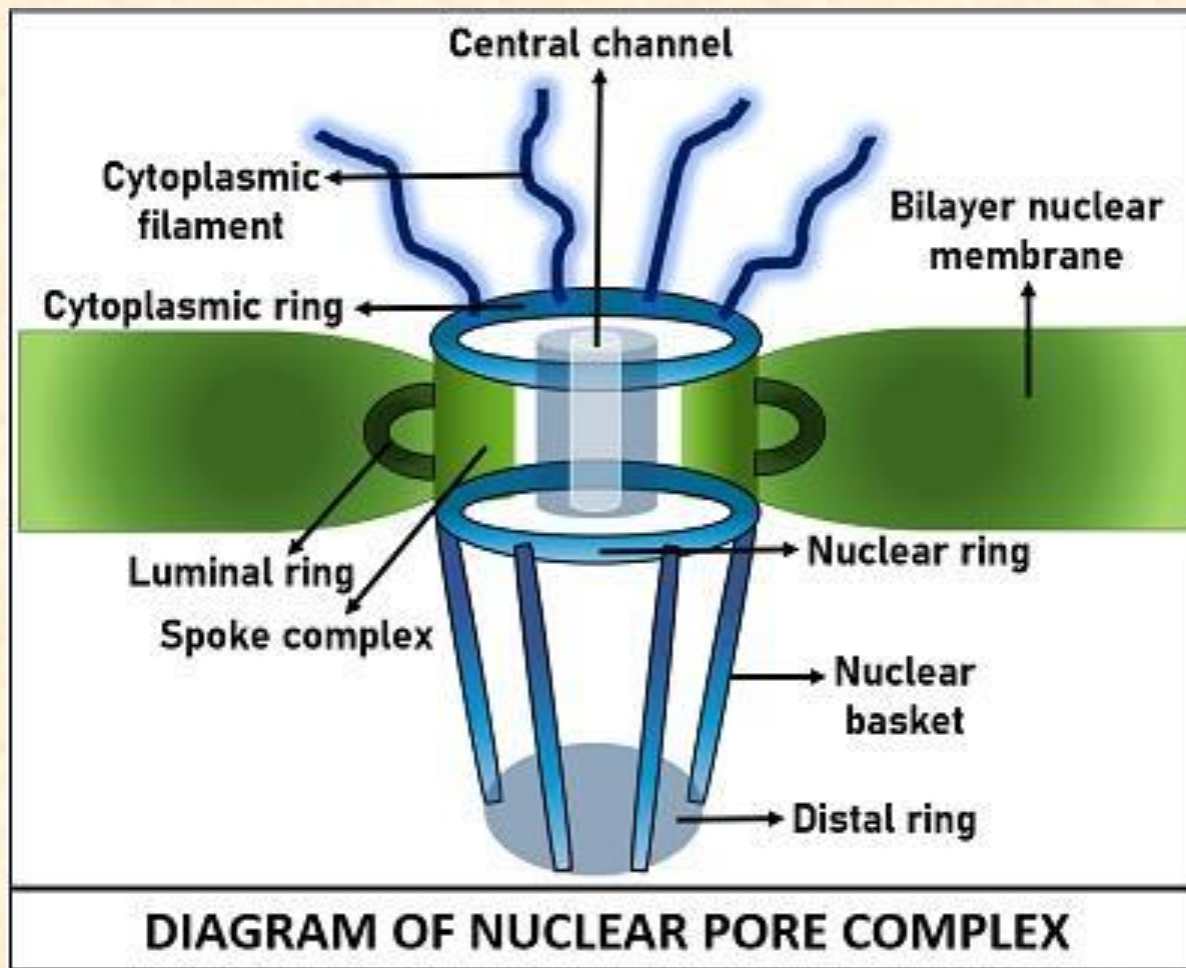
# Nuclear pores

- In certain regions of the nuclear envelope, the **outer and inner membranes are bridged and form openings called nuclear pores.**
- Through these openings, the exchange of ribosomal units, ions and numerous molecules between the cytoplasm and the nucleoplasm takes place.

