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Катедра за Хистологију и ембриологију

# CONNECTIVE TISSUE

**Week 3**

# CONNECTIVE TISSUE (CT)

Most abundant tissue

Connects everything

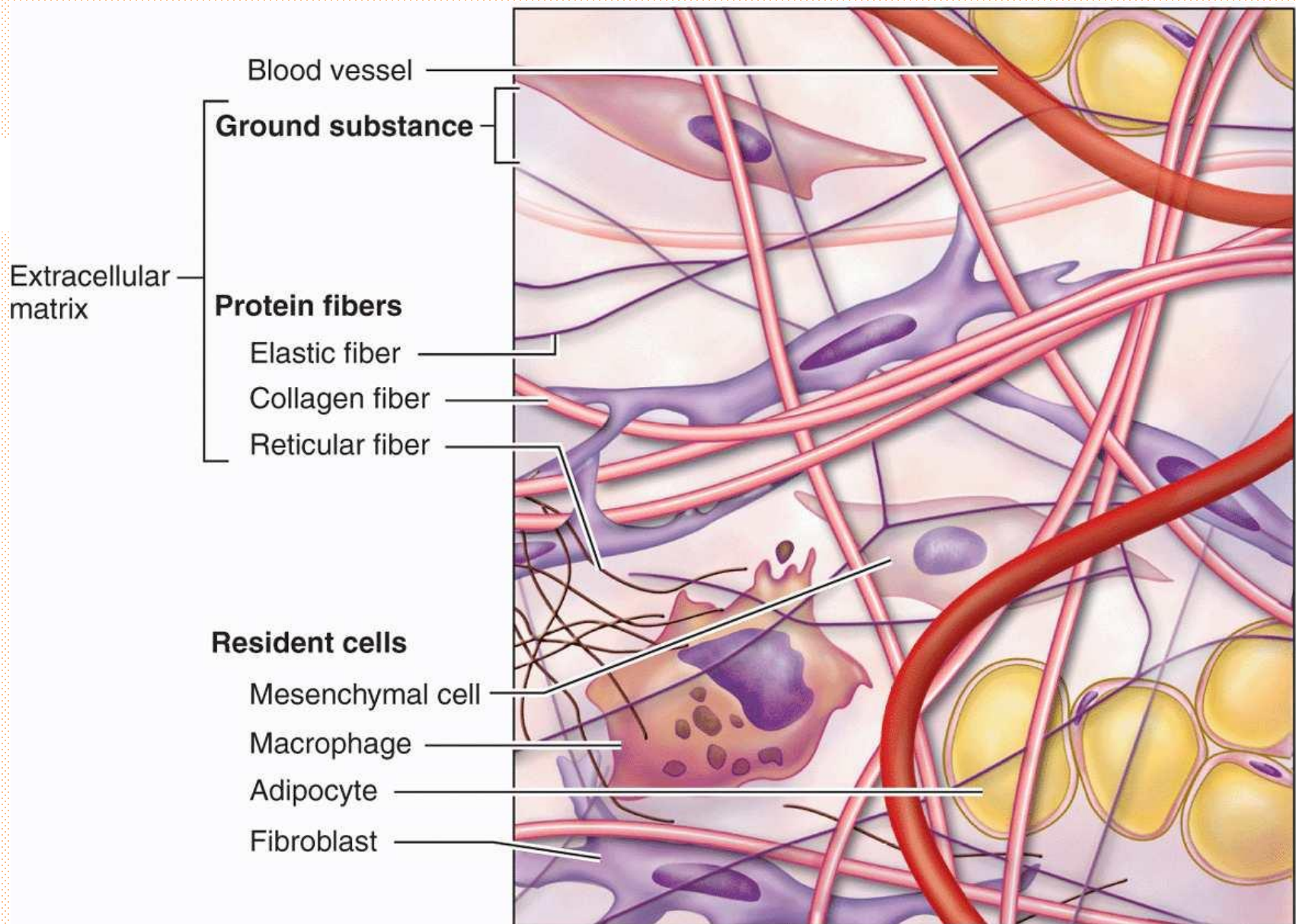
Connective tissue provides a matrix that supports and physically connects other tissues and cells together to form the organs of the body.

**Composition of connective tissue:**

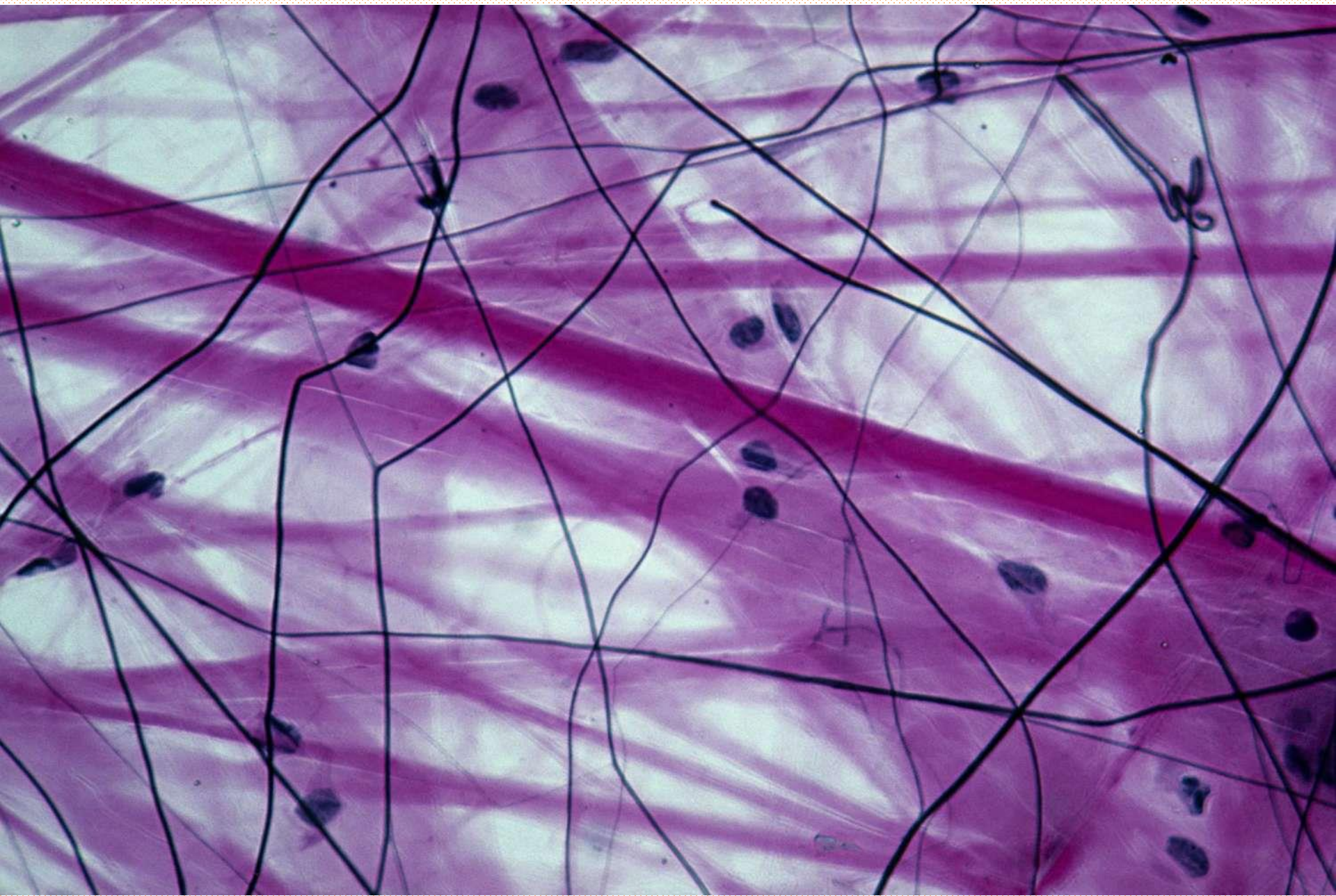
- **Cells**
- **Extracellular matrix (ground substance and protein fibers)**
  - collagen
  - reticular
  - elastic



# CT composition







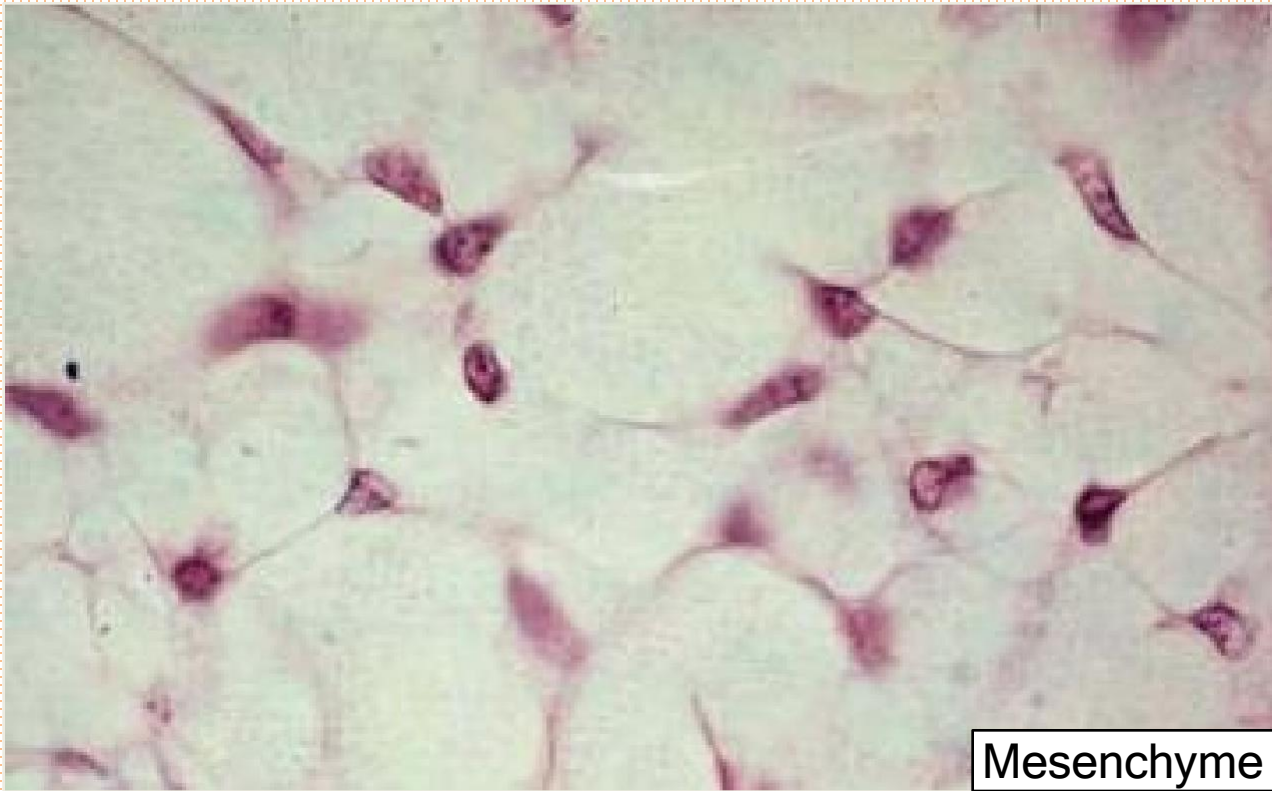


# Main roles

1. It provides support to organs by forming their stroma
2. It enables the exchange of gases and metabolites
3. It provides reparation of damaged tissues
4. **BATTLEFIELD** It participates in the body's defense through:
  - phagocytosis
  - immunoglobulin production
  - production of inflammatory mediators.

# Origin

All CT types originate from embryonic **mesenchyme**.

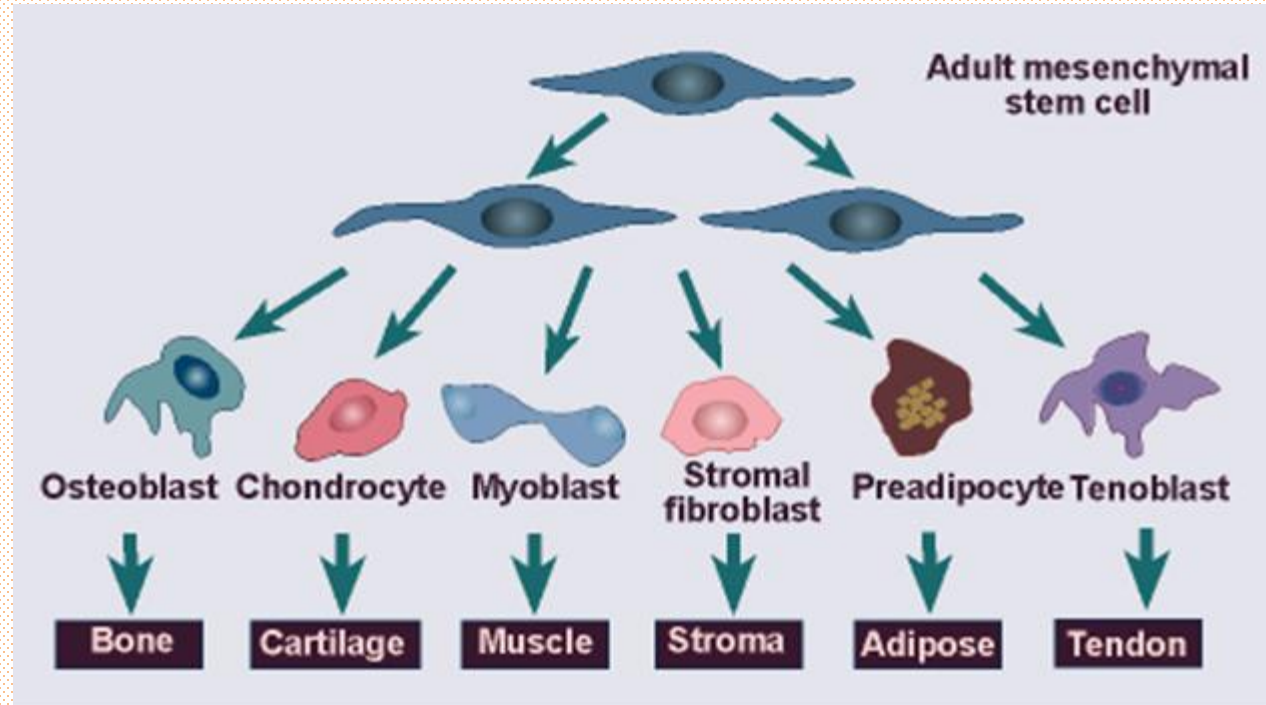


Mesenchyme



# Mesenchymal cell **can become**

- Bone marrow stem cells (blood cells)
- Fibroblast and fibrocyte
- Macrophage
- Osteoblast and osteocyte
- Chondroblast and chondrocyte.
- Reticular cell
- Adipocyte
- Mast cell
- Osteoclast



**CT cells**



# CT Cells

```
graph TD; A[CT Cells] --> B[Fixed]; A --> C[Mobile];
```

## Fixed

**Fibrocytes**

**Chondrocytes**

**Osteocytes**

**Adipocytes**

**Odontoblasts**

**Mesenchymal cells**

**Reticular cells**

## Mobile

### Leukocytes

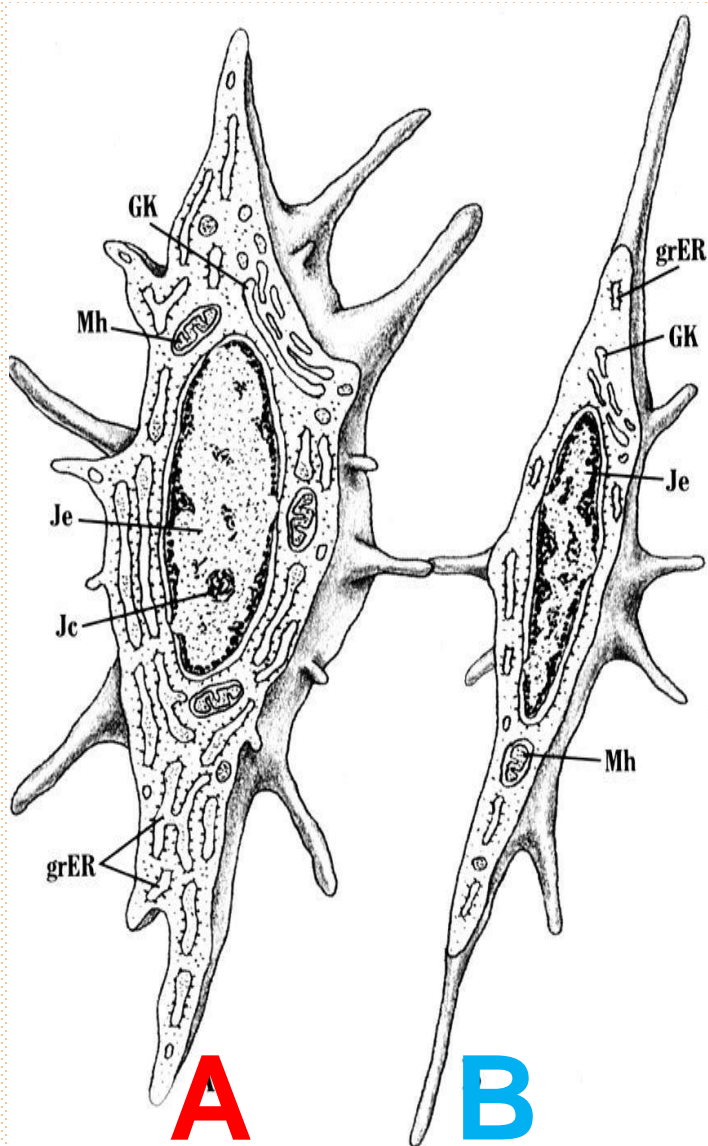
- neutrophil granulocytes
- eosinophilic granulocytes
- basophilic granulocytes
- monocytes
- lymphocytes

### Macrophages

### Plasma cells

### Mast cells

# Fibroblast and Fibrocyte



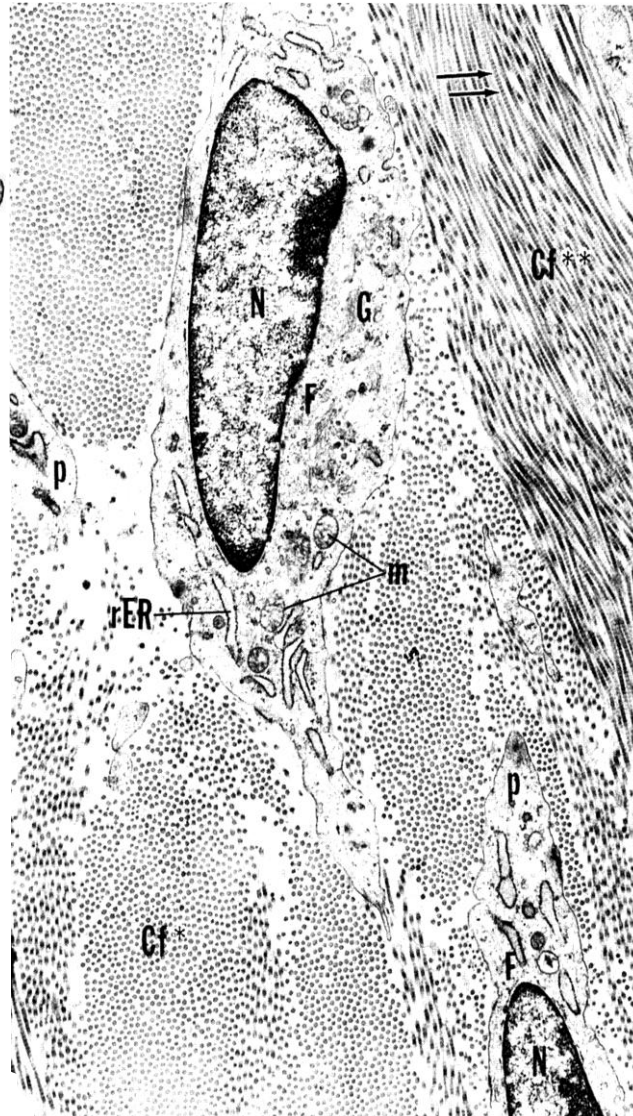
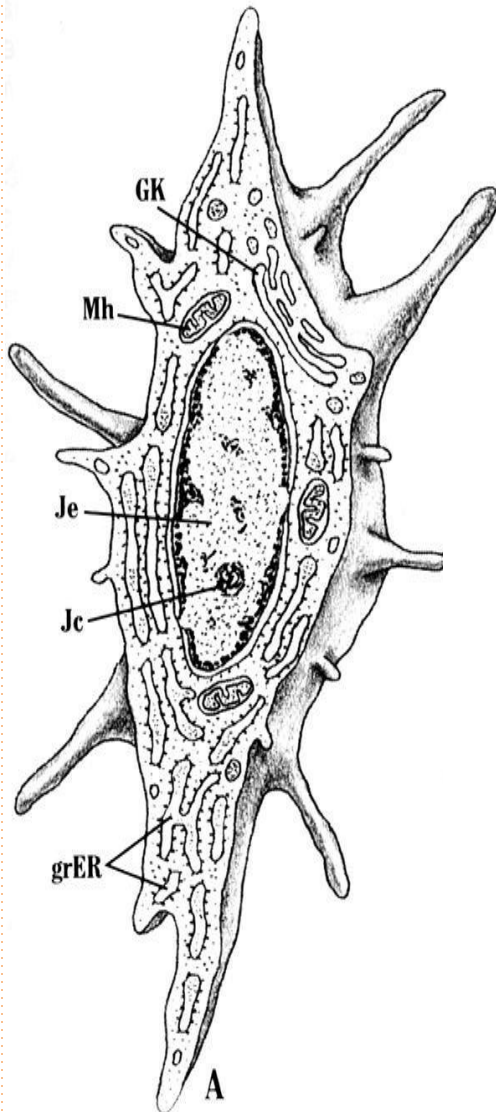
**A FIBROBLAST** most common cells in connective tissue proper

- synthesize and secrete collagen (the most abundant protein of the body) and elastin, which both form large fibers, as well as the GAGs, proteoglycans, and multiadhesive glycoproteins that comprise the ground substance
- fibroblast has abundant and irregularly branched cytoplasm, RER and a well-developed Golgi apparatus, with a large, ovoid, euchromatic nucleus and a prominent nucleolus

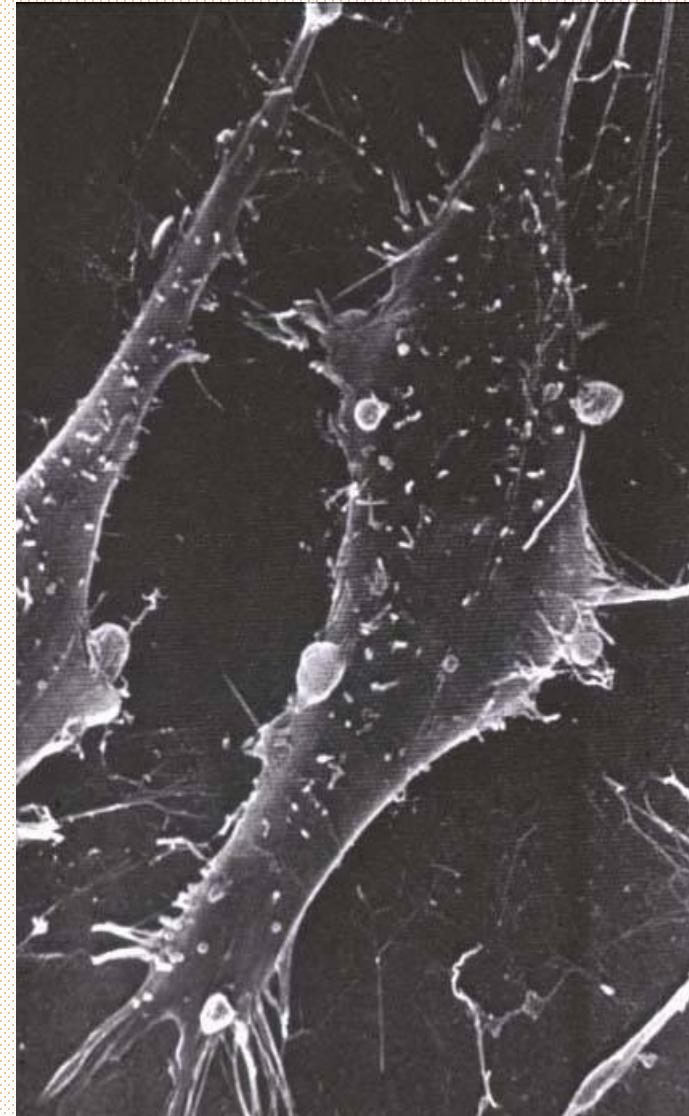
**B FIBROCYTE** is smaller than the active fibroblast, is usually spindle-shaped with fewer processes, much less RER, and a darker, more heterochromatic nucleus.



# Fibroblast



TEM

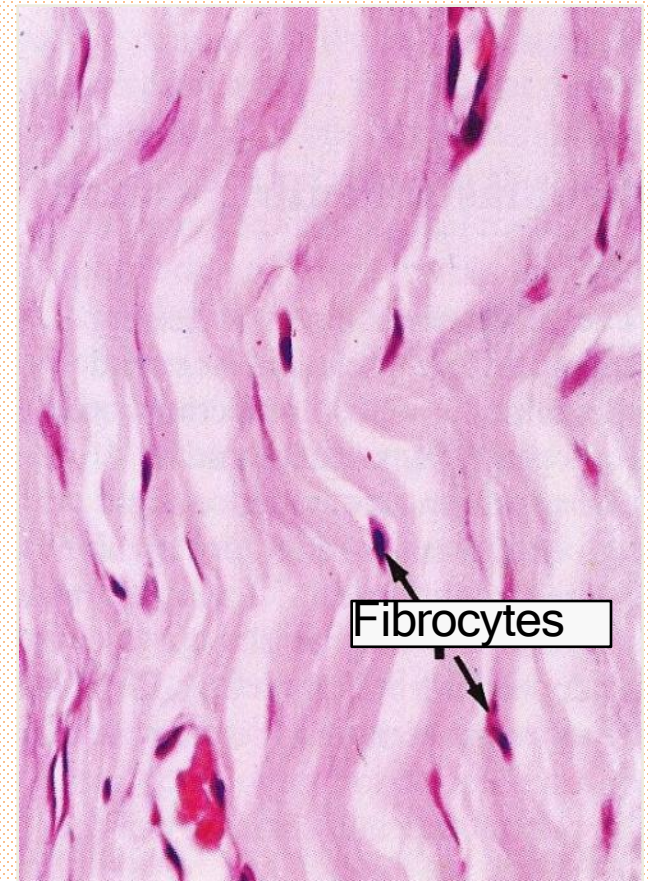
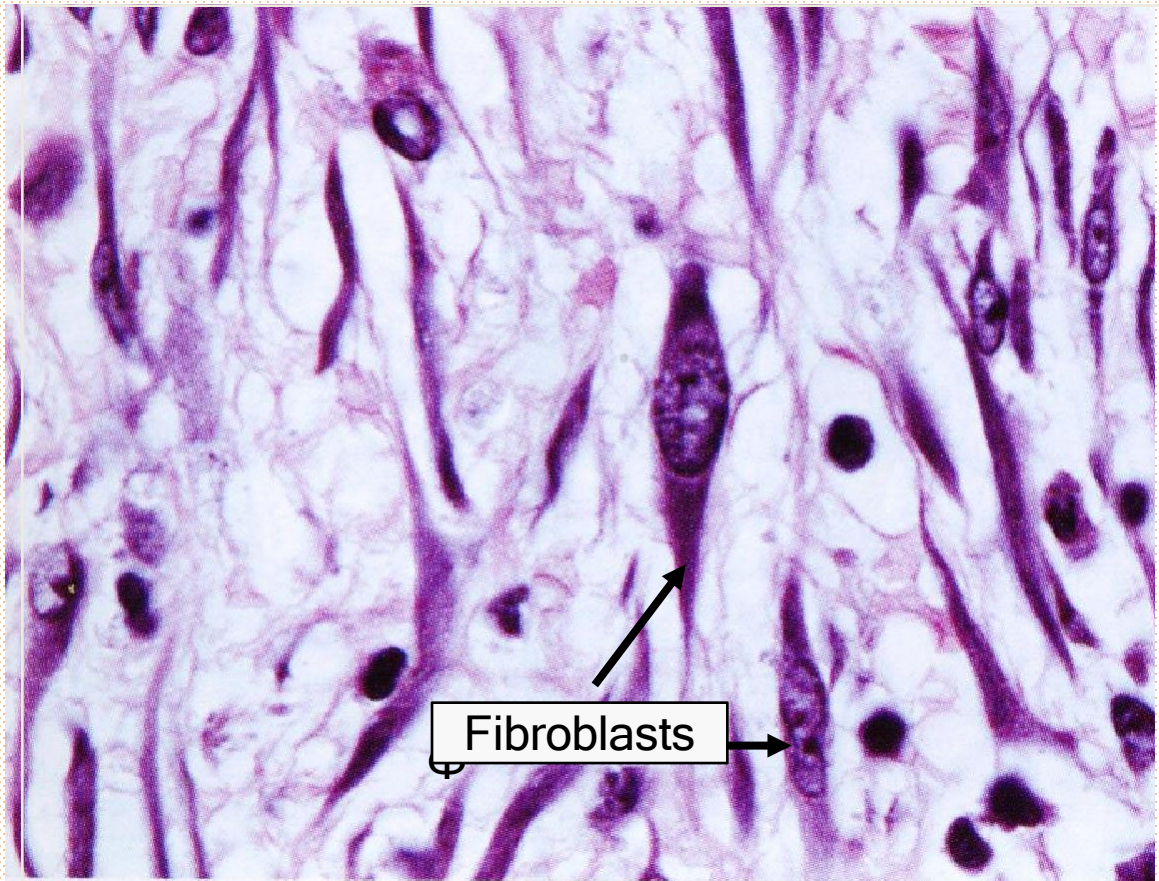


SEM



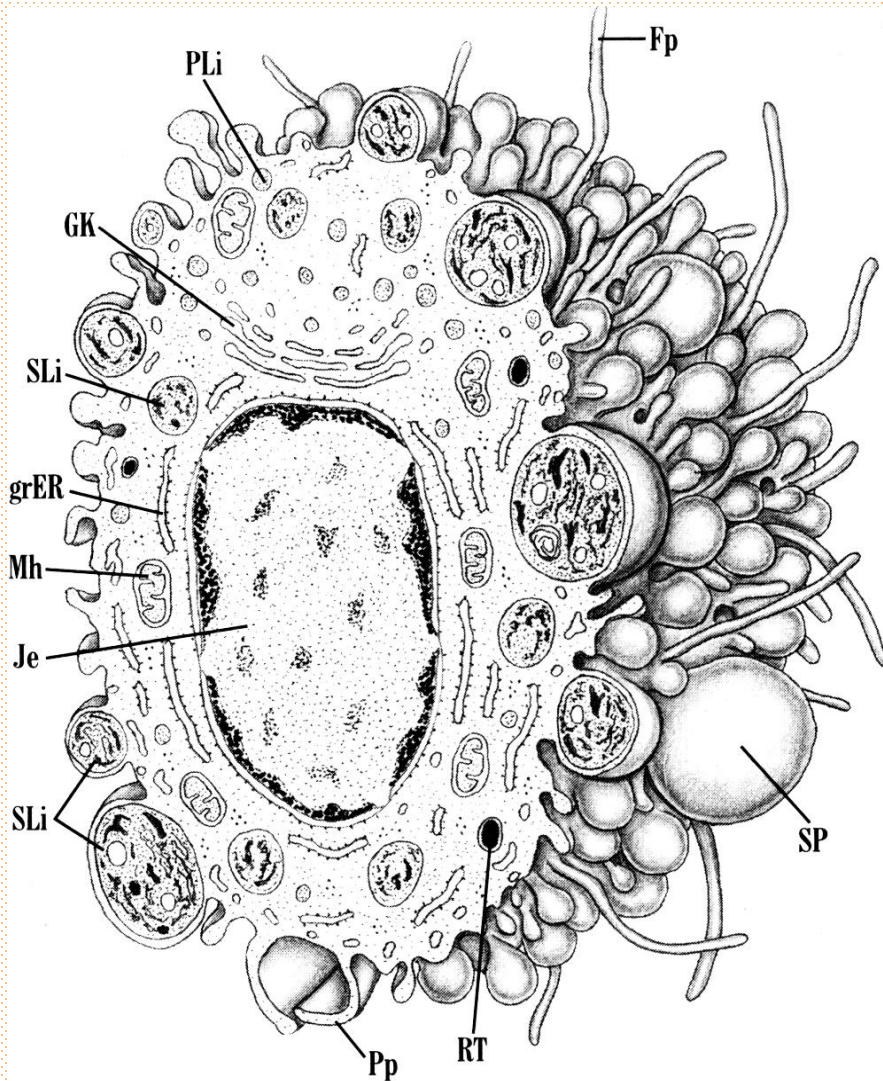
# Fibroblast and Fibrocyte

Stimulated by locally released growth factors, cell cycling and mitotic activity resume when the tissue requires additional fibroblasts

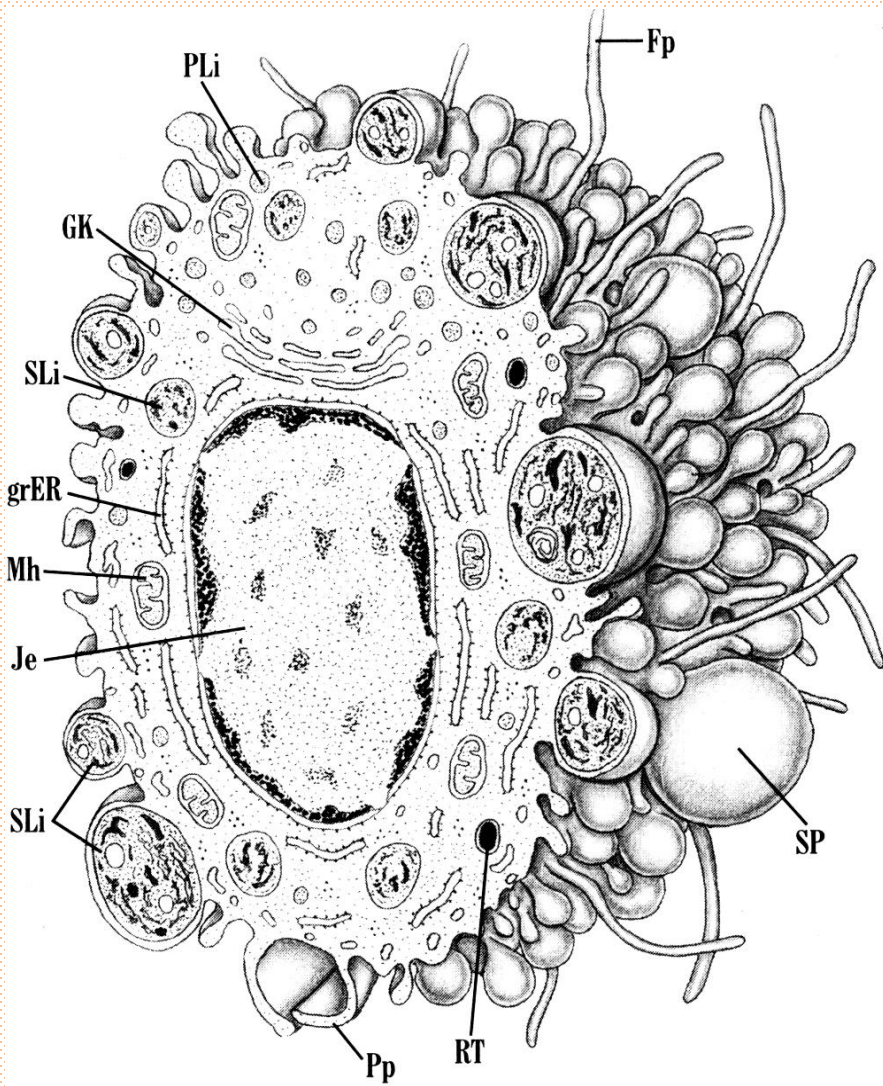




# Macrophage



- A macrophage is a mobile connective tissue cell that derives from monocytes.
- Size 15-30  $\mu\text{m}$ , lifetime about 2 months.
- By moving into the connective tissue, the monocyte undergoes phenotypic modification into a macrophage, acquires an oval shape with pseudopodia and filopodia on the surface.
- With pseudopodia, the macrophage captures and internalizes foreign particles in order to partially or completely break them down and then deposit them in lysosomes or expel them outside the cell.



