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HISTOLOGY, CYTOLOGY AND EMBRYOLOGY

CYTOLOGY AND EMBRYOLOGY

Practical manual

КИЇВСЬКИЙ УНІВЕРСИТЕТ 2024

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H69

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A guide for laboratory classes in the discipline "Histology, Cytology and Embryology" contains theoretical material and histological and cytological micrographs devoted to the modules of Cytology and Embryology. The guide is divided into three parts. The first part focuses on the cell's organisation, components, and membrane compartments. The second one describes central compartment organisation, which is the nucleus and principles of the organisation depending on the cell cycle. Finally, the last chapter explains the sequence of events during early embryonic developmental stages and the differentiation of cells and tissues. Each chapter concludes with various questions accompanied by medical models or descriptions to contextualise the application of the knowledge.

These questions are designed to assess the depth of understanding of the material and are intended for students of speciality 222 "Medicine" of the educational qualification level "Master".

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INTRODUCTION

In a guide for students specialising in "Medicine," the first section in the discipline "HISTOLOGY, CYTOLOGY AND EMBRYOLOGY" covers fundamental theoretical concepts and questions related to Cytology and Embryology. This section is divided into three chapters that provide information and contain created tasks and questions on histological methods of investigation, cell components and structures, and cell functions. Additionally, the third chapter delves into early embryonic development, cell differentiation, and tissue formation. Each chapter includes subchapters with materials and questions on cellular components, their functions, and developmental mechanisms.

The first chapter focuses on tissue processing stages, instruments and equipment, and the basic structure of cells in different organisms. After that, it covers the structural components of the cell envelope, its peculiarities, interactions, and functions. The next one discusses the organisation, interaction, function, and disturbances associated with the cytoskeleton. The organisation and role of the cytosol in cell behaviour, protein synthesis, energy turnover, and accumulation of substances are discussed further.

In the fourth part of chapter one, students will learn about the structure and functions of one-membrane organelles, including the rough and smooth endoplasmic reticulum, Golgi body, lysosomes, and peroxisomes. This chapter provides essential information about their morphology, functioning, involvement in cellular processes, and associated disorders. Additionally, students will explore the types of cells in the human body where each organelle is most developed and the reasons behind it. Further, students will delve deeper into the two-membrane organelle, mitochondria. This class emphasises its crucial role as the primary provider of ATP for the cell and addresses the potential risks associated with its functioning.

The second chapter focuses on the nucleus as the central compartment of the cell. It describes the surface apparatus, nucleoplasm, nuclear skeleton, chromatin, and nucleolus and their respective functions. Test tasks in this chapter cover typical disorders related to the nuclear lamina. Subsequently, the focus shifts to the

processes occurring during the cell cycle, encompassing mitotic division, growth, differentiation, DNA doubling, and preparation for the subsequent mitosis. This chapter also elucidates the changes that occur in the cell and the reorganisation of genetic material during prophase, metaphase, anaphase, and telophase.

Finally, the third chapter offers insights into embryogenesis and the development of an embryo, covering the pre-embryonic and embryonic periods of human life. It encompasses gametogenesis, characteristics of gametes, fertilisation, cleavage, gastrulation, neurulation, organogenesis, and extraembryonic organs. Furthermore, students are encouraged to respond to suggested questions, allowing them to assess their comprehension of the theoretical material and its practical application. This approach avoids subjective teacher assessment and establishes a solid foundation for subsequent course units, such as "Basic histology" and "Special histology."

Our own light and electron micrographs are used in the guide.

Chapter 1. CYTOLOGY

1. INTRODUCTION. MICROSCOPIC TECHNIQUE. TISSUE PROCESSING

1.1. Introduction

The history of knowledge about the cellular structure of various types of living organisms began in 1665 when Robert Hooke first described cells. This discovery became possible after the invention of glass and lens creation and the construction of the microscope.

A lot of researchers, such as Antonie van Leeuwenhoek, Marcello Malpighi, Nehemiah Grew, Jan Evangelista Purkinje, Robert Brown and others, described the microscopic structure of different parts of cells, organisms and tissues and collected data for further assumptions about the cellular structure of all living organisms.

In 1839, Matthias Jakob Schleiden and Theodor Schwann postulated "Cell Theory", which includes basic statements like "all organisms are composed of cells" and "cells are the unit of life". Rudolf Virchow added the third guideline: "All cells can arise only from pre-existing cells".

The improvement of the microscopic technique mostly caused all discoveries.

1.2. Microscopes

A microscope is a device used to investigate small objects, such as cells, in a magnified form. Microscope consists of optical and mechanical parts. The optical part includes a lens system that magnifies objects: eyepiece (synonym is the ocular), objective lenses, and condenser lenses for light focusing on the object. Microscopic light source = illuminator. The mechanical part unites lenses, object holder and operating knobs for focusing tissue and moving specimens (arm, stage, base, nose piece = revolving turret, coarse adjustment knob, fine adjustment knob, stage controls).

Magnification is how often an image of an object is increased compared to its original size. To calculate the magnification, multiply the magnification of the ocular lens by the magnification of the objective lens. For example, the magnification of the ocular lens is

10×, and the magnification of the objective lens is 20×. The total magnification of objects is 10×20 = 200 times.

Resolution is seeing two close points of objects as two separate points.

Several types of microscopes can be used to investigate various objects. A ***light microscope*** (bright field microscope) is used for the basic examination of the tissue after conventional staining (for example, with hematoxylin and eosin). ***Dark field microscopy*** is used for both unstained and stained objects, but the ***phase-contrast microscopy*** techniques only target the ***unstained*** phase objects. The dark-field microscopy (showing bright objects on a dark background) provides good contrast, enabling perfect magnification. The dark-field microscopy is used to examine crystals, for example, in synovial fluid and urine. The disadvantage of darkfield is its high sensitivity to dust. The phase-contrast microscopy techniques use the *interference* of light beams to give images with high contrast. In a ***fluorescence microscope***, from ultraviolet to the infrared wavelengths are used to illuminate the sample, activating the ability of specific molecules to fluoresce (vitamin A, some neurotransmitters and artificial antibodies bound to ***fluorochromes***). In an ***electron microscope (transmission and scanning)***, the electron beam is used for specimen illumination and gives magnification 100 times higher compared to a light microscope; it also has high resolution.

1.3. Tissue processing

After a tissue biopsy, several types of samples can be examined. Some examples are histological specimens, touch preparations, smears, and cell suspensions.

We will focus on general approaches to the histological specimen's preparation for the most common tissue samples.

Fixation. The tissue undergoes immediate fixation after removal from a patient's or experimental animal's body. This is the first stage of tissue processing. Physical and chemical fixatives can be applied. Freezing (used to assess lipid structure), drying, and heating are examples of physical fixation. Chemical fixators are usually a mixture of reagents. The most common fixator for light microscopy is a 3.7 % formalin solution; ***glutaraldehyde*** with ***osmium tetroxide*** solution is used for electron microscopy. Other fixators include acetone, methanol,

ethanol, picric acid, and others. Fixators protect the tissue from destruction and aim to preserve the original structure and solidify tissue.

Dehydration and clearing. Preparation of thin tissue sections (thickness 3–5 μm) requires embedding the tissue in paraffin or a plastic medium. Neither paraffin nor plastic medium are mixable with water. That is why water in tissue and cells is replaced by alcohol, called ***dehydration***. In this case, the tissue is transferred through solutions of increasing concentration of alcohols (70 %, 80 %, 90 %, 96 %, 100 %) until absolute alcohol concentration is reached. The next stage is the replacement of alcohol with an organic solvent (xylene, benzene, chloroform) – it is called ***clearing***. Organic solvents dissolve lipids, so this technique does not allow the investigation of lipid components of cells, such as plasma membrane phospholipids and lipid inclusions in cells. A frozen section is used for this purpose.

Imbedding. During the next sample preparation stage, the tissue is transferred to a mixture of organic solvent and paraffin at 37 ºC and liquid paraffin at 56 ºC and finally cooled to room temperature. The paraffin blocks are prepared.

Sectioning. The tissue paraffin block is cut on special equipment – a ***microtome***, which allows to cut sections with a thickness of 5 μm. A steel blade is used for this technique. A slice of tissue is transferred on a microscope slide. An unstained specimen is prepared.

Staining. Two dyes – hematoxylin and eosin (counterstain) are used in conventional specimen stains. The majority of dyes are water-soluble. The sample is ***deparaffinised*** (paraffin removal from tissue and cells) by immersing the sample in an organic solvent (xylene, benzene, or chloroform) followed by ***rehydration*** – transfer through descending concentrations of alcohol (100 %, 96 %, 90 %, 80 %, 70 %). The next stage is the staining process, which involves immersing the sample first in a hematoxylin solution and then in an eosin solution. Samples are covered with mounting medium and coverslips. A permanent specimen is prepared.

Dye characterization. *Hematoxylin* is the basic blue dye that stains the nucleus (DNA) and ribosomes (rRNA) in the cytoplasm dark blue. Examples of basic dyes – are methyl green, methylene blue, toluidine blue, and safranin. ***Eosin*** is the acidic pink-orange dye which stains the cell cytoplasm pink or red. Examples of acid dyes – are acid fuchsin, aniline blue, and orange G.

1.4. Basic structure of cells

As mentioned at the beginning of this chapter, all living organisms have cellular structures. According to the level of organisation, cells can be divided into ***prokaryotes*** and ***eukaryotes***. Prokaryotes have a simple organisation and do NOT have a nucleus and intracellular membrane organelles. The circular DNA molecule is located in the cytoplasm, and all chemical reactions also occur there. Prokaryotes can only be unicellular, have a plasma membrane envelope with a cell wall, can have flagella, and have a cytoplasm with prokaryotic ribosomes.

Eukaryotic cells have a ***nucleus*** with chromatin, surrounded by two membranes. Generally, the cell's envelope consists of a plasma membrane and over membrane and submembrane complex. Cells have non-membrane, one membrane and two membrane organelles. Non-membrane organelles include the cytoskeleton, centrioles, ribosomes, and proteasomes. One membrane organelle is a smooth, rough endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, and membrane-bound vesicles. Two membrane organelles are mitochondria and chloroplasts (plastids).

Eukaryotic cells can be animals or ***plant*** cells. Characteristic features of plant cells: they have ***cellulose*** cell wall over plasma membrane, sizeable ***central vacuole*** with cell sap, and two-membrane organelles: ***plastids*** (chloroplasts) with chlorophyll for photosynthesis and mitochondria. Plant cells do not have centrioles and can accumulate ***starch*** inclusions.

Animal cells have a glycocalyx as an over-membrane complex and a centriole, no plastids, and glycogen stored as a trophic inclusion. All other types of organelles are found in both animal and plant cells.

Laboratory tasks

Purpose: ***to get acquainted with the principles of microscopy. Master the basic microscopic structure of cells in various tissues.***

A principle of using a microscope

1. Set the objective with low power (4×, red label) in the working position.

2. Put the microscope slide with the specimen on the mechanical stage.

3. Switch on the microscope light.

4. Set the distance between the eyepieces according to your eye distance.

5. Look through the eyepieces and use a coarse adjustment to move the mechanical stage upward until the tissue is in overall focus.

6. Switch to the objective with 10× lens (yellow label) and focus on the tissue.

7. Switch to the objective with 40× lens (blue label) and focus the tissue.

8. Move the specimens and analyse them.

Practical task 1. Analyse a specimen of Frog Blood (Fig. 1)

1. Find the frog erythrocytes (Fig. 1). Look at the erythrocyte nuclei, erythrocyte plasma membrane, and cytoplasm. Analyse the cell size, the cell shape, and the nucleus shape, and pay attention to the cytoplasm colour.

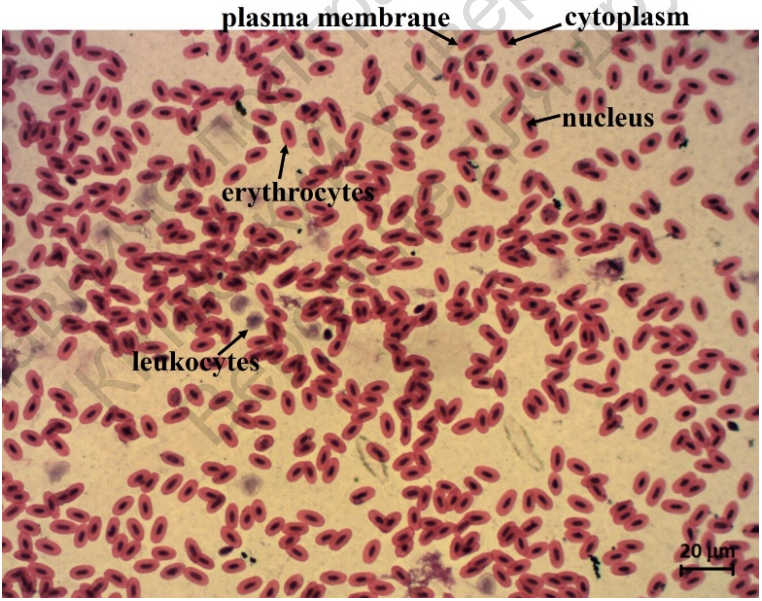


Fig. 1. Light micrograph. Frog Blood
(hematoxylin and eosin staining, ×400)

2. Find leukocytes. Analyse the size of cells, the shape of cells, the shape of nuclei, and the cytoplasm colour.

3. Draw frog blood cells in your Workbook and label the parts of the cells.

Practical task 2. Analyse a histological specimen of Rabbit Live (Fig. 2)

1. Look over the **Rabbit Live** tissue using an objective with 10× lens.

2. Switch to the objective with 40× lens. Find the liver cells (**hepatocytes**). Analyse the size of cells, the shape of cells, the shape of nuclei, and the cytoplasm colour.