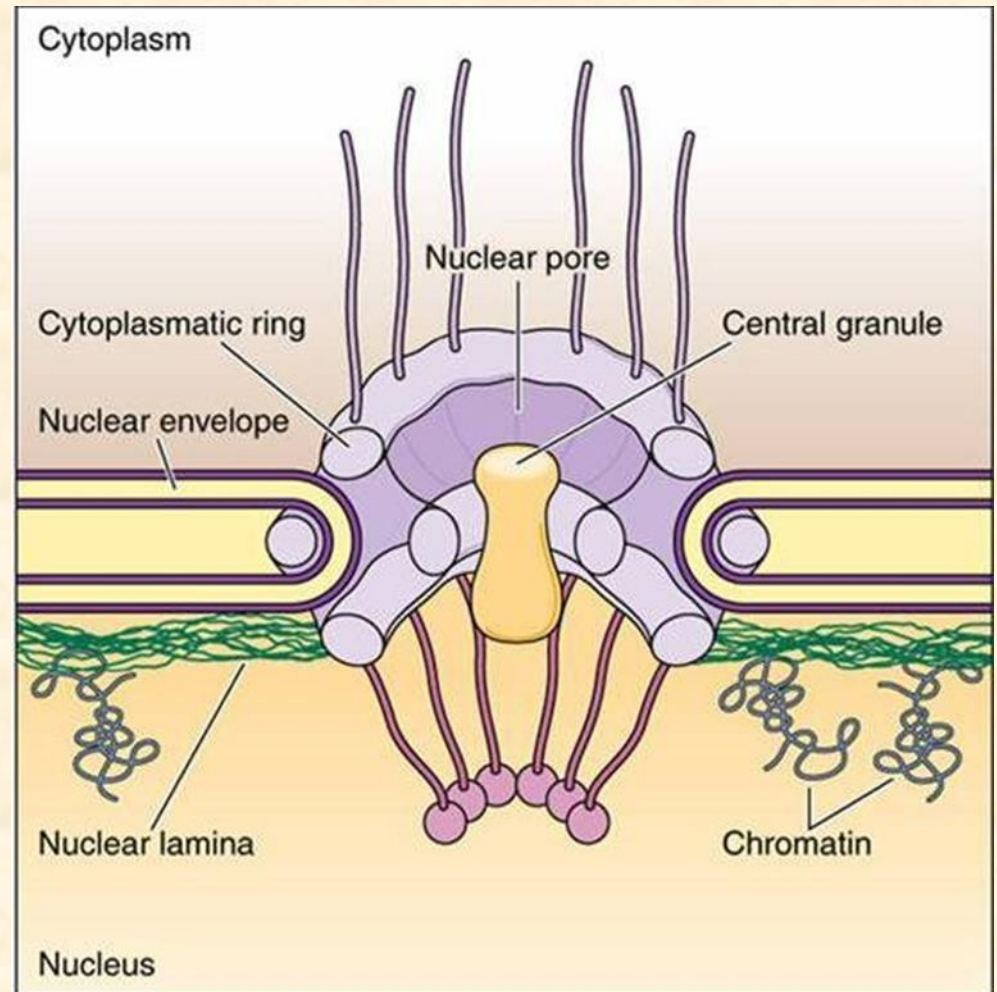




- A protein complex, called the **nuclear pore complex (NPC)**, is embedded in each nuclear pore, which regulates the exchange of substances between the nucleus and the cytoplasm.
- NPC is a complex similar to ion channels that can open and close as needed.



- Various nuclear proteins of a nuclear pore complex, called **nucleoporins**, display eightfold symmetry around a lumen.
- Ions and small solutes pass through the lumen by simple diffusion, but the nucleoporin complex regulates the movement of macromolecules between nucleus and cytoplasm.