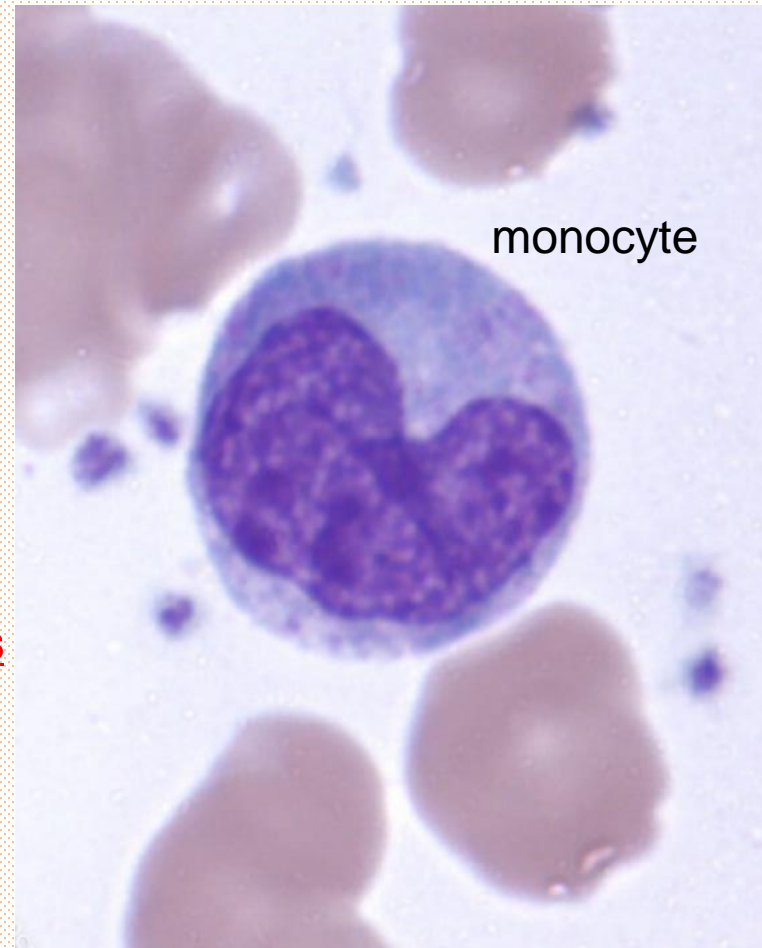
- The nucleus is heterochromatic, the organelles are well developed, and cytoplasm filled with vacuoles and lysosomes.

It belongs to the  
**Mononuclear Phagocyte System**

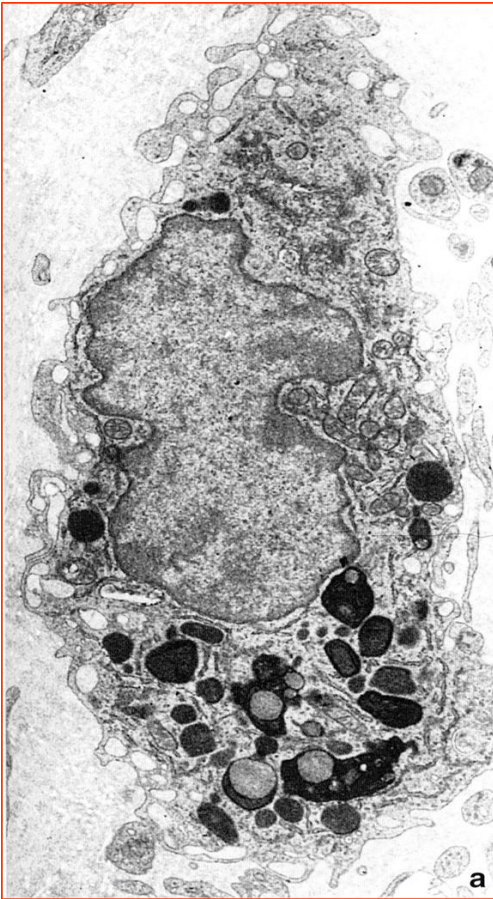
Roles:

- phagocytosis
- antigen presentation
- secretion of active substances (enzymes, inflammatory mediators)
- metabolic role (removal of erythrocytes).

- Mononuclear phagocyte system is a part of the immune system that consists of the **phagocytic cells located in reticular connective tissue**.
- These cells engulf and destroy bacteria, viruses, and other foreign substances and ingest worn-out or abnormal body cells.
- Mononuclear phagocytic cells are derived from precursor cells in the bone marrow - **monocytes and dendritic cells**, phagocytic cells that are released into the bloodstream.





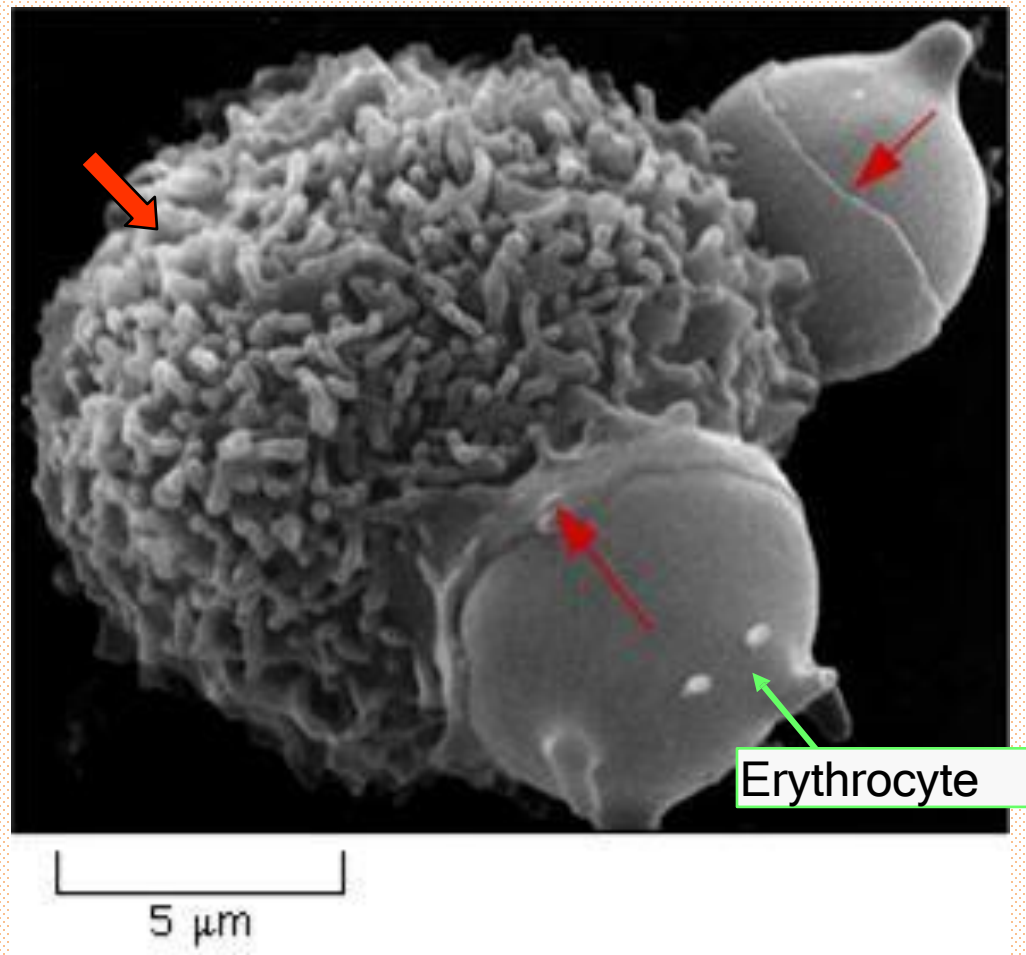
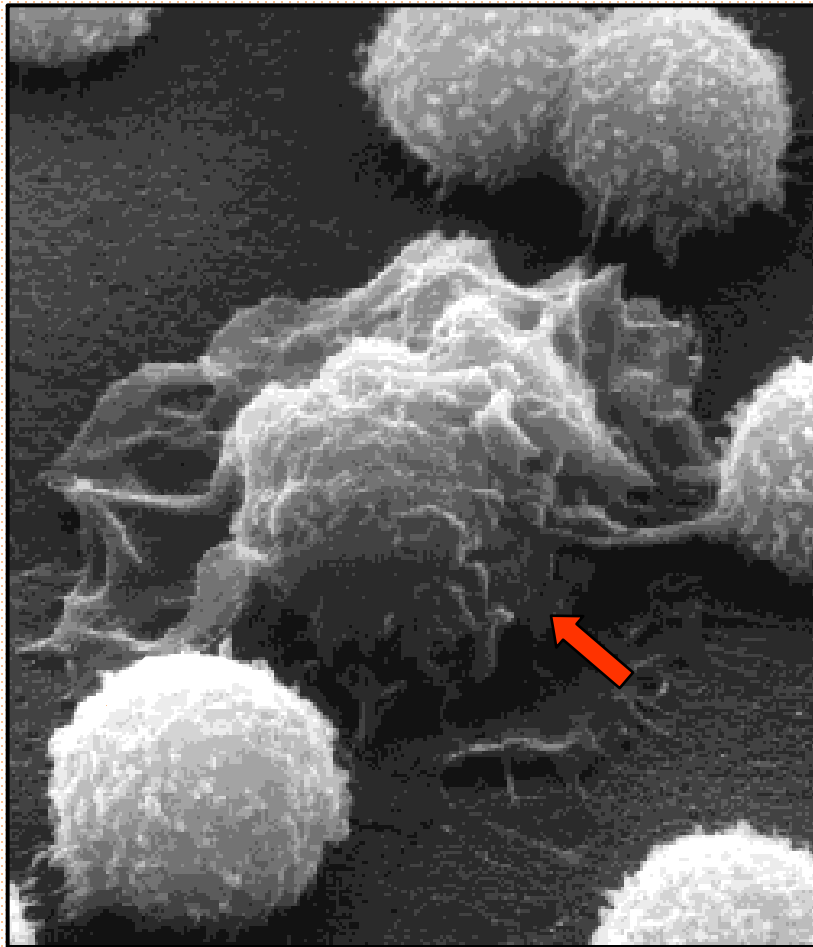


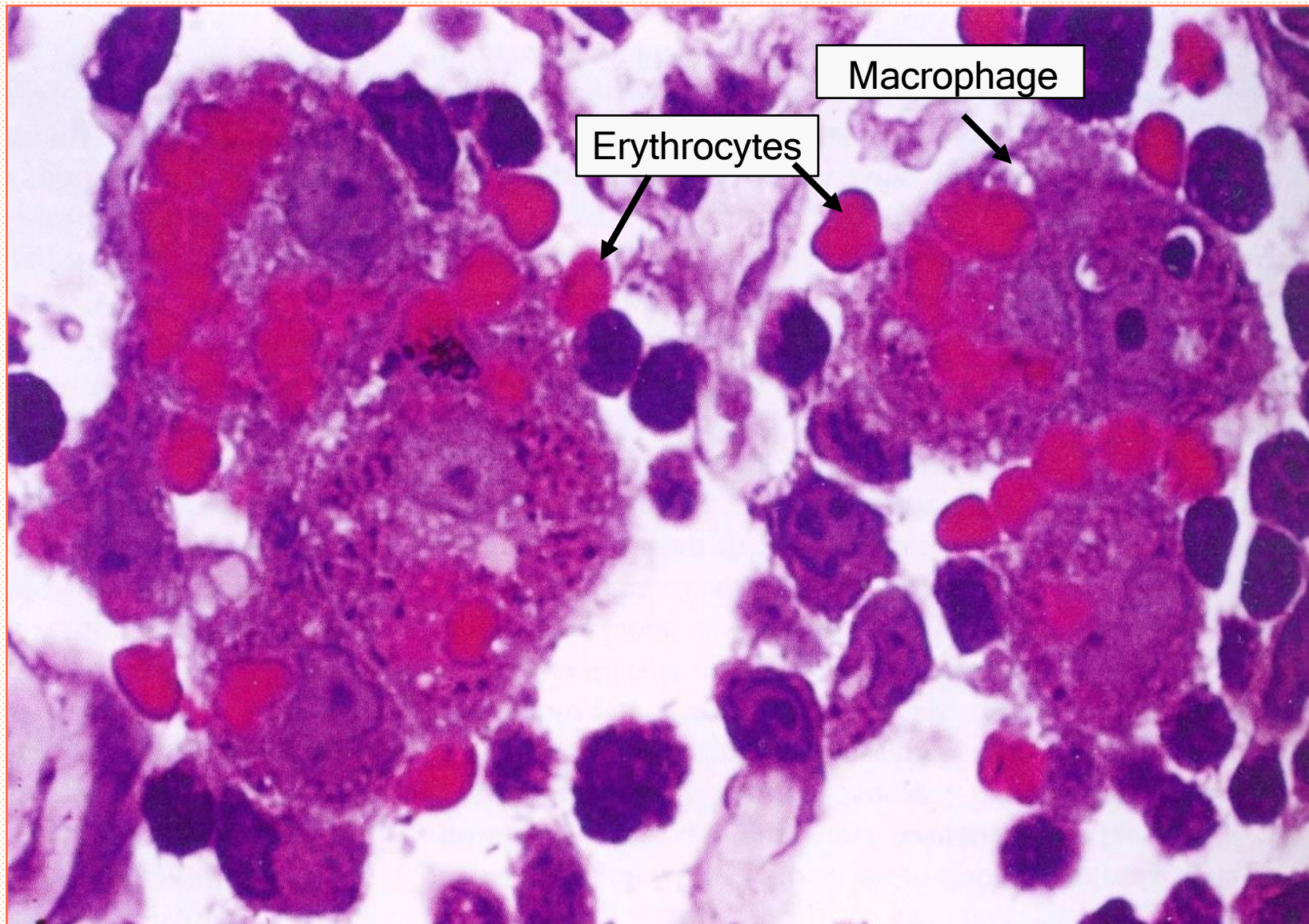
- Cells of the mononuclear phagocyte system differ in appearance and name because of their various locations. For example, **dendritic cells** are found in many tissues, including the lungs, the skin, and the gastrointestinal tract, as well as throughout the lymphatic system. **Histiocytes** are found in numerous subcutaneous tissues. **Kupffer cells** line the sinusoids of the liver. **Microglia** occur in nervous tissue, and alveolar macrophages are found in the air spaces of the lungs.



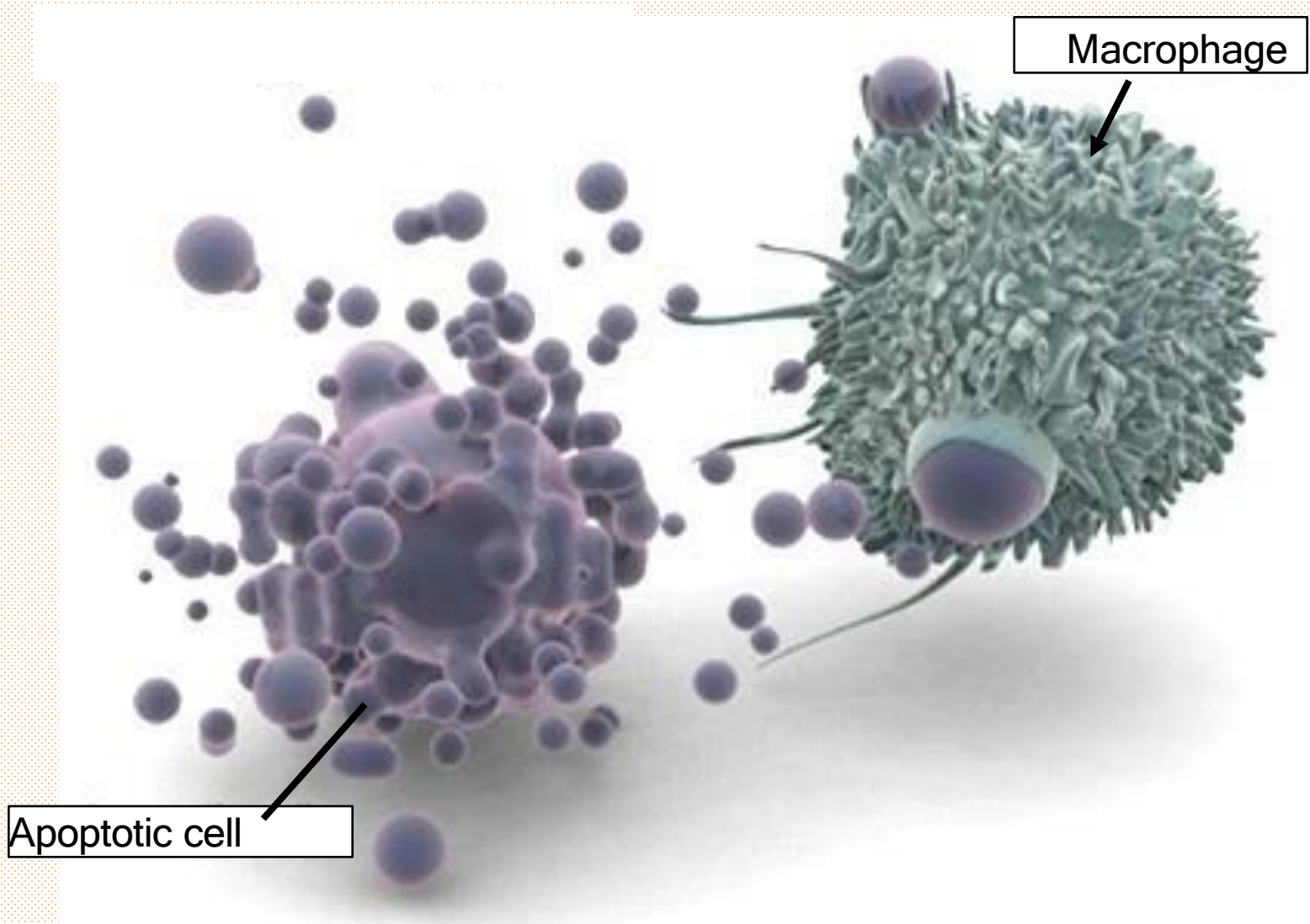
# Mononuclear Phagocyte System

Cell Name	Location
Adipose tissue macrophages	Adipose tissue
Monocyte	Bone Marrow/Blood
Kupffer cell	Liver
Sinus histiocytes	Lymph node
Alveolar macrophage (dust cell)	Pulmonary alveolus of Lungs
Tissue macrophages (Histiocyte) leading to Giant cells	Connective Tissues
Langerhans cell	Skin and Mucosa
Microglia	Central Nervous System
Hofbauer cell	Placenta
Intraglomerular mesangial cell <sup>[4]</sup>	Kidney
Epithelioid histiocyte	Granulomas
Red Pulp Macrophage (Sinusoidal lining cells)	Red pulp of Spleen
Peritoneal macrophages	Peritoneal cavity
Osteoclast	Bone



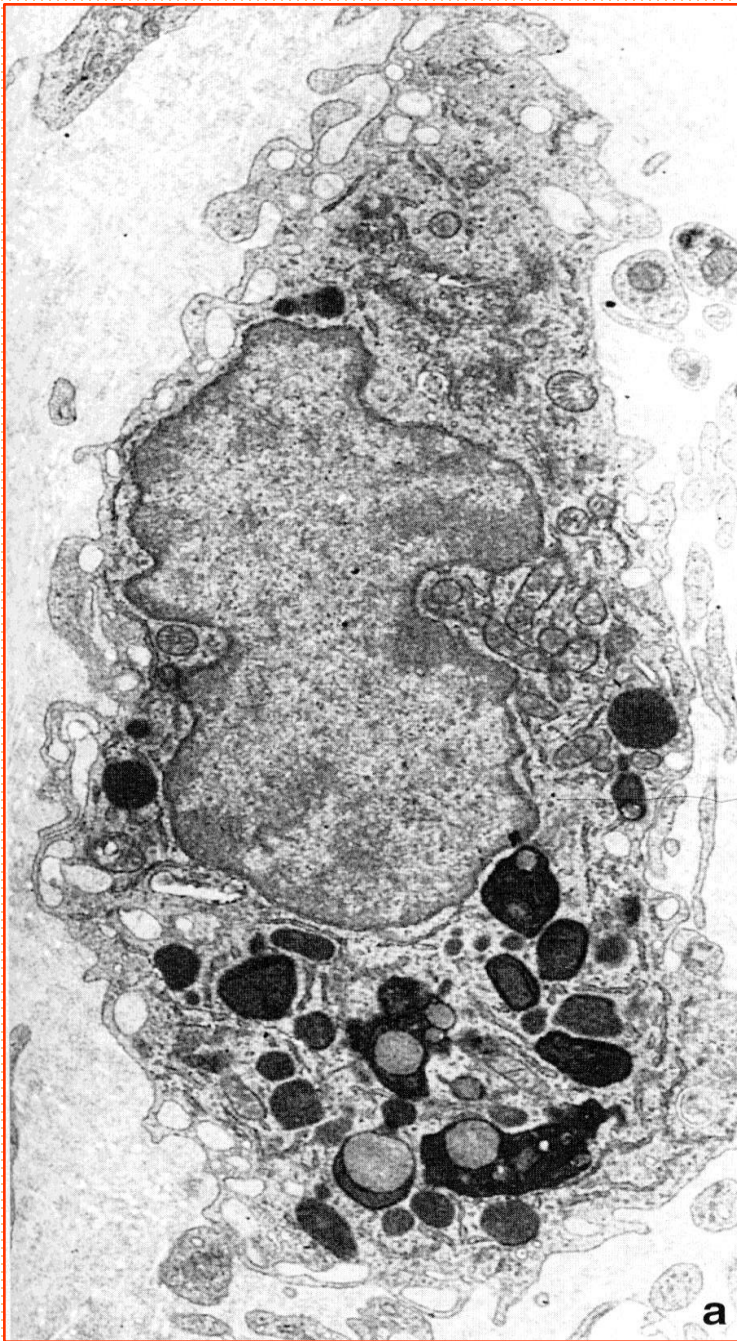




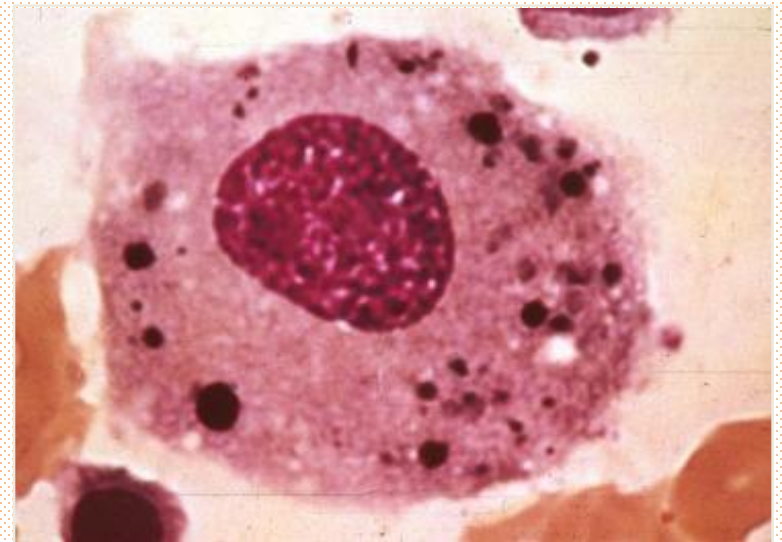
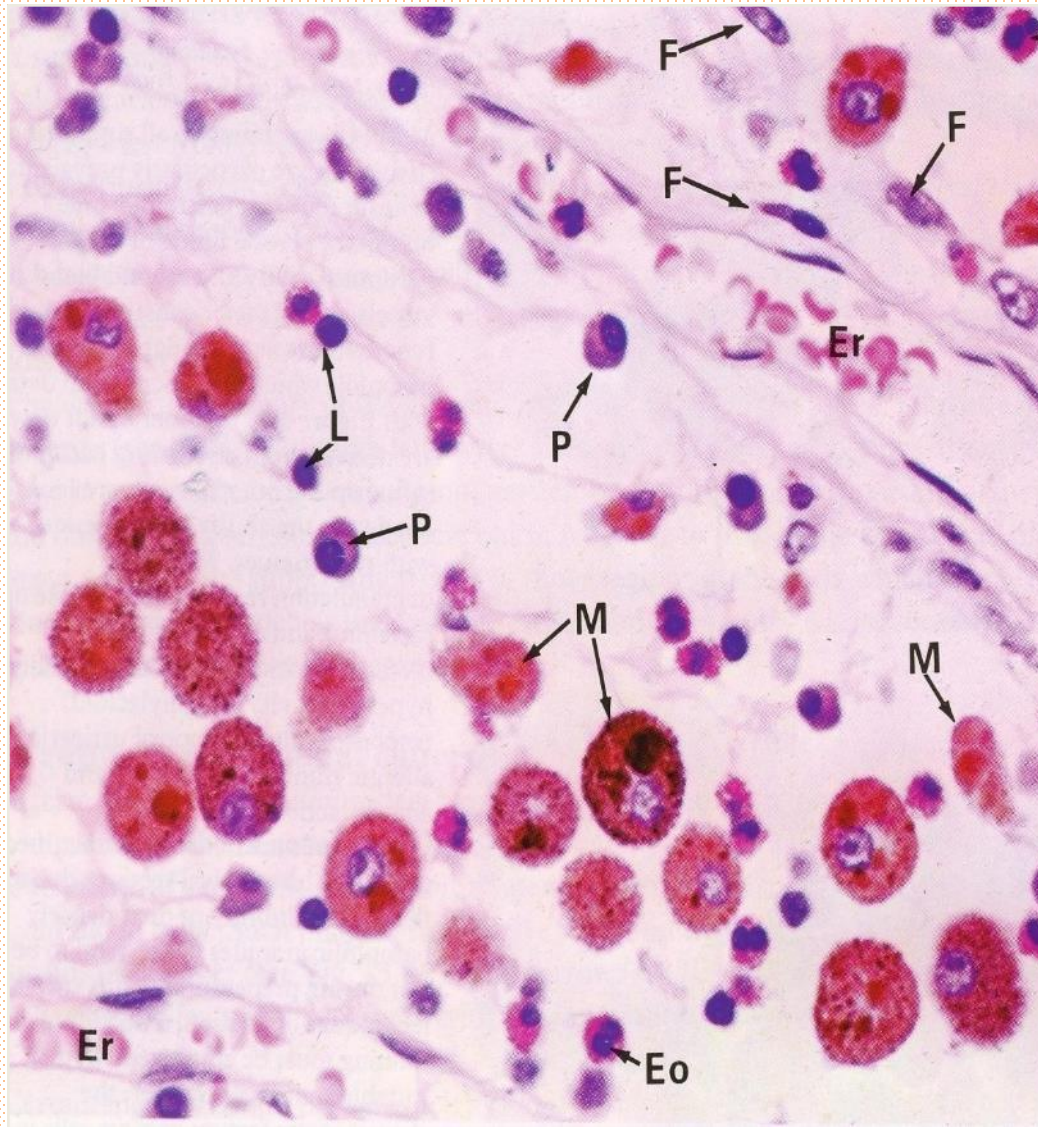




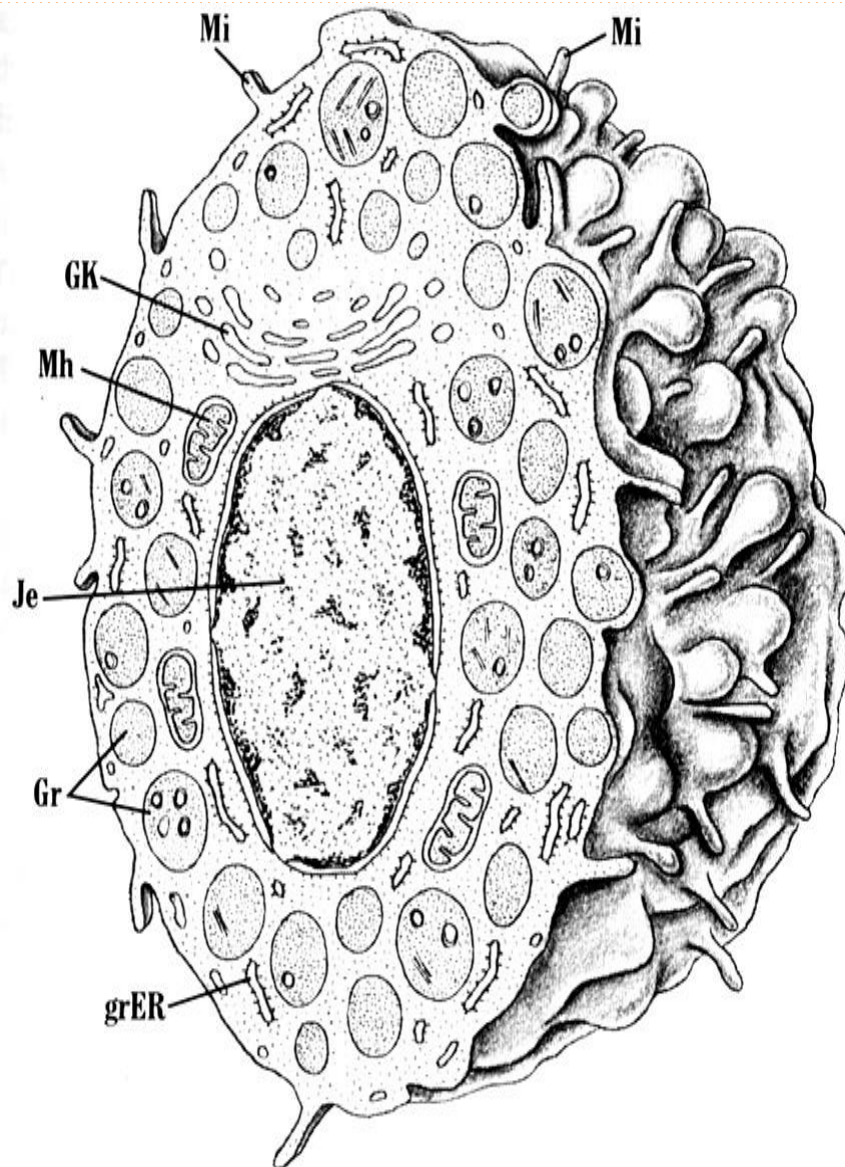
# Macrophage - EM







# Mast cell



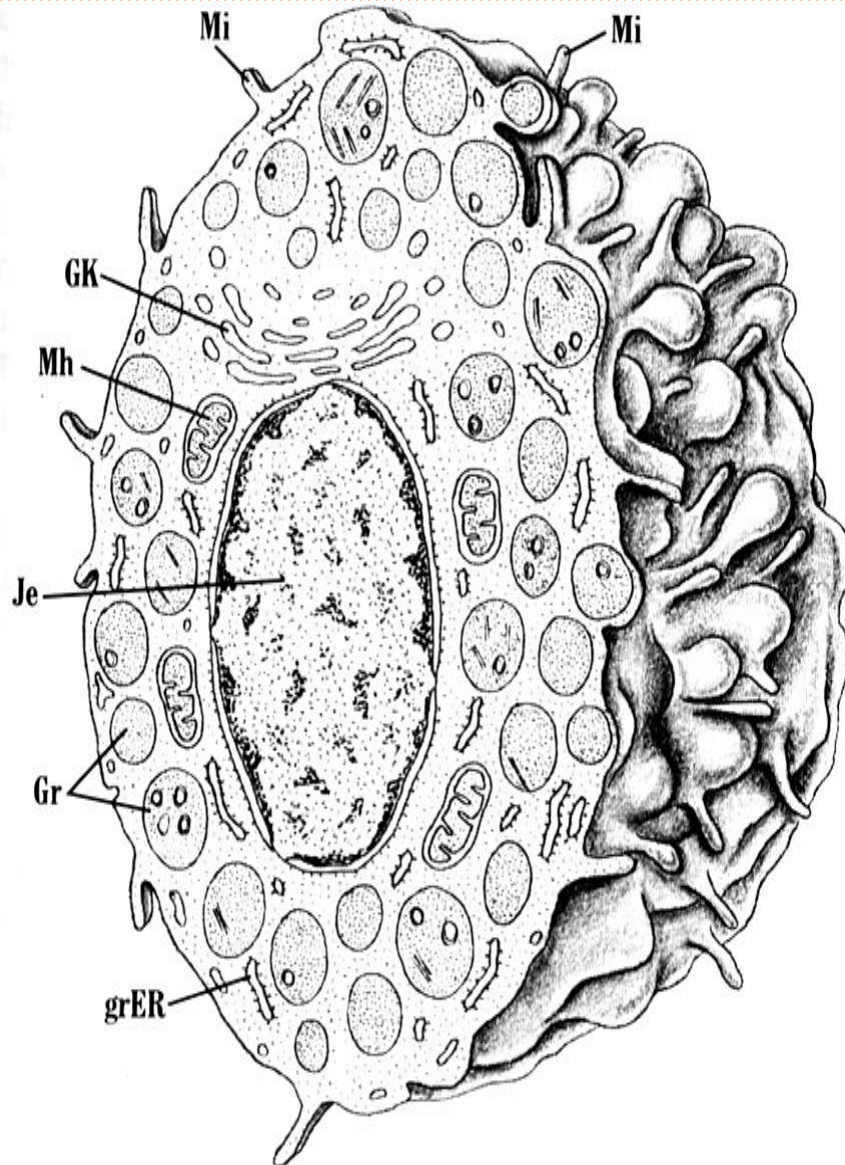
- A **mast cell** is a mobile connective tissue cell localized along blood vessels, especially in the dermis and respiratory mucosa.
- Mast cells make up about 10% of connective tissue cells and have a lifespan of several months.

