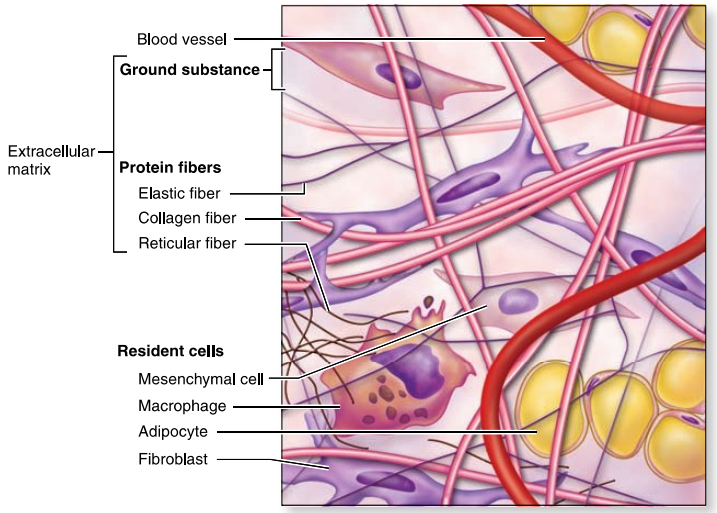
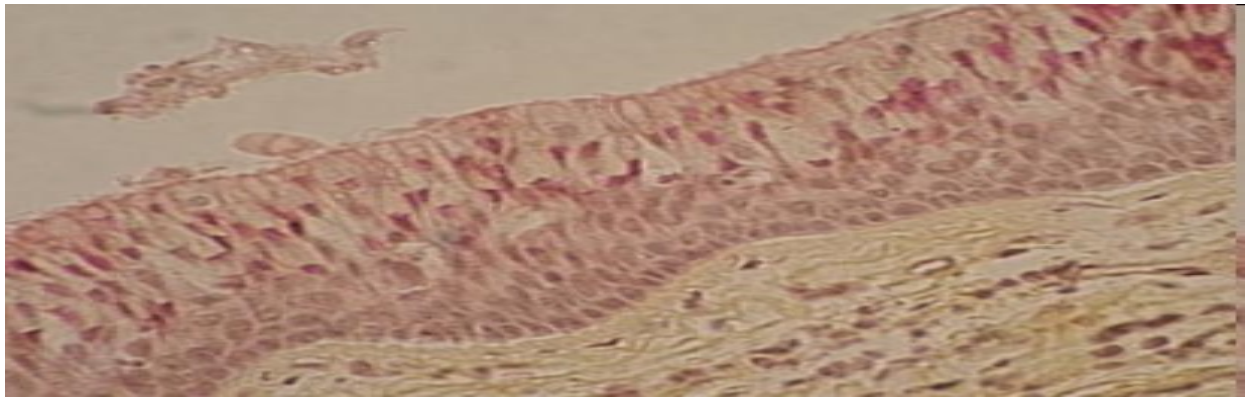
Connective tissue

- matrix that supports and connects other tissues and cells together
- major constituent of connective tissue is **the extracellular matrix**





Comparison of epithelium and connective tissue:
In the epithelium - the cells are close to each other, it is difficult to distinguish the number of layers and membranes of individual cells.
In connective tissue - there is a large amount of intercellular matrix and relatively few cells.

Connective tissue

Extracellular matrix includes:

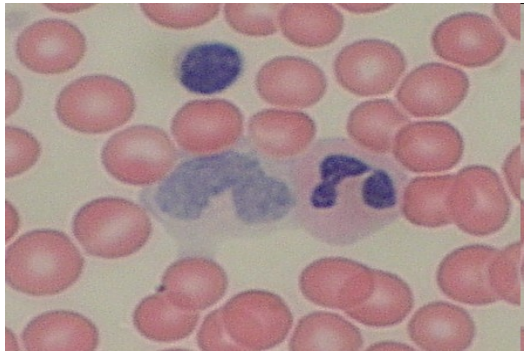
protein fibers - three main types: collagen, reticular and elastic fibers

ground substance - proteoglycans, glycosaminoglycans,
multiadhesive glycoproteins – fibronectin, laminin

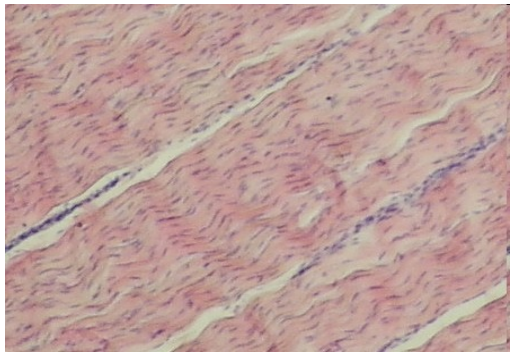
The ground substance contains a certain amount of water which allows the exchange of nutriens between cells and blood in vessels.

Extracellular matrix may have a thin, semi-solid or solid consistency.

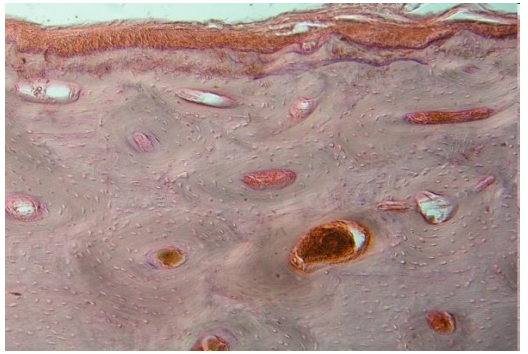
Components and consistency of the intercellular matrix, determine the properties of connective tissue



Blood smear

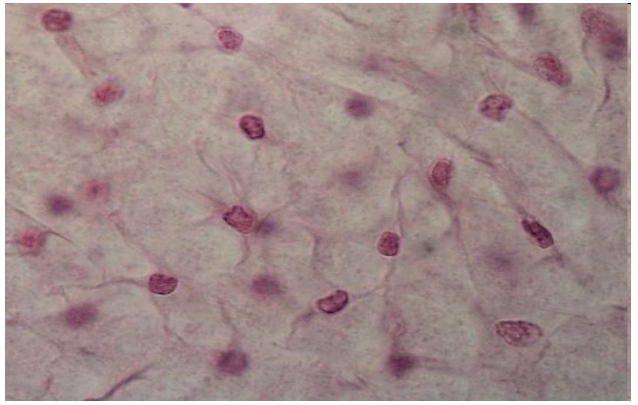


Tendon



Bone

Embryonic origin of connective tissues



All connective tissue originate from embryonic mesenchym

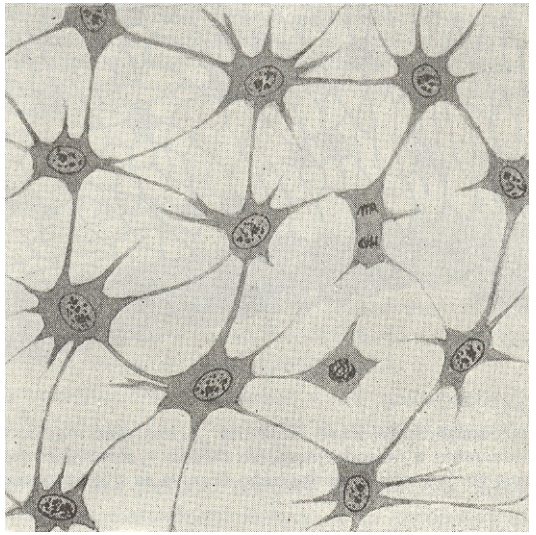
(develop from the middle layer of the embryo, the mesoderm)

Tissues developing from the mesenchyme:

connective tissue proper, cartilage, bone, blood, vascular endothelium, muscle

Mesenchymal cells:

- undifferentiated
- large euchromatic nuclei with prominent nucleoli, fine chromatin
- high level of syntetic activity
- spindle- shaped with cytoplasmic processes (protrusions)

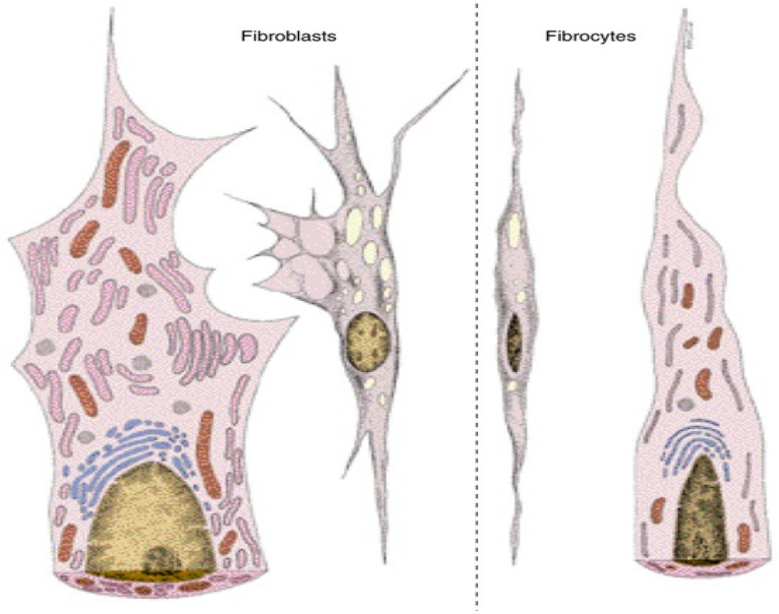


Cells of connective tissue

Two types of cells are generally found in connective tissues:

- permanent or fixed or resident cells (still present in tissue). These cells produce and maintain extracellular matrix (groung substance and fibers). For example, it belongs here fibroblast, chondrocyt, osteocyt.
- transient or (wandering) cells (blood leucocytes, macrophages, mast cells). These type of cells perform various function here for a period as needed and then usuually die by apoptosis. The main function of these cells is immune protection. These cells belong to the mononuclear phagocytic system

Cells of connective tissue



Fibroblasts, Fibrocytes

most common cells in connective tissue proper

produce and maintain all compounds of extracellular components

Fibroblast - metabolically more active form of this cell

more abundant cytoplasm, rough endoplasmatic reticulum,

well develop Golgi apparatus,

large euchromatic nukleus and prominent nucleolus

Fibrocytes - a highly differentiated, less metabolically active cell

smaller than an active fibroblast, ussually spindle shaped, much less rough ER, dark, heterochomatic nucleus