3. Find binucleated hepatocytes (liver cells).

4. Draw liver cells in your Work Book and label the parts of the cells and the binucleated cells.

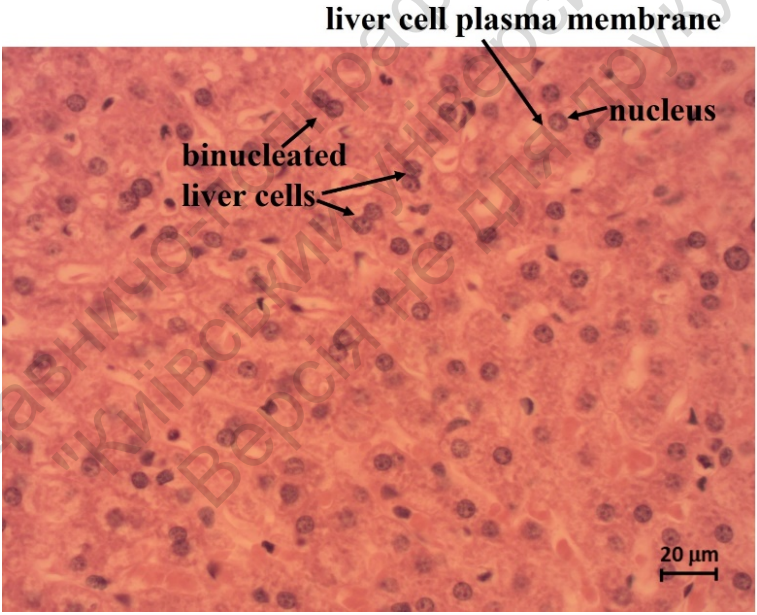


Fig. 2. Light micrograph. Rabbit Liver
(hematoxylin and eosin staining, ×400)

Control questions

1. Nephrectomy (kidney removal) was done on a 36-year-old patient due to a severe inflammatory process in the organ and loss of its function. A morphological examination of tissues is carried out to make a diagnosis. What is the first step of tissue processing?

A. clearing
B. staining
C. embedding
D. mounting
E. fixation

2. The pathologist is asked to analyse tissues acquired during surgery immediately. This information is essential to determine how the operation will proceed. What rapid type of physical tissue fixation can be used?

A. fixation using an acetone solution
B. fixation using a formaldehyde solution
C. fixation using a glutaraldehyde
D. fixation using an ethanol solution
E. freezing using a liquid nitrogen

3. A 16-year-old patient underwent splenectomy (removal of the spleen) due to excessive destruction of red blood cells. A morphological examination of the tissue is necessary to diagnose the disease. Histological specimens should be prepared for microscopic examination. What equipment is used to cut paraffin-embedded tissue?

A. blade
B. microscope
C. thermostat
D. automatic tissue processor
E. microtome

4. For diagnostic purposes, A skin neoplasm biopsy was performed on a 53-year-old patient. The tissue must be morphologically examined. The nucleus shape, specificity of staining, and structure are essential characteristics for tissue alteration assessment. What colour will the tissue be after staining with hematoxylin only?

A. pink cytoplasm
B. red nuclei
C. brown cytoplasm
D. brown nuclei
E. dark blue nuclei

5. The 28-year-old patient has a progression of myopathy (muscle weakness). A muscle tissue biopsy was made for diagnostic purposes. The tissue will be analysed using an electron micrograph. What type of dye is used to stain the specimen for electron microscopy?

A. haematoxylin
B. eosin
C. safranin
D. methylene blue
E. osmium tetroxide

6. After a tissue biopsy, preserving the sample from degradation and preparing thin tissue slices for microscopic evaluation are essential. The tissue must be condensed to facilitate the cutting process. What substance is used to condense tissue during the preparation of a histological sample?

A. immersion oil
B. mounting medium
C. alcohol
D. xylene
E. paraffin

7. What type of microscope is used to examine histological specimens after conventional hematoxylin and eosin stain?

A. light microscope
B. electron microscope
C. fluorescent microscope
D. polarising microscope
E. dark field microscope

8. What type of microscope is used to examine cytological specimens after immunofluorescence stain?

A. light microscope
B. electron microscope
C. polarising microscope
D. dark field microscope
E. fluorescent microscope

9. The medical laboratory scientist assesses a patient's bone marrow smear after fine-needle aspiration. First, he must focus the tissue at low magnification. What part of the light microscope should he use to bring the overall focus to the tissue?

A. condenser
B. objective
C. ocular
D. fine adjustment
E. course adjustment

10. The medical laboratory researcher assesses a patient's liver specimen after a tissue biopsy. After choosing a specimen for analysis at low magnification, further assessment is carried out at high magnification. What parts of the light microscope influence the magnification of an object?

A. condenser
B. objective
C. Ocular
D. Both A and B
E. Both B and C

11. The medical laboratory scientist assesses the vaginal smears of a patient. Many large epithelial cells and lactobacilli are visible during the microscopic examination. Lactobacilli are the most numerous bacteria situated in the healthy vagina of child-bearing women. What organelles can be found in bacteria (prokaryotes) and epithelial cells (eukaryotes)?

A. nucleus
B. mitochondria
C. lysosomes
D. rough endoplasmic reticulum
E. ribosomes

12. During a women's health screening, a 27-year-old woman underwent a cytological examination of smears from the vagina and cervix. The examination revealed the presence of many mature squamous epithelial cells and lactobacilli. What organelles are NOT found in bacteria/ prokaryotic cells (but can be found in epithelial/eukaryotic cells)?

A. plasma membrane
B. cytoplasm
C. ribosomes
D. all of the listed can be found in bacterial cell

13. The student was given two research samples during the exam: one containing animal cells and the other containing plant cells. Which characteristic is NOT specific to animal cells? The options are as follows:

A. plasma membrane
B. mitochondria
C. lysosomes
D. rough endoplasmic reticulum
E. starch deposition

2. CELL ENVELOPE. CYTOSKELETON STRUCTURE AND FUNCTION

2.1. Cell envelop

The cell envelope consists of the plasma membrane, over-membrane and submembrane complex and forms the boundary of a cell.

The plasma membrane comprises ***lipids*** and ***proteins*** in different proportions in different cells. The most abundant lipids in the membranes are ***phospholipids*** (which contain glycerol with a bounded phosphate group), which form a bilayer, ***glycolipids*** (containing sugar residues), ***sphingolipids*** (containing amino alcohol sphingosine) and cholesterol. Glycolipids and sphingolipids are mostly located in the outer leaflet of the plasma membrane bilayer, so they are located on the cell surface and form glycocalyx. It is essential in cell-cell recognition and cell-extracellular substance interaction, which are crucial in tissue organization and receptor function.

2.1.1. Phospholipids

Four main types of phospholipids constitute a phospholipid bilayer: phosphatidylcholine, sphingomyelin, phosphatidylserine and phosphatidylethanolamine. Each of the phospholipids is an amphipathic molecule: the polar part of it (***head*** = glycerol and phosphate group and radical) is charged and can interact with water (***hydrophilic*** = "love" water), the other one (tale = fatty acid = nonpolar) is noncharged and does not interact with water (***hydrophobic*** = "hate" water). In water, phospholipids spontaneously form a **bilayer**. In the bilayer, the polar head faces the external environment and cytosol and fatty acid tails towards each other. The external phospholipid layer is directed towards the external environment and is enriched by phosphatidylcholine and sphingomyelin. Phosphatidylserine and phosphatidylethanolamine are located mainly in the inner phospholipid layer, which faces the cytosol. The last two are very important for cell signalling.

Phospholipids undergo lateral movement and translocation from one lipid layer to another (flip-flop), and enzymes return them. The rigidity of the phospholipid bilayer is provided by ***cholesterol***, which is the sterol lipid specific for only animal membranes. Cholesterol

interacts with the polar head and fatty acids tails of the neighbouring phospholipids stabilises the structure and restricts their movement.

The phospholipid bilayer is semipermeable, permeable only to lipid-soluble substances and limits the diffusion of molecules.

2.1.2. Proteins

The plasma membrane proteins that can be embedded in the phospholipid layer and completely span it are called transmembrane proteins or ***integral*** proteins. They are tightly bound to the phospholipid bilayer. The function of these types of proteins can be the construction of membrane channels, transporters or linkers between cellular and extracellular structures. Peripheral protein is another type of protein that can be anchored to the phospholipid bilayer from the cytoplasmic or extracellular side of the cells. These proteins can be easily removed from the membrane. Membrane-bound enzymes can be such types of proteins.

2.1.3. Glycocalyx

The ***glycocalyx*** is an over-membrane complex that consists of oligosaccharides bound to lipids or proteins (glycolipids and glycoproteins). The glycocalyx is situated on the cell surface of animal cells. It forms receptors, an intercellular junctional complex, and contact between the cell and an extracellular matrix. Glycocalyx provides a concentration of some molecules, ions, water, and enzymes in close proximity to the cells. An example of complex glycolipids is gangliosides, which contain oligosaccharides with several sialic acid residues and provide a negatively charged cell surface. The nerve cell membrane is enriched on the gangliosides, which are up to 30-fold higher than other tissues and play a crucial role in nerve tissue function. Reduced production and levels of gangliosides can lead to developmental issues in the nervous system during both embryonic and postembryonic stages, as well as severe neurodegenerative disorders in adults.

2.2. Membrane transport

As mentioned above, the plasma membrane is semipermeable and forms a cellular barrier. The phospholipid bilayer is permeable only to lipid-soluble molecules (vitamin A, D, E, organic solvent xylene, benzene, chloroform), small polar molecules (water, ethanol) and

medications. Some gases (O_2, CO_2, NO) can move through the phospholipid bilayer by ***simple diffusion*** (without energy expenditure) according to the concentration gradient (from high concentration towards low concentration). Charged molecules (ions) and large molecules (glucose, amino acids) cannot move freely through the membrane.

Facilitated diffusion is the energy-independent transport of molecules of a specific size and conformation according to a concentration gradient through membrane ***channels*** and transmembrane proteins, forming a canal in the membrane. Channels transport different types of ions. The same kind of transport is carried out by ***carrier proteins***, which bind specific molecules on one side of the plasma membrane and transfer them to the other: the carrier proteins transport glucose, nucleosides, and amino acids.

Active transport. To maintain viability and change from an inactive state to an active state and vice versa, cells maintain different concentrations of various ions inside and outside the cell. For example, the cytoplasm has higher concentrations of K^+, H_2PO^{4-}, and HPO^{4-} ions compared to the extracellular space, where the concentrations of Na^+, Ca^{2+}, and Cl^- are higher. Membrane ***pumps*** use ATP energy and transport ions against a concentration gradient to keep their different concentrations outside and inside cells. Transport of one molecule is called ***uniport*** (glucose), two molecules in one direction are called ***symport*** (glucose and Na^+, amino acids and Na^+), and two molecules in the opposite direction are called ***antiport*** (Na^+ and K^+, H+ and K^+).

Vesicular transport. Some molecules (large molecules) are transported in membrane-bound vesicles, and this process depends on energy. Molecules uptake and the vesicle formation is called endocytosis; releasing substances from the vesicles is called ***exocytosis***. Receptor-mediated transport can be carried out in ***clathrin***-coated vesicles (for example, cholesterol undergoes such type of transport). Alternative or clathrin-independent transport can be by **caveolin**-coated vesicles (protein albumin undergoes such kind of transport). Liquid uptake and transport in membrane-bound vesicles is called ***pinocytosis.*** Engulf of particles and transport in membrane-bound vesicles is called ***phagocytosis; in this case,*** the vesicles are phagosomes.

2.3. Cell signalling

The plasma membrane provides extracellular signal recognition (binding) by membrane ***receptors*** and transmission signals from outside to inside the cells. The cytoplasmic part of the receptors can possess enzymatic activity or activate a chain of enzymes that propagates signals between different cellular compartments and modulates cellular metabolism, ion channels, activity of target genes, cytoskeleton arrangement, which regulates cell motility and others.

Proteins and phospholipids of the plasma membrane can undergo lateral movement, movement between phospholipid bilayer, rotate, and fatty acids of phospholipids can tilt, thereby forming an extremely dynamic structure. These properties of plasma membrane were described in "Singer-Nicolson Fluid-Mosaic Model of Membrane Structure" (1972) and its updates (2013).

What functions does the cell envelope perform?

1. A ***Barrier*** function, ***protection***. (It ***insulates*** the inner content of cells from the external environment).
2. The cell envelope forms the ***junctions*** between cells and the extracellular matrix in the tissue.
3. Provide ***selective permeability*** for substances which can move through the membrane.
4. Membrane transport.
5. ***Cell signaling***.
6. Separate cell compartments.

2.4. Cytoskeleton structure and function

Cells have a network of **non-membrane organelles** that performs various functions: provides mechanical support (maintains the shape of cells), participates in changing of cell's form during division, movement, phagocytosis, translocates organelles and vesicles inside cells and others. Three basic types of cytoskeletons exist in the cells: microfilaments, microtubules and intermediate filaments.

2.4.1. Microfilaments are fibrillar structures with polymerised globular actin proteins after binding ATP molecules. Magnesium ions assist this process. It is the thinnest cytoskeleton with a cross-sectional diameter of approximately 7 nm, which is why it is called a

microfilament. They are concentrated beneath the cell's plasma membrane and comprise its cortical layer. Microfilaments are dynamic structures in cells; they assemble and decompose during changes in cell functional state, and this process is called ***treadmilling***. Microfilaments are polar structures, with a "positive" end (which binds new globules of actin monomer) and a "negative" end (on this side, the disassembling of globular actin monomer occurs).