Types:

- **loose connective tissue mast cells** (heparin and neutral proteases are deposited in the granules)
- **mast cells of the mucosa** of the respiratory and digestive system (contain chondroitin sulfate and tryptase).



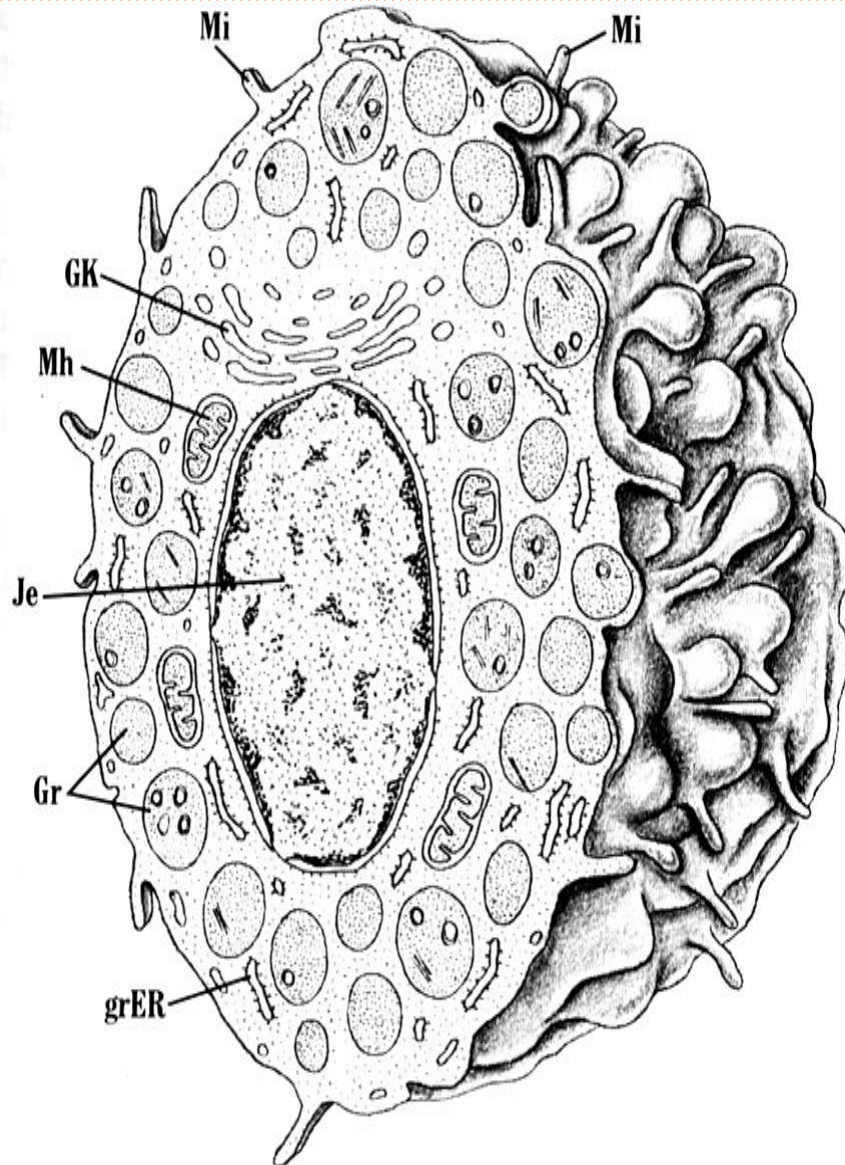
# Мастоцит



- **Appearance:** an oval cell with a diameter of 15-30  $\mu\text{m}$ , with numerous outgrowths on the surface and metachromatic granules in the cytoplasm.
- In the cytoplasm, it contains **metachromatic granules** up to 1  $\mu\text{m}$  in diameter.
- Some of the granules are filled with **heparin**.
- In addition to heparin, the granules also contain **histamine**, a strong vasodilator that increases the permeability of blood vessels

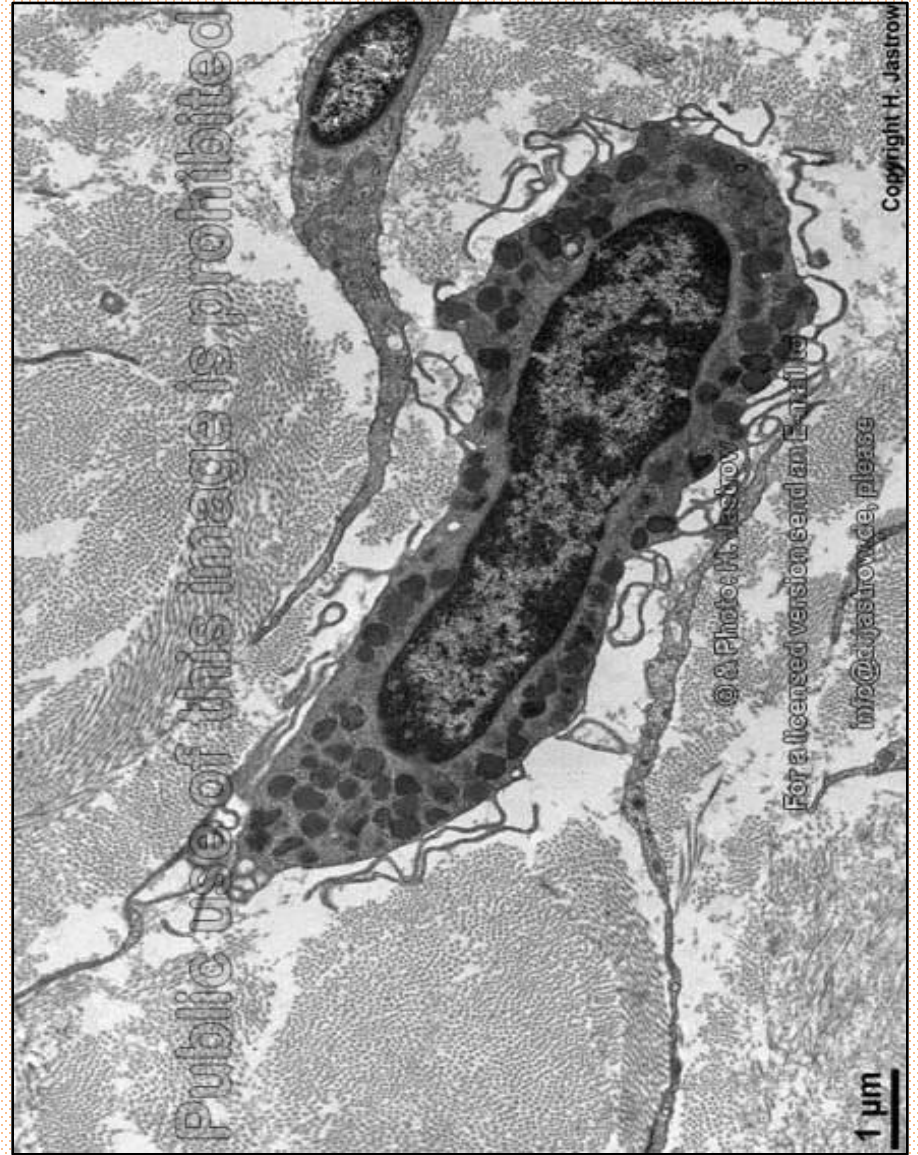
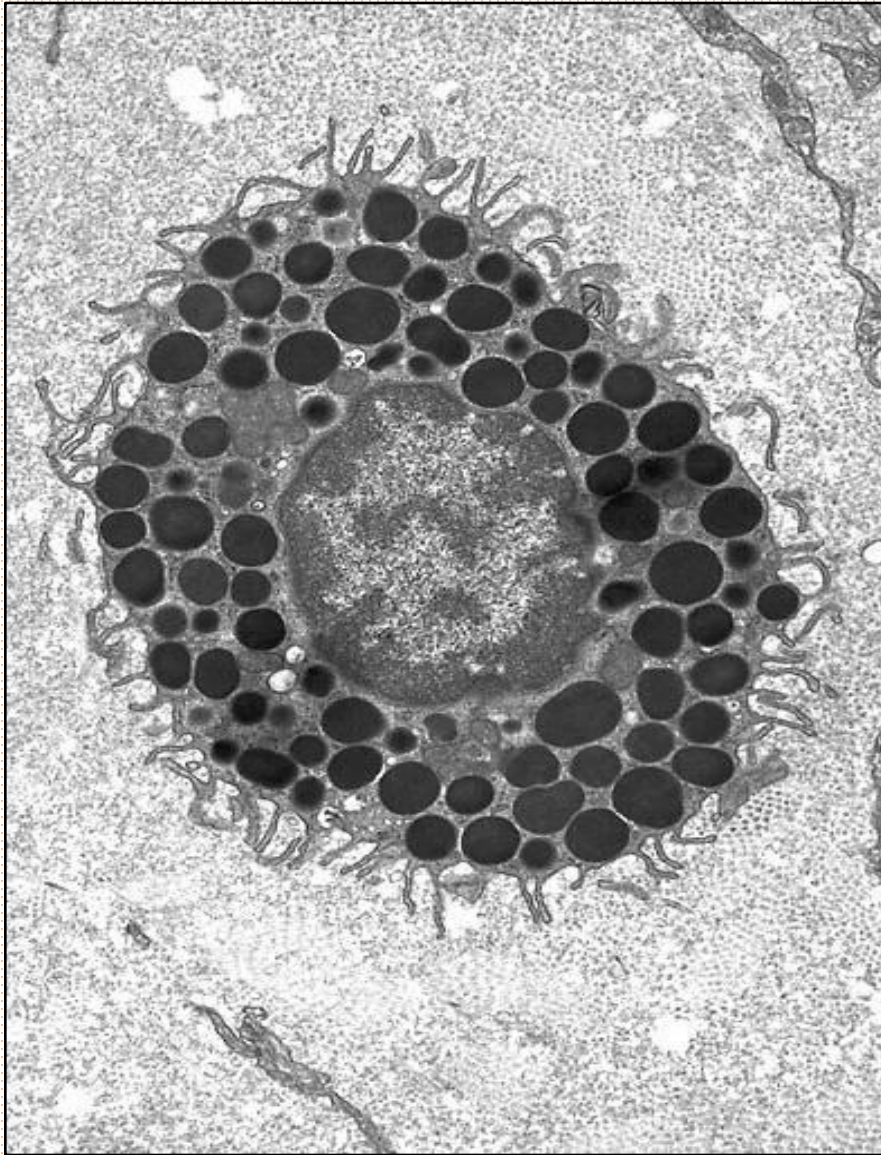


# Мастоцит



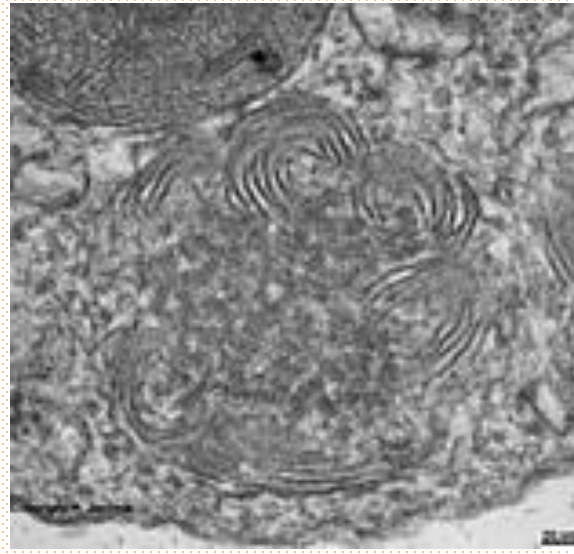
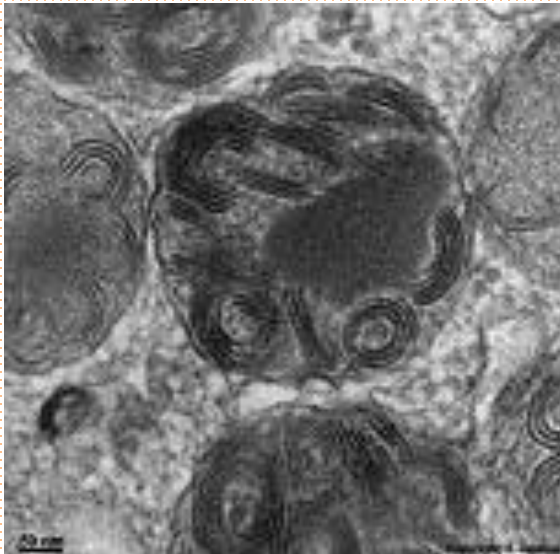
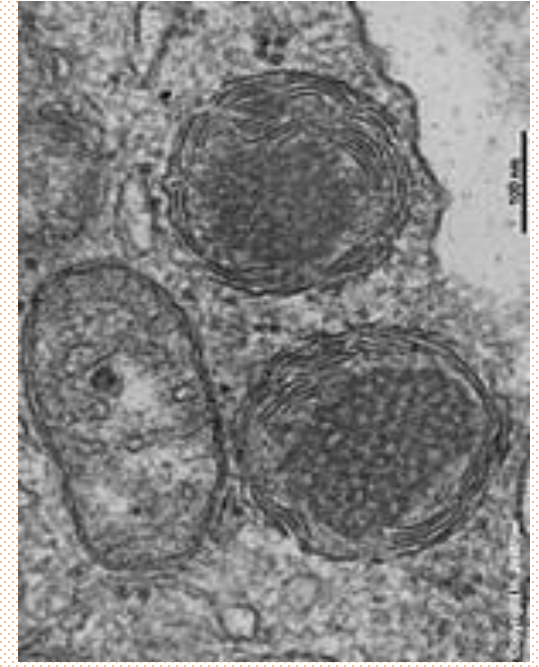
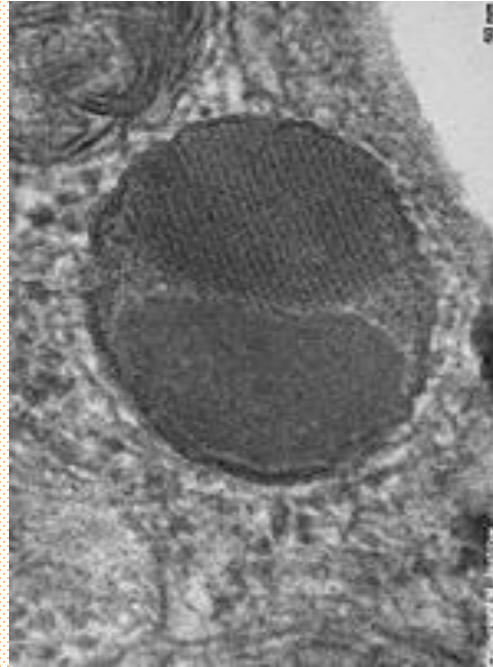
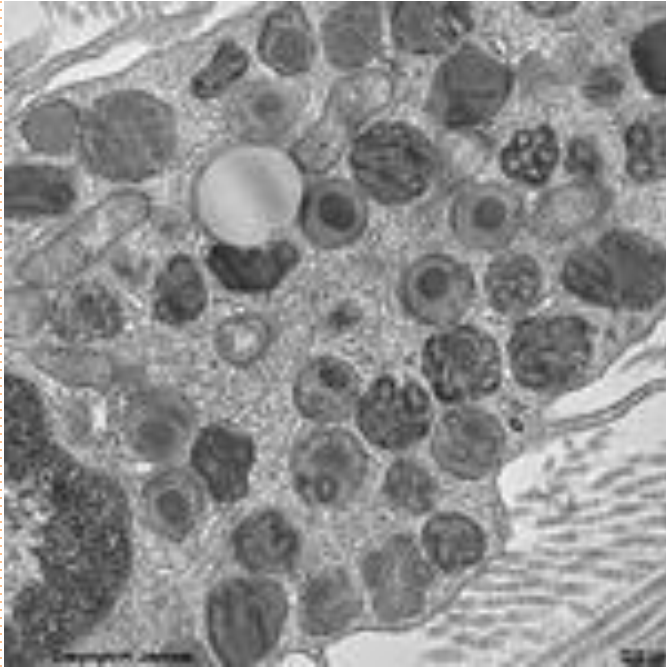
- They possess membrane bound IgE class antibodies. Antigen binding to IgE leads to massive degranulation and release of allergic mediators.
- Release of mediators stored in mast cells promotes the **immediate hypersensitivity reactions** because they occur within a few minutes after the appearance of an antigen in an individual previously sensitized to that antigen.

# Mast cell - EM



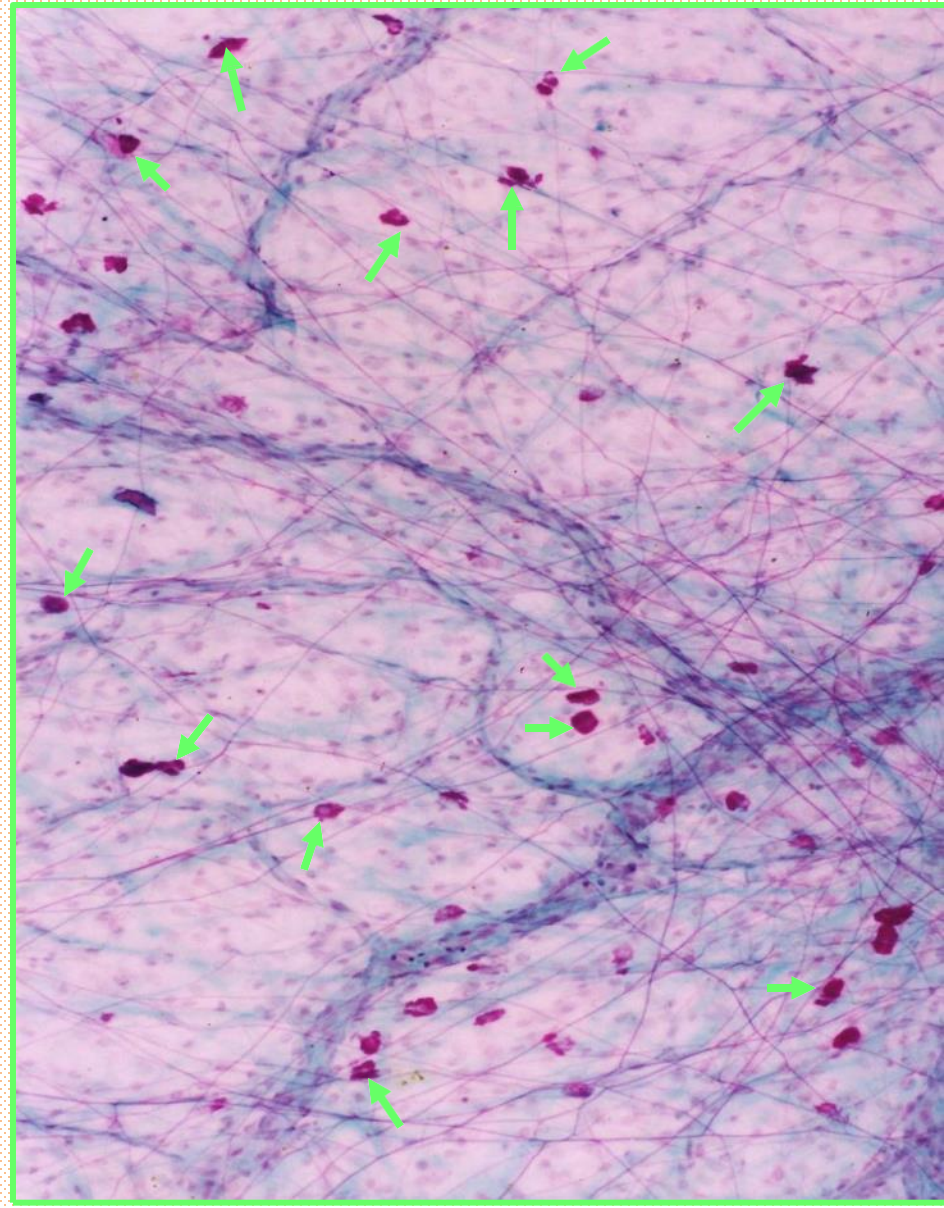
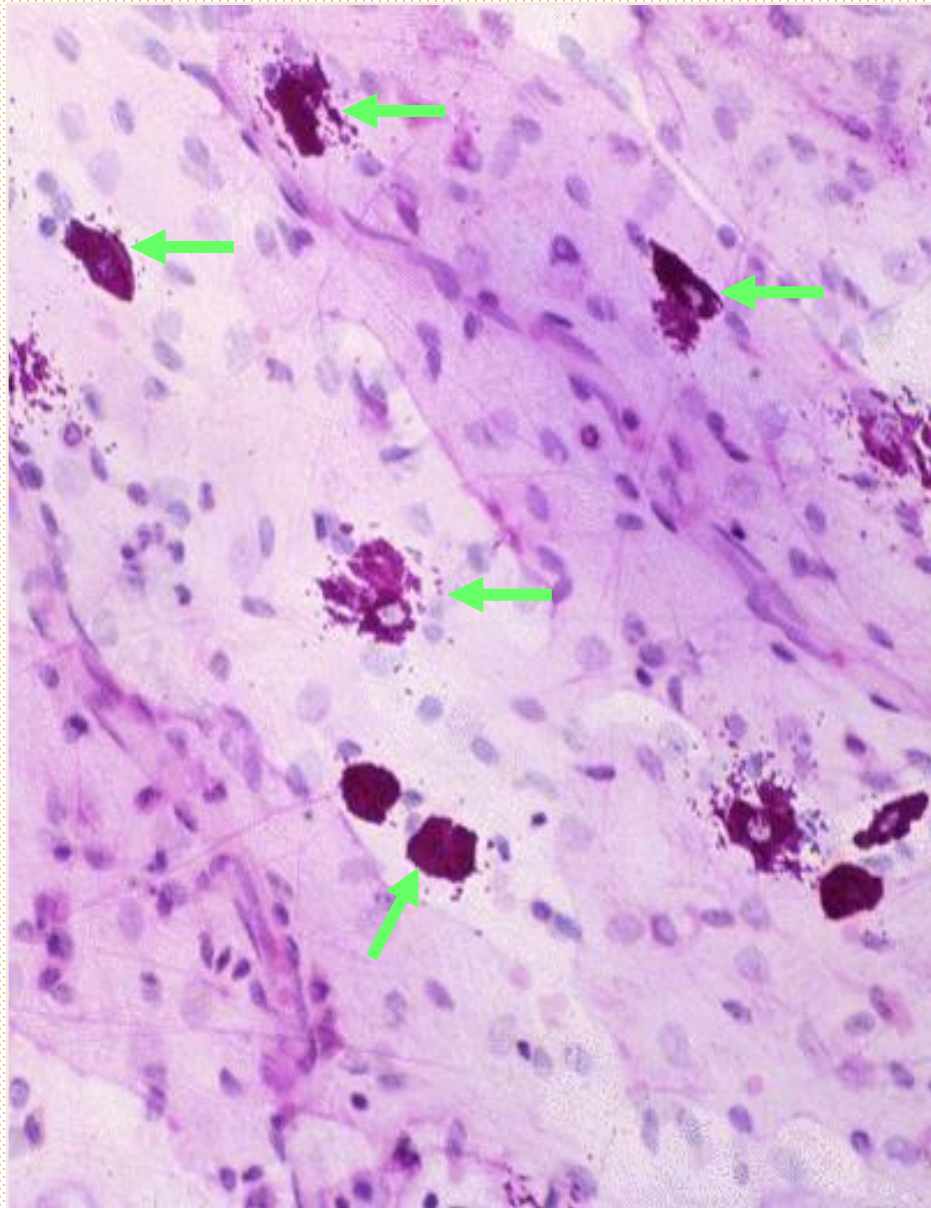


# Mast cell granules- EM

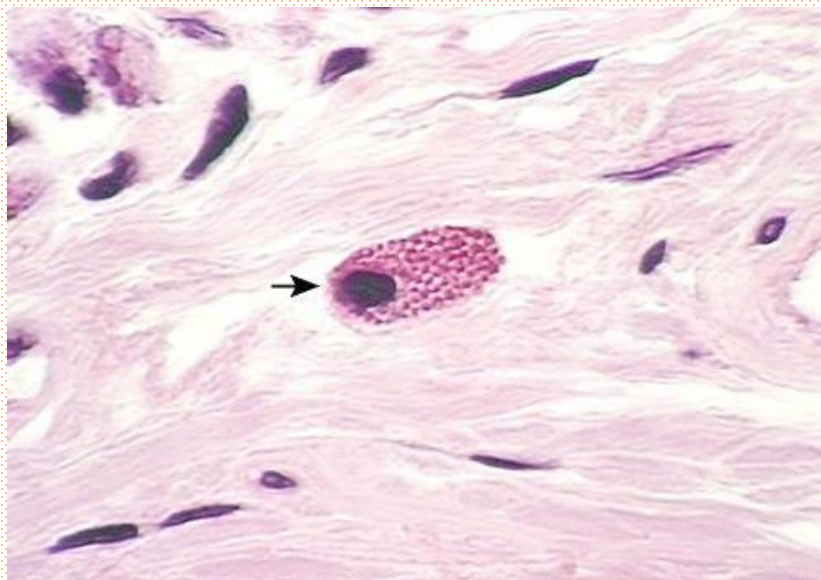
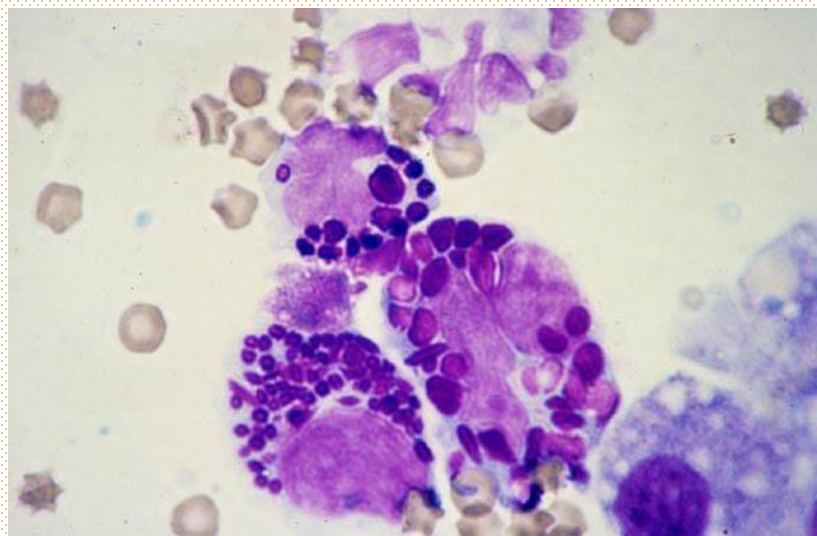
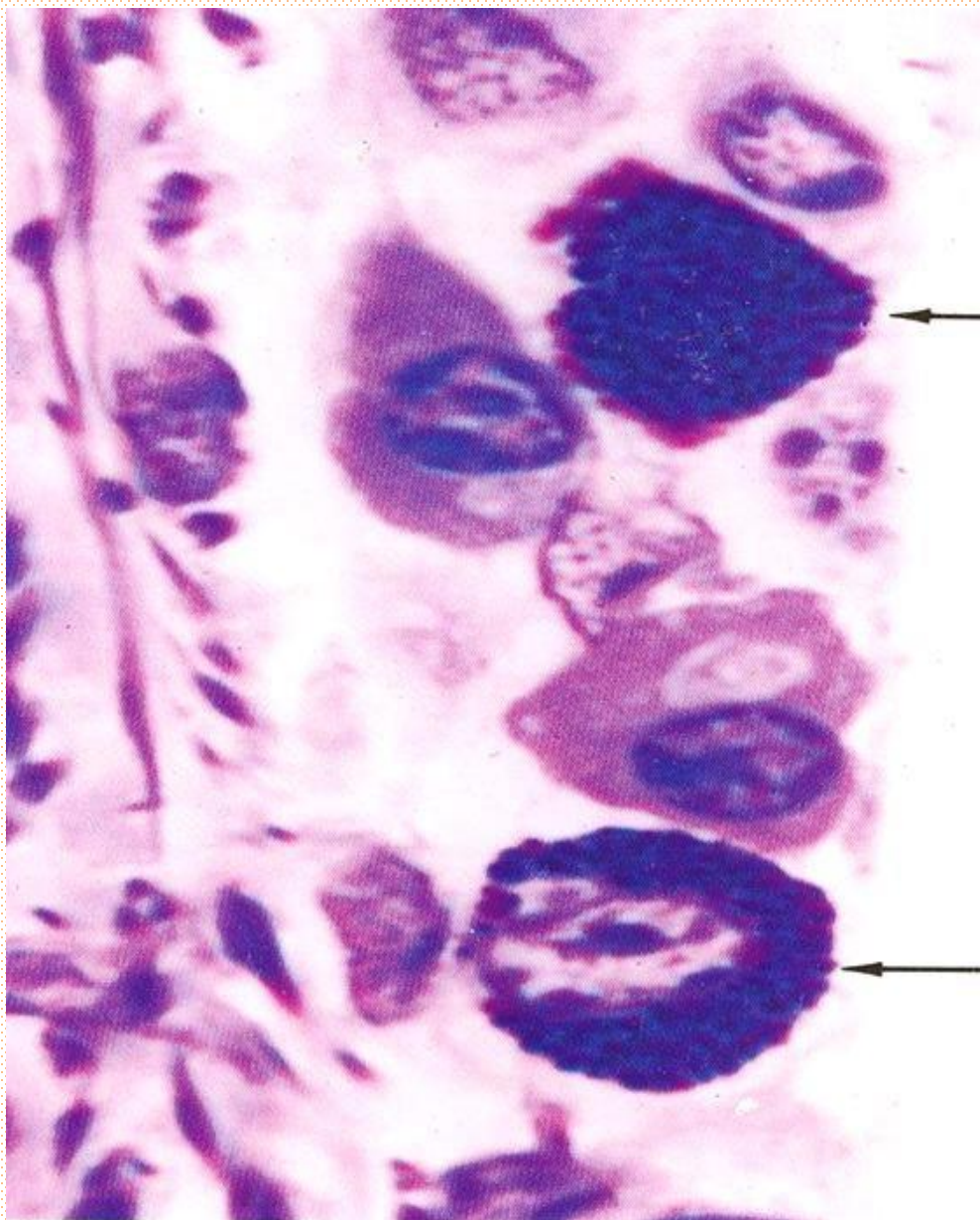