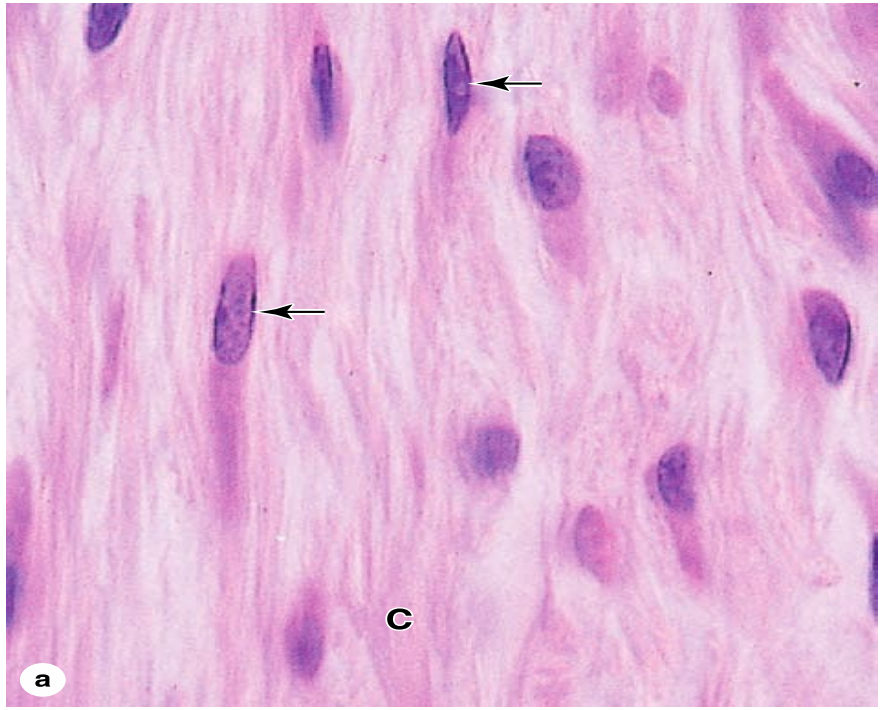
In adults, rarely undergo division.

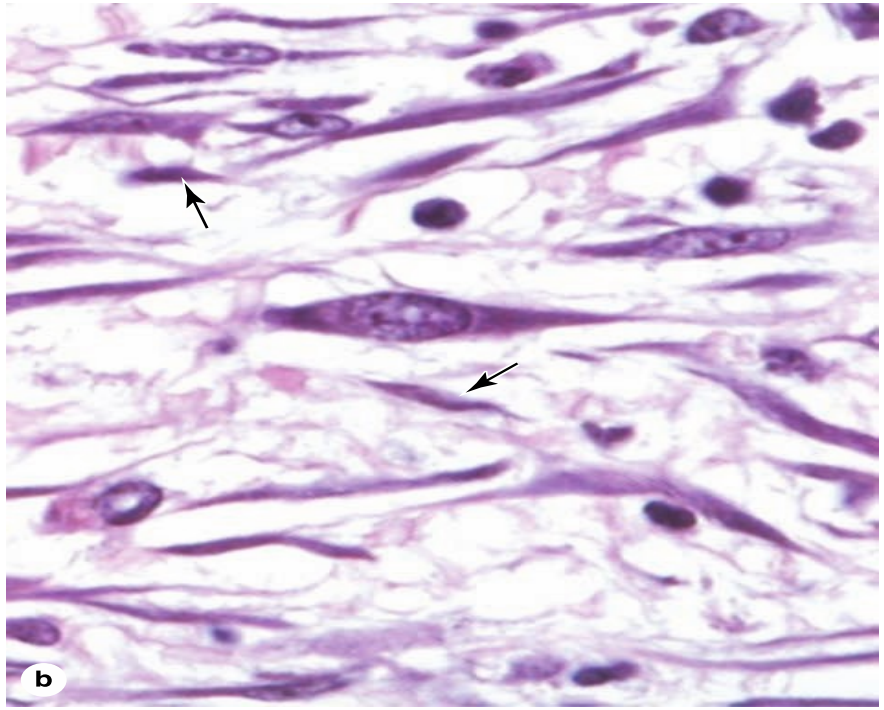
Some growth factors can act on fibroblasts and influence their metabolism.

Stimulated by growth factors, they can divide - tissue reparation, wound healing

Myofibroblast – fibroblast involved in wound healing, have contractile function and are enriched with actin

Cells of connective tissue (fibroblast, fibrocyte)





(a) Fibroblasts typically have large active nuclei and eosinophilic cytoplasm that tapers off in both directions along the axis of the nucleus, a morphology often referred to as "spindle-shaped." Nuclei (**arrows**) are clearly seen, but the eosinophilic cytoplasmic processes resemble the collagen bundles (**C**) that fill the ECM and are difficult to distinguish in H&E-stained sections.

(b) Both active and quiescent fibroblasts may sometimes be distinguished, as in this section of dermis. Active fibroblasts have large, euchromatic nuclei and basophilic cytoplasm, while inactive fibroblasts (or fibrocytes) are smaller with more heterochromatic nuclei (**arrows**). The round, very basophilic round cells are in leukocytes. Both X400. H&E.

Cells of the mononuclear phagocytic system

Cell Type	Major Locations	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow, pleural and peritoneal cavities	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing, and presentation
Kupffer cell	Liver (perisinusoidal)	Same as macrophages
Microglial cell	Central nervous system	Same as macrophages
Langerhans cell	Epidermis of skin	Antigen processing and presentation
Dendritic cell	Lymph nodes, spleen	Antigen processing and presentation
Osteoclast (from fusion of several macrophages)	Bone	Localized digestion of bone matrix
Multinuclear giant cell (several fused macrophages)	In connective tissue under various pathological conditions	Segregation and digestion of foreign bodies

Cells of connective tissue

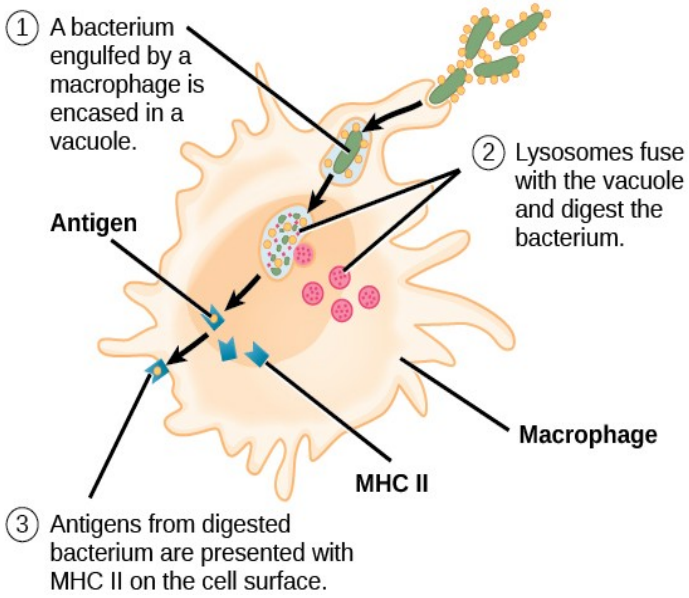
Macrophages

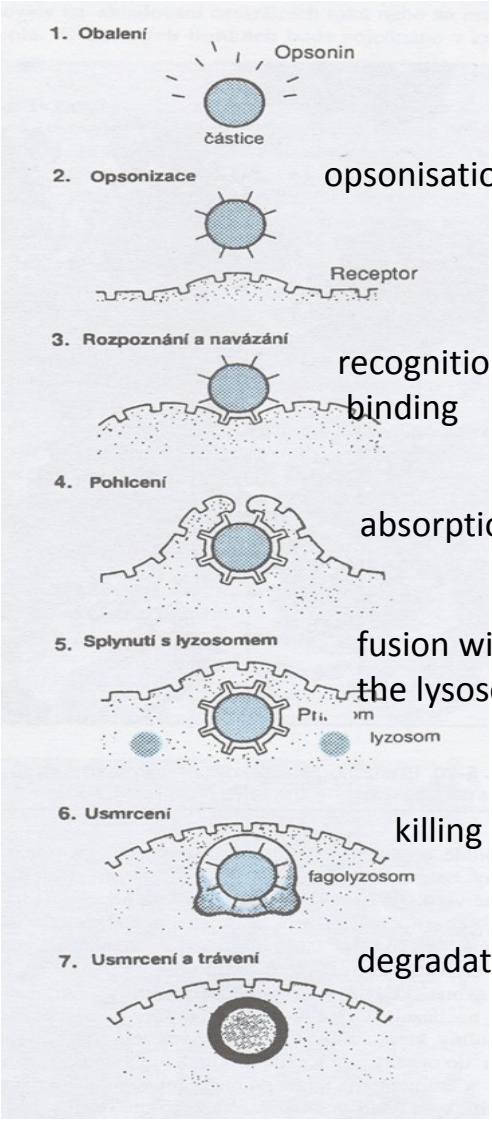
Highly developed phagocytic activity, active in removal of dead cells, tissue debris or patogens, are especially abundant at sites of inflammation

Size 10-30 m, excentically localed oval or kidney-shaped nukleus

Sometimes referred as histiocytes

They differentiate from monocytes that are in the blood and can pass through the walls of small venules into the tissues.