Microfilaments provide cell segregation at the end of mitotic division. A ***contractile ring*** is formed for cytokinesis. Polymerisation of actin filaments in the cell's equatorial plate, interaction with myosin and contraction assist cell separation. In addition, some types of muscle diseases, such as myopathy, are caused by mutations in the genes encoding the cytoskeleton, which subsequently disrupts the function of muscle cells.

Microfilaments are concentrated under the plasma membrane and form the ***cortex*** or cortical layer of the plasma membrane together with another cytoskeleton. Alteration of this layer disrupts vesicular transport. Microfilaments participate in the formation of cell junctional complexes. ***Adherens junctions*** provide attachment of actin microfilaments of neighbouring cells in cell-cell interaction and provide mechanical strength of junctional complex. Integral membrane proteins ***cadherins*** with assistant linker proteins vinculins and catenins connect microfilaments of cells. ***Focal adhesion*** equips cells to attach to the extracellular matrix with the participation of cellular integral protein integrins. Migration of cells in the tissue extracellular spaces or phagocytosis associated with the formation of cytoplasmic protrusions such as lamellipodia, filopodia (micro spikes), and pseudopodia with a core of polymerised microfilaments. Numerous finger-like protrusions microvilli have a parallel arranged polymerised actin core on the surface of active transporting cells, such as intestinal epithelial cells. The linker protein *alpha-actinin* or *spectrin* anchors microfilaments to the plasma membrane, ***villins*** and ***fimbrins*** *bunch* microfilaments, ***and filamins link*** microfilaments in three-dimensional grids.

2.4.2. Microtubules are hollow rods that are composed of polymerised globular protein ***tubulin***. Two tubulin subunits (alpha and beta) form a dimer, which assembles into a fibrillar protofilament.

13 protofilament molecules are assembled into hollow rods called microtubules with an outer diameter of 25 nm and an inner diameter of 14 nm. Like microfilaments, microtubules are polar structures, with a "plus" end where a new tubulin dimer is added and a "minus" end where tubulin breaks down. Unlike microfilaments, GTP is required for the polymerisation of microtubules. Another type of tubulin – *gamma*-tubulin, forms a ring and begins to grow a microtubule. A decrease in the concentration of the GTP-tubulin complex in the cytosol causes the disintegration of microtubules, and this process is called a catastrophe. The growth and decay of microtubules are called ***dynamic instability***. Some proteins (microtubule-associated protein = MAP2, tau protein) are associated with microtubules, stabilising their structure and allowing them to be bundled.

Microtubules serve as roads for the movement of organelles and vesicles in cells**.** Two motor proteins can move with the cargo along the microtubules: ***kinesin*** moves towards the "plus" end, and dynein moves towards the "minus" end.

Microtubules constitute the ***mitotic spindle*** during mitotic cell division. In cell culture, colchicine is added to stop microtubule polymerisation by blocking the attachment of new tubulin monomers. This makes it possible to analyse the morphology of chromosomes since they are in the highest condensation state.

A set of microtubules forms the core of a mobile protrusion on the cell's surface: a single ***flagellum*** or numerous ***cilia***. Flagellum and cilium have nine duplets of microtubules at the periphery and two central microtubules. Movement of cilia and flagellum is provided by dynein. Mutations in genes encoding dynein cause the development of immotile cilia and primary ciliary dyskinesia with subsequent development of respiratory and auditory disorders and infertility.

Tubulins constitute a non-membrane organelles **centriole**: hollow structures of nine triplets of microtubules on the periphery without the central microtubules. Their length is approximately 500 μm. Two centrioles are perpendicular to each other and form the cell centre or ***centrosome***. On the surface, one of two centrioles in the centrosome distal appendages are assembled, and this centriole is called the ***mother centriole***. The centrosome is the site of initiation of microtubule polymerisation, and distal appendages and gamma-tubulin are involved

in this process and are named Microtubule Organizing Centre. The basal body is a centriole derivative, has the same structure as the centriole, and provides cilium growth on the cell surface.

2.4.3. Intermediate filaments are the third primary type of cell cytoskeleton. Unlike microtubules and microfilaments, intermediate filaments are fibre proteins whose primary function is the mechanical support of cells. For different types of tissues, the protein of intermediate filaments is specific. For example, ***keratin*** is specific for ***epithelial*** tissue, ***desmin*** for ***muscle*** tissue, ***vimentin*** for mesodermal cells such as ***connective*** tissue, ***neurofilament*** for ***neural*** tissue, and ***nestin*** for nerve stem and progenitor cells. Antibodies that target these proteins are utilized in tumor diagnostics because tumors can arise from various tissue types, and cells often lose their specific morphological features, making diagnosis challenging. For instance, anti-keratin antibodies can stain cytokeratins in various types of cancer and their metastases. Specific subtypes of cytokeratins aid in distinguishing between different cancer subtypes. For example, cytokeratin 17 is highly expressed in squamous carcinomas, while cytokeratins 8 and 18 are prominent in adenocarcinomas. Neuroblastomas show positive staining with neurofilament antibodies. Vimentin serves as a marker for mesenchymal cells in epithelial-mesenchymal transition, a essential process in cancer metastasis development.

Intermediate filaments participate in the formation of the cell-cell junction ***desmosomes*** or cell-extracellular matrix junction ***hemidesmosomes***.

2.5. Plasma membrane and cell junctions

The plasma membrane and cytoskeleton are involved in the formation of cell junctions. Different classes of transmembrane proteins form various types of junctions. For example, ***cadherin*** family proteins are involved in forming ***tight junctions*** and ***desmosomes*** in intercellular joining, and ***integrin family proteins*** are made up of ***focal adhesion*** *and* ***hemidesmosomes*** that attach cells to the extracellular matrix.

According to the function, cell junctions can be classified as ***insulating*** (tight junction), ***adhesive junctions*** (desmosomes, hemidesmosomes, adherens junction, focal adhesion) and ***communicating junctions*** (nexus, synapse).

Tight junction or ***occluding junctions*** connect *actin microfilaments* of neighbouring cells through a chain of transmembrane protein occludins or claudins and the linker protein zonula occludens. The function of this type of junction is to insulate one part of intercellular spaces from the other.

Desmosomes belong to the anchoring junctions and connect *intermediate filaments* of adjacent cells by applying transmembrane proteins of the *cadherin* family (desmogleins and desmocollins) and cytoplasmic linker proteins desmoplakins. ***Hemidesmosomes*** hold cells on extracellular matrix proteins that include *integrins* and transmembrane proteins.

Focal adhesion anchors cells to the extracellular matrix with the participation of *actin microfilaments* and transmembrane protein *integrins.*

Nexuses are communication junctions composed of six subunits of the nexin protein and form canals between cells.

Synapses connect nerve cells (communicating junctions) and provide impulse transmission.

Clinical case 1. Cytoskeleton disorder

A twelve-month-old patient was admitted to the hospital due to a severe cough. History of disease revealed he had nasal obstruction at once he was born. During his first year, he had a cough with excessive mucus production, a practically permanent runny nose, and deep crepitations. Secretory otitis media and bronchiectasis were diagnosed after Computed Tomography. Puncture of the nasal epithelium was done. Basic analysis of the epithelium using light microscopy and electron microscopic analysis was performed. The protrusions of the apical surface of the epithelium were studied in detail. Surface structures consist of nine tubular duplets surrounded by plasma membrane. Two tubular duplets are located in the centre. Peripheral duplets of microtubules interconnected by two arms of motor protein dynein in a healthy person. Only one externally located arm was observed between the peripheral doublets in this patient. Primary

ciliary dyskinesia was presumed. Molecular analysis was done. Gene mutation in the dynein axonemal assembly factor 6 (which enables dynein intermediate chain binding activity and is located on the X-chromosome) was found. A sample of the parents' epithelium was analysed, and the maternal mutation was determined.

Conclusion. Primary ciliary dyskinesia was diagnosed.

Clinical case 2. Cell junction disorder

A sixty-year-old man who belongs to the Caucasian population visited the hospital due to interminable non-healing ulcers. A physical examination found that one-third of the patient's body had been covered by injured skin. The history of anamnesis clarifies the hips or/and, knees or/and, back or/and shoulders have persistent ulcerations after birth. The patient had used a variety of ointments with additives to moisture-altered areas to reduce the pain level and prevent bacterial load. A member of his family had a confirmed diagnosis of Epidermolysis bullosa. A complete blood count test was prescribed, and leukocytosis with neutrophilia was revealed. Microbiological analysis of tissue biopsies demonstrated a variety of Gram-positive bacteria that commonly colonise damaged skin. Indirect immunofluorescence, enzyme-linked immunosorbent assay and immunoblotting were done. Mixtures of autoantibodies against protein desmoglein and antibodies against BP230 hemidesmosome protein have been found.

Conclusion. Bullous pemphigoid coexisting with anti-desmoglein autoantibodies has been diagnosed. Supportive therapy is prescribed.

Laboratory tasks

Purpose: ***Master the microscopic structure of the surface apparatus of cells, plasma membrane, and cytoskeleton.***

Practical task 3. Analyse microscopic features of epithelial cells' surface apparatus (Fig. 3)

1. Look over the epithelial tissue using an objective with 10× lens.

2. Switch to the objective with 40× lens. Find the free surface of epithelial cells. Analyse the morphological appearance of cilia.

3. Find the localisation of the basal body.

4. Draw the liver cells in your Work Book and label the parts of cells, focusing on the cilia and the basal body.

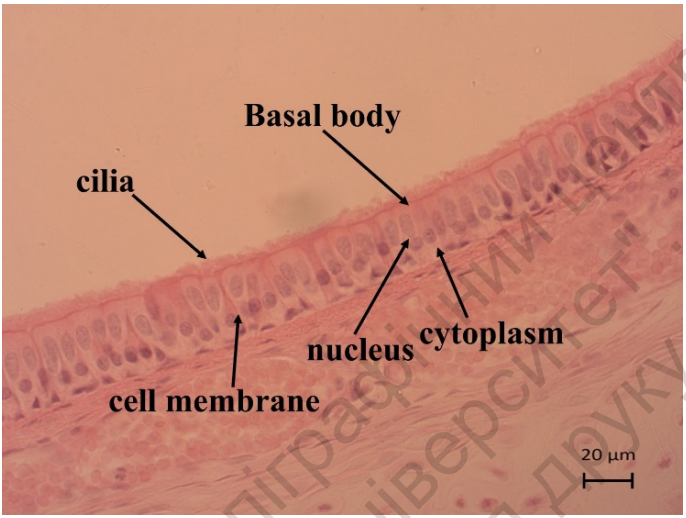


Fig. 3. Light micrograph.
Epithelial cells surface apparatus of the trachea: cilia
(hematoxylin and eosin staining, ×400)

Practical task 4. Analyse a microscopic feature of cilia or flagella axoneme (Fig. 4)

1. Look over the electron microscope image of a cross-section of cilia or flagella. Find plasma membrane and cytoplasm.

2. Analyze a skeleton organisation: the peripheral doublet of microtubules, A-tubule, B-tubule, dynein arms, and the central pair of microtubules.

3. Label the parts of cilia or flagella in your Workbook.

Practical task 5. Analyse microscopic features of neuron intermediate filaments: neurofibrilles in the histological specimen of the spinal cord (Fig. 5)

1. Look over the spinal cord cross-section using 10× objective. Find large motor neurons in the ventral horns. Find plasma membrane, cytoplasm nucleus, and nucleolus in neurons using 40× objective.

