MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION
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OF HIGHER EDUCATION N.I. PIROGOV RUSSIAN NATIONAL
RESEARCH MEDICAL UNIVERSITY

**Department of Histology, Embryology and Cytology at the Faculty of General Medicine**

# ESSENTIAL TEXTBOOK for practical classes in HISTOLOGY:

## tissues of the internal environment, muscle tissues, nervous tissue

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Essential textbook for practical classes in histology: tissues of the internal environment, muscle tissues, nervous tissue

The textbook contains essential guidelines for the students training in general histology under curricula of General Practice, Pediatrics and Dentistry.

## Contents

# Topic 1. TISSUES OF THE INTERNAL ENVIRONMENT. BLOOD SYSTEM

**Class objective:**

1. Gain understanding of the blood system and the blood as a tissue.
2. Learn morphology and functions of the formed elements of the blood and the lymph.
3. Identify erythrocytes, blood platelets and different types of white blood cells in a blood smear.
4. Learn the blood cell lineages and hematopoietic differons.

## *Task 1. Tissues of the internal environment (general overview)*

***Tissues of the internal environment*** have common features:

- Extracellular matrix predominates in volume over the cells;
- The cells are located apart from each other;
- These tissues usually regenerate well;
- They develop from mesenchyme (not necessarily of mesodermal origin).

*Classification of tissues of the internal environment is shown in Figure 1.*

***Tissues of the internal environment*** implement the following functions:

- support metabolic processes;
- maintain tissue homeostasis;
- provide immune defense.

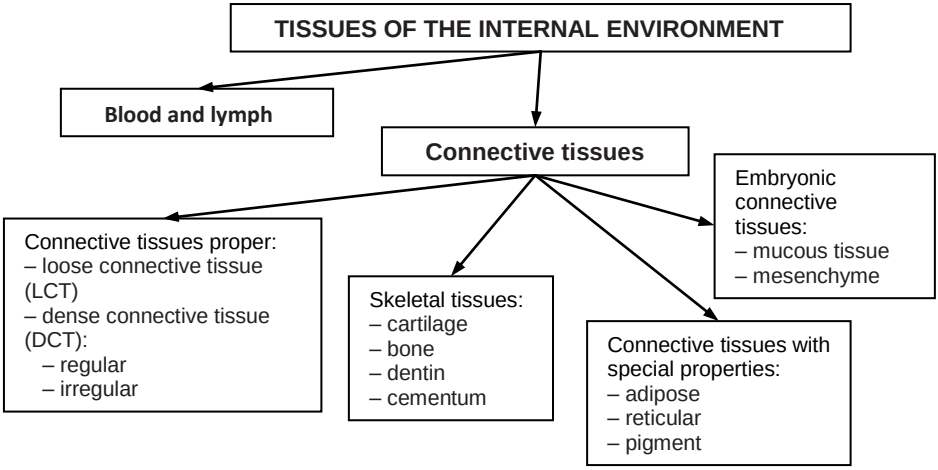


**Figure 1. Tissues of the internal environment**

**Exercises**

Answer the questions:

1. Give examples of tissues of the internal environment.
2. What are their common features?
3. At what locations in the body are they found?
4. From what embryonic sources do they develop?

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***Task 2. Blood system***

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***Blood system*** includes the blood and hematopoietic organs (red bone marrow, thymus, spleen and lymph nodes). It also includes diffuse lymphoid tissue found in other organs. All organs and structures of the blood system develop from mesodermal mesenchyme (except the thymus).

The blood constitutes 6–8% of the body weight, which corresponds to 4–6 liters in an adult. Up to 1.5 liters of the blood can be deposited in spleen, liver, intestines, lungs and venous plexuses.

***Functions of the blood***:

1) Blood is engaged in transport of substances with circulation, including transport of $O_2$ and $CO_2$ (*respiratory function*), nutrients (*trophic function*), hormones and other biologically active substances (*regulatory function*) and metabolic products (*excretory function*);
2) Blood participates in the maintenance of homeostasis (the constancy of the internal environment of the body) by regulation of osmotic equilibrium, acid-base balance and body temperature;
3) Blood and its system provide immune defense. They ensure innate immune responses, blood coagulation processes and adaptive immunity (cell-mediated and humoral).

***Functions of hematopoietic organs***:

1) maintenance of circulating formed elements (proliferation and differentiation of blood cell lineages, elimination of dead and damaged formed elements);
2) maintenance of immune homeostasis;
   - defense against microorganisms and foreign particles;
   - immune surveillance over own cells of the body;
3) temporary blood storage.

**Exercises**

1. Answer the questions:
   - Name components of the blood system.
   - What are the functions of hematopoietic organs?
2. List the functions of the blood — *Exercise № 39 in the Workbook*.

## *Task 3. Composition of the blood*

***Blood*** is a liquid tissue which circulates in blood vessels. It consists of *plasma* (55–60% by volume) and *formed elements*.

Volume percentage of formed elements in circulating blood is called *hematocrit* (abbreviated Ht or Hct). Reference ranges for Hct are 0.40–0.48 (40–48%) in men and 0.36–0.42 (36–42%) in women. Decreased Hct values indicate anemia; increased Hct values may indicate dehydration.

***Blood plasma*** is a liquid extracellular matrix of the blood. It is 90% water and its normal pH is 7.36. Dry mass of the plasma (7–10%) includes 6–8.5% of *proteins* and 1–3.5% of other organic and mineral compounds (*carbohydrates, lipids, salts* and *microelements*).

*Plasma proteins* include albumins, globulins and fibrinogen.

1) *Albumins* (approx. 60% of plasma proteins):
   - ensure oncotic pressure (colloid osmotic pressure) of the plasma;
   - bind and transport bilirubin, cholesterol, fatty acids, bile acid salts and other substances including pharmaceuticals;
   - provide a source of amino acids for nutrition.
2) *Globulins* (20–30% of plasma proteins):
   - $\gamma$ globulins (immunoglobulins) are engaged in immunity as *antibodies*;
   - $\alpha$ and $\beta$ globulins bind and transport lipids, hormones, vitamins and microelements.
3) *Fibrinogen* (4–8% of plasma proteins):
   - glycoprotein complexes transformed into insoluble *fibrin* during blood coagulation.

Defibrinated blood plasma (i.e. artificially depleted of fibrinogen under controlled laboratory conditions) is called *serum*.

*Erythrocyte sedimentation rate* (abbreviated ESR) reflects (a) protein composition of the plasma and (b) electric charge at the surface of erythrocytes. Reference ranges for ESR are 1–10 mm/h in men and 2–15 mm/h in women. ESR is increased in inflammatory, infectious and oncological diseases.

***Formed elements of the blood*** include *erythrocytes* (red blood cells), *leukocytes* (white blood cells) and *thrombocytes* (blood platelets).

***Hemogram*** (complete blood count, CBC, or blood index) is absolute numbers of formed elements per 1 liter of blood. Adult reference ranges for hemogram are:

1) erythrocytes $(3.9–5.5)\times10^{12}$/L in men and $(3.7–4.9)\times10^{12}$/L in women,
2) leukocytes $(4–9)\times10^{9}$/L,

3) thrombocytes (180–320)×$10^9$/L.

Pediatric reference ranges for hemogram are different. In newborns, they constitute:

1) erythrocytes (6–7)×$10^{12}$/L,
2) leukocytes (10–30)×$10^9$/L,
3) thrombocytes (200–300)×$10^9$/L.

By the age of 2 weeks, *erythrocyte* counts decrease to 'adult' values (approx. 5.0×$10^{12}$/L) and continue to decrease to reach the minimum of (3.1–4.5)×$10^{12}$/L at the age of 3–6 months (physiological anemia). After that, erythrocyte counts increase gradually to reach adult values by the age of puberty.

*Leukocyte* counts undergo age-related changes as well. By the age of 2 weeks, leukocytes drop to (9–15)×$10^9$/L (physiological leukopenia). Leukocyte counts reach adult levels by the age of 14–15 years.

## Exercises

Answer the questions:

1. Decreased pH of blood plasma (increased acidity of the plasma, acidosis) is a hallmark of certain pathologies. Which function of the blood is impaired? Which systems are responsible for the defect?
2. Analyze and interpret hemogram of a 47-year-old male patient:
   - erythrocytes 2.7×$10^{12}$/L;
   - leukocytes 6.8×$10^9$/L;
   - thrombocytes 129×$10^9$/L.
3. What contains more fibrinogen – plasma or serum?
4. Specify the type(s) of cell populations constituted by formed elements of the blood.

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### *Task 4. Erythrocytes and platelets*

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Erythrocytes (a.k.a. *red blood cells*, RBC) and thrombocytes (blood *platelets*) are post-cellular structures. ***Erythrocytes*** (a.k.a. red blood cells) are formed in the red bone marrow as a result of multistep process called *erythropoiesis*. Senescent or unfit erythrocytes are eliminated from circulation in the spleen, and also in the liver and red bone marrow. Each erythrocyte lives 100–120 days (from maturation to senescence).

Erythrocytes implement respiratory function by transporting $O_2$ and $CO_2$. They also participate in trophic, excretory, defense and regulatory functions of the blood by adsorbing and transporting antibodies, toxins and amino acids.

About 75% of red blood cells in a smear are *normocytes* — mature biconcave

erythrocytes 7–8 μm in diameter. The rest are *microcytes* (smaller than 6.0 μm, 12.5%) and *macrocytes* (bigger than 9.0 μm, 12.5%).

Abnormal size of erythrocytes (abnormal percentage of red blood cells of particular size) is called *anisocytosis*.

In adults, the blood contains *reticulocytes* — the under-differentiated precursors of erythrocytes. Reticulocytes contain *reticulofilamentous substance* composed of degrading Golgi apparatus, mitochondria, ribosomes and centriole; it can be revealed in reticulocytes by special staining. Reticulocytes normally constitute 0.2–1% of RBC (=2–10‰ *per mille*, parts per thousand, which corresponds to 2–10 reticulocytes per 1000 erythrocytes). Increased content of reticulocytes in the blood is typically associated with bleedings and hemolytic anemias. In newborns, *physiological anisocytosis* is observed, with prevalence of macrocytes, increased content of reticulocytes and occasional presence of the nucleated RBC precursors.

The shape of erythrocytes in a smear varies; 80–90% of erythrocytes in a normal smear are biconcave (*discocytes*). The aging forms may also be encountered in a smear, including spiculated (*echinocytes*, 6%), domed (*stomatocytes*, 2%) and spherical (*spherocytes*, 1%). The prevalence of abnormally shaped RBC in blood smears (characteristic of certain diseases) is called *poikilocytosis*. For instance, sickle-cell anemia (an inherited condition) is manifested by sickle-shaped erythrocytes.

Plasmalemma of erythrocytes is flexible and strong. It is also stretchable and resistant to many damaging factors. It carries Rhesus antigens (*Rh*), blood-group determinants and a variety of surface *receptors* to a wide range of proteins and chemicals. Its *integral* and *peripheral* proteins facilitate transport of substances across the membrane (ionic pumps, ionic channels, etc.) and secure the attachment of cytoskeletal elements. Erythrocyte plasmalemma contains 15 major proteins; over 60% of their mass is constituted by *spectrin, glycophorin, band 3, band 4.1* and *ankyrin* (Figure 2).

*Glycophorin* is a transmembrane receptor glycoprotein. Its oligosaccharide chains with sialic acid residues accumulate negative electrical charge.

*Band 3* is a transmembrane glycoprotein as well. Working as chloride-bicarbonate anion exchanger, it facilitates transportation of $CO_2$ by RBC.

*Spectrin* is the main cytoskeletal protein of erythrocytes. Its fibrils consist of α and β chains. Spectrin network maintains the shape of erythrocytes and withstands high pressure upon their passage through narrow capillaries. Spectrin forms nodal protein complexes with *actin filaments* and *band 4.1* protein, which links the spectrin network to *glycophorin*, whereas *ankyrin* links it to *band 3*.

The *cytoplasm of RBC* is oxyphilic and contains no organelles. It consists of water (60%), of *hemoglobin* (38%) and trace amounts of other substances (glucose, ATP and enzymes).

*Hemoglobin* (Hb) is the respiratory protein of the blood classified as chromoprotein. Hemoglobin has characteristic quaternary structure composed of polypeptide portion (*globin*) and the iron-containing non-protein portion (*heme*). In lungs, $O_2$ is added to the heme iron, which gives *oxygemoglobin*. In tissues, $O_2$ is abstracted from oxyhemoglobin, which is transformed back into the *reduced form* of hemoglobin. Globin chains of the reduced form can bind $CO_2$ molecules giving *carbaminohemoglobin*. Different types of hemoglobin include HbE (HbP), HbF, and notably HbA, which predominates in adults. The presence of small amounts of HbF (≤ 1.5% of the total Hb) in adults is normal.

Reference ranges of *hemoglobin concentration* are 130–160 g/L in men and 120–140 g/L in women. Reduced blood hemoglobin (*anemia*) may reflect a decrease in erythrocyte counts (*erythropenia*). It may also reflect low content of Hb in erythrocytes. Destruction of red blood cells within the circulation is called *hemolysis* (does not occur normally). Hemolysis results in leakage of hemoglobin into the plasma.

***Thrombocytes*** (blood *platelets*) are small non-nucleated fragments of *megakaryocytes* (giant precursor cells which reside in the red bone marrow).

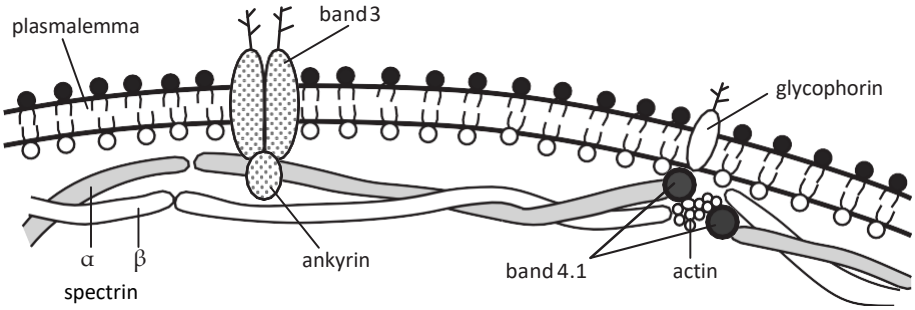


**Figure 2. Erythrocyte plasmalemma [1].**

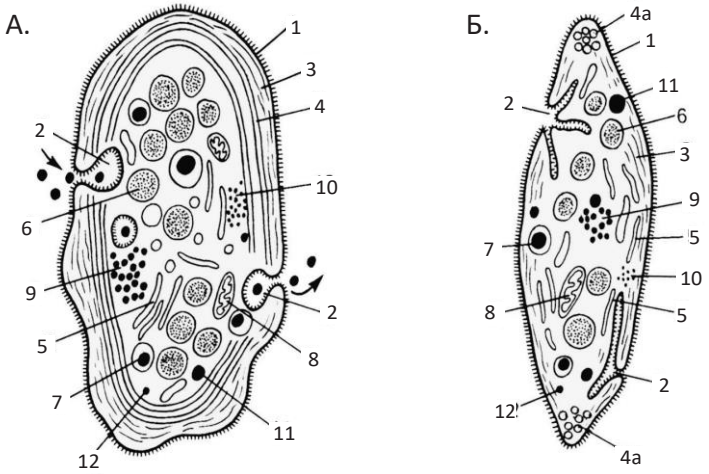


**Figure 3. Ultramicroscopic structure of thrombocytes [2].**
A — longitudinal section; B — transverse section.
1 — plasmalemma; 2 — open canalicular system; 3 — actin filaments; 4 — circular bundles of microtubules; 4a — cross-sectioned microtubules; 5 — closed canalicular system; 6 — α-granules; 7 — δ-granules; 8 — mitochondria; 9 — glycogen granules; 10 — ferritin granules; 11 — lysosomes; 12 — peroxisomes.

*Platelets* are 2–4 μm oval discs. Up to 70% of them circulate with the blood, the rest are stored in the spleen. A platelet lives 8 days.

The function of platelets is to prevent (alleviate) bleedings by releasing vasoconstrictors, forming temporary plugs and promoting coagulation.

A platelet is enclosed in plasmalemma covered by a thick layer of glycocalyx with numerous receptors which mediate *adhesion* and *aggregation* of platelets in the case of blood vessel damage. The content of a platelet consists of the transparent outer portion (*hyalomere*) and granular central portion (*granulomere*, Figure 3).

Hyalomere contains two distinct *canalicular systems* and cytoskeletal elements. Open canalicular system is connected to invaginations of the platelet membrane and ensures transport of nutrients and metabolites. Closed canalicular system, which consists of thin membranous channels filled with electron-dense content, ensures storage and release of calcium ions and prostaglandins required for blood coagulation.

Cytoskeleton of platelets consists of microtubules, microfilaments and intermediate filaments. Microtubules are located at the periphery in the form of a ring (*marginal annulus*) and maintain the shape of a platelet. Peripheral portion of the cytoplasm also contains actin, myosin, gelsolin and other contractile proteins which participate in the platelet contraction and clot retraction.

*Granulomere* contains mitochondria, glycogen inclusions, ribosomes, small cisternae of granular endoplasmic reticulum and Golgi apparatus. Most notably, granulomere contains granules.

The granules are classified as follows:

1) *α-granules* (alpha) are the largest granules (300–500 nm) filled with the matrix of moderate density. Contain blood clotting factors and proteins including fibrinogen, fibronectin and factor V;
2) *δ-granules* (delta) are granules of medium size (250–300 nm) with dense cores. Contain ATP, $Ca^{2+}$, serotonin and histamine;
3) *λ-granules* (lambda) are *lysosomes* and *peroxisomes*; they are small (200–250 nm) and contain lysosomal enzymes and peroxidase, respectively.

## Exercises

Solve the problems:

1. Deficiency of what chemical element is implicated in the development of anemia during pregnancy?
2. A patient is at increased risk of thrombosis. Which formed elements of the blood are implicated in the condition?

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### *Task 5. Leukocytes*

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***Leukocytes*** (a.k.a. *white blood cells*, WBC) are fully fledged cells. Leukocytes implement their functions in loose connective tissue (where they end up after exiting the circulation). Leukocytes are subdivided into *granular leukocytes* (granulocytes) and *agranular leukocytes* (agranulocytes). Mature *granulocytes* have segmented nuclei and characteristic granular cytoplasm, whereas *agranulocytes* have non-segmented nuclei and homogeneous cytoplasm.

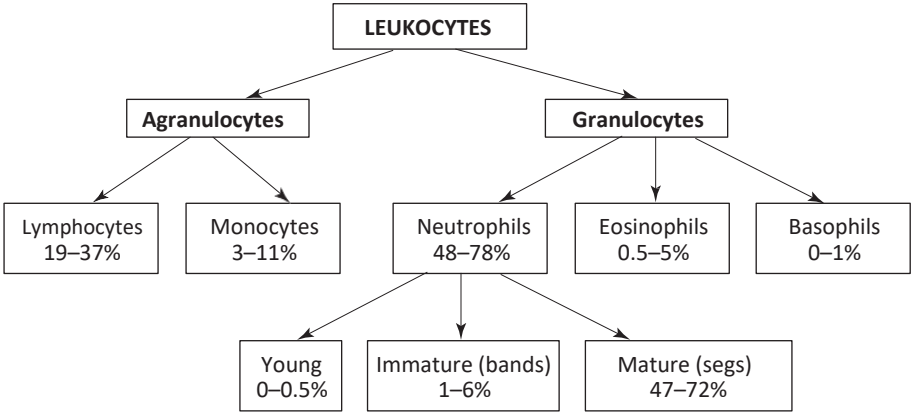


**Figure 4. Leukocyte formula**

*Leukocyte formula* (a.k.a. WBC differential, or leukogram, or leukocyte percentage index) reflects proportions of different types of WBC (Figure 4). Pediatric values for leukogram are different. Newborns have 65% neutrophils and 25% lymphocytes; by the age of 4–5 days, the proportions of neutrophils and lymphocytes converge. This effect is known as the *first physiological WBC crossover*. The proportion of lymphocytes continues to increase. Toddlers (1–2 year-olds) have 65% lymphocytes and 25% neutrophils in leukogram. Subsequent decrease in the proportion of lymphocytes and increase in the proportion of neutrophils leads to the *second physiological WBC crossover* at the age of 4 years. Gradual decrease in the proportion of lymphocytes and concomitant increase in the proportion of neutrophils continue till puberty.

*Granulocytes* are distinguished by staining of cytoplasmic granules. In *eosinophilic* (acidophilic) granulocytes, the granules are stained with eosin (bright pink, occasionally orange-red). In *basophilic* granulocytes, the granules are stained with basic dyes (deep blue or purple). *Neutrophilic* granulocytes show very fine granularity, a mixture of pink and purple, as their secondary granules are stained with both acidic and basic dyes. The granules of granulocytes differ by their size and ultrastructure (Figure 5). All granulocytes are capable of phagocytosis (neutrophils especially). All granulocytes contain *primary azurophilic granules* — these are lysosomes which contain lysosomal enzymes, myeloperoxidase and antimicrobial proteins.

Neutrophilic leukocytes (***neutrophils***) are subdivided into mature cells with *segmented* nuclei ('segs'), immature cells with *non-segmented* nuclei ('bands') and *young* neutrophils.

Mature neutrophils with segmented nuclei (*segs*) have condensed nuclei which consist of 2–5 irregularly shaped segments connected by thin junctions. In blood smears of female patients, one of the segments may have a projection in the shape of a drumstick, which corresponds to the permanently inactivated X chromosome (Barr body). Mature neutrophils are packed with tiny granules — these are predominantly specific (*secondary*) granules which contain lysozyme (destroys bacterial cell walls), lactoferrin (ensures binding of the bacteria), certain complement activators and destructive enzymes (collagenase, phospholipase).

Immature neutrophils (*bands*) have non-segmented nucleus resembling curved stick or band. *Young* neutrophils have more rounded nuclei.

In leukocyte formula, the percent of young neutrophils is conventionally written on the left, the percent of bands in the middle, and the percent of segs on the right. Accordingly, increased proportion of immature forms of neutrophilic granulocytes in a blood smear is called *'left shift'*. Left shift is usually indicative of enhanced hematopoiesis often related to acute inflammatory processes. Acute inflammation is associated with enhanced recruitment of young neutrophils and bands from the red bone marrow to circulation. Neutrophils actively participate in acute inflammatory reactions and kill bacteria by phagocytosis.

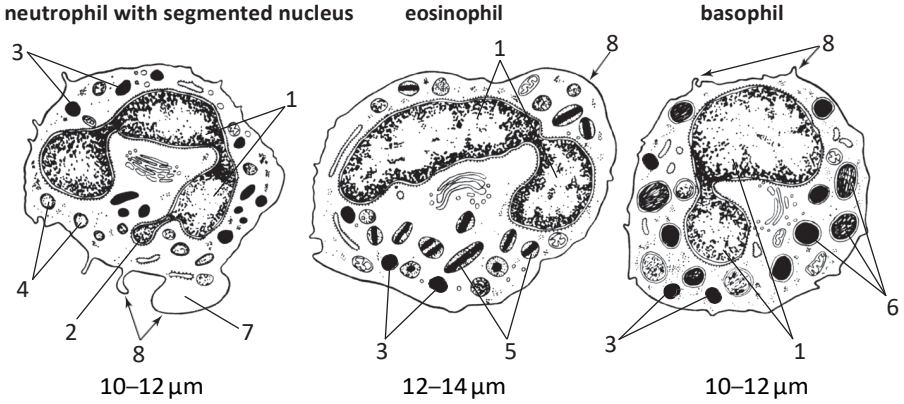


**Figure 5. Structure of granulocytes [2].**
1 — segments (lobes) of the nucleus; 2 — Barr body (a drumstick); 3 — non-specific granules (primary, azurophilic); 4 — specific granules of neutrophils; 5 — mature specific granules of eosinophil showing crystalloid bodies; 6 — specific granules of basophil (of variable size and density); 7 — peripheral zone (organelle-free); 8 — pseudopodia.

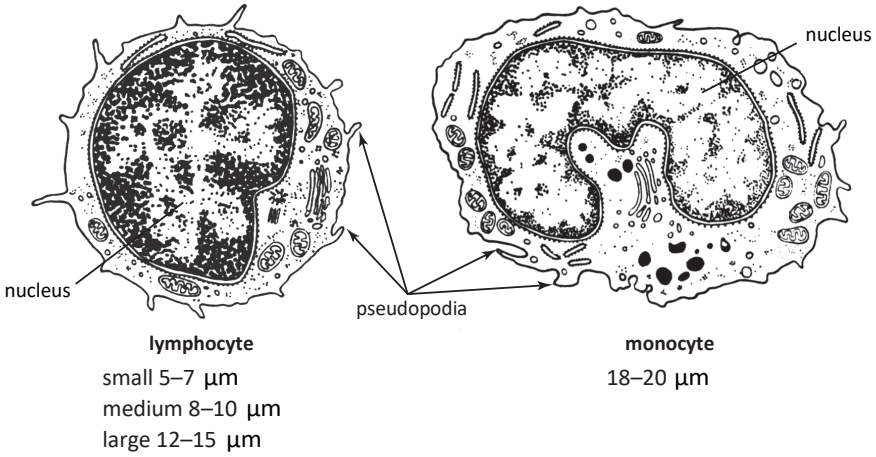


**Figure 6. Structure of agranulocytes [2]**

***Eosinophils*** have *bilobed* nuclei (consisting of two segments). Their cytoplasm is packed with specific eosinophilic granules (stained red with acidic dyes). Eosinophilic granules contain major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin and enzymes (peroxidase, histaminase, lysosomal hydrolytic enzymes), often in the form of electron-dense crystalloid bodies (not identifiable by light microscopy). Eosinophils are involved in allergic and inflammatory reactions; they also participate in elimination of parasites.

***Basophils*** are the least common WBC in a smear. Their cytoplasm is tightly packed with large metachromatic granules stained with basic dyes in deep blue, purple or magenta.

