ODESSA NATIONAL MEDICAL UNIVERSITY

Department of Histology, cytology and embryology

GENERAL

HISTOLOGY

(COURSE OF LECTURES)

ODESSA

CONTENTS

Theme 1. Cytology. The basics of general and comparative embryology

CYTOLOGY

Cytology *(Greek kytos "a hollow", -logia "study")* is the study of cells.

Cytology is the branch of life sciences that deals with the study of cells, derivatives of cells in terms of their structure, function, chemistry, reproduction and adaptation to various conditions of external environment.

All pathological processes in living organisms are accompanied by morphological and functional changes at the cellular level.

Cell is the basic structural and functional unit of all multicellular organisms. The processes we associate with the daily activities of organisms – protection, digestion, elimination of wastes, movements, reproduction and even death – are all reflections of similar processes occurring within each of the billion cells that constitute the organism.

Cell is surrounded by a plasma membrane that separates it from external environment. Cell can be divided into two major compartments: the cytoplasm and the nucleus. The cytoplasm contains organelles and inclusions in the aqueous gel called cytoplasmic matrix.

Cells of all living organisms are divided into two types:

1) eukaryotic cells – contain nucleus;
2) prokaryotic cells – do not contain nucleus.

Those organisms which are composed of eukaryotic cells are named eukaryotes, whilst those composed of prokaryotic cells are referred to as prokaryotes. Most of the living organisms including animals and humans are eukaryotes; the only two examples of prokaryotes are viruses and cyanobacteria.

CELL THEORY. NON-CELLULAR STRUCTURES

Cell theory is the theoretical foundation of biology and medicine; it reflects the quintessence of all concepts of structure and functioning of cell as a basic unit of living organisms.

Actual postulates of the cell theory

1. Cell is a basic structural unit of all living organisms. It is capable of reproduction, metabolic activity (consumption and production of energy), sensitivity, adaptation and modification.
2. Cells of different organisms have the similar general organization; use the similar mechanisms for synthesis, transport and reproduction. The variability of different cells is determined by specific functions they carry out.
3. The cell reproduction occurs only by a division of maternal cell.
4. Cells constitute the organism. Multicellular organisms are extremely complicated "ensembles" of various specialized cells that compose integrated systems of tissues, organs and systems of organs.

Non-cellular structures

In animal organisms, despite cells, there are also found non-cellular structures that are derivatives of cells.

Non-cellular structures are divided into two types:

1) nucleated;

2) unnucleated.

Nucleated non-cellular structures arise as a result of fusion of several cells or incomplete cell division. Such structures are symplasts and syncytia.

Symplast is a large structure which consists of cytoplasm and numerous nuclei. Symplasts are found in skeletal muscle and the trophoblast of placenta.

Syncytium arises as a result of incomplete cell division, when new-formed cells do not separate from the maternal one and remain connected by cytoplasmic bridges. Such temporary structures can be found at one of stages of male germ cells formation.

Unnucleated non-cellular structures are products which are synthesized by cells. The most common examples of such structures are fibers and ground substance of connective tissue, which are produced by cells called fibroblasts. The blood plasma is also referred to as unnucleated non-cellular structure.

It is necessary to mention that unnucleated cells are also found in human body. Such "cells", like red blood cells, platelets and keratinized cells of epidermis, are often called post-cellular structures, hence they have lost their nuclei in the process of their development. These cells carry out limited functions and are incapable of reproduction.

THE GENERAL STRUCTURE OF CELL

Eukaryotic cell consists of three major components:

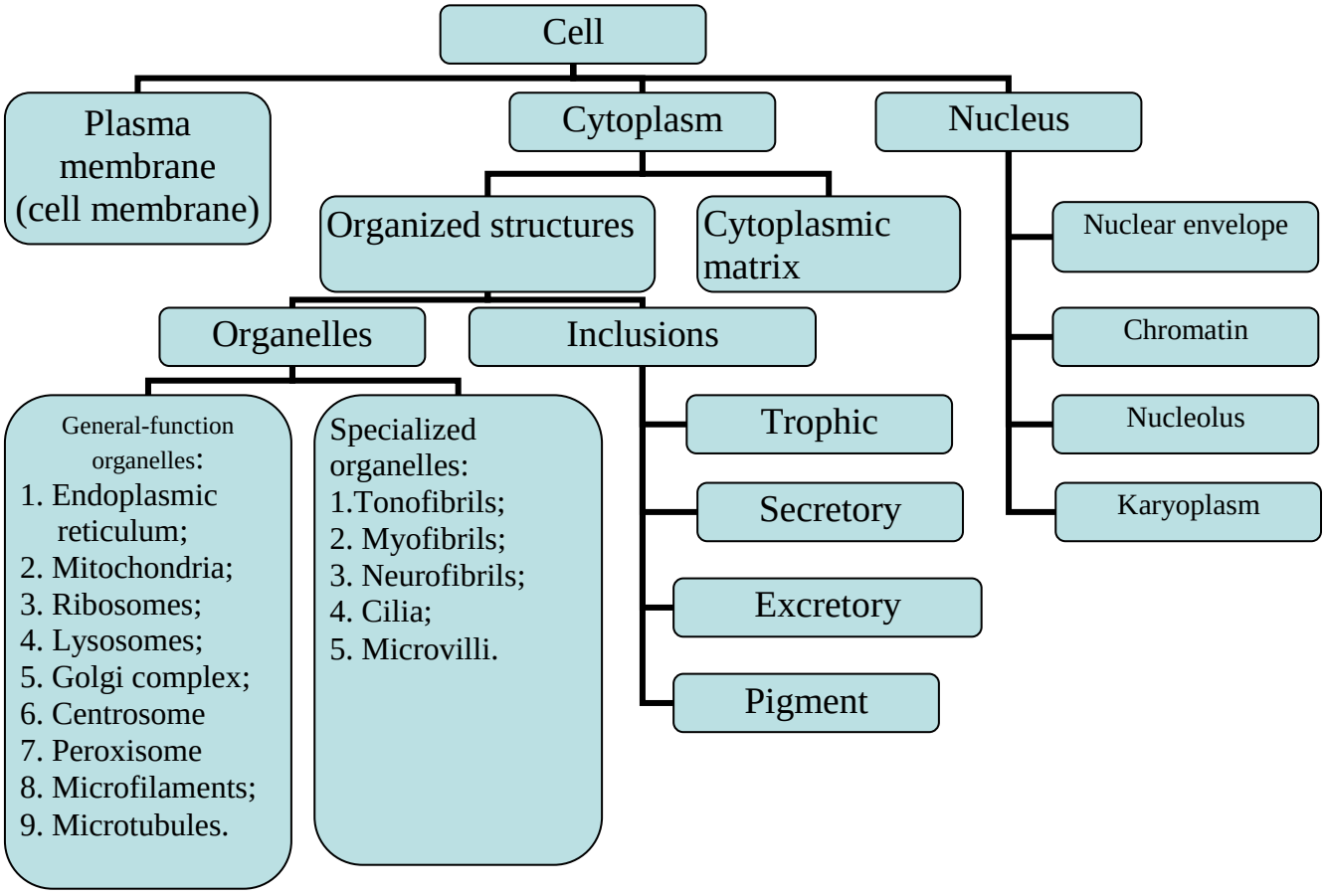
1. **Plasma membrane; 2. Cytoplasm; 3. Nucleus.**

Plasma membrane (plasmalemma, cell membrane) separates cell cytoplasm from neighboring cells or external environment.

Cytoplasm consists of cytoplasmic matrix and organized structures (organelles and inclusions).

Nucleus consists of nuclear envelope, karyoplasm, chromatin (chromosomes), and nucleolus.

All named components of cell interact with each other making it functioning as an integrated structure.



Scheme 1. Structural components of cell

PLASMA MEMBRANE

Plasma membrane (plasmalemma) represents the peripheral structure that forms an external covering of cell, and simultaneously separates and provides its interaction with external environment.

Structure

Plasma membrane consists of three layers:

1) external layer – glycocalyx;
2) lipid bilayer;
3) internal layer – cortical layer of cytoplasm.

Glycocalyx is formed by complexes of glycolipids and glycoproteins associated with the lipid bilayer. It is 3-4nm thick. Long branched carbohydrate chains form complexes with lipids and proteins that are the part of the lipid bilayer. The glycocalyx can be found in almost all animal cells. Polysaccharide chains of glycocalyx perform the function receptive apparatus, which helps cells "recognize" each other and interact with external microenvironment.

Lipid bilayer (biological membrane). The current interpretation of the molecular organization of the plasma membrane is reflected by the modified fluid-mosaic model devised by SJ Singer and GL Nicolson. According to this model the plasma membrane consists of phospholipid, cholesterol and protein molecules. The hydrophobic tails of phospholipids face each other, whilst the hydrophilic heads are, oppositely, pushed away from each other, thereby forming the lipid bilayer.

In most plasma membranes proteins constitute approximately half of the total membrane mass. Most of the proteins are embedded within the lipid bilayer or pass through it completely. Such proteins are called **integral** membrane proteins. The proteins of second type – semi-integral proteins- are partially embedded within the lipid bilayer. The third type of proteins is peripheral proteins, which are associated with lipid bilayer by strong ionic interactions. Hence the plasma membrane is dynamic structure the protein molecules can change their position within lipid bilayer depending on functional condition of cell.

Lability of components of the plasma membrane depends on number of cholesterol molecules it contains. Cholesterol molecules facilitate movements of proteins within lipid bilayer. The thickness of the lipid bilayer is about 5-7nm.

Cortical layer is the densest layer the plasma membrane (sometimes it is described as an outer layer of cytoplasm). This layer contains numerous microfilaments and microtubules that form a well-arranged network. Microfilaments and microtubules of the cortical layer form a cytoskeleton, provide movements of cell, and take part in exocytosis. The thickness of cortical layer is about 1 nm.

Functions of the plasma membrane

The main functions of the plasma membrane are:

1) delimiting;
2) transport;
3) receptivity;
4) formation of cell-to-cell junctions.

Delimiting and transport

Being separated from external environment, cell maintains its individuality; whilst due to the cell transport it can keep functioning. These two contradictory processes complement each other and provide maintenance of homeostasis of cell.

The transport of substances within and out of cell can be of two types: active transport and passive transport.

- Active transport uses energy of ATP to pump molecules against the concentration gradient (transport occurs from a low concentration of solute to a high concentration of solute).
- Passive transport does not require the ATP energy. It goes down the concentration gradient (from high to low concentration in order to maintain ionic equilibrium of cell).

Endocytosis is general term for process of transport in which substances **enter** the cell.

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

In general two main mechanism of endocytosis are recognized in the cell:

Phagocytosis – is an uptake and ingestion of large molecules like bacteria and particles of other cells.

Pinocytosis – is ingestion of fluid and small protein molecules via small vesicles.

- Endocytosis includes the following sequential stages:

1) Sorption – an ingested molecule binds to a receptive protein molecule of the plasma membrane.
2) Invagination of the plasma membrane and formation of vesicles.
3) Separation of transport vesicles from the plasma membrane (sometimes a few vesicles can fuse with each other);
4) Digestion of the ingested molecules using lysosomal hydrolytic enzymes.

In some cases the molecule ingested by one side of the plasma membrane is surrounded by a transport vesicle and just passes across the cytoplasm and then it is ejected through the opposite side of the plasma membrane. This process is called **cytopempsis**.

Exocytosis is a process by which the cell releases molecules to the extracellular space.

There are the following types of exocytosis recognized in the cell:

1) secretion;
2) excretion;
3) recretion;
4) clasmatosis.

Term ***secretion*** refers to an ejection of substances which are the products of synthesis of the cell. The products of secretion are essential for functioning of organs and systems of the organism.

Excretion is an ejection of toxic waste products, which need to be removed out of the organism.

Recretion is a process of removing molecules out of the cell, in which the chemical structure of ejected substance is not affected by the process of cell metabolism (water, mineral salts).

Clasmatosis is a process in which the cell ejects its own components (impaired organelles etc.).

- Exocytosis includes four sequential stages:

1) Storage of molecules produced by the cell for export in sacs and vesicles of the Golgi complex and their sorting and packaging into transport vesicles;
2) Intracellular traffic of the transport vesicles towards the plasma membrane;
3) Fusion of membrane of the transport vesicle with the plasma membrane;
4) Discharge of contents of the transport vesicles to the extracellular space.

Receptivity

A cell reception of external stimuli is provided by the specialized receptor proteins of its plasma membrane. A carbohydrate chain bounded to the receptor protein determines the selectivity of cell interaction with one or another stimulus. The complex processes of intercellular recognition and interaction are essential for living of multicellular organisms.

Cell-to-cell junctions (contacts)

The junctions between neighboring cells in tissues and organs of multicellular organisms are represented by complex specialized structures called cell-to-cell junctions.

In general, all cell-to-cell junctions are divided into three groups depending on their functional role:

1) Anchoring junctions
2) Occluding (tight) junctions
3) Communicating junctions

~ **The anchoring junctions** include a) simple junction; b) adherens junction; c) desmosome.

- Simple junction: the plasma membranes of two neighboring cells approach each other to the distance of 15-20 nm. Any specific structures of the cell cytoplasm are not involved in formation of this type of junction. Interdigitation is one of the examples of the simple junction.
- Adherens junction: the projections of the plasma membrane of one cell interlock with the invaginations of the plasma membrane of neighboring cell, providing the mechanical adhesion between adjoining cells. This type of cell-to-cell junction is widely found in epithelial tissue, where it provides the formation continuous cell layer. The neighboring cells remain distance of 10-20 nm.
- Desmosome is a junctional complex that represents localized spot-like adhesions of 0,5 μm in diameter arranged at the lateral sides of the plasma membrane. Electron microscopy reveals that the desmosome has a complex structure. On the cytoplasmic side of the plasma membrane of each of the adjoining cells is a disc-shaped structure consisting of very dense material called the desmosomal attachment plaque. This structure anchors intermediate filaments. The filaments appear to loop through the attachment plaques and extend back out into the cytoplasm. They are thought to play a role in dissipating physical forces throughout the cell from the attachment site.

~ **Tight (occluding) junction** is a zone, also named zonula occludens, in which the plasma membranes of adjoining cells come in close contact to seal off the intracellular space. The zonula occludens appears not as a continuous seal but as a series of focal fusions between two cells. At high resolution it was estimated that these focal fusions are created by globules of special integral proteins of adjoining cells, which are arranged in lines. These lines of globules can cross over, thereby

forming a network. From the side of cytoplasm this zone reveals numerous fibrils with diameter of 7 nm, which are oriented parallel to the surface of plasma membrane. The zone of such contact is impenetrable for molecules and ions. Tight junctions are usually found in epithelia, especially stomach and intestinal epithelia.

~ **Communicating junctions include**:

a) *gap junction* represents a communicating junction between adjoining cells through the special protein complexes – connexons, which form channels for transport of ions and molecules from cell to cell. The distance between the plasma membranes of two cells is 2-3nm. The area of the zone of connection measures 0,5-3 μm. Each connexon contains six subunits of an integral membrane protein connexin that is paired with a similar structure from the adjacent membrane. The pairs of connexons bridge the extracellular space between adjacent cells. The connexon in on cell membrane is aligned to dock with a corresponding connexon on a membrane of an adjacent cell, thus, allowing the communication between the cells. Such type of cell-to-cell junctions can be found in all types of tissues.

Synapses

Synapse is a special type of cell-to-cell junctions that allows only unidirectional transmission of impulses from one cell to another. This type of junction is characteristic only for the cells of nerve tissue.

MEMBRANES OF CELL

Biological membrane is a structural and functional unit of all cell membranes.

Structural and functional characteristics of biological membranes

All biological membranes represent thin (6-10nm) layers consisting of lipoproteins. The main chemical components of cell membranes are lipids (about 40%), proteins (about 60%), and, besides these, there are also found carbohydrates (about 5-10%).

Lipids constitute a big group of organic substances, which are almost not soluble in water (hydrophobic), and soluble in organic solvents and fats (lipophylic). The lipids of cell membranes are represented by phospholipids (glycerophosphates), sphingolipids, and steroid lipids – cholesterol.

The molecules of lipids of biological membrane are subdivided into two functionally different parts:

1) **Hydrophobic part** – unpolarized, uncharged, consists of fatty acids (tails);
2) **Hydrophilic part** – polarized, charged (heads).

In molecules of membrane proteins are usually also distinguished two parts:

1) polarized part – rich in charged amino acids;
2) unpolarized part – contains neutral amino acids (glycine, alanine, valine, leucine).

Depending on their location in relation to a lipid layer of membrane, the protein molecules are subdivided into:

1) integral proteins – totally embedded into the lipid layer;
2) semi-integral proteins – partially embedded into the lipid layer;
3) peripheral – attached to the outer surface of lipid layer.

On the basis of their biological function, the membrane proteins are subdivided into:

1) enzymes;
2) carrying proteins;
3) receptors;
4) structural proteins.

Carbohydrates are associated with membrane proteins (glycoproteins) or lipids (glycolipids). The amount of carbohydrates in membrane is the smallest one.

CYTOPLASM AND ITS COMPONENTS

The cytoplasm represents a complex colloid system which is composed of cytoplasmic matrix (hyaloplasm), organelles, and inclusions.

Cytoplasmic matrix is a complex colloid system which is composed of various biopolymers (proteins, nucleonic acids, polysaccharides). It may change from fluid (sol) to elastic (gel) and back again to being fluid.

♦ The cytoplasmic matrix consists of water with dissolved organic and inorganic substances, and a trabecular network of 2-3nm thick protein fibers.

♦ The function of cytoplasmic matrix is to form the environment into which all structures of cell are embedded, and provide chemical interaction between them.

A lot of types of intracellular transport occur through the cytoplasmic matrix (transport of amono acids, fatty acids, nucleotides, sugars). The cytoplasmic constitutes for 50% of the total volume of cytoplasm.

Organelles and inclusions. Organelles are permanent and essential microstructures of all cells, which provide performing of vital functions of cells.

On the basis of their size, the organelles are subdivided into:

1) microscopic – visible under the light microscope;
2) submicroscopic – distinguishable only under the electron microscope.

On the basis of presence of membrane, the organelles are subdivided into:

1) membrane-limited;
2) non- membrane-limited.

♦Membranous organelles: 1) mitochondria 2) lysosomes 3) peroxisomes 4) Endoplasmic reticulum 5) Golgi complex	♦Nonmembranous organelles: 1) ribosomes, 2) microfilaments, 3)microtubules, 4) centrosome.

Depending on their function, all organelles are subdivided into:

♦General-function organelles– Mitochondria, lysosomes, peroxisomes, Endoplasmic reticulum, Golgi complex, ribosomes, microtubules, Microfilaments, centrosome.	♦Special function organelles –are derived from general function organelles, and are found only in particular types of cells: Cilia, flagella, myofibrils (muscle cells), neurofibrils (nerve cells).

MEMBRANOUS ORGANELLES

Mitochondria

Mitochondria are general-function, membrane-limited, microscopic organelle.

- ♦ Sizes: 0,5μm thick, 1-10 μm long;
- ♦Shape: oval, elongated
- ♦ Structure: it is surrounded by two membranes of 7nm thick:

1) *Outer smooth membrane* separates mitochondria from cytoplasmic matrtix; appears as a closed sac.

2) Inner mitochondrial membrane forms folds, named crists, facing inside the mitochondrion, and encloses the inner content of mitochondrion – mitochondrial matrix. Mitochondrial matrix is an electron-dense substance, containing thin filaments of 2-3nm thick and granules with a diameter of 15-20 nm. The filaments represent the DNA molecules, the granules are mitochondrial ribosomes.

♦ Functions of mitochondria:

1. Synthesis and storage of ATP energy, which occurs as a result of oxidative phosphorylation.
2. Synthesis of proteins. Mitochondria possess its autonomic system of protein synthesis. They are the unique organelles which have molecules of their own DNA, which is free of histone proteins. The processes of formation of ribosomes and synthesis of proteins that are not encoded by nucleus also occur within the ribosomes. These proteins are used for mitochondrial enzyme systems.
3. Regulation of water balance.

Lysosomes

Lysosomes are submicroscopic, general-function, membranous organelles.

♦ Size: 0,2-0,4μm

♦ Shape: oval, spherical

♦ Structure: lysosomes contain proteolytic enzymes (it is known more than 60), which break down various biopolymers. These enzymes are enclosed in membranous sac, which prevents them from entering the cytoplasmic matrix.

The four types of lysosomes are distinguished:

1. Primary lysosomes;
2. Secondary lysosomes (phagolysosomes);
3. Autophagosomes;
4. Residual bodies.

Primary lysosomes are small (20-25) μm membrane-limited vesicles, filled with inactive hydrolytic enzymes (marker – acidic phosphatase).

Secondary lysosomes (phagosomes) are formed as a result of fusion between primary lysosomes and phagocytic vesicles. The enzymes of primary lysosome contact with biopolymers and break them down into monomers. The last ones are transported through

the membrane to the cytoplasmic matrix, where they are involved in several metabolic processes.

Autophagosomes are constantly found in plant and animal cells. Their structure remains that of the secondary lysosomes, the difference is that the vesicles contain fragments or even full cytoplasmic structures, like mitochondria, ribosomes, or glycogen granules.

Residual bodies contain the rests which were not totally dissolved, and small amount of hydrolytic enzymes.

♦Functions: digestion of biogenic macromolecules, modification of products, produced by a cell, using hydrolases.

Peroxisomes

Peroxisomes are submicroscopic, membranous, general-function organelles.

♦Size: 0,3-1,5μm.

♦ Shape: oval.

♦ Structure: peroxisomes are membrane-bounded bodies filled with granular matrix. The central part of the matrix of peroxisomes usually reveals crystalloid structures which consist of fibrils and tubules.

Peroxisomes are formed at the extended portions of cisterns of Endoplasmic reticulum. They are especially numerous in the cells of liver and kidneys. The matrix of peroxisomes is composed of oxidative enzymes, particularly catalase and other peroxidases. The catalase breaks down the hydrogen peroxide, which is toxic substance, thus protecting the cell.

♦Function: utilization of hydrogen peroxide, breakdown of ethanol, uric acid, oxidation of fatty acids.

Endoplasmic reticulum

Endoplasmic reticulum is a submicroscopic, general-function, membrane-bounded organelle.

The two types of endoplasmic reticulum are distinguished:

1) ***Smooth endoplasmic reticulum (sER)*** consists of short anastomosing tubules.

♦ Size: the diameter of tubules is 50-100 nm.

♦ Functions: lipid and steroid metabolism (synthesis of phospholipids, fatty acids, steroids, cholesterol), carbohydrate metabolism (glycogen metabolism); detoxification of noxious substances; sequestering of Ca^{2+} ions (in muscle tissue).

2) ***Rough endoplasmic reticulum (rER)*** appears as a series of interconnected, membrane-limited, flattened sacs called cisternae, with ribosomes attached to the exterior surface of the membrane.

♦ Size: the diameter of cisternae varies from 20 to 1000nm.

♦ Functions: due to the presence of ribosomes, rough endoplasmic reticulum carries out the function of protein synthesis (90%-for export, 10%- for cell itself). Rough endoplasmic is also involved in formation of membranous structures of the cell.

Golgi apparatus (complex)

Golgi apparatus is a microscopic, general-function, membrane-bounded organelle, which is responsible for the final modifications and formation of all substances produced by the cell.

♦ Structure: Golgi apparatus is represented by a series if stacked, flattened, membrane-limited sacs or cisternae, with a diameter of 25 nm, and tubular extensions. A stack of sacs and cisternae is named **dictyosome**.

Each dictyosome consists of densely packed 5-10 interconnected flattened sacs and cisternae, which are separated by a tin layer of cytoplasmic matrix. Each cistern has a changing thickness; in its central part the membranes are closely attached to one another, while the peripheral part forms extensions, named ampullae. Besides cisternae, numerous vesicles are also found in Golgi complex.

Each dictyosome consists of proximal (cis-face) and distal part (trans-face). The proximal part faces nucleus; the distal part – plasma membrane.