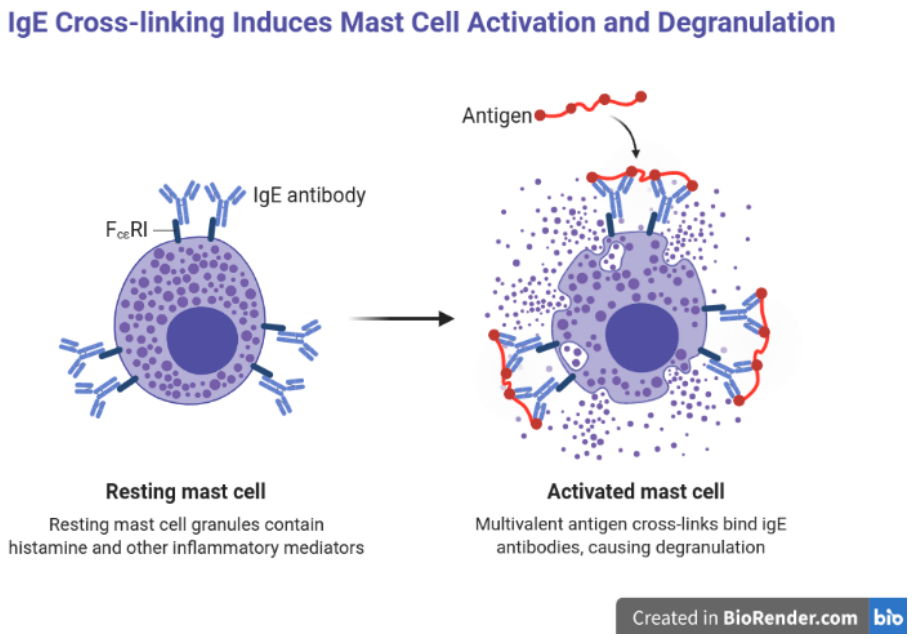
Cells of connective tissue

Mast cells

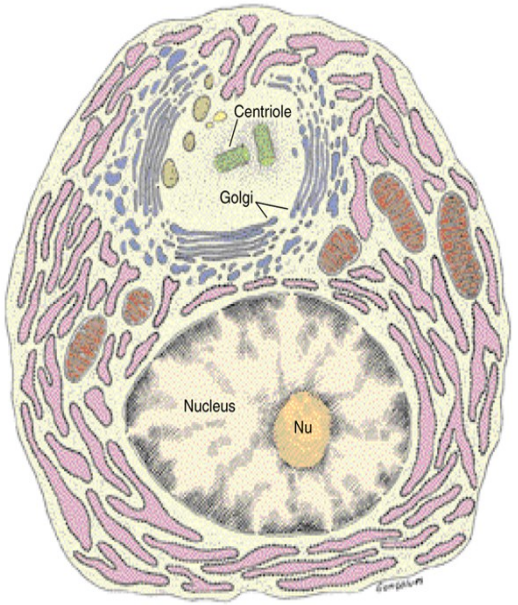
oval or irregular shaped, filled with basophillic secretory granules, which can obscure nukleus
secretory granules contain these substances: heparin (anticogulant), histamine (increase vascular permeability), serine proteases (activate mediators of inflammation), cytokines (affect leukocyte), chemotactic factors (attract leukocytes)

Mast cells are abundant around blood vessels in the skin and in the tissues of the digestive and respiratory tracts - in places with a higher risk of pathogen occurrence.

Mast cells bind IgE antibodies on their surface and in pathological conditions can participate in type I hypersensitive reactions

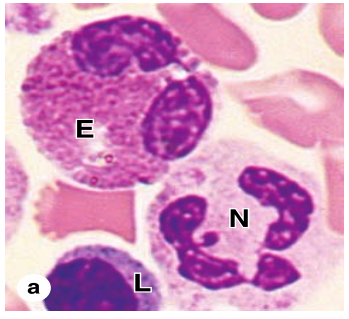


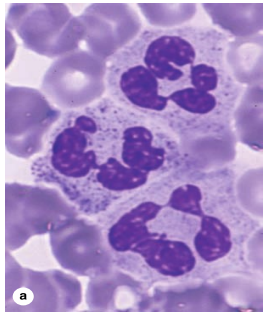
Cells of connective tissue

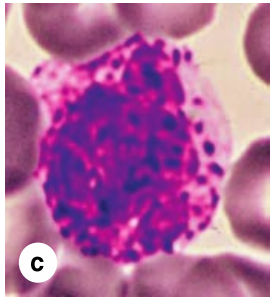


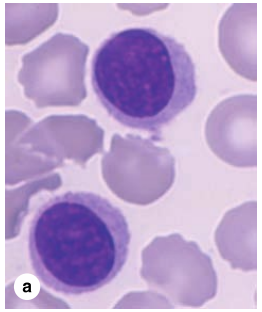
Plasma cells lymphocide-derived, antibody producting cells, ovoid, large cells, basophilic cytoplasm, rich in proteosynthetic apparatus, the nucleus contains both heterochromatin and euchromatin, darker and lighter areas.

Other types of leukocytes can also be found in the connective tissue their number increases during inflammation
neutrophils, eosinophils, several types of lymphocytes)









Fibers

Three types of fibers are found in connective tissues..

Collagen, elastic and reticular fibers

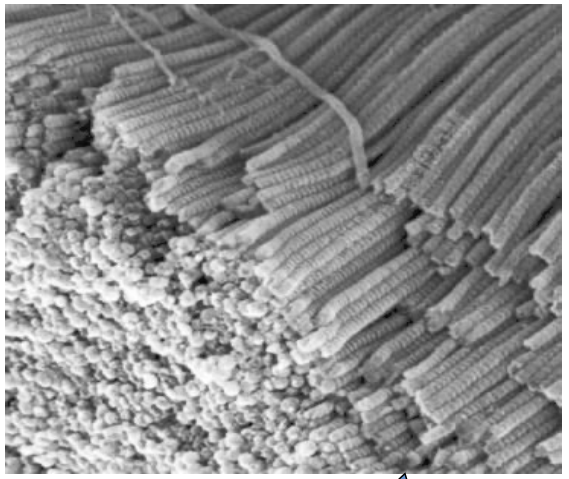
Reticular and collagenous fibers are composed of collagen, elastic are of elastin.

Fibers occur in different amounts according to the type of connective tissue.

The amount and type of fibers determines the properties of the tissue.

Collagen

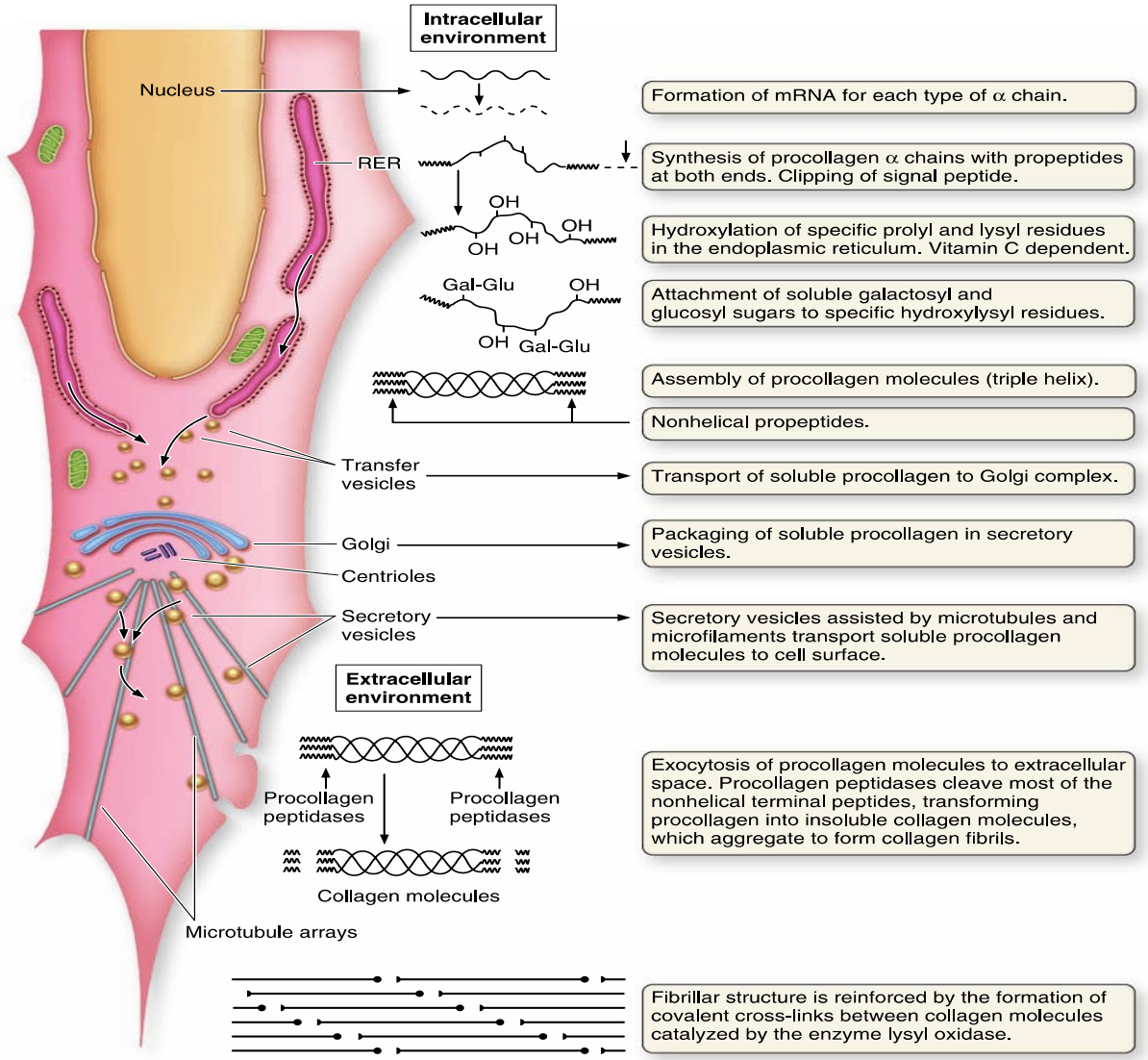
most abundant protein in the human body
representing 30% of the dry weight
mainly produced by fibroblast
there are 28 (5 the most important) types of collagen



Collagen fibrils forming a collagen fiber (next is bundle of collagen fibers) in a scanning electron microscope image. The basic structural units of collagen (three-helix size 300 nm) partially overlap during aggregation. This creates striations

Fibers, Collagen synthesis

- Collagen synthesis is catalyzed by several different enzymes.
- These enzymes must first be created in the cell by the process of proteosynthesis.
- The formation of collagen is therefore a very complicated process.
- There are a number of pathological conditions associated with collagen synthesis disorders (*scruvy – lack of vit C, which acts as cofactor of prolyl oxidase)*
- There is a so-called turnover of collagen in the tissues. Its intensity varies according to the type of tissue (*low in tendons, high in periodontal ligaments of teeth*). The degradation of collagen is initiated by enzymes called collagenases (*matrix metaloproteinases*).



Hydroxylation and glycosylation of procollagen α chains and their assembly into triple helices occur in the RER, and further assembly into fibrils occurs in the ECM after secretion of procollagen. Because there are many slightly different genes for procollagen α chains and collagen production depends on several posttranslational events involving several other enzymes, many diseases involving defective collagen synthesis have been described.