Basophils produce biologically active substances: heparin, histamine, prostaglandins, leukotrienes and serotonin. Under the action of allergens, specific granules of basophils merge with plasmalemma and the content is released into interstitium. This process, called *degranulation*, causes allergic reactions type I (the *immediate hypersensitivity reactions*). Degranulation of basophils stimulates blood flow, increases vascular permeability and facilitates mobilization of other granulocytes to the site of injury.

***Lymphocytes*** (Figure 6) are rounded cells with rounded, rather compact nucleus and a thin layer of cytoplasm containing azurophilic granules (lysosomes). Lymphocytes observed in smears can be small (5–7 μm in diameter), medium (8–10 μm) or large (11 μm and more).

Lymphocytes are subdivided into T lymphocytes (T cells), B lymphocytes (B cells) and normal killers (NK cells). Differentiation of T cells begins in the red bone marrow (with the rest of the formed elements) and continues in the thymus. Mature immunocompetent T cells are subdivided into cytotoxic T cells, T helpers, and regulatory T cells. Mature T cells are concentrated in lymph nodes and the spleen. They provide *cell-mediated immunity*: cytotoxic T cells kill genetically compromised cells (transplanted or transformed), whereas T helpers and regulatory T cells coordinate and modulate adaptive immune reactions.

*Humoral immunity* is a different branch of the adaptive immune reactions. The central role in humoral immunity is played by B lymphocytes. B lymphocytes (*B cells*) release specific proteins — immunoglobulins (a.k.a. γ-globulins, or *antibodies*) which specifically bind foreign molecules (*antigens*) and facilitate their elimination. Subpopulations of B cells and their derivatives include plasmocytes (*plasma cells* that produce antibodies) and memory B cells.

***Monocytes*** (Figure 6) are the largest leukocytes found in a smear. Their nuclei are less condensed than in lymphocytes and may have nucleoli. The cytoplasm is larger than the nucleus and contains small granules — lysosomes. Monocytes represent the *mononuclear phagocyte system*. They exit the circulation and differentiate into tissue macrophages which participate in immune reactions. Macrophages are capable of phagocytosis and antigen presentation; they produce of antibacterial substances and inflammatory signaling molecules. Under certain conditions, macrophages may also promote tissue repair.

All formed elements of the blood can be found in a blood smear (*Slide № 53*, azurII–eosin staining).

Most of the formed elements in a smear are *erythrocytes* stained pink with eosin. As erythrocytes are biconcave their central portion is thinner and therefore looks lighter. *Leukocytes* must be searched out carefully; the most common of them are *segs* recognized by deep-purple segmented nucleus and pale transparent cytoplasm of very fine, almost undistinguishable granularity. *Eosinophils* are packed with red granules; their nuclei are less condensed and usually bilobed (occasionally composed of three lobes or segments). *Basophils* are the least common formed elements. Basophils are tightly packed with metachromatic basophilic granules in various shades of deep blue and purple, which obscure the irregularly shaped nucleus.

*Lymphocytes* have round nucleus surrounded by a weakly basophilic rim of cytoplasm. The nuclei of lymphocytes are stained deep purple because the chromatin is mostly condensed. Small, medium and large lymphocytes are distinguished by their size and appearance of the nucleus. Small lymphocytes have condensed chromatin and a very narrow rim of cytoplasm. Medium lymphocytes have less condensed chromatin and a wider rim of cytoplasm. Large lymphocytes have loose nucleus and increased volume of cytoplasm.

*Monocytes* are large cells with a wide zone of light-blue cytoplasm and large, purple, irregularly shaped nucleus.

*Blood platelets* (thrombocytes) are small — about three times smaller than erythrocytes. In blood smears, they are stained pale-magenta and usually found in groups.

### Exercises

1. Slide № 53 *Human blood smear*. Examine the slide, draw formed elements and label the structures — *Exercise № 40 in the Workbook*.
2. *Ultrastructural organization of leukocytes*. Comprehend the scheme and label the structures — *Exercise № 41 in the Workbook*.
3. *Formed elements of the blood*. Complete the table — *Exercise № 42 in the Workbook*.

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### *Task 6. Hematopoiesis*

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***Hematopoiesis*** (a.k.a. ***haematopoiesis*** or ***hemopoiesis***) is the process of formation and replenishment of the formed elements of the blood. *Embryonic hematopoiesis* is development of the blood as a tissue. Postembryonic hematopoiesis is the process of physiological regeneration of the blood.

Development and differentiation of erythrocytes is called *erythropoiesis*. For granulocytes, the corresponding processes are called *granulocytopoiesis*, for platelets — *thrombocytopoiesis*, for monocytes — *monocytopoiesis*, for lymphocytes — *lymphocytopoiesis*, etc.

***Embryonic hematopoiesis*** can be subdivided into three main stages.

*Mesoblastic stage* begins with the emergence of blood cell progenitors in the mesenchyme of the *yolk sac wall*, amniotic stalk and chorion. These cells constitute the first generation of hematopoietic stem cells. In humans, mesoblastic hematopoiesis proceeds at the embryonic age of 2–8 weeks.

The next stage, *hepato-thymico-lienal*, begins at the embryonic age of 4–5 weeks. The central role in it is played by the liver — home for the second generation of hematopoietic stem cells. Hepatic hematopoiesis reaches its maximum at gestational age of 5 months and ends approximately at birth. Hematopoietic stem cells of hepatic origin colonize the thymus (the site of T lymphocytopoiesis), the spleen and the lymph nodes.

The final stage of embryonic hematopoiesis, *medullar*, is associated with emergence of the third generation of hematopoietic stem cells in the red bone marrow. Medullar hematopoiesis starts at the embryonic age of 9 weeks and comes into full force by full term. After birth, the red bone marrow becomes the sole central organ of hematopoiesis.

***Postnatal hematopoiesis*** (Figure 7) takes place in the red bone marrow and involves production of all formed elements of the blood. *Hematopoietic differons* comprise six classes of differentiating cells.

***Class I***, hematopoietic stem cells — *pluripotent* progenitors capable of differentiation into *any* blood cell lineage. Hematopoietic stem cells represent a self-sustaining population of high proliferative potential, which comes into action under certain stimuli (under normal conditions, hematopoietic stem cells divide rarely).

***Class II***, two types of *multipotent* progenitors derived from hematopoietic stem cells. One of them (*CFU-GEMM cells*, or *myeloid* progenitors) gives rise to granulocyte, erythrocyte, monocyte and megakaryocyte lineages. The second type of progenitors (*CFU-L cells*, or *lymphoid* progenitors) gives rise to lymphocyte lineages.

***Class III***, committed *oligopotent* and *unipotent* precursors (restricted *CFU-colony forming unit)* which differentiate from multipotent progenitors.

***Class IV***, precursors of particular blood lineages — *...blasts*.

***Class V***, differentiating cells which eventually lose proliferation capacity — *pro...cytes*. The process of maturation may involve several transitions between partially differentiated states. For example, *basophilic proerythrocyte* with abundant ribosomes engaged in hemoglobin synthesis eventually differentiates into *polychromatophilic proerythrocyte* and then *oxyphilic proerythrocyte*, which progressively lose the ribosomes and other organelles while accumulating hemoglobin. Granulocyte differons include *promyelocytes* with primary azurophilic granules, *myelocytes* with primary and secondary granules, and *metamyelocytes* which stop dividing and undergo condensation of the nucleus.

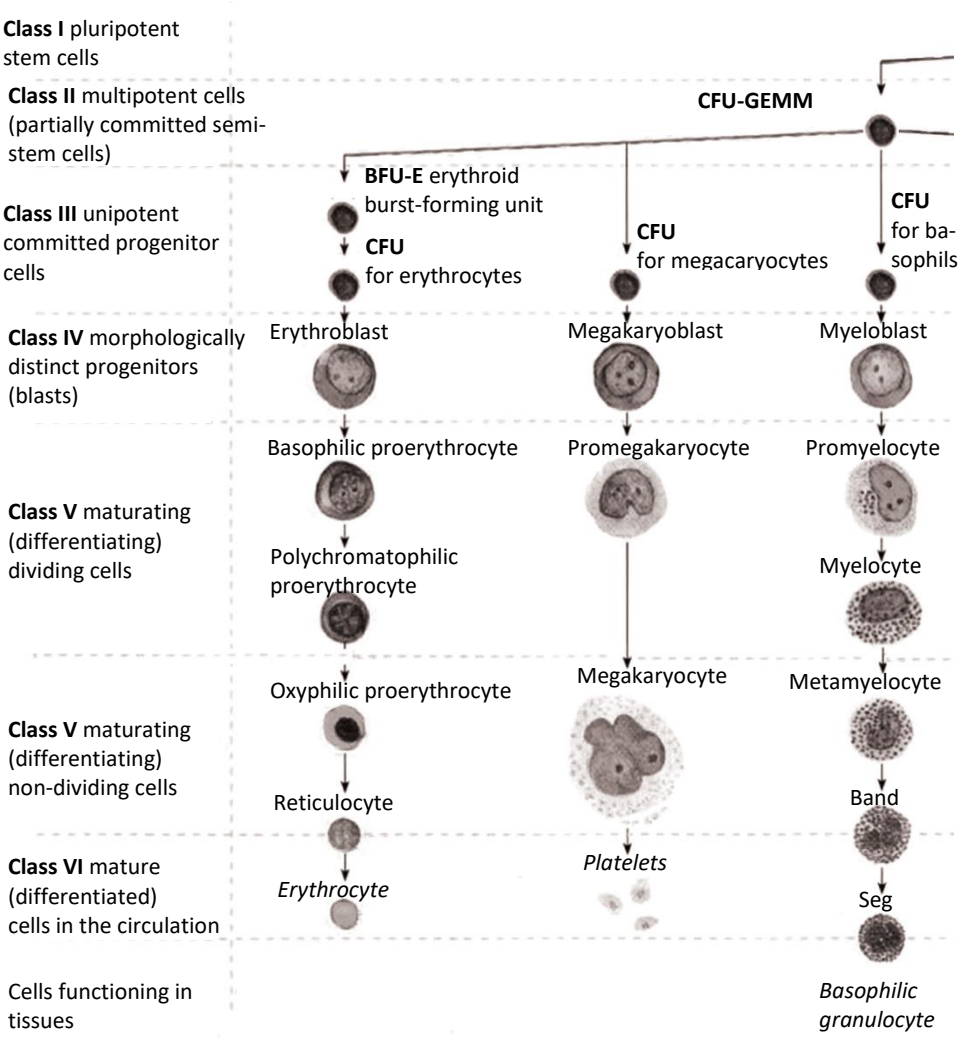
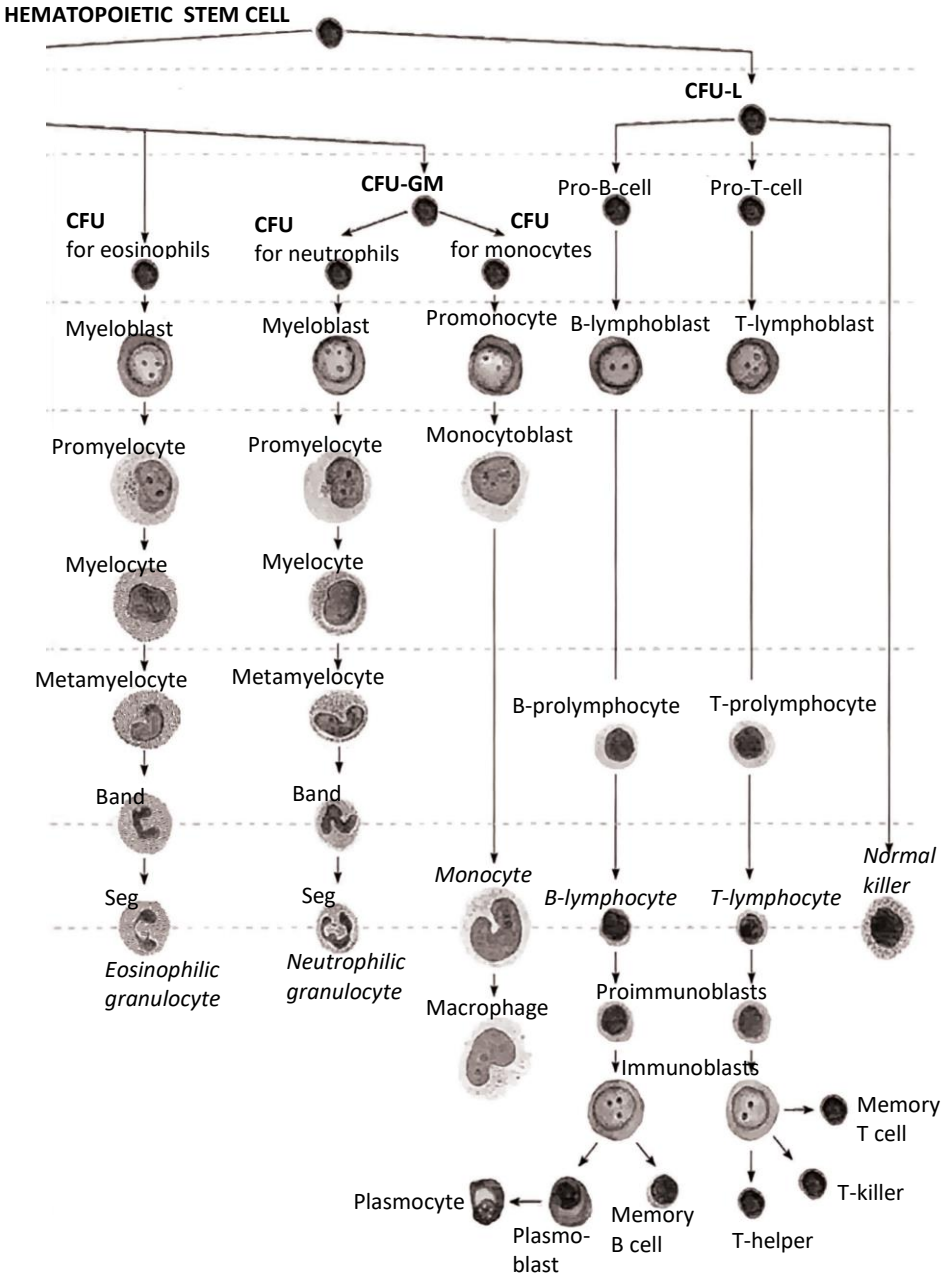



**Figure 7. Hematopoiesis [3]** *adapted with changes*.


HEMATOPOIETIC STEM CELL
CFU-L
CFU-GM
Pro-B-cell
Pro-T-cell
CFU
for eosinophils
CFU
for neutrophils
CFU
for monocytes
Myeloblast
Myeloblast
Promonocyte
B-lymphoblast
T-lymphoblast
Promyelocyte
Promyelocyte
Monocytoblast
Myelocyte
Myelocyte
Metamyelocyte
Metamyelocyte
B-prolymphocyte
T-prolymphocyte
Band
Band
Seg
Seg
Monocyte
B-lymphocyte
T-lymphocyte
Normal killer
Eosinophilic granulocyte
Neutrophilic granulocyte
Macrophage
Proimmunoblasts
Immunoblasts
Memory T cell
T-killer
Plasmocyte
Plasmo-blast
Memory B cell
T-helper

In the platelet differon, promegakaryocytes undergo polyploidization and their nuclei become lobular.

***Class VI*** are mature cells and post-cellular structures (*...cytes*) recruited from the red bone marrow to implement their functions elsewhere.

### Exercises

1. *Hematopoiesis*. Study the diagram, fill in the names of the classes and designate all cells and structures of hematopoietic differons — *Exercise № 43 in the Workbook.*

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### *Task 7. Lymph*

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***Lymph*** is a liquid tissue that flows in lymph capillaries and lymph vessels. The lymph is formed from interstitial fluids (which accumulate, because the efflux from blood microcirculation exceeds the re-absorption). Human body contains 1.5–2 liters of the lymph.

Lymph is composed of lymph plasma and formed elements. *Lymph plasma* has similar composition with the blood plasma, but contains lower levels of proteins (albumins, globulins, fibrinogen and enzymes). Lymph plasma also contains lipids, small organic molecules (amino acids, glucose, glycerol) and electrolytes. As the lymph contains fibrinogen, it is capable of coagulation.

Formed elements of the lymph are predominantly lymphocytes (90%); lymph also contains small numbers of monocytes, mature neutrophils, eosinophils, other cells and platelets. Total counts of formed elements in the lymph constitute $(5–35)\times10^9$/L. Red blood cells are typically absent from the lymph, unless the permeability of blood capillary walls is increased (in this case, red blood cells may get into the lymph).

Lymph that flows from different organs and tissues has specific composition that depends on metabolic and functional features of these tissues. For instance, the lymph that flows from the liver is rich in proteins, whereas the lymph flowing from the intestine after eating is rich in the absorbed emulsified fats. Upon passing the lymph nodes, the lymph becomes enriched with lymphocytes.

***Functions of the lymph*** are:

- maintaining constant composition and volume of interstitial fluids, returning proteins, electrolytes and water from peripheral tissues to circulating blood;
- regulatory —transport of hormones and other biologically active substances;
- metabolic — drainage of metabolic products;

- trophic — transport of digested nutrients, notably fats;
- defense — transfer of microorganisms, foreign particles and tumor cells from peripheral tissues to lymph nodes, where macrophages and lymphocytes are concentrated and plasma cells are formed. In lymph nodes, the lymph is cleared of damaged cells and foreign particles.

**Exercises**

Answer the questions:

1. Describe formation of the lymph and its circulation in the body.
2. What are its functions?
3. What is the cause of constant changes in its composition?

Homework: Connective tissues (connective tissues proper, connective tissues with special properties, embryonic connective tissues)

Pawlina W., Ross M.H. Histology: A text and Atlas: with Correlated Cell and Molecular Biology. Philadelphia etc.: Wolters Kluver: Lippincott Williams and Wilkins, 7-th edition, pp 156-193, 254-269

## Topic 2. CONNECTIVE TISSUES

**Class objective:**

1. Learn classification of connective tissues.
2. Learn structural and functional characteristics of their cellular component.
3. Learn principles of structural organization of the extracellular matrix (fibrous component and the amorphous ground substance).
4. Learn basic morphological characteristics of proper connective tissues and connective tissues with special properties.
5. Identify different connective tissues at the light microscopy level.

### *Task 1. General characterization of connective tissues*

***Connective tissues*** constitute a large group of tissues of mesenchymal origin (Figure 8). In musculoskeletal organs (tendons, ligaments, cartilages, bones) they carry basic functional load. In the walls of hollow organs, connective tissues work as scaffolds. In parenchymal organs, they constitute *stroma* which ensures compartmentalization and vascularization.

All connective tissues have:

- high content of extracellular matrix;
- discontinuous cellular component (the cells are located apart and typically lack permanent junctions);
- versatile functionality;
- mesenchymal origin.

***Functions of connective tissues***:

1) trophic — deliver nutrients to various tissue structures;
2) plastic — regulate the processes of tissue repair;
3) compartmentalization — provide general structural outline to the organs, modulate proliferation and differentiation of cells in various tissues;
4) mechanical — provide skeletal support for the body and fibrous scaffolds for many organs;
5) homeostatic —maintenance of stable internal environments, production of growth factors and paracrine substances;
6) metabolic — promote metabolite disposal;
7) defense — produce antimicrobials and inflammatory molecules, participate in immune reactions and debridement;
8) barrier — participate in the formation of blood-tissue barriers;
9) storage — serve as a depot of water, lipids and lipid-soluble substances (vitamins, hormones).

Connective tissues are composed of cells and extracellular matrix, which consists of *amorphous component* (a.k.a. ground substance) and *fibers* (collagen and elastic).

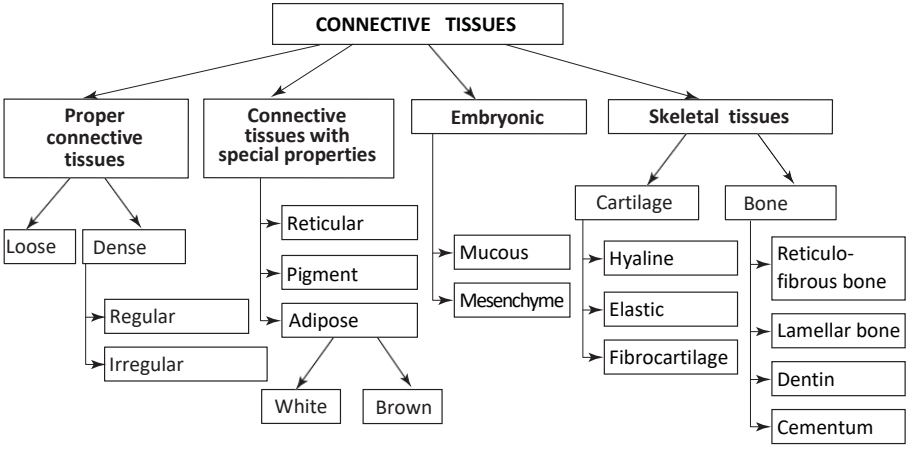


**Figure 8. Classification of connective tissues**

Classification of connective tissues reflects their cellular composition, characteristic types of intercellular interactions, abundance and composition of the ground substance, and certain functional properties.

## Exercises

Answer the questions:

1. Give examples of connective tissues.
2. What features do they have in common? In what respects do they differ?
3. What functions do they implement?

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### *Task 2. Proper connective tissues*

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***Proper connective tissues*** (also called *fibrous connective tissues*) are archetypal for this group. They have significant amounts of fibers in the extracellular matrix.

Two main ***types of fibers*** are collagen fibers and elastic fibers.

***Collagen fibers*** include *collagen fibers proper* and *reticular fibers*. Collagen fibers are composed of collagen, which is a protein. Each collagen molecule consists of three polypeptide chains ($\alpha$-chains) twisted into a spiral (Figure 9). Over 25 different collagen species have been identified, with diverse structure and properties.

*Collagen fibers proper* show very high mechanical strength and poor extensibility. These fibers are composed of *collagen type I*; in electron microphotographs, they look cross-striated.

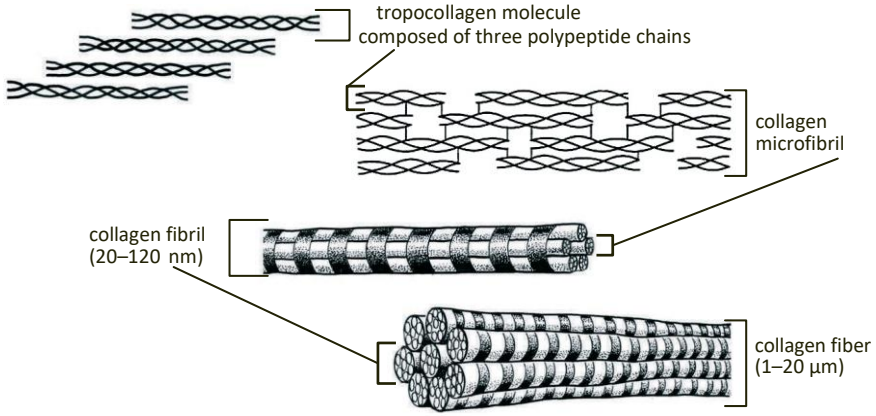


**Figure 9. Structure of collagen fibers [4]**

*Reticular fibers* are found in reticular tissue; they also participate in the formation of fibrous scaffolds for secretory and muscle cells. Reticular fibers are composed of *collagen type III*. These fibers are also referred to as *argyrophilic* because of their reactivity with silver salts.

Immature collagen fibers (*precollagen fibers*) are also argyrophilic.

***Elastic fibers*** confer the elasticity. They are responsible for the ability of tissue to stretch and then regain its original shape and length. Elastic fibers are thinner than collagen fibers and much weaker (torn easily). They are composed of elastin, which is also a protein. Immature elastic fibers are classified as *oxytalan* and *elaunin* fibers. *Oxytalan fibers* are composed solely of the microfibrils (constituted by fibrillar protein *fibrillin*), whereas *elaunin fibers* are composed of microfibrils with the equal amount of the amorphous *elastin*.

***Ground substance of the extracellular matrix*** in connective tissues is rich in proteoglycans, glycoproteins, blood plasma proteins, and ions. *Proteoglycans* consist of polypeptide chains linked to carbohydrate molecules — *glycosaminoglycans* (hyaluronic acid, chondroitinsulfates, heparin, etc.). These molecules show high water-binding capacity; this is very important for homeostasis. *Glycoproteins* include fibronectin and laminin, which mediate the interactions of cells with extracellular matrix and largely define motility, growth, adhesion and metabolic rates of the cells.

Depending on the proportion of fibers and ground (amorphous) substance in the extracellular matrix, proper connective tissues (also known as *fibrous connective tissues*) are classified into loose and dense connective tissues.

## Exercises

Answer the questions:

1. Characterize proper connective tissues.
2. Name the components of extracellular matrix in these tissues.
3. Specify the types of fibers in ECM, describe their structure and properties.

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### *Task 3. Loose connective tissue*

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***Loose connective tissue*** (LCT) contains a variety of cells, and its extracellular matrix is rich in ground substance (the fibers are relatively few).

Loose connective tissue is home of blood vessels and nerves in all organs. LCT constitutes the papillary layer of skin dermis, stroma of parenchymal organs, mucosa and adventitia of hollow organs.