♦Functions: Golgi apparatus functions in post-translational modification, sorting, and packaging of proteins, segregation and modification of lipids and carbohydrates, formation of polysaccharides and mucopolysaccharides, formation of primary lysosomes.

NONMEMBRANOUS ORGANELLES

Ribosomes

Ribosomes are submicroscopic, general-function, nonmembranous organelles, which represent elementary units of synthesis of protein and polypeptide molecules.

♦ Size of functioning ribosome: 25x20x20 nm.

♦ Shape: resembles mushroom

♦ Structure: ribosomes represent complex ribonucleoproteins, which are composed of proteins and RNA molecules in a ratio of 1:1.
The ribosome consists of two subunits:

a) small subunit;
b) large subunit.

Each subunit is composed of ribonucleoprotein cord, in which rRNA interacts with different proteins.

Ribosomes can form clusters connected with messenger RNA - polysomes. Solitary ribosomes and polysomes can also lie freely within the cytoplasm or be bounded to the membrane of rough ER. .

♦ Function – protein synthesis. Free ribosomes produce proteins for the cell itself (proteins of cell membranes, enzymes); while the ribosomes associated with rough ER produce proteins for export (secretion).

Centrosome

Centrosome is a microscopic, general-function, nonmembranous organelle which participates in formation of mitotic spindle and serves as the main microtubule-organizing center (MTOC).

This organelle is found in all somatic cells of animals and humans, except female germ cells (oocytes).

♦ Structure: centrosome is composed of two centrioles surrounded by centrosphere. In the cell, which is not preparing for division, the centrosome is found near the nucleus. A pair centriole is called diplosome.

Centrioles are built from circumferentially disposed nine microtubule triplets, which form a hollow cylinder of 0,2μm wide and 0,3-0,5 μm long. The triplets are connected via special structures- "handles". The "handles" are composed of protein ***dynenin***. Dynein is characterized by ATPase activity, which provides the movement of centrioles. The system of microtubules in centrioles can be described by a formula: (9x3)+0, showing the absence of microtubules in the center of centriole.

A maternal and a daughter centriole are distinguished in the diplosome. The end of daughter centriole is directed perpendicularly to the surface of maternal centriole. Each centriole is surrounded by fibrillar matrix, sometimes there are also found accessory structures, which are not connected to centrioles – satellites.

Centrosphere – is a cytoplasmic matrix which surrounds centrioles and lacks organelles. The centrosphere is penetrated by microtubules.

When the cell is preparing for division, the centrioles of diplosome move to the opposite ends of the cell, and each of them gives a rise to one new centriole. This process is called duplication.

♦ Functions: centrioles participate in induction, polymerization of tubulins and formation of microtubules. Before mitosis centriole is one of the centers of polymerization of microtubules of the mitotic spindle. Centriole – is a growth center of microtubules, and axoneme of cilia and flagella.

Microfilaments

Microfilaments are submicroscopic, general-function, nonmembranous organelles, which act as **cytoskeleton** of the cell.

Depending on their structure and functions the microfilaments are subdivided into three types:

1) Proper filaments;
2) Intermediate filaments.

• **Proper filaments** are present in virtually all types of cells and localized in the cortical layer of cytoplasm (immediately under the plasma membrane).

♦ Structure: proper filaments are thin filaments of 5-7nm in diameter which consist of proteins: actin, myosin, tropomyosin, α-actinin.

♦ Functions: proper microfilaments serve as intracellular contractile apparatus, which provides not only cell motility, but also intracellular movements, like flow of cytoplasm, movements of mitochondria and transport vesicles, and cytokinesis during cell division.

• **Intermediate filaments** are composed of proteins.

♦ Structure: intermediate filaments represent thin filaments which form bundles of 10-15nm in diameter. The cells of different types of tissues are characterized by the specific protein that composes their intermediate filaments. For example, intermediate filaments of epithelial cells are composed of keratin protein, those of the connective tissue – of desmin protein.

♦ Function: are responsible for maintenance of the shape of cell.

Microtubules

Microtubules are submicroscopic, membranous organelles, which build elastic and, at the same time, stable cytoskeleton.

♦ Structure: microtubules are built of globular proteins – tubulins, polymerizing in an end-to-end fashion, and thereby forming rounded

subunits of 5nm. The wall of microtubule is composed of densely packed subunits; 13 subunits form one circle of microtubule. The external diameter of microtubule measures approximately 24nm, the lumen of microtubule – 15nm. Microtubules compose highly organized organelles, like centrosome and basal bodies, are the principal components of cilia and flagella.

♦ Function: maintenance of cell shape, cell movement, movement of chromosomes during mitosis and meiosis, movement of cilia and flagella.

Cilia and flagella

Cilia and flagella are specialized organelles that can be found in particular cells of different organisms.

Cilia represent thin cylindrical projections of cytoplasm.

♦ Size: 200nm wide and 5-10μm high.

♦ Structure: cilium, from its basis to apex, is covered by a plasma membrane. The core of cilium is represented by axial filament (axoneme). Axoneme is a complex structure that consists predominantly of microtubules. The proximal part of cilia (basal body) is embedded into the cytoplasm. The diameters of axoneme and basal body measure approximately 150 nm.

Axoneme (axial filament) is composed of 9 microtubule duplets connected via "handles". A pair of central microtubules is located in the center of axoneme. A system of microtubules of axoneme is described by formula: (9x2)+2.

Basal body consists of 9 microtubule triplets connected via "handles". The system of microtubules of basal body is described by formula: (9x3)+0, as that of the centriole. Sometimes even two basal bodies, lying at the right angle to one another, can be found in the base of axoneme.

Axonemes and basal bodies are structurally interconnected: two microtubules of basal body's triplet are at the same time the microtubules of axoneme's duplets.

Flagella are thin cylindrical projections of cytoplasm, whose structure resembles that of the cilia.

♦ Size: diameter: 200nm, length: 150μm.

Like cilia, flagella are composed of axoneme and basal body.

♦ Functions: the cells that possess cilia and flagella are capable of movement. Immotile cells can move liquid or small particles by the movement of their cilia.

Inclusions

Cytoplasmic inclusions are temporary components of the cell, as they can appear or disappear depending on metabolic activity of the cell.

The following types of inclusions are distinguished:

1. Secretory;
2. Trophic;
3. Excretory;
4. Pigment.

Secretory inclusions are rounded structures of different shapes that contain biologically active substances formed as a result of secretory activity of the cell (hormones, secretions etc).

Trophic inclusions can include droplets of neutral fats, glycogen and protein molecules.

Excretory inclusions do not contain any enzymes or biologically active substances, and are waste products that should be removed out of the cell.

Pigment inclusions could be exogenous (carotene, dust particles, dyes) or endogenous (hemoglobin, hemosiderin, bilirubin, melanin, lipofuscin). Presence of pigment inclusions usually changes the color of tissue.

THE CELL NUCLEUS

The nucleus is a membrane-limited compartment that contains genetic information of the cell.

Structure and chemical composition of the nucleus

The nucleus consists of chromatin (chromosomes), nucleolus, karyoplasm (nucleoplasm) and nuclear envelope.

Most of cells which can undergo division possess only one nucleus. But there are also found cells with two (liver cells) and even several nuclei (osteoclasts of the bone tissue).

- Size – varies from 3-4 to 40 μm

Each type of cell is characterized by a constant ratio of the size of nucleus to the volume of cytoplasm. This ratio is called **index of Hertwig.** Depending on the index of Hertwig all cells are divided into two groups:

1. nuclear cells –are characterized by high index of Hertwig;
2. cytoplasmic cells – are characterized by low index of Hertwig.

- Shape of the nucleus varies among different types of cells. It can be spherical, bean-shaped, circular, segmented etc.

♦ Localization – the nucleus always has its distinct position in the cell cytoplasm. For example, in columnar cells of stomach it occupies the basal portion of the cytoplasm.

The nucleus can be found two functional conditions:

a) mitosis (the process of division)

b) interphase (the period between divisions).

During the interphase the nucleus of living cell appears empty and exhibits only the nucleolus. The other structures of the nuclei could be revealed only if it is affected by an injurious agent and undergoes paranecrosis (a reversible condition between life and death). From this condition the cell can return to life or die.

Dying cells have visible nuclear alterations. These include:

1) karyopyknosis – condensation of chromatin leading to shrinkage of the nucleus (it appears as dense basophilic mass);

2) karyorrhexis – fragmentations of nuclei;

3) karyolysis - the disappearance of nuclei due to complete dissolution of DNA.

Functions of the nucleus: 1) keeping and transfer of hereditary information; 2) control of cell growth, metabolism and reproduction.

Chromatin

Chromatin is a complex of DNA, RNA and proteins, which is responsible for the characteristic basophilia of the interphase nucleus.

Structurally chromatin is an analogue of chromosomes.

Two forms of chromatin are found in the nucleus:

1) heterochromatin;

2) euchromatin.

Heterochromatin is a highly **condensed** chromatin, which **stains** with hematoxylin and basic dyes. It is **evident** in the light microscope. Heterochromatin is divided into:

1) structural;

2) facultative.

Structural heterochromatin represents the areas of chromosomes that always remain condensed.

Facultative heterochromatin is capable of decondensation and can transform into euchromatin. Heterochromatin predominates in metabolically **inactive** cells.

Euchromatin is a **dispersed** form of chromatin. It is found during the interphase and represents **decondensed** areas of chromosomes. This type of chromatin **does not stain** with histological dyes and is **not evident** in the light microscope. Euchromatin indicates **active** chromatin – it can be used for reading and transcription of genetic information.

During mitosis all euchromatin becomes maximally condensed and forms chromosomes. In this period the chromosomes do not carry out any synthetic functions. The chromosomes can be found in functional conditions:

1) active (working) – when the chromosomes are decondensed and the processes of transcription and replication occur within the nucleus.
2) inactive – when the chromosomes are maximally condensed and carry out the function of distribution and passage of genetic information to the daughter cell.

In some cases the whole chromosome, even during the interphase, remains condensed. For example, one of the X-chromosomes of females is condensed at the early stages of embryogenesis and does not function. This X-chromosome was named the Barr body.

In different cells the sex chromatin appears differently:

1) in neutrophilic leukocytes it looks like a drumstick;
2) in epithelial cells of mucosa it is spherical structure adjacent to the nuclear envelope.

A series of electron-microscopic examinations revealed that the interphase chromatin is composed elementary chromosome fibrils with thickness of 20-25 nm.

The chemical composition of the chromatin fibrils includes complexes desoxyribonucleotids which are composed of:

1) DNA;
2) Specialized chromosome proteins;
3) RNA.

The ratio between amounts of DNA, proteins and RNA is 1:1,3:0,2. The DNA constitute 30-40% of chromatin mass. The total length of all DNA molecules in all chromosomes in one human cell is about 170 cm.

The proteins of chromatin constitute 60-70% of its dry mass. They include two types:

a) histone proteins;
b) non-histone proteins.

♦ *Histone proteins* are alkaline proteins, which contain basic amino acids (mostly lysine and arginine). Histone proteins are unequally distributed along the DNA molecule. One protein possesses 8 histone molecules which form nucleosome. These 10-nm-diameter particles represent the first level of chromatin folding and are formed by the coiling of the DNA molecule around a protein core. This step shortens the DNA molecule by approximately sevenfold relative to the unfolded DNA molecule.

♦ Non-histone proteins constitute 20% of the number of histone proteins. In the interphase nucleus non-histone proteins form a structural network called protein matrix. This matrix is a foundation that determines morphology and metabolism of the nucleus.

Besides chromatin, the nucleus also possesses perichromatin fibrils, perichromatin and interchromatin granules. These structures contain RNA and are found in almost all nuclei.

Perichromatin fibrils are 3-5 nm thick; perichromatin granules measure 45 nm in diameter; interchromatin granules - 21-25 nm in diameter.

Nucleolus

Nucleolus is the densest structure of nucleus, which is well-evident in unstained cell. Nucleolus is a derivative of chromosome, one of its locus with the highest concentration of the most transcriptionally active RNA. Nucleolus is not an independent structure or organelle.

♦ Size - 1-5 μm.

♦ Shape - spherical.

Examined under the electron microscope, nucleolus exhibit two morphologically different parts:

1) granular material (pars granulosa);

2) fibrillar material (pars fibrosa).

Granular material consists of granules with diameter of 15-20 nm. These granules represent the subunits of ribosomes. The granular material occupies the peripheral part of nucleolus.

Fibrillar material occupies the central part of nucleolus and represents the ribonucleoprotein cords of precursors of ribosomes.

The ultrastructure of nucleolus depends on the activity of RNA synthesis: if the RNA is actively synthesized the nucleolus contains numerous granules; if the RNA is not actively produced the nucleolus appears as dense basophilic fibrillar cord.

Nuclear envelope

The nuclear envelope consists of:

1. Outer nuclear membrane;
2. Inner nuclear membrane;
3. Perinuclear cisternal space of 20-60 nm width.

Each membrane of the nucleus is 7-8 nm thick. In general, the nuclear envelope appears as hollow double-layered sac, which separates the contents of nucleus from cytoplasm.

The outer nuclear membrane immediately contacts with the cytoplasm, resembles the membrane of the endoplasmic reticulum and, in fact, continues with the rER membrane. Polyribosomes are usually attached to the cytoplasmic side of the outer nuclear membrane. It's surface is not smooth and exhibits numerous projections, vesicles and tubules towards the cytoplasm.

The inner nuclear membrane is supported by rigid network of intermediate filaments (fibrous lamina). It contains several lamina-associated proteins that bind to chromosomes.

The two membranes of the envelope are perforated by **nuclear pores**. The nuclear pores are formed from the merging of the inner and outer membranes of the nuclear envelope. At numerous sites, the paired membranes are perforated by 70-80nm "openings" through the envelope. These opening are filled with complex globular and fibrillar structures. The complex of membrane perforations and these structures is known as nuclear pore complex.

The nuclear pore complex (NPC) is composed of about 50 different nuclear pore proteins (nucleoporines).

This central framework is inserted between the **cytoplasmic ring** and the **nuclear ring.** From the cytoplasmic ring, eight short **protein fibrils** protrude into the cytoplasm and point toward the center of the structure. The nucleoplasmic ring complex anchors a **nuclear basket** (or nuclear "cage" that resembles a fish trap) assembled from eight thin filaments joined distally by an adjustable **terminal ring**. The cylinder shaped central framework encircles the **central pore** of the NPC, which acts as a close-fitting diaphragm or gated channel. In addition, each NPC contains one or more water-filled channels for transport of small molecules.

♦Functions:

1. Barrier – separates contents of nucleus from contents of cytoplasm, provides selective transport of macromolecules between nucleus and cytoplasm.
2. Control of intranuclear order – fixation of chromosome material within the three-dimensional lumen of nucleus.

Karyoplasm

Karyoplasm is a fluid component of nucleus, into which all components of nucleus are embedded. Karyoplasm is a nuclear analog of cell cytoplasm.

Cell cycle

The cell cycle represents a self-regulated sequence of events that controls cell growth and cell division. For renewing cell populations, the goal of the cell cycle is to produce two daughter cells, each containing chromosomes identical to those of the parental cell. The cell cycle consists of two principal phases: the interphase, representing continuous growth of the cell, and the M phase (mitosis), characterized by the partition of the genome. The interphase is subdivided into three phases, G1 (gap1) phase, S (synthesis) phase, and G2 (gap2) phase.

The G1 phase is usually the longest and the most variable phase of the cell cycle, and it begins at the end of M phase. During the G1 phase, the cell gathers nutrients and synthesizes RNA and proteins necessary for DNA synthesis and chromosome replication.

In the S phase, DNA is replicated. Initiation of DNA synthesis marks the beginning of the S phase, which is about 7.5 to 10 hours in duration. The DNA of the cell is doubled during the S phase, and new chromatids are formed that will become obvious at prophase or metaphase of the mitotic division.

In the G2 phase, the cell prepares for cell division. During this phase, the cell examines its replicated DNA in preparation for cell division. This is a period of cell growth and reorganization of cytoplasmic organelles before entering the mitotic cycle.

The G2 phase may be as short as 1 hour in rapidly dividing cells or of nearly indefinite duration in some polyploid cells and in cells such as the primary oocyte that are arrested in G2 for extended periods.

There are some cells in the human which stay out of cycle – cells of G_0-period. They do not enter the S-phase of interphase, and do not divide.

There are the following types of such cells:

1. Stem cells – immature, low-differentiated cells which retain their ability to divide, but stay in a **G_0-period** for a long time.

2. Cells that lost their capacity of division but undergo specialization and differentiation. Such cells are subdivided into two types:

a) cells that started their differentiation and irreversibly lost their capacity of division (mature blood cells, cells of epidermis);

b) cells that even after differentiation can return to the cell cycle and divide (hepatocytes of liver).

3. Highly differentiated and specialized cell that irreversibly lose their capacity of division; their life span is the as the life of an organism (nerve cells).

MITOSIS

Mitosis is nuclear division plus cytokinesis, and produces two identical daughter cells during prophase, metaphase, anaphase, and telophase. Interphase is often included in discussions of mitosis, but interphase is technically not part of mitosis, but rather encompasses stages G1, S, and G2 of the cell cycle.

1 Prophase. Chromatin in the nucleus begins to condense and becomes visible in the light microscope as chromosomes. The nucleolus disappears. Centrioles begin moving to opposite ends of the cell and fibers extend from the centromeres. Some fibers cross the cell to form the mitotic spindle. The nuclear membrane dissolves, marking the beginning of prometaphase. Proteins attach to the centromeres creating the kinetochores. Microtubules attach at the kinetochores and the chromosomes begin moving.

2. Metaphase. Spindle fibers align the chromosomes along the middle of the cell nucleus. This line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.

3. Anaphase. The paired chromosomes separate at the kinetochores and move to opposite sides of the cell. Motion results from a combination of kinetochore movement along the spindle microtubules and through the physical interaction of polar microtubules.

4. Telophase. Chromatids arrive at opposite poles of cell, and new membranes form around the daughter nuclei. The chromosomes disperse and are no longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the partitioning of the cell may also begin during this stage. In animal cells, **cytokinesis** results when a fiber ring composed of a protein called actin around the center of the cell contracts pinching the cell into two daughter cells, each with one nucleus.

Chromosomes

During mitotic division, chromatin undergo condensation to form chromosomes. Each chromosome is formed by two chromatids that are joined together at a point called the centromere. The double nature of the chromosome is produced in the preceding synthetic (S) phase of the cell cycle, during which DNA is replicated in anticipation of the next mitotic division. The area located at each end of the chromosome is called the telomere. Telomeres shorten with each cell division. Recent studies indicate that telomere length is an important indicator of the lifespan of the cell. With the exception of the mature gametes, the egg and sperm, human cells contain 46 chromosomes organized as 23 homologous pairs (each chromosome in the pair has the designated X and Y. Females contain two X chromosomes; males contain one X and one Y chromosome. The chromosomal number, 46, is found in most of the somatic cells of the body and is called the diploid (2n) number. To simplify the description of chromosomal number and DNA changes during mitosis and meiosis, we use the lower case letter (n) for chromosome number and lower case letter (d) for DNA content.

ENDOMITOSIS

Endomitosis is mitosis taking place without dissolution of the nuclear membrane, and not followed by cytoplasmic division, resulting in doubling of the number of chromosomes within the nucleus.

MEIOSIS

Meiosis is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them. Meiosis includes two sequential mitotic divisions without interphase between them. The characteristic feature of meiosis is crossing over – an

exchange of genetic material between homologous chromosomes.

THE BASICS OF GENERAL EMBRYOLOGY

Embryology is the study of developmental process of embryo from the moment of fertilization until the moment of birth.

Medical embryology includes studies of molecular, cellular, and structural factors contributing to the formation of an organism; metabolic and functional characteristics of placental barrier (system mother-placenta-fetus); and causes of birth defects formation.

The principal aims of embryology

1. Investigation of various endogenous and exogenous factors and the role of microenvironment in development and functioning of gametes.
2. Investigation of mechanisms that control reproductive function and provide maintenance of homeostasis in human embryo.
3. Investigation of critical periods in human embryogenesis.
4. Cultivation of oocytes, embryos and their subsequent implantation in the endometrium of uterus.
5. Investigation of sources and mechanisms of tissues development.

Studying of embryology is impossible without having basic knowledge of comparative embryology, hence evolutionary the embryonic development of all mammals could be characterized by the similar principal stages, sequence and consistent patterns. For correct comprehension of the process of human individual development it is necessary to carry out analysis and compare the principal stages of human development with those of other animals.

It was estimated that the principal stages and consistent patterns of embryogenesis were established during the process of evolutional development of the mammals. At the same time some stages of human embryonic development are analogous to those of lower organized chordate animals; hence the process of human embryonic development is a result of long-term evolution and it partially reflects the features of development of other animals.

The idea of correlation between individual and historical (evolutional) development was proposed at the beginning of the XIX century by the eminent scientists K.Berr and F. Muller, who came to conclusion that a large group of animals at the early stages of development reveal much more common features than individual differences.

The quintessence of this idea is the Haeckel-Muller law (or Recapitulation theory), which proclaims that in developing from embryo to adult, animal go through stages resembling successive stages of evolution of their remote ancestors ("**ontogenesis recapitulates phylogenesis"**).

ONTOGENESIS

***Ontogenesis*-** is a process of individual development of an organism from the moment of fertilization until death.

Periods of ontogenesis

I Prenatal – period of intrauterine development (duration – 280 days);

a) initial period (early embryo) – first week

b) embryonic period (embryo) – 2^{nd}-8^{th} week, formation of primary cavity, organogenesis and appearance of heart beating on 21^{st} day

c) fetal (until birth) – placentation, differentiation of tissues.

II Postnatal – period of development after the birth;

a) early postembryonic

b) subsequent development, maturation, ageing and death.

Initial period include the following stages:

1) zygote – beginning of the DNA synthesis;
2) cleavage – beginning of synthesis of the all types of RNA;
3) morula – the cells are totipotential;
4) blastocyst - loss of totipotency; the cells are determined to formation of embryonic and extraembryonic structures;
5) gastrula – appearance of the germ layers and stem cells.

Ontogenesis is always preceded by progenesis hence the formation of new organism is impossible without formation and maturation of male and female gametes.

PROGENESIS

Progenesis – the process of formation, development and maturation of male and female gametes.

Gametes, unlike somatic cells, contain haploid number of chromosomes. All chromosomes of gametes are autosomes except the one which is referred to as sex gonosome.

Male gametes could contain X or Y-sex chromosome.

Female gametes could contain only X-chromosome.

The mature gametes are incapable to proliferation and characterized by low level of metabolism.

Male reproductive cells

Male gametes – spermatozoa are about 70 μm in size and capable of active movement (30-50mm per second in human). In human it is formed several thousand million spermatozoa.

♦*Spermatogenesis* – the process of formation and maturation of spermatozoa.

The structure of spermatozoa

The spermatozoon is composed of two principal parts: 1) head; 2) tail.

The head of spermatozoon contains dense nucleus with haploid number of chromosomes (22 autosomes and 1 sex chromosome). Depending on what kind of sex chromosome they possess, the spermatozoa are divided into two types:

1) androspem – carry Y-chromosome,
2) gynosperm – carry X-chromosome.

♦ The nucleus is rich in nucleoproteins and nucleohistones. The anterior wall of nucleus is covered by a vesicle called acrosome. The acrosome is a derivative of Golgi complex.

The acrosome contains a complex of enzymes, among which the most important are hyaloronidase and proteases (trypsin), which are crucial for dissolving ovum's coats.

Externally the head is surrounded by a plasma membrane.

The tail (flagellum) is composed of:

a) connecting piece, which contains **two centrioles** (proximal and distal). The axial filament (axoneme) extends from the distal centriole.

b) midpiece, which contain two central microtubules and nine pairs of peripheral microtubules spirally surrounded by several mitochondria (mitochondrial sheath);

c) tail piece contains axial filament, which resembles cilia;

d) end piece, which contains solitary contractive filaments.