# Nucleoplasm

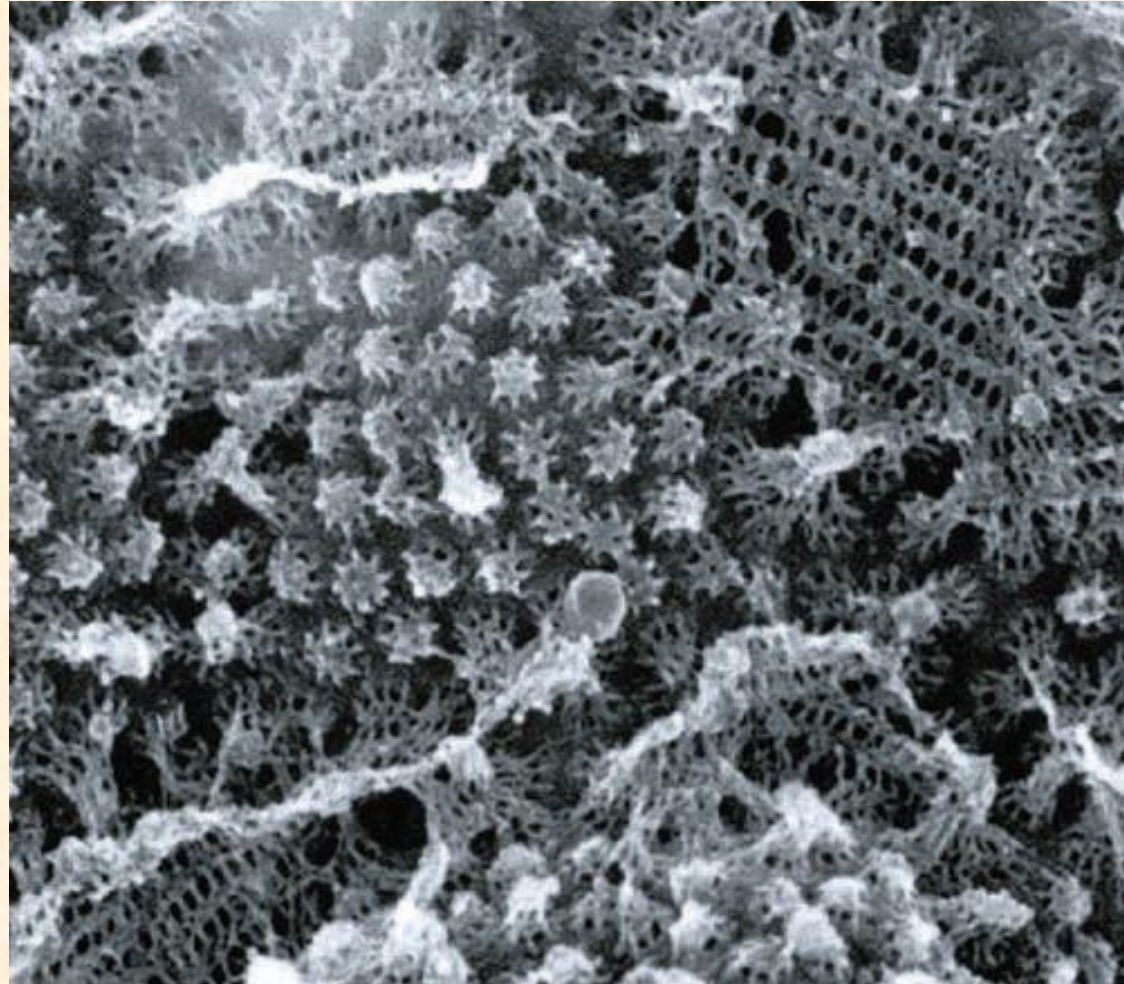
- Nucleoplasm is a semi-liquid substance, somewhat denser than cytosol, in which the nuclear skeleton, chromatin and nucleolus are submerged.
- Nucleoplasm contains water, ions, amino acids, proteins, various metabolites and complexes made of nucleic acids and polypeptides - ribonucleoprotein granules.
- The nucleoplasm is subject to constant changes in composition because a large number of its molecules and ions are in transit.



- Ribosomal subunits are transported through the nucleoplasm towards the cytosol, and nuclear proteins in the opposite direction.
- Nucleus proteins include various structural proteins, enzymes, proteins that enter the composition of chromatin, as well as functional proteins that control gene activity and the normal progression of the cell cycle.