2. Analyse a skeleton organisation: a bundle of neurofilaments in the neuronal processes in the cell soma.

3. Draw nerve cells in your Workbook and label the parts of cells, focusing on neurofilaments.

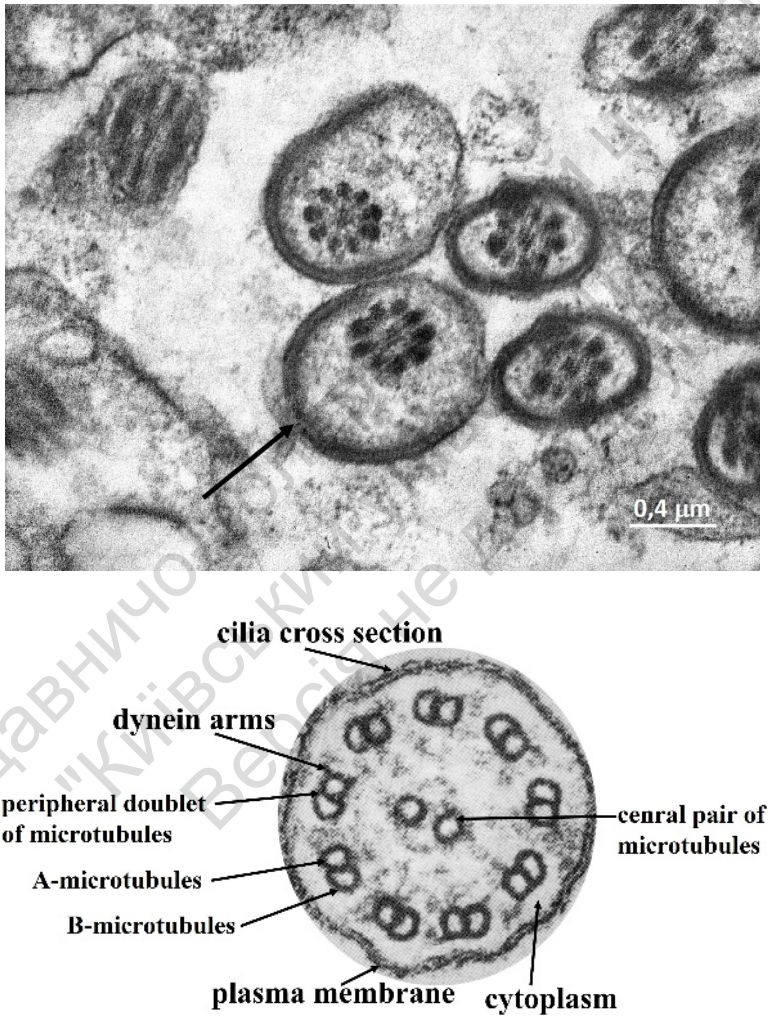


Fig. 4. Electron micrograph. Axoneme cross section

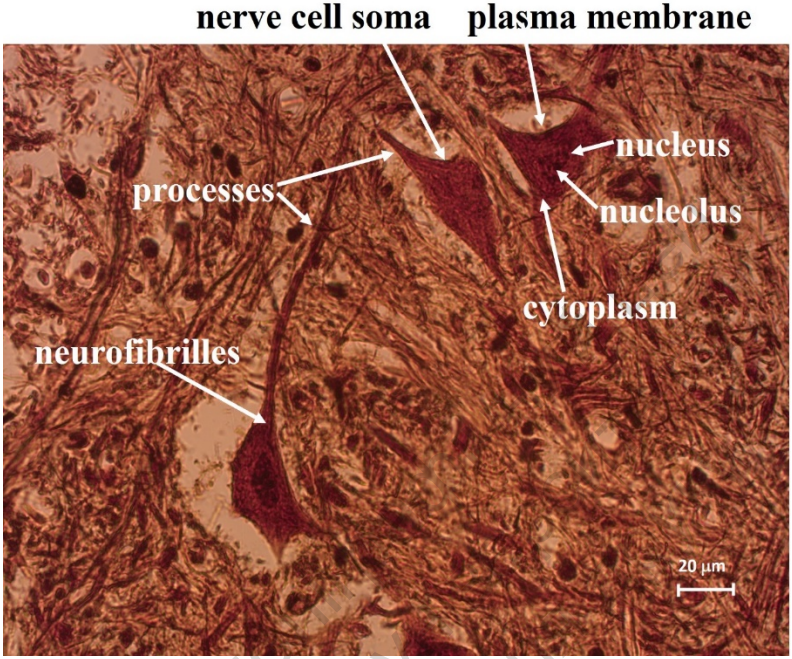


Fig. 5. Light micrograph. Spinal cord cross section. Neurofibrilles
(silver nitrate impregnation by Golgi and Ramon y Cajal, ×400)

Practical task 6. Analyse the microscopic features of the Desmosome (Fig. 6)

1. Look at the electron microscope image of the Desmosome. Find the plasma membrane of neighbouring cells, the cytoplasm.
2. Analyse a skeleton organisation: the intermediate filaments and dense cytoplasmic plaque.
3. Label the parts of the Desmosome in your Workbook.

Practical task 7. Analyse microscopic features of tight junction, a synonym for zonula occludens (Fig. 7)

1. Look at the electron microscope image of the Tight junction. Find the plasma membrane of neighbouring cells, the cytoplasm, and extracellular spaces.

2. Note! Transmembrane proteins occluding and claudin make junctional complex. **Actin filaments** strength Tight junctions.

3. Label the parts of the Tight junction in your Work Book.

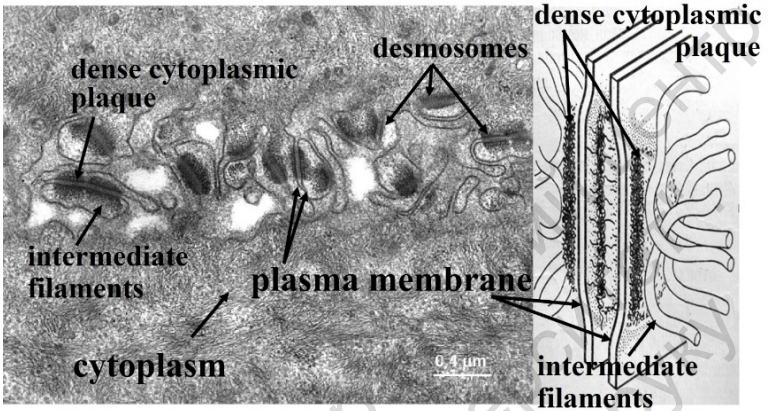


Fig. 6. Electron micrograph. Desmosome and diagram of desmosome organization

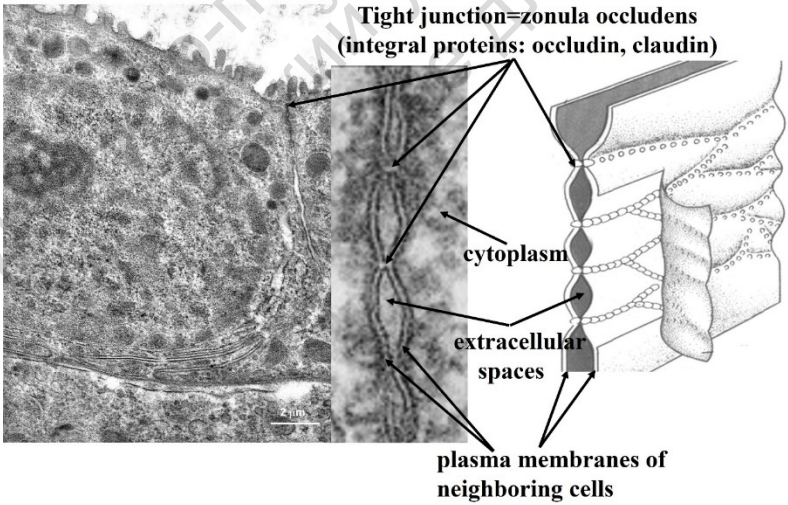


Fig. 7. Electron micrograph and Diagram. Tight junction

Practical task 8. Analyse a diagram of Gap junction; its synonym is Nexus (Fig. 8)

1. Look at the Nexus diagram. Find the plasma membrane of neighbouring cells, cytoplasm, and extracellular spaces.

2. Note! Six transmembrane proteins connexin make a complex with the channel in the central part.

3. Label parts of Nexus in your Work Book.

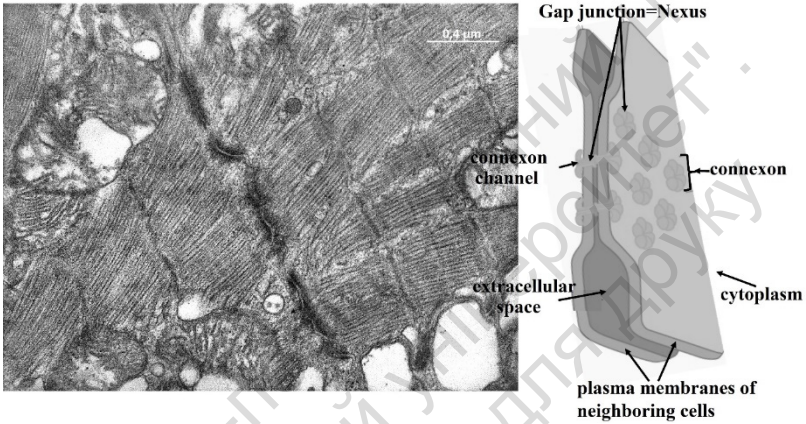


Fig. 8. Diagram. Gap junction (Nexus)

Control questions

1. When an experimental animal is exposed to a high dose of ionising radiation, a scientist analyses an electron micrograph of epithelial tissue. The scientist observes alterations in the structure of non-membrane, single- and double-membrane organelles. Which organelles within the cell lack a membrane?

A. lysosomes
B. rough endoplasmic reticulum
C. smooth endoplasmic reticulum
D. peroxisomes
E. ribosomes

2. The researcher examines electron micrographs of muscle tissue biopsies from volunteers who have undergone a series of sports workouts and have been given a new biologically active supplement. The analysis shows that the structure of single-membrane organelles has undergone significant development. Can you identify which cell organelles have a single membrane?

A. lysosomes
B. rough endoplasmic reticulum
C. smooth endoplasmic reticulum
D. peroxisomes
E. All of the listed

3. The 21-year-old patient has extreme polyuria (Excessive Urine Production). This condition is caused by the intake of Lithium medication for the treatment of the bipolar disorder. Lithium suppresses the expression of the aquaporin gene, reducing its mRNA production and transcription. The aquaporin protein is a water channel transporting water through the plasma membrane. What type of protein crosses the cell envelope?

A. peripheral protein
B. semi-integral protein
C. non-integral protein
D. non-peripheral protein
E. integral protein

4. A pregnant woman suffers from deep vein thrombosis of the lower extremities. Antiphospholipid syndrome (autoimmune disease) has been diagnosed. This disorder is associated with the production of anti-cardiolipin antibodies. Cardiolipin is one of the membrane phospholipids. How many layers do phospholipids form in cell membranes?

A. one
B. tree
C. four
D. five
E. two

5. Senescent red blood cells (RBC) in the RBC storage are associated with the degradation of membrane-bound carbohydrates, which consequently causes the removal of such cells from the

circulation by macrophages after transfusion. Membranous carbohydrates are attached to the plasma membrane:

A. on the one side of the membrane, facing the cytoplasm
B. on both sides of the membrane
C. between the layers of the lipid bilayer
D. between hydrophobic tails of lipids
E. on the one side of the membrane, facing the intercellular space

6. A 60-year-old man came to the clinic with a long-standing rash on his chest and legs that had worsened over the past few years. After examining biopsies of the affected skin, doctors diagnosed him with Darier disease. This condition is linked to a gene mutation that affects the formation of calcium-dependent transmembrane proteins in the cell junctional complex. Which cell junction links to the actin filaments of the cytoskeleton of two cells?

A. desmosome
B. hemidesmosome
C. gap junction
D. Connexon
E. adherent junction

7. Pemphigus vulgaris is an autoimmune disease characterised by the formation of autoantibodies against junctional proteins in cells, leading to skin blistering. Do you know which type of cell junction links the cytoskeletal intermediate filaments of two cells?

A. adherent junction
B. hemidesmosome
C. gap junction
D. tight junction
E. desmosome

8. In electron micrographs of the cell surface, microvilli are visible as finger-like protrusions that increase surface area. What type of cytoskeleton makes up the core of the microvillus?

A. tubulin
B. desmin
C. keratin
D. vimentin
E. actin

9. Some proteins anchor microfilaments to the intermediate filament's terminal web at the microvilli's bases to stabilise microvilli on the surface of cells. The same type of protein provides microfilaments with a fixture on the plasma membrane for cell shape stabilisation (erythrocytes). What kind of protein offers such binding?

A. fimbrin
B. filamin
C. Mini-myosin
D. Gelsolin
E. spectrin

10. Antibodies against intermediate filaments specific to certain tissue types are used to investigate the origin of cancer cells for diagnostic purposes. What type of protein constructs connective tissue intermediate filaments?

A. glial fibrillary acidic protein
B. protein of neurofilaments
C. desmin filaments
D. keratin filaments
E. vimentin filaments

11. The 16-year-old patient has significant swelling on the back. After the diagnostic puncture, cytological specimens were made. Some atypical cells were found in the slides. What type of antibody against intermediate filaments should be applied to identify epithelial cells?

A. antibody against actin
B. antibody against tubulin
C. antibody against myosin
D. antibody against vimentin
E. antibody against keratin

12. An 83-year-old man with Alzheimer's disease died. After the autopsy, the nervous tissue analysis revealed the presence of aggregates of proteins associated with the cytoskeleton in the neurons. These proteins block cell transport and ultimately damage nerve cell function. What type of protein is assembled into cylindrical structures and forms tubules of the cytoskeleton?

A. actin
B. tubulin
C. keratin
D. dynein
E. myosin

3. CYTOSOL STRUCTURE AND FUNCTION. INCLUSIONS

3.1. Cytoplasm and cytosol

The internal content of cells, which is located under the plasma membrane with the exception of the nucleus, is called ***cytoplasm***. ***Cytosol*** is a liquid part of cells without the nucleus and organelles. The cytosol is a viscous fluid containing 50 % to 90 % water, dissolved ions, and organic molecules. Organic molecules include glucose, polysaccharides (in animal cells, glycogen), amino acids, proteins, lipids (triglycerides), and fatty acids. The cytoskeleton anchors molecules and organelles in the cytosol. Polymerisation and depolymerisation of the cytoskeleton change the cytosol's fluidity and regulate the activity of various processes. The more liquid state of the cytosol is called a ***sol***, the more *viscous* state is called ***gel***. Cytosol has buffering properties.

Cytosol is the site of intermediate metabolism. For example, the initial stages of glucose degradation to pyruvate with the synthesis of the two energy molecules of ATP occur in the cytosol.

3.2. Cytosol and protein synthesis

Cytosol is a place for protein synthesis. Ribosomes carry out polymerisation of amino acids into a polypeptide chain called ***translation***. ***Ribosomes*** are non-membrane organelles with two subunits, large (60S) and small (40S). "S" is the sedimentation rate of structure; eukaryotic ribosomes are 80S, and prokaryotic ribosomes smaller are 60S (50S and 30S subunits). Each ribosomal subunit comprises a complex of proteins and ribosomal RNA (rRNA). The large number of ribosomes in cells provides basophilia of the cytoplasm; ribosomes react with the basic dye (for example, hematoxylin) and colour it in a bluish or purplish colour. In an electron micrograph, cells with active protein synthesis are darker (ribosomes are stained black with osmium tetroxide and provide black granular staining of the cytosol, where it accumulates).