The tail of spermatozoon, like its head, is surrounded by a plasma membrane.

The functions of spermatozoa

1. Fertilization of the ovum. By means of its tail the spermatozoa is capable to move in distinct direction determined by the specific substances which are produced by an ovum – *gynogamones.*

2. React on the chemical stimuli – *chemotaxis.*

3. Can move in opposite direction to the flow of fluid – *rheotaxis*.

4. In optimal conditions can remain their capacity to fertilization during 36-88 hours.

5. The favorable condition for spermatozoa is slightly alkaline environment.

Female reproductive cells

Female gametes – ova (oocytes) are developed in ovaries. In human there are produced several hundreds oocytes during the whole life.

The ovum is spherical cell, whose size can reach up to several cm. The characteristic features of ovum are big amount of cytoplasm and presence of yolk. The ovum, unlike spermatozoon, is not capable of movement.

Classification of ova

Depending on the amount of yolk the ova are divided into the following types:

1. Alecithal ova have no yolk in cytoplasm;

2. Oligolecithal ova have small amount of yolk in cytoplasm

a) primary oligolecithal – in primitive chordate, which are characterized by quick development via the stage of larva;

b) **secondary oligolecithal** – in mammals (placental) and **human**.

3. Mesolecithal ova contain moderate amount of yolk (amphibians).

4. Polylecithal ova contain enormous amount of yolk. (birds, reptiles, fishes).

Depending on the distribution of yolk the ova are classified into the following types:

1. Isolecithal – the yolk is uniformly distributed through the cytoplasm. Such distribution is usually characteristic for oligolecithal ova.

2. Centrolecithal - yolk is concentrated in the center of ovum’s cytoplasm.

3. Teleolecithal - are polylecithal ova, where the yolk is concentrated in one hemisphere (vegetal pole) and organelles remain in another hemisphere (animal pole).

The structure of ovum

The ovum of placental mammals is relatively small (50-150 μm). It is surrounded by zona pellucida and a layer of follicular cells, which provide its nutrition. The ovum is composed of coats, cytoplasm and nucleus.

♦ Coats – all ova are surrounded by the cytolemma (oolemma or primary coat), most of them also possess the secondary coat – a carbohydrate-protein coat, and some types of ova are covered by the tertiary coat – eggshell.

♦ Cytoplasm (ooplasm) contains nutritive material – yolk. Besides this the ooplasm stores a range of proteins: histones, ribosomal structural proteins, tubulin etc.

♦ Among other organelles, the rough ER exhibits strong development. The number of mitochondria is moderate. At the periphery of cytoplasm the Golgi complex and small cortical granules containing glycosaminoglycans are situated.

♦ Yolk – is a type of inclusion which appears as granules or larger globes and plates. The yolk is composed of phospholipids, proteins and carbohydrates. The structural unit of yolk is a complex of lipovitelin (lipoprotein) and phosvitin (phosphoprotein).

The ovum is characterized by cell polarity. The more yolk it contains the more conspicuous is its cell polarity. The pole of cytoplasm which contains yolk is called vegetal, and the pole which contains organelles and nucleus – animal.

♦ Nucleus possesses haploid number of chromosomes. Intensive synthetic processes (RNA, DNA synthesis) take place at the period of growth.

EMBRYOGENESIS

Embryogenesis – a period of intrauterine development of humans and animals which begins at the moment of fertilization, includes formation and development of all tissues, organs and system of fetus, and ends with a birth of child.

The embryonic development is phasic and accompanied by gradual quantitative and qualitative changes. The process of embryogenesis includes the following phases:

1) fertilization;
2) cleavage and formation of blastula;
3) gastrulation and differentiation of germ layers;
4) histogenesis (differentiation of tissues);
5) organogenesis (differentiation of organs);
6) formation of systems of organs.

Fertilization **–** is the process, by which male and female gamete fuse, which results in restoration of the diploid number of chromosomes and formation of unicellular embryo - **zygote**.

Fertilization is preceded by *insemination* – introduction of sperm into female reproductive tract in case of internal fertilization, or into the environment where the ovum is situated in case of external fertilization.

In human the fertilization normally takes occurs in the ampullary region of the uterine tube.

Spermatozoa are not able to fertilize the oocytes immediately upon arrival in the female genital tract, but must undergo capacitation and acrosome reaction to acquire this capability.

• Capacitation is a period of activation in the female reproductive that in human lasts approximately 7 hours. Much of this activation occurs in the uterine tube and involves epithelial interactions between the sperm and the mucosal surface of the tube. A significant role in this process is played by progesterone (the hormone of corpus luteum of ovary). Only capacitated sperm can undergo acrosome reaction.

• Acrosome reaction occurs after binding to zona pellucida of the oocyte and is induced by zona proteins. This reaction culminates in the release of enzymes needed to penetrate the zona pellucida, including acrosin- and trypsin-like substances.

• The phases of fertilization include:

1. Distant interaction is performed by several non-specific factors which facilitate the "meeting" of reproductive cells. Special chemical substances (gamones) are discharged by both spermatozoa and ovum. The female gamones are called gynogamones, the male gamones – androgamones. Gynogamones type I – are low-molecular non-protein substances which are believed to attract spermatozoa. Gynogamones type II – are species-specific proteins that cause gluing of spermatozoa in case of their reaction with complementary androgamone type II. Androgamones type I – being antagonists of gynogamones type I they depress sperm motility.

2. Contact interaction and penetration into the ovum is performed by mean of the acrosome. The enzymes required for penetration of corona radiata (hyaloronidase, trypsin) are released from the acrosome and destroy cell contacts between follicular cells (acrosome reaction). This phenomenon called denudation of the oocyte results in total dissolution of zona pellucida. The plasma membranes of ovum and spermatozoon fuse. The enzymes released from the acrosome dissolve the corona radiata and break down glycosaminoglycans of the zona pellucida. The separated follicular cells form conglomerate which follows the ovum while it moves via the uterine tube. The movement of fertilized ovum is provided by the peristaltic contraction and synchronic movements of cilia of the mucosa of the uterine tube.

3. Penetration of the ovum. Both the head and the tail of the spermatozoon enter the cytoplasm of oocyte. As a result of the release of cortical oocyte granules, which contain lysosomal enzymes, the oocyte membrane becomes impenetrable for other spermatozoa and the zona pellucida alters its structure and composition to prevent sperm penetration (cortical reaction). This reaction prevents polyspermy (penetration of more than one spermatozoon into the oocyte). After the penetration the head of spermatozoon makes a 180° degree turn; its nucleus becomes swollen and forms *male pronucleus*. The ovum's nucleus forms *female pronucleus*. Male and female pronuclei eventually come into close contact and lose their nuclear envelopes. The fusion of male and female pronuclei initiates the spiralization of chromosomes and formation of metaphase plate. A nucleus formed by two preexisting pronuclei is called **synkaryon**. The spermatozoon delivers centriole, which is essential for mitotic division of zygote, to the oocyte. In this way the zygote containing both maternal and paternal genes is formed.

At the same time the redistribution of cytoplasmic material of zygote and subsequent division of its cytoplasm into two zones (zone with high concentration of yolk granules and zone with high concentration of pigment granules) occurs. This phenomenon is called **ooplasmic segregation**. During further development each zone of the cytoplasm gives rise to distinct part of the organism. Such zones are called **presumptive zones**.

***Cleavage* –** is a series of mitotic divisions of zygote, which results in its transformation into multicellular organism called blastocyst. As the protein synthesis is inhibited, the cells, known as blastomeres, become smaller with each cleavage division. Due to the absence of the G_1-

period of interphase, the size of an embryo does not exceed that of the zygote. The cleavage lasts from 1st to 6th day of pregnancy.

The type of cleavage differs among different animals. It is determined by amount and distribution of yolk within an ovum.

Types of cleavage

Depending on the type of ovum, the following types of cleavage are distinguished:

1. Total equal cleavage is characteristic for primary oligolecithal, isolecithal ova (amphioxus).

2. Total unequal cleavage is characteristic for mesolecithal ova; hence the mitotic divisions of vegetal pole occur not so fast as that of the animal pole.

3. Partial or meroblastic cleavage is characteristic for teleolecithal ova. In such case only the apical part of ovum undergoes mitotic divisions (birds).

4. **Total asynchronous unequal cleavage** or holoblastic is characteristic for secondary oligolecithal isolecithal ova (mammals, **human**).

Approximately three days after fertilization the divisions of cells of an embryo result in formation of 16-cells morula. **Morula –** is a compacted aggregation of blastomeres which resembles mulberry.

Inner cells of the morula constitute the inner cell mass, and surrounding cells compose the outer cell mass. The inner cell mass gives rise to tissue of embryo proper, and the outer cell mass forms the trophoblast, which later contributes to the placenta.

About the time the morula enters the uterine cavity, fluid begins to penetrate through the zona pellucida into the intercellular spaces of the inner cell mass, which results in formation of cavity – blastocele. At this time the embryo is **blastocyst**. Cells of the inner cell mass, now called the **embryoblast**, are at one pole, and those of the outer cell mass, or **trophoblast**, flatten and form the epithelial wall of the blastocyst **(blastoderm)**. The zona pellucida has disappeared, allowing implantation to begin.

Types of blastula

1. Coeloblastula forms as a result of total equal cleavage. The blastoderm is formed of a single layer of cells. The blastocele is centric.(Amphioxus)

2. Amphiblastula is formed as a result of total unequal cleavage. The blastoderm is multi-layered. The blastocele is eccentric. (Amphibians)

3. Discoblastula is formed as a result of partial meroblastic cleavage. The blastocele is small and situated below the blastoderm. (Birds, reptiles).

Gastrulation **–** a period of embryogenesis that includes differentiation of the germ layers: ectoderm (outer layer), endoderm (inner layer), and mesoderm (middle layer).

Types of gastrulation

The process of gastrulation could be carried out in four main ways:

1. Migration – movement of part of blastomeres from the wall to the center of an embryo and formation of endoderm.
2. Invagination – inward of the wall of blastula into its center.
3. Epiboly – spread of an outside cell layer (where the mitosis is faster) to envelope a deeper layer (where the mitosis is relatively slow). It happens when the blastomeres of vegetal pole contain high amount of yolk (in amphibians).
4. Delamination – is a process accompanied by tangential splitting of the wall of blastula, which results in formation of two germ layers – primary ectoderm (outer) and primary endoderm (inner). Such type of gastrulation is characteristic for birds and mammals.

The types of gastrulation depend on preceded phases of development and amount of yolk in ovum. The vertebrates are characterized by combination of two or three types of gastrulation.

In the human the gastrulation lasts from 7^{th} to 17^{th} day of embryogenesis and includes 2 phases:

I phase ($7\text{-}14^{th}$ day) involves formation of inner (endoderm) and outer (ectoderm) germ layers. As a result of *delamination* the layer of cells that faces the blastocyst cavity splits out from the primitive node, thereby forming the primary endoderm (hypoblast). At the same time the cells of the primitive node which lie under the hypoblast undergo *cavitation* (due to the cumulation of liquid a cavity is formed in the center of the nodule; the cells which surround this cavity obtain epithelium-like shape – the amniotic vesicle is formed).

The opposite edges of the primary endoderm turn down, fuse together and form the yolk vesicle. The adjacent parts of both vesicles

(amniotic and yolk) form the embryonic disc, which gives rise to the body of an embryo.

Simultaneously with the formation of amniotic and yolk vesicles, beginning from the 8th day of development, occurs the differentiation of *extraembryonic mesoderm*, which gives rise to chorion and amniotic stalk (the basis of the future umbilical cord).

II phase lasts from 15th to 17th day of embryonic development and involves formation of the embryonic mesoderm. This process is realized migration of the cells of primary ectoderm in the space between two germ layers.

The space between germ layers is filled with the embryonic connective tissue – mesenchyme.

The mesenchymal cells migrate from mesoderm, endoderm and ectoderm. That is why two types of mesenchyme are distinguished:

a) endomesenchyme – originates from the endo- and mesoderm;

b) ectomesenchyme – originates from the ectoderm.

These two types of mesenchyme are morphologically indistinguishable, but they give rise to different structures:

- endomesenchyme – to the tissues of internal environment;
- ectomesenchyme – to auditory bones, the connective tssues of head.

The cell migration from the primitive node results in formation of axial chord of an embryo – chorda. At the end of the second week the hematopoietic cords and germs of primary blood vessels appear in the yolk sac.

The finger-like process – allantois, grows inwards the amniotic stalk of the intestinal endoderm. Blood vessels of the yolk sac grow inwards the wall of allantois and chorionic villi, which are supplied with maternal blood. Allantochorion, which is formed as a result of these processes, provides nutrition and gas exchange of fetus at this stage of development.

HISTO- and ORGANOGENESIS

Histo-organogenesis – is the process that involves differentiation and formation of tissue, organs and systems of organs of the fetus through several sequential stages: induction, determination, cell reproduction, migration, growth of cells, intercellular interactions, and cell death.

Induction – the process by which cells and tissues in embryo direct the development of adjacent cells and tissues. An example

of induction is the development of the eye lens from epidermis under influence of the eye cup, which grows toward the skin from the brain. As the eye cup comes into contact with any neighbouring epidermis, it transforms that particular region into a lens. The exact nature of the stimulus for lens induction is not known, although ribonucleic acid (RNA) has been implicated as a messenger.

Determination **–** is progressive restriction in developmental potential of the embryonic cells via blocking of particular components of genome.

Determination is a foundation of process of differentiation. Differentiation – is a process through which cells change to a more specialized type.

Cell reproduction **–** is the process by which cells divide to form new cells.

The process of renewal and restoration of biological object is called regeneration. There are distinguished three types of regeneration:

1) physiological – continuously occurs in healthy organism (renewal of blood, epithelial cell etc.);
2) reparative – occurs after injuries and trauma (healing of the wound);
3) pathological – normally does not occur; such type of regeneration is characteristic for cells of malignant tumors.

Migration **–** is the massive translation of cells from one location to another, which results in formation of tissues and organs.

Growth of cells – is a series of processes, which include development and organization of cells from the moment of their division until next division.

Intercellular interactions perform the leading role in differentiation of embryonic germ layers. For example, it was estimated that for development and existence of epithelium it is essential to be supported by connective tissue.

Cell death – is an unaltered process of termination of all its functions and interaction with external environment.

PROVISIONAL ORGANS

Provisional organs **–** are temporary organs, which are developed during embryogenesis, they are referred to as extraembryonic organs, which growth and development of an embryo.

The provisional organs include: 1) yolk sac; 2) amniotic sac; 3) serous coat; 4) allantois; 5) chorion; 6) placenta.

Yolk sac appears as a vesicle connected with the intestinal tube. Internally the wall of the yolk sac is lined with epithelium, externally – it is formed by connective tissue.

The formation of yolk sac is initiated at the stage of early gastrula, when there are distinguished the embryonic (intestinal) endoderm and the peripherally located extraembryonic yolk endoderm. The yolk sac maintains its connection with the intestinal tube via connecting stalk.

♦Functions: a) trophic; b) hematopoietic (7-8 weeks).

Amniotic sac – is a thin but tough transparent pair of membranes that hold a developing embryo. It takes part in secretion of amniotic fluid. The amniotic sac consists of two parts*: amniotic part* facing an embryo (fetus) and external part – *serosa*.

The amniotic sac originates from ectoderm and parietal layer of mesoderm, which firstly from amniotic folds growing towards the dorsal surface of an embryo. These folds surround an embryo and fuse, so the ectoderm and adjacent parietal mesoderm fuse with the analogous layers of the opposite side. Two layers of the folds give rise to two coats – *amniotic* (fluid-secreting) and *serous* (external).

The fluid secreted by the ectoderm of the amniotic coat contains proteins and carbohydrates; it acts as shock absorber and protects fetus from mechanical trauma.

♦ Functions: shock absorption; allows fetus to move freely.

Serous coat develops simultaneously with the amniotic coat. Hence it helps to supply an embryo with oxygen, it is considered to be a provisional respiratory organ. **It is not present in the human**.

Allantois begins its development in caudal region of an embryo proper as a projection of ventral wall of dorsal intestine, which is formed by endoderm and visceral mesoderm. The proximal part of allantois is situated along the yolk stalk; while the distal one invades the gap between the amniotic and serous coats. Allantois carries out the functions of excretion and gas exchange: the oxygen is delivered to an embryo via blood vessels which originate from the mesoderm of allantois; at the same time the waste products are excreted into allantois.

Recently it was estimated that at the early stages of the ontogenesis allantois performs the analogous function to the Bursa of Fabricius, i.e.

acts as main organ of B-lymphocytopoiesis. Allantois undergoes reduction after second month of the embryogenesis.

Chorion originates from the trophoblast and extraembryonic mesoderm. The trophoblast is primarily represented by a coat with primary villi, via which the connection with maternal organism is established. At the 2-3rd week of development the extraembryonic mesoderm appears, grow towards the trophoblast; and together they form the *secondary (epitheliomesenchymal) villi*. After this the trophoblast transforms into chorion.

Invading the mucosa of the uterus chorion takes part in formation in formation of placenta.

Placenta – is an organ that connects the developing fetus to the maternal organism.

♦ The development of placenta begins at the 3rd week, when the secondary epitheliomesenchymal villi are penetrated by blood vessels and transform into the *tertiary villi*. At the 6-8th weeks of development the differentiation of macrophages, fibroblasts and collagen fibers around the blood vessels takes place. The formation of collagen fibers in the chorionic villi is accompanied by the intensification of proteolytic activity of the trophoblastic epithelium (cytotrophoblast) and its derivate (syncytiotrophoblast).

The development of placenta initiates destruction of the uterine mucosa; and the histotrophic type of nutrition is substituted by the hematotrophic one. It means that now the chorionic villi are supplied with maternal blood discharged into lacunae from the disrupted vessels of the endometrium.

Placenta is composed of two parts: 1) maternal; 2) fetal.

Maternal part of placenta is formed by the area of uterine mucosa, which is invaded by the chorionic villi (**decidua basalis or placentalis**). The remainder of the decidua, which is free from the chorionic villi, is called **decidua parietalis.** Also, there is **decidua capsularis**, which grows over the embryo on the luminal side, enclosing it into the endometrium and surrounding the embryo together with the decidua basalis.

Fetal part of placenta is the chorionic portion of placenta, containing the fetal blood vessels. There are distinguished two types of chorion:

a) chorion frondosum

b) smooth chorion (leave)

The villi of the chorion frondosum grow inwards the endometrium of the decidua basalis.

The smooth chorion is a place of contact of the trophoblast with the decidua capsularis.

The most intensive development of placenta occurs from 3rd to 6th week of the embryogenesis.

The placentas of mammals are divided into four types according to their structure:

1) epitheliochorial; 2) desmochorial; 3) endotheliochrial; 4) hemochorial.

The **human placenta** is referred to as the **discoid desmochorial** one.

The structural and functional unit of placenta is called **cotyledon.** Each cotyledon represents the branches of the one *anchoring villium*.

The human placenta consists of about 200 cotyledons that are separated from each other by connective tissue septa, through which the arteries carrying oxygenated blood pass to the lacunae of the placenta. The blood outflow from the placenta occurs via the lacunar veins, which open into the lacunae. The wall of lacuna is composed of the endometrial connective tissue, covered by the amorphous substance – fibrinoid of Rohr.

The part of decidual membrane located between the chorion frondosum and the smooth chorion intimately grows together with the last one and forms the **chorionic plate**, which prevents the entrance of blood to the uterine lumen.

The placental barrier is composed of structures that separate the maternal and the fetal blood. It is composed of

1) epithelial cell and basal membrane of blood capillaries of the chorionic villi;
2) a layer of connective surrounding the blood capillaries; it is rich in macrophages and fibroblasts;
3) basal membrane of the chorionic villi;
4) syncytiotrophoblast;
5) fibrinoid of Langhans

Functions of placenta: 1. trophic; 2. excretory; 3. depositing; 4. endocrine; 5. respiratory; 6. defensive.

Umbilical cord – is a cord that contains blood vessels, which provide blood circulation between the fetus and placenta (two umbilical arteries and one umbilical vein) surrounded by a **mucous connective tissue** (Warton's jelly). It also contains the rests of yolk stalk and allantois. The mucous connective tissue contains great amount of the hyaluronic acid, which provides the turgor of the umbilical cord. There were revealed the tissue basophiles and the cells of Kaschenko-Gobfauer (protect the fetus from intrauterine infections) among the cells of the umbilical cord.

THE CRITICAL PERIODS OF DEVELOPMENT

The critical periods of embryogenesis – are the periods when the developing reproductive cells (progenesis) or the embryo (embryogenesis) are the most susceptible to the unfavorable factors.

Such periods are:

1) in progenesis – ovogenesis and spermatogenesis;
2) in embryogenesis – fertilization, implantation (6-8 day), placentation, gastrulation (3,8 week); period of intensive development of the brain (15-20 week); period of formation of the main systems of organs (20-24 week); parturition.
3) in postnatal ontogenesis – period of a newborn (until 1 year); period of sexual maturation (11-16 years).

Theme 2. Epithelial tissue

EPITHELIAL TISSUE

Epithelial tissue makes covering for body surfaces, lines body cavities and constitutes glands.

The characteristic feature of this tissue is that it is composed only of cells, named epithelial cells, and it is almost devoid of extracellular matrix.

The epithelial cells are connected to each other via different types of cell-to-cell junctions and form a solid layer that lies on the basal lamina. Under the microscope the basal lamina appears as homogenous plate of 1μm in thickness.

The basal lamina separates epithelium from underlying loose connective tissue.

The epithelial cells are characterized by cell polarity. It means that each epithelial cell has two cell domains – apical domain facing the

external environment, and basal domain lying on the basal lamina. The apical and the basal domain are different in their structure:

- The basal domain contains nucleus and main organelles;
- The apical domain may contain microvilli, brush border, cilia or flagella.

Epithelial tissue has a high capacity of both physiological and reparative regeneration due to its immediate contact with the external environment. The regeneration occurs due to the presence of stem cells, which are specific for each type of epithelium.

CLASSIFICATION OF EPITHELIA

Nowadays, there are two classifications of epithelia, which are based on its origin (phylogenetic classification) or its structure (morphological classification).

According to phylogenetic classification, we distinguish six types of epithelium:

1) **Skin epithelium** (epidermal) arises from ectoderm; structure: stratified and pseudostratified epithelium; localization: skin, oral cavity, esophagus, cornea, vagina.
2) **Intestinal epithelium** (enterodermal) arises from entoderm; structure: simple columnar epithelium; localization: stomach, small and large intestine; function: absorption.
3) **Kidney epithelium** (nephrodermal) arises from mesoderm; structure: simple cuboidal or columnar epithelium; localization: kidney tubules; Function: reabsorption of primary urine.
4) **Coelomic epithelium** arises from splanchnotomes of mesoderm; structure: simple squamos epithelium; function: delimiting and secretion.
5) **Ependymoglial epithelium** arises from neural tube; structure: simple epithelium; function: lines fluid-containing cavities of CNS;
6) **Angiodermal epithelium** (endothelium) arises from mesenchyme; structure: simple squamos epithelium; localization: lines blood and lymphatic vessels heart; function: protection, absorption.

MORPHOLOGICAL CLASSIFICATION

This classification is based on structural and functional characteristics of different types of epithelia. According to this classification we distinguish two types of epithelia: covering epithelia and glandular epithelia.

Covering epithelia, in its turn, is subdivided into

1.Simple epithelia:
a)
- squamos;
- cuboidal;
- columnar;

b) pseudostratified
- columnar

2.Stratified epithelia:
a) keratinized;
squamos;
b) non-keratinized:
- squamos;
- cuboidal;
- columnar;

c) transitional.

STRUCURE OF DIFFERENT TYPES OF EPITHELIA

I Simple squamos epithelium (endothelium and mesothelium)

Endothelium lines blood and lymphatic vessels, and also heart chambers. It represents a singular layer of squamos (flattened) cells – endothelial cells, which rest on the basal lamina.