Fibers

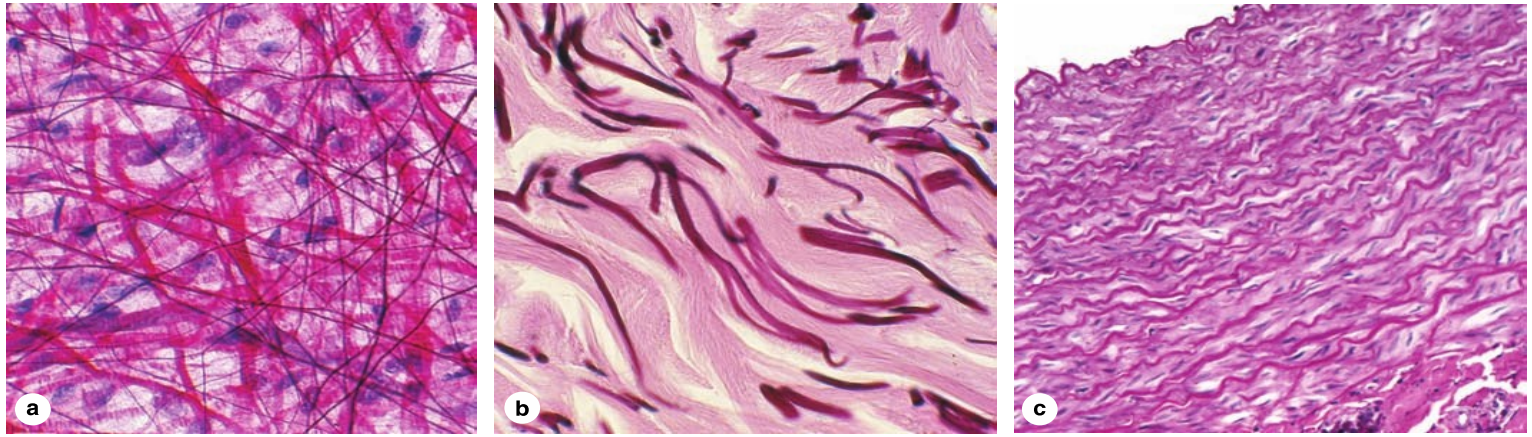
Elastic fibers

are thinner than collagen fibers

typically in organs where volume changes occur (aorta, lung tissue)

the main protein here is elastin

they are also called yellow due to the coloring of the tissues that contain them



Elastic fibers or lamellae (sheets) add resiliency to connective tissue. Such fibers may be difficult to discern in H&E-stained tissue, but elastin has a distinct, darker-staining appearance with other staining procedures.

(a) The length, diameter, distribution, and density of dark **elastic fibers** are easily seen in this spread preparation of nonstretched connective tissue in a mesentery. X200. Hematoxylin and orcein.

(b) In sectioned tissue at higher magnification, **elastic fibers** can be seen among the acidophilic collagen bundles of dermis. X400. Aldehyde fuchsin.

(c) Elastic lamellae in the wall of the aorta are more darkly stained, incomplete sheets of elastin between the layers of eosinophilic smooth muscle. X80. H&E.

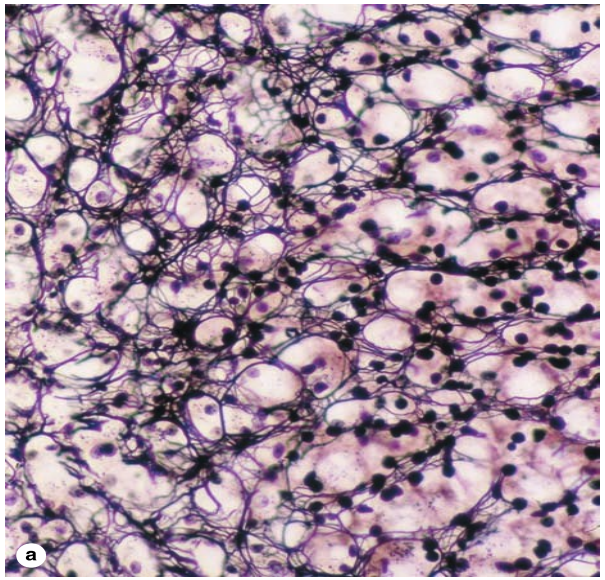
Fibers

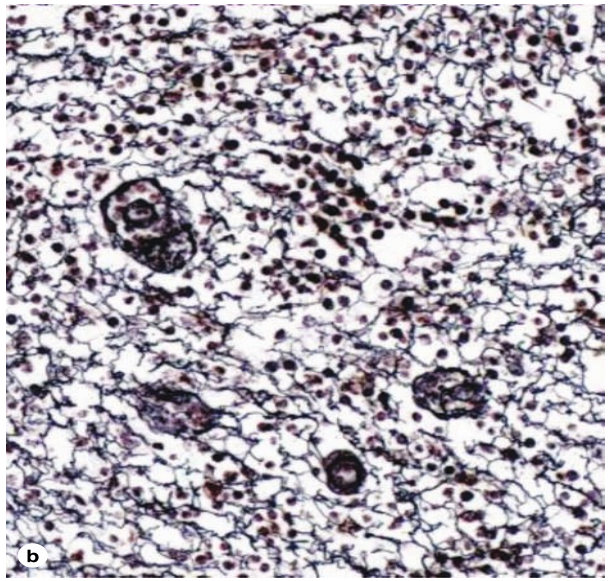
Reticullar fibers

consist of collagen type III, forms a network

typically occur in the immune organs, also in the reticular lamina of basement membrane and surround adipocytes, smooth muscle cells, nerve fibers, small blood vessels

are stained black by impragnation with silver salt.





In these silver-stained sections of adrenal cortex **(a)** and lymph node **(b)**, networks of delicate, black **reticular fibers** are prominent. These fibers serve as a supportive stroma in most lymphoid and hematopoietic organs and many endocrine glands. The fibers consist of type **III** collagen that is heavily glycosylated, producing the black argyrophilia. Cell nuclei are also dark, but cytoplasm is unstained. X100.

Types of connective tissue

Connective tissue proper:

1. Loose (areolar tissue)

consist of cells, ground substance and fibers in equal parts
forms the layer below the epithelium (including the epithelium of the skin),
filling space between fibers of muscle and nerve
is flexible and nor very resistent to stress

2. Dense

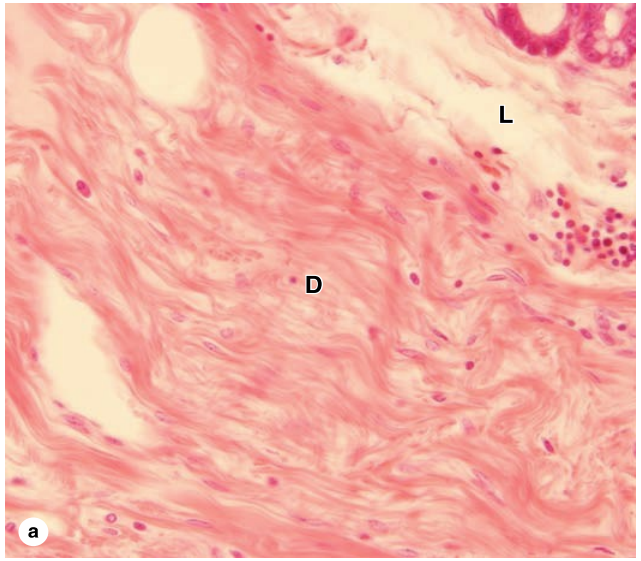
Higher proportion of collagen fibers – irregular or regular according to fiber arrangement

Irregular:

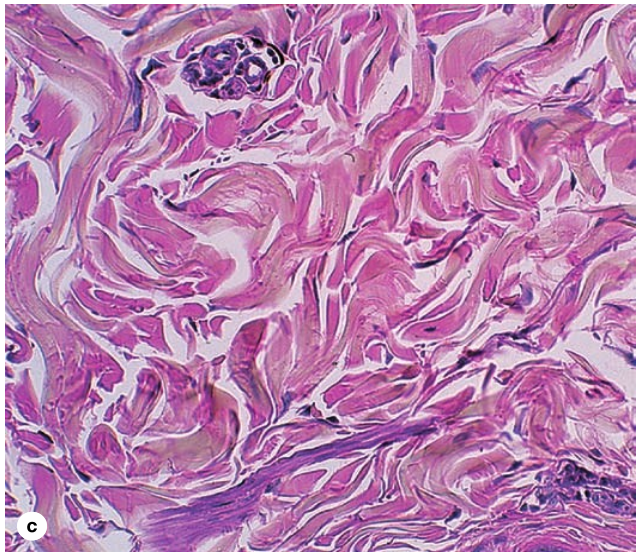
resistant to stress from all directions
deep dermis layer of skin, capsules around the organs

Regular:

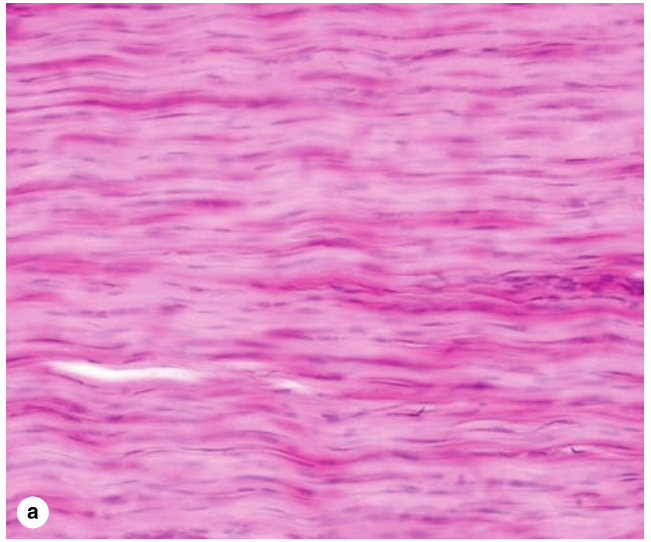
consists of fibroblasts and type I collagen bundles (minimum of ground substance)
resistant to stress from the same direction
tendons, aponeurosis, ligaments



L loose, D dense irregular connective tissue

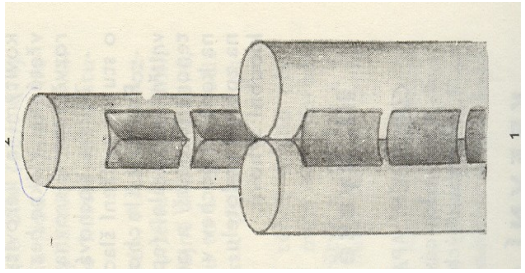


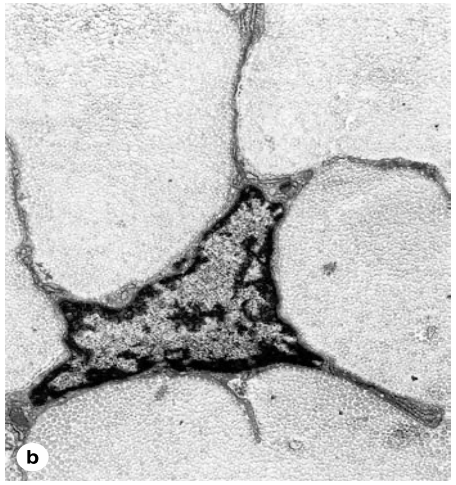
Dense irregular connective tissue



Dense regular connective tissue, tendon
Longitudinal section

Fibrocyte between bundles of collagen fibers on a transverse section of tendon, The cytoplasm forms a protrusion in the spaces between the fiber bundles.
In Czech, the name winged cells is used