# Mast cells





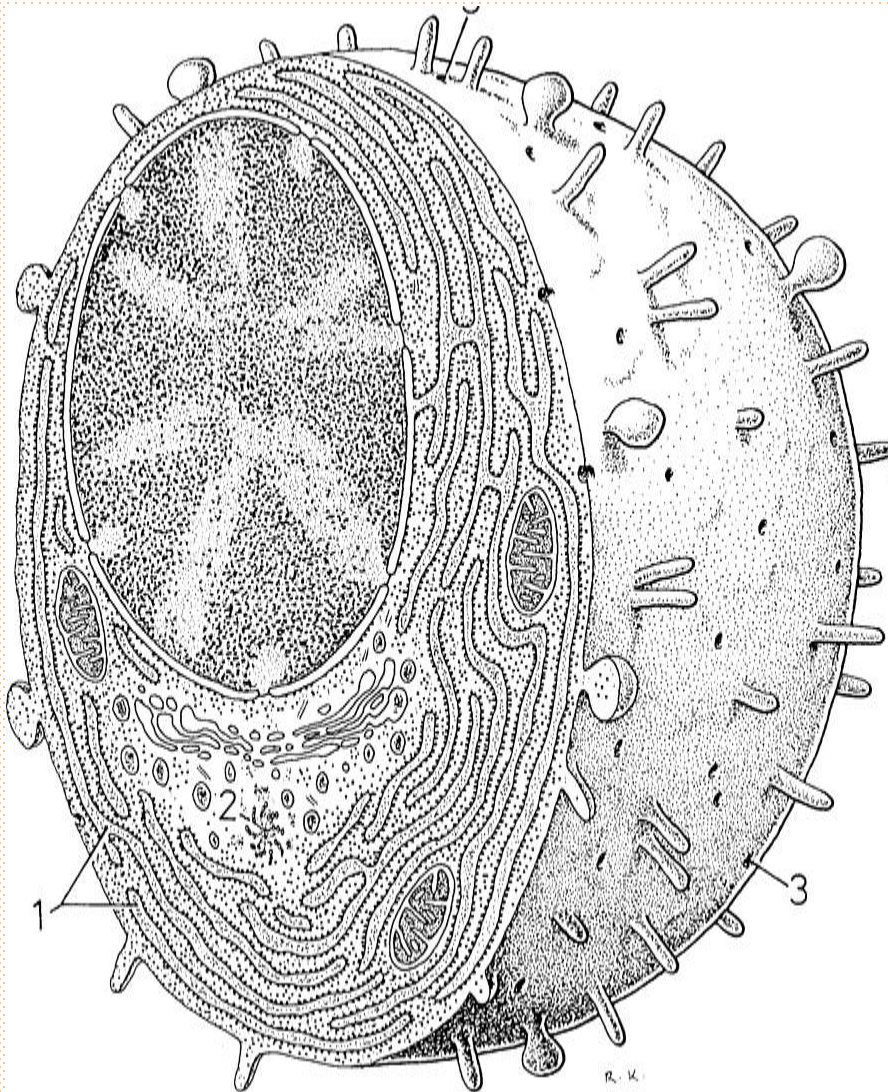


# Mast cell products

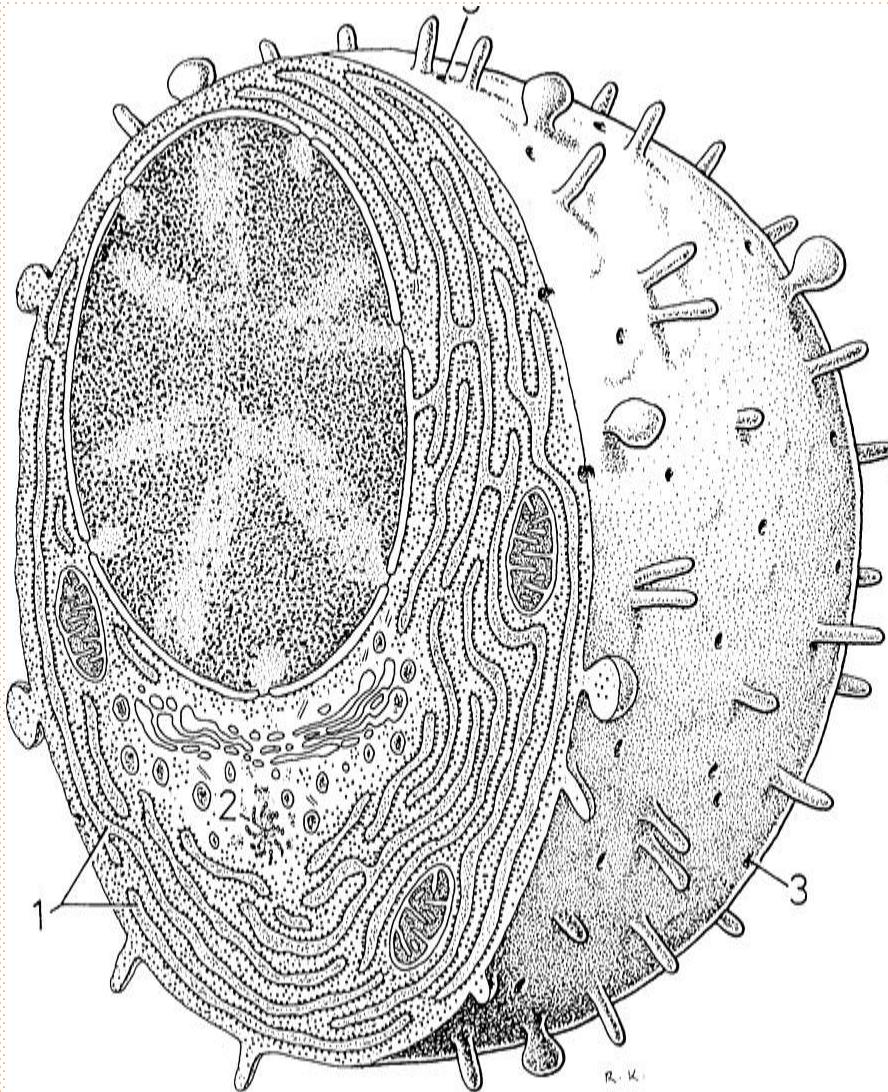
1. **Heparin**, a sulfated GAG that acts locally as an anticoagulant
2. **Histamine**, which promotes increased vascular permeability and smooth muscle contraction
3. **Serine proteases**, which activate various mediators of inflammation
4. **Eosinophil and neutrophil chemotactic factors**, which attract those leukocytes
5. **Cytokines**, polypeptides directing activities of leukocytes and other cells of the immune system
6. **Phospholipid precursors**, which are converted to prostaglandins, leukotrienes, and other important lipid mediators of the inflammatory response.



# Plasma cell



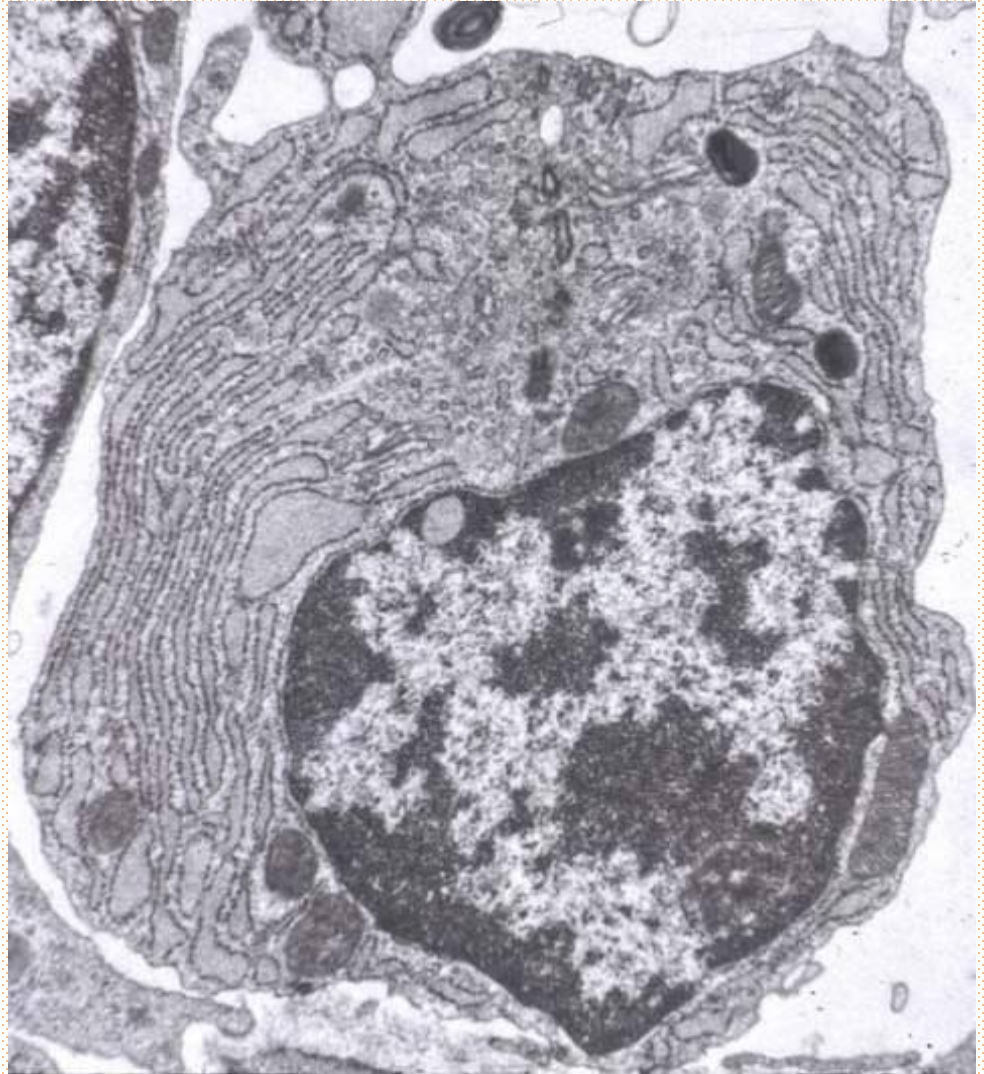
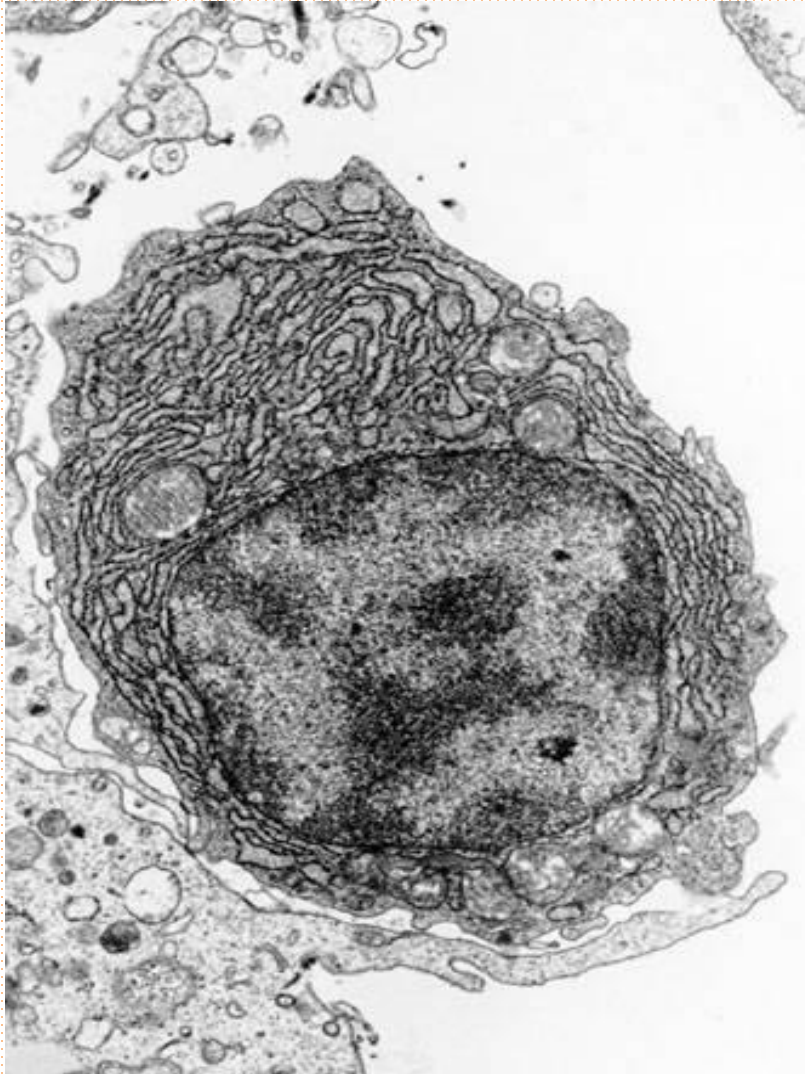
- A **plasma cell** is a mobile connective tissue cell that arises from a B lymphocyte after its antigenic stimulation.
- Life span 10-30 days.
- These cells are most represented in the connective tissue of the lymphatic organs, in the connective tissue of the mucous membrane of the respiratory and digestive tracts, as well as in the stroma of the exocrine glands.



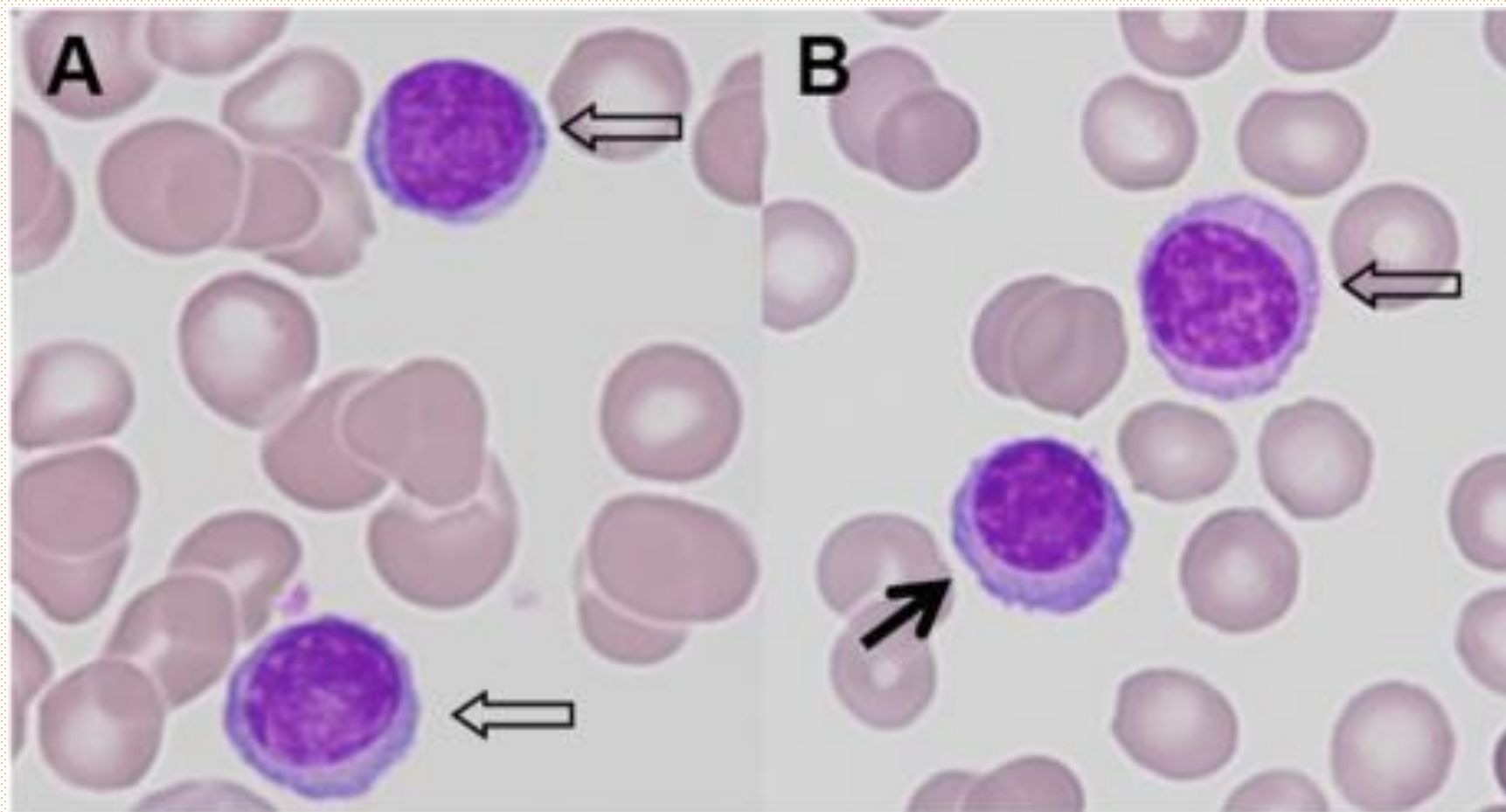
- Cell has a round or oval shape, 10-20  $\mu\text{m}$  in diameter, a round eccentric nucleus with a **characteristic chromatin arrangement**
- The bulky Golgi apparatus causes perinuclear lightening
- **Role: immunoglobulin secretion.**
- Sometimes **Russell's bodies** can be seen due to **excessive synthesis of Ig.**



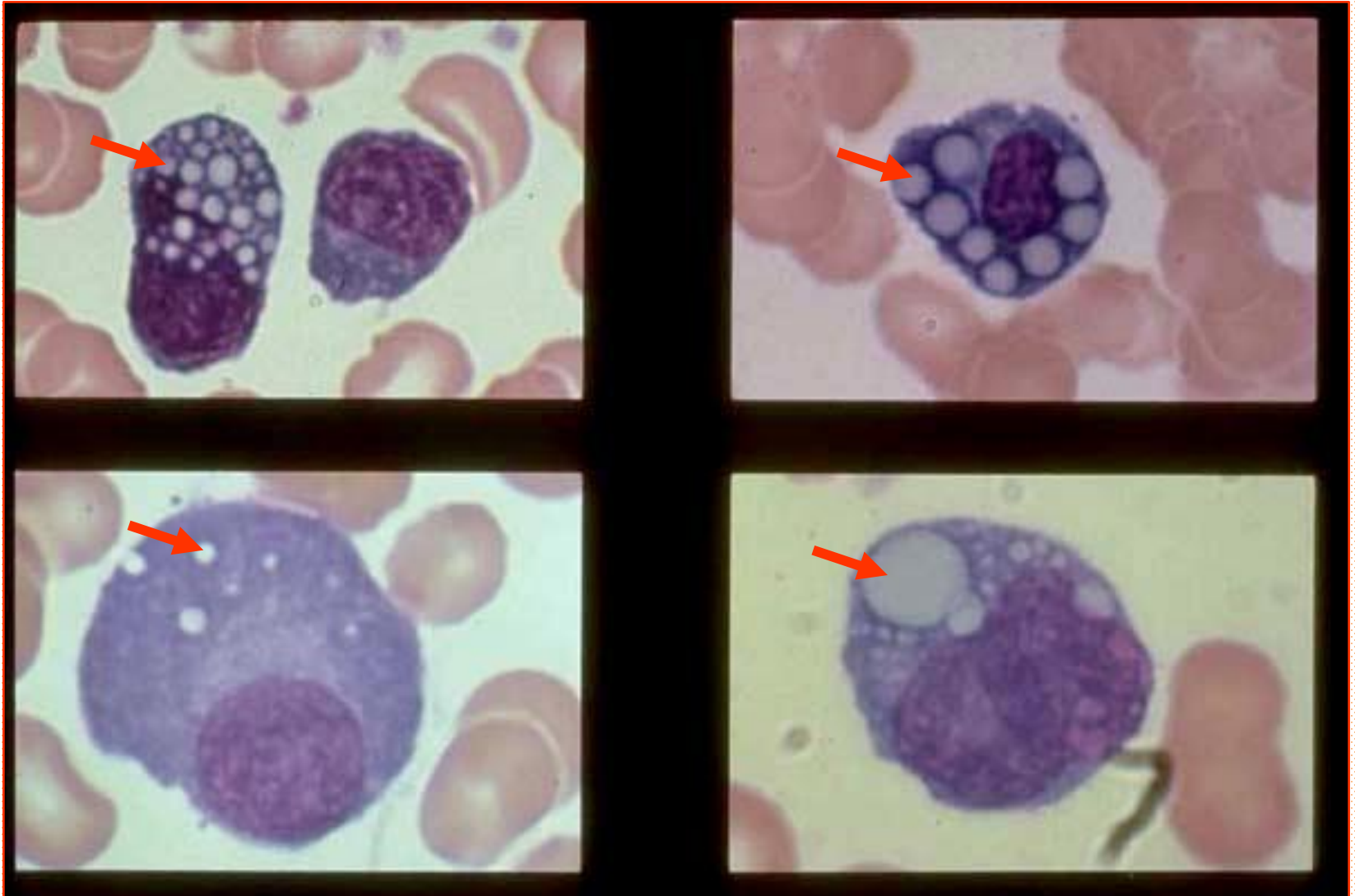
# EM





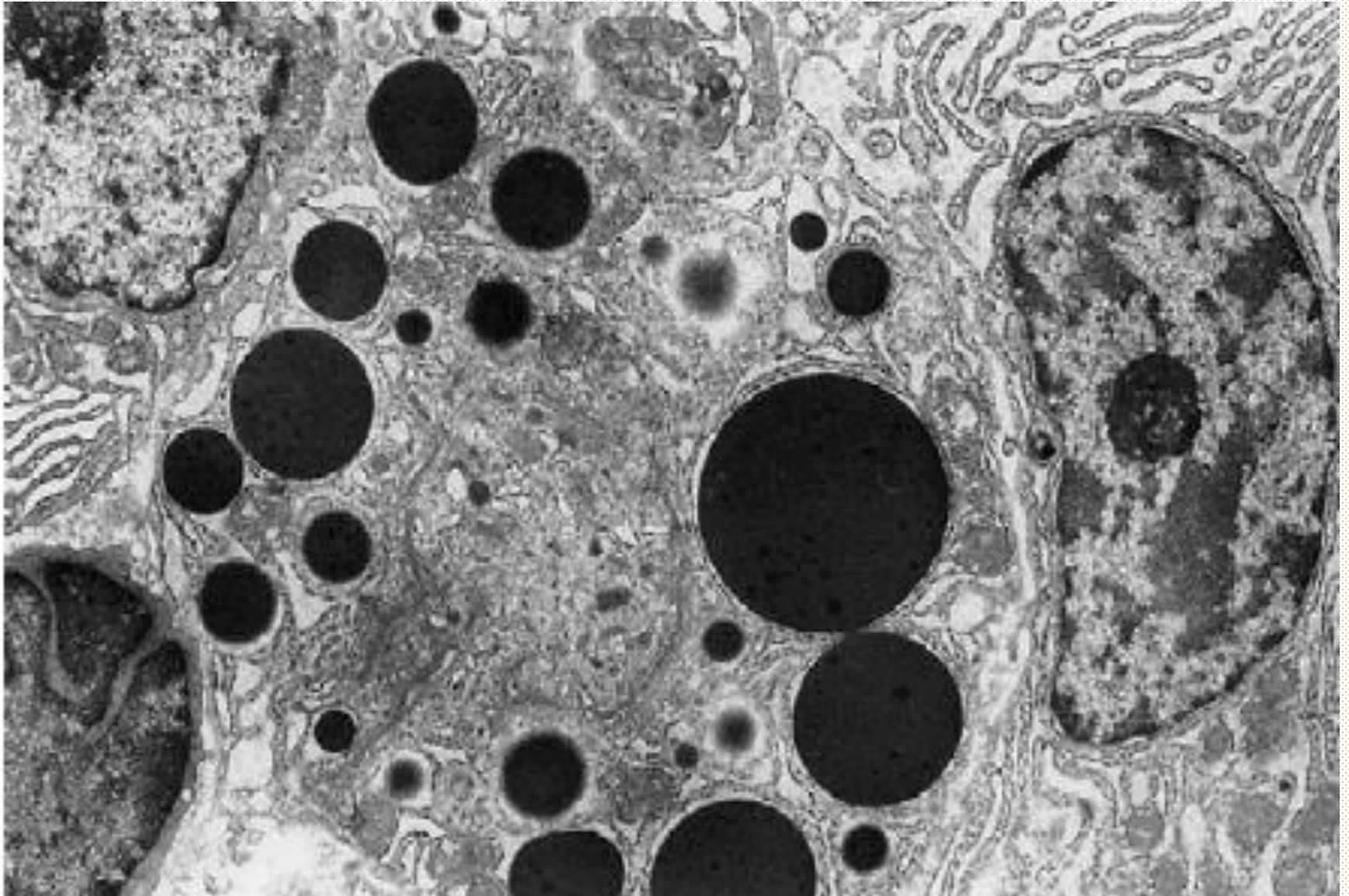


# Russell bodies





# Russell bodies- EM





# **Extracellular matrix**



# Extracellular matrix



```
graph TD; A[Extracellular matrix] --> B[Ground substance]; A --> C[Protein fibers]; B --> D[Glycosaminoglycans<br/>Proteoglycans<br/>Adhesive glycoproteins]; C --> E[Collagen fibers<br/>Elastic fibers<br/>Reticular fibers];
```

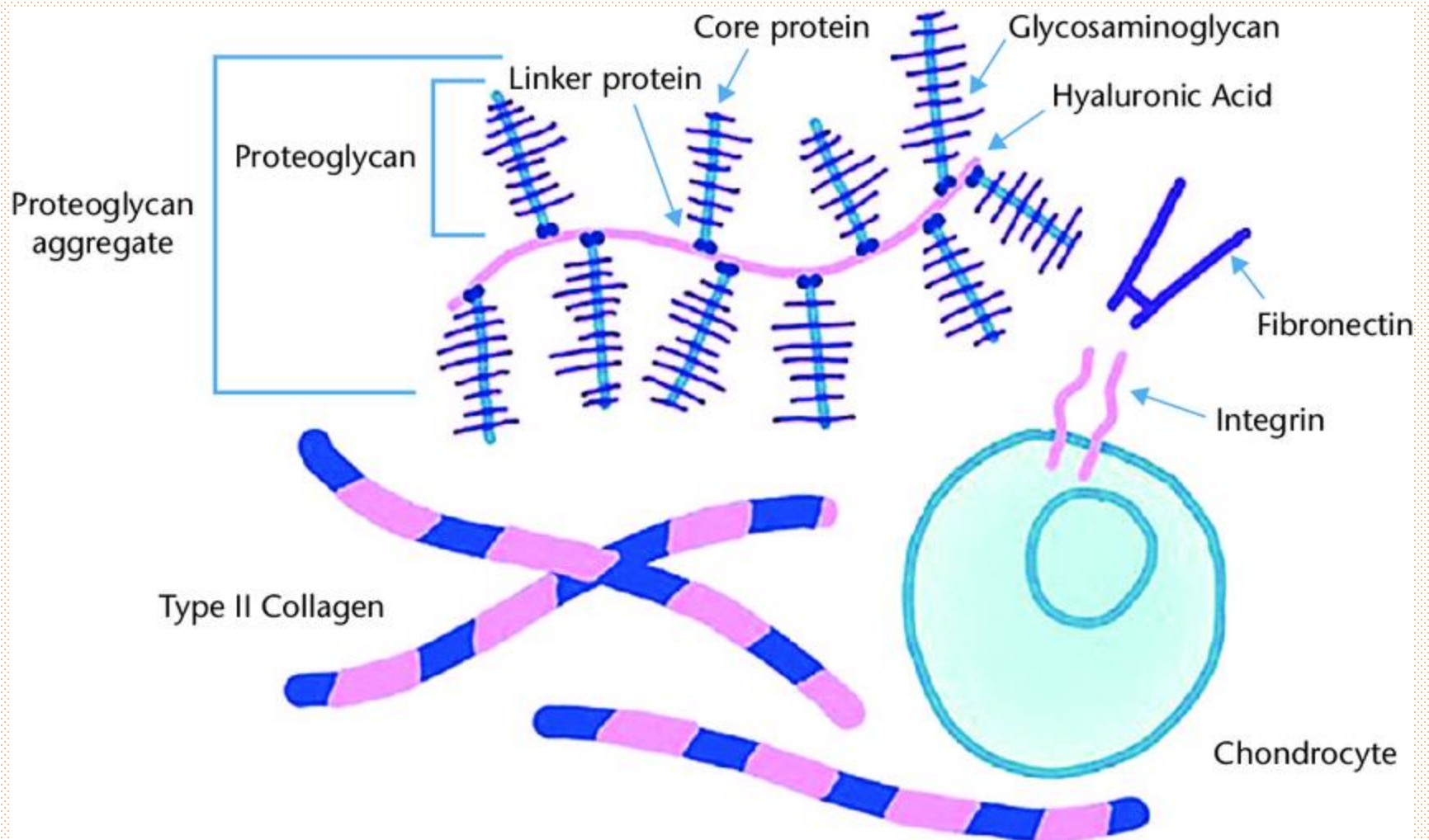
The diagram is a hierarchical flowchart. At the top is a box labeled 'Extracellular matrix'. Two arrows point down from this box to two separate boxes: 'Ground substance' on the left and 'Protein fibers' on the right. From the 'Ground substance' box, an arrow points down to a light blue box containing a list of three items: 'Glycosaminoglycans', 'Proteoglycans', and 'Adhesive glycoproteins'. From the 'Protein fibers' box, an arrow points down to a light blue box containing a list of three items: 'Collagen fibers', 'Elastic fibers', and 'Reticular fibers'.

## Ground substance

**Glycosaminoglycans**  
**Proteoglycans**  
**Adhesive glycoproteins**

## Protein fibers

**Collagen fibers**  
**Elastic fibers**  
**Reticular fibers**





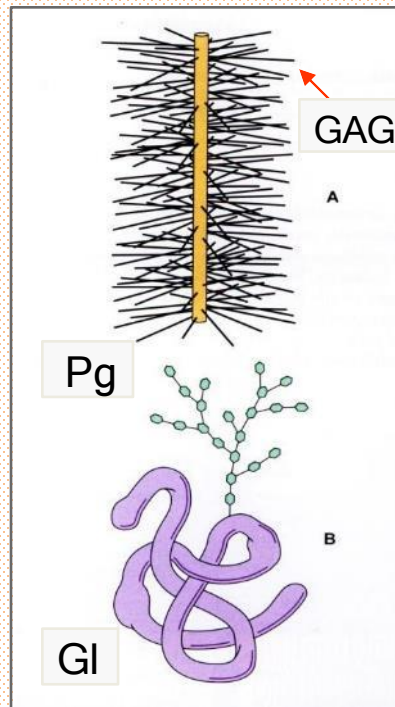
# Ground substance

## Proteoglycans

## Glycosaminoglycans

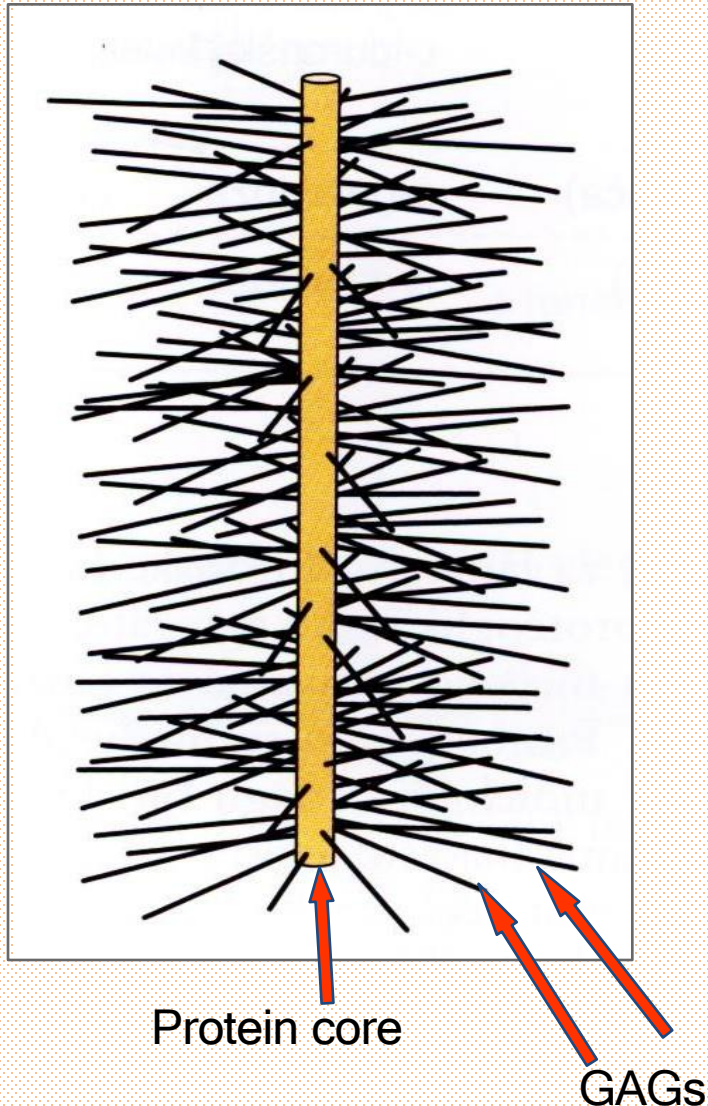
## Adhesive glycoproteins

Dermatan sulfate,  
Heparan-sulfate  
Chondroitin sulfates,  
Keratan sulfate,  
Heparan sulfate,  
Heparin  
Hyaluronic acid



Fibronectin Laminin  
Entactin  
Thrombospondin  
Chondronectin  
Osteonectin

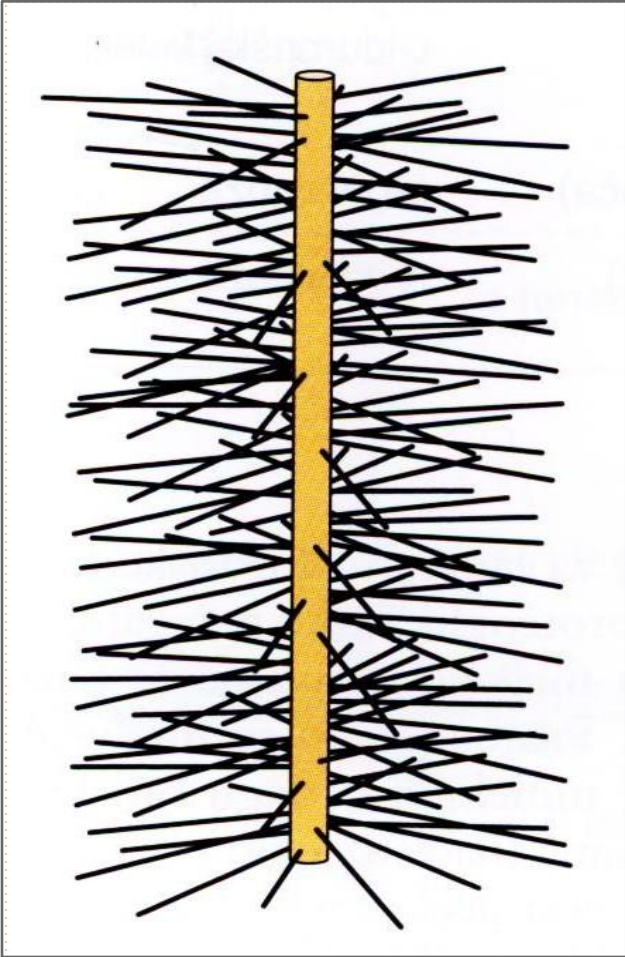
# Glycosaminoglycans



- **GAGs** are often labeled as mucopolysaccharides or mucins.
- GAGs are long polymers of repeating disaccharide units, usually a hexosamine and uronic acid
- GAGs act as **attracting anions/cations and bind tissue fluid.**
- All GAGs are sulfated, **except for LONGEST hyaluronic acid.**
- 80-90% of the molecular weight of glycoproteins falls on GAGs.