*Endothelial cells are polygonal cells with irregular borders; may possess 2-3 nuclei. Endothelial cells are relatively poor in organelles and contain pinocytotic vesicles in their cytoplasm.

♦ Function: transendothelial transport of oxygen, carbon dioxide and other substances; also participates in regulation of blood flow.

* Mesothelial cells are flattened, polygonal cells with irregular borders; may contain 2-3 nuclei. The apical domain of mesothelial cells possesses solitary microvilli.

♦ Function: secretion and absorption of serous liquid, prevents friction of inner organs.

II Simple cuboidal epithelium covers a part of the renal tubules (proximal and distal), excretory ducts of glands, small bronchi. The epithelial cells have equal height and width. The epithelial cells of proximal renal tubules possess brush border and basal striations. The basal striations appear because of high concentration of mitochondria in the basal domain of the cell.

♦ Function: reabsorption of substances from primary urine into the blood.

III Simple columnar epithelium are of three types:

1) *epithelium with a brush border* lines intestine, gallbladder.

2) *ciliated epithelium* lines oviducts; the cells possess cilia on their apical domain, which moves an egg towards the uterus.

3) *glandular epithelium* lines stomach; contains cells that are capable of production of mucous secretion and that's why were named

glandular cells. Along this these cells, the glandular epithelium contains endocrine cells, which regulate the activity of digestive tract.

IV Pseudostratified epithelium lines respiratory tract and some parts of reproductive system.

This epithelium includes four types of cells:

1) ciliated cells;
2) long and short basal cells;
3) goblet cells;
4) endocrine cells

♦ Basal cells are considered to be cambial (stem cells), which are capable of regeneration and differentiation into ciliated and goblet cells. Basal cells are wedge-shaped, their wide basal surface lies on the basal lamina, while their sharp apical surface inserts between the ciliated cells and does not achieve the surface of epithelium. .

♦ Ciliated cells are also wedge-shaped cells, their narrow basal surface lies on the basal lamina, while their wide apical surface, which possesses cilia, achieves the surface of epithelium.

♦ Goblet cells, hence their name, are goblet-shaped cells, which produce mucous secretion on the epithelial surface.

♦ Endocrine cells (their basal domain possesses granules) produce biologically active substances – hormones.

Pseudostratified epithelium is a type of simple epithelium; hence all epithelial cells lie on the basal lamina. Due to the different shapes of the cell, the nuclei are located at different levels, which makes this epithelium appear as stratified one.

V. Stratified squamos non-keratinized epithelium

Localization: cornea, oral cavity, esophagus, vagina, anal part of rectum.

This epithelium is composed of three layers of cells:

1) *Stratum basale* is composed of columnar epithelial cells, which lie immediately on the basal lamina. The cells of this include stem cell, capable of mitotic division; that's why is layer is also named cambial layer.
2) *Stratum spinosum* consists of polygonal epithelial cells that possess projections inserted between the apical portions of the underlying basal cells. These cells form several layers and possess projections that resemble spines.

3) *Stratum superficiale* is the outermost layer consisting of flattened cells.

VI. Stratified squamos keratinized epithelium covers the skin and is named **epidermis**.

The epidermis includes 5 layers, where occurs the transformation of epithelial cells into anucleate squamos cells filled with keratin protein. This process is called keratinization.

1) **Stratum basale** , also called stratum germinativum, is represented the single layer of cells, which rest on the basal lamina. It contains the stem cells from which new cells, the keratinocytes, arise by mitotic division. As new keratinocytes arise in this layer by mitotic division, they move into the next layer, thus beginning their process of upward migration. Besides the stem cells and keratinocytes, the stratum basale contains melanocytes, Merkel's cells, and Langerhans' cells.

The keratinocytes have a cuboidal or low columnar shape, basophilic cytoplasm and oval, rich in chromatin nucleus. Their cytoplasm contains keratine intermediate filaments and organelles; they also may contain various amounts of melanin.

The melanocytes – are pigment cells, which form processes branching towards the epidermal surface. On histological specimens the melanocytes are revealed by the silver impregnation method. The melanocytes synthesize melanin; their cytoplasm does not contain tonofibrils, but it is rich in ribosomes and melanosomes.

The Langenharns' cells are the type of macrophages, provide the immune defense. They are dendritic-appearing cells with cytoplasm containing Birbeck granules, which have an appearance of tennis racket. Langerhans' cells encounter and process antigens entering through the skin.

2) Stratum spinosum is formed by 5-10 layers of polygonal keratinocytes. Their cytoplasm contains tonofibrils, which take part in formatiom of desmosomes. The basal cells and cells of inner layers of stratum spinosum form the cambial zone of epidermis.

3) Stratum granulosum is formed by 3-4 layers of cells. At this layer filaggrin, a protein aggregating the keratin tonofilaments, is synthesized within the keratinocytes. As a result of aggregation of the keratin tonofilaments, the cytoplasm of keratinocytes stores the basophilic granules of keratohyaline. Involucrin and keratolinin form a protein layer under the plasmolemme, protecting it from action of hydrolytic enzymes of keratosomes and lysosomes, which are activated

by the Langenharns' cells. The appearance of keratohyaline granules is an indicative of the beginning of keratinization process.

4) Stratum lucidum is formed by 3-4 layers of squamos cells, within whose cytoplasm the nucleus and organelles are completely disrupted; the keratohyaline granules fuse into the refractile mass, which consists of aggregated keratin fibrils and amorphous matrix.

5) Stratum corneum of scores of layers of annucleate squamos cells largely filled with keratin filaments. Filaggrin provides the further aggregation of intermediate filaments within the keratinocytes. The keratinocytes store the air vesicles; the light cavity is appears at the site of nucleus. The intercellular lipid barrier and the keratinocytes provide impenetrability of the epidermis. The keratinocytes of stratum corneum are eventually sloughed off at the skin surface. The mechanism of desquamation is controlled by keratinosomes – the modificated lysosomes, which dissolve desmosomes and provide the separation of cornified cells from each other.

The stratum corneum is the layer that varies most in thickness, being thickest in thick skin. The thickness of this layer constitutes the principal difference between the epidermis of thick and thin skin.

The interrelated processes of differentiation and keratinization of keratinocytes constantly occur in the epidermis. These processes provide regular renewal of stratum corneum, which is characterized by mechanical and chemical resistance, high hydrolytic capability, poor heat conduction, and impenetrability for bacteria and their toxins.

VII. Transitional epithelium lines the urinary passages – renal pelvis, urethers, urinary bladder, and urethra. The epithelium begins in the minor calyces as two cell layers and increases to an apparent four to five layers in the ureter and as many as six or more layers in the empty bladder. However when the bladder is distended, as few as three layers are seen. This change reflects the ability of the cells to accommodate to distension.

Transitional epithelium consists of three layers of cells:

1. Basal layer consists of small rounded dark cells.
2. Transitional layer is composed of polygonal cells.
3. Superficial layer contains large dome-shaped cells, which possess 2-3 nuclei.

GLANDULAR EPITHELIUM. GLANDS.

Glandular epithelium consists of glandular cells that produce and discharge specific secretions on the surface of skin, mucous membranes, within the cavities of the inner organs (exocrine glands); or directly into the blood or lymph (endocrine glands).

The cytoplasm of glandular (secretory) cells exhibits numerous secretory inclusions and strongly developed rough Endoplasmic reticulum.

The glandular epithelium composes glands, which are subdivided into two big groups on the basis of the way the release secretion:

Exocrine glands release their secretion on the surface of epithelium. All exocrine glands are composed of two parts: 1) secretory portion (acinus); 2) excretory ducts	Endocrine glands do not possess excretory ducts and release thaeir secretion directly into the blood or lymph.

Besides this, glands are classified on the basis of their structure, type of secretion, and location in respect to the epithelial layer.

On the basis of location in respect to the epithelial layer, all glands are subdivided into:

1. **Endoepithelial glands** – do not exceed the borders of the epithelial layer. In the human, the only example of such glands is goblet cell, which is referred to as unicellular endoepithelial gland. The goblet cells are found within the epithelium of respiratory tract and intestine.
2. **Exoepithelial glands** are situated within the connective tissue that underlies the epithelial layer; they are connected with epithelial layers through the excretory duct. The exoepithelial glands are multicellular. Depending on the number of excretory ducts they are subdivided into **simple glands**, which possess only one excretory duct, and **compound glands**, which possess to or more excretory ducts.

   Simple glands, depending on the number of secretory portions, are subdivided into two types:

   1. Branched glands – possess two and more secretory portions;
   2. Non-branched glands – possess only one secretory portion.

Compound glands are always branched, as they possess several excretory ducts that end with several secretory portions.

♦ On the basis of the shape of secretory portion, glands are classified into:

1) tubular;
2) alveolar;
3) tubulo-alveolar.

Tubular – the shape of secretory portion is tube-like;

Alveolar- the shape of secretory portion is sac-like.

♦ On the basis of the mode of secretion, the glands are subdivided into:

1) merocrine;
2) apocrine;
3) holocrine.

Merocrine glands – the cell releases its secretion by exocytosis. The destruction of the secretory cell after discharge of secretion **does not** take place at all. Most glands release their product in this way.

Apocrine glands- the apical portions of cells are pinched off and lost during the secretory process. This results in a secretory product that contains a variety of molecular components including those of the membrane. (mammary glands).

Holocrine glands – the secretory breaks apart, the contents of the cell become the secretory product. (sebaceous glands).

Depending on the chemical composition of secreted product, the glands are subdivided into: serous, mucous, mixed (sero-mucous), sebaceous, and sweat glands.

Structure of glandular cells

The shape of glandular cells varies significantly and might change depending on the phase of secretion. Such cells usually possess quite large nucleus with rough surface.

The glandular cells that produce protein (serous) secretion contain strongly developed rough ER.

The glandular cells producing non-protein secretions (lipids, steroids) contain strongly developed smooth ER.

All types of secretory cells are characterized by presence of well developed Golgi complex, where transport vesicles are formed, and numerous mitochondria. The cytoplasm contains a big amount of inclusions. The glandular cells are also characterized by cell polarity.

PHASES OF SECRETION

Secretion represents the process, when the cell produces and releases some synthesized products.

The process of secretion includes 4 phases:

1. Absorption;
2. Synthesis and storage;
3. Release;
4. Restoration.

Absorption : the glandular cells absorb various substances, which are essential for synthesis of secretion (water, salts, ions, amino acids, monosaccharides, fatty acids), from blood and lymph.

Synthesis and storage: in the ER the absorbed substances are used for the synthesis of secretion (protein secretion – rough ER, non-protein secretion – smooth ER). The produced products are transported to Golgi complex, where they are modified, stored, and packed into transport vesicles.

Release: the secretory vesicles are released out of the cell by apocrine, holocrine or merocrine mode of secretion.

Restoration: the secretory cells return to their initial condition.

Usually these phases occur sequentially, constituting the secretory cycle, but sometimes they can occur simultaneously, which is characteristic for the spontaneous secretion.

Theme 3. Tissues of internal environment. Blood. Lymph.

Tissues of internal environment include blood, lymph and all types of connective tissue. Despite of a significant difference between each other, all these tissue can be referred to the one group because of their common origin, structure and functions.

♦ Origin: all of the tissues of internal environment are derived from mesenchyme.

Mesenchyme is an embryonic connective tissue. The mesenchyme structurally resembles a network, since the stellate-shaped or spindle-shaped mesenchymal cells contact with one another by their processes. Spaces between the cells are occupied by extracellular matrix. Density of the extracellular depends on intensity of metabolism. The

mesenchyme gives rise to blood, lymph and all types of connectives tissues.

The structural and functional characteristics vary in different kinds of connective tissue and depend on the physical and chemical properties of the extracellular matrix.

Classification of tissues of internal environment

All tissues of internal environment are divided into two main groups:

1. Fluid tissues. 2. Connective tissues

Fluid tissues include two types of tissues:

a) blood;

b) lymph.

Connective tissues are divided into the following types:

1. Connective tissue proper;
2. Skeletal connective tissue.

Connective tissue proper, in its turn, is divided into:

1) fibrous connective tissue;

2) connective tissue with special properties;

* Depending on the amount of fibers, the fibrous connective can be:

1) loose;

2) dense.

Loose fibrous connective tissue consists predominantly of cells and ground substance.

Dense fibrous connective tissue contains a great amount of fibers and, on the basis of their arrangement, is subdivided into:

a) reguar;

b) irregular.

Dense regular connective tissue – the fibers are arranged parallel to one another.

Dense irregular connective tissue – the fibers are randomly arranged, forming a network.

* Connective tissues with special properties include the following:

1. Reticular
2. Adipose
3. Pigment
4. Mucous

* Skeletal connective tissues include:

1. Cartilage
2. Bone tissue

BLOOD. ITS COMPOSITION AND FUNCTIONS. PLASMA.

Blood is a fluid tissue that circulates through the cardiovascular system. It constitutes approximately 5-9% of the total body mass (5-5,5litres).

Development: blood arises from mesenchyme. All blood cells arise from one common *pluripotential blood stem cell*. The further differentiation of the blood stem cell is determined by several factors:

a) microenvironment (reticular tissue of hematopoietic organs);
b) hemopoietins.

The population of blood cells is a constantly renewed system, where the mature (definitive) cells, after their death, are substituted by the newly-formed cells. The formation of new blood cells and destruction of the old ones are in physiological equilibrium, that's why the quantitative and qualitative composition of blood remains the same.

♦ Blood consists of cells and its derivatives and a protein rich fluid called plasma.

Blood cells and their derivatives include:

a) erythrocytes, also called red blood cells (RBCs);
b) leukocytes, also known as white blood cells (WBCs);
c) thrombocytes, also termed as platelets.

The relative volume of cells and plasma in whole blood is approximately **45% to 55%** respectively. This ratio is called **hematocrit**.

Functions of blood

1. Transport
2. Immune defense
3. Maintenance of homeostasis
4. Trophic
5. Respiratory

Transport and trophic functions: delivery of nutrients and other substances directly or indirectly to cells.

Respiratory function: delivering of oxygen from lungs to tissue and removing of carbon dioxide.

Immune defense: transport of humoral agents and cells of the immune system that protect the body from pathogenic agents (microorganisms

or cancer cells).

Maintenance of homeostasis: acts as buffer and participates in coagulation.

Plasma

Blood plasma is the liquid extracellular material that impairs fluid properties to blood. The blood plasma represents a colloid system which is composed of water (90-93%), organic (proteins: albumins, globulins and fibrinogen, and other organic substances - about 7%) and non-organic material (3%). The total concentration of mineral components constitute 0, 9% of the total plasma volume. The pH of the blood plasma is 7,36.

Plasma proteins:

1. Albumins (4%); functions: binds and transport hormones, metabolites and drugs; maintain osmotic blood pressure.

2.Globulins (1,1-3,1%); The globulins are of three types:

a) α - globulins;

b) β - globulins;

b) γ **- globulins** – are referred to as **antibodies**;

3. *Fibrinogen (0,2-0,4%)* is soluble in water, but in a series of cascade reactions with other clotting factors it is transformed into the insoluble protein fibrin. Due to this property, the fibrinogen is responsible for blood clotting and preventing of blood loss in case of damaging of the blood vessel. The blood plasma without fibrinogen is named **blood serum**.

Forming elements of blood

The blood cells and their derivates are traditionally referred to as forming elements of blood.

Erythrocytes (red blood cells) are immobile anucleate post-cellular structures devoid of nucleus and typical organelles. The main function of erythrocytes is to bind and transport oxygen and carbon dioxide. They carry out this function due to the specific iron-containing protein – **hemoglobin** (Hb).

Besides this, erythrocytes also provide transport of amino acids, antibodies, toxins and medicines by absorbing them on their plasma membrane.

The number of erythrocytes varies in males and females, and changes with ageing.

The number of erythrocytes per 1L of blood:

In males - $3,9 \times 10^{12}$ - $5,5 \times 10^{12}$;

In females - $3,7 \times 10^{12}$ - $4,9 \times 10^{12}$;

In newborns - $6,0 \times 10^{12}$ - $9,0 \times 10^{12}$;

In elderly people - up to $6,0 \times 10^{12}$.

Shape and structure

The major part of erythrocytes has a shape of biconcave discs. Such erythrocytes are called *discocytes*.

The discocytes account for 80% of total number of erythrocytes.

The electron microscope examinations revealed the erythrocytes of other shapes:

a) *planocytes* – flattened erythrocytes;
b) *stomatocytes* – domed-shaped erythrocytes;
c) *saddle-shaped* erythrocytes;
d) *spherocytes*- sphere-shaped erythrocytes;
e) *echinocytes* – spinous erythrocytes.

Echinoctytes and spherocytes are the old forms of erythrocytes.

The phenomenon of shape diversity of erythrocytes is named physiological poikilocytosis. Normally the percentage of abnormally shaped erythrocytes does not exceed 20% of total erythrocytes number.

If this percentage is higher, such condition, named pathological poikilocytosis, requires treatment.

♦ The shape of erythrocyte is maintained due to the structure of its plasma membrane, which contains specific structural proteins like β - *sialoglycoprotein, spectrin, and ankyrin within* the **cortical layer**.

♦ In the human the diameter of erythrocyte is 7,1 - 7,9 μm; the edge thickness is 2,0 – 2,5 μm, the central thickness is 0,8-1 μm.

The depression in the center of erythrocyte, named physiological excavation, maximizes the cell's surface area, which is an important attribute in gas exchange.

Normally 75% of circulating erythrocytes are of 7,1 - 7,9 μm in diameter (physiological anisocytosis). Besides them, two other forms of erythrocytes are found in blood – macrocytes and microcytes.

Macrocytes(12,5%) – are more than 8,0 μm in diameter.

Microcytes (10,5%) – are less than 6,0 μm in diameter.

The phenomenon when macrocytes and microcytes account form more than 25% of total number of erythrocytes is named pathological anisocytosis. The general surface area of erythrocyte constitute 125 μm.

♦Plasma membrane of erythrocyte is approximately 20 nm thick. The outer surface of the plasma membrane contains *phospholipids, sialic acid, antigenic oligosaccharides, and adsorbed proteins*.
The inner surface of the erythrocyte plasma membrane contains glycolytic enzymes, Na+ K+, ATP-ases, glycoproteins, hemoglobin.

Being semi-permeable, the erythrocyte's plasma membrane provides active transmembrane transport of ions of Na, K, O_2 и CO_2 , and other substances.

♦ Cytoplasmic matrix possesses numerous hemoglobin-containing granules. It consists of water (60%) and dry residue (40%). Hemoglobin constitutes 95% of the dry residue.

Hemoglobin is a complex protein which consists of four polypeptide chains of globin, and iron-containing heme group. It can bind oxygen (O_2) – oxyhemoglobin, or carbon dioxide (CO_2) – carbohemoglobin, thereby performing the respiratory function. The complex of hemoglobin with carbon monoxide (CO) is more stable than those with oxygen and carbon dioxide. The affinity between hemoglobin and carbon monoxide is approximately 300 times higher than the affinity between hemoglobin and oxygen so hemoglobin binds to carbon monoxide in preference to oxygen. So, carbon monoxide prevents hemoglobin from carrying oxygen to tissues, thereby causing death.
In the human the following types of hemoglobin can be distinguished:

a) **HbA** – prevalent in adults, accounts for 98% of total hemoglobin;
b) **HbF** – the principal form of hemoglobin in fetus. In adults accounts for 2%. Its capacity of binding oxygen is much higher that that of the HbA that enables HbF to provide tissues of the fetus with oxygen in conditions of mixed blood circulation. HbF production falls dramatically after the birth.

Mutations in genes encoding globin chains can cause disorders in hemoglobin production. Hence erythrocytes are devoid of mitochondria, the main way they produce energy is glycolysis – anaerobic oxidation of glucose followed by production of ATP and NADP.

The life span of erythrocyte is 120 days. Every day approximately 200 million erythrocytes are destroyed in the spleen. This destruction is accompanied with breakdown of hemoglobin into protein **globin** and iron-containing **heme group**. The released iron is used for hemoglobin synthesis in new erythrocytes. The globin is used for formation of bile acids in the liver.

Physiologically, along with the mature erythrocytes, in the blood are found 1-5% of young erythrocytes with lower concentration of hemoglobin – reticulocytes. Reticulocytes are stained with both basic and acidic dyes (polychromatic). Their cytoplasm reveals rests of mitochondria and endoplasmic reticulum which contain RNA.

Thrombocytes are anucleate fragments of cytoplasm that was separated from the giant cells of the red bone marrow – **megakaryocytes**.

*Size – 2-3 μm

* Number - 180 - 320 x 10^9 per 1L of blood

Structure

Thrombocyte consists of:

1) Hyalomere is the basis of thrombocyte;
2) Granulomere includes granules that form aggregations in the central or peripheral part of the cytoplasm.

Two types of granules are distinguished in thrombocyte:

1) dense, dark α-granules;
2) serotonin granules (δ-granules);
3) lysosomes and microperoxisomes (λ-granules).

- Granulomere contains also contains granules of glycogen and mitochondria.

- Hyalomere contains circularly arranged bundles of 10-15 microtubules that maintain the cytoskeleton of thrombocyte, and actin and myosin filaments.

Thrombocytes possess numerous processes of different size and thickness that take part in their aggregation and formation of thrombus.

The Romanovsky-Giemza staining reveals **5 types** of thrombocytes:

a) **young thrombocytes** with basophilic hyalomere and solitary azurophilic granules;
b) **mature thrombocytes** with slightly acidic hyalomere and numerous azurophilic granules;
c) **old thrombocytes** – dark, bluish-purple with dark-purple granules;
d) **degenerating thrombocytes** – with grayish- blue hyalomere and bluish-purple granules;

e) **giant thrombocytes** – their size is 2-3 times than usual one, possess pinkish-purple hyalomere and purple granules.

The life span of thrombocytes is 5-8 days.

Продолжительность жизни тромбоцита 5-8 дней.

♦ Function: take part in blood clotting. Thrombocytes discharge enzyme thromboplastin that catalyses transformation of soluble fibrinogen into insoluble fibrin. Aggregated thrombocytes also serve as a frame for the thrombus.

Leukocytes

Leukocytes are white, globe-shaped blood cells that possess nucleus and all organelles. Leukocytes can leave the blood stream and carry out their functions in connective tissue.

In adults the number of white blood cells per 1L is $3{,}8 \times 10^9$-9×10^9. Increase of total number of leukocytes in blood is called leukocytosis; decrease of total number of leukocytes in blood is referred to as leucopenia.

Classification

All leukocytes are classified into two general groups. The basis of this division is the presence or absence of prominent specific granules in the cytoplasm.

1. Leukocytes containing specific granules are classified as **granulocytes.**
2. Leukocytes that lack specific granules are classified as **agranulocytes.**

On the basis of granules' staining the **granulocytes** are subdivided into:

1) neutrophils;
2) eosinophils;
3) basophils.

The agranulocytes are of two types: lymphocytes and monocytes.

Granulocytes

I *Neutrophils*

♦ Neutrophils account for 65-70% of total leukocytes number. They measure 7-9 μm in fresh blood and 10-12 μm in blood smear.

♦ The cytoplasm of neutrophils contains granules. Each cell cam possess from 50 to 200 granules. Not whole cytoplasm is occupied by granules; a thin peripheral layer of cytoplasm is devoid of granules, as it

contains the elements of cytoskeleton. This layer provides the amoebic movements of the cell by formation of pseudopodia.

♦ Neutrophils possess two types of granules:

1) azurophilic (primary);
2) neutrophilic (secondary) - specific;

Azurophilic granules arise in early granulopoiesis and are named primary granules. They are less numerous than specific granules (10-20% of total amount of granules). Each granule is rounded or oval-shaped and measures 0,4-0,8 μm in diameter. These granules are the lysosomes of neutrophil and contain typical **acid hydrolases.**

Neutrophilic granules (specific) are named secondary as they increase in number with maturation of the cell. In mature neutrophil they account for 80-90% of total amount of granules. Neutrophilic granules are ellipsoidal, they measure 0,2-0,4μm. They contain acidic phosphatase, basic cationic proteins, lactoferrins, lysozymes, amino peptidases, and complement activators.