All types of proteins *start* polymerisation in the cytosol. Some of them complete their cytosol synthesis: these proteins will function in the *cytosol, nucleus, mitochondria* or *peroxisomes*. Others will continue their synthesis on the membrane of the rough endoplasmic

reticulum and will be packaged in vesicles. They are proteins that will be embedded in the *plasma membrane*, *lysosomal* enzymes, or *export proteins* (that will be released from cells, for example, extracellular matrix proteins, pancreatic enzymes, and hormones). Those proteins, which will complete their synthesis on the rough endoplasmic reticulum, have a short hydrophobic amino acids chain called a ***signal sequence***. In the cytoplasm, signal sequence binds signal particles, and complex translocates from cytosol and anchors on the rough endoplasmic reticulum membrane. Signal sequence is a complex of proteins and RNA.

Protein Synthesis passes several stages: initiation, elongation, termination and folding. Folding is a process of obtaining by protein three-dimensional conformation, which is accelerated and controlled by chaperons, foldase, and ***BiP*** proteins (**B**inding **i**mmunoglobulin **P**rotein). Old, altered or misfolded proteins bind a chain of small proteins ***ubiquitin*** and are degraded by the non-membrane organelles ***proteasome***. The proteasome is a protein complex which consists of a cylindrical degradation chamber with two regulatory subunits (caps).

3.3. Inclusions

Different substances can be accumulated in the cytoplasm; they are called inclusions. According to the inclusion functions, they are classified as ***trophic*** (*glycogen*, *lipids* (triglycerides), *yolk* (lipid-protein complex) in animal cells, *starch* and lipids in plant cells), ***excretory*** (lipofuscin is a gold-brown waste product of undigestible material, also called age pigment), ***pigment*** inclusions (colored products of cellular metabolism, such as melanin, pheomelanin (in person with red hear color), lipofuscin, haemoglobin and myoglobin), protective (melanin) and ***secretory*** (membrane-bound vesicles with secretory substances).

The inclusions can be categorized based on the **chemical composition**. In animal cells, carbohydrates are often represented by ***glycogen***, a form of glucose polymer that is intensively stored in highly active muscle and liver cells. Periodic Acid-Schiff staining and Best's carmine staining are used to observe and evaluate glycogen levels in cells. An imbalance in the activity of enzymes involved in glycogen metabolism can result in excessive accumulation of glycogen in cells, indicating a pathological condition. Different

patterns of glycogen storage in blood cell precursors can assist in the diagnosis of various types of leukemia. Examples of protein inclusion include enzymes in the digestive gland, which are accumulated in membrane-bound vesicles in the cell cytoplasm before being released.

Lipids and their derivatives are accumulated in cells in different-sized lipid droplets in their cytoplasm. Chemically, lipids are mostly triglycerides but can also be cholesterol derivatives in endocrine cells, which are specialised in steroid hormone secretion, sebum in sebaceous glands or vax in ceruminous glands. In white adipose tissue, one large lipid droplet accumulates in the cytoplasm; in contrast, in brown adipose tissue or other cells, numerous small droplets are scattered in the cytoplasm. Lipids products are clearly visible after staining with Sudan (orange, black) or osmium tetroxide.

Minerals deposition can be calcium ions (Ca^{2+}) or other polyvalent ions, such as Fe^{2+} or Cu^{2+} etc. They can be bound to protein as a transport or storage form (haemosiderin, metallothionein and glutathione complex) or are accumulated as mineral crystals due to pathological mineralization (calcium phosphate, calcium apatite, calcium oxalate crystals, etc). They mainly concentrated in cytosol, nuclei, smooth endoplasmic reticulum, lysosomes and mitochondria.

Hemosiderin is a brown pigment, an iron-containing complex which is an indigestible product of haemoglobin and ferritin. Hemosiderin is mostly found in the cytoplasm of macrophages, which digest altered and senescent erythrocytes and accumulate iron ions in complex with proteins to reduce iron toxicity.

Melanin is a brown, membrane-bound pigment produced by the oxidation of tyrosine by tyrosinase and its transformation into melanin granules in organelles called melanosomes in melanocytes.

Lipofuscin is a natural golden-brown pigment, a metabolic product that accumulates in all types of cells with age**.** That's why it's often referred to as the "aging pigment" or "wear-and-tear" pigment. It is classified as an excretory inclusion and is a complex of highly oxidized and covalently bonded proteins with lipids, sugar residues, and metal ions. Lipofuscin is mainly found in long-lived and metabolically active cells, such as skeletal muscle cells, cardiomyocytes, and neurons in their lysosomes. It does not affect cell viability and functional activity at low levels of accumulation, but it does alter their

activity significantly when a large amount is deposited and overload the cell volume.

The peculiarity of inclusion accumulation in various cells, their types, and their distribution pattern are widely used in disease diagnosis.

Clinical case 3. Inclusion disorder

An investigation of the influences of different approaches in muscle training on muscle lipofuscin accumulation and its relationship with antioxidant enzymes has been conducted.

Lipofuscin accumulation accompanies various conditions in various cells of the human body. Virus infections, ageing and inflammatory processes, and the toxic effect of multiple substances induce cellular accumulation of lipofuscin, an undigested product of complex proteins, lipids, polysaccharides, and membrane breakdown. Reactive oxygen species and free radicals are standard products of various types of inflammation, produced by immune cells to destroy invaders, but have damaging effects on surrounding molecules, cells and tissues and promote lipofuscin lay down.

Muscle puncture and muscle fibres (derivatives of cells) analysis were performed before and after resistance training. The results demonstrate no increase in lipofuscin accumulation and no disturbance in the antioxidant activity after two months of training.

In **conclusion**, the resistant training process does not alter metabolism and does not cause undigested material deposition in muscle cells.

Laboratory tasks

Purpose: ***Master the microscopic structure of cytosol organisation and inclusions.***

Practical task 9. Analyse a histological specimen of the Parotid salivary gland (Fig. 9)

1. Look over the epithelial tissue of the Parotid salivary gland using an objective with 40× lens.

2. Find the cell nucleus, plasma membrane, and cytoplasm. Focus on numerous protein **secretory** inclusions in the cytoplasm.

3. Draw tissue in your Workbook and label the parts of cells and secretory inclusions.

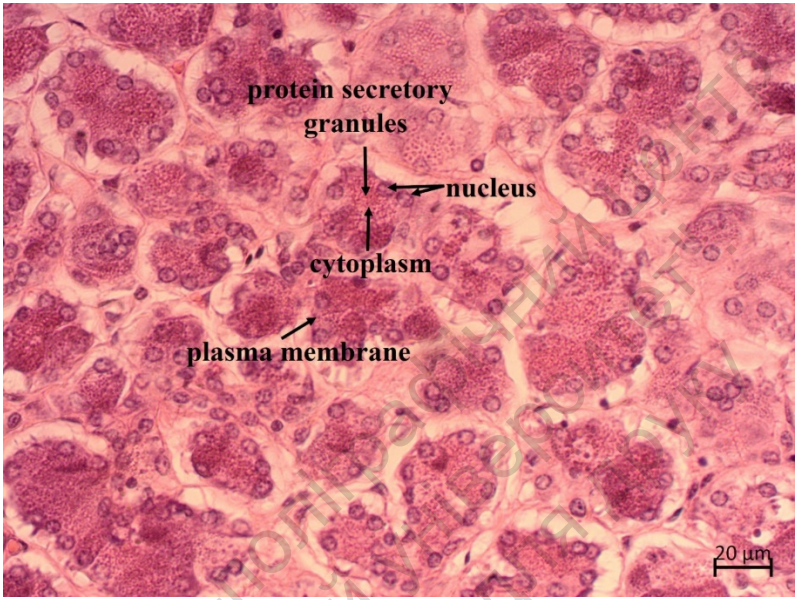


Fig. 9. Light micrograph. Parotid salivary gland. Secretory granules (hematoxylin and eosin staining, ×400)

Practical task 10. Analyse the histological specimen of Frog Blastula (Fig. 10)

1. Look over the Frog Blastula using an objective with 10× and 40× lenses.

2. Find the blastomere plasma membrane, blastomere cytoplasm, and blastomere nucleus. Focus on numerous **trophic *yolk*** (phospholipoproteins) inclusions in the cytoplasm.

3. Draw blastomeres in your Workbook and label parts of cells and trophic yolk inclusions.

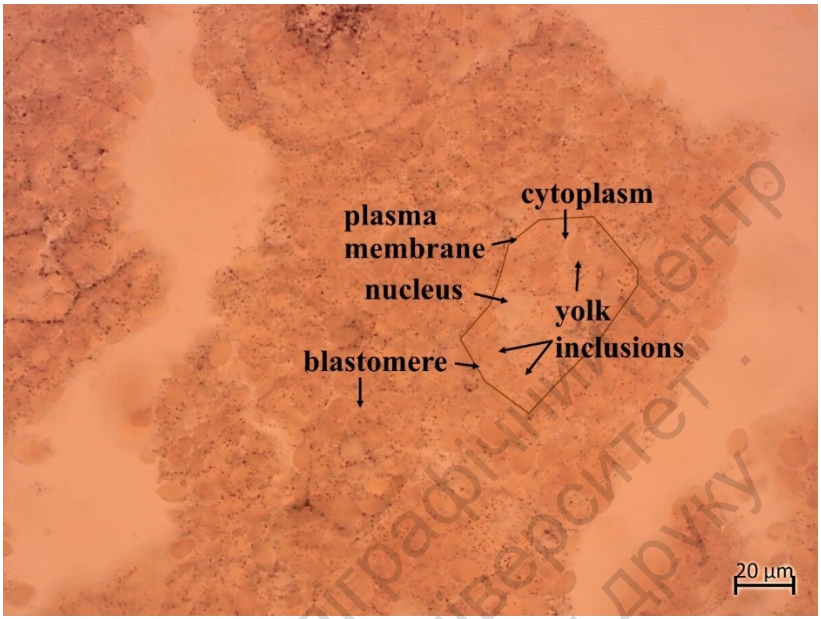


Fig. 10. Light micrograph. Frog blastula.
Yolk inclusions in the amphibian blastomere
(picric acid staining, ×400)

Practical task 11. Analyse a histological specimen of Rat liver (Fig. 11)

1. Look over the Rat liver tissue using an objective with 40× lens.
2. Find liver cells (hepatocytes), their plasma membrane, cytoplasm, and nuclei. Focus on numerous sites of non-stained **trophic *lipid*** inclusions (primarily triglycerides) in the cytoplasm after hematoxylin staining (Fig. 11, A).
3. Look over the micrograph of Rat liver tissue after **osmium tetroxide** staining and memorise the black staining of lipid inclusions (Fig. 11, B).
4. Draw hepatocytes in your Workbook and label the parts of cells and trophic lipid inclusions.

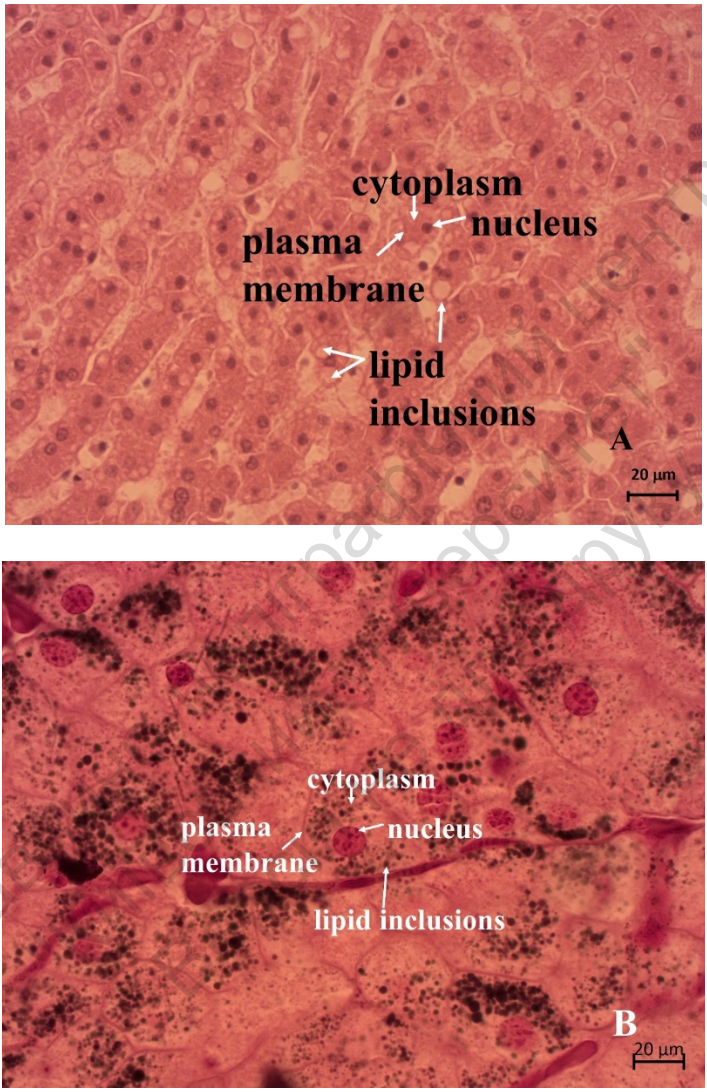


Fig. 11. Light micrograph
A) Human liver. Lipid inclusions in the liver cells
(hematoxylin and eosin staining, ×400).
B) Axolotl liver. Lipid inclusions in the liver cells
(osmium tetroxide and safranin, ×400)

Practical task 12. Analyse a histological specimen of Frog Skin (Fig. 12)