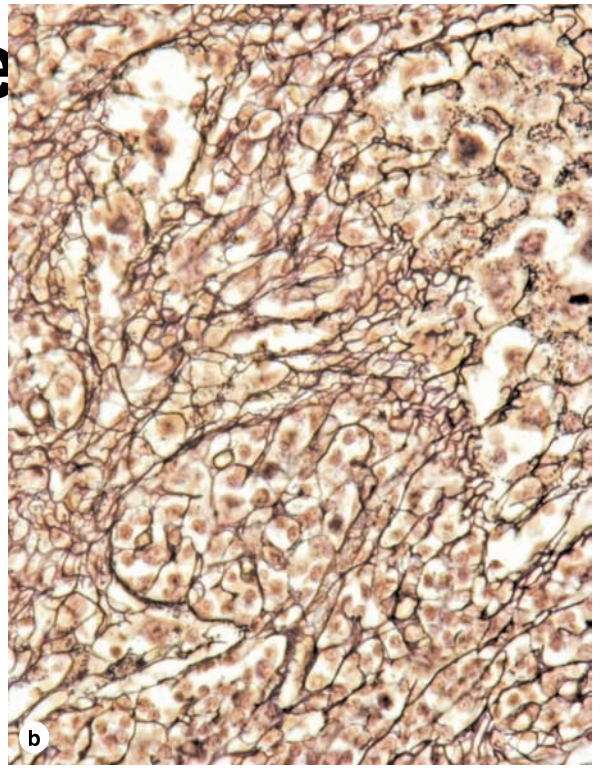




Types of connective tissue

Reticular tissue

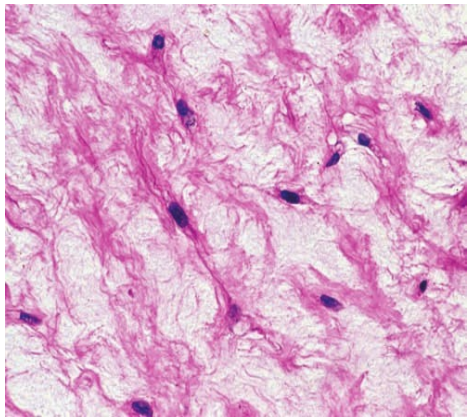
- Consist of type III collagen fibers (also known as reticullin) - produced by modified fibroblasts, called reticular cells
- Occurs in hematopoietic and lymphoid organs (bone marrow, lymph nodes, spleen), where it forms a network-like basic structure
- Contains many free cells, especially lymphocytes, as well as macrophages and dendritic cells



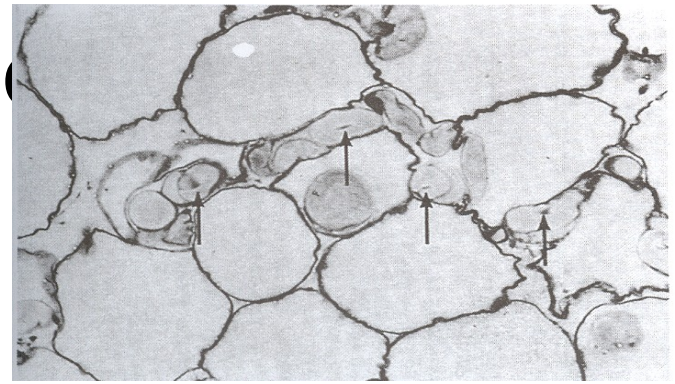
(b) The micrograph shows a silver-stained section of lymph node in which reticular fibers are seen as irregular black lines. Reticular cells are also heavily stained and dark. Most of the smaller, more lightly stained cells are lymphocytes passing through the lymph node. X200. Silver.

Mucoid tissue

- Component of fetal umbilical cord,
- Is called Wharton‘s jelly
- Contain fibroblasts, mesenchymal cells, few fibers,
- A lot of ground substance



Types of connective tissu



Adipose tissue

There are two major types: white and brown adipose tissue

White:

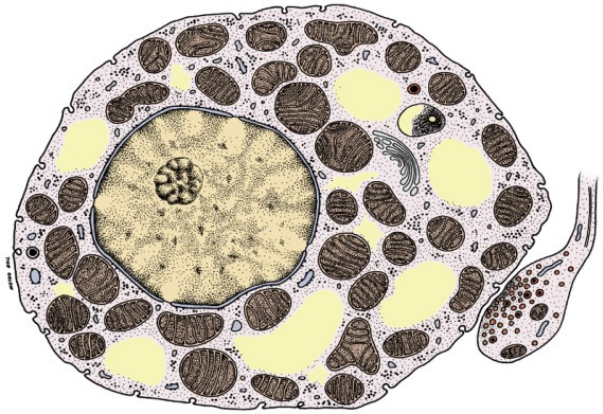
cells – adipocytes, size 50 – 150 µm, signet ring shape, one cytoplasmic droplet of fat-specialized for fat storage

Brown:

contain multiple lipid droples and abundant mitochondria, witch give this tissue a dark color.

adipocytes can produce heat by non-shivering thermogenesis.

They contain a special protein thermogenin, which disconnects the electron transport chain from oxidative phosphorylation. Protons accumulate in the intermembrane space of the mitochondria and their energy is converted into heat.



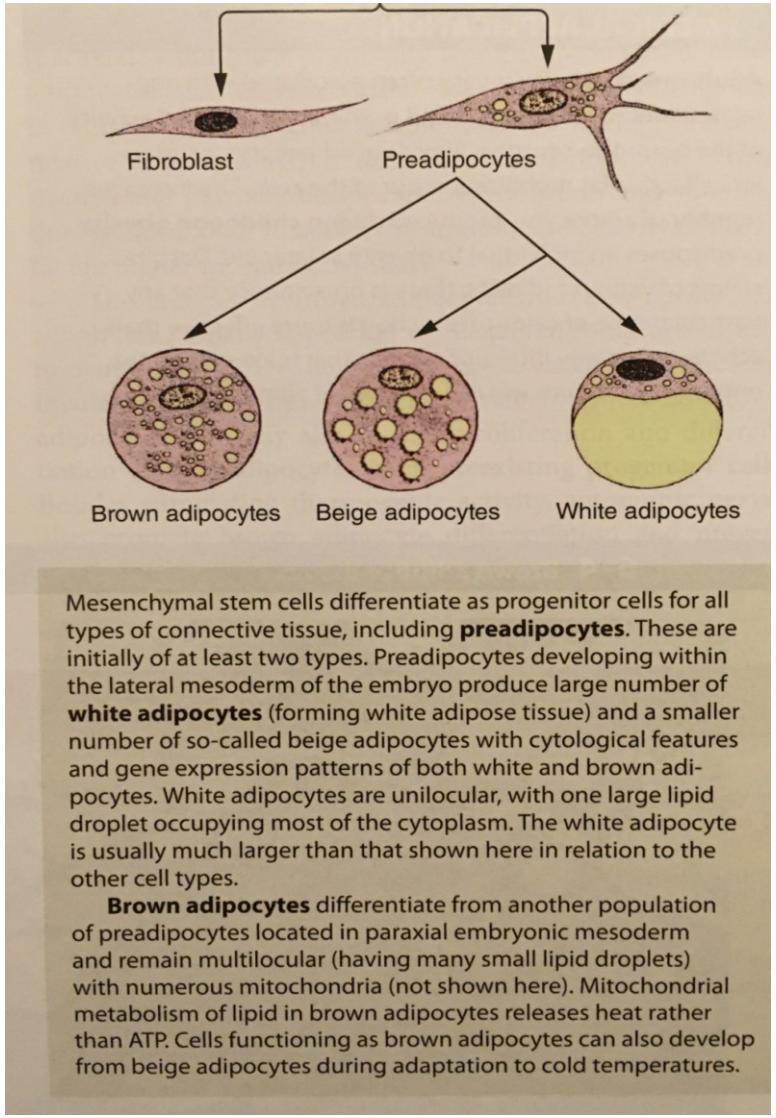
Types of connective tissue

Development of adipocytes:

The precursor is the mesenchymal stem cell.

First a preadipocyte is formed, from which white and beige and brown adipocytes develop.

Beige adipocytes have some characteristics of both white and brown. When the organism adapts to the cold, they can produce heat. They were discovered recently.



Mesenchymal stem cells differentiate as progenitor cells for all types of connective tissue, including **preadipocytes**. These are initially of at least two types. Preadipocytes developing within the lateral mesoderm of the embryo produce large number of **white adipocytes** (forming white adipose tissue) and a smaller number of so-called beige adipocytes with cytological features and gene expression patterns of both white and brown adipocytes. White adipocytes are unilocular, with one large lipid droplet occupying most of the cytoplasm. The white adipocyte is usually much larger than that shown here in relation to the other cell types.

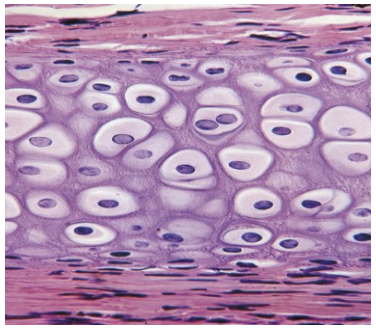
Brown adipocytes differentiate from another population of preadipocytes located in paraxial embryonic mesoderm and remain multilocular (having many small lipid droplets) with numerous mitochondria (not shown here). Mitochondrial metabolism of lipid in brown adipocytes releases heat rather than ATP. Cells functioning as brown adipocytes can also develop from beige adipocytes during adaptation to cold temperatures.