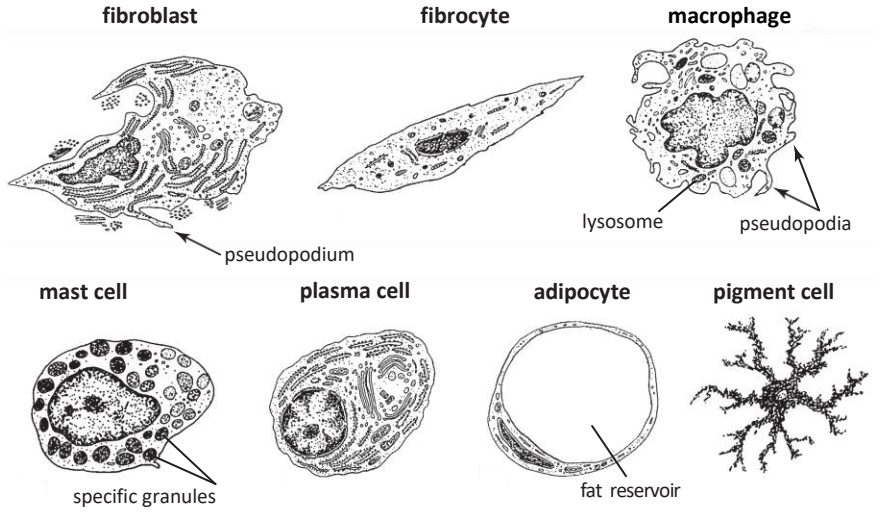


**Figure 10. Cells of loose connective tissue [5]**

The variety of cells in LCT can be subdivided into *resident cells* which are constantly affiliated with this tissue and *transient cells* (immigrants, or recruited cells) which arrive from local microcirculation to participate in certain processes and reactions.

Resident cells of LCT are shown in Figure 10.

***Fibroblasts*** synthesize structural proteins (procollagen, proelastin, fibronectin) and glycosaminoglycans, i.e. provide material for both the fibrous component and the ground substance of extracellular matrix. Fibroblasts can be functionally subdivided into low-differentiated (young) fibroblasts and mature fibroblasts (which produce collagen). At some point, fibroblasts stop dividing and become fibrocytes (long-living cells of very low metabolic activity). Well-developed granular endoplasmic reticulum and Golgi apparatus are prominent features in the ultrastructure of functionally active fibroblasts. Fibroblastic differons also include myofibroblasts and fibroclasts. Myofibroblasts are specialized cells that combine properties of fibroblasts and smooth muscle cells: they produce collagen like fibroblasts and also synthesize muscular contractile proteins (actin, myosin). Myofibroblasts participate in repair processes (e.g. wound healing).

Fibroclasts are cells of high phagocytic and hydrolytic activities, which participate in the destruction of collagen scaffolds for the purposes of tissue remodeling

***Tissue macrophages*** (*histiocytes*) have abundant lysosomes in cytoplasm. They implement phagocytosis, participate in immune reactions and secrete signaling molecules (which affect the numbers and functional activity of cells and the condition of extracellular matrix). Macrophages differentiate from blood monocytes.

***Mast cells*** (*tissue basophils* or *labrocytes*) have specific granules in the cytoplasm. They synthesize, accumulate and secrete biologically active substances: heparin, serotonin, histamine, dopamine and arachidonic acid derivatives (prostaglandins, leukotrienes). Mast cells regulate permeability of the ground substance and capillary walls. They are of hematopoietic origin as well.

***Plasma cells*** (plasmocytes) differentiate from B lymphocytes. They participate in the humoral immunity reactions by producing *antibodies* (a.k.a. gamma-globulins or immunoglobulins) that specifically bind foreign molecules (*antigens*, commonly associated with pathogens and their toxins). Plasmocytes have abundant cytoplasm with granular endoplasmic reticulum and prominent Golgi apparatus, observable as a perinuclear clearing ('light hof'). They are of hematopoietic origin as well.

***Adipose cells*** (adipocytes, lipocytes or fat cells) are recognized by a large drop of fat in the cytoplasm. These cells participate in lipid metabolism. They differentiate from multipotent adventitial cells.

***Pigment cells*** contain melanin, a pigment which protects the body from ultraviolet irradiation. These cells are of neural crest origin.

LFCT ensures exchange of substances between peripheral tissues and the blood. In LFCT, you can always find blood capillaries.

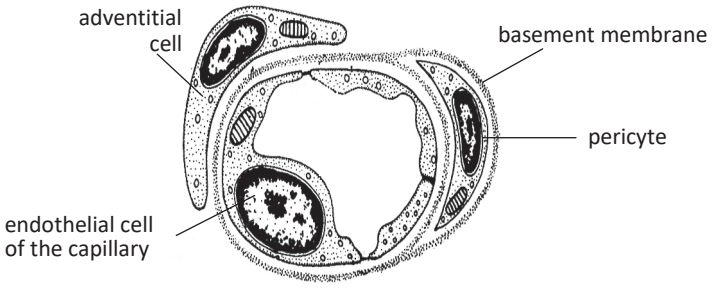


**Figure 11. Blood capillary [2]**

The capillaries consist of *endothelial cells*, *pericytes* and closely associated *adventitial cells* (all of them have mesenchymal origin).

***Pericytes*** are embedded in the basement membrane of the capillary. They have outgrowing processes with contractile filaments. Pericytes regulate the width of capillary lumen and participate in wound healing and vascular repair. They form gap junctions and adhesive junctions with endothelial cells and modulate their proliferation, migration, differentiation and survival (Figure 11).

***Adventitial cells*** are low-differentiated cells associated with microcirculatory blood vessels. They are capable of cell divisions and can differentiate into fibroblasts, myofibroblasts, fibroclasts and adipocytes.

Thus, LCT differons include fibroblastic, macrophage, lipoblastic, lymphocytic differons, and also the differons of mast cells, pigment cells, endothelial cells and pericytes. These cells are considered as resident.

In addition, LCT may harbor different types of ***leukocytes*** which arrive from circulation to participate in immune reactions. These cells are considered transient (non-resident).

LCT structure and cellular composition can be studied in *Slide № 55* comprising a whole-mount tissue specimen stained with iron hematoxylin.

A small fragment of loose tissue is stretched on a coverslip, fixed and stained. The resulting specimen has characteristically uneven thickness. Besides, the density and orientation of the fibers are affected by their partial dissolution.

*At low magnification*, pay attention to the low abundance of cells as related to the extracellular matrix. Observe the distribution and relative abundance of fibrous components. Select a transparent region for the study at higher magnification.

*At higher magnification*, observe cells and fibers against the background of transparent amorphous substance. *Collagen fibers* are thick and wavy; *elastic fibers* are thin, straight and branched. *Fibroblasts* and *macrophages* predominate among the cells. Fibroblasts have fuzzy outlines and light oval nuclei. Macrophages have smaller, more condensed nuclei which may look bean-shaped. The cytoplasm of macrophages is vacuolated ('foamy') and cell outlines are sharp. Leukocytes (especially lymphocytes), fibrocytes and mast cells can be also found in the slide. Lymphocytes typically have a very dense small nucleus and a minimal amount of cytoplasm. Fibrocytes are spindle-shaped cells with elongated, very dark nuclei. Mast cells (tissue basophils) are located along small vessels. They are large, rounded or oval in shape, with granulated cytoplasm.

**Exercises**

1. Study Slide № 55 *Loose connective tissue* (whole-mount specimen, not a section), label the structures in the scheme — *Exercise № 44 in the Workbook*.
2. *Structures of loose connective tissue*. Fill in the table — *Exercise № 45 in the Workbook*.
3. *Stages of collagen synthesis*. Study the scheme and label the structures — *Exercise № 46 in the Workbook*.

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***Task 4. Dense connective tissue***

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***Dense connective tissue*** (DCT) chiefly consists of fibers — the proportions of cells and ground substance are small. The cells are predominantly *fibrocytes*. The fibers are arranged in *bundles*.

Dense connective tissue can be *regular* or *irregular*. In ***regular DCT***, the bundles run in parallel. In ***irregular DCT***, the bundles are oriented randomly (chaotically, Figure 12). Irregular DCT is found in reticular layer of the dermis; regular DCT is found in tendons and ligaments.

Observe irregular DCT in *Slides № 60 and 61* (skin dermis, H&E).

*At low magnification*, find the prominent stratified squamous keratinized epithelium. It is underlied by a thin layer of LCT. This is papillary layer of the dermis characterized by dense vascularization and rich cellularity. Reticular layer of the dermis is found underneath the papillary layer. It is predominantly composed of *dense connective tissue*. Observe thick bundles of collagen fibers running in various directions and the dramatically low cellularity. Underneath the reticular layer, in hypodermis, observe lobules of round adipose cells with 'empty' lipid contents. Examination of the reticular layer *at higher magnification* reveals longitudinal, transverse and oblique sections of thick oxyphilic bundles of collagen. Spaces between the bundles contain ground substance and cellular elements, predominantly fibrocytes.

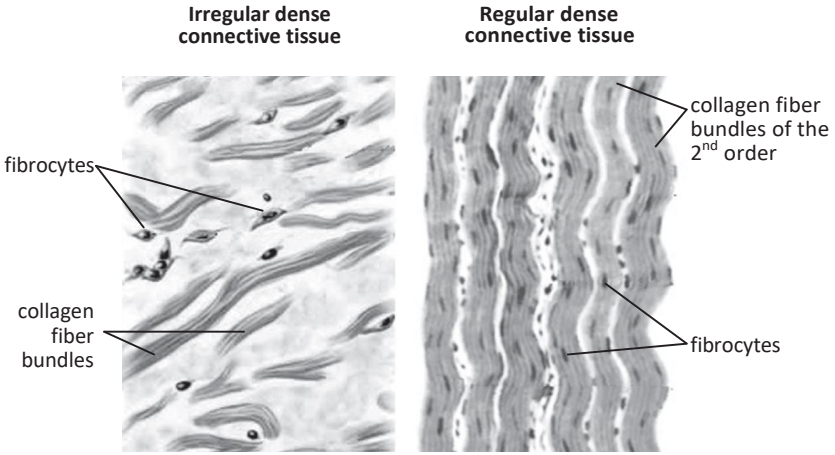


**Figure 12. Dense connective tissue [6]** *adapted with changes*

Observe regular DCT in *Slides № 62 and 62a* (respectively, longitudinal and transverse sections of a tendon, H&E).

*At low magnification*, observe the higher order tendon bundles separated by thin layers of LCT identified by its cellularity (cell nuclei are clearly visible, the cytoplasm is indiscernible). Select a bundle and study it *at higher magnification.* In the longitudinal section, observe the smallest (1st order) oxyphilic bundles of collagen running in parallel (which indicates that DCT is regular). Observe fibrocytes (*tendinocytes*, recognized by their elongated condensed nuclei) and a small amount of ground substance separating the 1st order bundles. In the transverse section, the bundles are cross-sectioned and look round, and the fibrocyte nuclei look triangular or star-shaped.

Tendon as an organ shows hierarchical organization. A group of bundles of the 1st order forms a bundle of the 2nd order enclosed in a thin layer of LCT — *endothendineum*. Bundles of the 2nd order are grouped in bundles of the 3rd order enclosed in a thicker layer of LCT — *perithendineum*. The entire tendon is coated in *epithendineum*. Epithendineum, perithendineum and endothendineum contain blood vessels and nerves.

**Exercises**

1. Study irregular DCT in Slides № 60 and 61 *Skin of a finger*.

2. Study regular DCT in Slide № 62 *Longitudinal section of a tendon* and Slide № 62a *Transverse section of a tendon*. Label structural elements in the corresponding schemes — *Exercise № 47 in the Workbook*.

### *Task 5. Connective tissues with special properties*

***Connective tissues with special properties*** include *reticular*, *adipose* and *pigment* tissues. These are tissues of a single cell type.

***Reticular tissue*** consists of reticular cells and reticular fibers. It constitutes the stroma of hematopoietic organs. In these organs, reticular cells are functional equivalents of fibroblasts. Reticular cells provide hematopoietic microenvironments and produce reticular fibers. Reticular cells can be identified by their large active nuclei. Their numerous outgrowing processes interlace with reticular fibers. Together they form a mesh which divides hematopoietic organ into hollow compartments harboring differentiating hematopoietic cells, lymphocytes and/or macrophages (Figure 13).

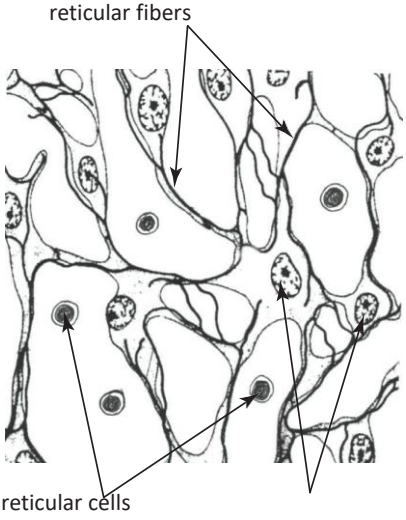


**Figure 13. Reticular tissue.**

***Adipose tissue*** provides accumulation and allocation of lipids. There are two types of adipose tissue — white and brown. Brown adipose tissue has much higher oxidative capacity than white adipose tissue.

***White adipose tissue*** is most abundant in hypodermis, omentum and mesentery. Its fat is rather easily mobilized upon starvation to cover the energy demands. However, white adipose tissue of the eye sockets, palms and soles is preserved during starvation, as its main function is to provide mechanical support. White adipose tissue is composed of *unilocular* fat cells (Figure 10).

Almost the entire volume of a white adipocyte is occupied by one large drop of fat, the rest of the cytoplasm forms a thin layer around it and the nucleus is pressed against the plasmalemma. White adipose tissue is organized in *lobules* separated by thin layers of LFCT. Solitary fibroblasts and mast cells can be found within the lobules. Each white adipose cell in a lobule is surrounded by its own network of reticular fibers and a network of blood and lymph capillaries.

***Brown adipose tissue*** is found at specific locations — between the scapuli, behind the sternum, along the spine and in the renal hilums. Newborns have well-developed brown adipose tissue; it is engaged in production of heat for thermoregulation. The heat is released by decoupling the oxidative processes in mitochondria from the ATP synthesis. Accordingly, brown adipose tissue is rich in mitochondria and the brown color is due to high amounts of the iron-containing mitochondrial enzymes, *cytochromes*. Brown adipocytes are *multilocular* fat cells. They are smaller than white adipocytes and their cytoplasm is packed with small droplets of fat. The nucleus, although located eccentrically, is not pressed against the plasmalemma.

***Pigment connective tissue*** resembles loose connective tissue in which pigment cells of neural origin predominate (Figure 10). It can be found in the iris and choroid of the eye.

Study white adipose tissue in *Slide № 61a* — whole-mount of omentum (sudan III–hematoxylin).

*At low magnification*, identify orange lobules of large and round adipose cells. They usually accompany blood vessels (which look like bluish cords). Study the structure of the *unilocular* white adipocytes *at higher magnification*. The specimen is whole-mount (not a section) and the cells overlap, which obscures their morphology. Find a single layer of fat cells at the edge of a lobule. Observe fat droplets, one per cell, stained yellow-orange with sudan (a fat-soluble dye). The flattened nucleus, small and blue, can be found at the periphery.

Study reticular tissue in *Slide № 59* — section of lymphatic node (H&E).

*At low magnification*, select a light area in the middle of the section. *At higher magnification*, observe huge numbers of lymphocytes, with small condensed nuclei enclosed in thin layer of basophilic cytoplasm. Find reticular cells — large, star-shaped cells with pale nucleus and pink cytoplasm. Reticular fibers are too thin and poorly stained with H&E to be observed in these sections.

## Exercises

1. Study connective tissues in Slides № 60 and 61 *Skin of a finger*. Draw LCT, DCT and adipose tissue. Label the structures — *Exercise № 48 in the Workbook*.

2. Study white adipose tissue in Slide № 61a *Whole-mount of omentum*. Identify the structures.
3. Study reticular tissue in Slide № 59 *Lymph node*. Make a schematic drawing and label the structures — *Exercise № 49 in the Workbook*.
4. *Reticular cell and reticular fibers*. Study the scheme and label the structures — *Exercise № 50 in the Workbook.*

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***Task 6. Embryonic connective tissues***

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***Embryonic connective tissues*** include *mesenchyme* and *mucous connective tissue*. These tissues are specific for the embryo and fetus; they cannot be found in the body after birth.

***Mesenchyme*** is a primitive connective tissue; it only exists at the early stages of development. Mesenchymal cells are stellate or spindle-shaped. Their fine branching processes form lacey networks but make no junctions. The extracellular matrix consists of amorphous material (hyaluronic acid) with a minimal amount of reticular fibers.

Although mesenchyme acquires specific structural identity of a tissue very early in development, its differentiation proceeds asynchronously and shows heterogeneous topography. Mesenchyme typically shows high rates of cell proliferation, production of fibers and tissue remodeling. *Primary mesenchyme* consists of cells that exit epiblast during the 2$^{nd}$ week of embryogenesis; these cells form extraembryonic mesoderm engaged in the formation of extraembryonic structures. *Secondary mesenchyme* is formed at later stages; it is engaged in the formation of organs and tissues of the embryo. Secondary mesenchyme has predominantly mesodermal origin; however, a number of structures develop from *neuromesenchyme* and *ectomesenchyme* which have ectodermal origin.

***Mucous connective tissue*** is abundant in extraembryonic organs and beneath the fetal skin. A typical example of mucous connective tissue is Wharton's jelly of the umbilical cord.

Mucous connective tissue contains large stellate cells immersed in the abundant jelly-like matrix composed predominantly of amorphous substance rich in hyaluronic acid with a small amount of thin collagen fibers.

**Exercises**

Answer the questions:

1. Describe the formation of secondary mesenchyme.
2. What mesodermal mesenchymal derivatives do you know?
3. What neuromesenchymal derivatives do you know? What is the origin of neuromesenchyme?

Homework: **Skeletal connective tissues**

Pawlina W., Ross M.H. Histology: A text and Atlas: with Correlated Cell and Molecular Biology. Philadelphia etc.: Wolters Kluver: Lippincott Williams and Wilkins, 7-th edition, pp 194-253

## Topic 3. SKELETAL CONNECTIVE TISSUES

**Class objective:**

1. Study microscopic and ultramicroscopic structure of cartilage and bone tissues and their components, learn their functions.
2. Identify these tissues by light microscopy.
3. Understand the basic stages of bone tissue histogenesis.

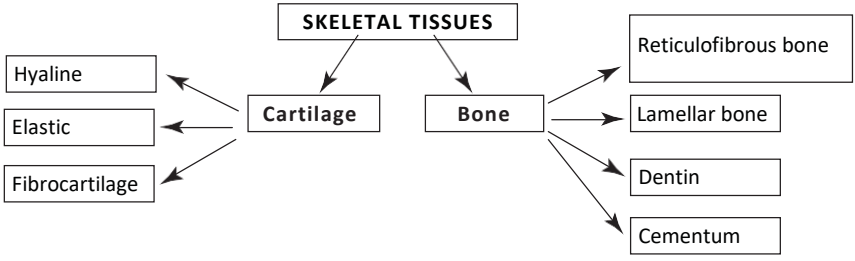


**Figure 14. Classification of skeletal connective tissues.**

### *Task 1. Skeletal connective tissues*

***Skeletal connective tissues*** include *cartilage tissues* and *bone tissues* (Figure 14).

Their ***functions*** are as follows:

1) spatial framework for the body, mechanical support of locomotion;
2) mechanical protection of the brain, spinal cord and internal organs;
3) bone tissues participate in storage and metabolism of calcium salts and phosphates.

The basic function of skeletal tissues, mechanical support for the body and its parts, depends on mechanical properties of extracellular matrix in these tissues. Skeletal connective tissues have solid extracellular matrix. Cartilage matrix is hydrated and resilient; bone matrix is mineralized and hard.

Skeletal connective tissues develop from *mesenchyme*, predominantly of mesodermal origin, although skeletal structures (and more generally, connective tissues) of the cranial region (head) develop from *neuromesenchyme*. Skeletal tissues undergo transitions (one tissue type is replaced with another) during histogenesis and regeneration.

## Exercises

Answer the questions:

1. What types of skeletal connective tissues do you know?
2. What are their locations in the body?
3. What are their functions?
4. What are their main structural properties?

### *Task 2. General overview of cartilage tissues*

***Cartilage tissues*** can be found in joints, intervertebral discs and respiratory airways. They give mechanical support, shape and protection to many structures. They provide morphogenetic basis for indirect osteogenesis.

*Cartilage tissues are characterized* by:

- low metabolic levels;
- high content of water in the extracellular matrix (up to 70–80%);
- avascularity (they lack blood vessels);
- resilience (certain extent of flexibility and reversible deformations).

Cartilage tissues have common structural organization. They consist of cells (chondrogenic cells, chondroblasts, chondrocytes and chondroclasts) and solid hydrated extracellular matrix.

The cells constitute growing population comprising two differons: the dominant *chondrocyte differon* (chondrogenic cells which give rise to chondroblasts which subsequently differentiate into chondrocytes) and the *chondroclast differon* which belongs to the macrophage system of the body. In cartilage tissues, it is represented by *chondroclasts* which differentiate from hematopoietic monocyte lineages.

***Chondrocytes*** are the main cell type of cartilage. They are sealed inside the extracellular matrix. More accurately, they occupy tight cavities (*lacunae*) either singly or in small groups. A group of chondrocytes in a lacuna is called *isogenous group*, as the whole group is produced by mitotic divisions of one cell. Basophilic zones of matrix around isogenous groups are called *territorial matrix*. Chondrocytes are engaged in synthesis of glycosaminoglycans and proteoglycans for the extracellular matrix.

***Chondroblasts*** are immature cells located in deep layers of *perichondrium*. In growing cartilage, chondroblasts have basophilic cytoplasm with granular endoplasmic reticulum and Golgi apparatus. Chondroblasts produce extracellular matrix of the cartilage; they divide and finally differentiate into chondrocytes. In adults, chondroblasts retain the capability of proliferation and differentiation, but stay inactive. Such cells can be recognized by elongated compact nucleus surrounded by small amount of weakly stained cytoplasm.

*Extracellular matrix of cartilage tissues* consists of ground substance and fibers. The ground substance is solid and resilient (due to the high content of water which resists compression). It contains proteoglycans, glycosaminoglycans, glycoproteins and mineral salts. Cartilage tissues are *avascular* — they lack blood vessels and receive their nutrients by diffusion from perichondrium. ***Perichondrium*** is the outer layer of connective tissue in cartilage (as an organ). Perichondrium consists of two layers: (1) the outer *fibrous layer* of dense fibrous connective tissue containing large blood vessels and (2) the inner *chondrogenic layer* containing chondrogenic cells, chondroblasts and their precursors (*prechondroblasts*).

***Chondrogenesis*** (formation of cartilage tissue *during the embryonic period*) proceeds as follows.

In *avascular* body regions of the embryo, skeletogenic mesoderm (derived from sclerotomes) undergoes differentiation into pluripotent cells of skeletogenic mesenchyme.

At the first stage of chondrogenesis, mesenchymal cells aggregate and form *chondrogenic islets*. Cells of the islets proliferate and differentiate into *chondroblasts* with prominent granular endoplasmic reticulum, Golgi apparatus and mitochondria. Chondroblasts subsequently differentiate into *chondrocytes*.

At the second stage of chondrogenesis, chondrocytes intensively produce *collagen type II* for the extracellular matrix (at this stage, cartilage matrix is *oxyphilic*).

At the third stage of chondrogenesis, chondrocytes progressively develop endoplasmic reticulum and Golgi apparatus. They produce large amounts of fibrillar proteins and chondroitin sulfates stained with basic dyes (at this stage, cartilage matrix is *basophilic*).

***Cartilage growth*** proceeds by two alternative mechanisms called appositional growth and interstitial growth.

*Appositional (peripheral) growth*, i.e. the growth by apposition *from the surface*, is facilitated by differentiation of chondrogenic cells located in the perichondrium. Chondrogenic cells differentiate into prechondroblasts and further into chondroblasts which produce matrix and eventually become chondrocytes. This process leads to sequential deposition of new layers of cartilage cells and surrounding matrix at the surface of the cartilage. Appositional growth of cartilage is a hallmark of the embryonic period and childhood; in adults, it is preserved as a latent capacity which comes into action only when the cartilage is damaged.

*Interstitial growth* proceeds *from the inside*. In this case, the increase in the mass of cartilage is due to synthetic activity of chondrocytes in isogenic groups: they produce matrix, and the cartilage volume increases. Isogenic groups can also increase in size, due to the residual ability of the cells to divide and expand the lacunae. Interstitial cartilage growth is typical of the embryonic period, but it may also occur during cartilage regeneration.

**Exercises**

Answer the questions:

1. What are the main structural features of cartilage tissues?
2. What are these tissues composed of?
3. What mechanisms of cartilage growth do you know? Describe these mechanisms.

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***Task 3. Cartilage tissues***

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***Cartilage tissues*** are subdivided into *hyaline cartilage*, *elastic cartilage* and *fibrocartilage*, which differ mainly by the structure and composition of the extracellular matrix (Figure 15).

***Hyaline cartilage tissue*** is found in costal cartilages, on the articular surfaces and notably at the junctions between epiphysis and diaphysis of the tubular bones (until cessation of their growth).

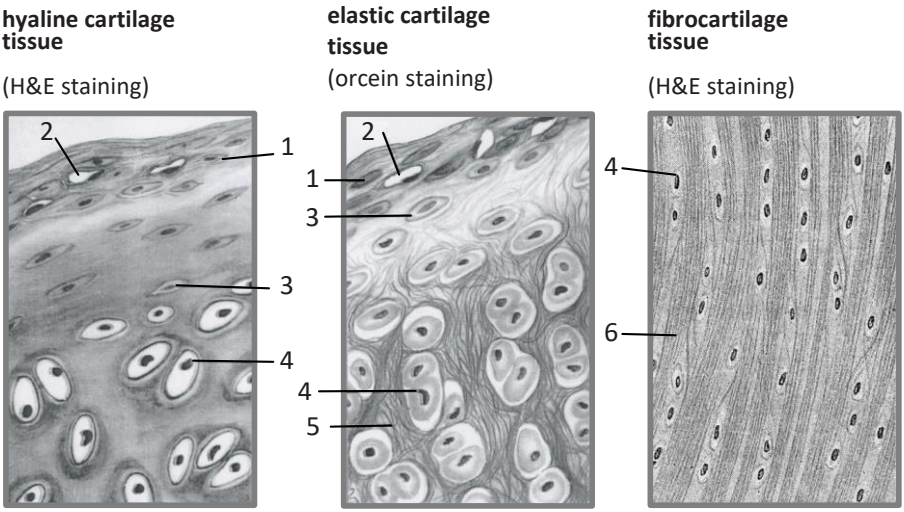


**Figure 15. Types of cartilage tissue.**

1 — chondrogenic cells in perichondrium; 2 — blood vessels of the perichondrium; 3 — immature (young) chondrocytes; 4 — isogenous groups of chondrocytes; 5 — elastic fibers; 6 — bundles of collagen fibers.