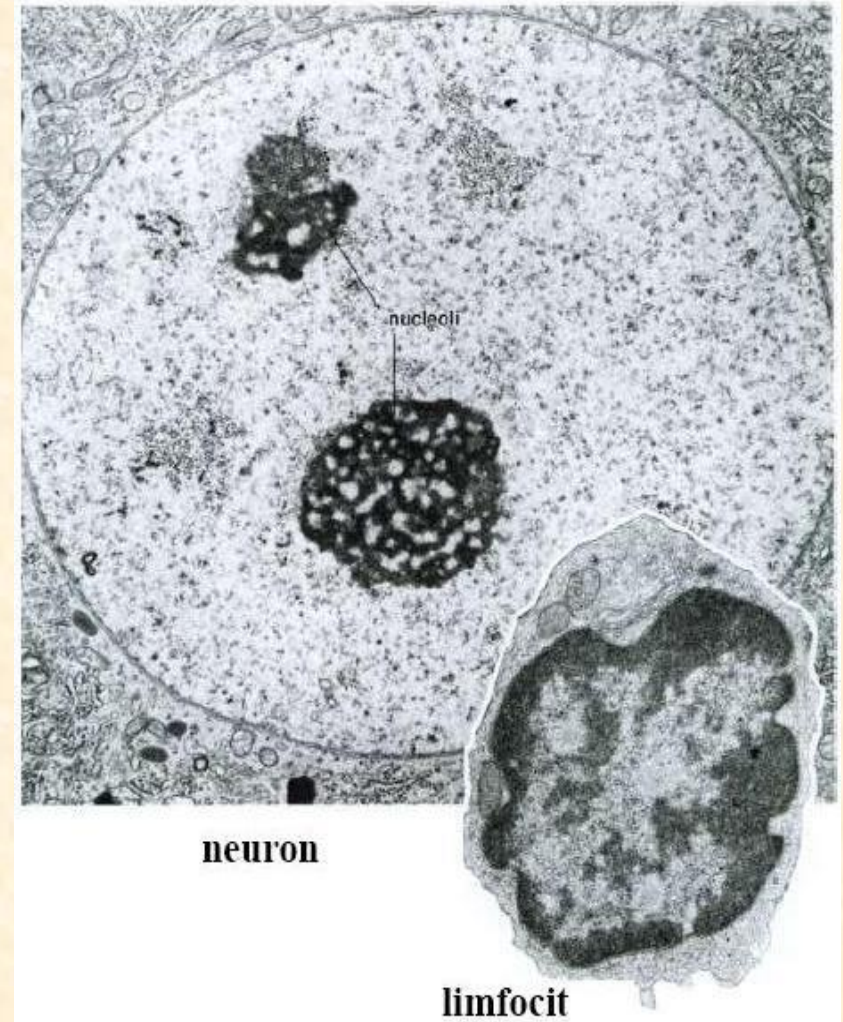
# Nuclear lamina

- Consists of the **intermediate filaments and microfibrils**.
- Closely associated with the inner nuclear membrane is a highly organized meshwork of proteins



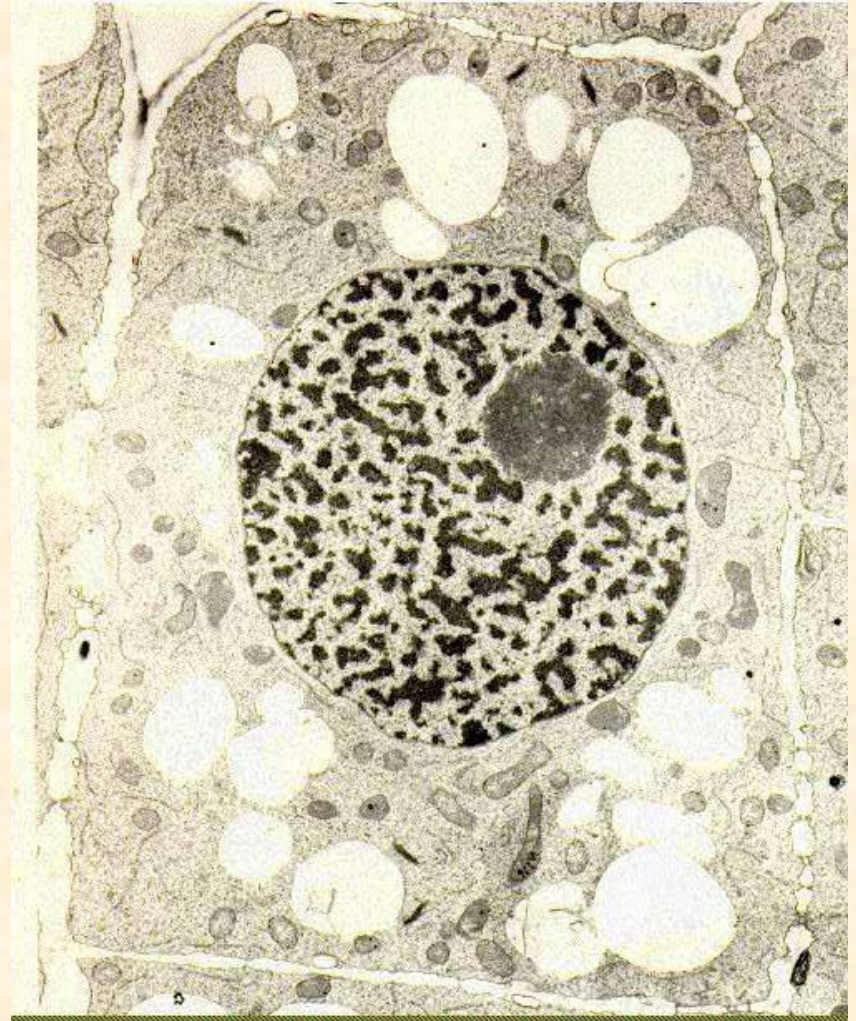
# Nucleolus

- Nucleolus is a component of the nucleus responsible for the **formation of ribosomal subunits**.
- The size and number of nucleoli depend on the type of cell and the degree of its activity.
- In cells that intensively synthesize proteins, as well as in cells that rapidly proliferate and grow (embryonic cells, cells of malignant tumors), nucleoli can occupy a quarter of the volume of the nucleus.