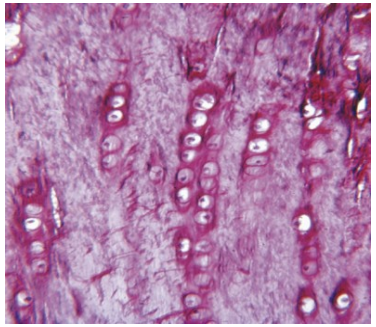
Cartilage

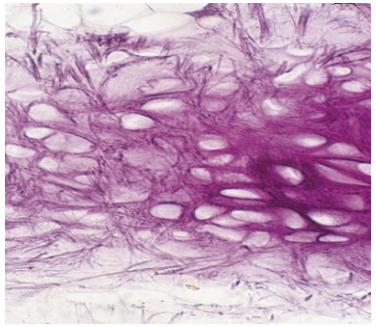
- The intercellular mass has a solid consistency, flexible, smooth on the surface, does not deform
- Important for the development of bones – ossification
- On the surface is dense connective tissue - the perichondrium (except of articular cartilage of joints). There are blood vessels in the perichondrium that do not penetrate the cartilage.
- Nutrition is realized by diffusion. For this reason cartilage is usually in thin layers so that diffusion is sufficient. Cells have small protrusions that increase the surface area for substance exchange.
- Cartilage contains a relatively large amount of bound water. Glycosaminoglycans that bind to core proteoglycans have many negatively charged groups. Na^+ ions, which are abundant in the tissues, bind to these negative charges. Water molecules than bind to sodium cations thanks to their free electron pairs.

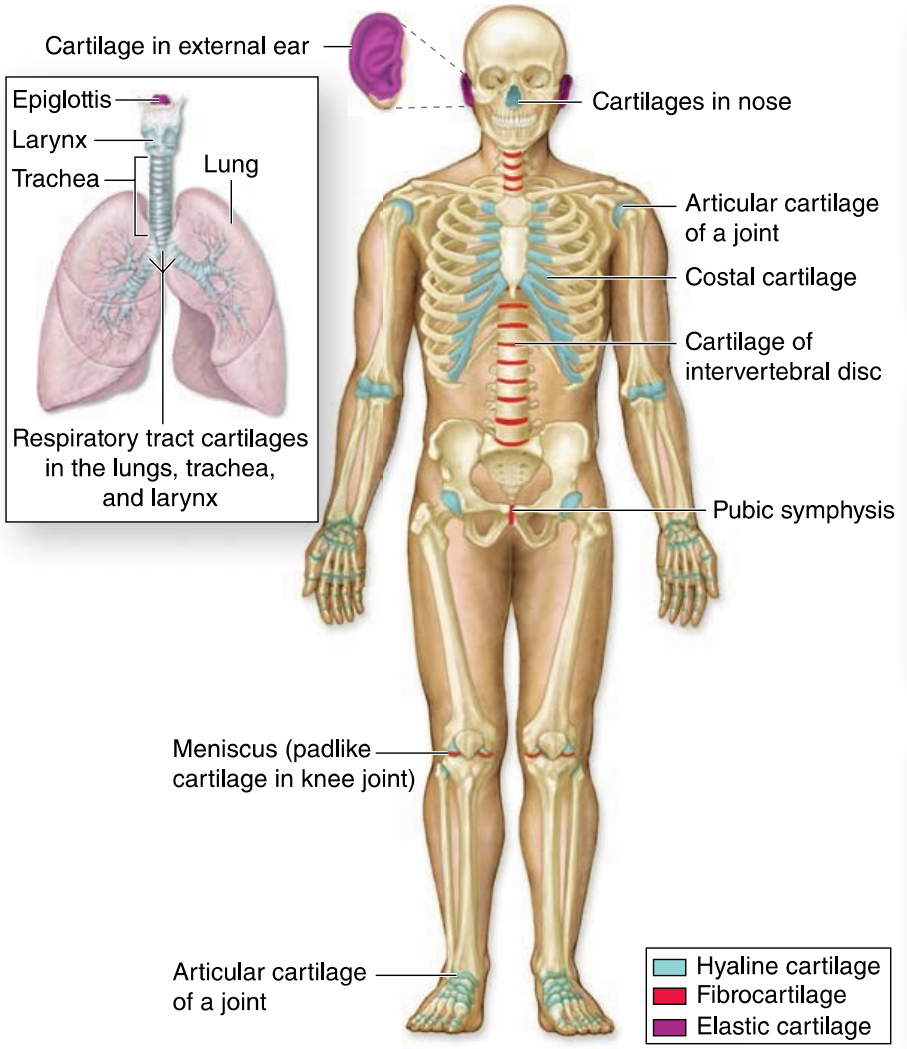
Cartilage

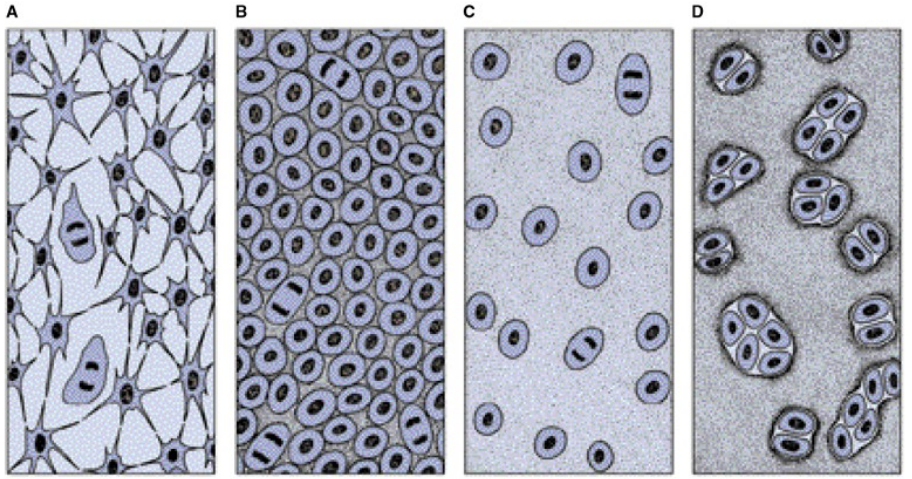
- Cells – chondroblasts - near the perichondrium, they divide and differentiate into chondrocytes – produce matrix components, located in matrix cavities called lacune.
- Cells originate from divisions of a single chondroblast - isogenous groups .
- Matrix contains type II collagen, hyaluronan and proteoglycans, glycoproteins, around the chondrocytes contains less collagen - territorial matrix.
- Cartilage growth is possible in two ways:
  interstitial (by mitotic division of chondrocytes in the inner part of the cartilag and appositional (by chondroblast differentiation from cells in the perichondrium)
- Cartilage repair is possible only in children, in adults is very limited, due to avascularity and low metabolism.


Hyaline

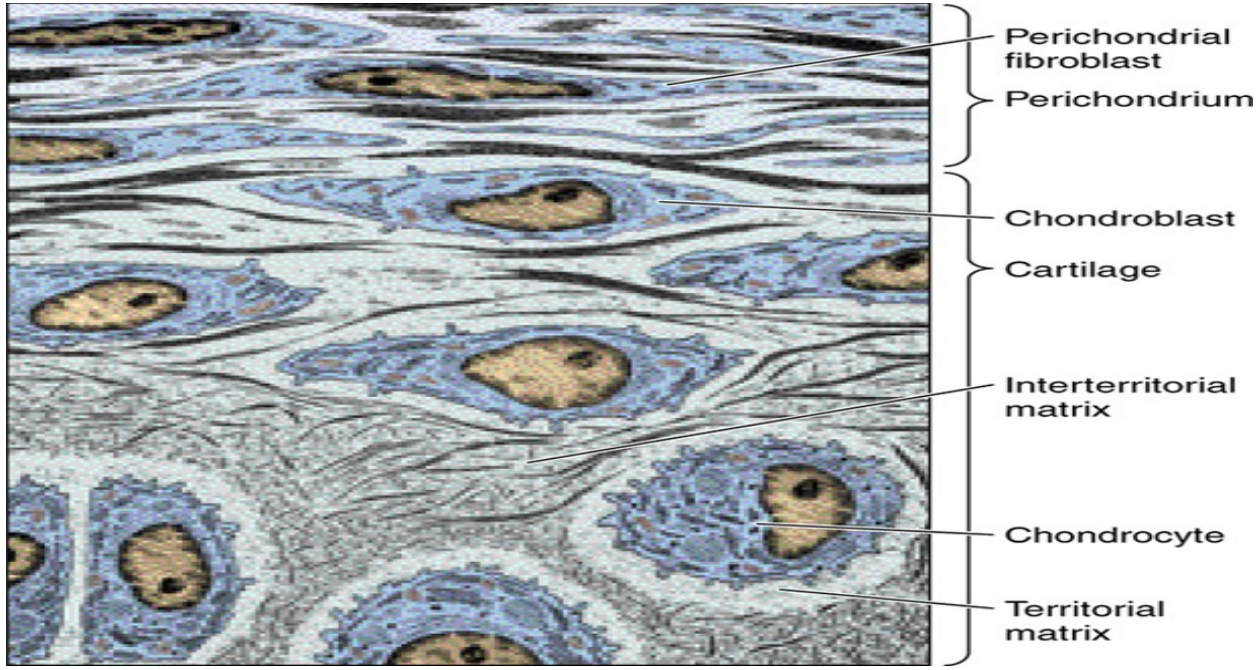

Fibrocartilage


Elastic





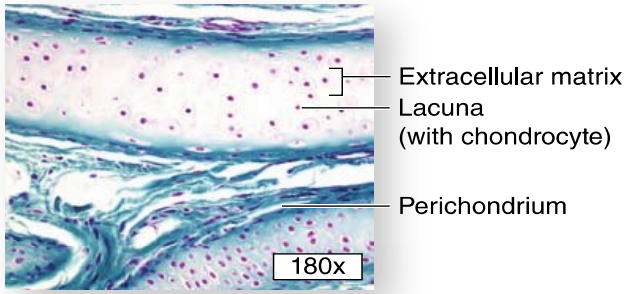
Chondrogenesis



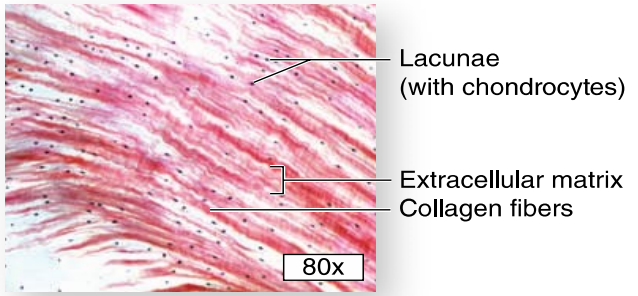
Cartilage

Types of cartilage:

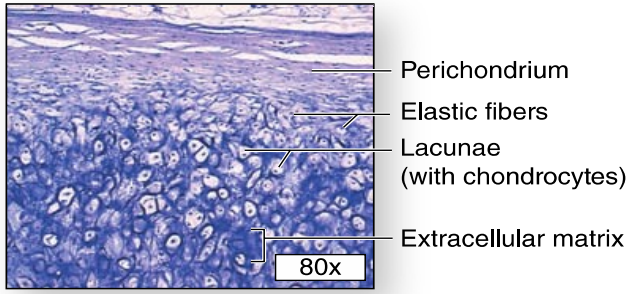
- Hyaline: most common, located in the articular surface, in certain parts of the respiratory system, junction of ribs and sternum, and epiphyseal plates of long bones (make possible bone growth).
- Fibrocartilage: transitional type between dense connetive tissue and cartilage, typically found in intervertebral discs, also contains fibroblasts, cells are axially arranged
- Elastic: similar to hyaline, but more flexible, contain an abundant network of elastic fibers, more resistant to degenerative processes, in auricule of the ear, external auditory canal, epiglottis, Eustachian tube.



b Hyaline cartilage



c Fibrocartilage



d Elastic cartilage

Bone

The intercellular mass is calcified by an inorganic compound – calcium hyproxyapatit is the most abundant.