*Hyaline cartilages* consist of hyaline cartilage tissue coated in perichondrium. Young chondrocytes (spindle-shaped immature cells) are located beneath the perichondrium. In deeper layers of the cartilage, the cells look more rounded. Upon division they do not drift apart, but stay close, forming isogenous groups of 3–8 chondrocytes. Confined to lacunae and pressed against one another, the cells of isogenous groups acquire irregular asymmetrical shapes. The cells are highly hydrated; they release glycosaminoglycans, which have acidic properties. In close proximity to isogenous groups, the matrix is rich in glycosaminoglycans and therefore stained basophilically. These basophilic areas around isogenous groups are called *territorial matrix*; they are separated by *interterritorial matrix*. The matrix also contains thin collagen fibers (chondrin fibers) composed of *collagen type II*. The ground substance is highly hydrated and contains proteoglycan conglomerates composed of sulfated glycosaminoglycans, glycoproteins and salts.

***Elastic cartilage tissue*** is found in auricles, epiglottis, corniculate and cuneiform cartilages of the larynx. It is elastic because up to 90% of the fibers in its extracellular matrix are branching elastic fibers which form a dense network (collagen fibers are also present but their role is minor). Isogenous groups of elongated chondrocytes are small (rarely contain more than four cells). Elastic cartilages have similar structural organization with hyaline cartilages.

***Fibrocartilage tissue*** combines structural features of dense fibrous connective tissue and hyaline cartilage. Fibrocartilage has much higher content of collagen fibers than hyaline cartilage. The collagen fibers form thick bundles, and flattened chondrocytes are located individually or in small groups between these bundles. Fibrocartilages lack perichondrium and get their nutrients from surrounding tissues. Accordingly, they are incapable of appositional growth (the only growth they are capable of is interstitial). Fibrocartilage tissue forms structural basis of pubic symphysis and intervertebral discs; it is commonly found at the cartilage-tendon junctions.

***Hyaline cartilage tissue*** can be observed in *Slide № 63* (cross-section of a rib, H&E).

*At low magnification*, find cross-section of the cartilage coated in *perichondrium*. Inside the perichondrium (which consists of two layers), observe the *cartilage tissue* composed of extracellular matrix and chondrocytes. The matrix looks homogeneous, because the thin collagen fibers and the amorphous substance have the same index of refraction, which makes the fibers invisible. *Young (immature) cartilage* zone is located immediately beneath the perichondrium; *mature cartilage* occupies the central portion of the section.

*At higher magnification*, observe the flattened nuclei of *prechondroblasts* and *chondroblasts* in the inner (chondrogenic) layer of perichondrium. Young cartilage zone contains small spindle-shaped *young chondrocytes* located singly. Mature cartilage zone contains *mature chondrocytes* lying in the cavities (lacunae). Mature chondrocytes form *isogenous groups* of 3–8 cells which descend from a single dividing cell. Histological fixation of the tissue may cause shrinking of chondrocytes and their detachment from the walls of lacunae. Each isogenous group has its own basophilic *territorial matrix*; the groups are separated by weakly oxyphilic *interterritorial matrix*.

***Elastic cartilage tissue*** can be observed in *Slide № 64* (section of auricle stained with *orcein*).

Examination of the slide *at low magnification* shows that elastic cartilage is very similar in structure to hyaline cartilage. The main difference is the structure of the extracellular matrix. In the elastic cartilage tissue, the matrix is interlaced with a network of elastic fibers, arranged chaotically and very well revealed by orcein staining (reddish-brown).

*At higher magnification*, examine the *perichondrium* and *chondroblasts. Isogenous groups* of chondrocytes can be observed in the central portion of the section; they look like columns of 2–4 cells. In elastic cartilage, isogenous groups are smaller than in hyaline cartilage (contain fewer cells) and the extracellular matrix is interlaced with a prominent network of elastic fibers which become thicker in mature layers. At the periphery of the cartilage, the cells (young chondrocytes) are located singly.

*Fibrocartilage tissue* can be studied in Slide № 65 (*section of intervertebral disc, H&E*).

*At low magnification*, find a lighter area in the middle of the section — this is *nucleus pulposus* of the intervertebral disc. The disc is inserted between the hyaline cartilage end-plates, located at the intervertebral surfaces of two adjacent vertebrae. Fibrocartilage of the intervertebral disc can be found *between nucleus pulposus and the hyaline cartilage tissue*. Fibrocartilage can be recognized by concentric bundles of collagen fibers interspaced with rows of chondrocytes. The transition of fibrocartilage to hyaline cartilage is noticeable (find it). From the outer surface, the disc and the vertebrae are wrapped in the ligament (the intensely oxyphilic dense fibrous connective tissue). On the vertebral sides of the hyaline cartilage, the brightly stained bone tissue of the vertebra with the elements of endochondral ossification and hematopoiesis may be found.

Fibrocartilage of the intervertebral disc should be studied *at higher magnification*. Observe the dense, weakly oxyphilic bundles of collagen running more or less in parallel, with the rows of single flattened chondrocytes between them. Isogenous groups of chondrocytes can also be found (most of them consist of two cells). At the margins of the disc close to the ligament, observe the concentric arrangement of the collagen layers in the fibrocartilage (hence the name *annulus fibrosus* — a ring of fibrous tissue).

## Exercises

1. Slide № 63 *Hyaline cartilage tissue. Cross-section of a rib, H&E*. Make a drawing and label the structures — *Exercise № 51 in a Workbook*.
2. Slide № 64 *Elastic cartilage tissue. Section of auricle (orcein)*. Make a drawing of the tissue at higher magnification and label the structures — *Exercise № 52 in the Workbook.*
3. Slide № 65 *Fibrocartilage tissue. Section of intervertebral disc, H&E*. Make a drawing of the tissue and label the structures — *Exercise № 53 in the Workbook.*

## *Task 4. General overview of bone tissues*

***Bone tissues*** form the skeleton, which belongs to the musculoskeletal (motor) system. Human skeleton provides spatial framework for the body, supports its motion, protects internal organs and works as a depot of calcium and phosphorus.

Bone tissues consist of cells (*osteogenic cells*, *osteoblasts*, *osteocytes* and *osteoclasts*) and mineralized (calcified) extracellular matrix. Inorganic substances (calcium salts, predominantly modified phosphates — *hydroxyapatites*) account for about 70% of the bone matrix and ensure its hardness and mechanical strength. Organic molecules (collagen, glycoproteins and proteoglycans) constitute about 30% of the bone matrix. *Ossein fibers* (predominantly composed of *collagen type I*) are prominent; they determine the flexibility of *decalcified* bone tissue.

Cells of bone tissue (Figure 16) represent two differons. One differon comprises *osteoblasts* and *osteocytes* (respectively, young and mature cells) formed by progressive differentiation of *osteogenic cells*.

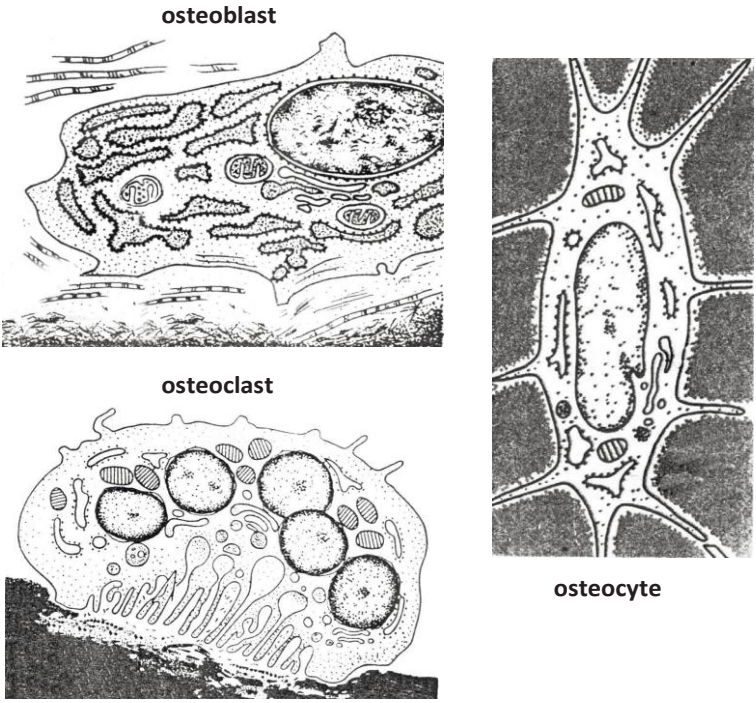


**Figure 16. Cells of bone tissue [2]**

The second differon comprises *osteoclasts* — highly specialized macrophages descending from hematopoietic stem cells.

***Osteoblasts*** are young dividing cells engaged in bone formation. They are located at the surface of bone structures. Osteoblasts can be identified as large cells with light rounded nucleus; their shapes vary. The cytoplasm is basophilic as it contains numerous ribosomes and granular endoplasmic reticulum; it also contains mitochondria and Golgi apparatus. *Osteoblasts* synthesize and secrete the components of bone matrix. They also participate in the bone matrix calcification. While producing the bone matrix, the cells literally seal themselves inside it and *differentiate into osteocytes*.

***Osteocytes*** are the major cell type of bone tissue; they are fully differentiated and incapable of division. Osteocytes are elongated cells with compact dark nucleus and weakly-basophilic cytoplasm containing few organelles. Osteocytes occupy individual cavities in the matrix, *lacunae*, which exactly repeat their shapes. The long cytoplasmic processes of osteocytes reach perivascular spaces of blood vessels in the osteons. All types of exchange between osteocytes and the circulating blood proceed via interstitial fluid of the perivascular spaces. Osteocyte processes occupy numerous anastomosing channels (bone *canaliculi*, which connect the lacunae with perivascular spaces) and form gap junctions enabling the transfer of low molecular weight substances between the cells. Osteocytes participate in the bone matrix maintenance.

***Osteoclasts*** are very large *multinucleated* cells (up to several dozen nuclei is contained in one cell). Their function is *destruction* of bone matrix (including the destruction of calcified cartilage during osteogenesis). Osteoclasts are highly specialized *macrophages* formed by fusion of monocytic precursors (hence their giant multinucleated appearance). The cytoplasm contains a variety of organelles, notably Golgi apparatus and lysosomes. Mitochondria are concentrated in the cytoplasmic portion distant from the bone. At their contact surface with bone matrix, osteoclasts form numerous finger-like processes which constitute the *ruffled border*. Osteoclast creates acidic environment at the ruffled border. The acidity promotes dissolution of the mineral component of the matrix. Lysosomal proteases, also released by osteoclast, become activated in the acidic environment and destroy the organic component of the bone matrix. The resulting local destruction of bone tissue can be observed as a *resorption lacuna* beneath the osteoclast.

Bone tissue is the formative tissue of the skeleton. Both *compact* (cortical) and *spongy* (cancellous, trabecular) bone matters are built of bone tissue. The bones are coated in *periosteum*. In tubular bones, epiphyseal surfaces (sites of joints) are coated in *articular cartilage*.

***Periosteum*** consists of two layers: the outer *fibrous layer* of dense irregular fibrous connective tissue and the inner *osteogenic layer* of loose fibrous connective tissue. Osteogenic layer contains osteogenic cells. The whole thickness of periosteum is stitched with Sharpey's fibers — strong collagen fibers which attach the periosteum firmly to the bone. Periosteum protects the bone and participates in the nutrition, development, growth and regeneration of the bone tissue. Periosteum contains a number of receptors.

Nutrition of the bone tissue is provided with blood flow from periosteum via the extensive network of blood vessels located in the perforating Volkmann's canals and central (Haversian) canals of the osteons.

## Exercises

1. *Cellular elements of bone tissue*. Study the scheme and label the structures — *Exercise № 57 in the Workbook*.

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### *Task 5. Bone tissues*

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***Bone tissues*** are subdivided into:

1) reticulofibrous bone (primary);
2) lamellar bone (secondary);
3) dentin;
4) cementum.

***Reticulofibrous bone tissue*** has chaotic arrangement of bundles of thick collagen fibers interspersed with relatively abundant cellular elements (osteocytes). This type of bone tissue is formed primarily, whenever the bone develops or regenerates, to be subsequently replaced with mature (lamellar) bone tissue. Extracellular matrix of reticulofibrous bone is mineralized (similarly with the matrix of lamellar bone).

***Lamellar bone tissue*** is mature bone tissue. Its structural units are ***bone lamellae*** (thin plates of mineralized bone matrix with osteocytes at the interfaces). Within each lamella, thin collagen fibers are oriented in parallel and usually at right angles with the fibers in adjacent lamellae.

Bone lamellae build both *compact* (cortical) and *spongy* (cancellous, trabecular) bone matters. *Spongy bone* consists of bone trabeculae separated by large inter-trabecular spaces. Each trabecula is formed by irregularly shaped, overlapping bone lamellae. *Compact bone* consists of densely and regularly arranged bone lamellae organized in *osteons* (Figures 17 and 18). Osteonic organization is the hallmark of compact bone reflecting its hierarchical morphology and mode of functioning. Osteon is therefore considered as structural-and-functional unit specifically of compact bone.

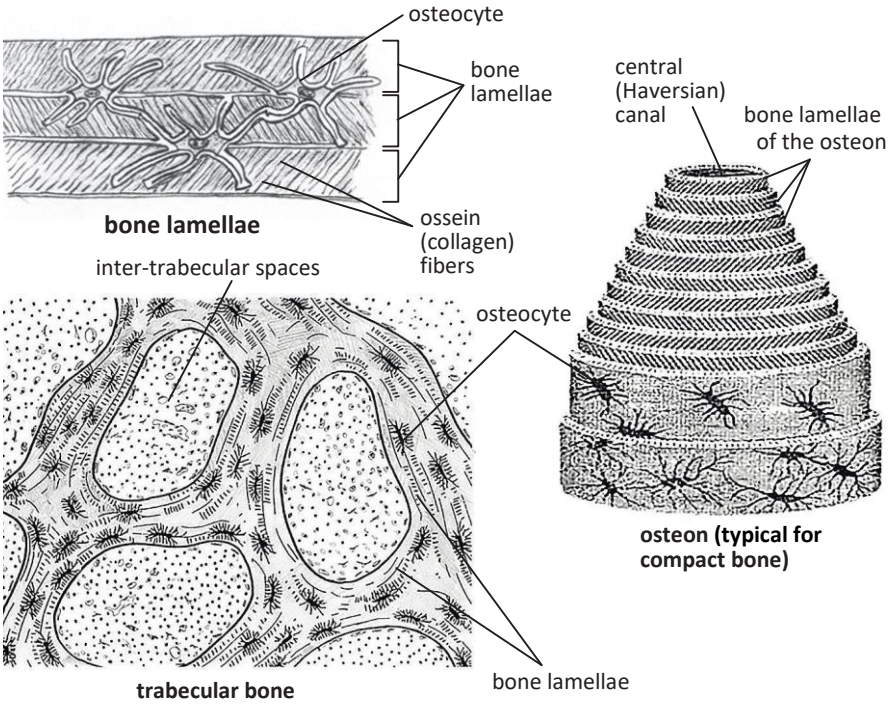



**Figure 17. Lamellar bone tissue**

***Dentin and cementum*** are structurally similar to the bone tissue.

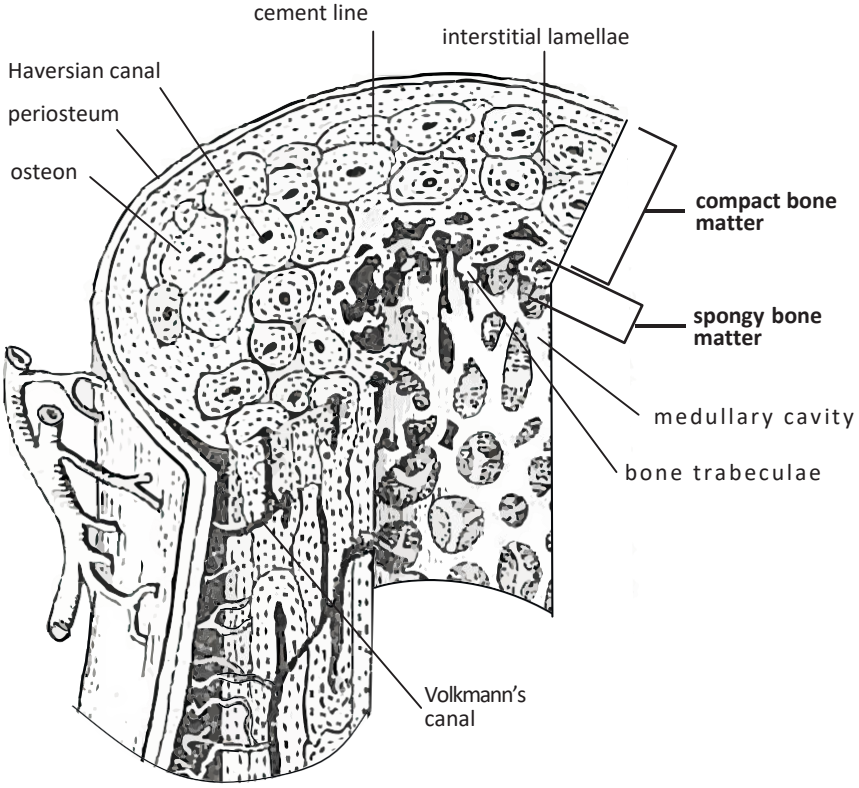
*Dentin* has neither its own cellular elements nor blood vessels. It is entirely composed of collagen fiber bundles interspersed with ground substance. It only contains cytoplasmic processes of odontoblasts (a.k.a. *dentinoblasts*), the bodies of which are located outside dentin.

*Cementum* is also composed of collagen fiber bundles and ground substance; it may contain cells (*cementocytes*) or their processes only.

Observe lamellar bone tissue in *Slide № 67* representing cross-section of a tubular bone diaphysis (Schmorl's staining).

*At low magnification*, identify the layered structure of diaphysis including periosteum, outer circumferential lamellae, osteonic layer, inner circumferential lamellae, trabecular bone and endosteum. Periosteum is the external layer (stained green yellow or brown). Inner circumferential lamellae can be observed beneath the periosteum.

**Tubular bone diaphysis (3D scheme)**




**Layered structure of diaphysis (shown in cross-section)**

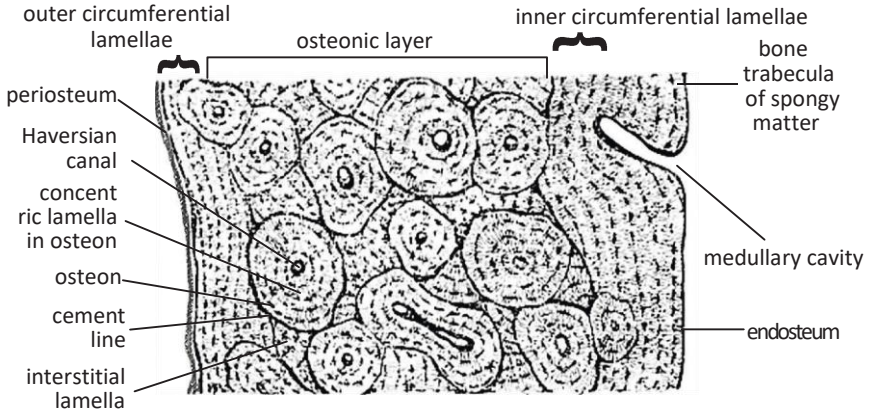



**Figure 18. Microanatomy of diaphysis [7]**

Beneath the inner circumferential lamellae, observe the prominent osteonic layer which consists of *osteons* — cylindrical units composed of concentric lamellae (inserted one into another). Each osteon has its central (Haversian) canal, occasionally with a preserved section of blood vessel with blood elements. Each osteon is confined within its border with other osteons — the *cement line*. Interstitial lamellae fill the spaces between osteons. Inner circumferential lamellae surround the *medullary cavity* partially compartmentalized by the trabeculae of spongy bone. *At higher magnification*, observe osteocytes in lacunae wedged between adjacent concentric lamellae along their circumference. Long cytoplasmic processes of osteocytes occupy numerous bone canaliculi oriented perpendicularly to the lamellae.

## Exercises

1. Specify the locations of osteogenic cells — *Exercise № 56 in the Workbook.*
2. Slide № 67 *Lamellar bone tissue. Cross-section of tubular bone diaphysis (Schmorl's staining).* Study the scheme and label the structures — *Exercise № 54 in the Workbook.*
3. Understand a schematic drawing of cross-sectioned osteon, label the structures, indicate orientation of ossein fibers in the bone lamellae — *Exercise № 55 in the Workbook.*

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***Task 6. Osteogenesis***

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Development of bone tissue *during the embryonic period* proceeds by two different mechanisms:

1) *direct osteogenesis* proceeds directly from mesenchyme;

2) *indirect osteogenesis* requires cartilage model which develops from mesenchyme and is subject to ossification.

*Postembryonic* development of bone tissue is basically growth and physiological regeneration.

***Direct osteogenesis*** a.k.a. *intramembranous ossification* (Figure 19) gives rise to the bone derivatives of branchial arches (e.g. the mandible). The process begins during the 1st month of embryogenesis and includes the following stages:

1) osteogenic cells accumulate and form *osteogenic islets* composed of proliferating and differentiating cells; the islets become *vascularized*;

2) cells of osteogenic islets differentiate into osteoblasts and start producing unmineralized organic matrix (*osteoid*);

3) mineralization of the osteoid: the matrix becomes impregnated with calcium salts, osteoblasts differentiate into osteocytes and *reticulofibrous bone tissue is formed*;

4) bone remodeling: reticulofibrous bone tissue is eventually destroyed and *replaced with lamellar bone tissue.*

Indirect osteogenesis a.k.a. *endochondral ossification* (Figures 20 and 21) begins during the 2nd month of embryogenesis and ends by 18–25 years of life. Its stages are as follows:

1) *Cartilage model* in the shape of future bone is formed from mesenchyme by conventional hyaline cartilage histogenesis (Figure 20A).

2) *Perichondral bone cuff* forms in the inner layer of perichondrium. Under enhanced oxygenation, precursor cells differentiate into osteoblasts instead of chondroblasts. The osteoblasts start to produce bone matrix. The newly formed reticulofibrous bone tissue encircles the diaphysis of the cartilage model like a cuff, and the overlying perichondrium gradually turns into periosteum (Figure 20B).

3) Formation of the *endochondral bone in diaphysis* is a consequence of perichondral ossification. The perichondral bone tissue disrupts the delivery of nutrients to the underlying cartilage thus causing its dystrophy and promoting mineralization. The process is augmented by the ingrowth of blood vessels accompanied by mesenchymal cells which form osteogenic islets inside the deteriorating cartilage. Reticulofibrous endochondral bone tissue can be distinguished from the perichondral bone by the presence of residual calcified cartilage matrix which is used by osteoblasts as a scaffold for the endochondral bone matrix deposition.

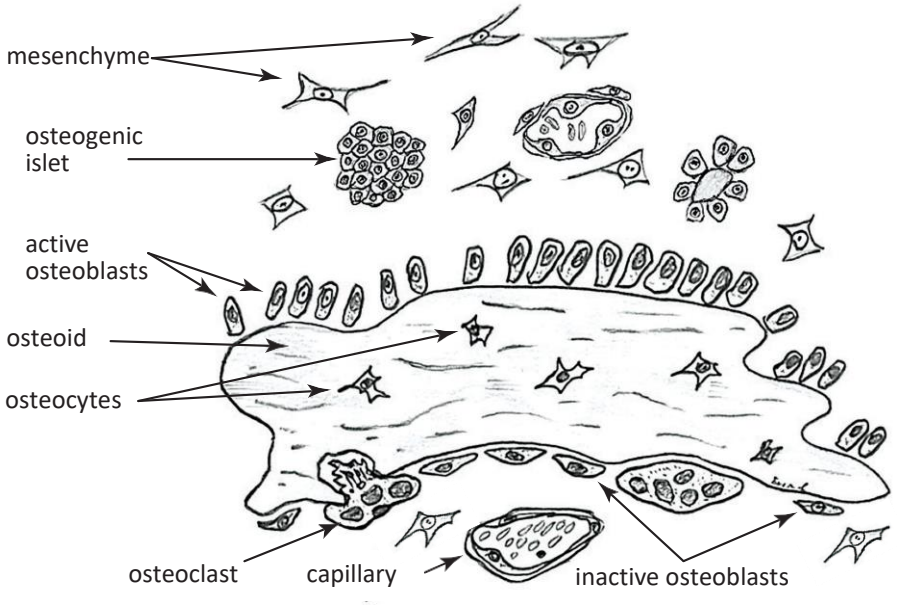


**Figure 19. Direct osteogenesis**

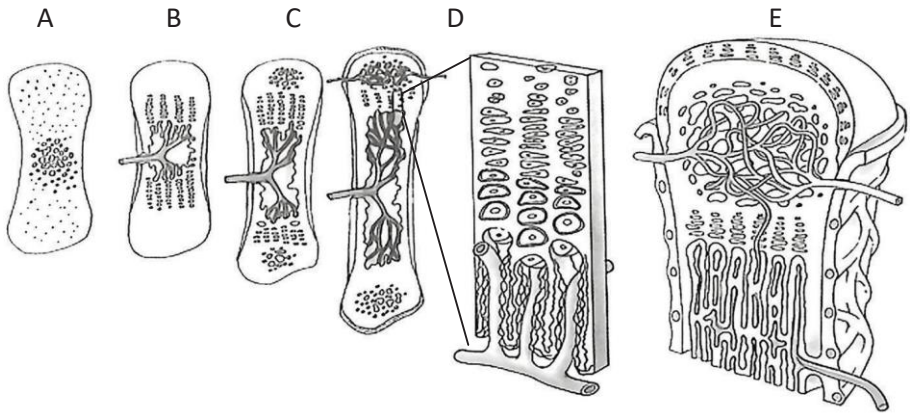


**Figure 20. Stages of indirect osteogenesis [8]**

The ingrowing blood vessels also bring osteoclasts to the endochondral ossification area. Osteoclasts start to destroy the endochondral bone matrix and the residual calcified cartilage. Partial destruction of the forming endochondral bone by osteoclasts promotes formation of medullary cavities. Mesenchymal cells penetrate into these cavities with the ingrowing blood vessels and differentiate into the bone marrow stroma subsequently colonized by hematopoietic stem cells (Figure 20 C).