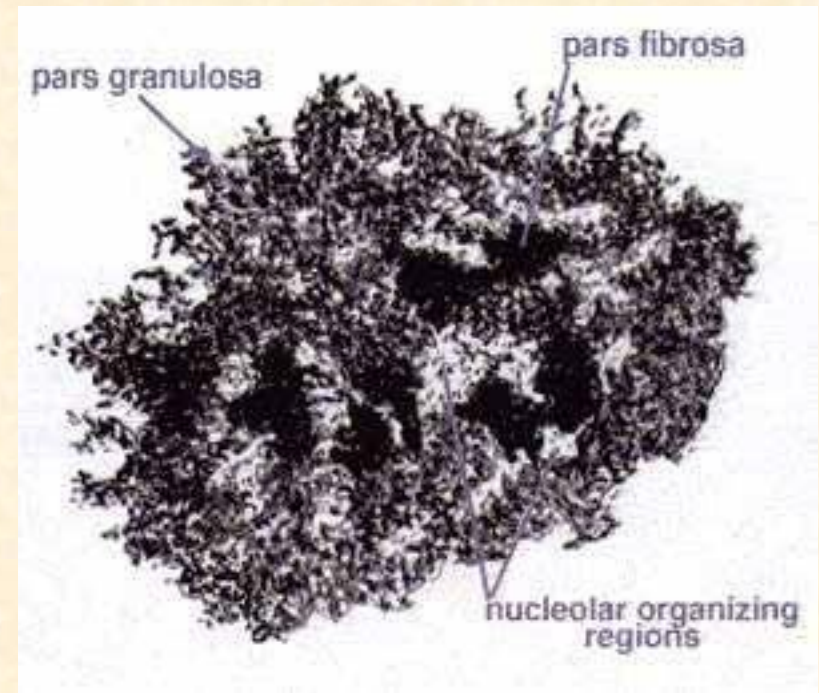
- The nucleolus is usually located eccentrically, and is often attached to the inner nuclear membrane, which is a favorable position for the rapid transport of its products into the cytoplasm.
- Does not have its own envelope.
- On light microscopy, it is observed in the form of a spherical basophilic corpuscle with a diameter of about  $1\mu\text{m}$ .



Chromosomal regions with the **genes for rRNA** one or more nucleoli in cells requiring intense ribosome production for protein synthesis during growth or secretion

Ultrastructural analysis of an active nucleolus reveals two regions

- **granular component** (contains ribosomal subunits)
- **fibrillar center** (the part of the DNA strand that is not transcribed)



# Cell cycle

The cell cycle has four distinct phases: mitosis and periods termed G1 (the time gap between mitosis and the beginning of DNA replication), S (the period of DNA synthesis), and G2 (the gap between DNA duplication and the next mitosis).owing nuclear pores.

- ❖ The growth and maintenance of cell populations of multicellular organisms is achieved through the division of existing cells.
- ❖ Cell divisions enable the development and growth of the organism, and in adult individuals, the replacement of damaged and functionally worn out cells is ensured.
- ❖ There are two types of cell division: **mitosis and meiosis**.
- ❖ Mitosis is present in somatic cells, while meiosis takes place during gametogenesis.