Cells

- Osteocytes: in lacunae, between bone matrix layers (lamelle), have cytoplasmatic processes
- Osteoblasts: produce the organic component of the intercellular matrix and control the process of mineralization
- Osteoclasts: large multinucleated cells involved in removing bone matrix and remodelation of bone tissue

Periosteum:

on the outside, dense connective tissue. Bundles of collagen fibers called Sharpey fibers penetrate the bone and bind the periosteum tightly to the bone.

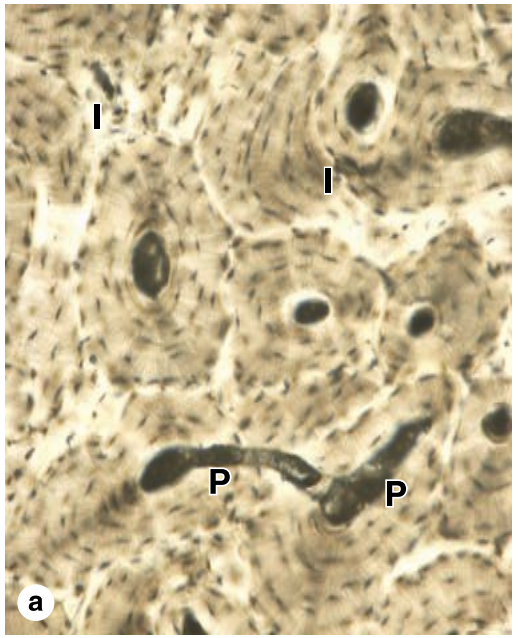
The inner layer contains osteoprogenitor cells, with the potential to divide and differentiate into osteoblasts. Important in fracture healing.

Endosteum:

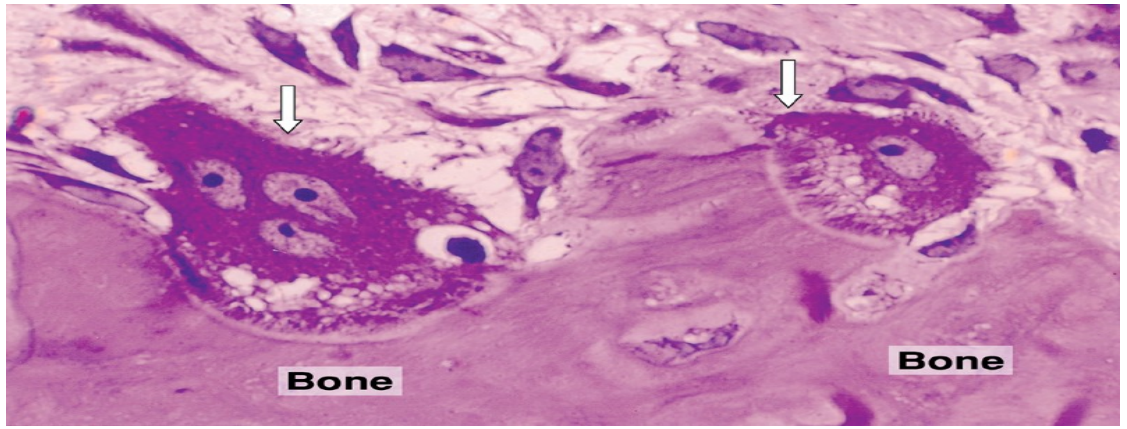
thin layer of osteoprogenitor and covering cells and a small amount of collagen fibers

Bone

Bone can be observed as groung bone (after grinding) or on decalcified, stained sections.
Decalcification is realized by maceration of the bone in acid.

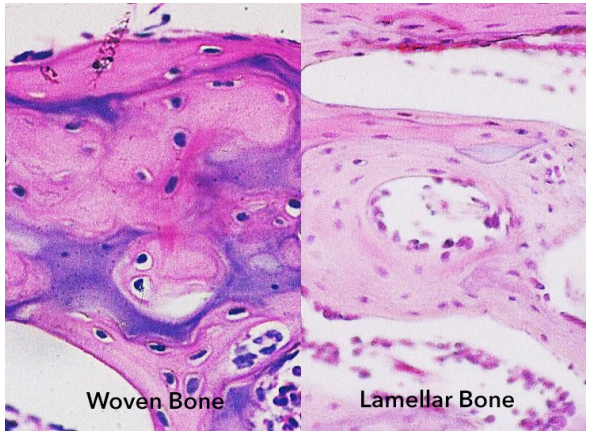


P - Volkmann canals
I – Intersticial lamellae



Arrows indicate osteoclasts

Types of bone



- Woven (Fibrous) bone:

random disposition of colagen I fibers and lover mineral content

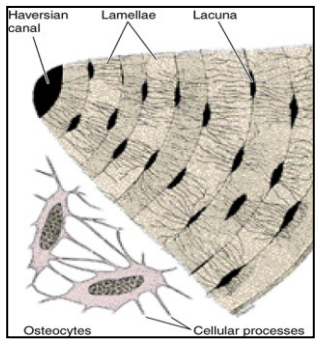
it appears first during embryonic development and during reparative processes, and is then replaced by lamellar bone

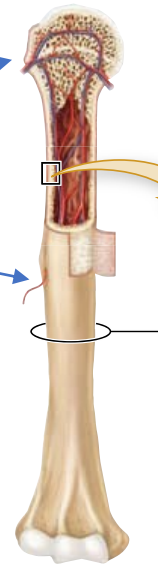
- Lamellar bone:

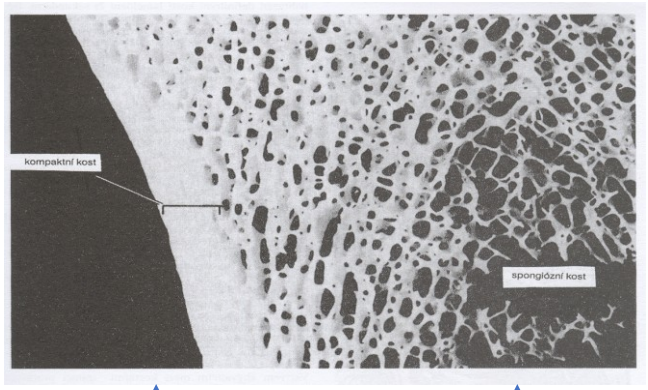
consists of lamellae 3-7 µm in size, organized as:

parallel sheets - cancellous or spongy bone

concentrically around a central canal – compact bone



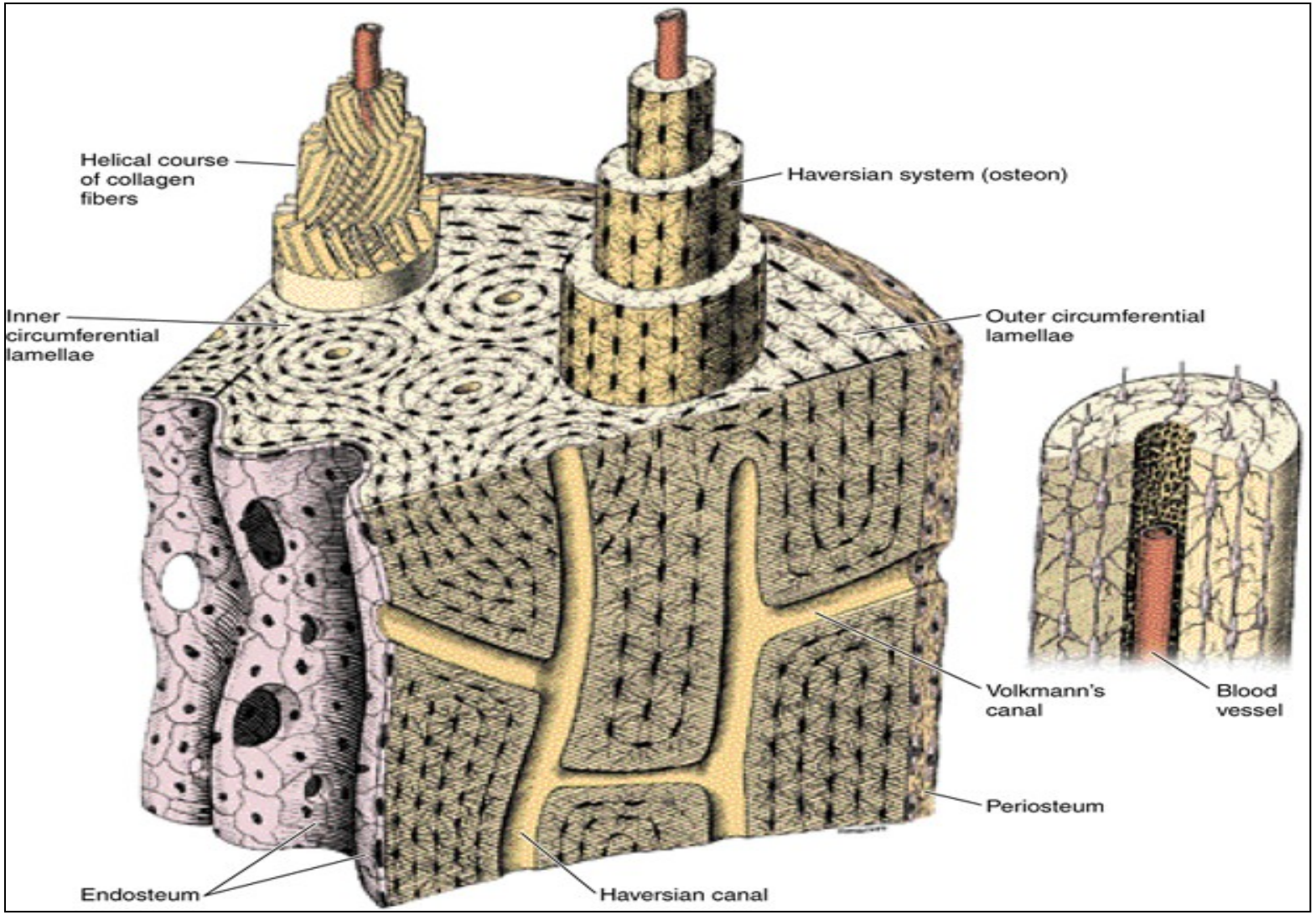




Compact bone

Spongy bone

Compact bone structure

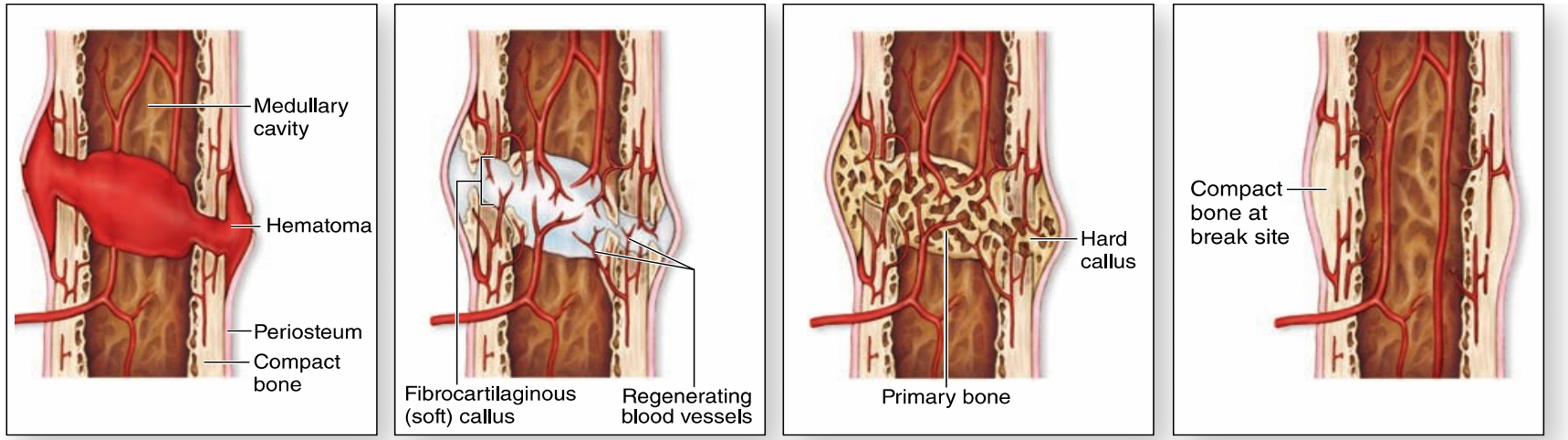


Summary of bone types

Type of Bone	Histological Features	Major Locations	Synonyms
Woven bone, newly calcified	Irregular and random arrangement of cells and collagen; lightly calcified	Developing and growing bones; hard callus of bone fractures	Immature bone; primary bone; bundle bone
Lamellar bone, remodeled from woven bone	Parallel bundles of collagen in thin layers (lamellae), with regularly spaced cells between; heavily calcified	All normal regions of adult bone	Mature bone; secondary bone
Compact bone, ~80% of all lamellar bone	Parallel lamellae or densely packed osteons, with interstitial lamellae	Thick, outer region (beneath periosteum) of bones	Cortical bone
Cancellous bone, ~20% of all lamellar bone	Interconnected thin spicules or trabeculae covered by endosteum	Inner region of bones, adjacent to marrow cavities	Spongy bone; trabecular bone; medullary bone

Bone

Fracture healing: osteoprogenitor cells in the periosteum and endosteum are important in fracture healing. First, an undifferentiated highly cellular mass is formed (callus). Callus is similar to fibrocartilage. Blood vessels grow into this tissue and osteoblasts differentiate. Then woven bone is formed, which is then remodeled into lamellar type bone.



(a) A fracture hematoma forms.

(b) A fibrocartilaginous (soft) callus forms.

(c) A hard (bony) callus forms.

(d) The bone is remodeled.

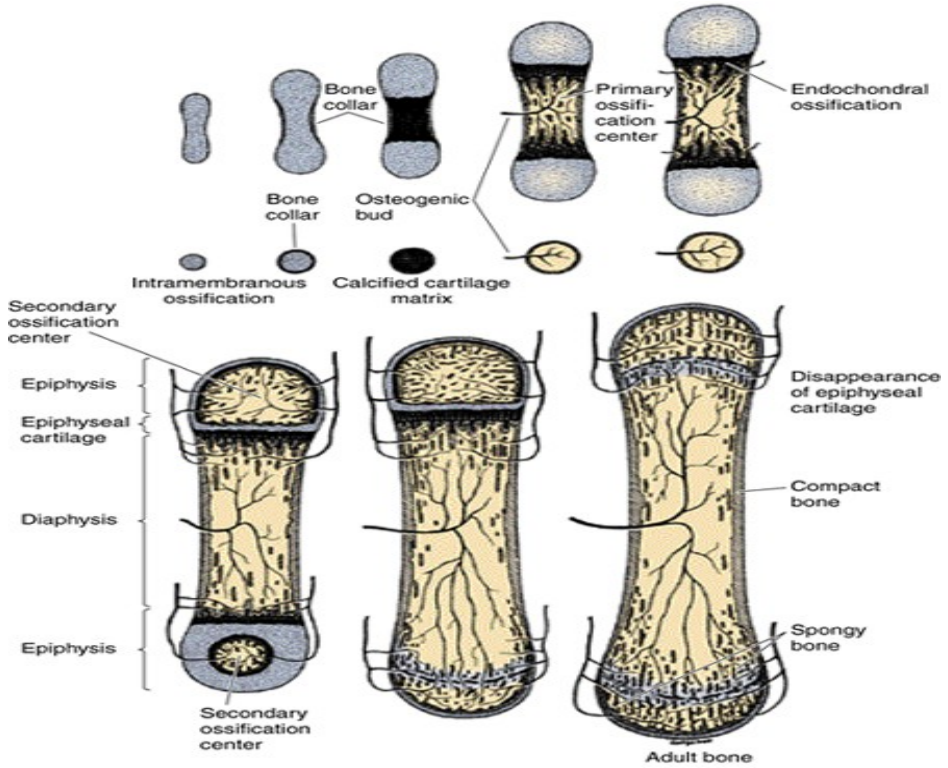
Ossification

Bone development occurs by one of two processes:

- Intramembranous ossification – osteoblasts differentiate directly from mesenchyme
- Enchondral bone develops from the hyaline cartilage that forms the base of the bone

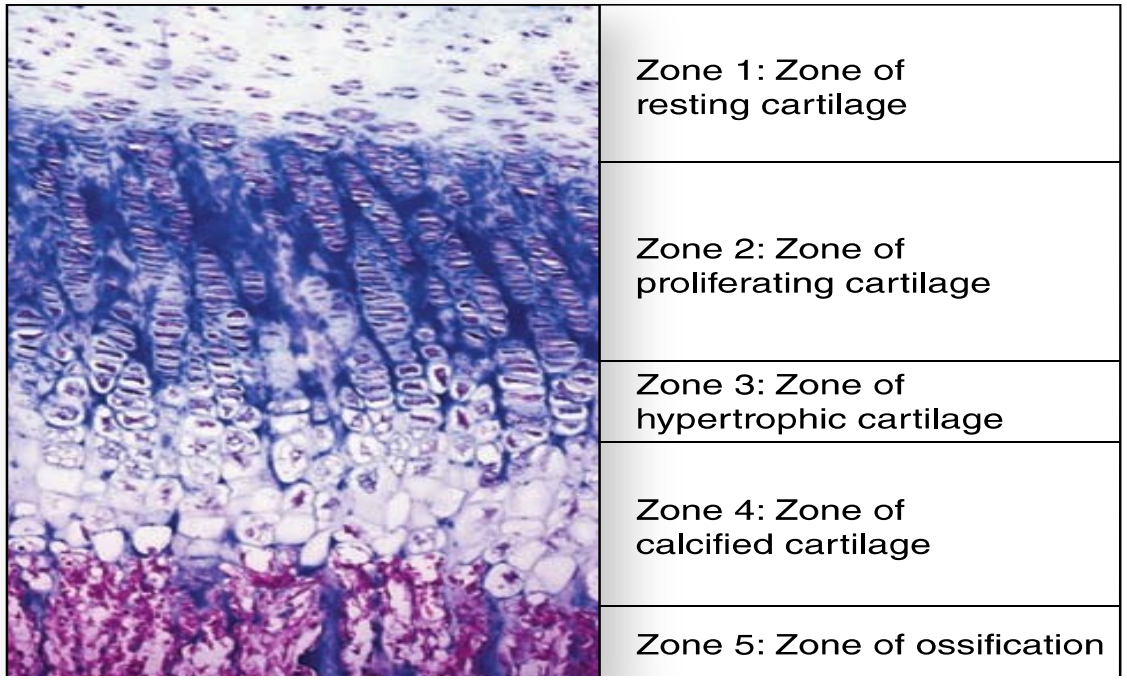
This process has several steps:

First a bone model is created from cartilage. Further, a bone collar and a primary ossification center in the diaphysis are formed. Cartilage in this place begins to degenerate due to lack of oxygen and nutrients. Cavities are created in it, into which blood vessels and osteoprogenitor cells from the periosteum penetrate. These differentiate into osteoblasts and form the intercellular bone mass. In the same way bone is formed in secondary ossification centers in the epiphyses. In this way, primary bone (woven) is created, which is later remodeled into a secondary type.



Epiphyseal plate

The area between the diaphysis and the epiphysis, where even after birth there is cartilage and bone growth takes place here.
At the same time the cartilage is transformed into bone.
Cartilage remains here, until human growth is completed. This cartilage gradually ossificated in the same way as ossification occurs in embryonic development. At the same time, bone growth takes place in the longitudinal direction by the division of chondrocytes. We can therefore study the ossification process on this plate even after birth.



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