4) Formation of the *endochondral bone in epiphysis* is generally similar to the ossification processes in diaphysis. Epiphysis is home to the *secondary ossification foci*, where reticulofibrous bone tissue develops (Figure 20 D and E). *Metaphyseal growth plate* (Figure 20 E, Figure 21) is formed at the border between the still cartilaginous epiphysis and the expanding ossified area in diaphysis. Metaphyseal growth plate has distinct *proliferative zone* where dividing chondrocytes show the characteristic *stacked coins* appearance. It is followed by *hypertrophic zone* where chondrocytes undergo degradation by swelling. It eventually turns into *zone of calcified cartilage* adjacent to the ossified zone in the diaphysis. In opposite direction from the growth plate, the remaining distal portion of epiphysis is occupied by *resting zone* of unmodified hyaline cartilage which will eventually enter the ossification process. In tubular bones of humans, proliferative zones of secondary ossification are preserved in active state until the age of 20 years.

5) Replacement of reticulofibrous bone tissue with lamellar bone tissue as a result of *bone remodeling*. The lytic activity of osteoclasts leads to formation of cavities in bone matrix. When these cavities expand to a certain size and become penetrated by blood vessels, osteons are formed by the activity of osteogenic cells located in the adventitia of the vessels.

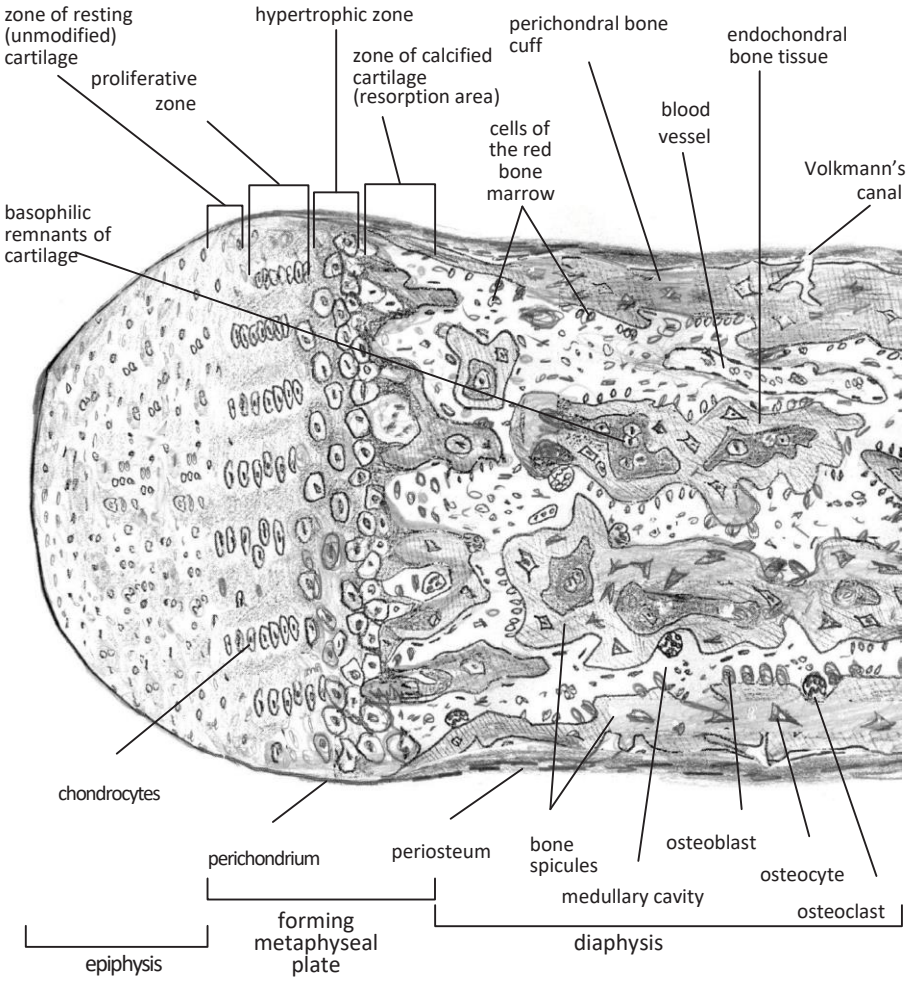


**Figure 21. Indirect osteogenesis**

Bone remodeling is a lifelong process; it does not end with the replacement of reticulofibrous bone tissue with lamellar bone tissue but gives rise to secondary, tertiary osteons, etc. The remnants of destroyed early osteons can be observed in the form of interstitial bone plates. Periosteum promotes the gradual increase in the diameter of tubular bones by appositional growth.

***Bone repair*** results from the activity of osteogenic cells in periosteum, endosteum and osteonic canals. The sequence of events in bone repair generally corresponds to the sequence of events in osteogenesis.

The process of direct (intramembranous) ossification can be observed in *Slide № 68* (H&E).

*At low magnification*, find bone spicules. They are composed of reticulofibrous bone tissue stained bright pink. Observe mesenchyme surrounding the bone spicules. *At higher magnification*, observe the rich cellularity of bone spicules. Their surface is densely packed with basophilic cells. These cells are *osteoblasts*, and their shape correlates with their activity (cylindrical cells are active, flattened cells are inactive). An unstained layer of unmineralized matrix (*osteoid*) can be observed between active osteoblasts and the eosinophilic bone matrix of the spicules. *Osteocytes* can be observed embedded in the bone matrix within bone spicules. Find *osteoclast* — a giant basophilic cell with multiple nuclei. Observe *resorption lacuna* at the site of contact of osteoclast with the bone. Try to observe the *ruffled border* by slightly pulling down the condenser.

The process of indirect (endochondral) ossification can be observed in *Slide № 69* (H&E).

Examine the diaphysis of the developing tubular bone. Find the *perichondral bone cuff* located immediately beneath the periosteum. Its bone matrix is stained pink with eosin, whereas osteoblasts and the osteocyte nuclei are basophilic. In the central portion of the diaphysis, observe zones of endochondral ossification concentrated around purplish or bluish fragments of the calcified cartilage matrix, representing the process of *endochondral bone formation*. The endochondral bone spicules are composed of the newly formed bone surrounding the calcified remnants of cartilage. Each spicule therefore contains some basophilic remnants of the calcified cartilage matrix at its core and the nascent (freshly formed) bone tissue at its periphery (the bone matrix stained pink with eosin). *Osteoblasts* and *osteoclasts* can be observed at the surface of bone spicules. The prospective red bone marrow can be observed within the forming medullary cavities.

At the border between diaphysis and epiphysis called *metaphysis*, find *resorption area* where calcified cartilage undergoes destruction and becomes replaced with bone tissue. Proceeding from this area in the direction of epiphysis, observe *hypertrophic zone* with swollen chondrocytes looking like transparent bubbles. It should be noted that hypertrophic zone corresponds to the frontier of the perichondral bone formation and the transition of perichondrium into periosteum. From hypertrophic zone, proceed further in the direction of epiphysis to observe *proliferative zone* with dividing chondrocytes of characteristic *stacked coin* appearance. A major portion of the epiphysis at this stage is occupied by *resting* (unmodified) hyaline cartilage.

**Exercises**

1. Slide № 68 *Direct osteogenesis*. Make a labeled drawing of the specimen — *Exercise № 58 in the Workbook*.

2. Gain understanding of direct osteogenesis, list its stages and make a brief description for each stage — *Exercise № 59 in the Workbook.*
3. Gain understanding of indirect osteogenesis, list its stages and make a brief description for each stage — *Exercise № 60 in the Workbook*.
4. Slide № 69 *Indirect osteogenesis*. Make a labeled drawing of the specimen at higher magnification — *Exercise № 61 in the Workbook.*

Homework: **Muscle tissues**

Pawlina W., Ross M.H. Histology: A text and Atlas: with Correlated Cell and Molecular Biology. Philadelphia etc.: Wolters Kluver: Lippincott Williams and Wilkins, 7-th edition, pp 314-355

## Topic 4. MUSCLE TISSUES

**Class objective:**

1. Learn classification of muscle tissues.
2. Learn morphological and functional characterization and embryonic sources of different muscle tissues (smooth, skeletal and cardiac).
3. Understand muscle contraction mechanism.
4. Identify different muscle tissues by light microscopy.

### *Task 1. Muscle tissues*

***Muscle tissues*** are unified by their distinct ability to contract, which is directly associated with their function. They differ by embryonic origin and structure.

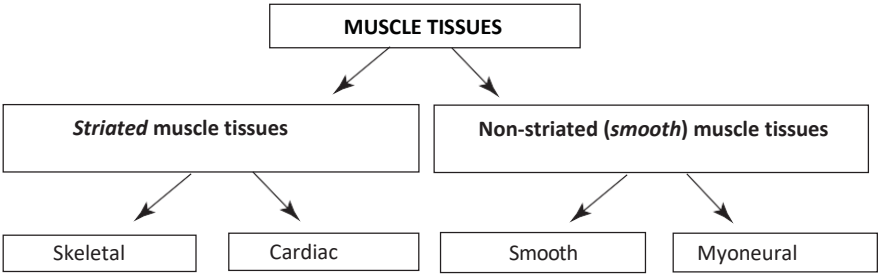


**Figure 22. Morphogenetic classification of muscle tissues**

Muscle tissues ensure all types of *motion*, including locomotion, respiratory motions, voluntary movements (e.g. fine motor skills and articulation of sounds), and the motion of internal organs (heartbeat, peristalsis).

In addition to their basic function — ensuring motion through *contractility* muscle tissues have several other functions including:

- mechanical protection;
- shape of the body;
- participation in thermoregulation;
- participation in nutrition and nutrient storage (trophic function).

Basic ***morphological properties of muscle tissues*** are as follows:

1) elongated shape of structural elements (muscle cells or muscle fibers);
2) the presence of basement membrane associated with plasmalemma;
3) the presence of specific structural elements in the cytoplasm, including:
   - longitudinally oriented myofibrils and myofilaments — highly specialized cytoskeletal structures that ensure contractility (*contractile elements*);
   - numerous *mitochondria* nearby the contractile elements;
   - inclusions of glycogen, lipids and myoglobin, which serve as an *energy reserve* for muscle contraction;
   - structures that ensure accumulation, storage and release of *calcium ions* required for the muscle contraction (smooth endoplasmic reticulum, caveolae).

Classification of muscle tissues is given in Figure 22.

***Striated muscle tissues*** harbor myosin and actin myofilaments which constitute *myofibrils* — permanent (i.e. constantly present in the cell) structural elements of the contractile apparatus. Myofibrils have highly ordered arrangement of the actin and myosin myofilaments organized in *sarcomeres* considered as structural-and-functional units of myofibrils. In adjacent myofibrils, the boundaries between sarcomeres are located *at the same level*, hence the observable *cross-striation* of the tissue.

***Smooth muscle tissues*** harbor *depolymerized myosin*, which polymerizes and becomes capable of interactions with actin only at the moment of contraction in the presence of calcium ions. Smooth muscles exhibit no cross-striations.

All voluntary movements of human body are produced by skeletal muscle tissue. Other muscle tissues (cardiac, smooth, myoneural) ensure involuntarily contractions.

Embryonic sources of muscle tissues are diverse (Figure 23).

**Figure 23. Histogenetic classification of muscle tissues**

| Types of muscle tissue | Embryonic source of origin | Examples |
|---|---|---|
| Striated skeletal (*somatic*) | Somite myotomes | Muscles of the extremities, torso, tongue |
| Striated cardiac (*coelomic*) | Myoepicardial plate (belongs to visceral layer of the lateral plate mesoderm) | Myocardium of the heart |
| Smooth (*mesenchymal*) | Mesenchyme | Muscles in the walls of hollow organs and blood vessels |
| Smooth myoneural | Neural tube | Muscles of the iris |

## Exercises

Answer the questions:

1. What muscle tissue types do you know?
2. What are their common features, and in what aspects do they differ?
3. What functions do they implement?
4. From what embryonic sources do they develop?
5. Specify the locations of different muscle tissues within the body.

### *Task 2. Striated skeletal muscle tissue*

***Structural-and-functional unit*** of skeletal muscle tissue is called ***muscle fiber***. Muscle fibers are 1–20 cm long and about 50 μm in diameter. Skeletal muscle fiber consists of myosymplast and cambial cells — myosatellitocytes. The fiber is coated in basement membrane (Figure 24).

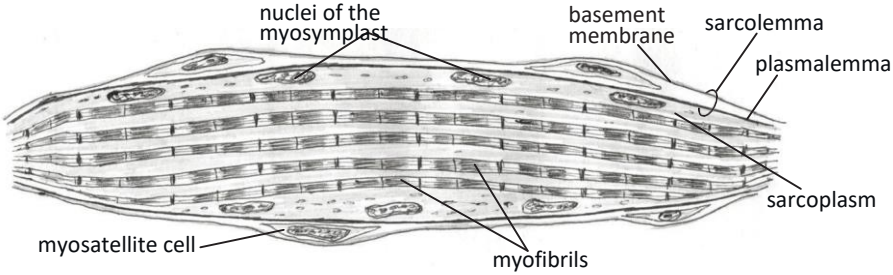


**Figure 24. Muscle fiber**

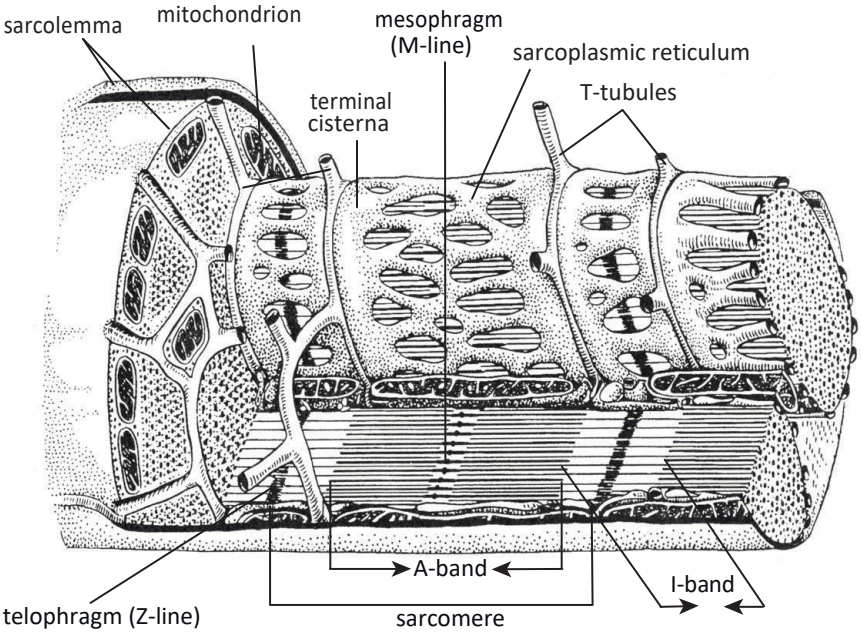


**Figure 25. Ultramicroscopic structure of myosymplast [2]**

The terms used for the *morphological description of muscle fibers* include:

- sarcoplasm — cytoplasm of the muscle fiber;
- sarcolemma — the outer membrane (basement membrane + plasmalemma);
- sarcoplasmic reticulum — agranular endoplasmic reticulum of the muscle fiber.

The inner volume of ***myosymplast*** is occupied by longitudinally oriented *contractile elements* — ***myofibrils***, composed of highly regular arrangements of *actin and myosin myofilaments*. Each myofibril is surrounded by longitudinally oriented and anastomosing channels of agranular endoplasmic reticulum (*sarcoplasmic reticulum*). Myofibrils are interspersed with numerous mitochondria. The multiple oval nuclei of myosymplast are located at the periphery of the fiber, immediately beneath the plasmalemma. Sarcolemma of the myosymplast forms deep branching invaginations — *T-tubules* (Figure 25). Each T-tubule is fixed between two L-tubules (cisternae of *sarcoplasmic reticulum* — *the modified agranular endoplasmic reticulum*). Together they form *a triad* — two L-tubules (cisternae of sarcoplasmic reticulum) plus one T-tubule (invagination of sarcolemma). L-tubules accumulate calcium ions ($Ca^{2+}$) required for the muscle contraction.

Characteristic cross-striation observed at the light microscopy level is due to the alternation of light and dark areas (bands) in myofibrils.

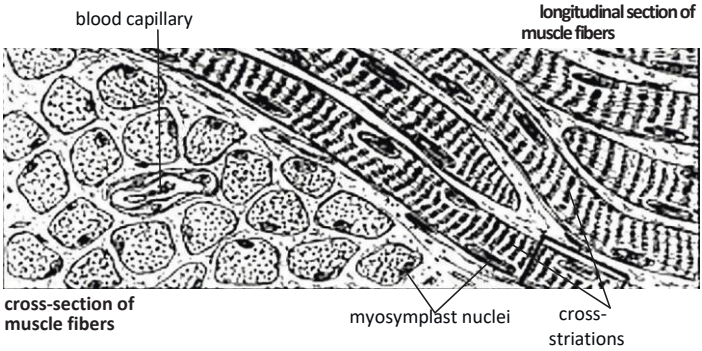


**Figure 26. Striated skeletal muscle tissue (as observed by light microscopy)**

In polarized light, dark bands of myofibrils show birefringence and are therefore designated *anisotropic* (A-bands). Light bands of myofibrils do not show such property and are therefore designated *isotropic* (I-bands, Figure 26).

***Structural-and-functional unit of myofibril*** is called ***sarcomere***. Sarcomeres represent the highly ordered parallel arrangement of thin (actin) and thick (myosin) myofilaments (Figure 27).

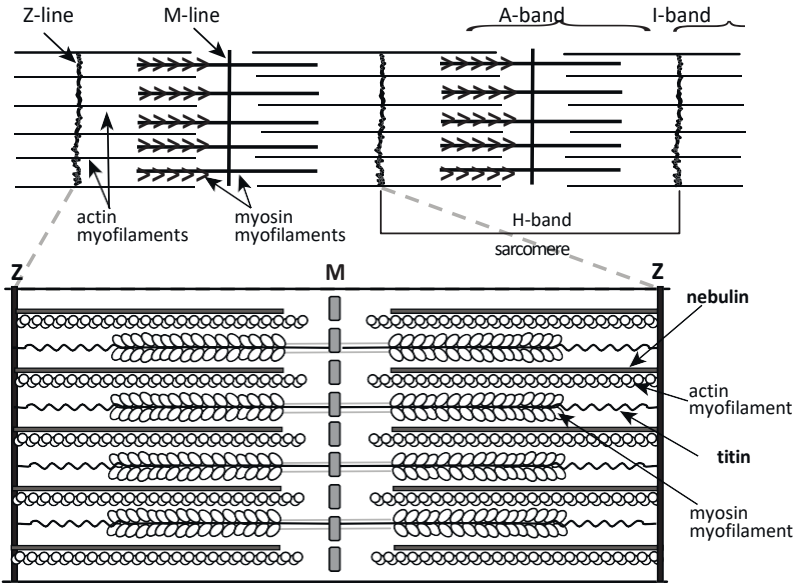


**Figure 27. Structure of sarcomere**

I-bands of sarcomeres contain thin actin myofilaments only, whereas A-bands contain both thick and thin myofilaments. The middle portion of A-band has lighter area (H-band) containing thick myofilaments only. Actin myofilaments are attached to *telophragm* (Z-line) via *α-actinin*, *desmin* and *vimentin*. Actin myofilaments are accompanied by *nebulin* molecules which provide additional connection of thin filaments with Z-lines.

Central portions of thick myosin myofilaments are fixed to the *mesophragm* (M-line) by *C-protein* and *myomesin*. Distal portions of thick myofilaments are directed towards Z-lines and have thin actin myofilaments inserted between them. Myosin myofilaments do not reach Z-lines, but are physically connected with Z-lines by *titin* protein molecules.

The *α*-actinin complexes in Z-lines of adjacent myofibrils are connected by intermediate filaments, which approach the inside of plasmalemma and firmly attach myofibrils to cytoskeletal elements in the cortical layer. The alignment of Z-lines of myofibrils in a fiber by physical attachment brings their sarcomeres 'in phase' and thus creates the observable striation of the fiber.

The structure of sarcomere can be described by a formula:

$$\frac{1}{2}\mathrm{I} + \mathrm{A} + \frac{1}{2}\mathrm{I}$$

***Histogenesis of striated skeletal muscle tissue*** proceeds in several stages (Figure 28).

Stage 1 is the stage of *myoblasts* derived from myogenic stem cells of somite myotomes. The majority of myogenic stem cells differentiate into actively proliferating myoblasts. A smaller fraction is turned into myosatellitoblasts which eventually differentiate into myosatellitocytes.

Stage 2 is the stage of *myotubules*. Myoblasts aggregate in long chains and fuse, forming tubules. Myofibrils start to emerge in the cytoplasm.

Stage 3 is the stage of *myosymplasts*. Myotubules increase in volume the numbers of myofibrils in them progressively increase.

Stage 4 is the stage of *mature muscle fibers.* The myofibrils grow thicker and shift to the central position. The nuclei migrate to peripheral position, centrioles and granular endoplasmic reticulum degrade, while mitochondria increase in size and number. Myosatellitocytes occupy their positions at the surface of symplast to become enclosed in common basement membrane with the symplast — the muscle fiber is formed.

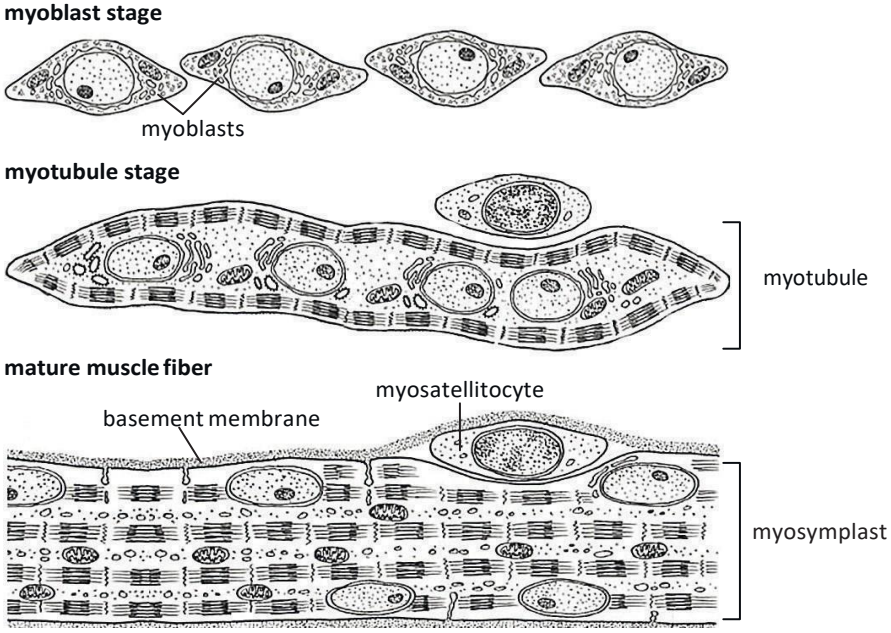


**Figure 28. Histogenesis of cross-striated skeletal muscle tissue [2]** *adapted with changes*

***Skeletal muscle as an organ*** comprises bundles of muscle fibers. The whole muscle is coated in *epimysium* — a capsule of dense fibrous connective tissue. Bundles of muscle fibers inside the muscle are coated individually in *perimysium* composed of loose fibrous connective tissue. Each muscle fiber is coated individually in *endomysium* — a subtle layer of loose fibrous connective tissue with nerve(s) and capillaries.

Skeletal muscle tissue can be observed in *Slide № 70* (H&E).

*At low magnification*, find the region of skeletal muscle with the fibers sectioned longitudinally and transversely. In longitudinal sections, observe multiple basophilic oval nuclei located at the periphery of the fiber. Almost entire volume of the fibers is occupied by myofibrils stained oxyphilically. *At higher magnification*, find a distinctly cross-striated region of the fiber, with clearly discernible dark anisotropic bands (A-bands) and light isotropic bands (I-bands). In transverse sections of muscle fibers, observe myofibrils as distinct dark dots. Observe loose connective tissue filling the spaces between the muscle fibers.

## Exercises

1. Slide № 70 *Striated skeletal muscle tissue of esophagus.* Make a drawing of the tissue at higher magnification and label the structures — *Exercise № 62 in the Workbook.*
2. *Structural organization of myofibril*. Study the scheme and label the structures — *Exercise № 63 in the Workbook*.

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### *Task 3. Muscle contraction mechanism*

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***Muscle contraction*** results from interactions between actin and myosin, formation of bridges between them and displacement of thin (actin) myofilaments relative to thick (myosin) myofilaments consistently with the ***sliding filament model***.

Thick myofilaments consist of *myosin* (300–400 molecules in each thick myofilament). Each myosin molecule consists of double globular head and long tail; it shows the hinge-like flexibility, so that the head and the proximal portion of the tail are capable of rotation. Myosin molecules lie in parallel with the heads concentrated at distal portions of the filament and pointing towards its termini. The central portion of the filament (M-line area) consists of tails only. Myosin heads have ATPase activity (Figure 29).