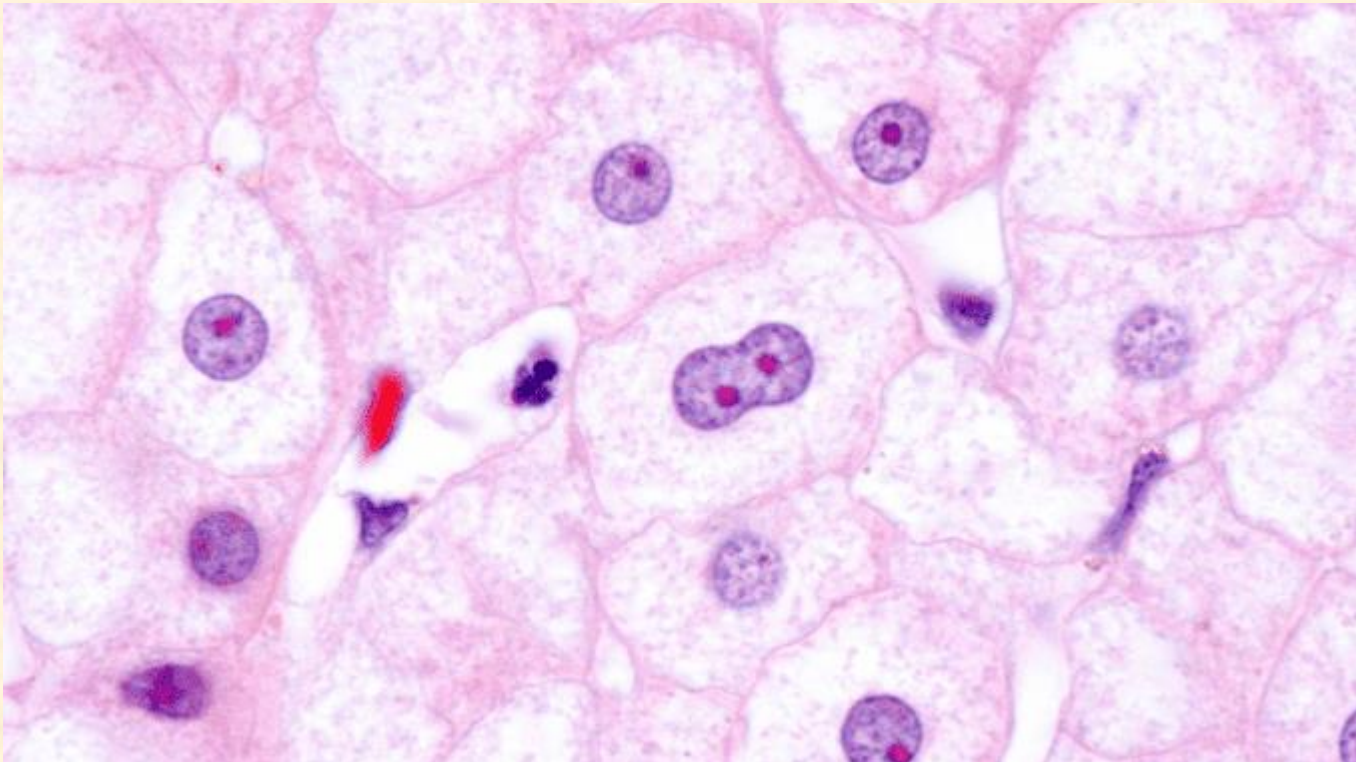


# Amitosis and endomitosis

- These are **not cell divisions in the true sense**.
- They imply only an **increase in the amount of genetic material** that can be accompanied by the division of the nucleus, but not by the division of the cytoplasm.