♦ The cytoplasm of neutrophils possesses poorly developed organelles, small amount of mitochondria, sometimes reduced elements of Endoplasmic reticulum can be found. It also usually contains glycogen and lipid inclusions. The Romanovsky-Giemza stain gives the granules both pink and purple color.

♦ The nuclei of neutrophils contain dense chromatin. Wide regions of heterochromatin are located chiefly at the periphery of the nucleus; regions of euchromatin are located primarily at the center of the nucleus. In woman, the Barr body (the condensed, single, inactive X-chromosome) forms a drumstick-shaped appendage on one of the nuclear lobes. Neutrophils possess multilobal nuclei, which are variable in shape; thus, they are also called polymorphonuclear neutrophils.

Mature neutrophils possess two to four lobes of nuclear material joined by thinner nuclear strands. These neutrophils are referred to as **segmented neutrophils**. They account for 49-72% of total number of leukocytes.

Band neutrophils are immature cells accounting for 1-6% of total number of leukocytes. These neutrophils possess S-shaped or horseshoe-shaped nucleus.

Metamyelocytes (young neutrophils) are characterized by bean-shaped nucleus and account only for 0-0,5% of total number of leukocytes.

Neutrophils are motile cells that can leave the bloodstream and migrate to the site of inflammation in the connective tissue. They are capable of **phagocytosis.**

Neutrophils also produce chalones – specific substances that inhibit the DNA synthesis in granulocytes and regulate the processes of proliferation and differentiation of leukocytes. The life span of neutrophils is about 8 days; they circulate in the bloodstream for 8-12 hours, after that they migrate in the connective tissue and perform their functions.

II *Eosinophils*

♦ The diameter if eosinophil in the fresh blood is 9-10 μm; in the blood smear – 12-14 μm. They account for 1-5% of total number of leukocytes.

♦ The cytoplasm contains two types of granules:

1) Specific granules are oval or polygonal in shape, measuring about 0,5-1,5μm. Specific granules of eosinophils exhibit intense acidophilia due to the containing of **major basic protein that is rich in arginin**. Specific granules also contain histaminase, arylsulfatase and collagenase.
2) Azurophilic granules are lysosomes. They contain variety of the usual lysosomal acid hydrolases and other hydrolytic enzymes which function is destruction of parasites and hydrolysis of antigen-antibody complexes.

♦ Three types of eosinophils are distinguished

a) segmented;
b) band eosinophils;
c) young eosinophils.

The nuclei of segmented eosinophils usually consist of two lobes joined by thin strands. Band and young eosinophils are rarely found in blood; they resemble neutrophils of the same stages of maturation. The nuclei of eosinophils contain predominantly heterochromatin, the nucleoli are not distinguishable. Eosinophils are less motile than neutrophils.

Functions. Eosinophils take part in defensive reactions against foreign proteins, and allergic reactions. They are capable of phagocytosis and breakdown of histamine by the enzyme histaminase. The number of eosinophils in blood increases in case of parasites

invasion and allergic reactions. The capacity of phagocytosis in eosinophils is lower than that of neutrophils.

III. Basophils measure 9μm in diameter in the fresh blood and 11-12 μm in the blood smear. They account for 0,5-1% of total number of leukocytes.

♦ Cytoplasm contains large, rounded or polygonal, basophilic granules with diameter of 0,5-1,2 μm.

The granules are characterized by metachromasia due to the containing of acidic glycosaminoglycan – **heparin**.

Metachromasia is an ability to change the initial color of dye. Besides heparin, the granules also contain **histamine.**

The density of granules can be different that reflects different degree of maturity and functional conditions. Besides specific granules, basophils also contain primary azurophilic granules that represent lysosomes. The cytoplasm possesses all general-purpose organelles.

♦ The lobules of the nuclei of basophils are not very conspicuous. The nuclei of basophils are stained less intensively than those of neutrophils and eosinophils.

♦ Functions of basophils are determined by their capacity of metabolism of histamine and heparin. They take part in regulation of blood clotting (heparin is anticoagulant) and permeability of blood vessels (histamine). They also take part in immunological reactions, especially allergic reactions. Due to the presence of receptors to antibodies on their plasma membrane, they can react on antigen-antibody complexes by discharging of histamine. Histamine, by increasing of permeability of blood vessels, causes the symptoms of allergic reaction (itch, redness, and edema). Besides these, histamine causes spasm of smooth muscles of the small bronchi, and is involved in pathogenesis of the bronchial asthma. Basophils also produce eosinophils-chemotaxis factor. Eosinophils take part in inactivation of histamine, thereby stopping allergic reactions. Basophils are practically incapable of phagocytosis.

Agranulocytes

Lymphocytes account for 19-37% of total number of leukocytes. Their size varies significantly from 4,5 to 10 μm. That's why three types of lymphocytes are distinguished on the basis of their size:

a) small – 4,5-6,0 μm in diameter;

b) medium – 7-10 μm in diameter;

c) large – more than 10 μm in diameter.

Lymphocytes are characterized by intensively stained, rounded or bean-shaped nucleus and relatively small amount of basophilic cytoplasm. The cytoplasm of lymphocytes contains small amount of azurophilic granules (lysosomes).

The electron microscope examination revealed four types of lymphocytes in adults:

1) small light lymphocytes;
2) small dark lymphocytes;
3) medium lymphocytes;
4) plasma cell (lymphoplasmocytes).

Small light lymphocytes measure about 7 μm in diameter. The nucleus occupies more space than the surrounding cytoplasm. The nucleus is rounded; heterochromatin is concentrated at the periphery of the nucleus.

The cytoplasm contains relatively few ribosomes and polysomes, lysosomes, poorly developed Golgi complex, mitochondria, but numerous vacuoles and vesicular bodies. The organelles are concentrated near the nucleus. Small light lymphocytes account for 70-75% of total number of lymphocytes.

Small dark lymphocytes measure of 6-7 μm in diameter and account for 12-13% of total number of lymphocytes. The nucleus occupies even more space than that of small light lymphocytes. The chromatin appears dense; the nucleolus is relatively big.

A thin layer of dense dark cytoplasm surrounds nucleus, contains numerous ribosomes, a few mitochondria. Other organelles are found rarely.

Medium lymphocytes measure of about 10 μm in diameter and account for 10-12% of total number of lymphocytes. They possess bean-shaped nucleus with visible finger-like projections of nuclear envelope. The chromatin is less condensed, the regions with heterochromatin are revealed only near the nuclear envelope; the nucleolus is well distinguished.

The cytoplasm contains elongated canaliculi of endoplasmic reticulum, free ribosomes and polysomes. The centrosome and Golgi complex are usually situated near the site of invagination of the nuclear envelope. Lysosomes and mitochondria are not numerous.

Plasma cells are characterized by canaliculi of the rough ER that concentrically surround the nucleus. They account only for 1-2% of total number of lymphocytes.

Three functionally distinct types of lymphocytes are present in the human body:

1. T-lymphocytes;
2. B-lumphocytes;
3. NK – cells.

T-lymphocytes (thymus-dependent) arise from the blood stem cell in the red bone marrow and provide reactions of cell-mediated immunity and regulation of humoral immunity.

The life span of T-lymphocytes can last for several (even several decades) years. They account for 80% of total number of lymphocytes.

Three types of T-lymphocytes have been identified:

1. Cytotoxic T-killer cells;

2. T-cells that regulate humoral imminity:

- T-helper cells;
- T-suppressor cells.

T-killer cells are effector cells of the cell-mediated immunity. They are responsible for rejection of allografts and elimination of tumor cells.

T-helper cells initiate and encourage the production of antibodies by B-lymphocytes.

T-suppressor cells inhibit the production of antibodies by B-lymphocytes. They act via production of specific soluble substances – **lymphokines**, which is initiated by action of antigens.

T-memory cells keep the information about previously circulating antigens.

B-lymphocytes arise from the blood stem cell in the red bone marrow. No distinctive morphological differences were revealed between T- and B-lymphocytes. B-lymphocytes possess stronger developed rough ER, whilst T-lymphocytes possess more lysosomes. T-lymphocytes are relatively smaller; possess smaller nucleus with more heterochromatin.

The plasma membrane of B-lymphocytes contains various receptors to different antigens.

♦ Function: provide humoral immunity by producing antibodies (immunoglobulins).

The definitive form of B-lymphocyte is plasma cell.

NK (natural killer) cells are programmed during their development to kill certain virus-infected cells and some types of tumor cells. They also secrete an antiviral agent, interferon.

Monocytes measure 9-12 μm in fresh blood and 18-20 μm in blood smear. Monocytes are the part of the macrophagic system of the body. The cells of the macrophagic system arise from the precursors of monocytes of the red bone marrow, circulate with the bloodstream for 36-104 hours and then migrate into the connective tissue and maturate there.

♦ The cytoplasm is less basophilic than that of lymphocytes. The Romanovsky-Giemza staining gives it light-blue color. The peripheral part of the cytoplasm is densely stained, as it contains numerous fine azurophilic granules (lysosomes). Monocyte possesses finger-like projections, phagocytic vacuoles, pinocytotic vesicles, short canaliculi of the rough ER and small mitochondria.

♦ The nuclei of monocytes are variable in shape: bean-shaped, horseshoe-shaped, lobulated, with diverse projections or invagination etc. The nuclei can possess one or more nucleoli. The chromatin is scattered through the nucleus.

Monocytes account for 3-11% of total number of leukocytes.

Functions: As it leaves the bloodstream, monocyte differentiate into macrophage and carry out its specific functions.

Lymph is a yellowish fluid consisting predominantly of proteins that circulates through the lymphatic vessels. It is composed of lymph plasma and forming elements.

The lymph plasma composition is similar to that of the blood plasma, but contains fewer proteins. It contains more albumins than globulins. Most of the proteins are enzymes, like diastase, lipase, and glycolytic enzymes. It also contains neutral fats, simple sugars, NaCl, Na_2CO_3, and complexes containing Mg, Ca and Fe.

Forming elements include predominantly lymphocytes (98%), and also monocytes.

Three types of lymph are distinguished:

1. Peripheral lymph – flows from tissues to lymph nodes;
2. Transitional lymph – outflows from lymph nodes;
3. Central lymph – the lymph of the thoracic duct and the right lymphatic duct.

Lymph is formed in lymphatic capillaries of tissues and organs under the influence of different factors that include osmotic and hydrostatic pressure.

Theme 4. Tissues of internal environment. Connective tissues.

Connective tissues are the tissue of internal environment which are characterized by wide range of cells and strong development of the extracellular matrix.

The physical properties and chemical composition of the extracellular matrix determines the functions and features of each type of connective tissue.

Functions of connective tissues

1. Mechanical and supporting: form capsule and stroma of the inner organs;
2. Protective: mechanical protection (fascia, bones, cartilage), provide cell-mediated and humoral immunity by phagocytosis and production of antibodies;
3. Participates in healing of wounds, regeneration and reactions of adaptation to changing conditions of external environment.
4. Nutritive: provides nutrition for surrounding tissues, regulates metabolism and maintenance of homeostasis.

Classification

All connective tissues are subdivided into:

1) Connective tissue proper (fibrous connective tissue);
2) Skeletal connective tissue (cartilage, bone).

- Depending on the amount of fibers, the fibrous connective tissue is subdivided into
  1) loose connective tissue;
  2) dense connective tissue.

Loose connective tissue contains more cells and ground (amorphous) substance.

Dense connective tissue is composed predominantly of fibers and, depending on their organisation , is subdivided into:

1) dense **regular** connective tissue;
2) dense **irregular** connective tissue

In dense regular connective tissue the fibers are laid down parallel to one another.
In dense irregular connective tissue the fibers are laid down in different directions to each other, forming a network.

* The types of connective tissues with special properties are:

1. Reticular tissue;
2. Adipose tissue;
3. Pigment tissue;
4. Mucous tissue.

LOOSE CONNECTIVE TISSUE

Loose connective tissue is composed of cells and extracellular matrix. It is found in all tissues and organs, as it accompanies blood and lymphatic vessels.

Cells of connective tissue

Fibroblasts are the most numerous group of cells, capable of production of fibrillar and globular proteins (collagen, elastin), glycosamonoglycans (sulfated and non-sulfated), proteoglycans, glycoproteins, which are then released in the extracellular matrix.

In emryogenesis the fibroblasts arise from the mesenchymal cells, after the birth – from stem cells.

The histogenetic lineage (differon) of fibroblasts includes:
stem cells → semi-stem cells-precursors→ young (immature) fibroblats→ mature firoblasts (functioning) → fibrocytes (definitive cells), and also myofibroblasts and fibroclasts.

Young fibroblasts are of 20-25 μm in size. The young fibroblast possesses rounded nucleus with small nucleolus, and basophilic karyoplasm, rich in RNA. The cytoplasm contains numerous ribosomes. Endoplasmic reticulum and Golgi complex are poorly developed. The cell **does not** possess distinctive cytoplasmic processes. These fibroblasts are characterized by very low level of protein synthesis and secretion. They are capable of mitotic division.

Mature fibroblasts – in the specimen their size can account for 40-50μm. The nucleus is oval, contains 1-2 large nucleoli. The cytoplasm contains well developed rough ER, which at some sites even contacts with the plasma membrane. The Golgi complex, which appears as tubules and cisterns, is distributed throughout the cell. The cortical layer

of cytoplasm contains microfilaments of 5-6nm thick that are composed of actin- and myosin-like proteins. These microtubules enable the fibroblasts to movement. Active synthesis of components of the extracellular matrix (collagen and elastic proteins, glycosaminoglycans, and proteoglycans) occurs within the fibroblasts.

In usual conditions, the fibroblasts reveal weak motility and low phagocytic activity. Their motility is activated in case of binding to the supporting fibrillar structures (fibrin, connective tissue fibers) with a help of fibronectin –glycoprotein which is responsible for adhesion of cells to extracellular matrix.

Fibrocytes are definitive forms of fibroblasts. Shape: spindle-shaped with prominent wing-like (pterygoid) processes. The cytoplasm contains small amount of organelles, vacuoles, lipids, and glycogen. In these cells the synthesis of collagen and other substances is dramatically reduced.

Myofibroblasts are the cells functionally related to the smooth muscle cells, but, unlike the smooth muscle cells, their cytoplasm possesses strongly developed rough ER. These cells are especially numerous and active at the sites of healing of wounds and in the uterus during pregnancy.

Fibroclasts are the cells of fibroblast differon, which are characterized by extremely high phagocytic and hydrolytic activity. They contain numerous lysosomes. The fibroclasts participate in dissolving of the extracellular matrix at the period of involution of an organ.

Macrophages are the cells of hematogenic origin, which are capable of active phagocytosis. The macrophages of two types are distinguished: fixed macrophages and wandering macrophages.

Size: 10-15μm.

Shape: rounded, elongated, polygonal.

The nucleus is smaller than that of the fibroblasts, but it is denser, as it contains more heterochromatin.

The cytoplasm is basophilic, heterogeneous, contains numerous lysosomes, phagosomes, pinocytotic vesicles, rough ER, Golgi complex, mitochondria.

The plasma membrane forms both deep folds and long projections, which helps these cells to uptake foreign particles. Because of the presence of receptors to cancer cells, erythrocytes, T-and B-

lymphocytes, and immunoglobulins on the surface of their plasma membrane, the macrophages actively participate in immune reactions.
Functions: phagocytosis, synthesis and secretion of biologically active substances (interferon, lysosyme, pyrogens, prostheses, acidic hydrolases). The important role is performed by mediators- monokines: interleukin –I, which activates the DNA synthesis in lymphocytes; factors, stimulating the production of antibodies by B-lymphocytes; factors, stimulating the differentiation of T- and B-lymphocytes; factors, activating T-helper cells and cytolytic factors.
Development: the macrophages arise from the stem cell of blood (red bone marrow).

Macrophage system is a powerful defensive system, participating in both general and local defensive reactions. The macrophage system is regulated by local mechanisms and by nerve and endocrine system as well.

The macrophage system includes all cells which are capable of uptake and breakdown of foreign particles, old or dead cells, bacteria etc:

1) macrophages (histiocytes) of loose connective tissue;
2) stellate cells of sinusoidal capillaries of liver (Kupffer's cells);
3) fixed and wandering macrophages of hemopoietic organs;
4) lung macrophages (dust cells);
5) macrophages of inflammatory exudates;
6) osteoclasts;
7) foreign-body giant cells;
8) glial macrophages of the nerve tissue.

All named cells are capable of phagocytosis, possess receptors to immunoglobulins on the surface of their plasma membrane, and arise from monocyte-precursors of the red bone marrow or monocytes of the blood.

Besides of their capacity of phagocytosis, fibroblasts, reticulocytes, endothelial cells, and neutrophils are not included in the macrophage system.

Plasma cells

Size: 7-10μm.
Shape: rounded or oval.
The nucleus is not big, located eccentrically. It contains condensed chromatin, which gives the nucleus the characteristic pattern of **"spoke wheel"**.

The cytoplasm is basophilic, contains well developed rough ER and numerous ribosomes. The nucleus is surrounded by a light area of cytoplasm – perinuclear zone. In the perinuclear zone the centrioles surrounded by the cisterns Golgi complex are found.
Functions: synthesis of immunoglobulins (antibodies), thereby providing specific humoral immunity.
The plasma cells arise from B-lymphocytes of blood.

Tissue basophils (mast cells)

Size: 4-14μm in width, up to 22μm in length.

Shape: variable (rounded, oval, irregular, polygonal); may possess short wide processes that enable them to amoeboid movements.
The nuclei are relatively small, contain densely packed chromatin. The cytoplasm exhibits numerous mitochondria, small amounts of smooth and rough ER, well developed Golgi complex. There are also found membrane-bounded granules of 0,2-0,8μm. These granules contain heparin (30%) and histamine (10%). The matrix of the granules is composed of proteins (chimase) and heparin, which from a stable network that bounds histamine via ionic bonds. The granules also contain chondroitin sulfate, hyaluronic acid, and, in some animals, serotonin.

Functions: regulate local homeostasis of connective tissue, participate in regulation of blood clotting, increase permeability of bood-tissue barriers, and participate in inflammatory and allergic reactions.

Adipocytes (fat cells). The diameter of fat cell can account for 120μm. Shape: spherical. Almost all inner volume of adipocyte is filled with a droplet of neutral flat (triglycerides). The cytoplasm and organelles form a thin layer that surrounds the fat droplet. Organelles are situated near the elongated nucleus and include both types of endoplasmic reticulum, Golgi complex, mitochondria.
Functions: depositing of fat, thermoregulation, maintenance of water balance.

Pigment cells possess short processes of varying shape. The cytoplasm is filled with pigment – melanin. Pigment cells arise from crests of neural tube.

Adventitial cells are low-specialized cells which accompany blood vessels. Shape: flattened or spindle-shaped. Nucleus – oval. Cytoplasm – slightly basophilic, the organelles are underdeveloped. Adventitial cells can give rise to fibroblasts, fibroclasts, myofibroblasts, and adipose cells.

FIBRILLAR STRUCTURES

Collagen fibers in loose connective tissue are laid in different directions and appear as wavy, spirally twisted rounded or flattened strands of 1-10μm thick. They form bundles of 150 μm thick.

Levels of organization of collagen fiber

The collagen molecules are built of three polypeptide chains (α-chains) of pre-collagen which twist in spiral within the cell. This is the first, molecular, level of organization.

The second, supramolecular, level of organization is represented by aggregated and cross-linked via hydrogen bonds molecules of collagen – protofibrils. A bundle of linked protofibrils compose microfibrils.

The third, fibrillar, level of organization – glycosaminoglycans and glycoproteins surround bundles of microfibrils, thereby forming fibrils. The fibrils exhibit cross-striations due to the arrangement of amino acids in secondary cross links.

Several fibrils aggregated together compose collagen fiber – the fourth level of organization.

12 types of collagen exist:

I type of collagen – connective tissue of skin, bones, cornea, sclera, arteries;
II type – hyaline and fibrocartilage, corpus vitreous;
III type – skin derma of fetus, wall of large blood vessels, reticular fibers;
IV type – basal lamina, capsule of lens;
V type – near the cells which produce it;
VI- VII type – is named microfibrillar;
VIII- XII type – endothelium, cartilage, corpus virtues.

Elastic fibers

Elastic fibers endow connective tissue with flexibility and elasticity. Shape of the fibers is rounded or flattened.

Structure

The main structural component of elastic fiber is globular protein – elastin, which is produced by fibroblasts. The molecules of elastin appear as globules with a diameter of 2,8 nm – the first, molecular, level of organization. In the extracellular matrix they are connected in chain of 3-3,5 nm thick, which is called elastin protofibril – the second level of organization. Protofibrils surrounded by glycoproteins from 8-10nm

wide microfibrils – the third level of organization. Elastic fiber (the fourth level of organization) represents cylinder, which center is filled with amorphous component and surrounded by microfilaments.

Mature elastic fibers contain 90% of elastin and 10% of microfilaments.

Reticular fibers

Reticular fibers are the type of collagen fibers, as they are built type III collagen. They contain an increased number of carbohydrates, which are produced by reticular cells and form a three-dimensional network – reticulum.

Ground substance of extracellular matrix

Ground substance of extracellular matrix accounts for 20% of total body mass and represents jelly-like hydrophilic mass with changing density and chemical composition. The ground substance is mainly produced by fibroblasts.

Chemical composition: water, proteins, polysaccharides, mineral substances. Polysaccharides are represented by glycosaminoglycans which are of two types:

a) sulfated – heparin sulfate, chondroitin sulfate, dermatan sulfate etc.
b) non-sulfated – hyaluronic acid.

The amount of ground substance varies in different types of tissues.

Functions : regulation of water metabolism, ion balance, cell adhesion etc.

DENSE CONNECTIVE TISSUE

Dense irregular connective tissue is characterized by abundant fibers and few cells. Dense irregular connective tissue contains mostly collagen fibers. Cells are sparse and are typically of a single type, the fibroblast. This tissue also contains relatively little ground substance. Because of its high proportion of collagen fibers, dense irregular connective tissue provides significant strength.Typically, the fibers are arranged in bundles oriented in various directions (thus, the term irregular) that can withstand stresses on organs or structures.

Skin contains a thick layer of dense irregular connective tissue called the reticular ayer (or deep layer) of the dermis. The reticular layer provides resistance to stretching forces from different directions.

Dense regular connective tissue is characterized by ordered and densely packed arrays of fibers and cells. Dense regular connective tissue is the main functional component of tendons, ligaments, and aponeuroses. As in dense irregular connective tissue, the fibers of dense regular connective tissue are the prominent feature, and there is little ground substance. However, in dense regular connective tissue, the fibers are arranged in parallel array and are densely packed to provide maximum strength. The cells that produce and maintain the fibers are packed and aligned between fiber bundles.

• **Tendons** are cordlike structures that attach muscle to bone. They consist of parallel bundles of collagen fibers. Situated between these bundles are rows of fibroblasts called tendinocytes.In H&E–stained cross sections of tendon, the tendinocytes appear stellate. The substance of the tendon is surrounded by a thin connective tissue capsule, the epitendineum, in which the collagen fibers are not nearly as orderly. Typically, the tendon is subdivided into fascicles by endotendineum, a connective tissue extension of the epitendineum. It contains the small blood vessels and nerves of the tendon.

• **Ligaments,** like tendons, consist of fibers and fibroblasts arranged in parallel. The fibers of ligaments, however, are less regularly arranged than those of tendons. Ligaments join bone to bone, which in some locations, such as in the spinal column, requires some elasticity.

• **Aponeuroses** resemble broad, flattened tendons. Instead of fibers lying in parallel arrays, the fibers of aponeuroses are arranged in multiple layers. The bundles of collagen fibers in one layer tend to be arranged at a right angle to those in the neighboring layers. The fibers within each of the layers are arranged in regular arrays; thus, aponeurosis is a dense regular connective tissue.