Thin myofilaments are composed of *actin* and two important regulatory proteins — *troponin* and *tropomyosin*.

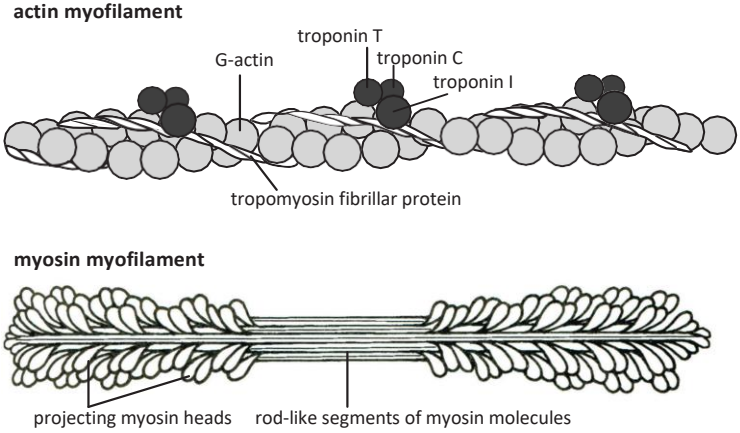


**Figure 29. Structure of myofilaments [9]**

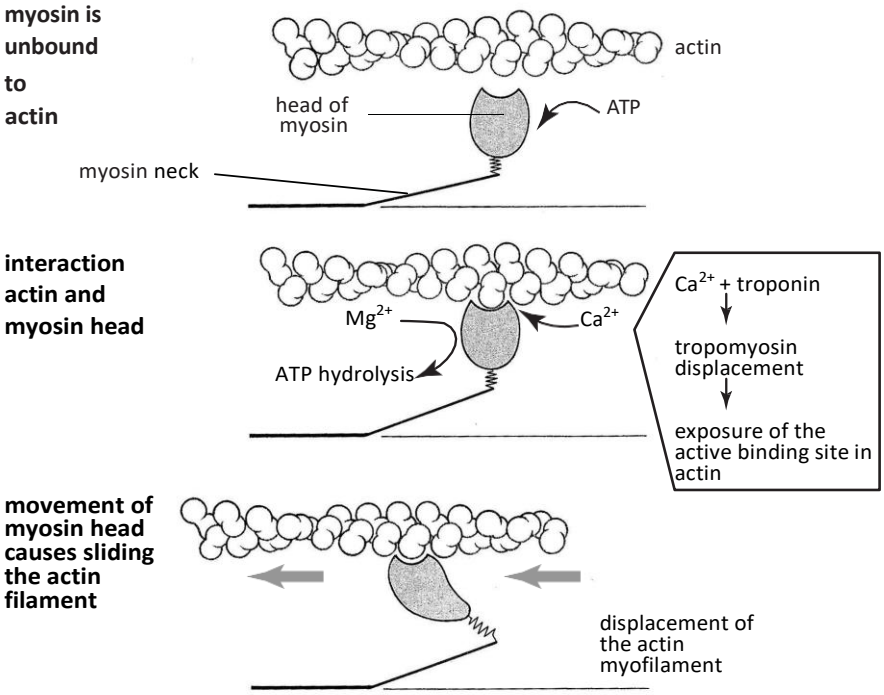


**Figure 30. Muscle contraction mechanism [2]**

Globular actin molecules (arranged one-by-one in threads) form structural backbone of the filament and provide the binding sites for myosin. In the absence of calcium ions, these sites are effectively hindered by long molecules of *tropomyosin*. The globules of calcium-binding protein *troponin* are positioned at regular intervals along the filament (Figure 29).

In the relaxed state of the muscle, the interaction between thick and thin myofilaments is impossible, as the myosin-binding sites in actin filaments are blocked by troponin.

Arrival of the action potential at a neuromuscular junction causes depolarization of sarcolemma. The wave of depolarization propagates into T-tubules and facilitates the opening of calcium channels in L-tubules. Accumulated calcium ions flow from L-tubules to cytosol. Binding of calcium ions to troponin triggers a cascade of conformational changes in thin filaments. Tropomyosin shifts from its blocking position and the myosin binding sites become exposed. The availability of binding sites immediately promotes formation of bridges between myosin heads and actin molecules. Formation of a bridge promotes release of ADP and concomitant bending of the myosin head towards the tail. Upon this bending, the head pulls the bound actin molecule (and the whole thin myofilament) towards the M-line (Figure 30).

Binding of new ATP molecule to the head causes disruption of the bridge. Subsequent hydrolysis of the ATP molecule restores the relaxed position of the head relative to the tail. The cycle is repeated at a rate of 500 times a second.

Thus, muscle contraction results from sliding of thin myofilaments along thick myofilaments in sarcomeres. *The lengths of actin and myosin filaments are constant*. The size of A-band is constant (equal to the length of thick filaments, Figure 31). The sliding of myofilaments causes narrowing of both I-band and H-band and a decrease in the length of sarcomere.

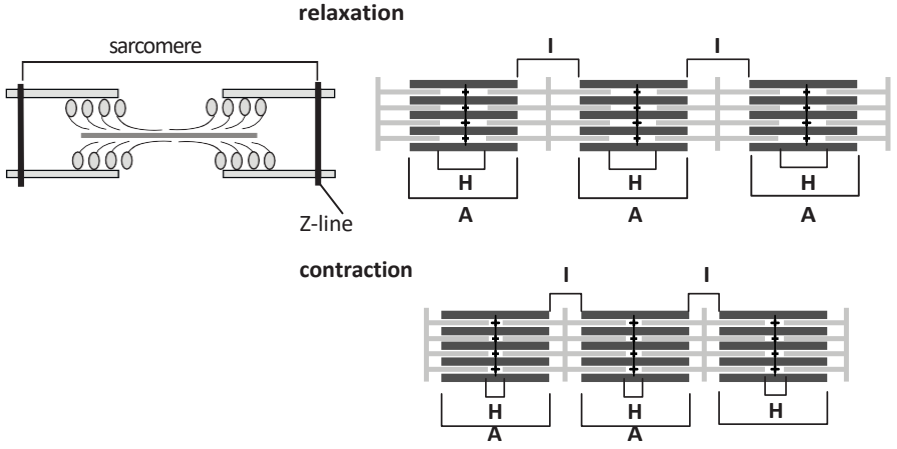


**Figure 31. Relative positions of different components in sarcomere during muscle contraction [10]** *adapted with changes*

## Exercises

Answer the questions:

1. Describe the mechanism of muscle contraction.
2. Which myofilaments mediate triggering of muscle contraction?
3. Which myofilaments mediate production of energy for contraction?
4. Which myofilaments changed their lengths during muscle contraction?
5. A patient has been diagnosed with muscular dystrophy. Would a genetic consultation make sense? Substantiate your opinion.

### *Task 4. Striated cardiac muscle tissue*

***Cardiac muscle tissue*** is found in the muscular layer of the heart wall (*myocardium*) and occasionally in the proximal portions of great vessels connected to the heart. Cardiac muscle tissue has *cellular* structure. Its main competence is making spontaneous rhythmic contractions (a type of involuntary muscle contractions).

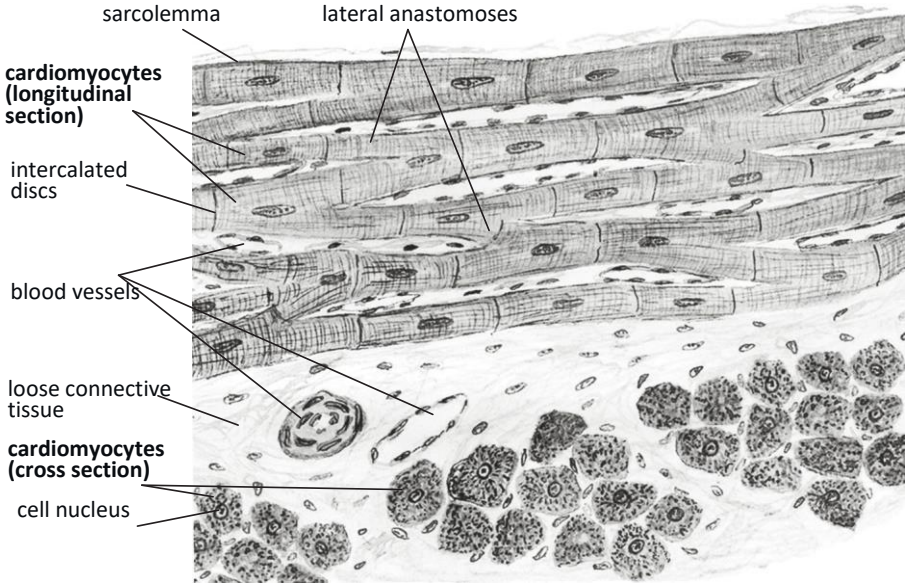


**Figure 32. Striated cardiac muscle tissue [3]**

Cardiac muscle tissue is composed of cells (***cardiomyocytes***) connected by intercalated discs and forming a three-dimensional network of branching and anastomosing *functional cardiac muscle fibers* interspersed with thin layers of loose fibrous connective tissue containing blood vessels and nerves (Figure 32).

Cardiomyocytes can be subdivided into three types (Figure 33):

1) working cardiomyocytes (also known as typical or contractile);
2) atypical cardiomyocytes (pacemaker, transitional and conducting);
3) secretory cardiomyocytes.

***Typical*** (***'working'***) ***cardiomyocytes*** are cylindrical or branching cells, 100–150 μm in length and 10–20 μm in diameter. Each of them contains one or two nuclei positioned in the middle of the cell and cytoplasm with prominent contractile apparatus. The plasmalemma is coated in basement membrane. The contractile apparatus consists of myofibrils, which may fuse with one another. Myofibrils are interspersed with numerous mitochondria. Myofibrils bend around the nucleus, making a clearance of perinuclear cytoplasm containing more mitochondria and the Golgi complex. Similarly with skeletal muscle, cardiomyocyte sarcolemma forms characteristic deep invaginations — T-tubules. By contrast with skeletal muscle, every T-tubule contacts *a single* L-tubule of sarcoplasmic reticulum thus forming a ***diad*** consisting of one L-tubule and one T-tubule (Figure 34).

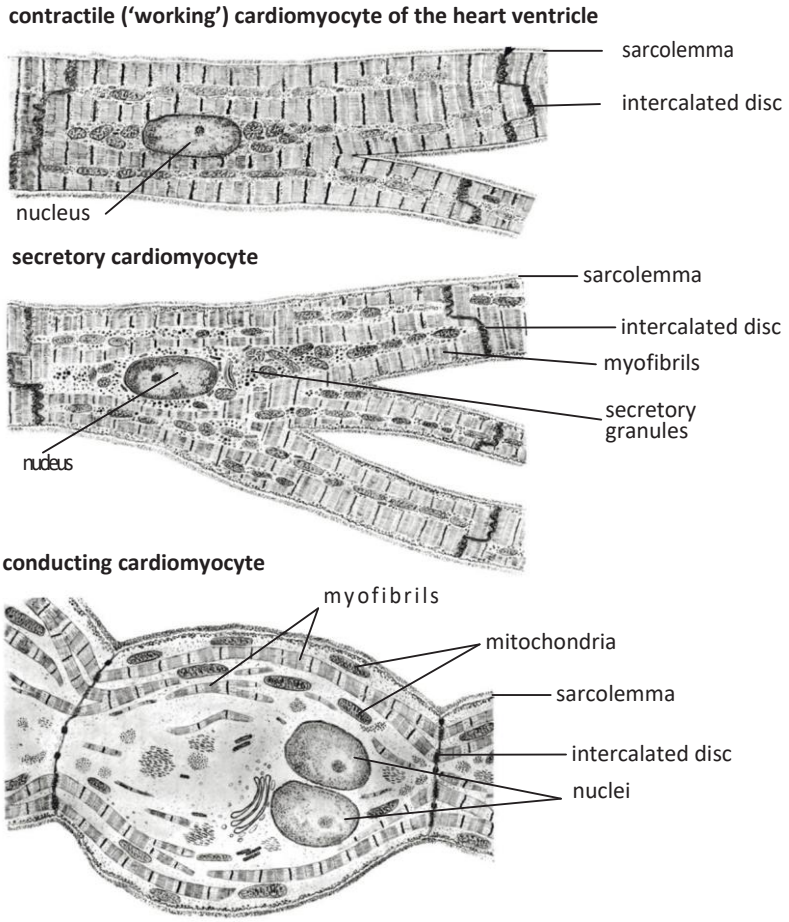


**Figure 33. Ultrastructural organization of cardiomyocytes of different types [3]**

Working cardiomyocytes constitute the bulk of myocardium. They make a dense network of interconnections to act as multicellular *functional fibers*. The junction area between adjacent cardiomyocytes is called *intercalated disc*. This area comprises spot desmosomes, belt desmosomes and gap junctions. *Spot desmosomes* ensure mechanical strength of the heart muscle by preventing the disruption of physical connections between adjacent cardiomyocytes. *Gap junctions* ensure the unity of ion-mediated signaling throughout the functional fibers composed of multiple cardiomyocytes. Belt desmosomes (*adherens junctions*) provide the anchorage to myofibrils of adjacent cardiomyocytes and staple them together, thus contributing to the fiber-like overall organization and functionality of the cardiac muscle tissue (Figures 32 and 35).

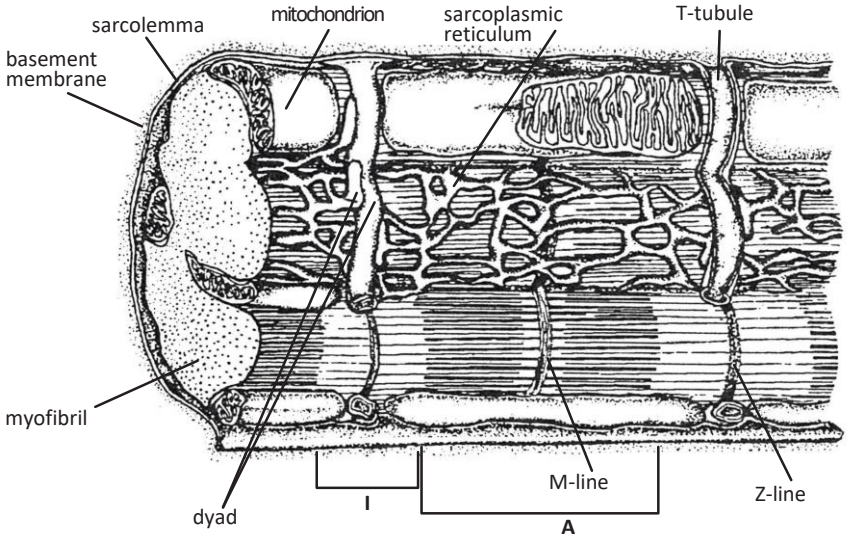


**Figure 34. Sarcotubular system of cardiomyocyte [1]**

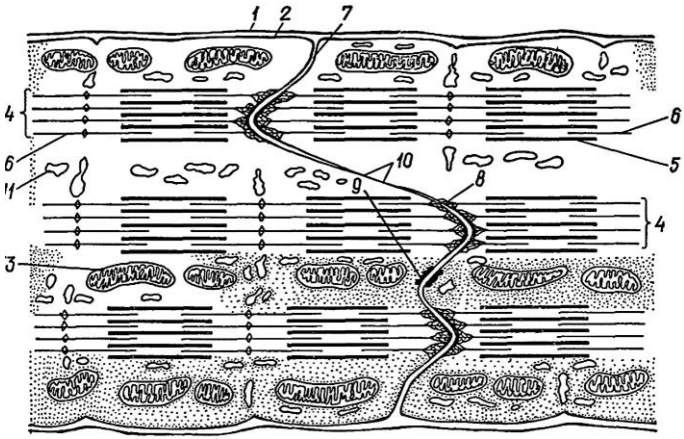


**Figure 35. Ultrastructural organization of the intercalated disc area between adjacent cardiomyocytes [11]**

1 — basement membrane; 2 — sarcolemma; 3 — mitochondrion, 4 — myofibril, 5 — myosin myofilament, 6 — actin myofilament, 7 —intercalated disc, 8 — belt desmosome, 9 — spot desmosome, 10 —gap junction (nexus).

***Atypical cardiomyocytes*** form *conduction system of the heart*. They have large nuclei and scarce myofibrils arranged chaotically and therefore showing no cross-striations. The sarcoplasm is rich in mitochondria and glycogen inclusions. Atypical cardiomyocytes generate electrical impulses, conduct them and convey them onto working cardiomyocytes. Atypical cardiomyocytes include:

1) pacemakers (P-cells, primary pacemakers of the sinoatrial node);
2) transitional (secondary pacemakers of the atrioventricular junction and the bundle of His);
3) conducting cardiomyocytes of the Purkinje fibers.

***Secretory*** (***endocrine***) ***cardiomyocytes*** are found in the atria. They have branched appearance and a moderately developed contractile apparatus. These cells contain electron-dense membranous secretory granules in perinuclear sarcoplasm. These granules are filled with atrial natriuretic peptide — a hormone that stimulates excretion of sodium and water with urine. The effect is accompanied by vasodilation and causes a decrease in the arterial blood pressure.

The structure of cardiac muscle tissue can be observed in *Slide № 71* (H&E).

*At low magnification*, find cardiomyocytes sectioned longitudinally and transversely. *At higher magnification* observe cardiomyocytes with the nuclei in central positions. Cardiomyocytes are connected to one another by lateral anastomoses (at their sides) and intercalated discs (at their ends). The intercalated discs are not the easy thing to observe in this slide. To see them, pull down the condenser and adjust the fine focus carefully. Intercalated discs are observed as thin semitransparent lines (plates) across the fiber. In cardiomyocytes, myofibrils are oriented in parallel. The alternation of isotropic and anisotropic bands located at the same level results in the observable cross-striation. In transverse sections, the myofibrils are visible as dark dots. The chains of cardiomyocytes (functional fibers of the heart muscle) are interspersed with the layers of loose fibrous connective tissue.

The structure of cardiac muscle tissue and Purkinje fibers can be observed in *Slide № 71* (iron hematoxylin staining).

Observe a fragment of the heart wall comprising the layers of endocardium and myocardium. *At low magnification*, find transverse sections of conducting cardiomyocytes — large pale cells arranged in bundles (Purkinje fibers) located beneath the endocardium. They are surrounded by loose fibrous connective tissue containing characteristic white adipose cell clusters. In conducting cardiomyocytes, find the nuclei located centrally or slightly shifted to the periphery of the cell. Conducting cardiomyocytes have fuzzy outlines due to the high content of short myofibrils.

The bulk of myocardium is constituted by working cardiomyocytes which are significantly smaller than conducting cardiomyocytes. Longitudinal sections of working cardiomyocytes have distinct cross-striated appearance. Intercalated discs and anastomoses can be easily observed in this slide. In transverse sections of working cardiomyocytes, observe the centrally located nuclei and the dotted appearance of the cytoplasm comprising numerous transversely cut myofibrils. *At higher magnification*, find blood capillaries in loose connective tissue which surrounds working cardiomyocytes.

**Exercises**

1. Slide № 71 *Striated cardiac muscle tissue*. Make a drawing of the tissue and label the structures — *Exercise № 64 in the Workbook*.
2. Study the scheme of intercalated disc and label the structures — *Exercise № 65 in the Workbook*.
3. Slide № 71a *Conduction system of the heart (iron hematoxylin)*. Make a drawing of the tissue and label the structures — *Exercise № 66 in the Workbook*.
4. Compare different types of cardiomyocytes — complete the table, *Exercise № 67 in the Workbook*.

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***Task 5. Smooth muscle tissue***

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***Smooth muscle tissue*** is found in the walls of hollow organs (blood vessels, urinary bladder, intestine). The tissue lacks cross-striations. It is *innervated by the autonomic nervous system* and provides involuntarily contractions.

Structural-and-functional unit of smooth muscle tissue is ***smooth muscle cell*** (smooth myocyte, Figure 36). Smooth muscle cells are spindle-shaped. The centrally located elongated nucleus is surrounded by cytoplasm with organelles. The cell is enclosed in sarcolemma (plasmalemma+basement membrane). Perinuclear area of the cytoplasm contains Golgi complex, sarcoplasmic reticulum, ribosomes, numerous mitochondria. Diminutive bulbous invaginations of plasmalemma (*caveolae*) accumulate calcium ions. Myofilaments are not organized in myofibrils. Thin myofilaments consist of intertwined chains of globular actin — similarly with thin filaments in striated muscles. In smooth muscle cells, actin filaments form a three-dimensional network: their termini are fastened to the termini of other thin filaments, cytoskeletal elements and plasmalemma by α-actinin and vinculin proteins found in *dense bodies* (located in cytosol) and *dense plaques* on the inside of plasmalemma. The same structures provide attachment sites for intermediate filaments of the cytoskeleton.

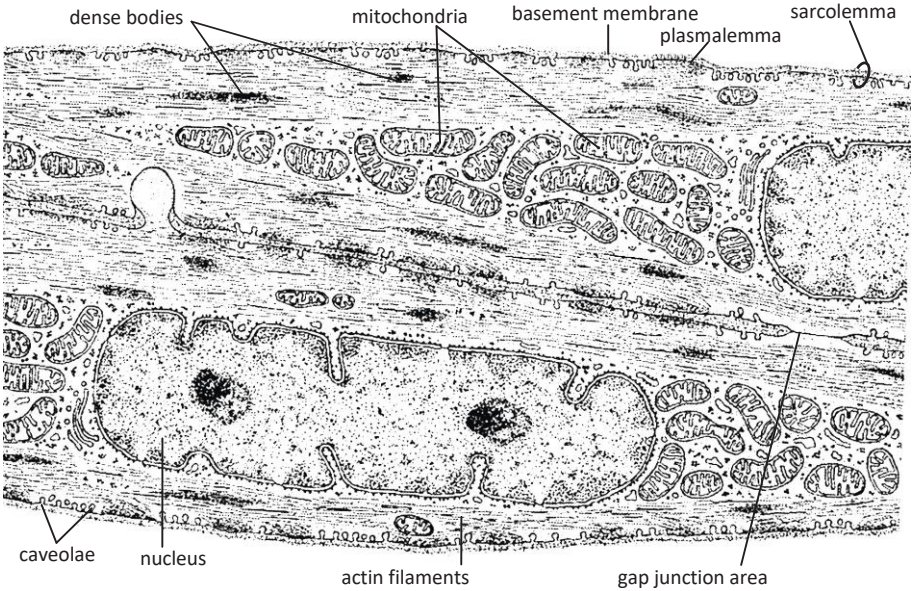


**Figure 36. Ultramicroscopic structure of smooth muscle cell [9]**

Importantly, in relaxed smooth muscle cells, myosin is *depolymerized*. The available myosin monomers (*micromyosin*) are found in cytosol, nearby the actin filaments.

Smooth muscle cells are coated in the discontinuous basement membrane, which leaves a certain portion of plasmalemma exposed, so that adjacent muscle cells form gap junctions (nexuses) with each other.

In addition, the cells are joined together by interdigitations and spot desmosomes. The basement membranes of smooth muscle cells are interlaced with elastic and reticular fibers, which ensure functional coordination of the cells in higher-order morphological structures. Smooth muscle tissue is usually organized in bundles or layers of smooth muscle cells (Figure 37). Smooth muscle cells synthesize proteoglycans, glycoproteins, procollagen and proelastin, which give rise to the amorphous and fibrous components of extracellular matrix.

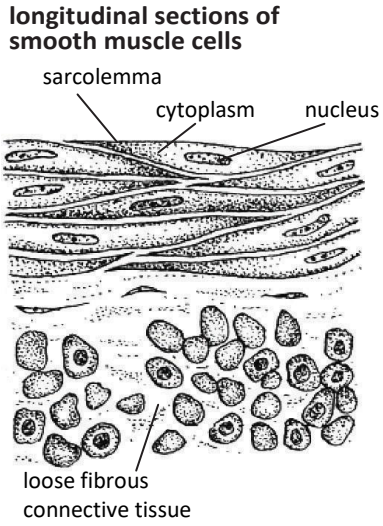


**Figure 37. Smooth muscle tissue at the light microscopy level**

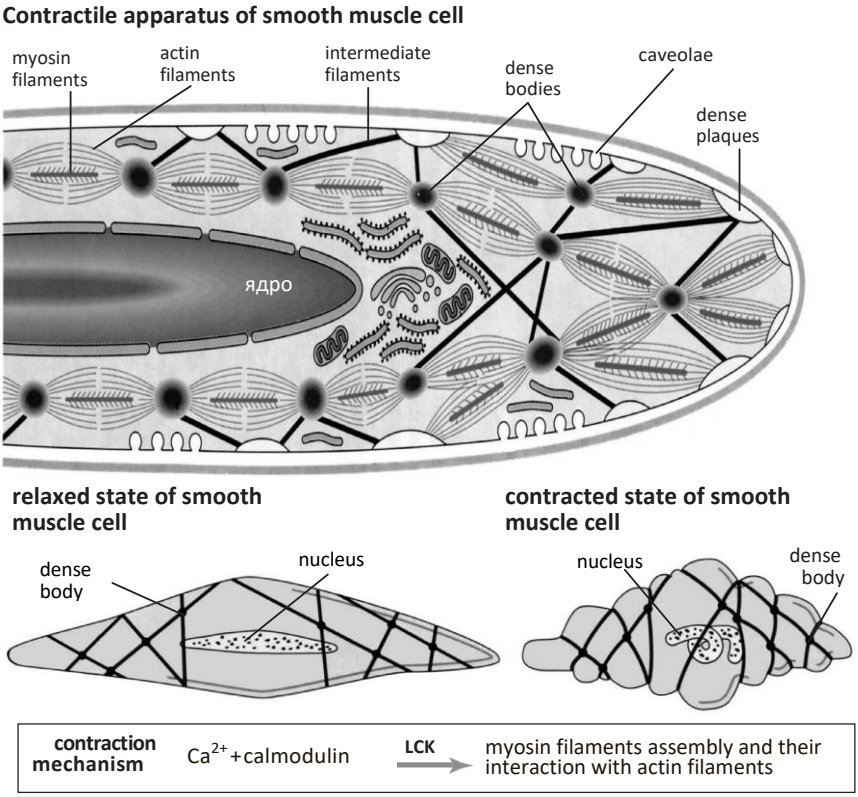


**Figure 38. Contractile apparatus of smooth muscle cell and its engagement in smooth muscle contraction [12, 13]**

***Contraction of smooth muscle cells*** results from interaction of actin filaments with myosin. A neurotransmitter interacts with its receptor at the surface of plasmalemma thereby altering its permeability. The contraction is induced by the resulting *influx of calcium ions from caveolae* and sarcoplasmic reticulum into the cytoplasm. The interaction of calcium ions with *calmodulin* protein triggers *phosphorylation of myosin light chains* by the enzyme called *myosin light-chain kinase* (LCK). Myosin phosphorylation results in its polymerization. In the temporary myosin myofilaments of smooth muscle, myosin 'heads' are distributed evenly along the filament, which expands the contact area with actin myofilaments and facilitates the interaction. When the filaments come into sliding motion, thin actin filaments pull dense bodies and dense plaques they are attached to. The pulling force is conveyed onto sarcolemma and the entire smooth muscle cell becomes contracted (Figure 38). Withdrawal of the activation signal (neurotransmitter) causes a reduction in the cytoplasmic concentration of calcium.