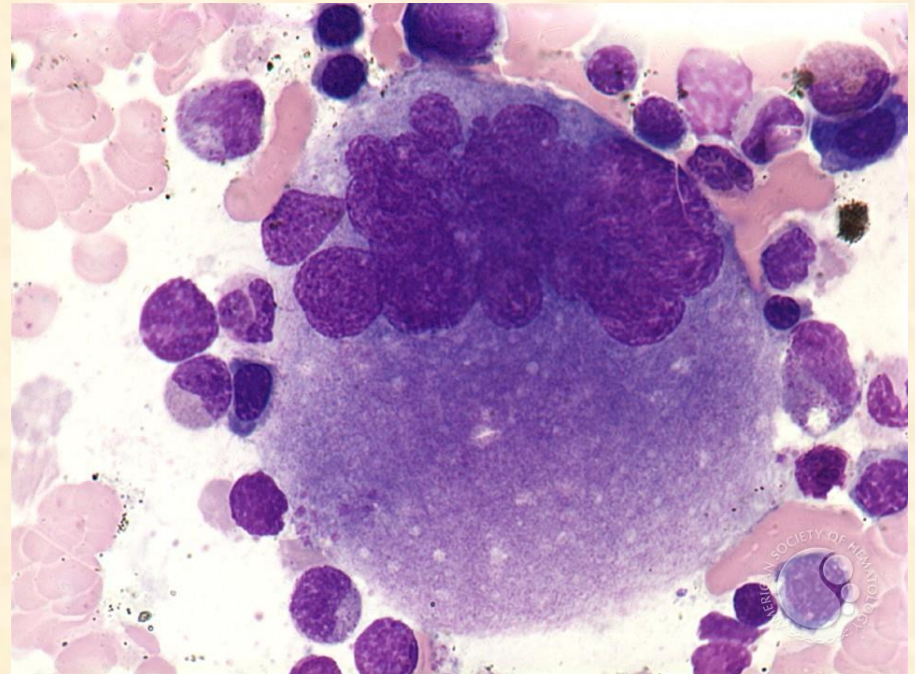
# Amitosis

- It represents the division of the nucleus without visible morphological signs characteristic of mitosis.
- The number of chromosomes doubles, but cytokinesis is absent, so the **newly formed cell has two nuclei** (hepatocytes, ganglion cells).



# Endomitosis

- The process of multiple doubling and splitting of chromosomes into chromatids without separating them.
- This phenomenon is called **polyploidy** (megakaryocytes).
- In this way, the amount of DNA is doubled (tetraploid), quadrupled (octaploid)...
- With the increase of the nucleus, the cytoplasm also becomes more voluminous.





# Proliferation and differentiation

- **Proliferation** is the production of new cells through serial divisions of existing cells.
- Different cells have a different ability to proliferate, which is **inversely proportional to the degree of maturity** (specialization) of the cells.
- Unspecialized cells proliferate rapidly and continuously.
- Specialized cells proliferate only in case of loss of part of their own population.
- Certain highly specialized cells do not have the ability to divide.

- **Differentiation** represents the maturation of cells and their progeny during several consecutive cell cycles.
- In the process of differentiation, stem (non-specialized) cells give rise to differentiated cells specialized to perform one, and sometimes more, specific functions.
- Between stem and differentiated cells there are several generations of increasingly specialized cells called transitional cells.

According to the degree of maturity and the level of mitotic activity, the cells of the human body can be classified into three categories:

- **Stem** (high proliferative potential)
- **Transit amplifying cells** (are in transit along the path from the stem cell niche to a differentiated state, while still amplifying by mitosis)
- **mature** (differentiated) cells



# Stem cells

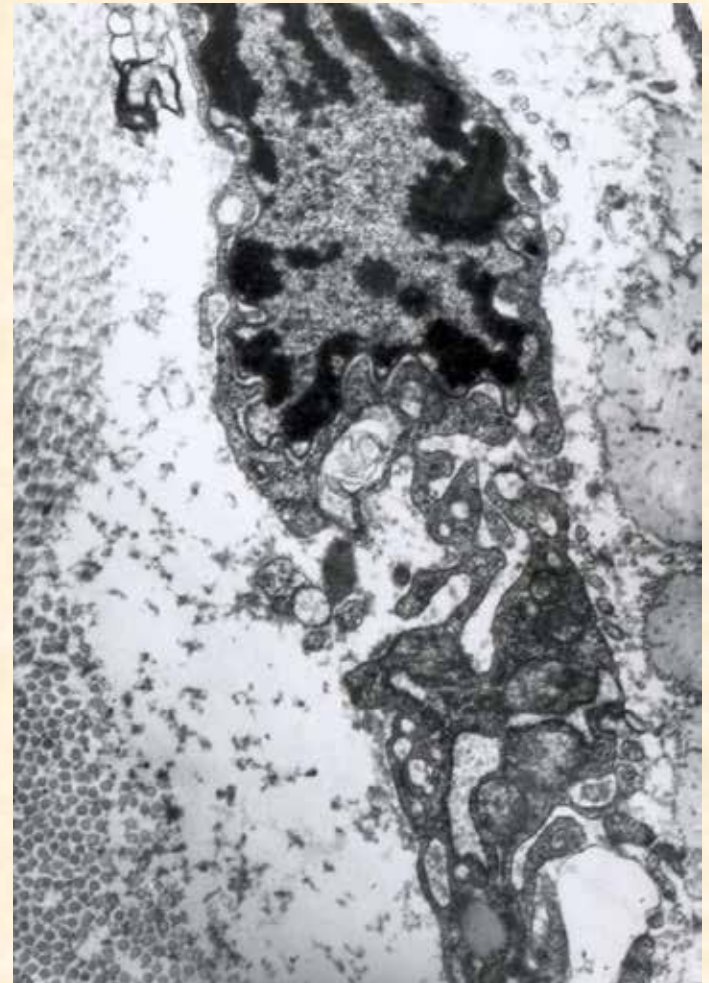
- Many stem cells divide infrequently and the divisions are asymmetric; that is, one daughter cell remains as a stem cell, while the other becomes committed to a path that leads to differentiation
- Their pronounced mitotic activity results in **continuous symmetric** (division into two identical daughter cells) or **asymmetric divisions** (two daughter cells that differ from each other).
  - In **symmetric division**, newly formed cells can be of the same or slightly higher degree of specialization than the parent cell.
  - In **asymmetric division**, one daughter cell is identical to the parent cell (it serves as a reserve for the self-preservation of stem cells), while the other daughter cell moves in the direction of morphological and functional specialization - differentiation.