1. Look over the specimen of Frog Skin using an objective with 40× lens.

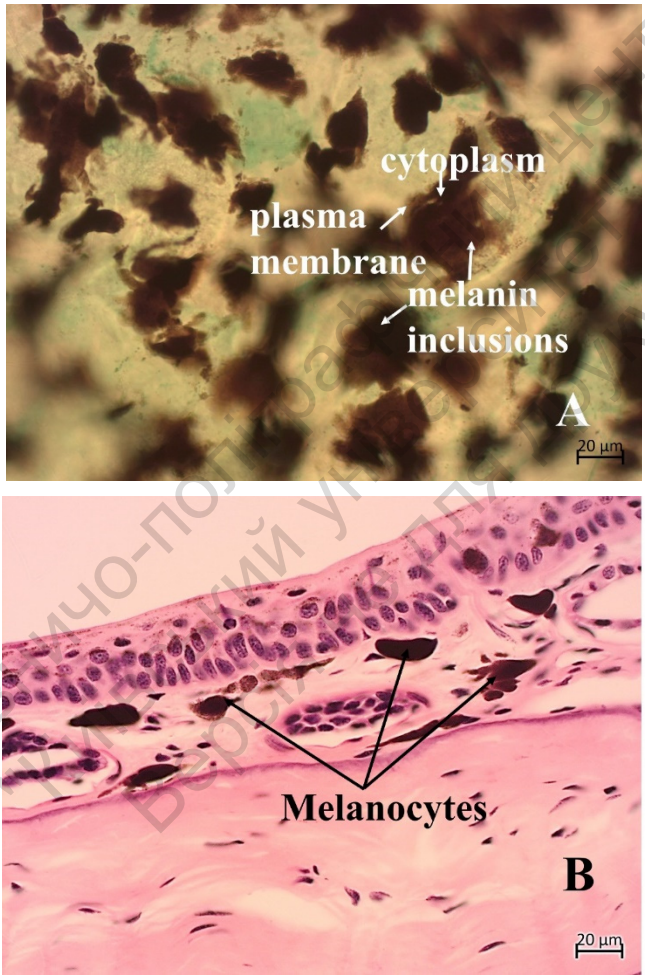


Fig. 12. Light micrograph. Frog Skin. Melanin inclusions in melanocytes.
A) Epidermis, surface view (non-stained, ×400).
B) Cross section of skin (hematoxylin and eosin staining, ×400)

2. Find melanocytes, their plasma membrane, and cytoplasm in the specimen of the surface epidermis of skin (Fig. 12, A) and in the cross-section of skin (Fig. 12, B). Focus on numerous **pigment and protective *melanin*** inclusions in the cytoplasm and their natural **brown-black** appearance.

3. Draw melanocytes in your Workbook, label the parts of cells and pigment and protective ***melanin*** inclusions in cytoplasm.

Practical task 13. Analyse a histological specimen of Rat liver after Best's staining (Fig. 13)

1. Look over the Rat liver tissue using an objective with 40× lens.

2. Find liver cells (hepatocytes), their plasma membrane, cytoplasm, and hepatocyte nucleus. Focus on numerous **trophic *glycogen*** inclusions in the cytoplasm after Best's staining.

3. Draw hepatocytes in your Workbook and label the parts of cells and trophic glycogen inclusions.

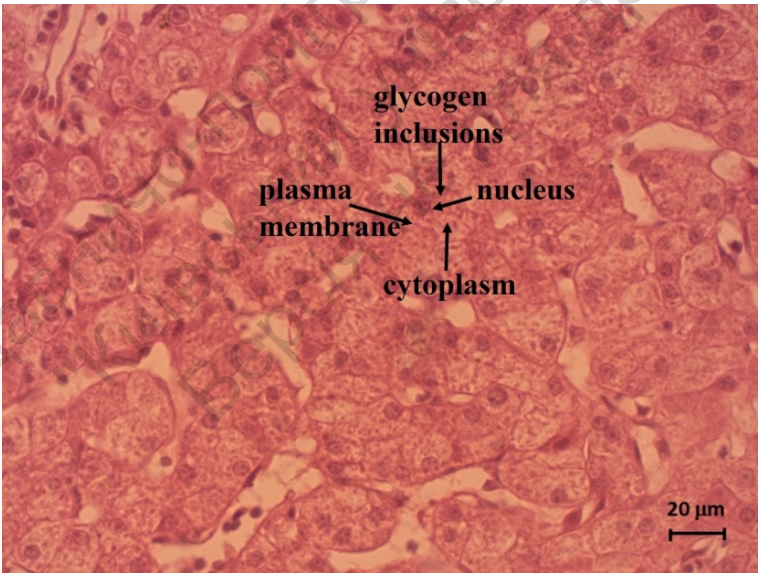


Fig. 13. Light micrograph. Human liver.
Glycogen inclusions in the liver cells
(Best's stain: carmine and hematoxylin staining, ×400)

Practical task 14. Analyse a Human heart specimen after hematoxylin staining (Fig. 14)

1. Look over the Human heart specimen using an objective with 40× lens.

2. Find **heart** cells (cardiomyocytes), cardiomyocytes plasma membrane, their cytoplasm, and nucleus. Focus on numerous **excretory *lipofuscin*** inclusions in the cytoplasm and their natural **golden-yellow** appearance.

3. Draw cardiomyocytes in your Workbook, label the parts of cells and excretory lipofuscin inclusions in the cytoplasm.

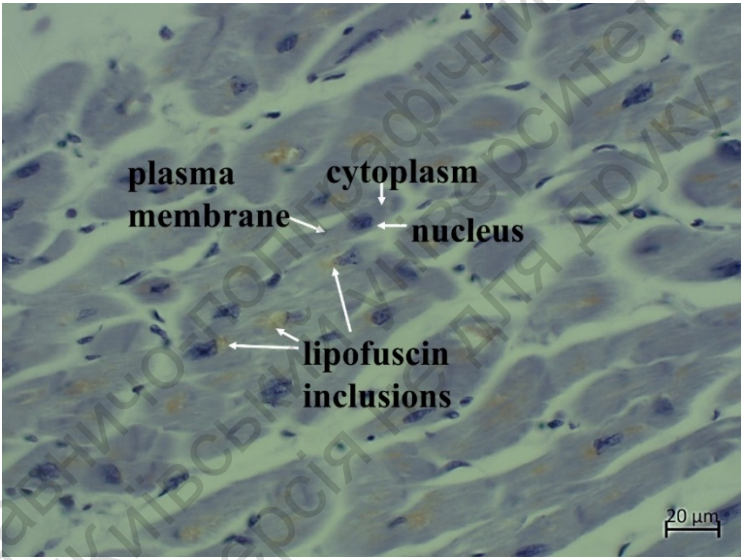


Fig. 14. Light micrograph. Human heart. Lipofuscin inclusions in the heart cells
(hematoxylin staining, ×400)

Control questions

1. In the histological specimen of a liver tissue biopsy from an 87-year-old individual, numerous granular yellow-gold pigments are observed in the cytoplasm of hepatocytes in each cell. These spots are

accumulations of excretory inclusions. What type of inclusion in human cells is excretory inclusion?

A. melanin
B. fat inclusion
C. haemoglobin
D. yolk
E. lipofuscin

2. During different stages of cell cycles, trophic inclusions accumulate in the cell to provide metabolic support. In the cytoplasm of oocytes, a specific type of inclusion accumulates to support the metabolic needs of the early embryo development stage following fertilisation. What kind of inclusion accumulates?

A. Melanin
B. Hemoglobin
C. Myoglobin
D. Glycogen
E. Yolk

3. The unstained touch preparation of the 28-year-old patient's skin biopsy revealed aggregates of large cells with large droplets in the cytoplasm. In a healthy person, lipid inclusions primarily accumulate in the cells. Which molecule mainly accumulates in the form of lipid inclusions?

A. mucus
B. phosphatidylinositol
C. glycogen
D. phosphatidylcholines
E. triglycerides

4. The unstained touch preparation of the 62-year-old patient's skin biopsy revealed aggregates of cells with numerous dark brown inclusions in the cytoplasm. What type of inclusions refers to them?

A. glucose
B. mucus
C. myosin
D. melatonin
E. melanin

5. Some inclusions were revealed in the cytoplasm of muscle cells after periodic acid Schiff staining in the histological specimen of

a 23-year-old patient's muscle tissue biopsy. Polysaccharides are a type of trophic inclusion.

A. haemoglobin
B. melanin
C. triglyceride
D. starch
E. glycogen

6. In Alzheimer's, patients experience a buildup of proteins in the nerve cell cytosol because of impaired protein degradation. What non-membrane organelle is responsible for breaking down altered, misfolded, or old proteins in the cytosol?

F. ribosome
G. nucleosome
H. signal recognition particle
I. chaperone
J. proteasome

7. Patients with Parkinson's disease accumulate abnormal proteins in the cytosol of nerve cells. Protein synthesis and acquiring an active conformation take place in several stages. During what stage does protein obtain a specific three-dimensional conformation?

K. transcription
L. proteolysis
M. chaperone
N. translation
O. folding

8. Fibroblasts actively synthesise and release pro-collagen protein during tissue reparation. The synthesis of all types of proteins goes through several stages. What kind of RNA molecule maintains information about the sequence of amino acids in a polypeptide chain and serves as a template for protein synthesis?

A. tRNA
B. rRNA
C. siRNA
D. heterogeneous nuclear RNA
E. mRNA

9. Obtaining a three-dimensional conformation of a protein (folding) regulates its functional activity. What type of protein

regulates the proper folding of other proteins and accelerates this process in cells?

A. ubiquitin
B. caveolin
C. myosin
D. tubulin
E. chaperones

10. Fibroblasts actively synthesise and release pro-collagen protein during skin growth. What is a specific marker for proteins synthesised in the rough endoplasmic reticulum and then released from the cell?

F. importin
G. ubiquitin
H. exportin
I. symporter
J. signal sequence

4. NON-MEMBRANE AND MEMBRANE ORGANELLES

Organelles are permanent structures inside the cell, providing its general and specific functions.

Based on their structure, they are divided into non-membrane, single-membrane, and double-membrane organelles.

4.1. Non-membrane organelles

Non-membrane organelles do not include lipid bilayers in their structure.

Free ribosomes comprise two subunits (large and small) – a combination of proteins and RNA. They are produced in the nucleus, pass through the nuclear pores to the cytoplasm, and are assembled during protein synthesis. The function of ribosomes is to translate proteins to meet the needs of the cell itself.

Microfilaments consist of the globular protein actin, which is assembled using the energy of ATP. They provide movement of cells and organelles, as well as phagocytosis.

Microtubules are tubular structures made of dimers of the globular protein tubulin, which assemble using the energy of GTP. They support the cell's shape and work as "rails" to transport organelles.

Intermediate filaments are thread-like structures within cells that provide mechanical support and help maintain the structure of the cell. These filaments are composed of various types of proteins. Each type of intermediate filament is specific to certain tissue types. For instance, keratin is a marker for epithelial tissue, desmin for muscle tissue, vimentin for connective tissue, and neurofilament for nerve tissue.

The cell centre (centrosome) is formed by nine triplets of microtubules ($9 \times 3 + 0$). It participates in cell division and the formation of microtubules.

Proteasomes are cylindrical protein complexes with a "core" of four folded rings forming a central pore with catalytic subunits and two regulatory subunits like a cap-covered catalytic one. They are responsible for the elimination of old or damaged proteins.

4.2. Single-membrane organelles

Single-membrane organelles are surrounded by a single lipid bilayer.

4.2.1. Granular/Rough endoplasmic reticulum (contains ribosomes)

It is connected to the outer nuclear membrane and has many tubules and cisterns. The rough endoplasmic reticulum belongs to one-membrane organelles because it is restricted by one lipid bilayer. It is a chain of interconnected tubules and sacs called **cisternae**. A system of vesicles pinched off from the endoplasmic reticulum or fused with them is called a vesicular-tubular cluster and functionally associated with them. It is well developed in all cells with high synthetic activity and makes the cytoplasm basophilic due to the high concentration of different types of RNA. This organelle provides translation of lysosomal enzymes, integral proteins of the plasma membrane, and synthesis and modifications of secretory proteins.

Nissl's substance/tigroid is a highly developed rough endoplasmic reticulum in neurons, which ensures the permanent production of neurotransmitters.

All proteins that will be synthesised on the endoplasmic reticulum begin their synthesis in the cytoplasm from the so-called signal sequence, consisting of nearly ten hydrophobic amino acids. Further, this signal sequence binds to the signal-recognition particle and anchors by a receptor-mediated mechanism on the membrane of the rough endoplasmic reticulum. An endoplasmic reticulum integral membrane protein ***translocator*** transports the synthesised protein into the lumen of the reticulum.

Synthesis and post-translational modifications followed by folding and sorting in the lumen of rough endoplasmic reticulum occur. Protein N-glycosylation is an important posttranslational modification that starts in the endoplasmic reticulum. Peptide glycosylation increases their solubility. Proteins with the general name of chaperones speed up the folding process. Essential chaperones are calnexin, calreticulin and binding immunoglobulin protein (BiP). Violation of their functions causes the accumulation of misfolded proteins and accompanies the development of neurodegenerative, muscular and metabolic diseases.

After appropriate protein synthesis and folding in the rough endoplasmic reticulum, they are packaged into vesicles with marker coat protein complex II (COPII), which is transported towards the Golgi apparatus with subsequent modification and processing. If proteins must return to the endoplasmic reticulum, they are packaged into vesicles with ***coat protein complex I*** **(*COPI*)**.