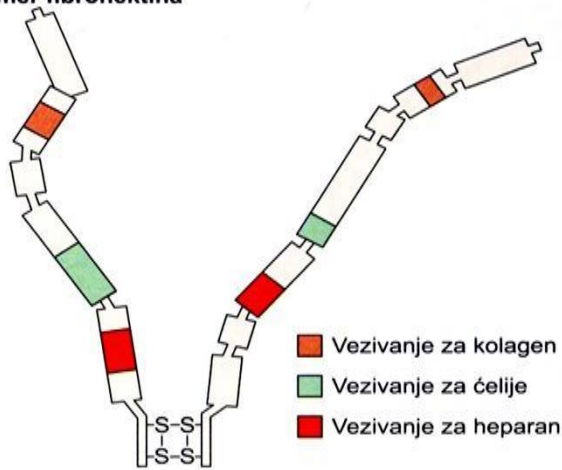
# Proteoglycans



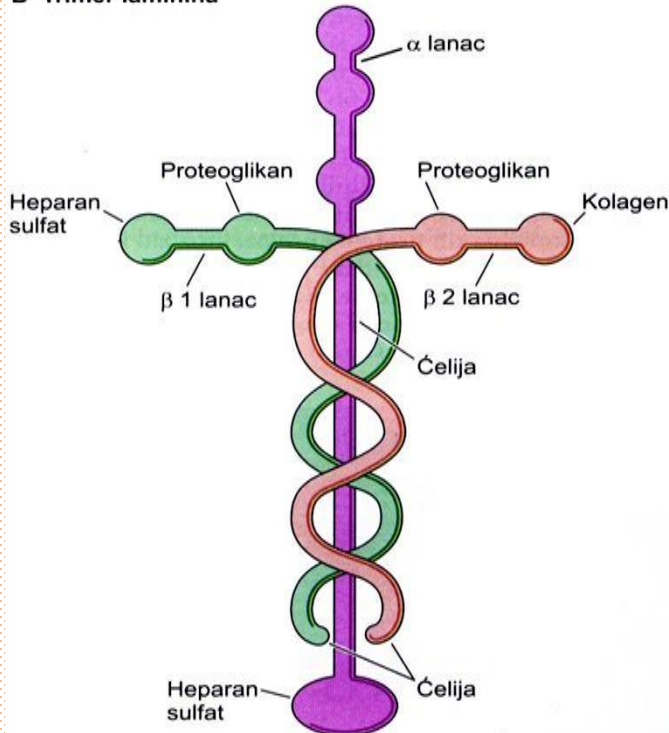
- **Proteoglycans** are highly sulfated (acidic) glycosaminoglycans with a centrally placed protein core.
- They are synthesized by fibroblasts, mast cells, and in certain conditions, smooth muscle cells with a synthetic phenotype.
- The degree of polymerization of these large molecules determines the degree of viscosity of the base substance.
- Most abundant are **aggrecan and perlecan**
- **RETAINS TISSUE FLUID (WATER)**

# Adhesive glycoproteins

A Dimer fibronektina



B Trimer laminina



- (Multi)-Adhesive glycoproteins are involved in **cell-ECM** interactions.
- Polysaccharide chains in proteoglycans are branched.
- Adhesive glycoproteins **have binding sites for transmembrane proteins** of cell membranes, **then for collagen fibers** of the extracellular matrix and finally, **for glycosaminoglycan** ground substances.
- The most important proteoglycans: **fibronectin, laminin, entactin, thrombospondin, chondronectin and osteonectin.**

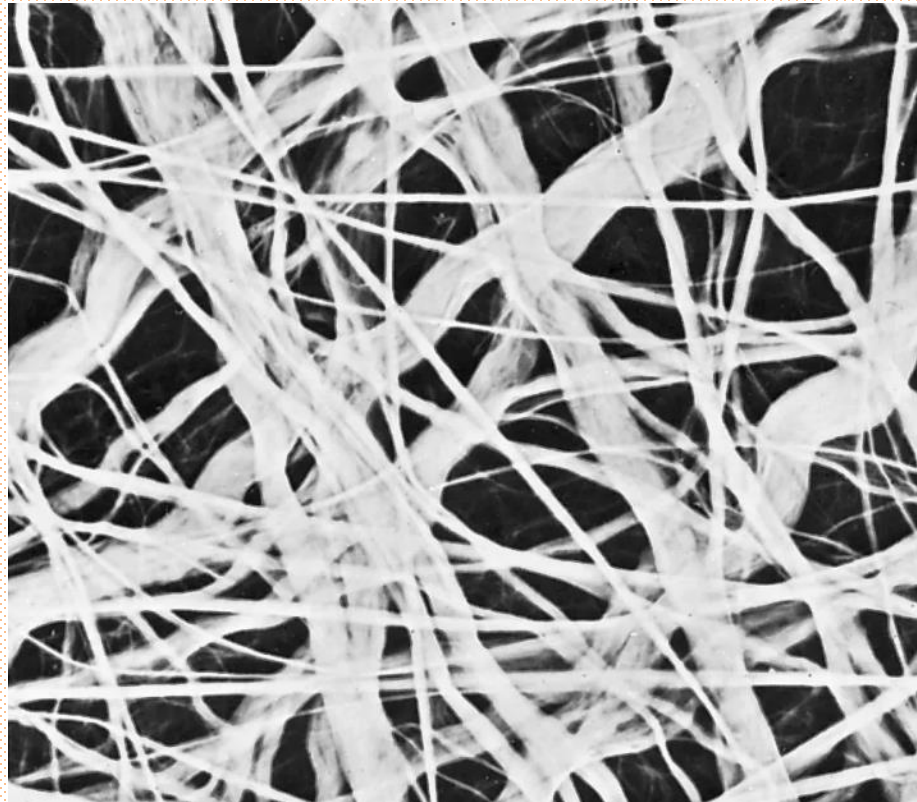


# CT Fibers

**COLLAGEN** (rope)

**RETICULAR** (net)

**ELASTIC** (bangy jump)



# Collagen fibers

- Collagen fibers are the most common connective tissue fibers that represent **elements of strength** in the tissue, providing resistance to mechanical pressure and stretching forces. Representing **30% of human dry weight**.
- They are made of **collagen protein**, which in its composition has three polypeptide  $\alpha$  chains spirally twisted around each other, forming a triple helix.
- Collagen is synthesized by fibroblasts, but also by other fixed cells of connective tissue, as well as reticular cells and smooth muscle cells with a synthetic phenotype.
- There are **over 25 types of collagen** that differ by small variations in the composition of the  $\alpha$  chain. The first seven types are best studied.

## **Type I Collagen**

accounts for more than 90% of the total collagen content in the body

bone

ligament

tendon

meniscus

annulus of intervertebral disks

skin

healed cartilage

scar tissue

nerves

## **Type II Collagen**

articular (hyaline) cartilage

nucleus pulposus of intervertebral disks

type II collagen has a very long half life

## **Type III Collagen**

found in skin and blood vessels

## **Collagen types**

Fibrillar (I and II)

Sheet-forming IV

Anchoring VII



### **Type IV Collagen**

found in basement membranes

### **Type V, VI, IX Collagen**

occur in small amounts in articular cartilage

### **Type VII and VIII Collagen**

basement membrane (epithelial)

### **Type X Collagen**

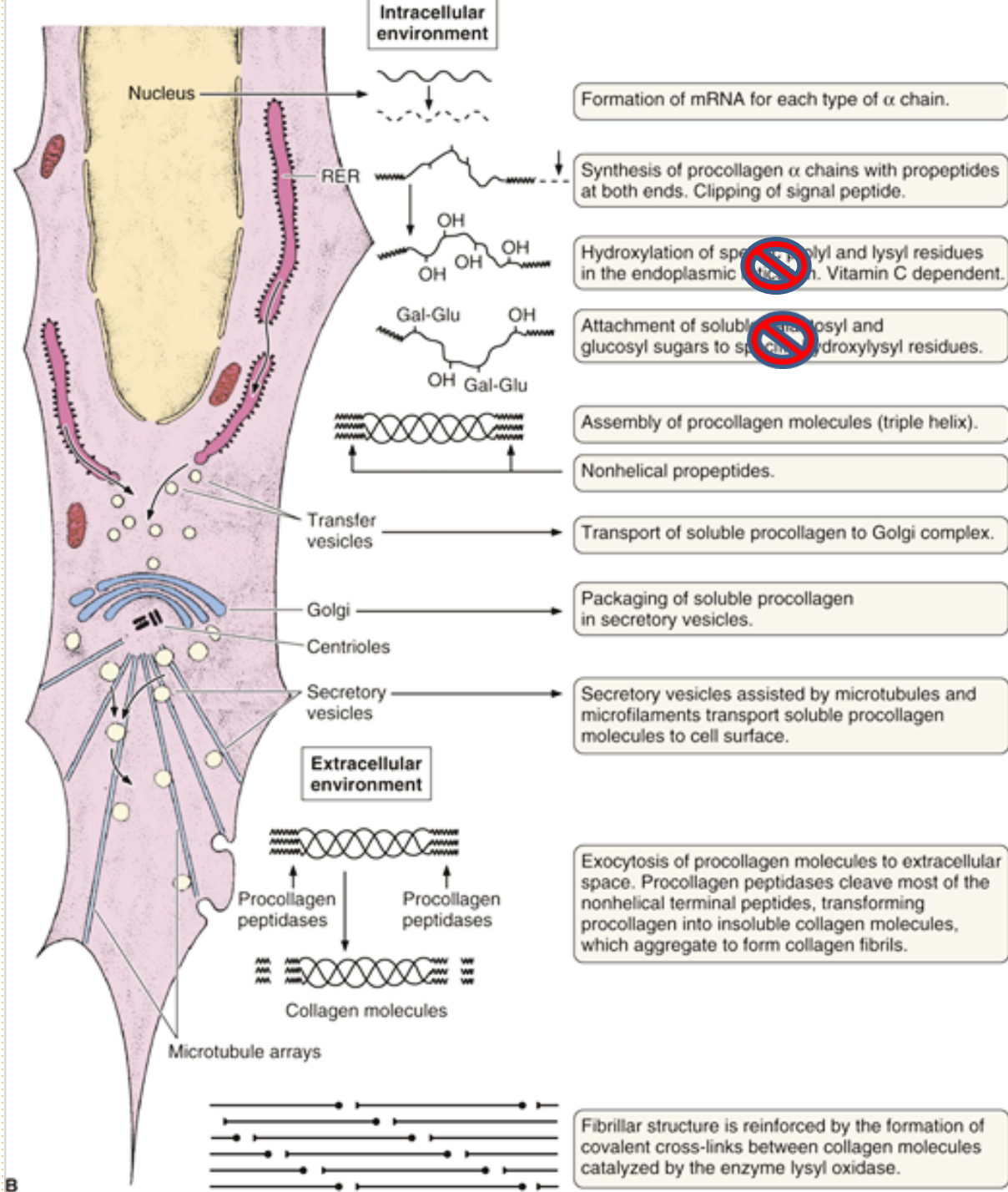
is found in the deep calcified layer of cartilage produced only by hypertrophic chondrocytes during enchondral ossification (growth plate, fracture callus, heterotopic ossification)

### **Type XI Collagen**

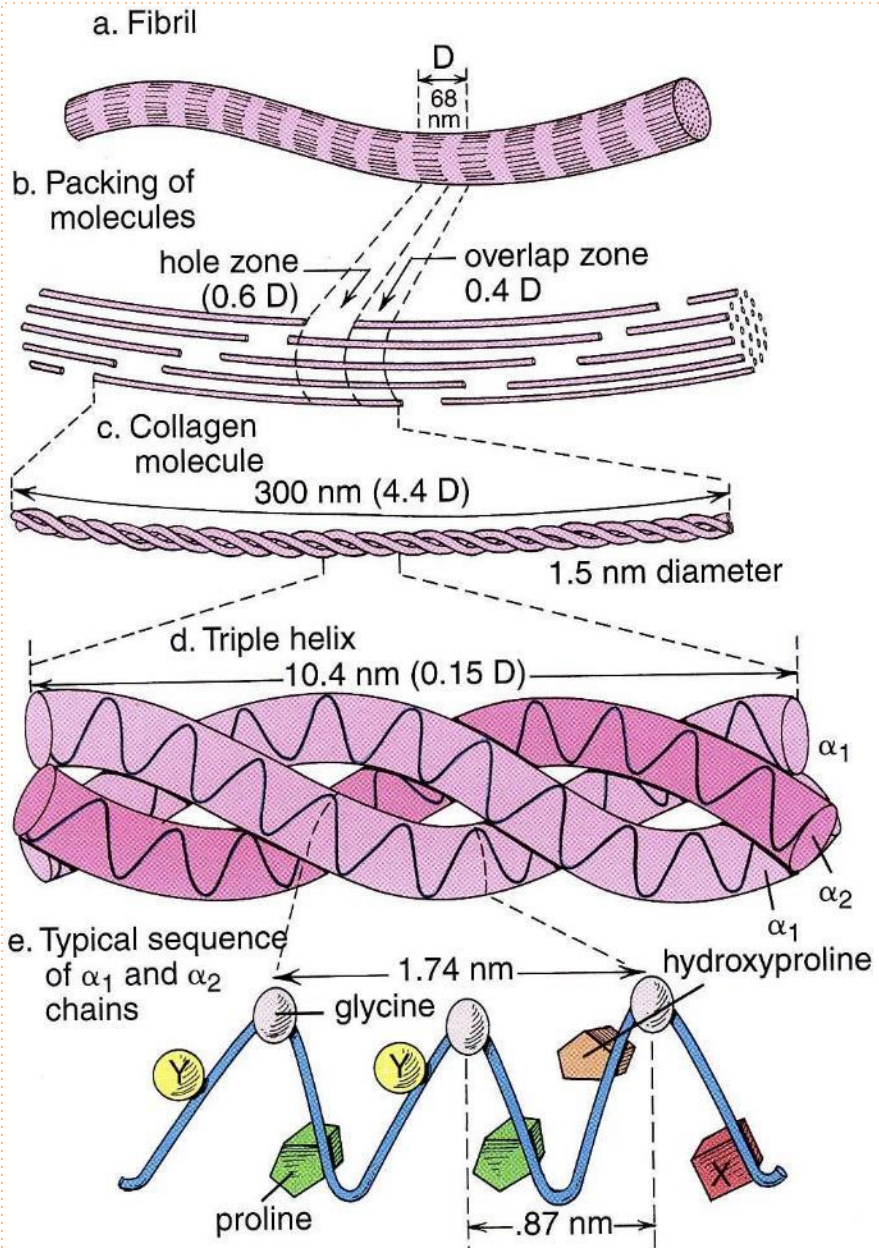
an adhesive with the function of holding the collagen lattice together in cartilage

## **Collagen turnover**

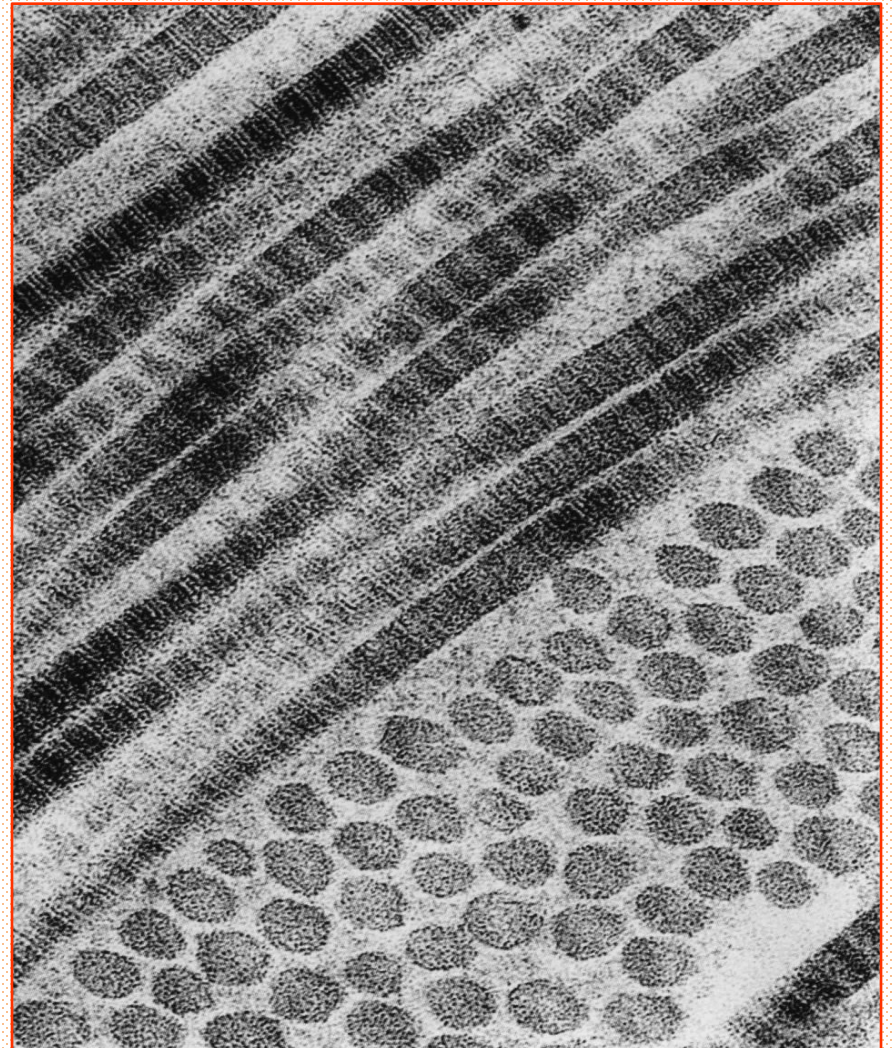
Specific enzymes called **collagenases** clip collagen fibrils or sheets in such a way that they are then susceptible to further degradation by nonspecific proteases





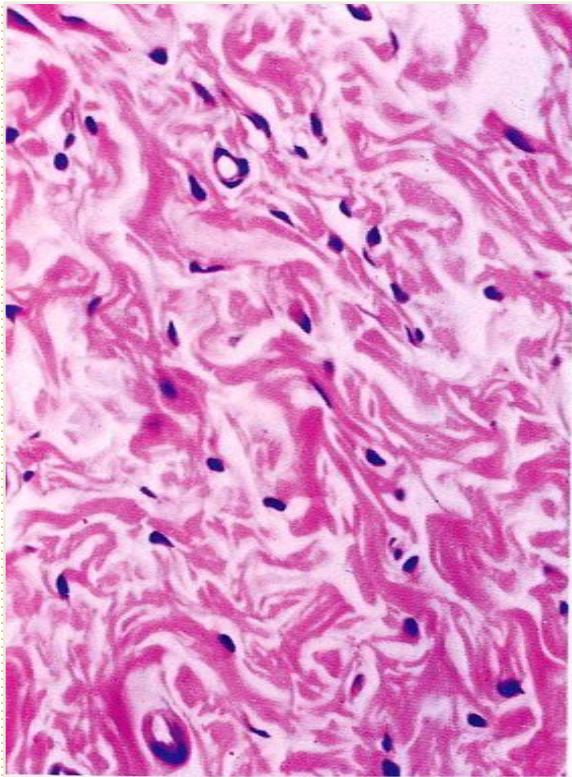


- 67 nm striation

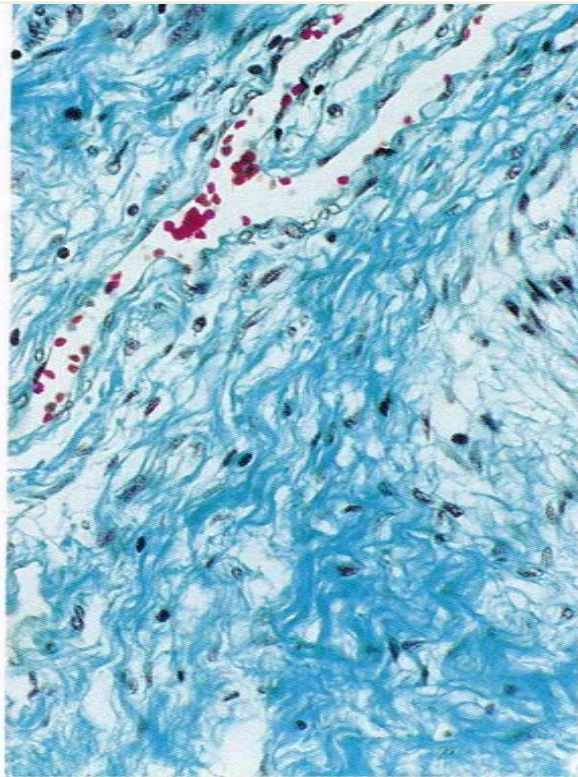




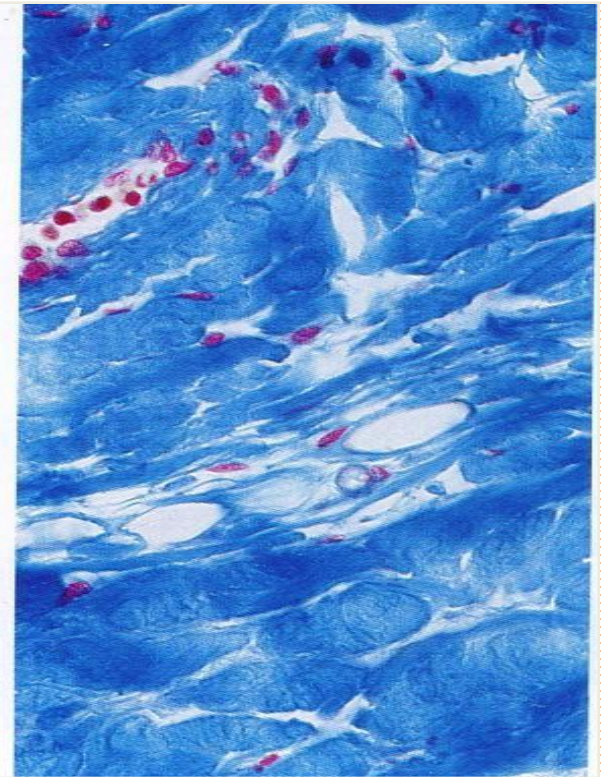
# Special staining



H/E



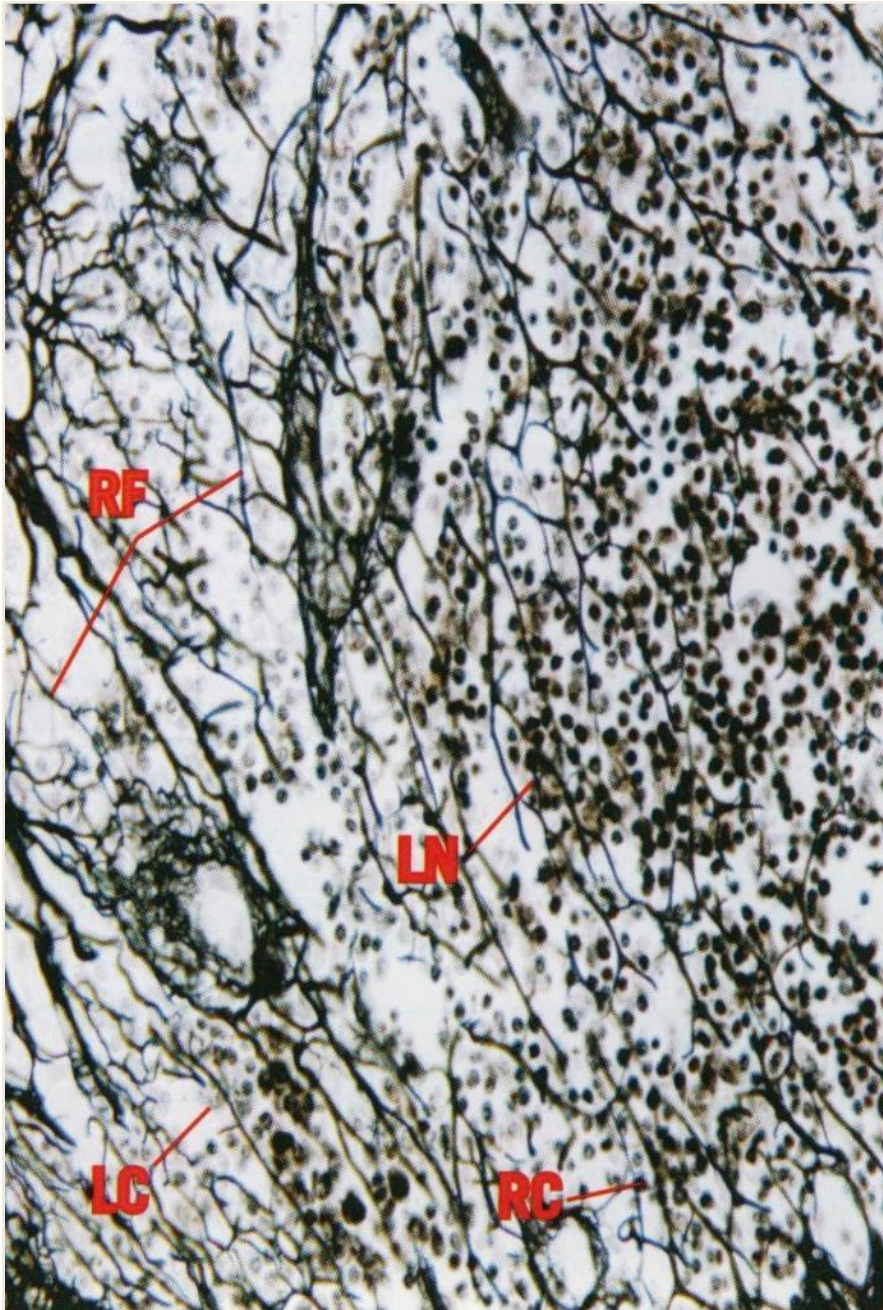
Masson-trichrome



Azan

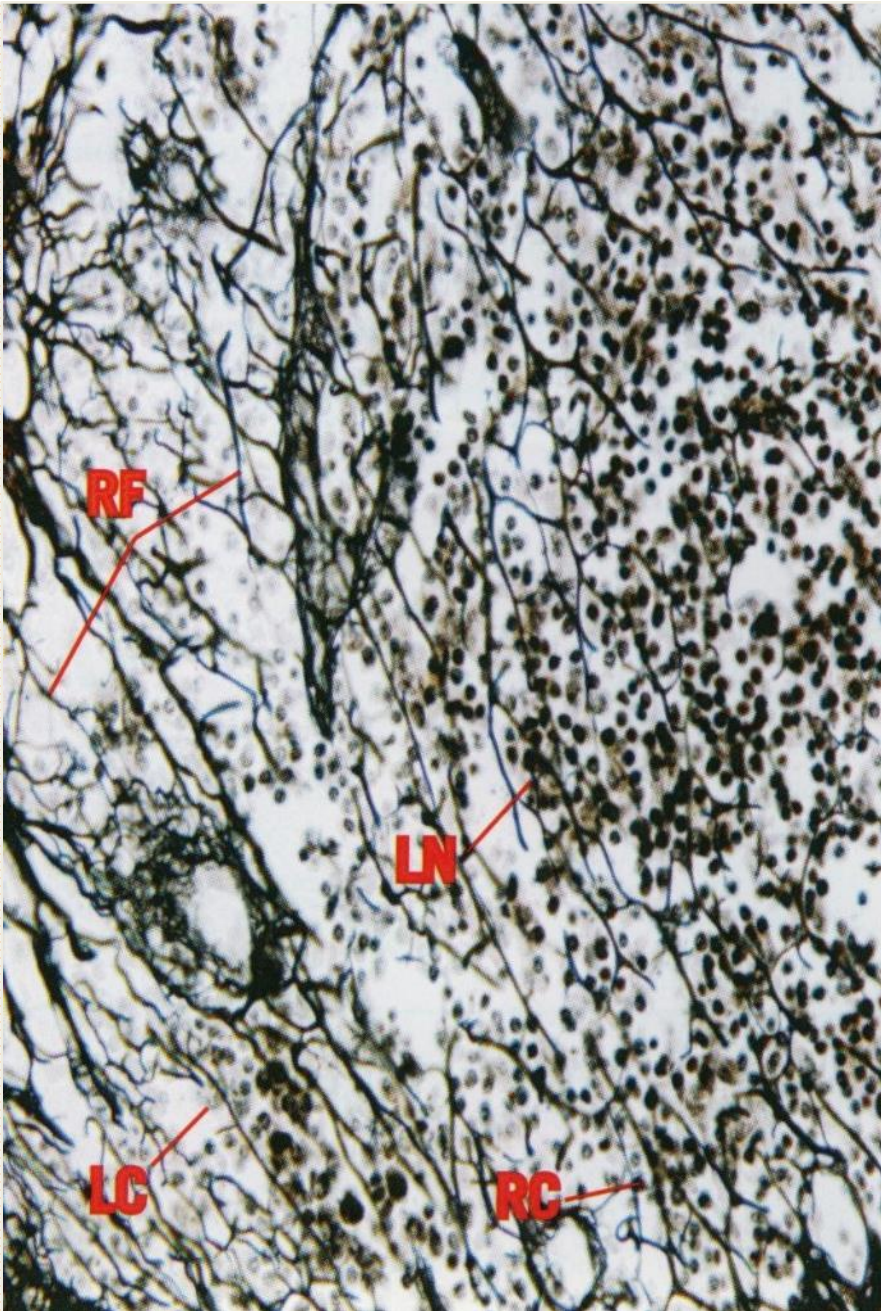


# Reticular fibers



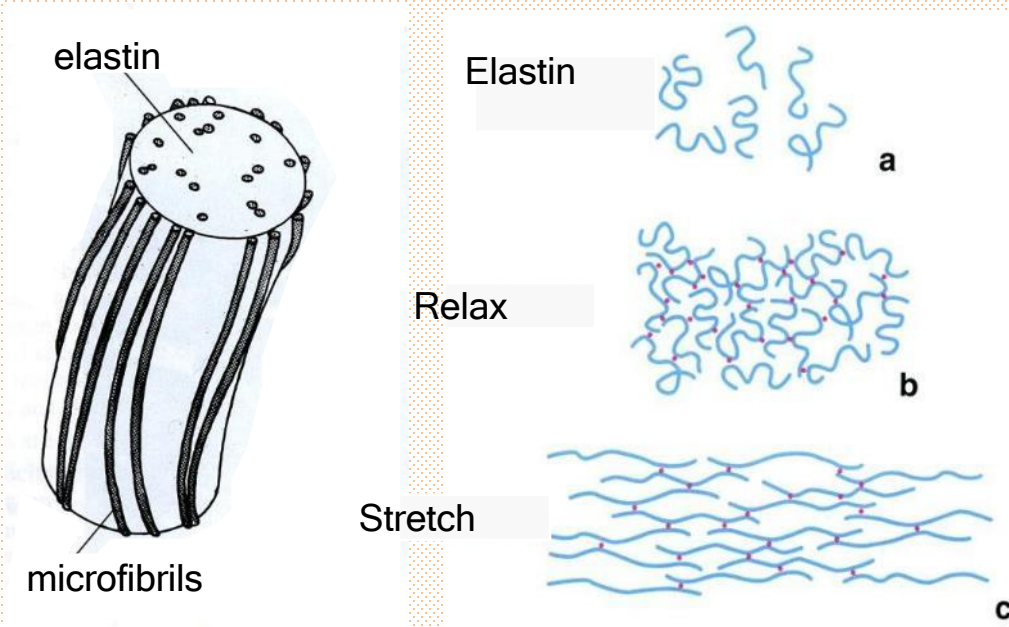
- Fiber diameter 0.5-2  $\mu\text{m}$ .
- Made of **collagen type III**.
- Located in tissues associated with stronger type I collagen fibers.
- They form a supportive three-dimensional **network**, which supports the cells.
- They are shown by PAS and Gomori staining (argyrophilic fibers).