CONNECTIVE TISSUES WITH SPECIAL PROPERTIES

These types of connective tissues are characterized by predomination of particular cell types, which determine the name of the tissue.

Reticular tissue is a type of connective tissue which forms stroma of hemopoietic organs and microenvironment for developing blood cells.

Structure: it is composed of reticular cells and reticular fibers. Reticular cells and reticular fibers, anastomosing with each other via cell processes, form an interconnected network. Fibers and cells form a loose reticulum, hence the name of the tissue.

Reticular fibers by their chemical composition resemble collagen fibers, but they are less thick and anastomosing. Reticular fibers are revealed after a silver impregnation, that's why they are also called argirophilic fibers.

There are distinguished proper reticular fibers and pre-collagen fibers.

Proper reticular fibers – definitive, are composed of III type collagen.

Pre-collagen fibers – are precursors, immature type of collagen fibers.

Reticular fibers contain more sulfur, lipids and carbohydrates than the pro-collagen ones.

Reticular cells are subdivided into:

a) reticular fibroblast-like cells;

b) phagocytic cells (arise from monocytes);

c) low-differentiated (immature) cells.

Adipose tissue is an aggregation of fat cells (adipocytes). Two types of adipose tissue are distinguished:

c) white adipose tissue;

d) brown adipose tissue

White adipose tissue is situated under the skin, especially in area of abdominal wall, buttocks, and hips, where it constitutes hypoderm; and also in omentum, mesentery and retroperitoneal area.

Structure: white adipose tissue is composed of fat cells (adipocytes) which contain one single fat drop. Fat cells are closely attached to each other and form lobules of different shapes and sizes. Tiny spaces between adipocytes are occupied by fibroblasts, lymphoid elements, tissue basophils, and also thin collagen fibers lying in different directions.

The lobules are separated from each other by layers of loose connective tissue.

Brown adipose tissue is found in newborns and some animals in the area of neck, shoulder blades and other.

Fat cells of brown adipose tissue contain numerous small fat inclusions within the cytoplasm. They have a brown color due to the presence of iron-containing pigments – cytochroms of mitochondria. Here are found much more mitochondria than in fat cells of white adipose tissue. That's why the oxidative capacity of fat cells of brown

adipose tissue is approximately 20 times higher than that of fat cells of white adipose tissue.

Mucous tissue is found only in umbilical cord of fetus (Wharton's jelly). It consists of mucocytes (fibroblast-like cells), loose network of collagen fibers (appear only in third trimester of pregnancy), and jelly-like ground substance rich in hyaluronic acid.

Function: protection of umbilical vessels from clamping.

Pigment tissue contains a lot of pigment cells- melanocytes. It is found in iris of the eye, skin of nipples of mammary gland, scrotum, anus, birthmarks.

Theme 5. Skeletal connective tissues. Cartilage. Bone.

Skeletal connective tissues include cartilage and bone tissue, which take part in regulation of water-salt balance, carry out supporting, protective and mechanical functions.

CARTILAGE

Cartilage is avascular connective tissue that is found in several organs of the respiratory system and intervertebral discs. Cartilage consists of cells (chondrocytes) and extensive extracellular matrix.

Cartilage is composed of 75% of water, 10-15% of organic component, and 4-7% of inorganic salts. Collagen constitutes 50-70% of dry mass of cartilage.

Classification of cartilage

Three types of cartilage are distinguished on the basis of structural and functional characteristics of their extracellular matrix:

1. Hyaline cartilage;
2. Elastic cartilage;
3. Fibrocartilage.

♦ Functions: the cartilage is well adapted o bear weight, especially in joints; it is a key tissue in the development of fetal skeleton.

Chondrogenesis

Chondrogenesis is a process of cartilage development. It takes place in the embryonic period and in the postembryonic period in case of regeneration.

Embryonic chondrogenesis

The cartilage arises from mesenchyme – embryonic connective tissue. The chondrogenesis includes three stages:

♦ *First stage* – **formation of chondrogenic islet.** The process cartilage development begins with the aggregation of chondroprogenitor mesenchymal cells to form a mass of rounded closely apposed cells. Such aggregations are called chondrogenic islets. The chondroprogenitor cells lose their cytoplasmic processes, their nucleus becomes rounded. These changes result in the cell becoming a chondroblasts, which will give rise to a cartilage by producing its matrix. Numerous free ribosomes and rough ER appear in the cytoplasm of chondroblasts.

♦ *Second stage* – **formation of primary cartilage.** The chondroblasts start to produce extracellular matrix containing fibrillar proteins (collagen of the III type), which results in their differentiation into the primary chondrocytes. The newly formed extracellular matrix exhibits distinctive oxyphyllia.

♦ *Third stage* – **differentiation of cartilage.** In this stage the primary chondrocytes differentiate into secondary chondrocytes and obtain capacity of synthesis of sulfated glycosaminoglycans (chondroitin sulfate), which form complexes with the collagen proteins (proteoglycans).

The mesenchyme surrounding the developing cartilage gives rise to perichondrium that consists of the outer fibrous and the inner cellular (cambial) layers.

• Cartilage is capable of two types of growth :appositional and interstitial.

♦ *Appositional growth*, the process that forms new cartilage at the surface of an existing cartilage. The cells of the inner (cambial) layer of perichondrium differentiate into chondroblasts and chondrocytes, which produce extracellular matrix. The chondroblasts progressively move apart while they produce matrix. When they are completely surrounded by a matrix material they produce, they become chondrocytes.

♦ *Interstitial growth*, the process which forms new cartilage within an existing cartilage mass. The chondrocytes lying within their lacunae retain their ability to divide, that's why the daughter cell occupies the same lacuna as the maternal cell does. Such types of grows takes place in the embryonic development of cartilage and in case of its regeneration.

Physiological regeneration of cartilage is provided by the activity of chondrocytes. The chondrocytes produce chondromucoid, collagen and elastin.

With continued secretion of the cartilage matrix moves the cells

away from their source of nutrition – perichondrium. Due to the lack of nutrition the chondrocytes, finally, lose their ability to divide; some of them even dye.

Cells of cartilage

Chondroblasts are young cells that are capable of proliferation and synthesis of the cartilage extracellular matrix.

Shape: polygonal, flattened.

Origin: arise from chondroprogenitor cells that, in their turn, arise from the mesenchymal stem cells. Stem cells, chondroprogenitor cells, chondroblasts and chondrocytes form one common differon.

Cytoplasm possesses strongly developed ER (both rough and smooth), Golgi complex, and a big amount of RNA. The cytoplasm of chondroblast exhibits basophilia.

In the process of development the chondroblasts become chondrocytes. The chondroblasts are responsible for the appositional growth of cartilage.

Chondrocytes are the main cells of cartilage.

Shape: oval, rounded or polygonal.

Localization: within the cavities of cartilage matrix (lacunae). Isogenous group – the group of chondrocytes situated in one common lacuna. The chondrocytes of one isogenous group arise as a result of division of one maternal cell. There distinguished three types of chondrocytes in one isogenous group:

Ist type of chondrocytes predominates in young developing cartilage. These chondrocytes are capable of **mitotic division**, which allows considering them as the source of reproduction of the isogenous groups. These cells are characterized by a high nuclear-cytoplasmic index. Their cytoplasm contains numerous vacuoles, well-developed Golgi complex, mitochondria and free ribosomes.

IInd type of chondrocytes is characterized by a decreased nuclear-cytoplasmic index, less active DNA synthesis; but increased RNA synthesis, strongly developed Golgi complex that is responsible for **synthesis and secretion** of glycosaminoglycans and proteoglycans into the extracellular matrix.

IIIrd type of chondrocytes is characterized by a low nuclear-cytoplasmic index, strong development and regular arrangement of rough ER. The cells of this type retain ability to produce and secrete proteins, but their capacity of synthesis of glycosaminoglycans decreases dramatically.

Extracellular matrix of cartilage is composed of organic material: proteins (predominantly collagen of II type), lipids, glycosaminoglycans and proteoglycans. Cartilage has the highest concentration of proteoglycans.

The fibers orientation within the cartilage matrix is determined by the direction of the lines of force.

The extracellular matrix that surrounds the lacuna is more intensively basophilic and is referred to as **territorial matrix**.

Hyaline cartilage

Localization: the wall of trachea, bronchi, sites of junctions of ribs to sternum, articular surfaces, and epiphyseal plates.

Structure: Externally the hyaline cartilage is covered by perichondrium.

The perichondrium consists of two layers: 1) outer fibrous layer; 2) inner cellular layer.

- The outer fibrous layer is formed by dense irregular connective tissue with a network of blood vessels;
- The inner cellular layer is predominantly composed of cells – *chondroprogenitor cells and chondroblasts*.

Immediately under the perichondrium the young spindle-shaped chondrocytes are situated. The long axis of these young chondrocytes lies along the long axis of the cartilage itself.

The chondrocytes found in deeper layers of cartilage become oval or rounded in shape and form the isogenous groups. The young chondrocytes and the isogenous groups are surrounded by the extracellular matrix containing high concentrations of chondromucoid and collagen fibers (II type of collagen).

- However not all kinds of hyaline cartilage have the same structure. The hyaline cartilage of articular surfaces (articular cartilage) is not covered by perichondrium. The articular cartilage is composed of three poorly distinguished zones: 1) outer zone; 2) medium zone; 3) deep zone.

The outer zone contains young immature cells.

The medium zone possesses large oval or rounded cells which form columns. The columns are directed perpendicularly to the articular surface.

The deep zone is composed of calcified cartilage; only here the blood vessels are found.

Elastic cartilage

Localization: external ear, external acoustic meatus, auditory tube, epiglottis.

- Structure: In general, the structure of the elastic cartilage is similar to that of the hyaline cartilage. It is covered by perichondrium. The cells lay in lacunae and form isogenous groups.

Unlike hyaline cartilage, the extracellular matrix of elastic cartilage possesses **elastic fibers** along with collagen fibers.
Anastomosing with each other, the elastic fibers form a dense branching network and continue with the fibers of the perichondrium.

Besides this, elastic cartilage contains less lipids, glycogen and chondroitin sulfate than hyaline cartilage. Unlike hyaline cartilage, which calcifies with ageing, the matrix of elastic cartilage does not calcify during the ageing process.

Fibrocartilage

Localization: intervertebral discs, slightly movable joints, certain places where tendons attach to bones.

Structure: the extracellular matrix consists of parallel bundles of collagen fibers, which continue with the fibers of hyaline cartilage. The chondrocytes of elastic cartilage form rows. There is no surrounding perichondrium in fibrocartilage. Extracellular matrix of fibrocartilage is characterized by presence of both type I and type II collagen fibers.

BONE TISSUE

Bone tissue is a specialized form of connective tissue that is characterized by a highly mineralized extracellular matrix.

Bone tissue is composed of cells (osteoblasts, osteocytes and osteoclats) and extracellular matrix (osseomucoid). The extracellular matrix consists of 70% of inorganic substances, principally calcium phosphate. Organic component is represented by proteins and lipids, which constitute bone matrix. Such combination of organic and inorganic components produces an extremely hard tissue capable of providing mechanical support.

Functions

1. support – due to its hardness and strength bone tissue provides mechanical support and movements of body;
2. protection – bone tissue protects entire organs from injuries;
3. storage site for calcium and phosphate.

Classification

Two types of bone tissue are distinguished depending on their structure and physical properties:

1. Woven bone
2. Lamellar bone

Woven bone (immatute) is characterized by irregular interlacing arrangement of ossein fibers (collagen type I), which are surrounded by calcified osseomucoid. Osteocytes lie in lacunae within osteomucoid between bundles of collagen fibers. Such type of bone is characteristic for skeleton of a developing fetus; in adults areas of woven bone are common in the alveolar sockets, sutures of skull and where tendons insert into bones.

Lamellar bone (mature) is characterized by a strictly parallel arrangement of bundles of collagen fibers and formation of bone lamellae.

According to the orientation of bone lamellae it is distinguished two types of lamellar bone: 1) compact bone; 2) spongy bone.

Compact bone (dense) is characterized by the absence of cavities. The diaphyses of long bones are built from this type of bone.

Spongy bone (cancellous) is characterized by the bone lamellae forming a meshwork of thin anastomosing spicules – trabeculae. The spaces within the bone meshwork are occupied by bone marrow. Spongy bone forms flat bones and epiphyses of long bones.

Development of bone

Mesenchyme is a source of development of bone tissue. During the embryonic development of bone the formation of two cell differons takes place.

The first cell differon – osteoprogenitor cell, osteoblast, osteocyte.

The second cell differon – hematogenic cells: stem cell of blood, common myeloid progenitor, granulocyte/monocyte progenitor,

osteoclast precursor, osteoclast.

It is distinguished an embryonic and post-embryonic formation of bone.

The embryonic formation of bone (osteohistogenesis) is classified as direct (intramembranous ossification) and indirect (endochondral ossification).

1. Direct osteohistogenesis – development of bone directly from mesenchyme.
2. Indirect osteohistogenesis – a cartilage model serves as a precursor of future bone.

The post-embryonic bone formation takes place in case of regeneration of bone or ectopic osteohistogenesis.

Embryonic osteohistogenesis

Direct (intramembranous ossification) is characteristic for development of woven bone during formation of flat bones of skull and occurs during first month of embryonic development. It is characterized by formation of primary membrane osteoid bone, which is then impregnated with salts of Calcium and Phosphorus.

Intramembranous osiffication includes four stages:

1) Formation of osteogenic island;
2) Osteoid stage;
3) Calcification of extracellular matrix, formation of woven bone;
4) Formation of secondary spongy bone.

1)Formation of osteogenic island. The focal proliferation and aggregation of mesenchymal cells starts at the sites where future bone is destined to form. It results in formation of osteogenic island, which then penetrated by blood vessels.

2) Osteoid stage. The cells of osteogenic islands differentiate into osteoblasts, which start to produce the organic bone matrix (osteoid) - oxyphilic extracellular matrix with collagen fibers. As the matrix is produced and the amount of collagen fibers increases, the osteoblasts within bone matrix become separated from one another, but they still remain connected to each other by their thin cytoplasmic processes. The components of bone matrix also include mucoproteins that harden the collagen fibers in a dense mass. When the osteoblasts become

embedded into the bone matrix they differentiate into osteocytes. Then more of the surrounding mesenchymal cells proliferate, differentiate into osteoblasts and add more matrix. This process is called **appositional growth of bone**.

3) Calcification of the extracellular bone matrix. The osteoblasts produce enzyme called phosphatase that brakes down the glycerophosphate taken from blood into sugar and phosphoric acid. Phosphoric acid reacts with calcium salts contained in ground substance and collagen fibers of the bone matrix, thereby forming the hydroxyapatite crystals. The important role in bone matrix calcification is carried out by the matrix vesicles with diameter of 1μm which contain active alkaline phosphatase and pyruvate phosphatase. The significant role in the process of calcification also belongs to the osteonectin – the glycoprotein that binds calcium and phosphorus salts to collagen.

The result of calcification is formation of bone spicules or trabeculae which anastomose with one another and form a wide network. The space between the spicules is occupied by loose connective tissue with blood vessels.

By the finishing of osteogenesis numerous osteoprogenitor cells and fibers appear around the future bone. A portion of connective tissue immediately surrounding the bone spicules is transformed into the periosteum that provides nutrition and regeneration of bone. Such bone, formed at early stages of embryonic development, is called woven or membrane or primary spongy bone.

5) Formation of secondary spongy bone (lamellar). This stage is accompanied by destruction of particular portions of primary bone and its penetration by blood vessels. This process, both in embryonic and postembryonic periods, involves osteoclasts.

The mesenchyme surrounding blood vessels differentiate into new osteoblasts which produce bone lamellae. The collagen fibers in each lamella are laid down parallel to one another, but in different direction to adjacent lamella. Due to that the blood vessel becomes surrounded by concentric bone cylinders insert in one another (primary osteon), the formation of woven bone stops and it is substituted by the lamellar bone.

At the side of periosteum and endosteum there are formed the outer and inner circumferential bone lamellae which surround the whole mass of the bone.

By the described mechanism the formation of flat bones occurs.

Formed in the embryonic period the bone is remodeled (the old osteons are destroyed and the new ones are formed) during the whole future life.

Indirect osteogenesis

Indirect osteogenesis includes 4 stages:

1. Formation of hyaline cartilage model.
2. Perichondral ossification.
3. Endochondral ossification.
4. Epiphyseal ossification.

Formation of hyaline cartilage model occurs at the 2nd month of the embryonic development. It also begins with proliferation and aggregation of mesenchymal cells at the site of the future bone. The mesenchymal cells differentiate into chondroblasts and the hyaline cartilage model with the general shape of the future bone is formed. The cartilage model is covered by perichondrium. Once established, the cartilage model grows by interstitial and appositional growth. **Perichondral ossification.** The process of bone formation is initiated at the diaphyseal region of cartilage model. The perichondreal cells in the midregion of diaphysis of cartilage model differentiate into osteoblasts and form there the intramembranous (woven) bone. This cuff of bone around the cartilage model is called bony collar**.** The bony collar alters the nutrition of cartilage resulting in dystrophic changes in the midregion of cartilage model. The chondrocytes enlarge; their surrounding cartilage matrix is resorbed. With the death of chondrocytes much of the matrix breaks down and neighboring lacunae become confluent, producing a large cavity.

The unchanged distal portions of diaphysis continue growing. The chondrocytes located at the boundary of epiphysis and diaphysis are arranged in columns oriented in the same direction with the long axis of future bone.

It must be mentioned that the chondrocytes in column undergo two opposite processes simultaneously:

1) proliferation and growth in distal regions of diaphysis;
2) dystrophy (hypertrophy) and death in the midregion of diaphysis.

The hypertrophic chondrocytes begin to synthesize alkaline phosphatase that is responsible for calcification of cartilage matrix, resulting in its basophilic staining and fragility. With the expanding of blood vessels

and appearance of osteoblasts perichondrium is transformed into periosteum. Blood vessels with surrounding mesenchyme, osteoprogenitor cells and osteoclasts grow through the thin diaphyseal bony collar and vascularize the calcified cartilage. Osteoclasts discharge the hydrolytic enzymes that destroy the calcified cartilage matrix. The cavities appeared after the destruction of cartilage are now occupied by osteoblasts, which form here the bone tissue.

Endochondral ossification is the process of bone formation within the cartilage model (diaphyseal ossification center).

Due to the activity of osteoclasts the endochondral bone is destroyed and the large cavities appear. These cavities are the future site of red bone marrow location. The hemopoietic stem cells gain access here via the blood vessels and give rise to the red bone narrow including all the blood cell lineages.

At the same time the periosteum gives rise two new and new bone lamellae, which expand in length and with forming a dense bone. The concentric bone lamellae are formed around blood vessels forming the primary osteons.

Epiphyseal ossification is the process of formation of ossification centers in epiphysis. It is preceded by differentiation and hypertrophy of chondrocytes and calcification of cartilage matrix.

Between the epiphyseal and the diaphyseal ossification it is formed the epiphyseal plate, which consists of 3 zones:

1) zone of reverse cartilage;
2) zone of columnar cartilage (proliferation);
3) zone of hypertrophy.

When the epiphyseal and diaphyseal ossification centers become confluent the bone growth stops. In human it happens at the age of 20-25 years.

Cells of bone tissue

The bone tissue contains three types of cells:

a)osteocytes;
b) osteoblasts;
c) osteoclasts.

Osteocytes are predominant definitive cells of bone tissue, which have lost their capacity of division.

Shape: elongated, possess processes;

Size: 15×45 μm;
Nucleus: relatively compact, round;
Cytoplasm: less basophilic, poorly developed organelles.
Localization: occupies bone lacuna, which conforms to the shape of the cell.

Osteoblasts are differentiated bone-forming cells that secrete bone matrix.

Shape: cuboidal, pyramidal, or polygonal;
Size: 15-20 μm
Nucleus: round or oval in shape, eccentrically located, may contain one or more nucleoli.
Cytoplasm: markedly basophilic, exhibits well-developed rough Endoplasmic reticulum, mitochondria, Golgi complex, big amount of RNA.

Osteoclasts are derived from the hemopoietic progenitor cells; they are responsible for bone and cartilage resorption.

Shape: irregular, rounded;
Size: up to 90 μm;
Nucleus: from 3 to several decades;
Cytoplasm: markedly acidophilic, contains numerous lysosomes and mitochondria.

At the site of adhesion to the resorbed bone the osteoclast exhibits two distinct zones:

1) ruffled border;
2) clear zone (sealing zone).

Ruffled border is the part of the cell in direct contact with bone. It contains numerous deep plasma membrane infoldings forming microvillous-like structures that increase the surface area for the exocytosis of hydrolytic enzymes.

Clear zone is a ring-like perimeter of cytoplasm adjacent to the ruffled border that demarcates the area of bone which is being resorbed. It contains actin microfilaments, but lacks other organelles.

The peripheral cytoplasm of the osteoclast contains numerous small vesicles and vacuoles, mitochondria, and lysosomes; rough ER is poorly developed. It is suggested that osteoclasts discharge CO_2, whilst the enzyme carboanhydrase uses it produce the acid H_2CO_3 that dissolves Calcium salts and destroys the organic matrix of bone. As a result of osteoclast activity the lacuna is formed at the site of its

attachment to a bone.

Extracellular matrix of bone tissue

The extracellular matrix of bone tissue consists of ground substance impregnated with inorganic salts, and bundles of collagen fibers.

The ground substance contains small amounts of chondroitin sulfuric acid and large amounts of citric acid that binds calcium to the organic bone matrix. The ground substance of bone tissue also contains regularly arranged hydroxyapatite crystals and amorphous Calcium phosphate. Bone tissue contains more than 30 microelements (Cu, Sr, Zn, Mg, Ba etc.)

The collagen fibers form bundles. They are built of the type I collagen. In the primary (woven) bone the collagen fibers are randomly scattered, whilst in the mature (lamellar) bone they are regularly arranged.

The structure of long (tubular) bones

The long bones are predominantly composed of the lamellar bone tissue (except tubercles).

Anatomically the long bone consists of central part – diaphysis and peripheral endings – epiphyses.

The diaphysis of the long bone is composed of three layers:

1) periosteum;
2) osteonal layer;
3) endosteum.

- Periosteum consists of the outer fibrous layer formed of collagen fibers, and the inner cellular layer containing osteoprogenitor cells and osteoblasts. The periosteum provides nutrition, appositional growth and regeneration of the bone.
- Osteonal layer (the bone proper) is separated from the periosteum by outer circumferential laminae and from endosteum by inner circumferential laminae.
- *The outer circumferential lamellae* do not form close rings around the diaphysis; they are overlapped by succeeding lamellae. **Perforating canals (Volkmann's canals)** are channels in lamellar bone which perforate periosteum and outer circumferential lamellae to reach the osteonal canal. Besides this, perforating collagen fibers (Sharpey's fibers) also enter bone tissue through the periosteum.

- *The inner circumferential lamellae* are distinguished only at the sites where the compact bone immediately bounds with the marrow cavity. If the compact bone continues with the spongy bone, the inner circumferential lamellae continue with the spongy bone lamellae.

Osteonal layer. The osteonal layer is composed of cylindrical units called **osteons or Haversian systems.** The osteon, as a ***structural unit of compact bone***, is a bony tube with a diameter of 20-300 μm.

Each osteon consists of 5-20 concentric lamellae of bone matrix which surround the central (Haversian) canal that contains vascular and nerve supply. The lacunae with osteocytes are located within the bone lamellae. The processes of osteocytes lying in canaliculi anastomose with each other via gap junctions. The system of canaliculi opens to the osteonal canal, thereby serving for passage of substances between the osteocytes and blood vessels.

The collagen fibers in the concentric lamellae in an osteon are laid down parallel to one another in any given lamella but in different directions in adjacent lamellae. This arrangement imparts great strength to the osteon.

Between the osteons are remnants of previous concentric lamellae called **interstitial lamellae**.