Under reduced calcium concentration, myosin undergoes depolymerization and the contraction stops. The elasticity of cytoskeleton returns the muscle cell into relaxed state.

Smooth muscle tissue of the intestine can be observed in *Slide № 72* (H&E staining).

*At low magnification*, identify the muscular layer in the wall of intestine and find a location where smooth muscle cells are sectioned in different orientations. The cytoplasm of smooth muscle cells is intensely oxyphilic. *At higher magnification*, observe smooth muscle cells in longitudinal and transverse sections. The spindle-shaped smooth muscle cells are positioned closely. In longitudinal sections, they look elongated, with the thin rod-shaped nuclei in the middle. In transverse sections, both the cells and their nuclei look rounded. Because of the spindle shape with pointed termini, the diameter of transverse sections varies and the nuclei can be observed only in some of them (other cells look 'enucleated'). Smooth muscle cells are surrounded by networks of collagen and elastic fibers interlacing the basement membrane; however, these lacey networks are poorly distinguishable from the oxyphilic cytoplasm of the cells. Observe loose fibrous connective tissue interspersing bundles and layers of smooth muscle.

### Exercises

1. Slide № 72 *Smooth muscle tissue in the wall of small intestine*. Make a drawing of the tissue and label the structures — *Exercise № 68 in the Workbook.*
2. *Contractile apparatus of a smooth muscle cell*. Study the scheme and label the structures — *Exercise № 69 in the Workbook.*
3. *Comparative characterization of muscle tissues* (complete the table) — *Exercise № 70 in the Workbook.*

Homework: **Nervous tissue**

Pawlina W., Ross M.H. Histology: A text and Atlas: with Correlated Cell and Molecular Biology. Philadelphia etc.: Wolters Kluver: Lippincott Williams and Wilkins, 7- th edition, pp 356-381

## Topic 5. NERVOUS TISSUE

**Class objective:**

1. Learn morphological features and functional properties of nervous tissue.
2. Learn the elements of nervous tissue, their functional significance and embryonic sources.

3. Study microscopic and ultramicroscopic structure of neurons, glial cells, nerves and nerve endings.
4. Gain understanding of the reflex arc organization.

## *Task 1. General overview of nervous tissue*

***Nervous tissue*** is responsible for:

- perception of stimuli from the external and internal environments, processing of these stimuli into complex excitation patterns, conduction and transmission of the excitation to other neurons or non-neural effector cells;
- synthesis of biologically active substances including hormones;
- trophic influence on other tissues.

*Nervous tissue* consists of nerve cells (*neurons*) and *neuroglia*.

***Neurons*** are basic components of nervous tissue responsible for its specific functions. ***Neuroglia*** supports the viability and functioning of neurons; the functions of neuroglia can be classified as architectonic, barrier, trophic, secretory and protective.

Nervous tissue constitutes the nervous system and develops from neuroectoderm. Its early morphogenesis includes formation of neural plate, neural groove and neural tube. During the neural tube closure, a fraction of cells from the apical portion of neural folds escape and form ***neural crest*** — a transient structure, two symmetrical strips of cells on the sides of the neural tube. Neural crest eventually dissociates into neuromesenchyme which migrates to specific destinations in the developing embryo to produce a number of derivatives. In the cranial area, characteristic thickenings of the ectoderm (*neural placodes*) are formed at the sides of neural tube (Figure 39).

Neural tube gives rise to all neurons and glial cells of the central nervous system (CNS) *except microglia*.

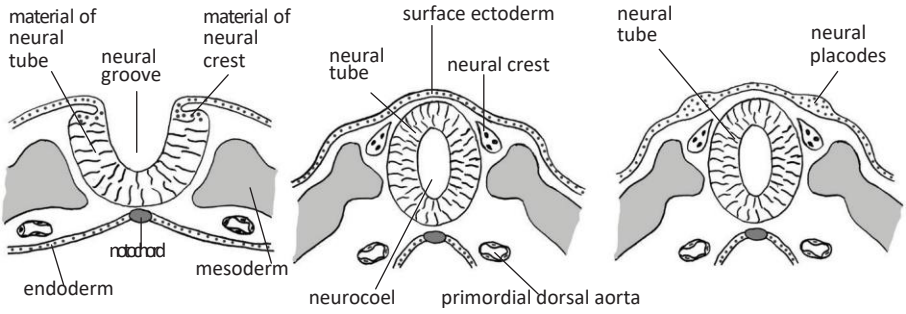


**Figure 39. Embryonic development of nervous tissue**

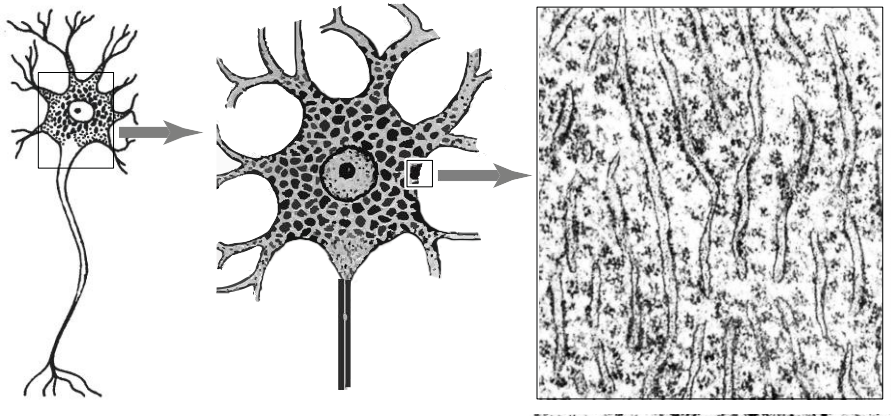

**Figure 40. Tigroid bodies in cytoplasm of a neuron**

Neural crest (*neuromesenchyme*) gives rise to neurons and glial cells of the peripheral nervous system (PNS) which includes dorsal root ganglia, autonomic ganglia and peripheral nerves. It also gives rise to the structures of arachnoid mater and pia mater, chromaffin cells of adrenal medulla, skin melanocytes and sensory cells. Neural placodes give rise to the cranial nerve ganglia V, VII, IX and X. *Microglial cells* of CNS have mesodermal origin: they are descendants of hematopoietic (monocytic) cell lineages. Neuroblastic differon includes neuroblasts, immature neurons and mature neurons. Glioblastic differons include glioblasts and glial cells. Neuronal cell populations are *static*, whereas glial cell populations are *expanding*.

## Exercises

Answer the questions:

1. What elements of nervous tissue do you know? What are their functions?
2. Are neurons capable of cell divisions in the postnatal period?

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### *Task 2. Neurons*

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***Neurons*** are highly specialized cells of nervous system responsible for the perception of stimuli from external and internal environments, processing of these stimuli, generation and conduction of signals in the form of electrical impulses and transmission of these signals to other neurons, muscle cells or secretory cells. The transmission is accomplished through release of specific substances (*neurotransmitters*) which bind to receptors at the surface of target cells.

Each neuron consists of the body (*perikaryon*) and processes — dendrites and one axon. Dendrites collect the excitation signals and ensure their conduction to the neuron body for accumulation and integration, whereas the axon ensures propagation of the resulting electrical output. The excitation travels along the axon in the form of *action potential* (nerve impulse) and is finally transmitted onto target cells via *axon terminals*.

A neuron can have many dendrites, but only *one* axon. Neuron, as any cell, consists of the nucleus and cytoplasm, and is enclosed in plasmalemma. The major portion of cell volume is constituted by its processes. The nucleus with prominent nucleoli occupies central position in the perikaryon. Plasmalemma is engaged in reception, generation and conduction of excitation signals. The structure of cytoplasm in perikaryon is different from its structure inside the processes.

Cytoplasm of the perikaryon is rich in organelles: endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes. Neuron-specific cytoplasmic structures, which can be observed at the light-microscopy level, include chromatophilic substance (Nissl bodies) and neurofibrils.

***Chromatophilic substance*** of the cytoplasm (*Nissl bodies*, basophilic bodies, *tigroid substance*) is observable in neurons stained with basic dyes (methylene blue, toluidine blue, hematoxylin, etc.). Nissl bodies represent accumulations of granular endoplasmic reticulum cisternae, free ribosomes and polysomes. Nissl bodies in the form of clumps and granules can be observed in perikaryons and dendrites, but is never observed in axons nor their initial segments (*axonal hillocks*, Figure 40).

Cytoskeletal elements found in the cytoplasm of neurons include intermediate filaments (*neurofilaments*), microtubules (sometimes called *neurotubules*) and actin microfilaments. Bundles of neurofilaments in association with microtubules can be observed as filamentous moieties in silver-stained neurons — the patterns known as *neurofibrils*. Intermediate filaments implement mechanical function — they are responsible for the shape of perikaryon and processes. Actin filaments participate in morphogenesis (e.g. define the shape of growth cone during neurite outgrowth). Microtubules and their associated proteins ensure cytoplasmic transport of substances and structures, especially in axons.

***Axonal transport*** is subdivided into *anterograde transport* (from the body of a neuron outward along the axon) and *retrograde transport* (along the axon to the body) (Figure 41). The transported substances are packed in vesicles driven along the axon by interactions with cytoskeletal elements (chiefly with microtubules via the microtubule-associated motor proteins — *kinesin* and *dynein*). Axonal transport is calcium-dependent. ***Anterograde axonal transport*** is subdivided into slow (1–5 mm/day) and fast (100–500 mm/day). *Slow anterograde axonal transport* ensures transportation of cytoskeletal elements and enzymes. It is needed for the maintenance of axonal cytoplasm (*axoplasm*) in mature neurons. It also supports neurite outgrowth in developing or regenerating neurons.

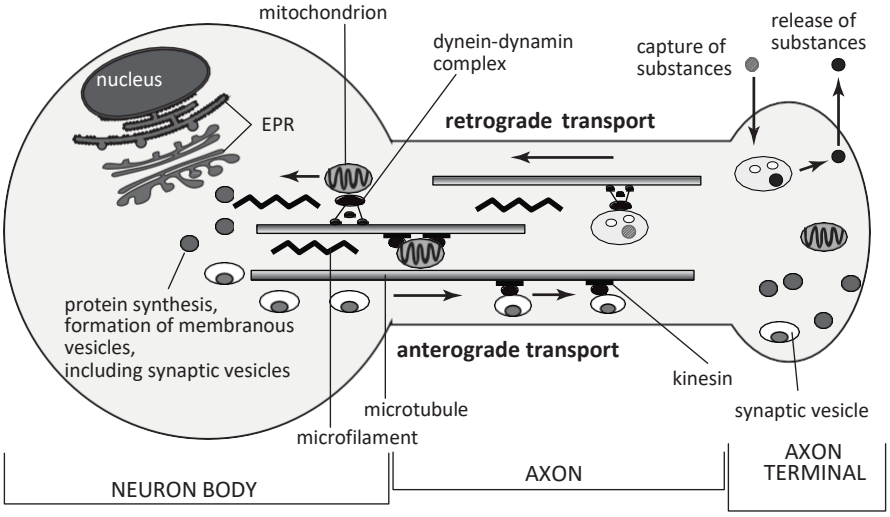


**Figure 41. Axonal transport [14]**

*Fast anterograde axonal transport* ensures transportation of endoplasmic reticulum cisternae, mitochondria and neurotransmitter-containing vesicles. ***Retrograde axonal transport*** (100–200 mm/day) ensures removal of worn-out elements from axon terminals for lysosomal degradation, turnover of the vesicles and mitochondria.

***By morphology*** (Figure 42), the neurons are subdivided into:

- multipolar — with multiple processes (*the majority of neurons are multipolar*);
- unipolar — with one axon and no dendrites (*are typical for embryogenesis*);
- bipolar — with one axon and one dendrite (found in retina of the eye, spiral ganglion of the inner ear);
- pseudounipolar — with one axon and one dendrite which have common initial segment (found in dorsal root ganglia).

*Chromatophilic substance* (Nissl bodies) in the multipolar neurons of the spinal cord can be observed in *Slide № 77* (Nissl staining).

Chromatophilic substance is rich in ribonucleoproteins. It is therefore highly basophilic and binds intensely with methylene blue or toluidine blue.

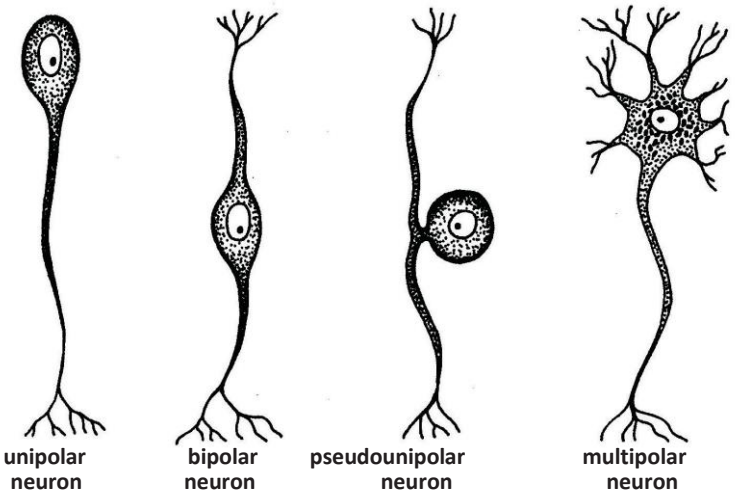


**Figure 42. Morphological classification of neurons [1]**

In the spinal cord, *neurons* (cell bodies, dendrites and initial segments of axons) are found in the gray matter which occupies the central portion of the organ and is characteristically butterfly-shaped in cross-sections. *At low magnification*, find a big multipolar neuron stained blue. *At higher magnification*, study its nucleus with active chromatin (looks 'empty') and prominent nucleolus. Observe the basophilic clumps of Nissl bodies in the perikaryon and dendrites; pay attention to their absence from the axon and axonal hillock.

*Neurofibrils* in motor neurons of the anterior horns of the spinal cord can be observed in *Slide № 76* (staining by silver impregnation).

The impregnation with silver salts stains nucleoli and neurofibrils dark brown. The nucleus looks pale and empty. *At low magnification*, find a big neuron in one of the anterior horns of the spinal cord. *At higher magnification*, study its nucleus with prominent nucleolus and observe neurofibrils in the cytoplasm. Pay attention to the reticular morphology of neurofibrils in the cell body as compared to the parallel orientation of neurofibrils in the processes.

## Exercises

1. *Types of neurons* — complete the table, *Exercise № 71 in the Workbook.*
2. Slide № 77 *Tigroid substance (Nissl bodies, chromatophilic substance) in multipolar neurons of the spinal cord* and Slide № 76 *Neurofibrils in multipolar neurons of the spinal cord*. Make drawings of neurons, label the structures — *Exercise № 72 in the Workbook.*

## *Task 3. Neuroglia*

***Neuroglia*** is a collective term for heterogeneous group of cells in nervous tissue. Glial cells provide structural and functional support to the neurons. Glia of the central nervous system originates from the neural tube, whereas glia of the peripheral nervous system originates from the neural crest.

***Glial cells*** *of the central nervous system* typically have long branching processes. By size, these cells are subdivided into *macroglia* and *microglia*. Macroglia includes *astrocytes*, *ependymocytes* and *oligodendrocytes* (Figures 43 and 44).

***Astroglia*** is a collective name for *astrocytes* — the cells of stellate shape, with pale oval nucleus and intermediate filaments composed of the *glial fibrillary acidic protein* (GFAP) used as a marker of astrocytes. Astroglia implements architectonic, barrier and trophic functions. Astrocyte processes have feet-like extensions (*podia*) which form insulating coatings around neurons and synapses. Astrocyte processes reach basement membranes of blood capillaries and form perivascular membranes around the capillaries — the basis of the blood-brain barrier. Astrocytes capture various substances from blood and convey them to neurons; they scavenge the excessive extracellular potassium and neurotransmitters, which accumulate in the extracellular spaces after intense neuronal activity. In addition, the podia of astrocytes form *glia limitans* — a protective sheet at the surface of the brain (the glial limiting membrane of the brain). Astrocytes are connected by gap junctions with each other and also with the cells of oligodendroglia and ependymal glia.

*Astrocytes are subdivided* into protoplasmic astrocytes and fibrous astrocytes. *Protoplasmic astrocytes* are located in the gray matter of the central nervous system; their processes are not very long but thick and highly branched. *Fibrous astrocytes* are predominantly located in the white matter; their processes are thin, long and weakly branched. Astrocytes are capable of proliferation and migration in the postnatal period. In the case of brain damage, they fill in the defect and form glial scars.

***Ependymal glia*** (ependyma) consists of cubic or cylindrical cells which jointly constitute a single-layered lining of the ventricles of the brain and central canal of the spinal cord. Ependyma includes *ependymocytes* proper, *tanycytes* and *choroidal ependymocytes*.

*Ependymocytes* make a single-layer lining of brain ventricles and central canal. Their apical surfaces comprise motile cilia immersed in liquor (the cerebrospinal fluid, CSF) and promoting its circulation.

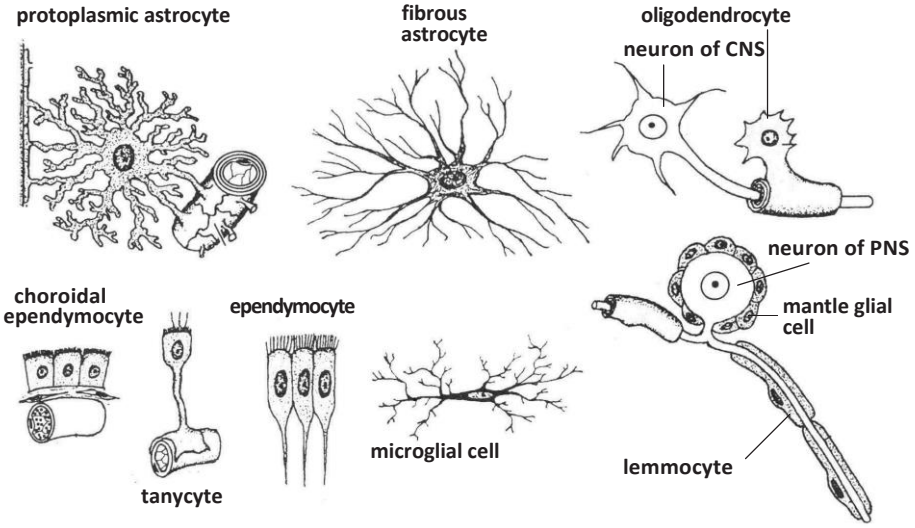


**Figure 43. Types of glial cells [1]** *adapted with changes*

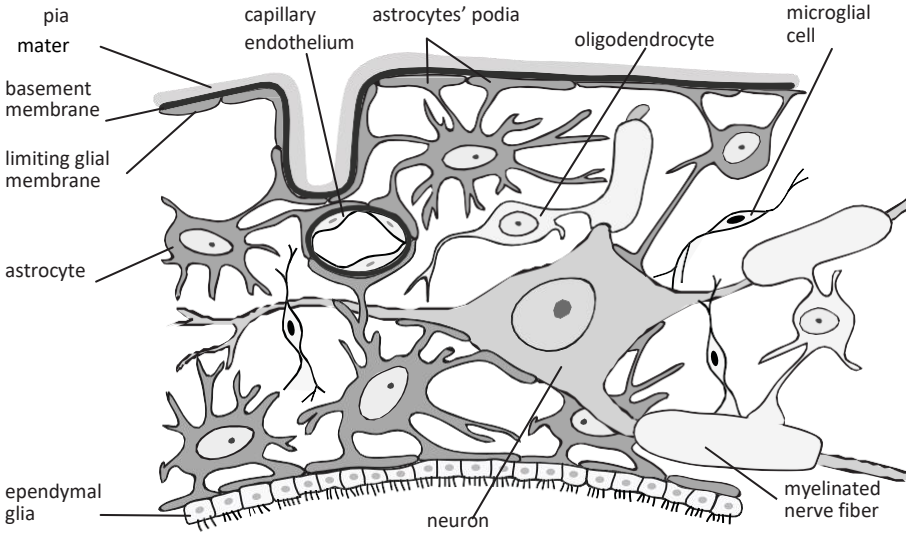


**Figure 44. Locations of glial cells in the central nervous system**

Some ependymocytes have long basal processes (one per cell) reaching the surface of the brain and incorporating into glia limitans. *Tanycytes* are specialized cells with microvilli and occasional cilia at the apical surface and a long basal process with a foot-like ending that wraps around a blood capillary. Tanycytes capture substances from the liquor and transfer them to the capillary lumina thus ensuring communication between the liquor and the blood. *Choroidal ependymocytes* are found in choroid plexuses of the brain; they are engaged in production of the liquor and participate in the blood-liquor barrier.

***Oligodendroglia*** (*oligodendrocytes*) are rather small cells; their processes are few and not very long. *Oligodendrocytes* are located in both gray and white matters of the central nervous system. Glial cells of PNS (*lemmocytes* of peripheral nerves and *mantle cells* of ganglia) resemble oligodendrocytes and may be considered as their subtypes.

Oligodendrocytes participate in formation of myelinated and unmyelinated nerve fibers. They modulate conduction of nerve impulses and participate in repair of neuronal damage by phagocytosis of distal portions of the damaged axons and myelin. Oligodendrocytes contribute to insulatory, trophic, architectonic and protective functions of neuroglia.

***Microglia*** consists of small stellate cells which belong to macrophage system of the body. Microglial cells implement defense function and are otherwise known as *glial macrophages.*

*Mantle glial cells of PNS* in ensemble with pseudounipolar neurons of a dorsal root ganglion (spinal ganglion) can be observed in *Slide № 85a* (staining by silver impregnation).

*At low magnification*, find large round cells with light, empty-looking nuclei. These are pseudounipolar neurons located in groups at the periphery of the ganglion. Their processes can be observed very well, thanks to the special staining by impregnation with silver salts. Find a neuron with observable initial segment of the process branching dichotomically at some distance from the perikaryon. *At higher magnification*, study the layer of small mantle cells surrounding the neurons. The cytoplasm of glial cells is scarce and almost invisible, but their rounded nuclei can be observed very well.

## Exercises

1. *Neuroglia* — complete the table, *Exercise № 73 in the Workbook*.
2. Answer the questions:
   - What types of neuroglia do you know? What are their embryonic sources?
   - Describe structural features and location of astrocytes, ependymocytes and oligodendrocytes. What specific functions do they implement?
   - Characterize the microglia.

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### *Task 4. Nerve fibers*

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***Nerve fibers*** consist of cellular processes of neurons coated in glial membranes. Nerve fibers are subdivided into *myelinated* and *unmyelinated*.

***Unmyelinated nerve fibers*** predominantly belong to the autonomic nervous system. They have *low conduction velocities* (i.e. propagation of nerve impulses along these fibers is *slow*). Unmyelinated nerve fibers have glial cells (*lemmocytes*) at their core. These lemmocytes hold insulated neuronal processes (*axial cylinders*) in multiple folds of their cytoplasm. As an axial cylinder (usually axon) contacts lemmocyte surface, the lemmocyte wraps around it. The converging cytoplasmic edges come together and form *mesaxon* — a duplicated area of plasmalemma with the membranes tightly pressed against one another (Figure 45) and the axial cylinder sealed beneath. A single lemmocyte can harbor 10–20 insulated axial cylinders; the whole structure resembles a multi-wire cable (and is sometimes referred to as *cable-type nerve fiber*). The fiber is enclosed in basement membrane from outside.

***Myelinated nerve fibers*** are found in both central and peripheral nervous systems; they are characterized by *high conduction velocities*.