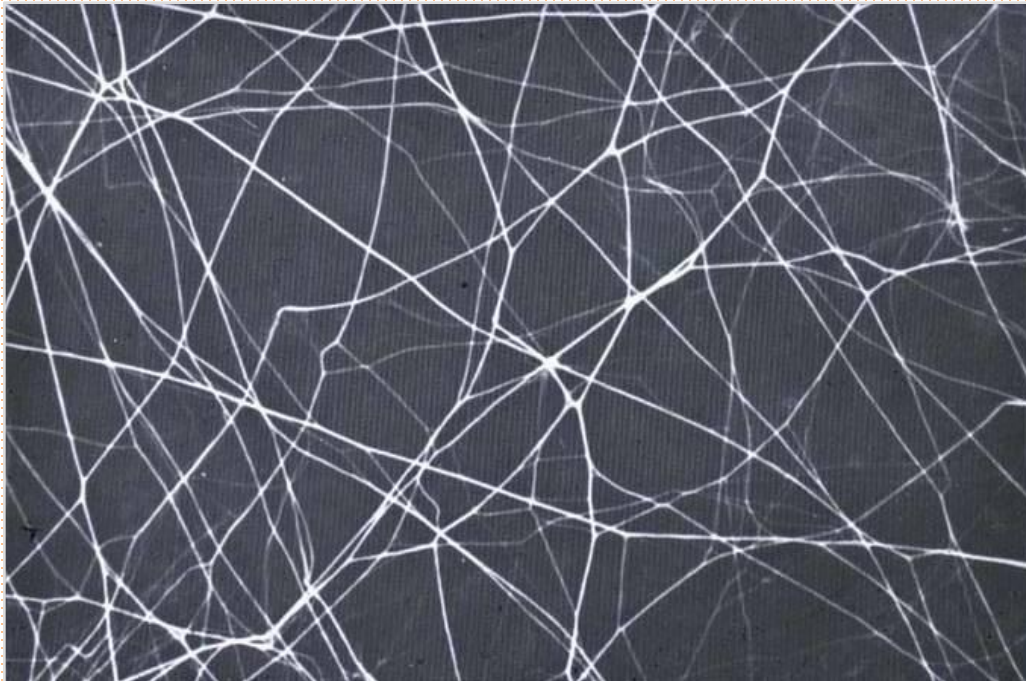
- They are present in lymphatic organs, around glandular acini, fat and muscle cells.
- Type III collagen is often called "fetal" collagen, because fetal tissue contains more collagen III compared to adult tissue in the same place.

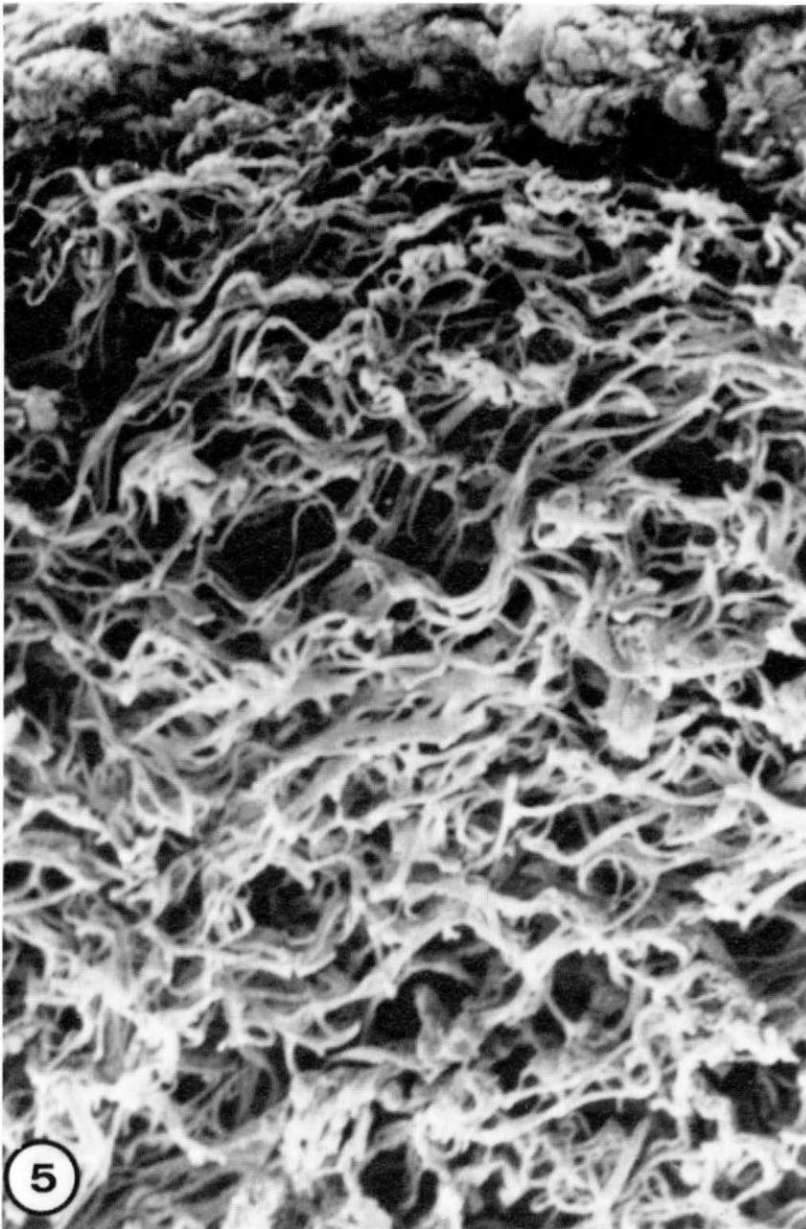




# Elastic fibers

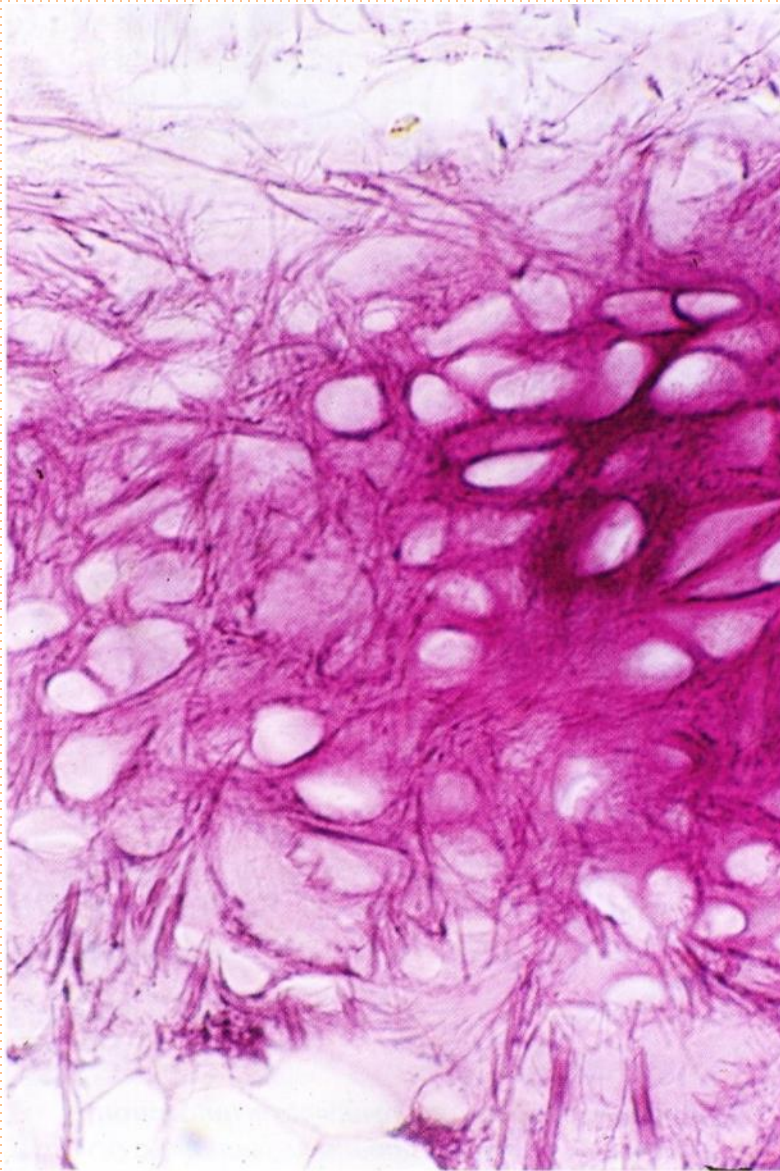
- **Elastic fibers** represent the elements connective tissue that ensure the elasticity of tissues and organs.
- These are thin and branched fibers.
- Composition: **protein elastin + fibrillin (microfibrils)**
- During the synthesis, microfibrils are initially formed, and then within them the bundle is embedded with amorphous elastin.



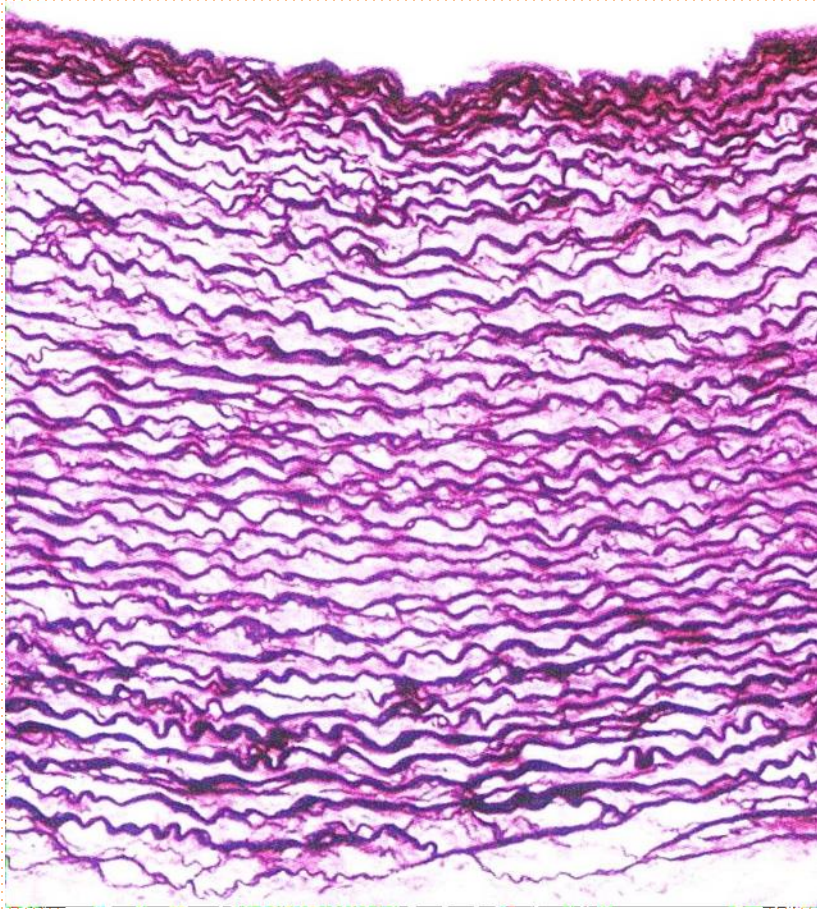


- Immature elastic fibers contain a larger amount of microfibrils, while with age, the amount of elastin increases, which results in the loss of tissue quality and elasticity.
- Elastin contains specific amino acids **desmosine** and **isodesmosine**.
- Fibers stretch up to 150%.
- **Oxytalan and elaunin** fibers are immature forms of elastic fibers.
- Elastic fibers are synthesized by fibroblasts and smooth muscle cells of the synthetic phenotype.

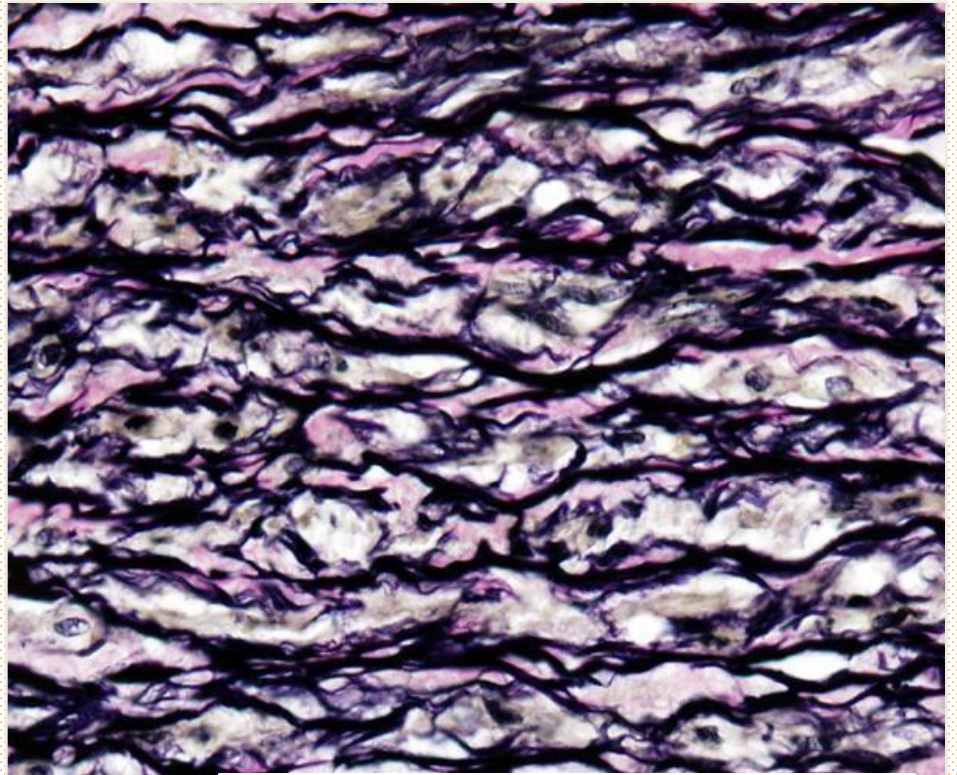








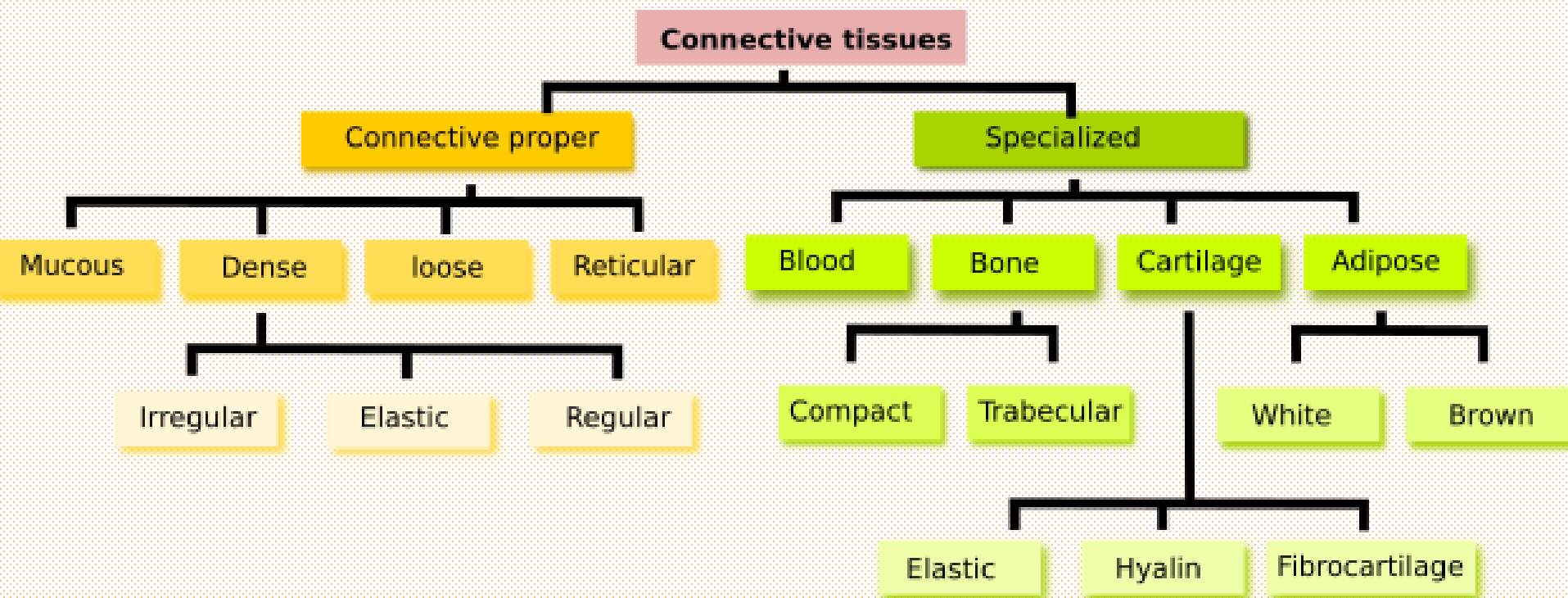
**Weigert stain**



**Verhoeff - van Gieson stain**

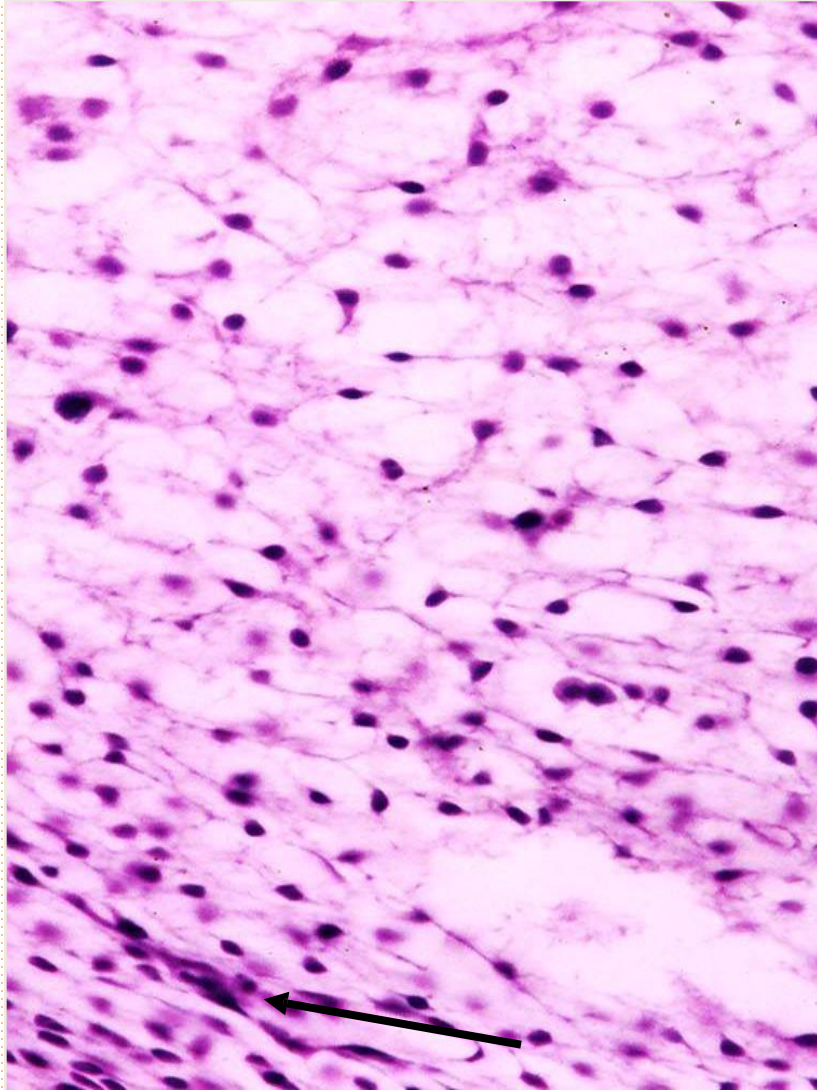


# **TYPES OF CONNECTIVE TISSUE (CT)**





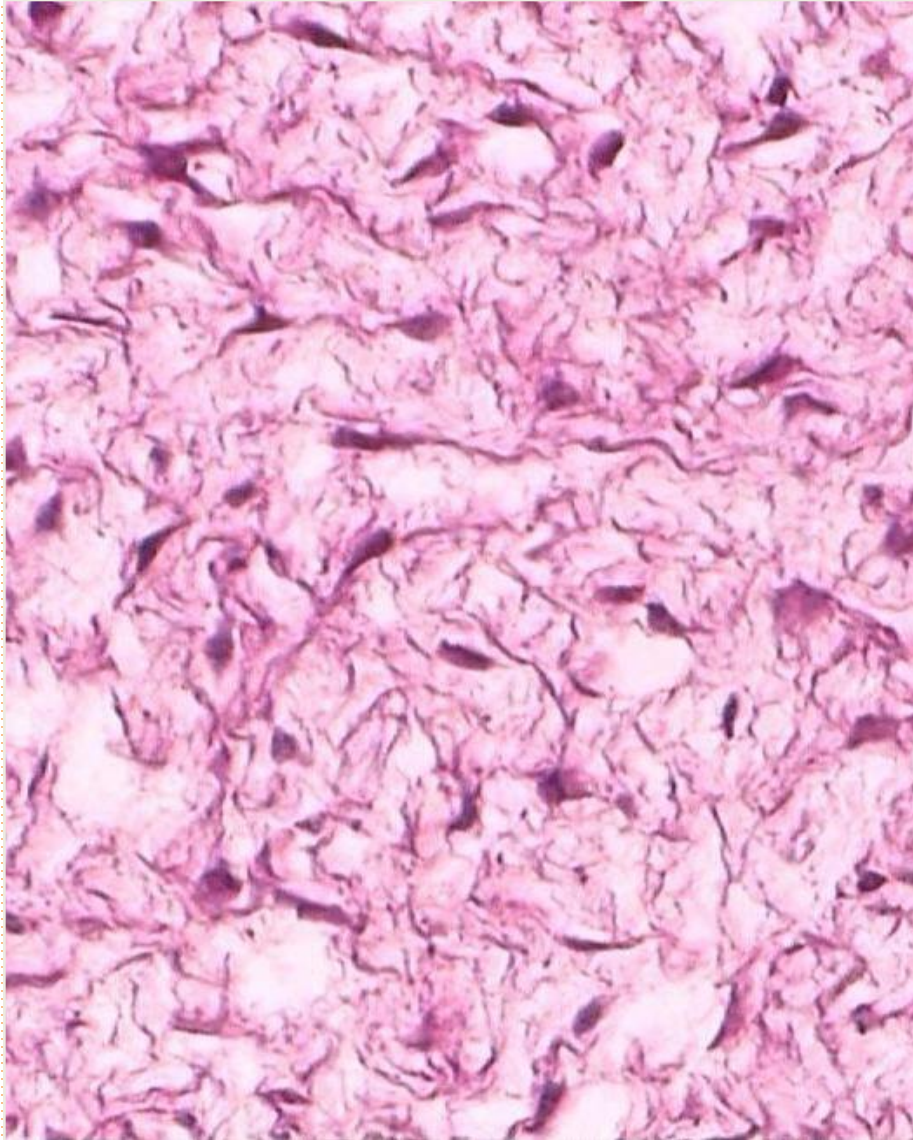
# Mesenchyme



## Reminder!!

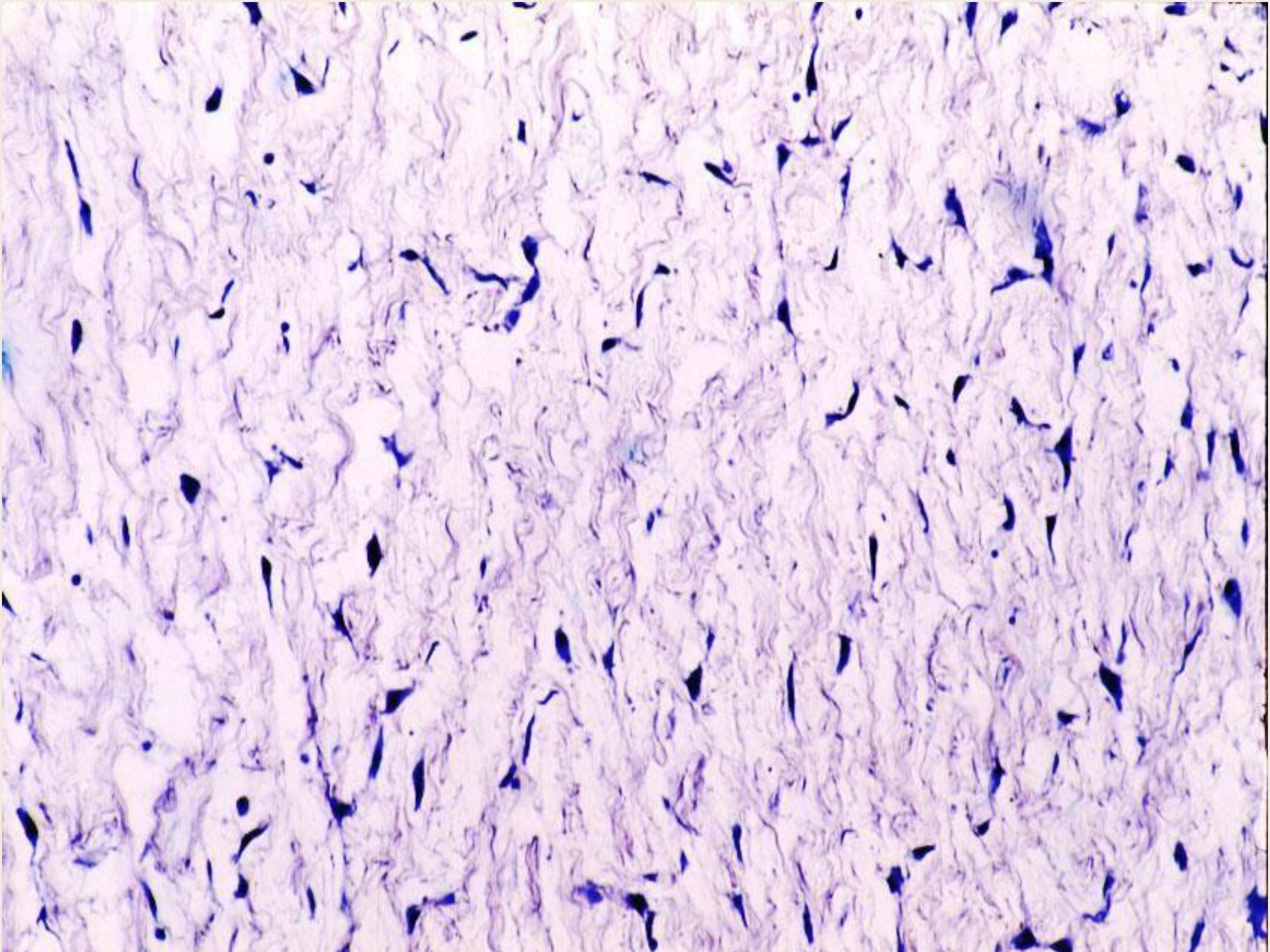
- **Mesenchyme** represents the original, embryonic connective tissue that it mostly originates from the mesoderm.
- Present only in the embryonic period.
- Mesenchymal cells are characterized by high proliferative potential and frequent mitoses.

# Mucous (muroid) CT



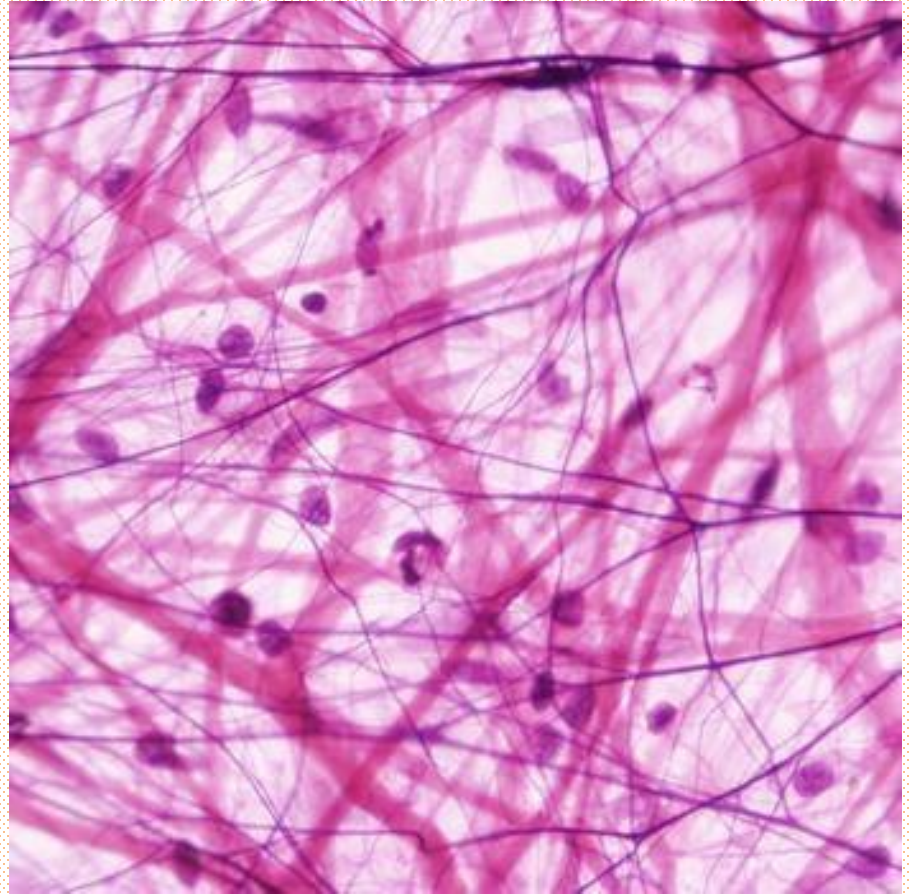
- Main component of the fetal umbilical cord, where it is referred to as [Wharton's jelly](#).
- Abundant ground substance composed of hyaluronan, mucous CT is gelatinous, with sparse collagen fibers and scattered fibroblasts
- Many mesenchymal stem cells, which are being studied for their [potential in regenerative medicine](#)





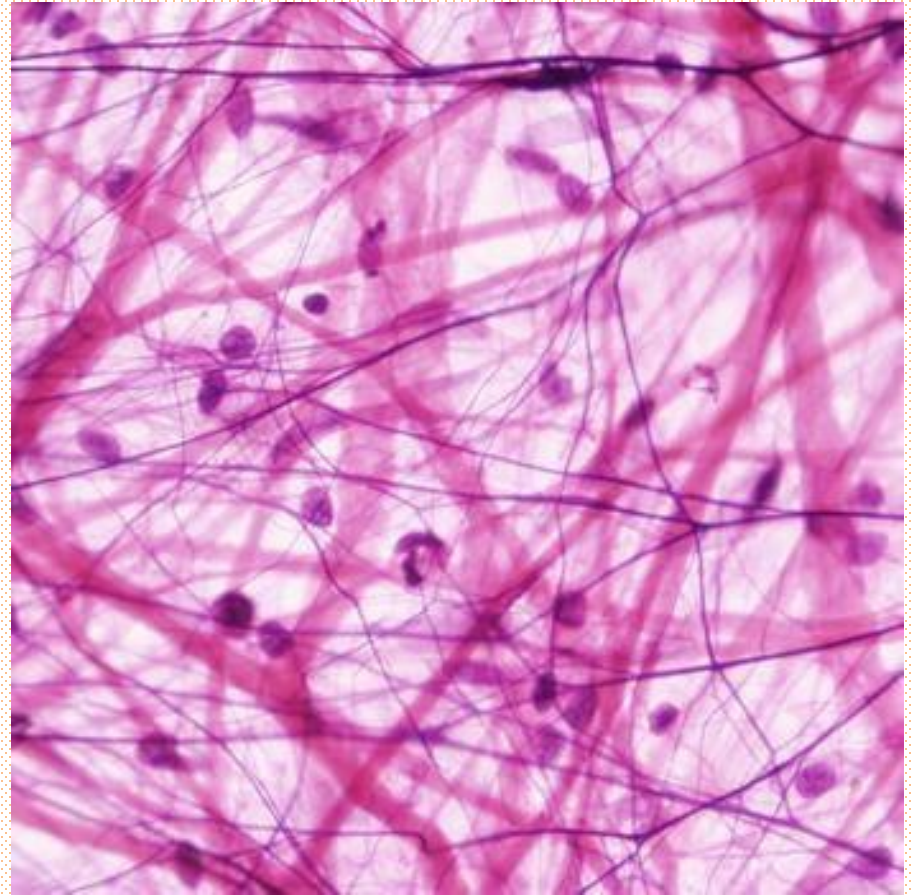
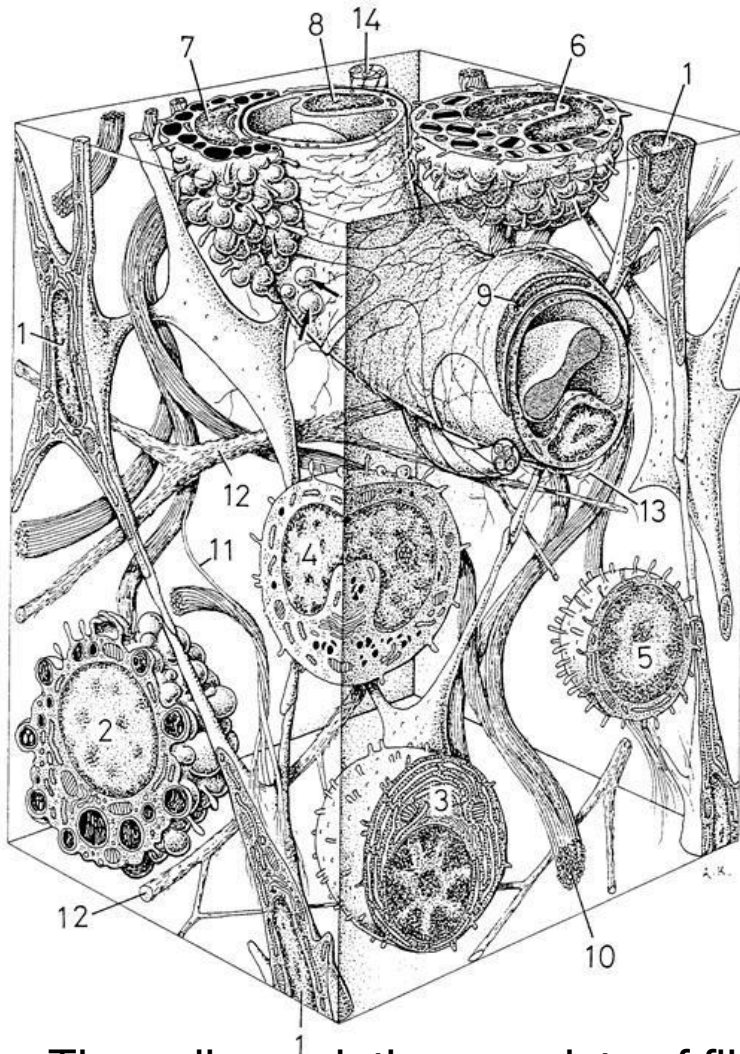


# Loose (areolar) CT



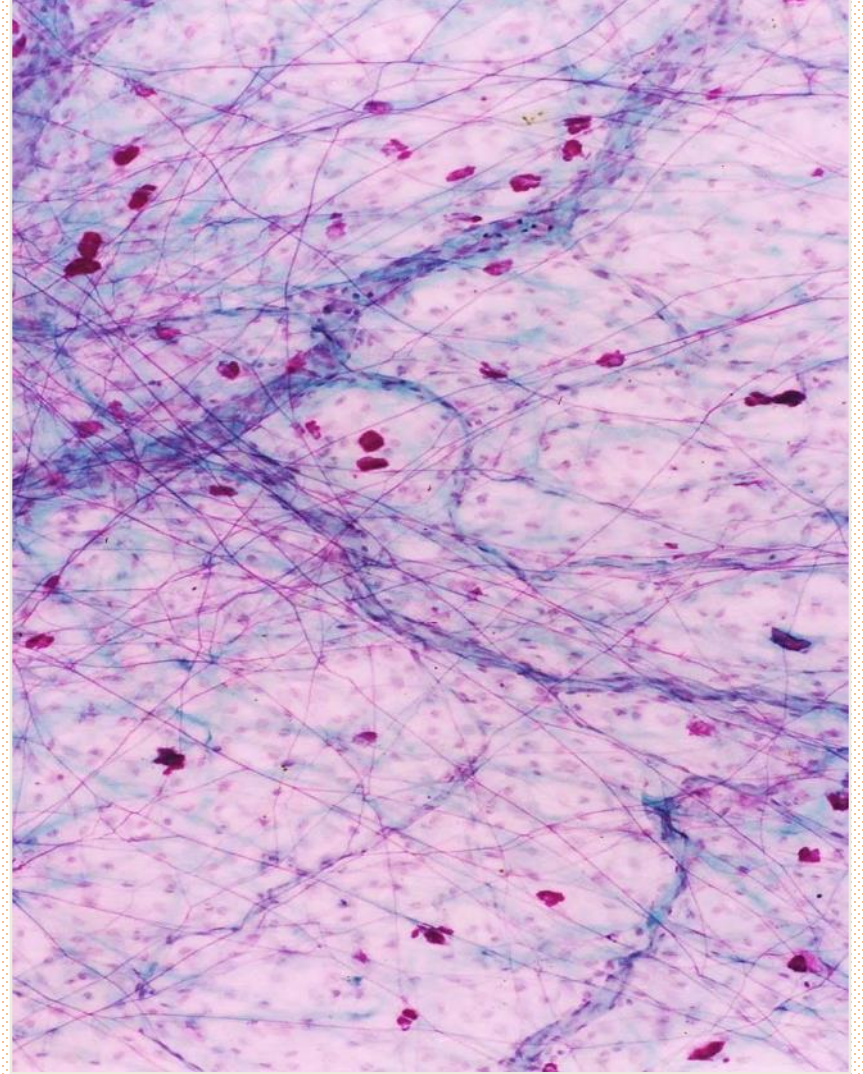
- It is found in the dermis, in the mucous membranes under the epithelium, blood and lymphatic vessels, etc.
- It contains a multitude of cell types, an extensive intercellular substance, and all three type of connective fibers in a loose arrangement.