* **Endosteum** is a lining tissue of bone facing the marrow cavity. It is composed of fibrous connective tissue containing osteoblasts and osteoclasts.

* **Bone epiphysis** is composed of spongy bone. Externally it is covered by periosteum. Under the periosteum are the layer of circumferential lamellae and osteonal layer. In the depth of epiphysis the bone lamellae form a network of trabeculae. The spaces between these trabeculae are filled with reticular tissue and hemopoietic cells.

Growth of long bones

The growth of long bones in length is provided by the presence of the epiphyseal cartilaginous plate, within which the two opposite processes occur simultaneously:

1) destruction of the epiphyseal cartilage;
2) continuous proliferation of the cartilage cells and formation of the new cartilage.

Three zones are distinguished in the epiphyseal cartilage:

a) zone of reverse cartilage;
b) zone of proliferation (zone of columnar cells);
c) zone of hypertrophy.

* Zone of reverse cartilage consists of the isogenous groups of chondrocytes, which exhibit no active proliferation or cartlage matrix production. This zone connects the cartilaginous plate with the bone. Between the bone and the cartilage are blood capillaries.

* Zone of proliferation consists of cartilage cells which undergo division and organize into columns.

* Zone of hypertrophy contains greatly enlarge (hypertrophic) cartilage cells. The hypertrophied cells begin to degenerate and the cartilage matrix becomes calcified. The calcified cartilage situated nearest the diaphysis is in direct contact with the connective tissue of the bone marrow cavity. In this region, small blood vessels and accompanying connective tissue invade the space previously occupied by the dying chondrocytes. The invading blood vessels are the source of osteoprogenitor cells, which will differentiate into bone-producing cells (osteoblasts).

When an individual achieves maximal growth, proliferation of new cartilage within the epiphyseal plate terminates. The cartilage that has already been produced in the epiphyseal plate continues to undergo changes that lead to the formation of new bone until there is, finally, no remaining cartilage. At this point the epiphyseal and diaphyseal marrow cavities become confluent. The elimination of the epiphyseal plate is called **epiphyseal closure**.

The growth of bone in width is provided by the proliferation of the osteogenic layer of periosteum.

Immature (woven) bone

Immature bone is the major bone type in developing fetus. In adults it is found in the alveolar sockets of the oral cavity and where tendons insert into bone.

The collagen fibers form dense interlacing fibers.

The matrix of immature bone has more ground substance than does of the matrix of mature bone. The osteocytes lying in lacunae tend to be randomly arranged.

Periosteum forms the external covering of immature (woven) bone.

Mature (lamellar) bone

Lamellar bone consists of the bone lamellae, which are composes of osteocytes and mineralized ground substance with collagen fibers.

Theme 6. Muscle tissue.

Muscle tissue (textus muscularis) is a specialized tissue, whose structural elements are capable of contraction, which is accompanied by changes in the membrane potential. Contraction, as a process of change of shape of the contractile elements, occurs due to the interaction between actin and myosin filaments with the participation of Calcium ions and other structural proteins.

Sources of development

Five different sources of development can give rise to the muscle tissue. Depending on its origin muscle tissue is classified into five different histogenetic types:

1) somatic type **–** originates from myotomes of mesoderm (skeletal muscle)

2) coelomic type **–** originates from ventral mesoderm (cardiac muscle)

3) visceral type **–** originates from mesenchyme (smooth muscle of inner organs)

4) neural type **–** originates from the neural tube (smooth muscle of the iris)

5) epidermal type **–** originates from skin ectoderm (myoepithelial cells of sweat, mammary, salivary and lachrymal gland)

Morphofunctional classification of the muscles

1. Smooth muscle, in which the cells do not exhibit cross-striations.

2. Striated muscle, in which the fibers exhibit cross-striations due to the arrangement of the actin and myosin myofilaments.

THE SMOOTH MUSCLE

The smooth muscle of mesenchymal origin

Histogenesis. The stem cells and the cells-precursors of the

smooth muscle cells are suggested to be related to the cells-precursors of the connective tissue fibroblasts. Like the fibroblast, the smooth muscle cells of mesenchymal origin produce glycosaminoglycans and collagen.

Localization – the wall of the hollow organs (alimentary tract, respiratory tract, urinary tract, reproductive tract, blood and lymphatic vessels), the capsule of spleen and lymph nodes.

Structural and functional unit – **smooth muscle cell**.

The smooth muscle is fusiform cell, which could be from 20 to 500 μm in length and from 5-8 μm in diameter. In the uterus, endocardium, aorta, urine bladder are found the smooth muscle cells which possess processes. During the contraction the smooth muscle cell becomes round or even spiral.

The plasmalemma of the smooth muscle cells forms numerous invaginations – pinocytic vesicles and **caveolae**, through which the Calcium ions enter the cytoplasm. The cytoplasm is acidophilic. The general-function organelles are concentrated at each end of the nucleus. There could be found inclusions of lipids, carbohydrates and pigments. The well developed **general-function organelles** of the smooth muscle cells are smooth Endoplasmic reticulum and mitochondria.

The specialized organelles are represented by the actin and myosin myofilaments.

The actin (thin) myofilaments form the three-dimensional network within the cytoplasm of the smooth muscle cell. The sites of attachment of the actin myofilaments to the cytoplasm are called **dense bodies**. These structures are distributed throughout the cytoplasm and are composed of the protein α- actinin. The actin myofilaments are more numerous than the myosin. Besides the contractile protein actin, the thin filaments are composed of the structural proteins – tropomyosin, caldesmon, calponin.

The myosin (thick) filaments are longitudinally arranged within the cytoplasm of the smooth muscle cell. During the contraction the actin filaments are pulled over the entire length of the myosin filaments. The process of the smooth muscle contraction is initiated by the phosphorylation of the myosin, which depends on concentration of the Calcium ions. The regulation of Calcium concentration is provided by the special protein – **calmodulin**. The complex of Calcium and calmodulin activates the enzyme that phosphorylates the myosin. Only the phosphorylated myosin is capable to interaction with the actin.

Due to the molecular interactions with the myosin, the actin

filaments slide towards each other and, being attached to the plasmalemma, change the shape of the smooth muscle cell.

Each smooth muscle cell is surrounded by a basal lamina, which contains holes. Due to these holes the gap junctions – **nexus** are formed between two adjacent smooth muscle cells. The smooth muscle cells are surrounded by thin collagen, elastic and reticular fibers which form the reticular network – **endomysium.** The groups of 10-12 smooth muscle cells are surrounded by a connective layer containing blood vessels and nerve fibers.

The smooth muscle of epidermal origin

The myoepithelial cells of this tissue originate from the skin ectoderm.

Localization – sweat, salivary, mammary, lachrymal glands. Having the same precursors as the secretory epithelial cells of the named glands, the myoepithelial cells immediately adjoin the secretory cells and have a common basal lamina.

Shape – stellate or basket-like. The myoepithelial possess cytoplasmic processes, which surround the secretory portions of the glands and **stimulate the discharge of secretion**. The contractile apparatus of the myoepithelial cells is organized similarly to that of the smooth muscle cells of mesenchymal origin.

The nucleus and organelles are situated in the center of the cell cytoplasm.

The ***regeneration of the myoepithelial cells is possible*** due to the low-differentiated cells of the epidermal origin.

The smooth muscle of neural origin

The smooth muscle cells of neural origin originate from the neural tube.

Localization – constitute two muscles of the iris – m.sphincter pupillae and m.dilatator pupillae (iris dilator muscle, iris sphincter muscle).

The smooth muscle tissue as a part of organs

The smooth muscle cells are collected in bundles, which are separated by thin layers of the loose connective tissue (perimysium). Such bundles form the whole muscle, which is covered by thicker layer of the connective tissue (epimysium). The connective tissue layers contain blood vessels, nerve fibers, which supply and innervate the

smooth muscle cells.

Localization – the walls of the hollow organs (respiratory system, alimentary canal, reproductive system, blood vessels).

STRIATED MUSCLES

T he cardiac muscle tissue

Histogenesis. The source of development of this tissue is the *myoepicardial plate* (symmetric areas of the visceral layer of splanchnotome). The most cells of the myoepicardial plate give rise to the cardiac muscle cells, whilst the rest of the cells undergo differentiation into the mesothelial cells of the epicardium).

There are distinguished three types of the cardiac muscle cells:

1) contractile

2) conducting

3) secretory

The structure of the contractile cardiac muscle cells

Shape – elongated, cylindrical

Size – 100-150 μm.

The contractile cardiac muscle cells adjoin each other, thereby forming chains which compose so-called functional fibers of 10-20 μm in length. The specialized cell-to-cell attachment of the cardiac muscle cells is called ***intercalated disc***. Some cardiac muscle cells in a "fiber" may join two or more cells through intercalated disc, thereby forming a branched fiber.

The lateral surface of the cardiac muscle cells is covered with basal lamina.

*Nucleus (*may be 2) is oval in shape, occupies the central portion of cytoplasm. In most cases nuclei of the smooth muscle cells are polyploid.

Cytoplasm contains general-function organelles including Golgi complex, centrosome, poorly developed rough ER, lysosomes. A highly developed **smooth ER** forms anastomosing **tubules of the L-systems**. Besides these, the cytoplasm exhibits inclusions of glycogen, lipids and **myoglobin**. There are also found numerous mitochondria, which from chains surrounding the specialized organelles – **myofibrils**.

The structure of myofibrils

The myofibrils are composed of regularly arranged actin and myosin filaments, which represent the contractile proteins. For structuring of the actin and myosin filaments there are present the specialized structures – *telophragm (Z-line) and mesophragm (M-line).*

♦ Telophragm is a network of protein molecules, which are attached to the plasmalemma. On a longitudinal section of the cardiac muscle cell the telophragms appear as about 100 nm thick lines, which were named Z-lines. A portion of the myofibril located between two Z-lines is called **sarcomere**.

♦ In the middle of sarcomere it is located the mesophragm (M-line). The myosin (thick) filaments are restricted to the central portion of sarcomere (M-line). The actin (thin) filaments are attached to the Z-line. Each myosin filament is accompanied by six actin filaments.

A segment of the sarcomere, where are found only myosin filaments is called H-band (light zone). The segment of sarcomere, where both actin and myosin filaments are found is called A-band.

Two neighboring segments of two adjoining sarcomeres, separated by the Z-line and containing only actin filaments compose the I-band.

Such names of the bands were given because of the different light refraction. In polarizing microscopy the A-bands are birefringent (alter polarized light in two planes). Being doubly refractive, the *A*-bands are dark *(anisotropic)*. The *I*-bands are monorefringent (do not alter the plane of polarized light) and called *isotropic*.

The myofibrils are surrounded by mitochondria and smooth ER. The mitochondria are quite large and form a dense three-dimensional network around at the area of I-band.

At the level of telophragm the plasmalemma of the cardiac muscle cell forms deep invaginations named transverse tubules (T-tubules). The T-tubules in the cardiac muscle include not only the invagination of the plasmalemma, but also the invagination of the basal lamina. The T-tubules provide fast conduction of the action potential to each myofibril, thereby providing the synchronous contraction. Along the myofibrils there are found cisterns of the smooth ER, which anastomose with each other and approach the T-tubules.

As previously noted, the intercalated disc is the attachment site between cardiac muscle cells. The intercalated disc includes several types of cell-to-cell contacts: interdigitations, adhering junctions, desmosomes and gap junctions (nexus). The transverse component of intercalated disc is represented by adhering junctions and desmosomes.

The adhering junction serves as the site at which the actin filaments from the neighboring cardiac muscle cell anchor. The desmosome helps prevent the cells from pulling apart under the strength of contraction.

Gap junctions (nexus) constitute the lateral component of the intercalated disc. Gap junctions provide ionic continuity between adjacent cardiac muscle cells, thus allowing informational macromolecules to pass from cell to cell.

The structure of conducting cardiac muscle cells

Size – are significantly larger than the contractile cells (100 μm in length, 50 μm in width).

Cytoplasm contains the all general-function organelles. The mitochondria are evenly distributed through the cytoplasm.

Myofibrils are not numerous and concentrated at the periphery of the cytoplasm.

Plasmalemma does not form T-tubules.

The conducting cardiac muscle cells are connected via intercalated discs, but they are more primitively organized that those of the contractile cardiac muscle cells.

The conducting cardiac muscle cells are organized into nodes and include the following types:

1) Pacemaker cells (SA node) –generate nerve impulse;

2) Transitional cells (AV node);

3) Cells of the bundle of His and Purkinje fibers.

The conducting cardiac muscle cells initiate, regulate and coordinate the heartbeat by conducting impulses to the contractile cardiac muscle cells.

The regeneration of the cardiac muscle is believed to be impossible because of lack of the stem and progenitor cells.

The molecular mechanisms of muscle contraction

When the muscle is relaxed the Calcium ions are deposited in the tubules of its smooth ER. The action potential spreads through the plasmalemma and T-tubules and initiates releasing of the Calcium ions. The Calcium ions interact with the special regulatory proteins of the myofibrils – *troponin and tropomyosin*. The troponin and tropomyosin surround the actin filament and mask the myosin-binding site on the actin molecule. The interaction with Calcium ions makes the troponin and tropomyosin molecules open the myosin-binding sites on the actin molecule, which initiates the movement of actin filaments along the myosin filaments. As a result of such movement of the actin filaments

(as they are attached to the Z-lines), the sarcomere shortens. When a muscle contracts, each sarcomere shortens and becomes thicker, but the myofilaments remain the same length.

The process of muscle contraction requires energy of the ATP. The heads of myosin are capable of binding the ATP molecules and, oppositely, breaking down the ATP.

SKELETAL MUSCLE

The skeletal muscle tissue originates from cells of myotomes of the dorsal **mesoderm**. The skeletal muscle progenitors differentiate into types of cells.

1) Myoblasts which fuse together and form a multinucleated syncytium – myotubes. The myotubes undergo further differentiation into the myosymplasts.
2) Satellite cells, which do not fuse with other myoblasts.

The structure of muscle fiber

Muscle fiber is the structural and functional unit of the skeletal muscle tissue. The muscle fiber is composed of myosymplast, satellite cells and basal lamina.

When viewed in a cross section, the mature multinucleated muscle fiber reveals a polygonal shape with a diameter of 10-150 μm. The length of each muscle fiber is the same as the length of the whole muscle.

Sarcolemma ("sarcos"-meat) of muscle fiber consists of two layers. The inner layer is the plasma membrane of myosymplast. The outer layer is represented by a basal lamina with reticular and thin collagen fibers woven into it.

Myosymplast is covered by a plasma membrane, which forms T-tubules through that the action potential is conducted.

The myosymplast may possess up to several thousands nuclei. The nuclei are situated below the plasma membrane; they are elongated, contain nucleoli and small amounts of heterochromatin.

The cytoplasm of myosymplast was named sarcoplasm. The sarcoplasm contains three groups of well-organized structures:

1) general-function organelles;
2) specialized organelles – **myofibrils**;
3) inclusions – lipids, carbohydrates, pigment.

- The general-function organelles surround nucleus. The

mitochondria are large and numerous. The rough endoplasmic reticulum (rER) is poorly developed. The smooth ER is extremely well developed and here it was named ***sarcoplasmic reticulum***. The sarcoplasmic reticulum represents the system of tubules and flattened cisterns which surround the myofibrils. It forms a collar-like structure around the sarcomere. The terminal cisterna of the sarcoplasmic reticulum forms L-tubule which serves as reservoir for Ca^{2+}.

Between two neighboring L-tubules there is located one transverse tubule (T-tubule). The T-tubule in the skeletal muscle is an invagination of plasma membrane of myosymplast. Also located around the myofibrils are large number of mitochondria and glycogen granules, both of which are involved in providing the energy necessary for contraction.

♦ The myofibrils are located along the muscle fiber; they are 1-2 μm thick and extend the full length of the muscle fiber.

The structure of myofibril

Myofibrils are composed of bundles of myofilaments. Cross-striations are seen in preparations of striated muscle examined with a phase contrast or polarizing microscope as alternating light and dark bands. These bands are termed the **I-band** (isotropic) and the **A-band** (anisotropic).

In H&E- stained preparation the A-bands are more intensively stained than the I-bands. In the center of each A-band there is a thin dark line called telophragm or **Z-line**. In the center of each A-band there is a less dense light region named **H-band**. Furthermore, the H-band is bisected by a narrow dense line called **M-line** (mesophragm).

The structural and functional unit of myofibril is **sarcomere**. Sarcomere is the region of myofibril bounded by two Z-lines (telophragms). The length of each sarcomere is 2-3nm.

Sarcomere is the basic contractile unit of striated muscle. The sarcomere is composed of ***actin (thin) and myosin (thick) myofilaments***. The arrangement of thick and thin filaments gives rise to the density differences which produce the cross-striations of the myofibril.

~ The actin (thin) filaments attach to the Z-line, compose the I-band and extend into the A-band the edge of the H-band. The thin filaments are composed of the contractile protein actin and structural proteins troponin and tropomyosin. They are approximately 5nm in

diameter and 1 μm in length.

~ The thick (myosin) filaments are found only in the A-band. They are composed of the contractile protein myosin. The thick filaments are 1,5 μm long and 10-12 nm in diameter.

Each myosin filament is accompanied by two actin filament.

Satellite cells

Satellite cells are interposed between the plasma membrane of myosymplast and external basal lamina. The plasma membrane of the satellite cells attaches to that of the myosymplast. Each satellite cell has a single nucleus with a dense chromatin network. Their cytoplasm contains evenly distributed mitochondria and ER. The Golgi complex and centrosome surround the nucleus. The specialized contractile organelles are absent in the satellite cells.

THE TYPES OF SKELETAL MUSCLE FIBERS

The free types of skeletal muscle are **red muscle fibers** (type I), **intermediate muscle fibers** (type IIa), and **white muscle fibers** (type IIb).

Three types of the fibers are found in any given skeletal muscle; their proportion depends on the functional role of the muscle.

Red muscle fibers appear red in fresh specimens because of large amounts of myoglobin and cytochrom complexes. They are characterized by slow myosin ATPase reaction velocity. These fibers are slow-twitched and fatigue-resistant.

Intermediate muscle fibers. Besides high myosin content and numerous mitochondria the intermediate fibers also contain large amounts of glycogen and are capable of anaerobic glycolysis. Such fibers are both quite fast-twitched and fatigue-resistant.

White muscle fibers appear light pink in fresh specimens and contain less myoglobin and fewer mitochondria than two other types of muscle fibers. They have a low level of oxidative enzymes, but exhibit high anaerobic enzyme activity and store a considerable amount of glycogen. The white muscle fibers are fast-twitched and fatigue-prone.

THE SKELETAL MUSCLE AS AN ORGAN

A skeletal muscle consists of striated muscle fibers held together by connective tissue.

The individual muscle fiber is surrounded by a delicate layer of connective tissue called **endomysium.** Reticular and collagen fibers of the endomysium interweave with fibers of the sarcolemma.

Perimysium is a thicker connective tissue layer that surrounds a group of fibers to form a bundle.

Epimysium is the sheath of dense connective tissue that surrounds the whole muscle. The major vascular and nerve supply of the muscle penetrates the epimysium.

Theme 7. Nerve tissue.

Nerve tissue (textus nervosus) is one of the four principal tissues of the body. It provides regulation of activities of organs and tissues and their interaction with external environment. It is composed of nerve cells (neurons) and supporting cells (neuroglia).

Histogenesis

Nerve tissue is derived from a dorsal thickening of **ectoderm** – neural plate. Neural plate is sequentially transformed into neural groove and then into neural tube. Neural tube is separated from overlying epidermal ectoderm. A part of cells is included neither in the epidermal ectoderm nor in the neural tube and remains located between them as a loose aggregation of cells – neural crest. The cells of neural crest start to migrate in both lateral and ventral directions, thereby forming the nuclei of cranial nerves, neurons of spinal and autonomic ganglia, neurolemmocytes (neuroglia), pigment cells.

The thickenings of ectoderm at the sides of head (neural placodes) give a rise to ganglia of the V, VII, IX and X pairs of cranial nerves, as a result of cell migration from neural placode.

At the early stages of embryogenesis the neural tube is referred to as pseudostratified neural epithelium formed by ventricular and neuroepithelial cells.

Ventricular cells are cylindrical in shape and connected with each other via gap junctions; they possess apical processes, which reach the lumen of neural tube. The basal portions of these cells adjoin the subpial limiting membrane. The cyclic migration of nuclei is characteristic for the ventricular cells: the nuclei of premitotic cells lie deep, during prophase they approach the surface, karyokinesis takes place near the ventricular surface, and the nuclei of daughter cells again return into the depth.

The proliferative activity of ventricular cells constantly decreases

during embryonic development, and it completely disappears after the birth.

Morphologically similar ventricular cells undergo differentiation into different types of cells of mature nerve tissue. A part of them gives a rise to neurons, another part – to glial cells (ependymal cells, astrocytes and oligodendrocytes).

In that parts of brain where the histogenesis is especially intensive the ventricular cells lose cylindrical shape and capacity for migration of nuclei, but remain high proliferative activity. Such cells are referred to as subventricular and neurogerminative (cambial) cells. Further they give a rise some types of neurons and glial cells. Subventricular and extraventricular cells exist during some time after birth.

NEURONS. CLASSIFICATION. STRUCTURE.

Nerve cell (neuron) is morphological and functional unit of nerve tissue. Depending on their morphological and functional features neurons of different parts of nervous system vary from each other.

Two classifications of neurons exist: a) morphological; b) functional.

Morphological classification

According to this classification neurons are classified on the basis of the number of processes extending from the cell body:

1. Unipolar – have only one process, an axon. In human body such neurons are not revealed. Only the neuroblasts are referred to as unipolar neurons.
2. Bipolar – have two processes: axon and dendrite. These neurons are rare and associated with organs of special senses; they are found within the retina of the eye and the ganglia of the vestibulocochlear nerve of the ear.
3. Pseudounipolar – have one process that divides close to the cell body into two branches. In human nervous system such neurons are found within spinal ganglia.
4. Multipolar – have numerous processes, one of them is an axon, the all others are dendrites. The most of neurons of human body are the multipolar ones.

Functional classification

Depending on their function, neurons are classified into the following:

1. Sensory neurons (receptive or afferent);
2. Interneurons (intercalated or associative);
3. Motor neurons (efferent).

Sensory neurons generate nerve impulse under the influence of external or internal stimuli.
Interneurons form a communicating and integrating network between sensory and motor neurons.
Motor neurons transmit impulses to tissues of effector organ (skeletal muscle, smooth muscle, glands).

Structure of neurons

♦ Neurons show variation in size, from 4-6 μm in granular layer of cerebellar cortex to 130 μm – giant pyramidal cells in cerebral cortex (Betz cells). Neurons consist of cell body (perikaryon) and processes.

♦ Although neurons demonstrate great variation in shape, presence of processes is the characteristic feature for all of them. Given that the neurons' processes provide transmission of nerve impulse from one part of the body to another, their length varies from several micrometers to 1,5 meters. The neurons' processes are of two types: axon and dendrites. Neurons have only one axon, usually the longest process (up to 1,5m) extending from the cell, which transmits impulses away from the cell body. Most of neurons usually have many dendrites, shorter branching processes which transmit impulses from the periphery towards perikaryon.

♦ Nucleus of neuron is round and usually centrally located. The nucleus is euchromatic, containing 1 or sometimes 2-3 prominent nucleoli. Most neurons possess only one nucleus, multinucleated neurons are very rare and found only among ganglia of autonomic nervous system. For example in prostatic gland and cervix of uterus the neurons possessing up to 15 nuclei are found.

♦ Cytoplasm contains the following organized structures:

1) special organelles;
2) general-function organelles;
3) inclusions – carbohydrates (glycogen), pigments (melanin, lipofuscin), several secretions (in neurosecretory cells).