# Cell death

- **Necrosis and apoptosis**
- **Necrosis** is manifested in extreme non-physiological conditions.
- It represents a passive type of cell death, which occurs due to the collapse of cellular homeostatic mechanisms.
- **Apoptosis** and autophagy occur both during physiological and pathological conditions.
- During apoptosis and autophagy, the cell actively participates (energy is required for their development)
- The term **programmed cell death or cell suicide** is often used for apoptosis and autophagy.

# Apoptosis

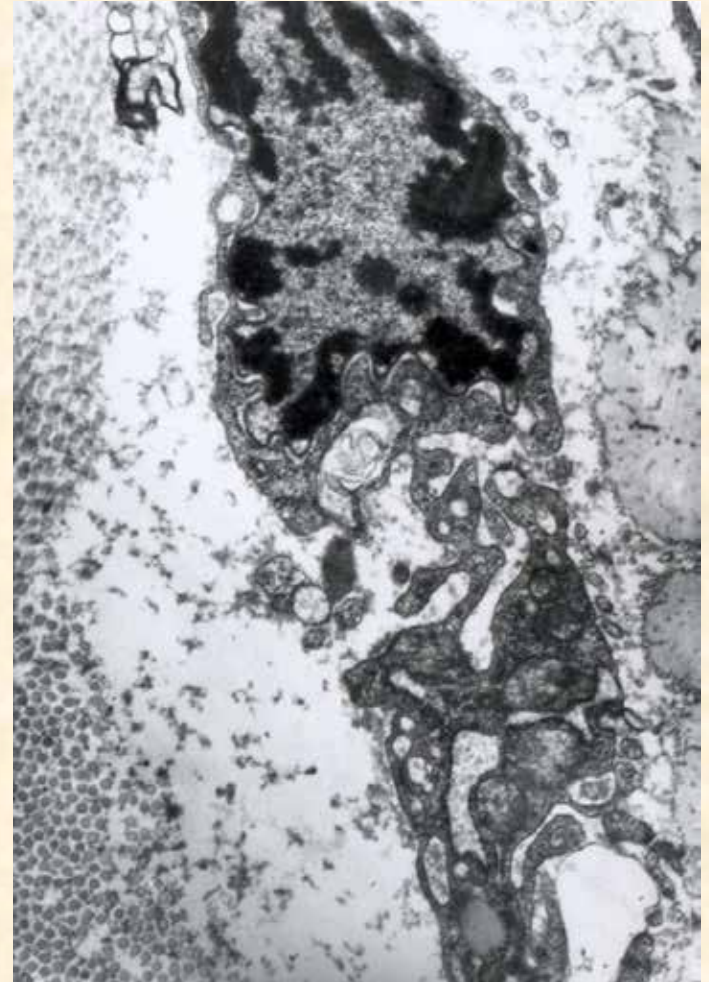
- Apoptosis is a rapid, highly regulated cellular activity that shrinks and eliminates defective and unneeded cells
- It results in small membrane-enclosed apoptotic bodies, which quickly undergo phagocytosis.
- Apoptotic cells do not rupture and release none of their contents, unlike cells that die as a result of injury and undergo necrosis.





Apoptosis is controlled by cytoplasmic proteins in the **Bcl-2** family, which regulate the release of death-promoting factors from mitochondria

- Bcl-2 proteins release cytochrome c into the cytoplasm where it activates enzymes caspases resulting in protein degradation throughout the cell.
- Endonucleases are activated, which cleave DNA between nucleosomes into small fragments.
- Destruction of the cytoskeleton and chromatin causes the cell to shrink quickly, producing structures with darkly stained pyknotic nuclei



- The plasma membrane of the shrinking cell undergoes dramatic shape changes, such as “blebbing,” as membrane proteins are degraded and lipid mobility increases.
- Remnants of cytoplasm and nucleus separate as very small apoptotic bodies