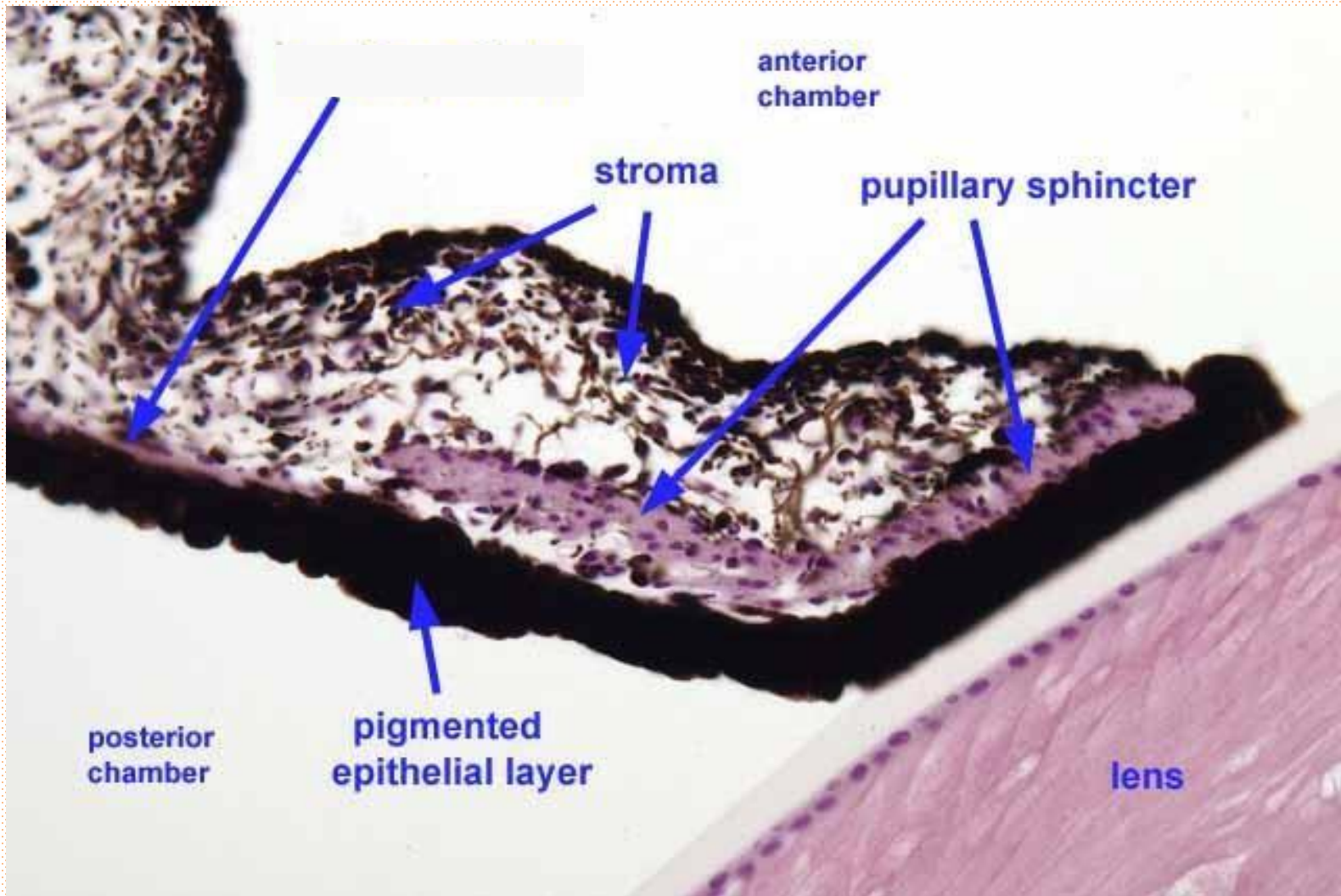
- The cell population consists of fibroblasts, but also a large number of wandering cells whose number and composition change during inflammatory and allergic reactions.
- The tissue is flexible and less resistant to mechanical stress.







# Pigmented CT

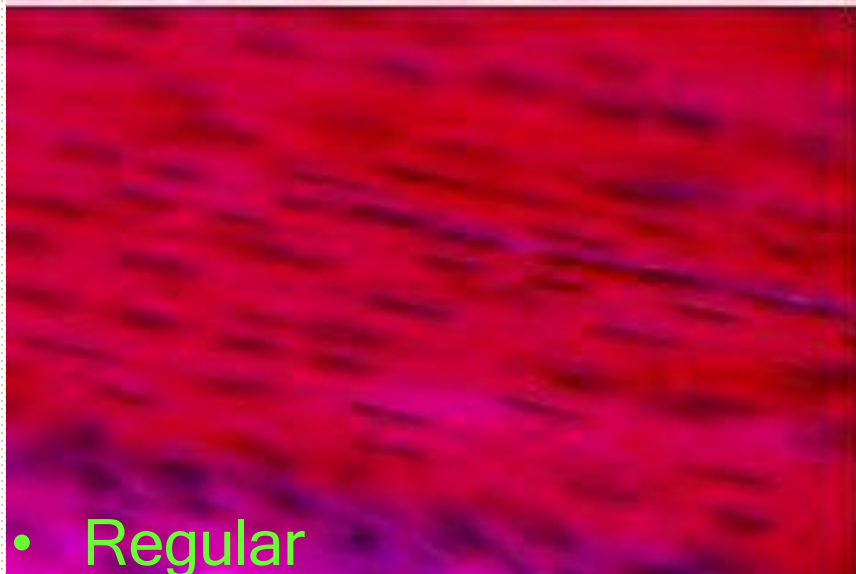


- Variant of loose CT with a lot of **pigment cells**.
- It is located in the **lens, ciliary body and part of the choroid**.

# Dense connective tissue



• Irregular



• Regular

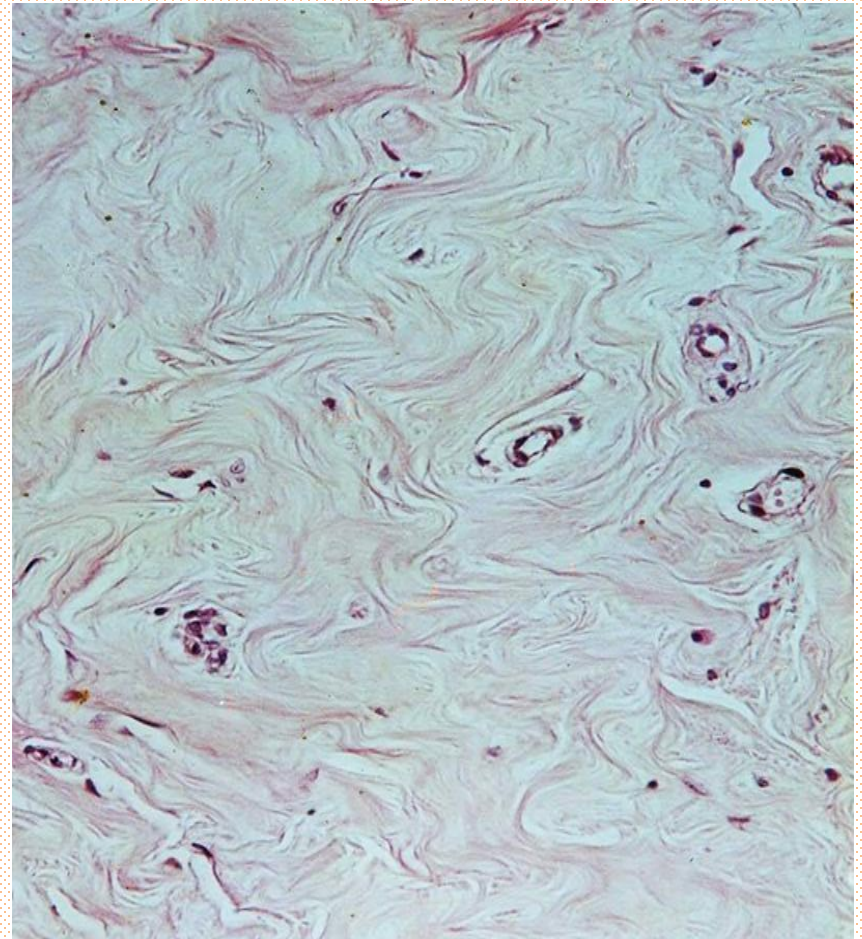
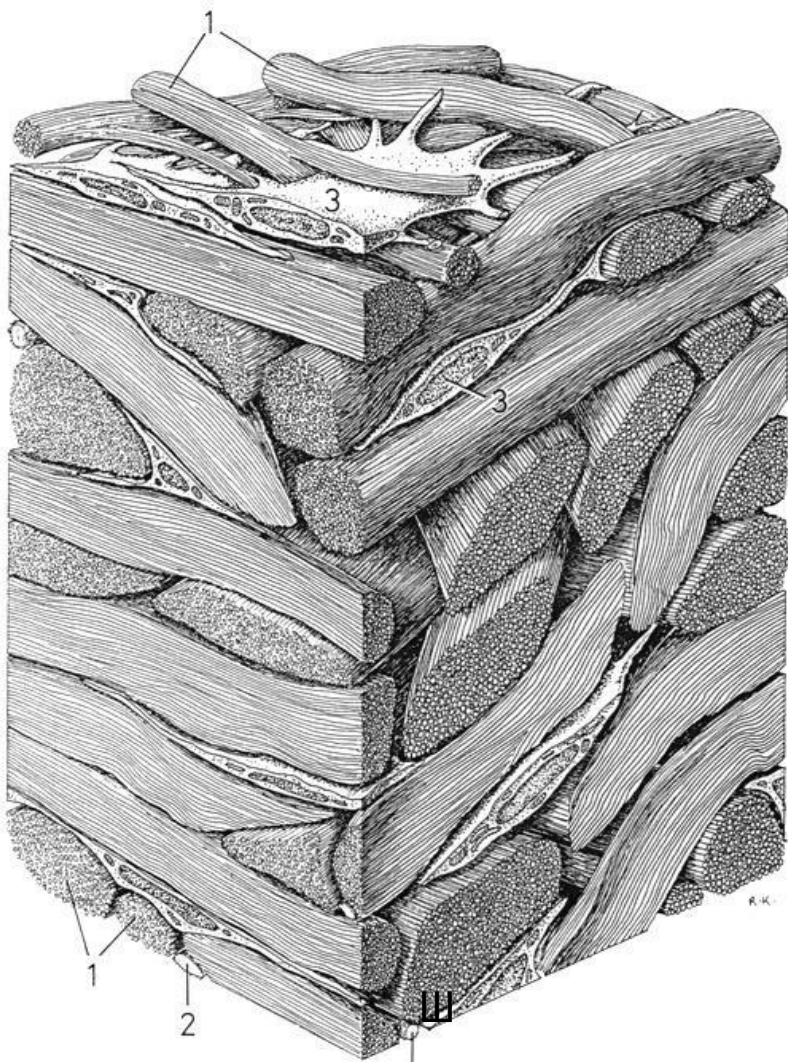
- Dense connective tissue has similar components as loose connective tissue, but with fewer cells, mostly fibroblasts, and a clear predominance of bundled type I collagen fibers over ground substance.

- irregular and regular

- **Irregular** contains bundles of collagen fibers directed in **different directions** with little intercellular substance and rare fibroblasts and macrophages in between.
- **Regular** dense connective tissue contains properly oriented collagen and elastic fibers. Depending on which fibers dominate, the tissue is divided into regular **collagenous and elastic connective tissue**.



# Irregular



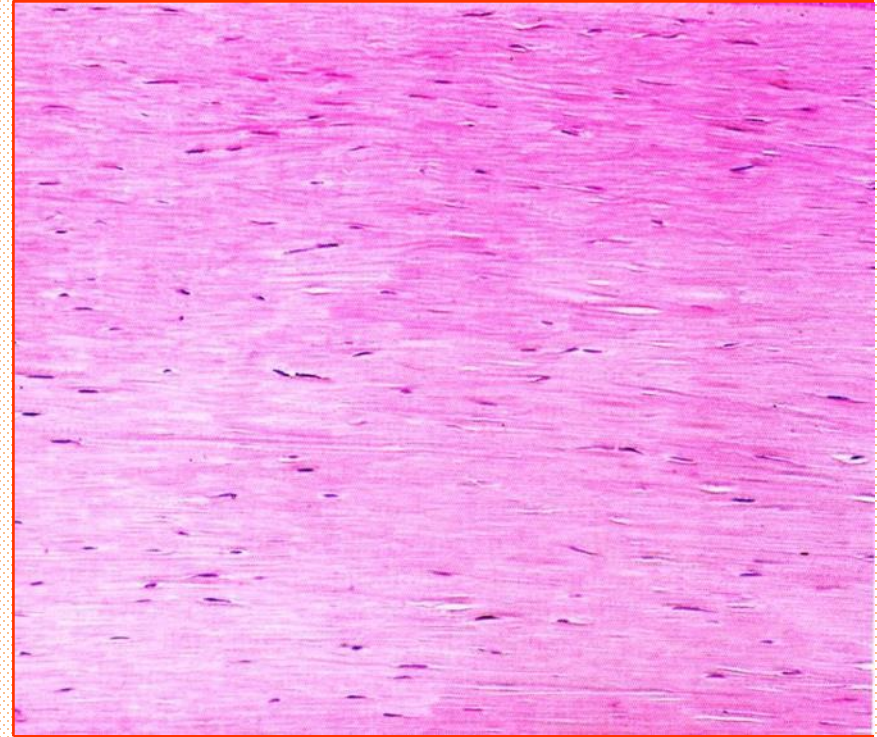
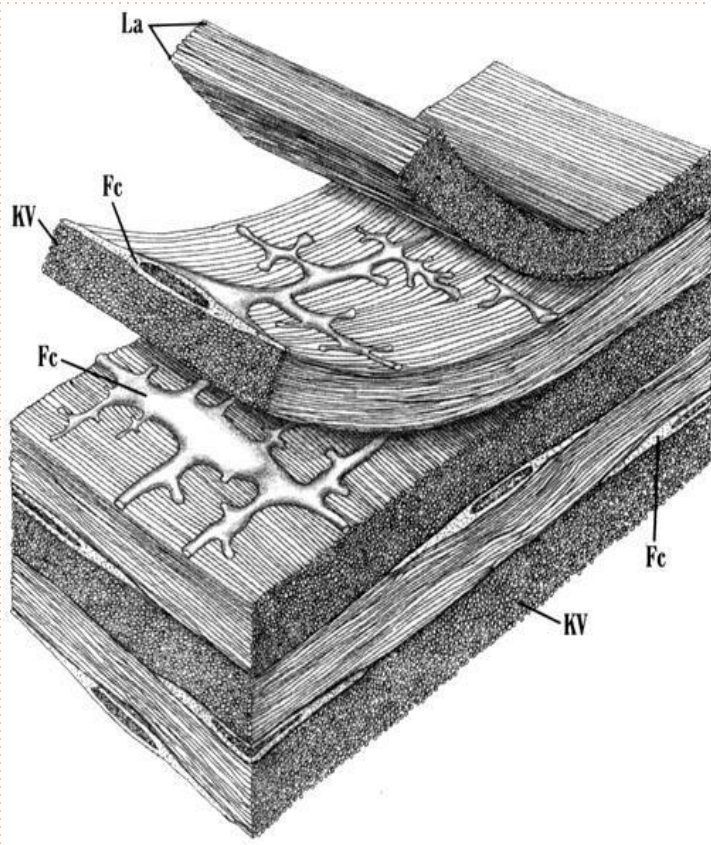
- It is found in the submucosa of the stomach and intestines, in the depth of the dermis, around larger nerves, in the capsule and septa of numerous organs, etc.

# Regular

- Regular collagen dense connective tissue can be composed of:
- bundles of collagen fibers that have a parallel arrangement, such as is the case with **tendons and ligaments**,
- bundles or densely packed collagen fibers, where the fibers in one layer are oriented parallel, but the layers are stacked at different angles, which is the case with **fascia, aponeurosis, stroma of the cornea, periosteum, perichondrium, centrum tendineum of the diaphragm, as well as the dura mater and skeleton of heart valves**.
- Between the collagen fibrils there is very little basic substance and rare fibrocytes that are specifically called tendons **tendinocytes**.

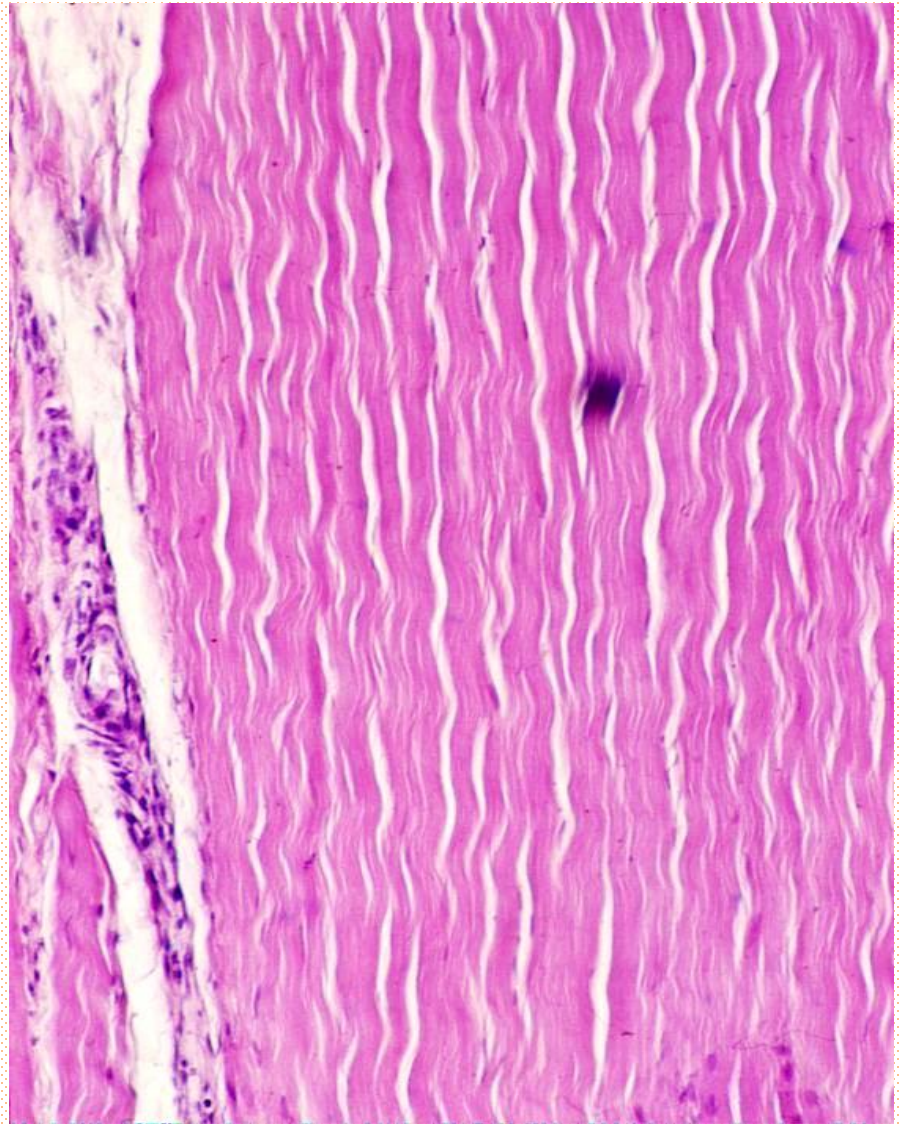
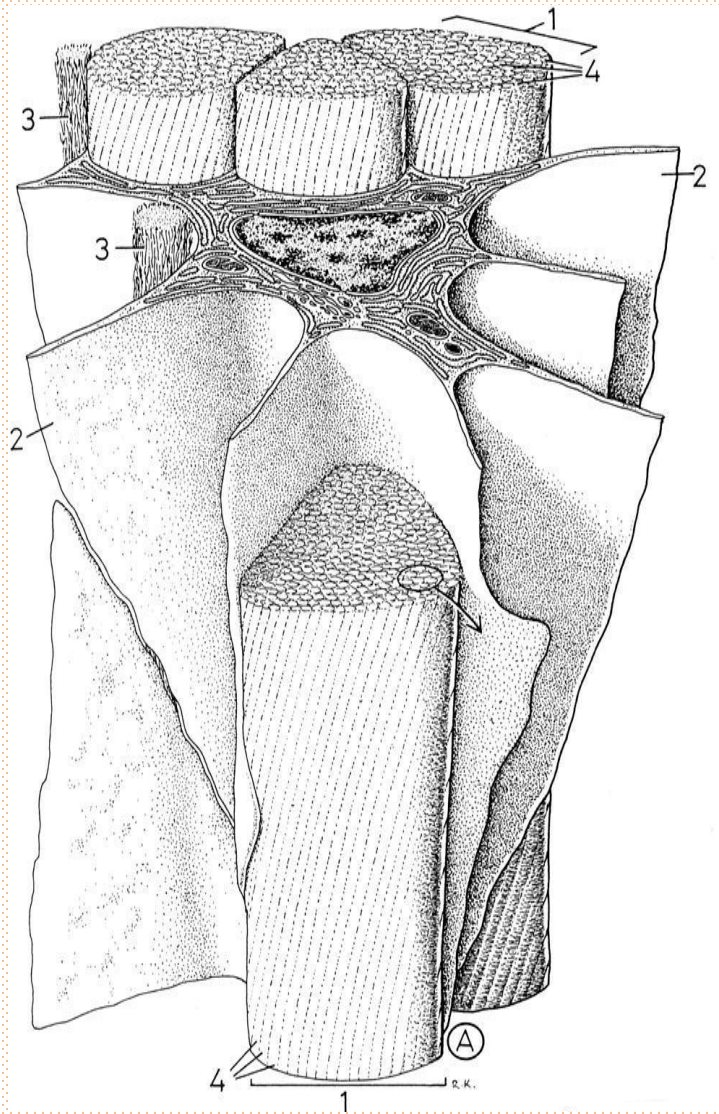


# Regular



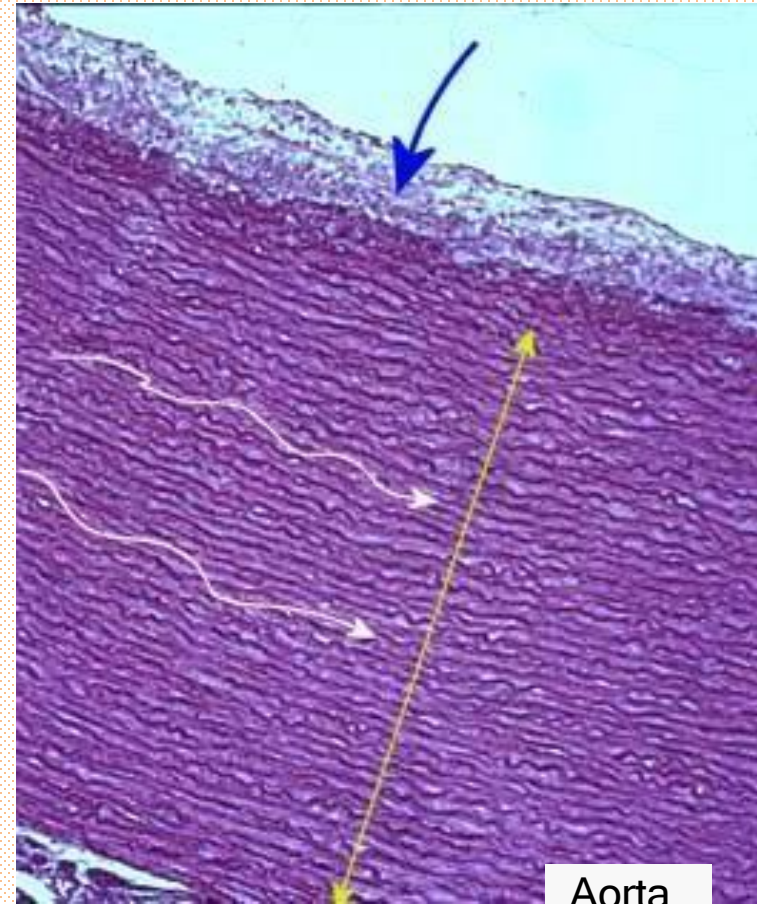
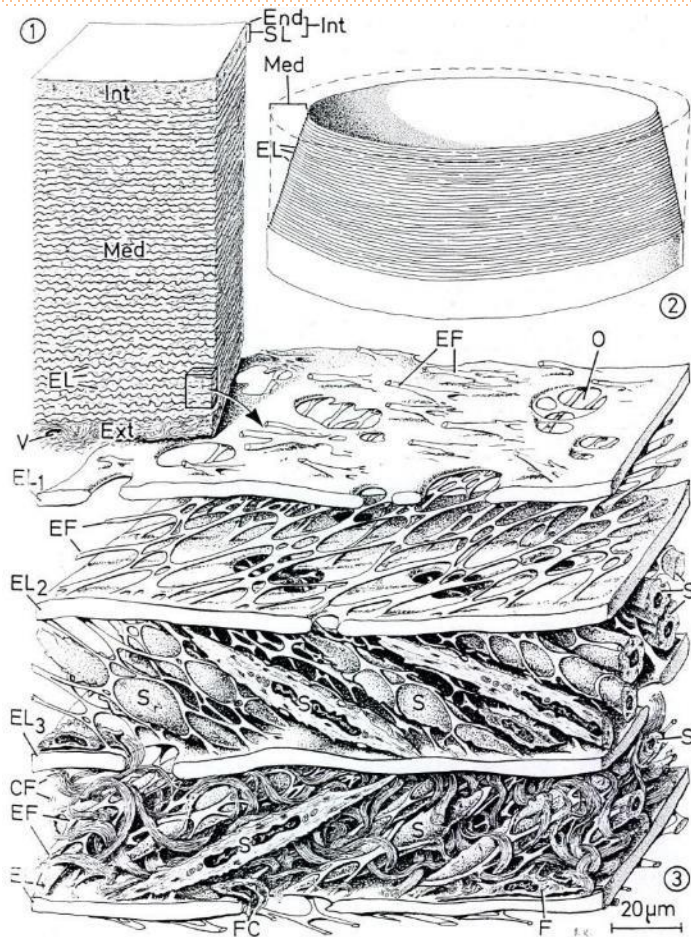


# Tendon





# Regular dense CT – elastic



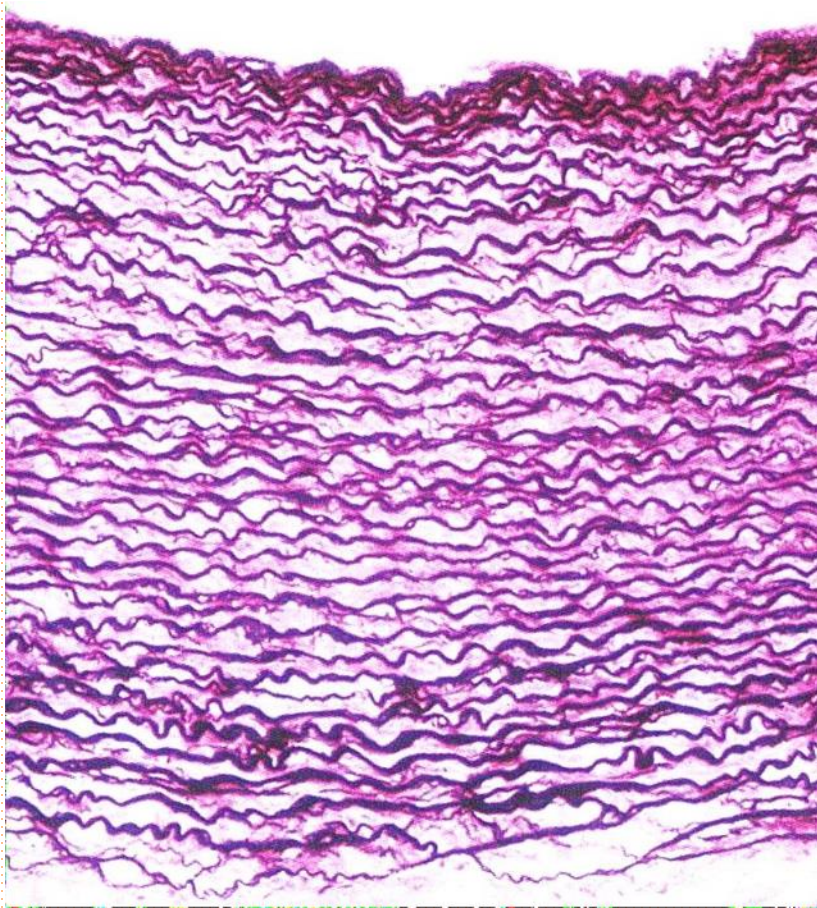
- Regular elastic dense connective tissue contains thick elastic fibers, between which there is a little ground substance with rare fibrocytes and a little reticular and collagen fibers.