4.2.2. Agranular/Smooth endoplasmic reticulum (does not contain ribosomes)

It consists of many interconnected tubules, sucs and cisterns. Smooth endoplasmic reticulum synthesises lipids, steroids, and carbohydrates and their modifications and transport. In muscle cells, it specialises in Ca^{2+} depots (belonging to fast depot) and is called sarcoplasmic reticulum. For this, it contains proteins *calreticulin* and *calsequestrin*, which bind Ca^{2+}, providing its concentration in an inactive form in the endoplasmic reticulum lumen. Activation stimuli cause the release of Ca^{2+} from the endoplasmic reticulum into the cytosol, resulting in muscle cell contraction or transition from an inactive to an active state in other csells. Moreover, *calreticulin* and *calnexin* (chaperones) bind to oligosaccharides and misfolded proteins, pushing them to degradation. In liver cells, the smooth endoplasmic reticulum is responsible for detoxification. Cytochrome P450 is an enzyme found in the smooth endoplasmic reticulum. It converts insoluble toxins such as metabolites, waste products, and drugs into soluble forms, allowing their release from the cells and finally with urine from the body. Liver cells have a highly developed smooth endoplasmic reticulum due to the high detoxification activity of hepatocytes. Endocrine cells, which specialise in the secretion of steroid hormones, such as cells of the adrenal glands and Leydig cells, have a well-developed smooth endoplasmic reticulum. The *smooth endoplasmic reticulum* ensures the ***acidophilic properties*** of the cell cytoplasm.

4.2.3. Golgi apparatus

Camillo Golgi identified the Golgi complex in 1889 when he investigated neurons. However, in 1954, observers Dalto and Felix described the ultrastructure using transmission electron microscopy. And they implemented the term Golgi apparatus.

Golgi apparatus consists of a series of flat cisterns (dictyosomes) which are expanded on the periphery. It is located in the cytoplasm next to the endoplasmic reticulum and the nucleus. Transport vesicles with different substances are continuously fused with the cis compartment facing the nucleus, continue modification in the intermediate compartment and are released through the trans compartment and tubule-vesicular trans-Golgi network facing the membrane. Osmium tetroxide intensively stains the cis-compartment of the Golgi complex. The Golgi body's functions are modifying substances (proteins, oligosaccharides, lipids), sorting, and packaging into vesicles for delivery to points of destination. Glycoproteins, glycolipids, and proteoglycans are widely represented in the glycocalyx, and their composition varies between different cell types and tissues. The negatively charged acetylneuraminic acid is added to the glycocalyx oligosaccharides in the Golgi's trans-compartment. The glycocalyx arrangement controls the cell-cell and cell-matrix interactions. The Golgi apparatus synthesises the sphingomyelins and glycosphingolipids. Ceramide is primarily synthesised in the endoplasmic reticulum, which is then bound to the lipids in the Golgi apparatus. The surface antigens of blood groups are products of the activity of the Golgi complex. Very low-density lipoproteins (apolipoproteins are the lipid transport form) are assembled in the Golgi apparatus. Glycosylation of mucin in the cis- and mid-compartments followed by packaging into mucin granules in the trans-Golgi, a constantly active process in mucus-producing goblet cells, and for this, the Golgi apparatus is highly developed in these cells.

The Golgi is also responsible for the production of primary lysosomes. Mannose-6-phosphate is a marker of lysosomal enzymes that are added to them in the Golgi cis-compartment.

4.2.4. Lysosomes are one-membrane vesicles that are divided into different types based on their morphology and processes happening in them. The primary lysosome may fuse with the phagosome (a result of phagocytosis), thus forming a secondary lysosome where hydrolytic enzymes digest the substrate. Undigested remnants, surrounded by a membrane, form residual bodies. The main function of lysosomes is breaking down phagocytosed particles and ageing organelles. This process is called ***autophagy*** and plays a crucial

role during cell differentiation in cases of intense functional activity, starvation, cell senescence, and death.

Lysosomes contain hydrolytic enzymes that are active at acidic pH 5. As mentioned above, hydrolases are synthesised in the rough endoplasmic reticulum, and inside COPII vesicles, they are moved toward the Golgi complex. Acidic pH provides a lysosomal membrane proton pump. Inclusion = ***I – cell disease*** is an inherited disease with defective phosphotransferase activity. This enzyme transfers phosphate to mannose residues in lysosomal enzymes, and disruption of its activity causes their release from cells instead of moving them to lysosomes. As a result, mucolipidoses are developed.

Mutations in the genes encoding lysosomal enzymes cause the development of a wide range of diseases, the general name of which is ***lysosomal storage diseases***. Enzyme defects cause the accumulation of undigested materials in lysosomes, and such organelles occupy the cells' internal spaces and violate their functions. There are some lysosomal storage disease examples. Sphingolipidoses are a group of lysosomal lipid storage disorders caused by enzyme inactivation for ceramide and sphingolipid metabolism (Farber disease). Galactosialidosis is neuraminidase or/and β-galactosidase insufficiency and the storage of oligosaccharides and lipids in the lysosomes (Fabry and Schindler diseases). GM2 gangliosidosis with hexosaminidase deficiency leads to Tay–Sachs disease. Niemann–Pick disease is induced by sphingomyelinase lack. Glycogen storage disease provokes glycogen accumulation in muscle and nerve cells and develops Pompe disease.

4.2.5. Peroxisomes are round vesicles containing oxidising enzymes: oxidases and peroxidases. Urate oxidase forms the electron-dense zone – crystalloid core in the peroxisomes. These enzymes produce H_2O_2 as a product oxidising reaction, which is toxic to cells. Other peroxisomal enzymes, such as catalase, break down H_2O_2 into water and oxygen and detoxify these toxic substances for cells. Peroxisomes participate in betta-oxidation of fatty acids and utilisation of lipid oxidation products. Peroxisomal enzymes are synthesised in the cytosol on the polyribosomes, and carrier receptor-proteins ***peroxins*** transport them into peroxisomes. The number of

peroxisomes increases in cells by their division. Peroxisomes provide the metabolism of fatty acids. For this, they contain beta-oxidation enzymes. Peroxisomes are involved in alcohol detoxification, converting ingested alcohol to acetaldehyde by transferring hydrogen to oxygen molecules. After toxins influence, the number of peroxisomes in cells increases by their division after enlargement.

4.3. Double-membrane organelles – Mitochondria

Mitochondria are the energy stations of the cell because their main function is the production of ATP. Since they provide cells with energy, it is convenient to observe them in actively functioning cells: liver hepatocytes, intestinal enterocytes, kidney tubule epitheliocytes, neurons and muscle cells. Altman staining (aniline, acid-fuchsin contrasted with picric acid) is used to visualise mitochondria.

Mitochondria belong to double-membrane organelles because they are bounded *outer* and *inner membranes* separated by *intermembrane space*. The inner space of mitochondria is called a ***matrix***. The outer membrane is smooth and highly permeable due to embedded integral proteins – porins. The inner membrane forms protrusions into the matrix – ***cristae*** containing enzymes of the electron transport chain and ATP synthetase; it is practically impermeable. The impermeability of the inner mitochondrial membrane provides a specific phospholipid ***cardiolipin*****,** which is a marker of the inner mitochondrial membrane. Intermembrane space accumulates H^+ ions for further ATP synthesis, ***apoptosis-inducing factor*** *(AIF)* and ***cytochrome c***, which are important triggers of apoptosis (programmed cell death). The inner space of mitochondria is filled with a matrix – an internal environment that contains Krebs cycle enzymes (citric acid cycle), fatty-acid beta-oxidation enzymes and mitochondria's own protein synthetic apparatus. Mitochondria has its own circular DNA molecule, which encodes enzymes of oxidative phosphorylation pathway, mitochondrial ribosomal RNAs and transfer RNAs. The presence of its own synthetic apparatus testifies in favour of the hypothesis that mitochondria are aerobic prokaryotes incorporated in the first eukaryotic cells for symbiotic coexistence. Moreover, they have mitochondrial ribosomes that structurally resemble prokaryotic ribosomes (have smaller sizes of ribosomal

subunits than eucaryotes: large 50S and small 30S). However, not all mitochondrial proteins encode their own DNA. Some of them (up to 99 %) are encoded by nuclear DNA, synthesised by cytosolic ribosomes and transported into mitochondria through the ***translocase of the outer mitochondrial membrane (TOM)*** and ***translocase of the inner mitochondrial membrane (TIM)*** pore complexes. The encoding of most mitochondrial proteins by nuclear DNA is a counterargument in the symbiotic theory of the mitochondria origin.

Mitochondria have their own detoxification enzymes to prevent peroxide toxicity (glutathione peroxidase, peroxiredoxins, catalases) and reactive oxygen species toxicity (superoxide dismutase (Mn-SOD)). Mitochondria play a role in alcohol detoxification by facilitating the second stage of alcohol metabolism through the mitochondrial isoform aldehyde dehydrogenase, which operates in the mitochondrial matrix. Mitochondria accumulate polyvalent ions, such as Ca^{2+} (belonging to slow depot), Fe^{2+}, etc, in the form of electron-dense **matrix granules**. So, three cellular organelles provide detoxification: smooth endoplasmic reticulum, peroxisomes and mitochondria.

The number of mitochondria in cells increases through their division (additional features of the symbiotic theory of the mitochondria origin).

Clinical case 4. Golgi apparatus disorder

A one-and-a-half-year-old child was admitted to a hospital. Some delay in neurological development was observed. Physical examination revealed "coarse" facial features, short neck, regular head support on the neck, thin skin on the face and neck, marked generalised symptomatic hyperplasia of the gingiva in the upper and lower jaw, bleeding gums, funnel-shaped deformation of the chest, normal elbows, hands with reduced grip, and thick skin with Xeroderma, difficulty moving up the arms above the head. Joint problems, multiple signs of dysostosis, and gingival hyperplasia suggested some metabolic disease. Complete blood tests showed an increase in the leukocyte count. Microscopic findings were reported in some cells, including pathological cytoplasmic inclusions. The activity of some leukocyte enzymes and their biochemical tests in blood serum were carried out. Cytochemical tests demonstrated

decreased Arylsulfatase A (lysosomal enzyme) activity in leukocytes. On the contrary, Arylsulfatase, an Acid Sphingomyelinase, and Iduronate sulfatase were elevated in serum. The patient was directed to a genetic consultation. Gene tests revealed a mutation in the GNPTAB gene encoding N-acetylglucosamine-1(GlcNAc-1)-phosphotransferase.

The formation of lysosomes is closely related to the activity of the rough endoplasmic reticulum and the Golgi apparatus. N-acetylglucosamine-1(GlcNAc-1)-phosphotransferase catalyses the first step in generating mannose-6-phosphate, a marker that is required recognition for addressing efficient soluble lysosomal enzymes in lysosomes. Defects in GlcNAc1-phosphotransferase lead to poor distribution of various lysosomal enzymes and the accumulation of macromolecules in the lysosomes not degraded and the reason for mucolipidosis type II development (**inclusion cell disease** = I – disease). It is a rare autosomal recessive disease within inborn errors of metabolism caused by reduced activity of the N-acetylglucosamine-1-phosphotransferase due to mutations in the gene GNPTAB encoding this enzyme.

Conclusion. Type II mucolipidosis was diagnosed in this patient.

Clinical case 5. Lysosomal disorder

A two-year-old patient was admitted to the hospital with a recent onset of repeated grand mal seizures with a duration of up to 3 minutes, a dry cough without mucus release and a low level of periodic fever. The child had a long medical history due to severe developmental delays and refusal to eat. He had recurrent hospital admissions caused by analogous symptoms. During Physical examination, the child demonstrated a Coma Scale score of 8 out of 15, reduced muscle tone and exhaustion of the lower limbs, quick reflexes, and weakened plantar reactions. His breathing was rapid and shallow, and wheezing in the lower part of the left lung and eye twitching were observed. These features gave presumption of a potential respiratory infection. Antibiotic treatment was prescribed.

The complete blood count was in the normal range with a slight elevation of lymphocytes. Liver and renal state biochemical markers correspond to the healthy level, except for some elevation of aspartate

aminotransferase activity. Magnetic resonance imaging of the brain showed markedly progressive atrophy of the brain and deep demyelination of the white matter. An ophthalmologist examination was carried out, and a ***retinal cherry red spot*** was reported.

Ultrastructural studies of cultured fibroblasts derived from patients revealed numerous lamellar inclusions in the lysosomes. The targeted biochemical test of hexosaminidase demonstrated the absence of enzyme activity in the serum. Cytochemical examination of **hexosaminidase** activity in leukocytes revealed negative staining.

Conclusion. Gangliosidosis (Tay-Sachs disease) was diagnosed.

Tay-Sachs is an inherited, destructive neurological disorder due to mutation in the gene encoding enzymes **hexosaminidase**. Hexosaminidase is a lysosomal complex group of isoenzymes (glycoprotein nature) that cleaved N-acetylgalactosamine and/or N-acetylglucosamine from the glycoproteins, glycolipids, and glycosaminoglycans which are component of glycocalyx. So, Hexosaminidase broke down ganglioside (sialic acid-bonded glycosphingolipids), an essential component of the nerve cell membrane. Its turnover is constant during cell development and functioning. Hexosaminidase inactivity due to gene mutation causes accumulation of undigested lipid products in lysosomes in various cell types (nerve, leukocytes, fibroblasts, etc.) and violates their development and function. The most pronounced complications of its deficiency are neurological disorders. Tay-Sachs disease has a progressive course, but symptomatic treatment has improved the patient's condition, although ongoing monitoring remains necessary.

Clinical case 6. Mitochondrial disorder

A 19-year-old student was admitted to the hospital due to suspected deep vein thrombosis. The patient complained of an increase in muscle pain, swelling and progressive bilateral weakness of the lower limbs for one month.

During the physical examination of the patient, bilateral drooping and falling of the raised eyelids (and this sign has been observed in the patient since childhood), restriction of eye movements, bilateral swelling of the knee joints and moderate weakness of the muscle groups

of the lower limbs with an absence of tendon reflexes, accompanied by foot drop. The neurologist determined the weakness of the proximal muscles with an exaggeration of the weakness of the distal muscles.