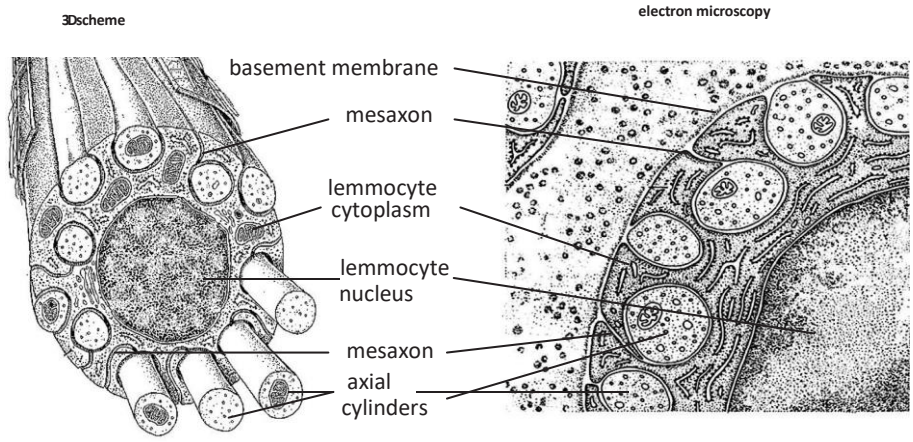


**Figure 45. Unmyelinated nerve fiber [15, 16]**

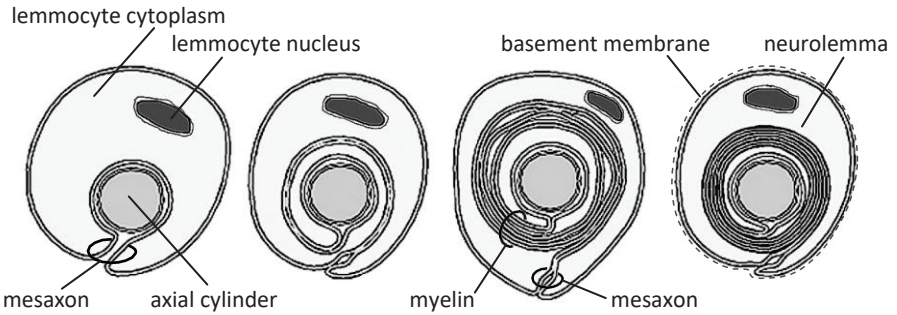


**Figure 46. Myelination in PNS**

Myelinated nerve fibers are usually thicker than unmyelinated. Each of them has single axial cylinder at its core. This axial cylinder is wrapped in myelin sheath formed by continuous multiple wrapping of the *thinly flattened cytoplasmic edge of glial cell* around the axial cylinder. As the myelin sheath predominantly consists of lemmocyte *plasmalemma*, it is stained intensely with *osmic acid*. Myelin sheaths are formed by *oligodendrocytes* in the central nervous system and by *Schwann cells* (a.k.a. *neurolemmocytes* or *lemmocytes*) in the peripheral nervous system.

In the *peripheral nervous system*, the enormously extended mesaxon grows around the axial cylinder wrapping it progressively in multiple layers (Figure 46). As the number of layers increases, the cytoplasm inside them degrades – the layers become increasingly thin. Mature myelin sheets are almost entirely composed of plasmalemma, with tiny portions of cytoplasm between adjacent membranes preserved at certain locations only. These portions of residual cytoplasm in myelin (*Schmidt-Lanterman incisures*, Figures 47 and 48) can be identified by their resistance to osmium staining. The outer layer of the myelin sheath in mature fibers is called *neurolemma*; this layer is thick as it contains the lemmocyte nucleus and perinuclear cytoplam. The whole fiber is enclosed in basement membrane.

Importantly, each lemmocyte covers a single segment of the fiber. The regions of the fiber between adjacent myelinated segments are left unmyelinated. These unmyelinated regions (*nodes of Ranvier*) maintain depolarization of the same amplitude over the whole length of the fiber. Electron microscopy of the nodal area reveals a bulbous expansion of the axial cylinder and cytoplasmic interdigitations of adjacent lemmocytes. Myelination insulates the surface of axial cylinder and thereby enforces propagation of the electrical excitation via axoplasm thus greatly increasing conduction velocity. Conduction of the excitation by myelinated fibers is *saltatory*, as membrane depolarization occurs at the nodes of Ranvier only.

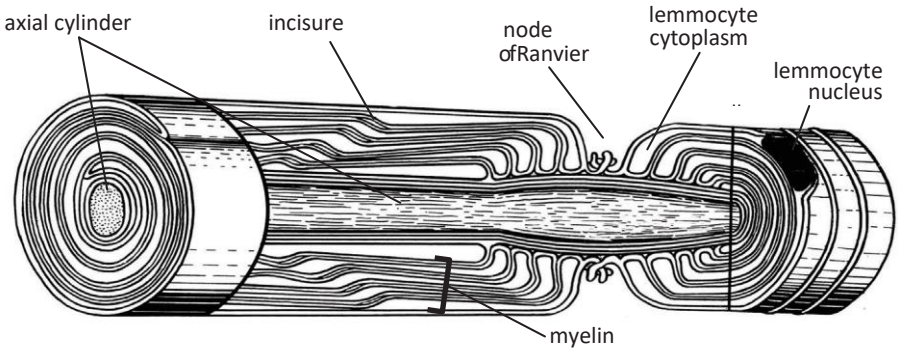


**Figure 47. Structure of myelinated nerve fiber [2]**

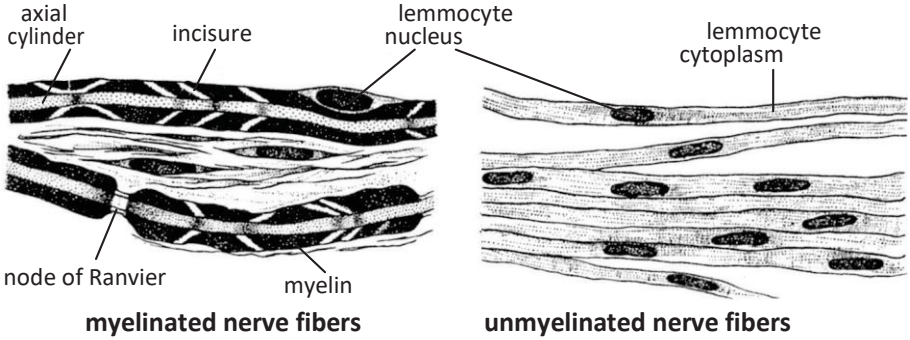


**Рис. 48. Myelinated and unmyelinated nerve fibers in PNS [2]**

In the *central nervous system*, myelination is responsibility of *oligodendrocytes*. One oligodendrocyte can be involved in myelination of different fibers, as each of its processes covers one internodal segment (Figure 49). In CNS, myelinated fibers have no incisures and are not enclosed in basement membranes.

Myelinated and unmyelinated nerve fibers from *peripheral nerve* can be observed in *Slide № 79* (staining: osmic acid and carmine).

Osmic acid stains myelin black because it is so rich in lipids. *At low magnification*, find a single separated myelinated nerve fiber. *At higher magnification*, observe the pale axial cylinder surrounded by dark myelin layer with the *nodes of Ranvier*, which look like constrictions across the fiber, and *Schmidt-Lanterman incisures*, which look like narrow oblique slots. Neurolemma is best observed with slightly lowered condenser: it looks like a lucid stripe at the periphery of the fiber and is especially distinct at the nodes.

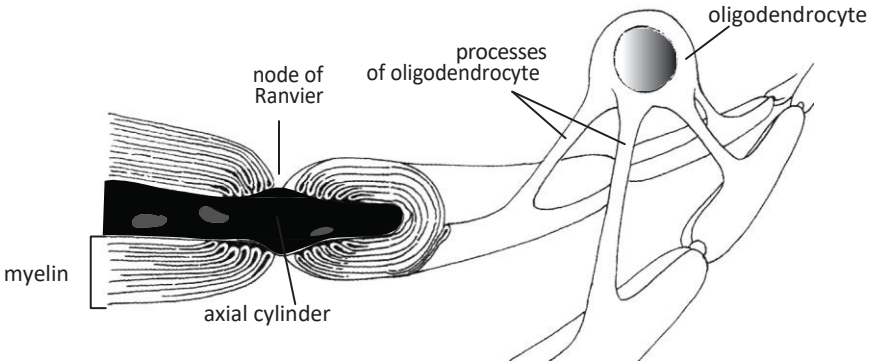


**Figure 49. Formation of a myelinated nerve fiber in the central nervous system [8]**

*At low magnification*, identify unmyelinated nerve fibers. Observe them *at higher magnification* as gray threads with elongated nuclei of lemmocytes stained red or pink. Lemmocyte membranes, mesaxons and axial cylinders are very subtle and cannot be distinguished in this slide.

**Exercises**

1. Study the scheme of myelinated nerve fiber. Label the structures — *Exercise № 74 in the Workbook.*
2. Study the scheme of myelinated and unmyelinated nerve fibers. Label the structures — *Exercise № 75 in the Workbook.*

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***Task 5. Nerve endings***

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***Nerve endings*** are morphologically distinguished specializations at the ends of nerve processes. Nerve endings are subdivided into three functional groups:

1) *axon terminals* on neurons (interneuronal synapses) ensure electrical communication between the neurons;
2) *efferent* (*effector*) nerve endings are *modified axon terminals* which transmit the excitation signals onto effector organs (muscles, glands). Include *motor nerve endings* (notably *motor end plates* at neuromuscular junctions) and *secretory nerve endings*;
3) *afferent* (*receptor*, or *sensory*) nerve endings receive stimuli from the external and internal environments and transform these stimuli into excitation; these endings belong to *dendrites*.

***Synapses*** are subdivided into *chemical synapses* and *electrical synapses* depending on the mechanism of transmission**.**

***Electrical synapses*** resemble gap junctions (*nexuses*); they ensure bidirectional exchange of signals in the form of ionic currents. In humans and other mammals, *electrical synapses are typical for embryogenesis* and play important role in the establishment of interneuronal connections.

***Chemical synapses*** transmit the excitation by release of specific molecules — *neurotransmitters*. Chemical synapse ensures unidirectional transmission of the excitation.

***Chemical synapse*** consists of:

1) *presynaptic zone* — synaptic terminal of an axon; contains synaptic vesicles, cytoskeletal elements (neurotubules and neurofilaments), mitochondria and characteristic specialization of plasmalemma (*presynaptic density*);
2) *synaptic cleft* contains neurotransmitters released from synaptic terminal to bind their receptors at the opposite (postsynaptic) side of the cleft;
3) *postsynaptic zone* belongs to other neuron or innervated non-neuronal cell; it contains receptors to neurotransmitter and associated protein networks (*postsynaptic density*).

Human nervous system comprises a variety of neurotransmitters. Synaptic transmission at each particular synapse is always mediated by the same type of neurotransmitter, e.g. acetylcholine (in cholinergic synapses) and norepinephrine (in noradrenergic synapses). *Cholinergic synapses* include parasympathetic synapses, preganglionic sympathetic synapses, neuromuscular junctions and a number of synapses in CNS. *Noradrenergic synapses* include postganglionic sympathetic synapses and a number of synapses in CNS.

Plasmalemma of synaptic terminals is rich in voltage-dependent calcium channels. When the wave of depolarization reaches the terminal, these channels open, and the resulting influx of calcium promotes the release of neurotransmitter by exocytosis.

*Presynaptic membrane* is a region of plasmalemma (axolemma) of the presynaptic neuron (which conveys the signal). Presynaptic membrane has electron-dense patches where neurotransmitter is released by exocytosis.

*Synaptic cleft* lies between the pre- and postsynaptic membranes separated by a 20–30 nm distance and tethered to one another with synaptic cell adhesion molecules.

*Postsynaptic membrane* is a region of plasmalemma of the postsynaptic neuron or non-neuronal cell. Postsynaptic membrane contains receptors and ionic channels and shows distinct specialization (*postsynaptic density*) because it provides anchorage to huge macromolecular complexes responsible for the postsynaptic processing of the signal.

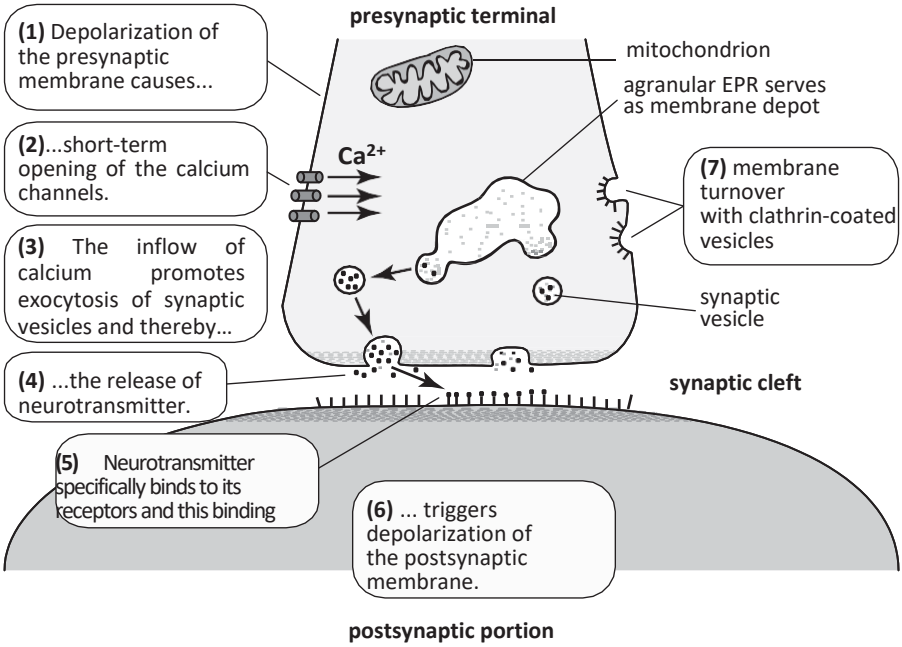


**Figure 50. Synaptic transmission mechanism [13]** *adapted with changes*

Figure 50 illustrates the order of events at a chemical synapse:

- depolarization reaches synaptic terminal;
- it triggers the opening of voltage-dependent calcium channels thus promoting *influx of calcium ions* to the terminal;
- the local increase in axoplasmic calcium facilitates *exocytosis of synaptic vesicles* — neurotransmitter is released into synaptic cleft;
- *neurotransmitter molecules bind their cognate receptors* at the opposite side of the cleft (on the outer surface of the postsynaptic membrane);
- the binding of neurotransmitter causes *opening of ionic channels* in the postsynaptic membrane thus promoting *generation of excitatory or inhibitory postsynaptic potentials*;
- *neurotransmitter is withdrawn* (through re-uptake by presynaptic terminal, uptake by glial cells, diffusion or enzymatic degradation) and the transmission stops.

***Efferent (effector) nerve endings*** are subdivided into *motor nerve endings* and *secretory nerve endings*.

***Motor nerve endings*** belong to either autonomic or somatic efferent neurons (the bodies of which reside, respectively, in autonomic ganglia and spinal cord).

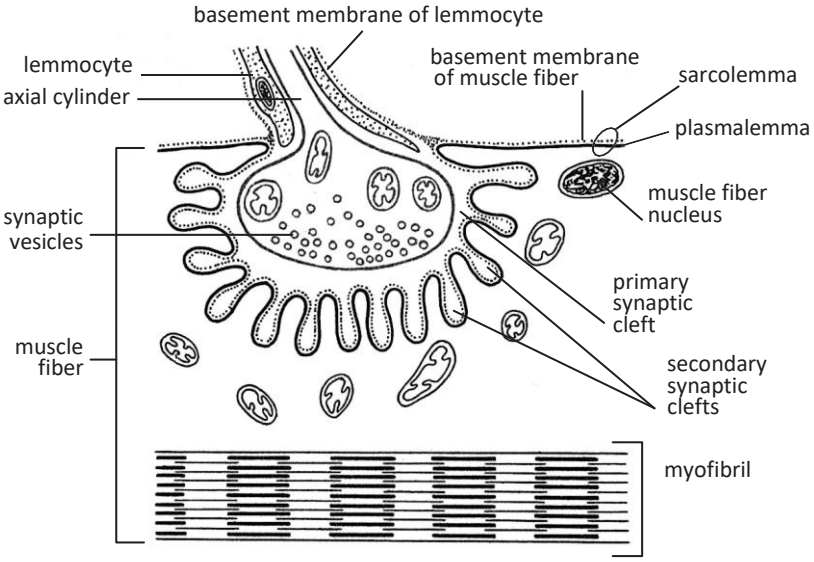


**Figure 51. Motor end plate [17]**

Efferent neurons convey the excitation signal onto tissues in effector organs.

Motor nerve endings in striated skeletal muscles are called *neuromuscular junctions* (a.k.a. neuromuscular synapses, or *motor end plates*). ***Motor end plate*** consists of a terminal axonal branch of somatic motoneuron and specialized postsynaptic region of skeletal muscle fiber (Figure 51). The myelinated fiber undergoes thinning of the myelin layer while approaching the junction. The basement membrane of lemmocytes is fused with the basement membrane of the muscle fiber at the site of junction. Presynaptic membrane of the axon terminal is separated from the muscle fiber by synaptic cleft filled with ground substance. The neurotransmitter is *acetylcholine*. Multiple folds of sarcolemma at the site of junction serve as secondary synaptic clefts; their function is to amplify the signal by increasing the postsynaptic surface and thus the number of available acetylcholine receptors. Sarcoplasm of muscle fiber at the site of junction lacks cross-striations but typically contains mitochondria and nuclei.

In *smooth* muscles, *motor nerve endings* look like multiple nodules (varicosities) of axons (which belong to neurons of the autonomic nervous system, Figure 52).

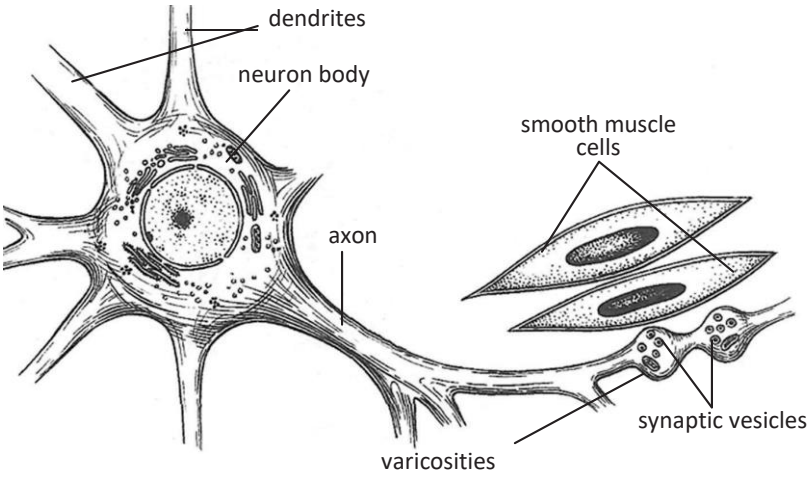


**Figure 52. Secretory-type motor nerve ending in smooth muscle tissue [2]**

Varicosities contain adrenergic or cholinergic presynaptic vesicles. However, the action of released neurotransmitter on the target cells in this case is *diffuse*, and no characteristic postsynaptic excitatory profiles are generated.

*Secretory nerve endings* have similar structure. They look like bulbous extensions of axons (which belong to effector neurons of the autonomic nervous system). These extensions contain synaptic vesicles, predominantly cholinergic.

***Receptors*** are highly specialized nerve endings which belong to dendrites of sensory neurons (pseudounipolar or bipolar neurons of spinal ganglia and cranial nerve ganglia). Receptors are dispersed in the body and respond to stimuli from both external and internal environments. Accordingly, receptors are subdivided into exteroceptors and interoceptors.

*Exteroceptors* generate electric potentials in response to stimuli from external environment — auditory, visual, olfactory, gustatory, and tactile.

*Interoceptors* respond to stimuli from the internal environment of the body. They include *viscероceptors* (responsive to stimuli from internal organs) and *proprioceptors* (responsive to stimuli from musculoskeletal apparatus).

By ***modality of the stimuli*** that can be transformed into excitation signals, receptors are subdivided into chemoreceptors, mechanoreceptors, baroreceptors, thermoreceptors (heat, cold), nociceptors (pain), etc.

By ***histological structure***, *sensory nerve endings* (receptors) are subdivided into *free* and *non-free* (the latter can be *encapsulated* or *non-encapsulated*, Figure 53).

*Free nerve endings* belong to dendrites of sensory neurons.

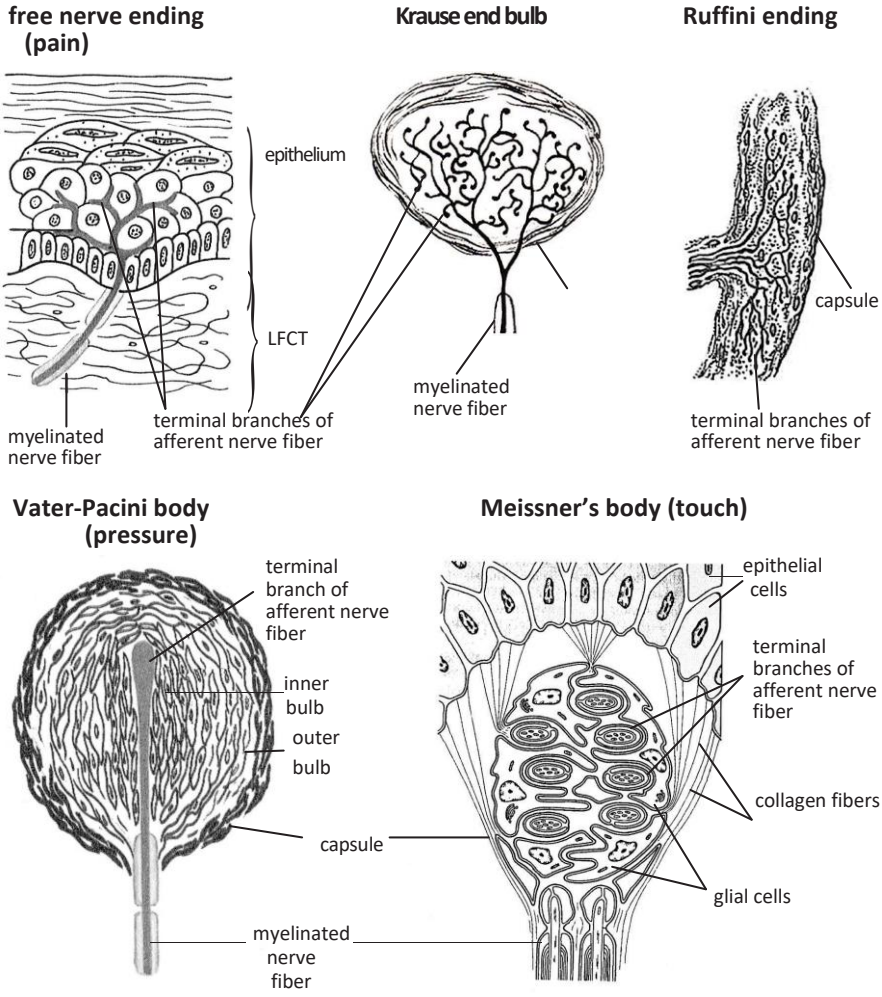


**Figure 53. Sensory nerve endings [2, 9, 17]**

*Non-free sensory nerve endings* contain glial cells and/or specialized non-neural cells. Nerve endings of this type are typically mechanoreceptors.

Non-free sensory nerve endings with connective tissue capsules (*encapsulated*) include lamellar bodies of Vater-Pacini, Meissner's corpuscles, Ruffini endings, Krause end bulbs, Golgi tendon organs, and neuromuscular spindles.

## Exercises

1. Complete the table on receptor nerve endings —*Exercise № 76 in the Workbook.*
2. Draw a labeled scheme of interneuronal synapse — *Exercise № 77 in the Workbook.*
3. Label the scheme of a motor end plate — *Exercise № 78 in the Workbook*.

### *Task 6. Reflex arc*

***Reflex arc*** is a path of excitation from a sensory nerve ending through the central nervous system to the site of actuation in effector organ. *Reflex arc consists of neurons connected by synapses*.

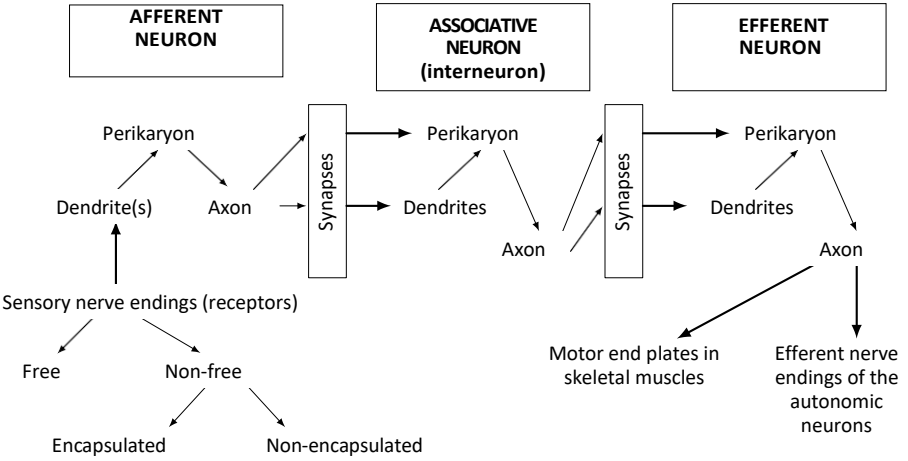


**Figure 54. General scheme of reflex arc**

***Functional classification of neurons*** is about their roles in producing, processing and actuation of nerve impulses. *By their roles in reflex arcs*, neurons can be *afferent*, *associative* or *efferent* (Figure 54).

1) *Sensory* (*afferent*) neurons receive stimuli from external or internal environment and transform them into nerve impulses. The excitation is generated in afferent nerve endings (formed by dendrites) and conducted along sensory (afferent) nerve fibers. Sensory neurons are *pseudounipolar* or *bipolar*. Their bodies are located is sensory ganglia.

Axons of sensory neurons enter the central nervous system and convey the excitation onto other neurons.

2) *Associative neurons* (a.k.a. *interneurons*) constitute middle component of reflex arcs. They ensure processing of the signals by *interneuronal communication*. About 99.98% of neurons in the nervous system are interneurons. They are *multipolar*. Their axons convey the excitation onto other interneurons and/or onto efferent neurons.
3) *Efferent* neurons convey the excitation onto effector organs and structures (muscles, glands). Efferent neurons are *multipolar*. Their axons form efferent nerve fibers.

**Exercises**

1. Understand the schemes of reflex arcs and label their elements — *Exercise № 79 in the Workbook.*
2. Answer the questions:
   - What is reflex arc? Name its components.
   - Specify morphological types of afferent, associative and efferent neurons.

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