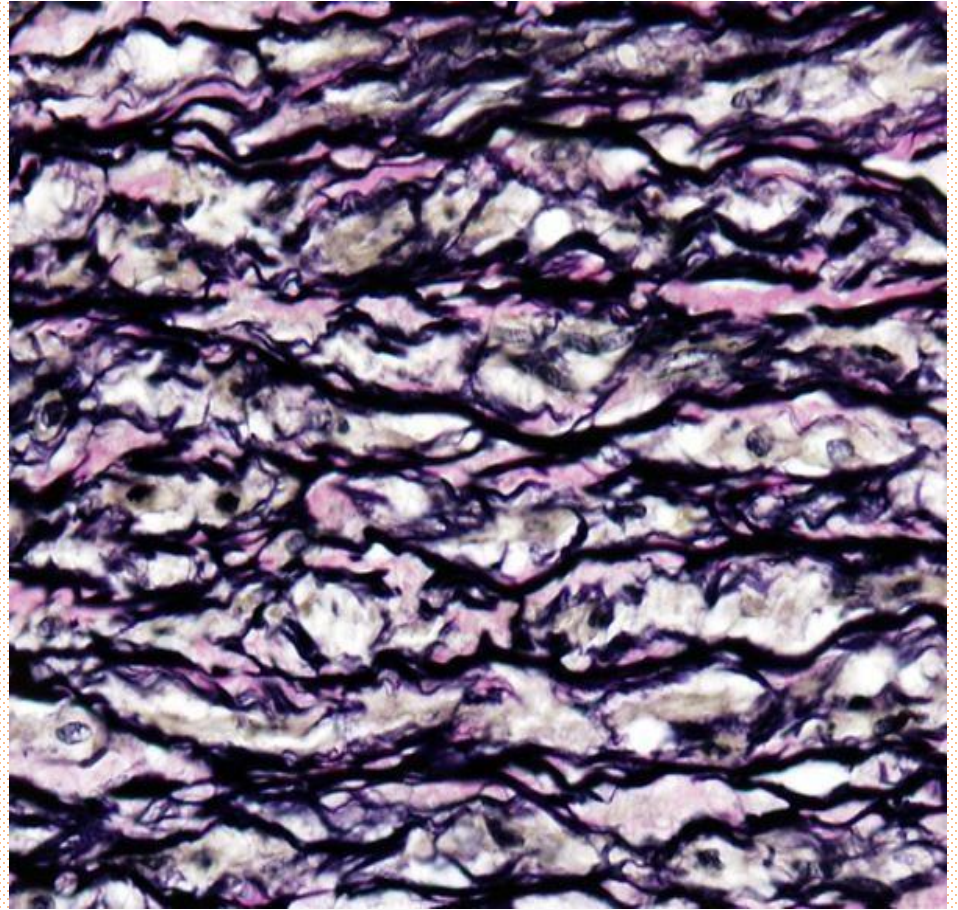




# Aorta

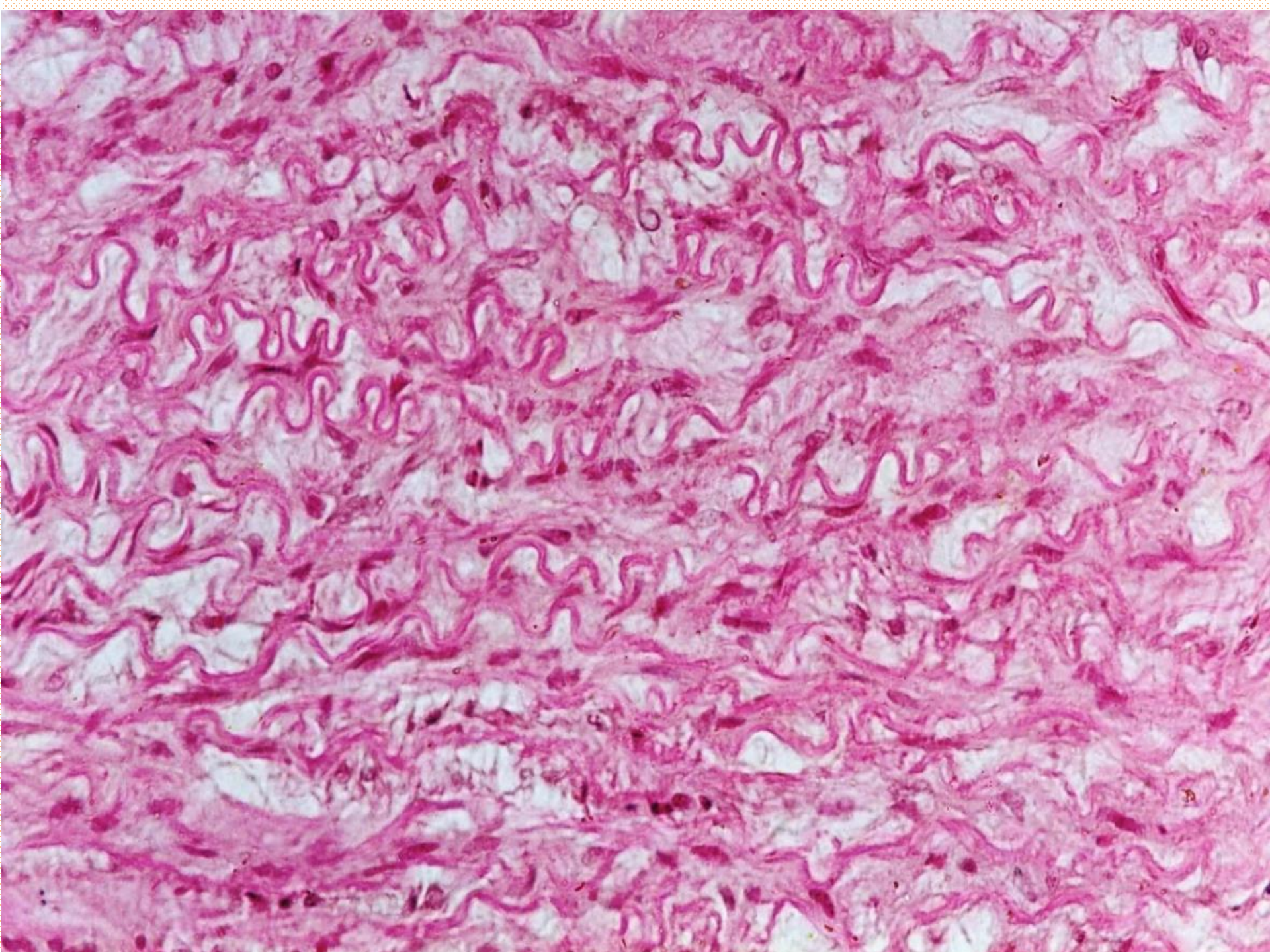


Weigert staining



Verhoeff - van Gieson staining



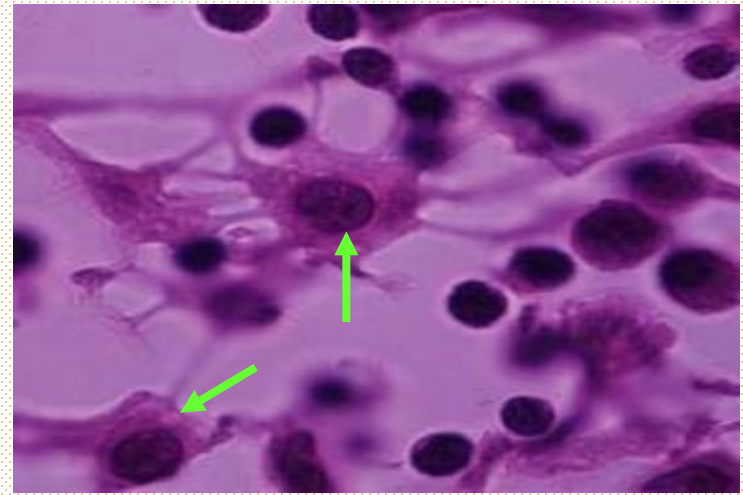
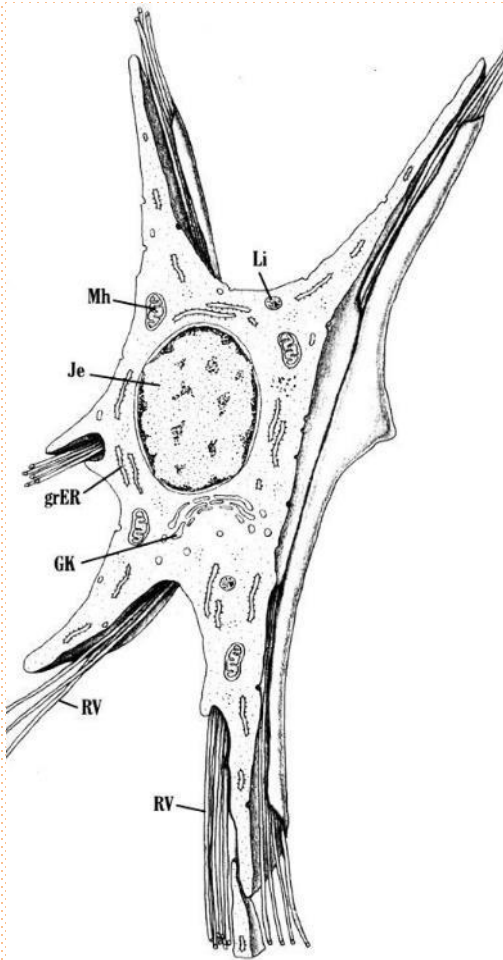




# Reticular (hematopoietic) CT

- Specialized in blood cell production and lymphocyte maturation.
- The tissue is highly cellular and has a soft consistency.
- It is found in hematopoietic and lymphatic organs
- **Myeloid type** found in the bone marrow dominated by immature blood cells
- **Lymphatic type** found in the thymus, spleen, lymph nodes and mucosa of the digestive tract. It is dominated by lymphocytes.
- Myeloid and lymphoreticular types contain reticular cells and reticular fibers and are often referred to by one name - **reticular connective tissue**.

# Reticular cells



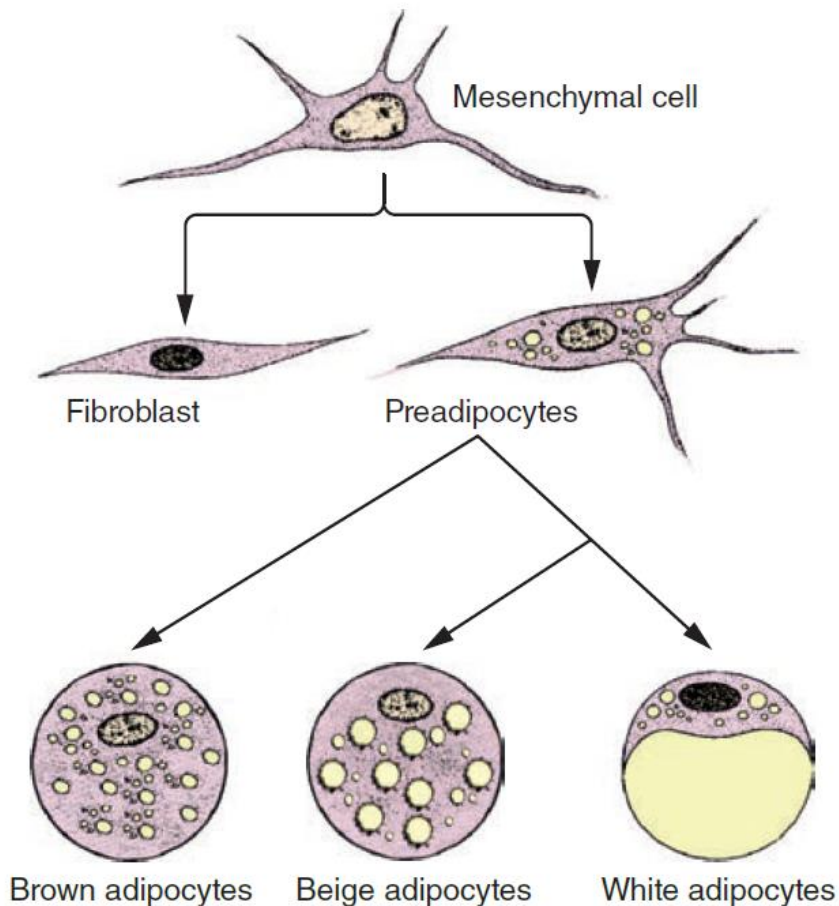
- They have a stellate body and long extensions that wrap the reticular fibers.
- In the cytoplasm, a well-expressed system of rER cisterns and the Golgi apparatus, which participate in the synthesis of **collagen III (reticular fibers)**.
- Part of the reticular cell population resembles fibrocytes, but the other part resembles macrophages.



# Fat tissue (adipose)

- Adipose tissue is a highly specialized **cellular connective tissue** dominated by cells specialized for lipid accumulation.
- It is built mainly from cells **ADIPOCYTES**, while the ECM is less represented.
- Fat tissue accounts for about 12-15% of body mass in an adult, and 20-25% in a woman.
- Can be **white and brown adipose tissue**.
  - White adipose tissue is specialized for fat storage
  - Brown adipose tissue release heat and function to warm the blood

**FIGURE 6–3** Development of white and brown fat cells.



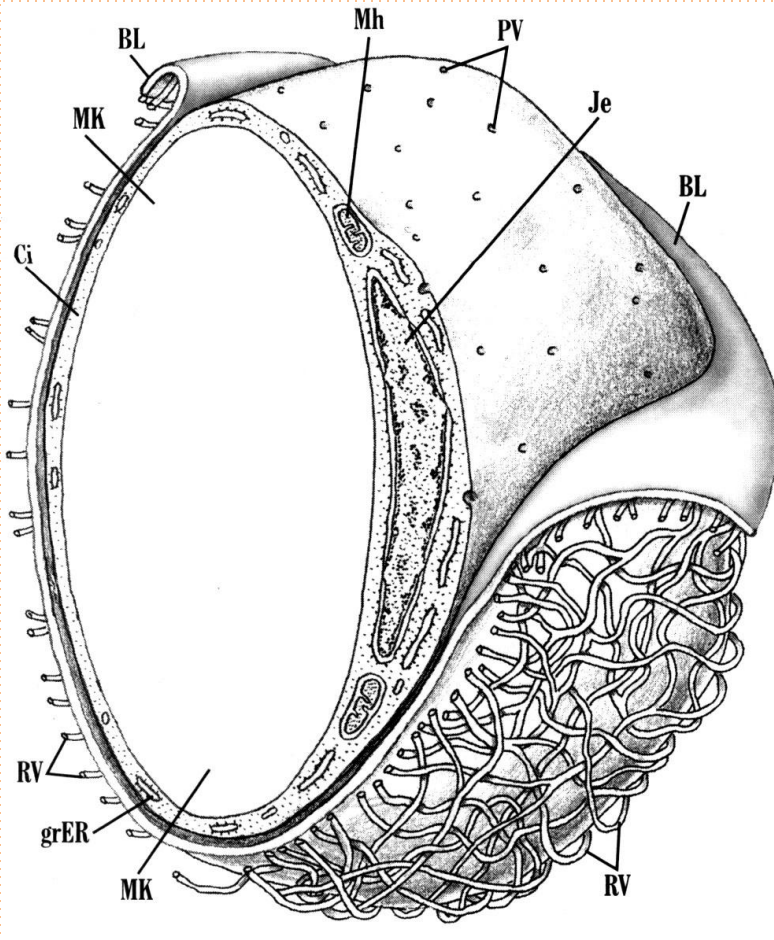
Mesenchymal stem cells differentiate in **preadipocytes** and later **white adipocytes** (forming white adipose tissue)

Small number of so-called **beige adipocytes** with cytological features and gene expression patterns of both white and brown adipocytes.

**Brown adipocytes** differentiate from another population of preadipocytes and remain **multilocular**

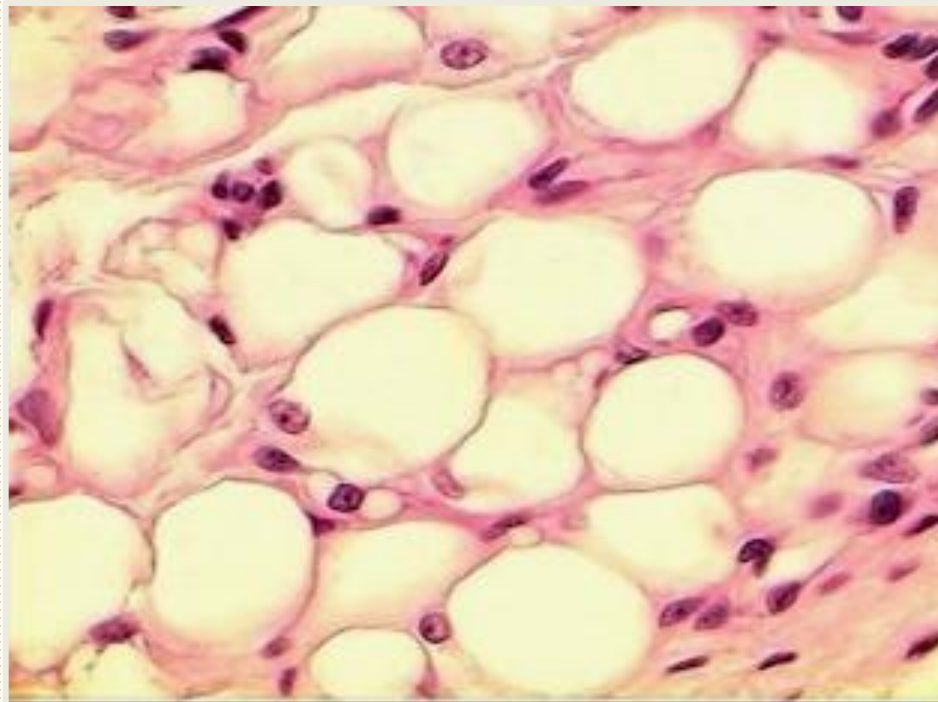
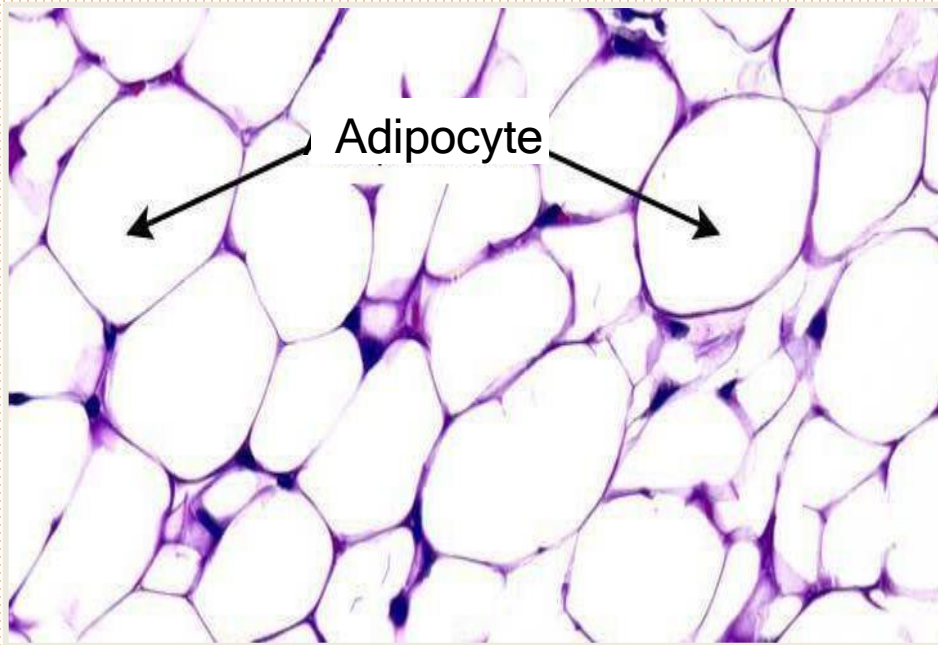


# White adipocyte



- White adipocyte is a round cell, diameter 50-150  $\mu\text{m}$ , surrounded by an external lamina.
- Organelles are weakly expressed, and the largest part of the cell is occupied by a faty droplet. The nucleus is pushed to cell periphery.
- With the single large droplet of triglycerides, white adipocytes are also called unilocular.

# White adipose CT



Roles of adipose tissue:

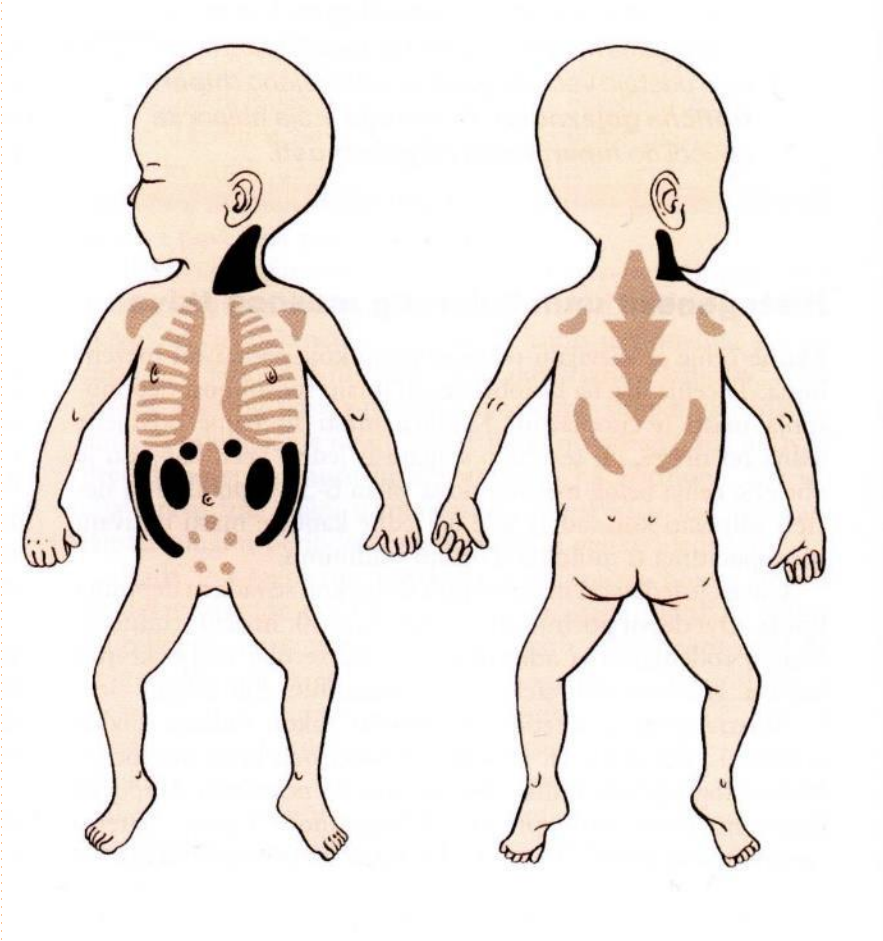
- energy depot
- mechanical role (shock adsorbent)
- thermal insulator
- thermoregulator
  - endocrine role

Adipocytes secrete hormones

leptin, adiponectin,  
angiotensinogen and  
resistin, as well as cytokines  
called adipokines.

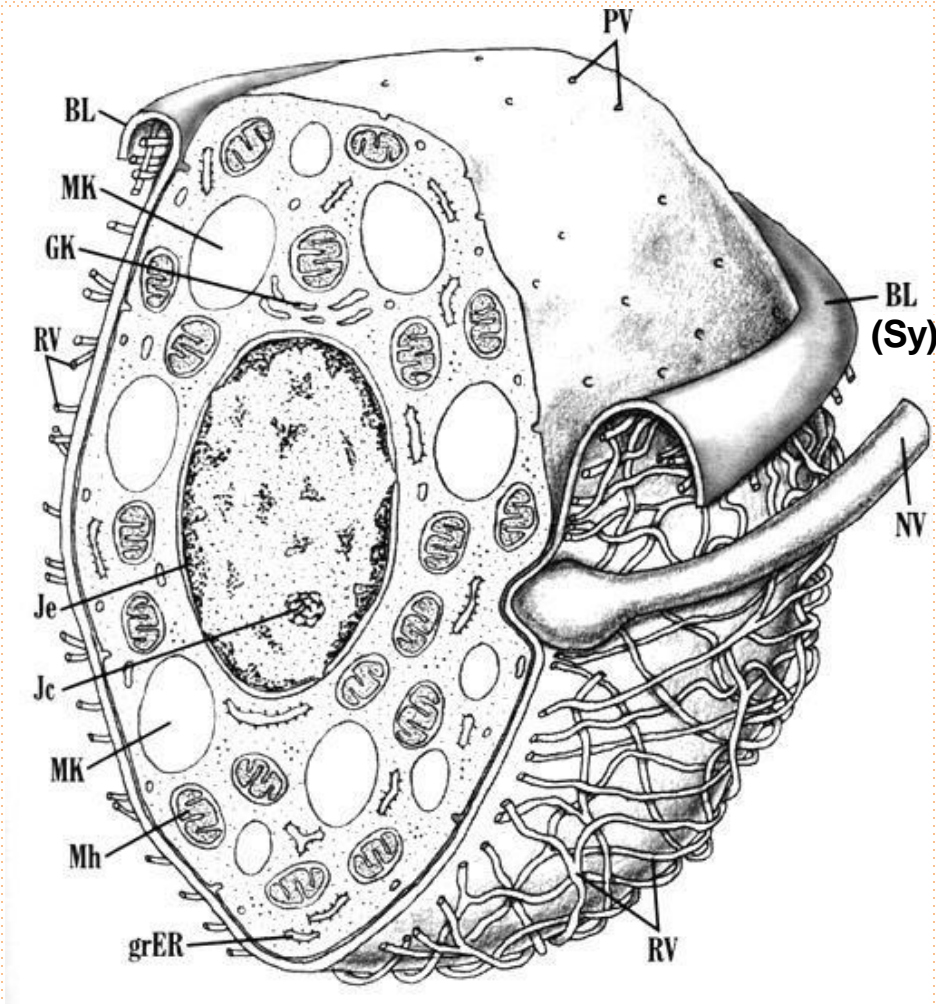


# Brown adipose CT



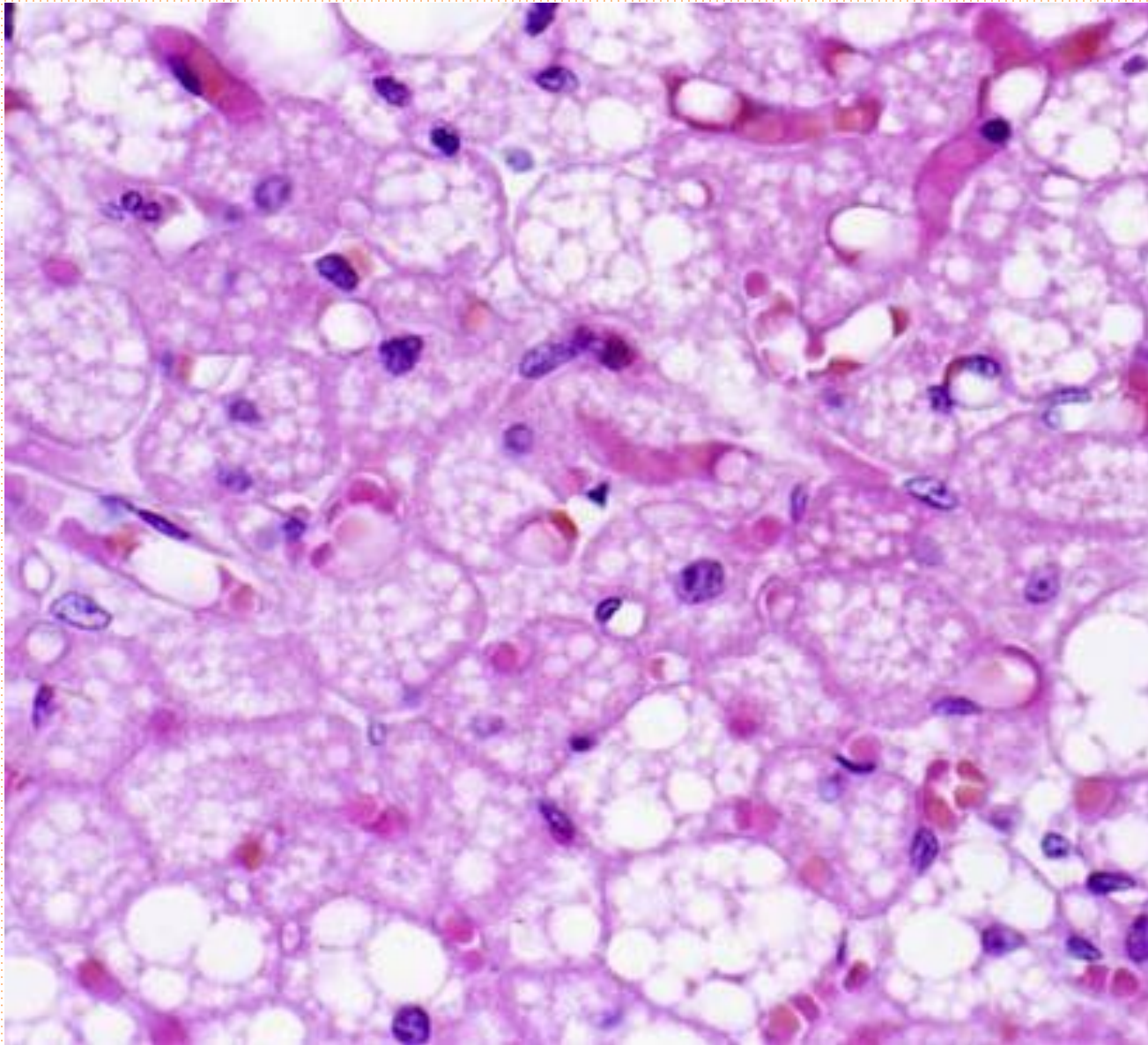
- It is located in the neck, mediastinum, abdomen
- Brown fat comprises up to 5% of the newborn body weight but smaller amounts in adults.
- Adipocytes of this tissue contain primarily **many small lipid droplets (they are multilocular)**
- In cytoplasm - many mitochondria and a central nucleus.
- Mitochondria of brown adipocytes use fat for thermogenesis rather than ATP synthesis, using **thermogenin**

# Brown adipocyte



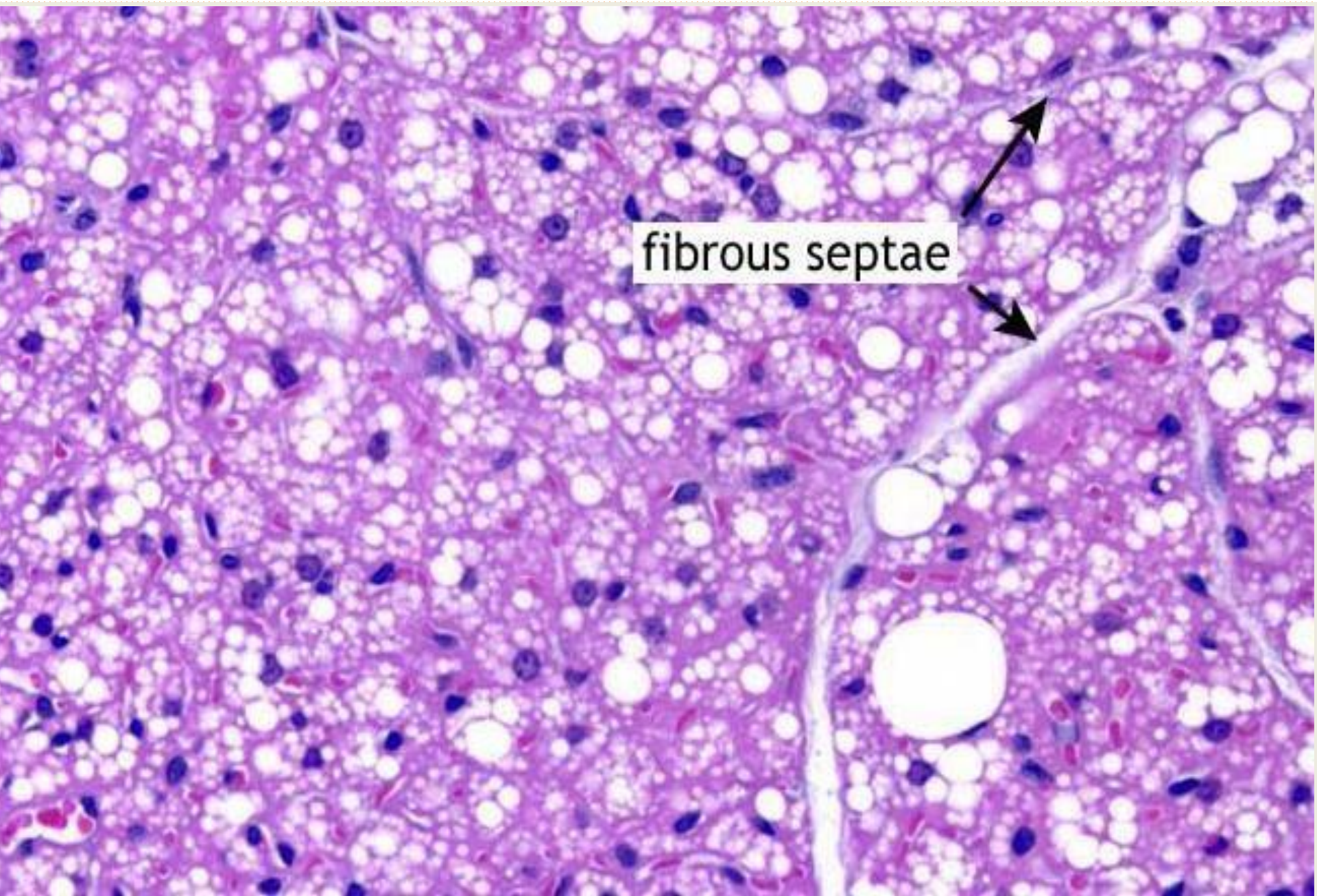
- It is specialized in energy production ("chemical heater").
- Cells are smaller than in white FT, polygonal
- Unmyelinated fibers are in direct contact with the cell





- The dark color of the tissue comes from the high **cytochrome** concentrations and a **rich network of capillaries**.





fibrous septae