Special organelles of neurons are *Nissl substance* and *neurofibrils.*

Nissl (chromatophilic) substance corresponds to a rough endoplasmic reticulum (rER). When the nerve tissue is stained with aniline dyes, the Nissl substance is revealed in cytoplasm of neuron as an aggregation of basophilic granules of various sizes (Nissl bodies). It occupies perikaryones and dendrites, and is absent within axons and in the area of cell body, which the axon extend from (axonal hillock). The Nissl bodies reveal a high content of ribonucleoproteins. Under the electron microscope examination it was revealed that each Nissl body corresponds to a stack of rER. The degree of the Nissl bodies' arrangement varies among different types of neurons. They are the most regularly arranged in neurons of spinal ganglia. In motor neurons of spinal cord the granules of the Nissl substance are large and surround the nucleus. In sensory neurons of spinal ganglia the Nissl substance appears as a powdered granularity. The Nissl substance is considered to be an indicator of neuron's functional activity.

Because the synthetic activity of the neuron is concentrated in the nerve cell body, the continuous movement of cytoplasm is required to convey enzymes essential for synthesis of neurotransmitters from perikaryon to axon terminals. Such type of transport is called *slow axonal transport*, which occurs at the speed of 1-3 mm per day.

The *fast axonal transport* (5-10 mm per 1 hour) is possible in two directions: from the nerve cell body to the axon terminal *(anterograde)* and to the nerve cell body from the axon terminal *(retrograde).* Through the *fast anterograde transport* the membrane-limited organelles and low-molecular-weight proteins are carried to the axon terminal. While the *fast retrograde transport* serves for carrying the same materials as well as other molecules endocytosed by axon terminal to nerve cell body.

Dendritic transport (3mm per 1 hour) appears to have the same characteristic for the dendrites as axonal transport does for the axon, provides transport of enzymes (for example acetylcholinesterase, which breaks down the neurotransmitter acetylcholine).

Neurofibrils are revealed in the nerve tissue when it is impregnated with silver. Neurofibrils appear as thin filaments of 0,3-0,5μm in diameter, in the perikaryon they form a dense meshwork, while in axon and dendrites they have parallel arrangement. When observed

with electron microscope the neurofibrils are estimated to be composed of bundles of the neurofilaments of 6-10nm in diameter and the neurotubules of 20-30nm in diameter. The neurotubules and neurofilaments are referred to as the cytoskeleton of the neuron.

General-function organelles of neurons

The Golgi apparatus of the neuron has a usual ultrastructure.

The centriole of the neuron is situated between the nucleus and the dendrites.

The mitochondria are found in the perikaryon as well as in the axons and dendrites, at the nerve terminals they are especially numerous.

The neurons also reveal numerous lysosomes and ribosomes within their cytoplasm.

Neurosecretory cells are neurons, which are specialized predominantly in synthesis and secretion of biologically active substances. The secretions of theses neurons are referred to as neuroregulators taking part in interaction between nervous and endocrine system.

Such neurosecretory cells are found in hypothalamus. They have several morphological characteristics:

1) they are large neurons;
2) the Nissl substance is located at the periphery of the cell body;
3) in the cytoplasm of the axons and dendrites the secretory granules containing proteins and sometimes lipids and polysaccharides are found;
4) contain irregular-shaped nuclei, which is the sign of high anabolic activity.

SUPPORTING CELLS OF THE NERVE TISSUE: THE NEUROGLIA

Neuroglia forms the environment that surrounds neurons and carries out supportive, delimiting, protective and trophic functions.

Such morphological peculiarities of capillaries of the nerve tissue as continuous endothelial lining and thick dense basal lamina provide the selectivity of exchange between the nerve tissue and blood. Besides this the glial cells (predominantly astrocytes) stretch their processes from

blood capillaries to neurons, thereby delimiting neurons from immediate attachment to the wall of blood vessels. The endothelium and basal lamina of blood capillaries and the astrocytes together form the **blood-brain barrier.**

Neuroglia includes cells which belong to two different histogenetic lineages:

1) Macroglia (glial cells);
2) Microglia (glial macrophages).

Macroglia

The cells of macroglia include 1) ependymal cells, 2) astrocytes, 3) oligodendrocytes.

Ependymal cells form the epithelium-like lining of the fluid-filled cavities of CNS (central canal of the spinal cord, ventricles of the brain).

Ependymal cells are the first glial cells which are differentiated from the glioblats of the neural tube. On the internal surface of neural tube their elongated cell bodies from the epithelium-like lining, carrying out the delimiting and supportive functions. The ependymal cells facing the lumen of the neural tube possess up to 40 cilia on their apical surface. These cilia are considered to facilitate the movement of cerebrospinal liquor. From the basal portion of the ependymal cells the long processes extend, then branch along the whole neural tube and form its supportive apparatus. These processes are involved in formation of *superficial glial limiting membrane*, which separates the content of the neural tube from other tissues.

After the birth the ependymal cells gradually lose their cilia; the cilia remain only in some parts of the CNS (aqueduct of cerebrum).

At the area of posterior comissure the ependymal cells carry out the secretory function forming the “subcomissural organ”, which discharge the secretion which is suggested to take part in regulation of water balance.

The ependymal cells which cover choroid plexus of the brain ventricles are cuboidal in shape, in newborns they possess cilia, which are reduced later. The basal cell surface exhibits numerous infoldings of cytoplasm with large mitochondria, lipid and pigment inclusions.

Astrocytes are the largest of the neuroglial cells. They are stellate in shape and possess numerous widely branched processes.

Two kinds of astrocytes are identified:

1) protoplasmic;
2) fibrous.

Protoplasmic astrocytes

- Localization – gray matter.
- Size – 15-25 μm, possess short but widely branching processes.
- Nucleus – large, oval, light.
- Cytoplasm – contains small amount of rER cisterns, free ribosomes and microtubules, rich in mitochondria.
- Function – delimiting and trophic.

Fibrous astrocytes

- Localization – white matter.
- Size – up to 20 μm, possess 20-40 long and relatively straight processes. These processes, which are referred to as glial fibers, form a dense network – supporting apparatus of the brain. Attaching to the blood vessels and to the brain surface, the astrocytes' processes by their terminal dilatations form the perivascular glial limiting membranes.
- Cytoplasm – under the electronic microscope examination appears light; contains not numerous lysosomes and elements of rough ER; is filled with numerous fibrils of 8-9 nm in diameter, which form bundles and extend into the processes.
- Nucleus – large, light; the nuclear envelope sometimes forms deep folds; the karyoplasm is characterized by homogenous electronic density.
- Function – supporting and isolation of neurons from external influences.

Oligodendrocytes are the most numerous and polymorphic group of the glial cells responsible for the synthesis of myelin in the CNS.

- Localization – surround the nerve cell bodies in the central and peripheral nervous system, are found as a part of sheaths of nerve fibers and nerve endings.
- Size – very small.
- Shape – different parts of the nervous system are characterized by different shapes of the oligodendrocytes. The cell bodies of oligodendrocytes give a rise to short slightly branched cell processes.
- Cytoplasm – its density is approximately the same as that of the neurons' one; doesn't contain neurofilaments.
- Function – carry out the trophic function by taking part in metabolism of the neurons. Provide formation of the sheaths around both myelinated and unmyelinated nerve fibers (in such case are called **neurolemmocytes or Schwann cells**). Take part in maintenance of the water-salt balance, regeneration and degeneration of the nerve fibers.

Microglia

Microglia is an aggregation of small cells possessing 2-3 processes, which contain short secondary and tertiary branches. These cells are capable to amoebic movements.

The nuclei are elongated or triangular in shape; are rich in chromatin.

In case of irritation of microglial cells they change their shape, pull their processes into and become round-shaped. Recently the microglial cells were proved to be able to produce proteins – immunoglobulins (antibodies). This all indicates on the fact that the microglial cells belong to the macrophage system.

NERVE FIBERS. CLASSIFICATION. STRUCTURE

Nerve fibers are represented by the nerve cells' processes covered with sheaths. Depending on the structure of the sheath the nerve fibers are divided into two main groups:

1. Myelinated.
2. Unmyelinated.

Both of them include nerve cell's processes, which is located in the center and called axial cylinder, and sheath formed by the oligodendrocytes (neurolemmocytes or Schwann cells).

Myelinated nerve fibers consist of the *axial cylinder, myelin sheath, neurolemma and basal lamina.*

- Diameter in cross section – 1-20 μm.
- Localization – Central nervous system, peripheral nervous system.

The axial cylinder is referred to as the nerve cell's process (axon or dendrite). The axial cylinder consists of neuroplasm covered by a membrane – axolemma.

Neuroplasm is the cytoplasm of the nerve cell which contains longitudinally oriented neurofilaments and neurotubules. The neuroplasm possesses mitochondria, which are densely concentrated near the nodes of Ranvier.

Axolemma is the continuation of the plasmalemma of neuron. Axolemma provides conduction of the nerve impulse. The speed of the nerve impulse conduction via thick myelinated nerve fiber varies from 5 to 120 m/s.

Myelin sheath is a tube from 0,3 to 20 μm thick which covers the

axial cylinder throughout all its length. The axonal hillock and the terminal arborizations of axon are not covered by myelin. The myelin sheath is also absent at nodes of Ranvier. The node of Ranvier is represents the junction between two adjacent oligodendrocytes (Schwann cells). The part of nerve fiber between two sequential nodes of Ranvier is called internodal segment. The length of internodal segments varies from several micrometers to several millimeters. The size of the node of Ranvier is 0,25 – 1 μm.

Hence the myelin is composed of about 80% lipids it is intensively stained by the osmic acid into dark brown color. In this case the whole nerve fiber appears as homogenous cylinder, which reveals light lines, located on a distance from each other – myelin incisures (Schmidt-Lanterman clefts).

During formation of the myelin nerve fiber the axial cylinder initially lies in a groove on the surface of the oligodendrocyte, thereby forming a deep fold. This double fold of the plasmalemma of oligodendrocyte is called mesaxon. Myelin sheath formation is initiated when the Schwann cell mesaxon surrounds the axon. A sheetlike extension of the mesaxon then wraps around the axon in a spiraling motion. Under the electron microscope examination each whorl of mesaxon appears as a light area about 8-12 nm in depth, which corresponds to the lipid bilayers of two layers of plasmalemma of the oligodendrocyte. The myelin incisures (Schmidt-Lanterman clefts) correspond to those sites where the whorls of mesaxon are moved apart by the cytoplasm of oligodendrocyte (Schwann cell).

Numerous oligodendrocytes participate in formation of one nerve fiber. They contact with each other at the sites of the nodes of Ranvier. One internodal segment corresponds to one oligodendrocyte.

On a longitudinal section near the node of Ranvier the area where the whorls of mesaxon successively contact with the axial cylinder are visible. The sites of attachment of the innermost whorls of mesaxon are the furthermost from the node, while the all subsequent whorls gradually approach it. This is because of stratification of the mesaxon which takes place during the growth of the axial cylinder and the oligodendrocytes; that's why the first layers of mesaxon are shorter than the subsequent ones. The edges of two adjacent Schwann cells form annulate processes of 50 nm in diameter at the site of the node of Ranvier.

Neurolemma – is peripheral portion of the nerve fiber, which

contains the nuclei and cytoplasm of the oligodendrocytes.
Basal lamina forms the external covering of myelinated nerve fiber. It is associated with the longitudinally oriented dense cords of collagen fibrils, which don't interrupt at the nodes of Ranvier.

Unmyelinated nerve fibers

Unmyelinated nerve fibers are predominantly found in the autonomic nervous system.

♦ The diameter of fibers is 1-4 μm. They are significantly thinner than the myelinated fibers.

The unmyelinated nerve fibers are composed of the axial cylinder, neurolemma and basal lamina.

The neurolemma consists of the densely aggregated neurolemmocytes (oligodendrocytes), which form cords. These cords exhibit oval nuclei, which are located on a distance from each other.

The axial cylinder represents the nerve cell process. It invaginates into the plasmalemma of the neurolemmocyte; thereby the neurolemmocyte encloses the axial cylinder like a muff. The plasmalemmae of neurolemmocytes surround the axial cylinders and occlude forming deep folds.

Occluded at the area of the fold, the sites of plasmalemma of neurolemmocyte form double membrane called mesaxon. Hence the plasmalemmae of the neurolemmocytes are extremely thin, it is impossible to view the cells' boundaries and mesaxon. That's why the neurolemma appears as continuous cord of the cytoplasm, which covers the axial cylinder.

The nerve fibers of the inner organs usually contain not only one, but several (10-20) axial cylinders in the cord of the neurolemmocytes. Such unmyelinated nerve fibers are called the fibers of cable type.

Externally the unmyelinated nerve fiber is covered by basal lamina.

The speed of nerve impulse conduction in unmyelinated nerve fiber (1-2 m/s) is much lower than in the myelinated one (120 m/s). This is explained by the fact that in unmyelinated nerve fiber the wave of depolarization continuously spreads along the all length of the plasmalemma of the nerve fiber. In myelinated nerve fibers the conduction of nerve impulse is described as "jumping" from node to node. This process is called salutatory conduction. The myelin sheath

around the fibers does not conduct the nerve impulse, the electric impulse can only occur at the node of Ranvier, where the plasmalemma lacks a myelin sheath.

REGENERATION OF THE NERVE CELLS AND NERVE FIBERS

The nerve cells (neurons) form constant (non-dividing) cell population. The neurons are capable only to intracellular physiological regeneration, which is represented by continuous rebuilding of structural proteins of their cytoplasm.

However the nerve cells' processes and peripheral nerves, in case of their alteration, reveal regenerative capacity. Initially they undergo degeneration. At this stage, which lasts approximately 24 hours, the acute activation of neurolemmocytes (oligodendrocytes) is observed. Their cytoplasm exhibit the increased number of free ribosomes, polysomes and developing endoplasmic reticulum. The myelin sheath is no more revealed as separated structure. The following 3-4 days are characterized by the intensive proliferation and enlargement of the neurolemmocytes. At the end of a second week the myelin sheaths and some portions of the axial cylinders are resorbed. The resorption is performed by the connective tissue macrophages as well as by the cells of microglia.

The axial cylinders form clavate expansions (growth cones) at their terminals. These growth cones help the axial cylinder to grow into the neurolemmocytes of the peripheral portion of the nerve. The speed of such growth is approximately 1-4mm per day. Renewal of the myelin sheath and terminal structures takes place a little bit later.

NERVE ENDINGS

Absolutely all nerve fibers posses their terminal apparatus which is called nerve ending.

Classification of nerve endings

Depending on their functional role the nerve endings are divided into three principal groups:

1. Effector:
a) motor;
b) secretory.
2. Receptive (affector or sensory)
3.Terminal

Effector nerve endings

Motor nerve endings are represented by the terminal apparatus of axons of the somatic and autonomic nervous systems. The nerve impulse via these nerve endings is transmitted to the tissues of working (effector organ).

The motor nerve endings in the skeletal muscles are called neuromuscular endings. The neuromuscular endings are the terminals of axons of the motor neurons of anterior horns of the spinal cord. The neuromuscular ending includes the following components:

1) terminal branches of the axial cylinder of the nerve fiber;
2) specialized site of the muscle fiber.

As the nerve fiber approaches the muscle fiber, it looses the myelin sheath and penetrates the muscle fiber involving its plasmalemma. The plasmalemma of the axonal terminal branches is separated from the plasmalemma of the muscle fiber by a cleft of approximately 50 nm wide. This gap is called synaptic cleft.

At the site of contact the plasmalemma of the muscle fiber forms numerous folds which are referred to as secondary synaptic clefts.

The terminal branches of the nerve fiber in neuromuscular synapse are rich in mitochondria and presynaptic vesicles filled with the neurotransmitter – acetylcholine.

The muscle fiber at the site of contact with the nerve ending doesn't exhibit typical striation and is characterized by an abundance of mitochondria and aggregation of round and oval nuclei. The sarcoplasm together with mitochondria and nuclei forms the postsynaptic part of synapse.

In case of excitation the acetylcholine is released from the vesicles and transported through the presynaptic membrane to the synaptic cleft. Then the neurotransmitter binds to its specific receptor on the postsynaptic membrane, which leads to the depolarization (excitation) of the postsynaptic membrane.

The postsynaptic membrane of the neuromuscular ending contains the enzyme called acetylcholinesterase. This enzyme breaks down the molecule of acetylcholine and limits the time of its action.

The motor nerve endings in the smooth muscle have much more primitive structure. The thin bundles of axons or their terminals penetrate the spaces between the smooth muscle cells and form expansions (varices), which contain cholinergic and adrenergic presynaptic vesicles.

Secretory nerve endings are terminal expansions of the axons which contain presynaptic vesicles usually filled with acetylcholine.

Receptive nerve endings are scattered throughout all over the body and percept various stimuli from external environment as well as from inner organs.

~Exteroceptors percept stimuli from external environment.
~Interoceptors percept stimuli from inner organs and tissues of the body.

Proprioceptors are referred to as a type of interoceptors. They represent the sensory nerve endings in muscles and tendons which are involved in regulation of movements and muscle tone.

Depending on nature of the percepted stimulus all sensory nerve endings are classified as mechanoreceptors, baroreceptors, chemoreceptors, thermoreceptors, nociceptors etc.

Based on their structure the sensory nerve endings are divided into **free** and **non-free**. Free nerve endings are represented **only** by the terminal branches of the axial cylinder, while non-free nerve endings retain all components of the nerve fiber (branches of the axial cylinder, myelin sheath, glial cells).

- Non-free nerve endings could be enclosed in connective tissue capsule, in such case they are referred to as ***encapsulated.***
- Non-free nerve endings which are not enclosed in a capsule are called ***non-encapsulated***.

The sensory nerve endings of epithelial, connective and muscle tissues are characterized by the several distinctive features.

The epithelial tissues are endowed with *free* nerve endings. As they approach the epithelial layer, the myelinated nerve fibers loose the myelin sheaths and their axial cylinders penetrate and branch between the epithelial cells.

The free receptors of epidermis perform the function of perception of temperature and pain stimuli. Network of free dermal nerve endings also surround most hair follicles and attach to their outer root sheath. They are particularly sensitive to hair movements and serve as mechanoreceptors.

The stratified epithelium owes nerve endings which include not only nerve terminals but also modified epithelial cells – ***tactile epithelial cells of Merkel***, which serve as mechanoreceptors. These cells are distinguished from other epithelial cells by the light cytoplasm, flattened nucleus and presence of osmiophilic granules, which are 65-180 nm in

diameter.

The basal domain of the Merkel cell together with adjoining nerve endings form receptive terminal structures – ***Merkel discs***.

Connective tissue also posses wide range of receptive nerve endings, which are divided into ***non-free encapsulated, non-free non-encapsulated and neurotendinous spindles***.

Despite their variability, the encapsulated receptors of connective tissue are always composed of branching axial cylinder and glial cells which are surrounded by a connective tissue capsule.

The encapsulated nerve endings of connective tissue include ***Pacinian corpuscles, Golgi-Mazzoni bodies, Meissner's corpuscles and end-bulbs of Krause***.

♦ **Pacinian corpuscles** are large ovoid structures of 0,5x2mm in size.

They are composed of a myelinated nerve ending surrounded by a capsule structure. The nerve enters the capsule at one pole with its myelin sheath intact. The myelin is retained for one or two nodes and is then lost. The unmyelinated portion of the axon extends toward the opposite pole from which it entered, and its length is covered by a series of tightly packed, flattened Schwann cell lamellae that form the inner core of the corpuscle. The remainder or bulk of the capsule, the outer core, is formed by a series of concentric lamellae. Pacinian corpuscles respond to **pressure and vibration**. They are found in the deeper dermis and hypodermis (especially in the fingertips), in connective tissue in general, and in association with joints, periosteum, and internal organs.

♦ **Golgi-Mazzoni bodies** are smaller than Pacinian corpuscles; possess thinner capsule and relatively large inner core. They are found in skin, serous and mucous tunics of inner organs. Golgi-Mazzoni bodies serve as **baroreceptors**.

♦ **Meissner's corpuscles** are oval structures that measure about 50-100 μm. They are composed of oligodendrocytes oriented perpendicularly to the long axis of the corpuscle. As it penetrates the corpuscle, the nerve fiber looses its myelin sheath and branches into several terminals which contact with the glial cells. A thin connective tissue capsule is predominantly composed of collagen fibers. Meissner's corpuscles are found in the papillary layer of hairless skin. They serve as **touch** receptors which are particularly responsive to low-frequency

stimuli.

♦ **End-bulbs of Krause**. Entering the capsule the nerve fiber looses myelin sheath, and then ends as a bulb-like expansion and branches, thereby forming a network of unmyelinated nerve fibers. The capsule is extremely thin. End-bulbs of Krause are found in connective tissue of tongue and external genitalia. They perform the role of **mechanoreceptors**.

♦ **Neuro-tendon spindles** (**Golgi tendon organ**) are formed by the myelinated nerve fibers of 15 μm in diameter. As they approach the collagen fibers of tendon, the nerve fibers lose their myelin sheath, give numerous branches and surround the tendon bundles.

♦ **Ruffini corpuscles** have the similar structure. They are found in deep layers of the derma and the hypoderm; are the most numerous within the skin of feet.

The Golgi tendon organs and the Ruffini corpuscles serve as mechanoreceptors.

Neuromuscular spindles

The neuromuscular spindle is composed of 10-12 thin and short striated muscle fibers, surrounded by a connective tissue capsule. Such fibers are called **intrafusal**. At their endings these muscle fibers possess actin and myosin filaments which do contract. The receptive part of the intrafusal muscle fiber is represented by the central **non**-contractive part.

There are two types of intrafusal muscle fibers:

a) nuclear bag fibers;

b) nuclear chain fibers.

The neuromuscular spindle contains 1-3 nuclear bag fibers, which possess numerous nuclei in their central dilated part.

And there are 3-7 nuclear chain fibers. They are two times thinner and two times shorter than the nuclear bag fibers. The nuclei in the nuclear chain fibers are found along the whole receptive zone.

Externally the connective tissue capsule is surrounded by the striated muscles, which form the outer capsule. Such muscles are called extrafusal.

The intrafusal muscle fibers are innervated by the two types of afferent nerve fibers: a) primary; b) secondary.

♦ The primary nerve fibers are of 17μm in diameter; they form the annulospiral nerve endings on the nuclear bag fibers as well as on the

nuclear chain fibers.

♦ The secondary nerve endings are of 8μm in diameter; they form the flower-spray endings on the both sides of the annulospiral nerve endings. Both of these nerve endings react on the change in length of the muscle fibers.

INTERNEURON SYNAPSES

The synapse is composed of three parts: presynaptic part, postsynaptic part and synaptic cleft.

There are distinguished two types of synapses according to the type of transmission of the nerve impulse:

1) chemical;
2) electrical.

Depending on their localization the synapses are divided into:

1) Axosomatic – between the axon of one neuron and the nerve cell body (perikaryon) of another one.
2) Axodendritic – between the axon and the dendrite.
3) Axoaxonic – between the axon of one neuron and the axon of another one. Such synapses inhibit the nerve impulse transmission.

The structure of chemical synapse

The presynaptic part is formed by the extended terminal branch of the axon that transmits the nerve impulse. It is covered by the presynaptic membrane; contains numerous mitochondria and presynaptic vesicles, containing neurotransmitter – acetylcholine (cholinergic synapses), noradrenalin (adrenergic synapses). The function of neurotransmitter could be performed by various biologically active substances: dopamine, glycine, gamma aminobyturic acid (GABA), glutamine acid, substance P, serotonin, histamine etc. Dopamine, glycine, gamma aminobyturic acid (GABA) are inhibitory neurotransmitters.

The synaptic cleft is 20-30 nm wide and filled with the tissue liquor. The neurotransmitter is released here from the vesicles of the presynaptic part.

The postsynaptic part is covered by the postsynaptic membrane, which contains receptors to the neurotransmitter.

THE REFLEX ARCH

The reflex arch is represented by a chain of neurons, connected with each other via synapses, which provide the conduction of the nerve impulse from the receptor (sensory neuron) to the motor (effector) nerve ending on a working organ.

The most primitive reflex arch consists of 2 neurons – the sensory and the motor one. In most cases there are several interneurons (associative) between them.