Tachycardia was determined, and functional tests of cardiac workup revealed a diminished ejection fraction that proved systolic heart failure. An endomyocardial biopsy was carried out. Foci of lymphocyte accumulation were found to demonstrate myocarditis development.

Laboratory tests of muscle damage showed an increase in the level of creatinine kinase in serum (muscle enzyme that reflects muscle cell injury and their releasing into the bloodstream), elevated level of cardiac muscle violation such as brain natriuretic peptide, common serum markers for muscle damage are myoglobin, troponin and lactic acid and reduced serum bicarbonate. Magnetic resonance imaging of the heart did not find any features of myocardium alteration or infiltrative cardiac disease. Different types of autoimmune and infection tests were negative.

So, multiple disorders of the skeletal and cardiac muscles were detected.

A rectus femoris muscle biopsy was performed, and histological specimen examination with the application of different types of staining was carried out. Cytochemical staining for succinate dehydrogenase (mitochondrial enzymes of the citric acid cycle = Krebs cycle, which provides oxidation of succinate to fumarate) was performed to assess mitochondrial function. Some abnormal localisation and hyperactivity in some muscle cells = fibers were found. In addition, additional staining for cytochrome c oxidase (an enzyme of the mitochondrial respiratory chain) showed an adverse reaction in some muscle fibres, accompanied by hyper-staining in other cells. Gomori trichrome stain application revealed the dispersed presence of ragged-red fibres (cells with red rim (site of mitochondria localisation) and irregular sarcoplasm).

Genetic analysis revealed a mitochondrial DNA fragment deletion.

Conclusion. According to muscle biopsy data, mitochondrial myopathy with active and chronic courses was diagnosed.

Mitochondrial myopathy is a rare congenital disorder with the rarest onset in adults. The key to establishing a diagnosis is the importance of interdisciplinary evaluation of such an unusual clinical case with diverse multisystem involvement.

Laboratory tasks

Purpose: ***Master the microscopic structure of the single-membrane organelles: rough endoplasmic reticulum, Golgi apparatus, lysosomes and two-membrane organelle: mitochondria.***

Practical task 15. Analyse a specimen of Nerve tissue (Fig. 15)

1. Look over the Nerve tissue specimen using an objective with 40× lens.

2. Find the nerve cells (neurons), nerve cell soma, plasma membrane, cytoplasm, nucleus and nucleolus. Focus on numerous deep blue **Nissl bodies = Chromatophilic substances = Tigroid substances**, which are highly developed cisternae of the Rough/granular endoplasmic reticulum.

3. Draw nerve cells in your Workbook, label the parts of cells and **Nissl bodies =** rough/granular endoplasmic reticulum in the cytoplasm.

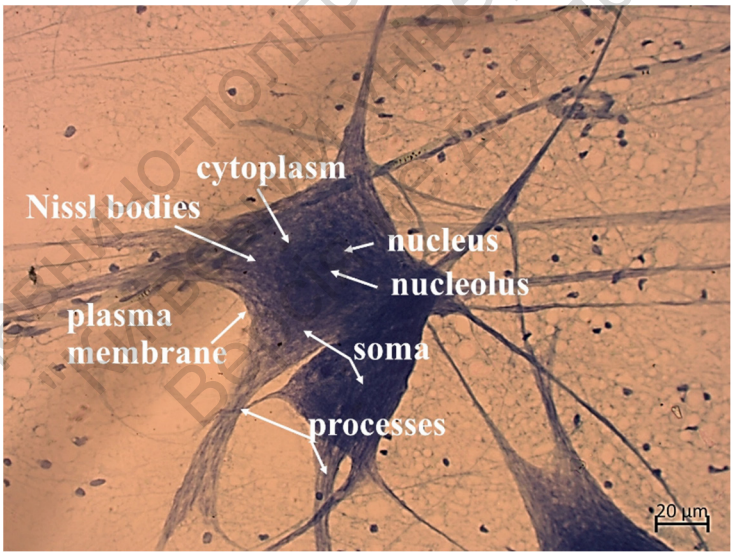


Fig. 15. Light micrograph. Nerve tissue.
Nissl substance (Tigroid substance) in the nerve cells
(Nissl's Methylene Blue staining, ×900)

Practical task 16. Analyse a Rough endoplasmic reticulum in the Electron micrograph (Fig. 16)

1. Look over the structure of the Rough endoplasmic reticulum in the Electron micrograph. Find rough endoplasmic reticulum membranes, tubules cavity, and ribosomes.

2. Label the structural elements of the rough endoplasmic reticulum in your Workbook.

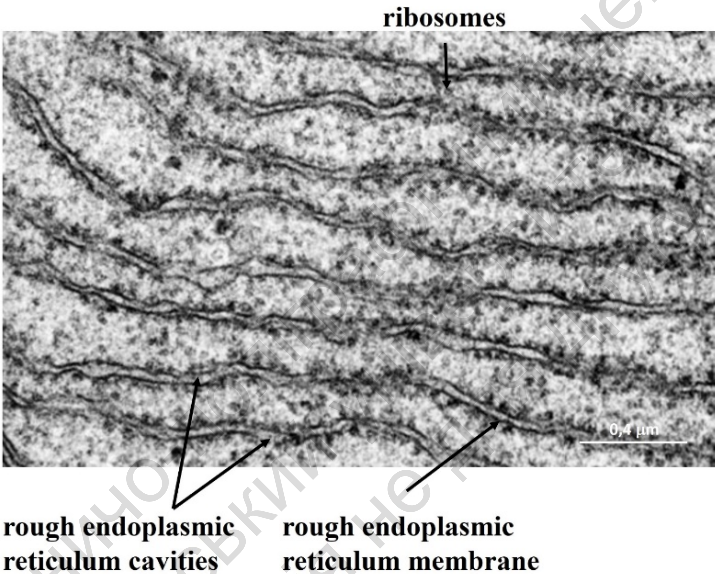


Fig. 16. Electron micrograph. Rough endoplasmic reticulum

Practical task 17. Analyse the light micrograph of the Spinal ganglia histological specimen after osmium tetroxide staining (Fig. 17)

1. Look over the micrograph of Spinal ganglia histological specimen using 40× lens.

2. Find the neurons, neuron plasma membrane, neuron cytoplasm, neuron nucleus and nucleolus. Focus on the numerous **Golgi apparatus** in the cytoplasm.

3. Draw a neuron in your Workbook, and label the parts of the cells and **Golgi apparatus** in the cytoplasm.

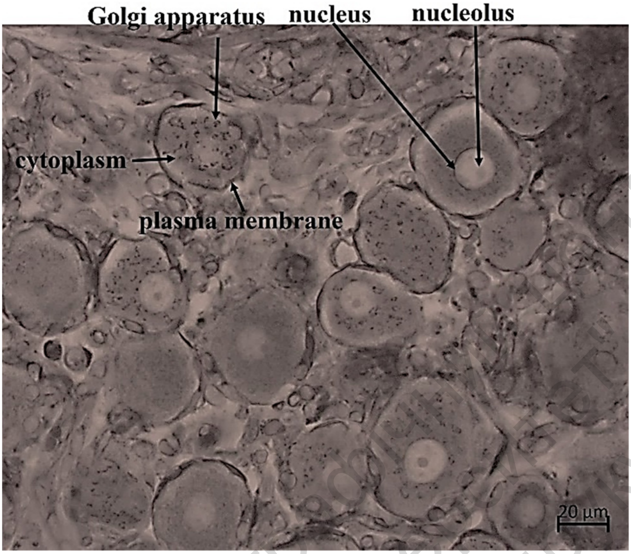


Fig. 17. Light micrograph. Golgi apparatus in neurons
(osmium tetroxide staining, ×400)

Practical task 18. Analyse the Electron micrograph of the Golgi apparatus (Fig. 18)

1. Look over the structure of the Golgi apparatus in the Electron micrograph. Find the cis-pole, trans-pole.
2. Find the cis-compartment, the intermediate compartment, the trans-compartment, the nucleus.
3. Label the structural elements of the Golgi apparatus in your Workbook.

Practical task 19. Analyse the light micrograph of the Epithelial tissue histological specimen after Altmann's staining (Fig. 19)

1. Look over the micrograph of the Epithelial tissue histological specimen using an objective with 40× lens.
2. Find the epithelial cells, the basement membrane, the epithelial cell plasma membrane, the epithelial cell cytoplasm, and the epithelial cell nucleus. Focus on the numerous **dark brown/red mitochondria** in the cytoplasm.
3. Draw epithelial cells in your Workbook and label the parts of the cells and **mitochondria** in the cytoplasm.

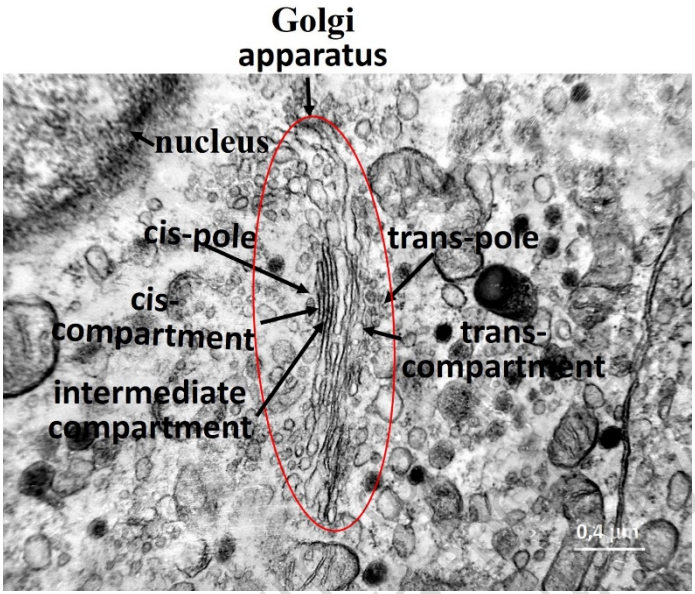


Fig. 18. Electron micrograph. Golgi apparatus
(osmium tetroxide staining, ×400)

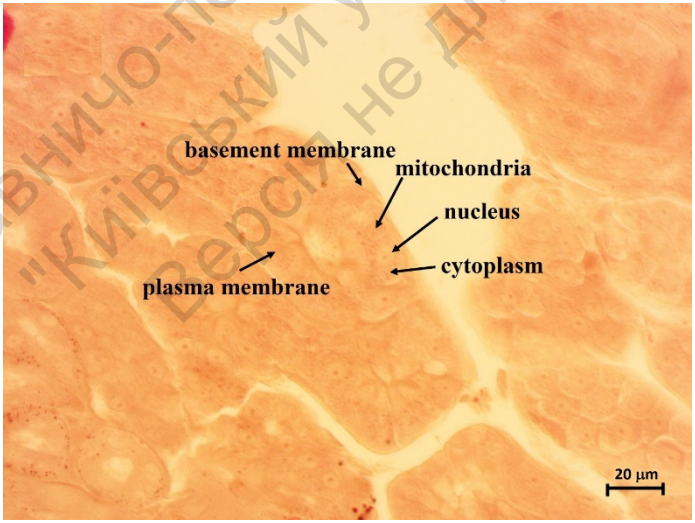


Fig. 19. Light micrograph. Epithelium. Mitochondria in epithelial cells
(Altmann's staining, ×400)

Practical task 20. Analyse the Electron micrograph of mitochondria (Fig. 20)

1. Look over the structure of **mitochondria** in the Electron micrograph.

2. Find the cytoplasm, the outer mitochondrial membrane, the inner mitochondrial membrane, the intermembrane space, the mitochondrial cristae, and the mitochondrial matrix.

3. Label the structural elements of mitochondria in your Workbook.

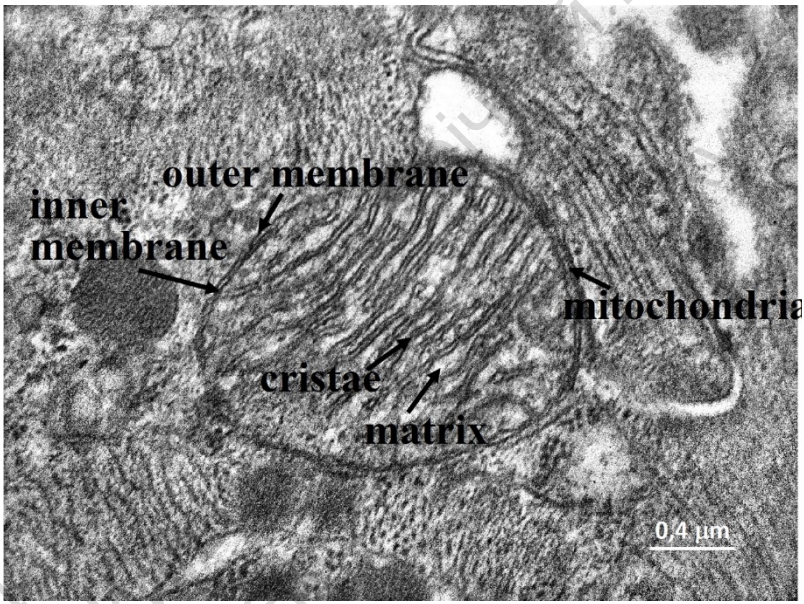


Fig. 20. Electron micrograph. Mitochondria

Practical task 21. Analyse a light micrograph of Connective tissue histological specimen after supravital trypan blue staining (Fig. 21)

1. Look over the micrograph of the Connective tissue histological specimen using an objective with 40× lens.

2. Find histiocytes, their plasma membrane, cytoplasm, and nucleus. Focus on the numerous **dark blue lysosomes** in the cytoplasm.

3. Draw connective tissue with histiocytes in your Workbook and label the structural elements of cells and **lysosomes** in the cytoplasm.

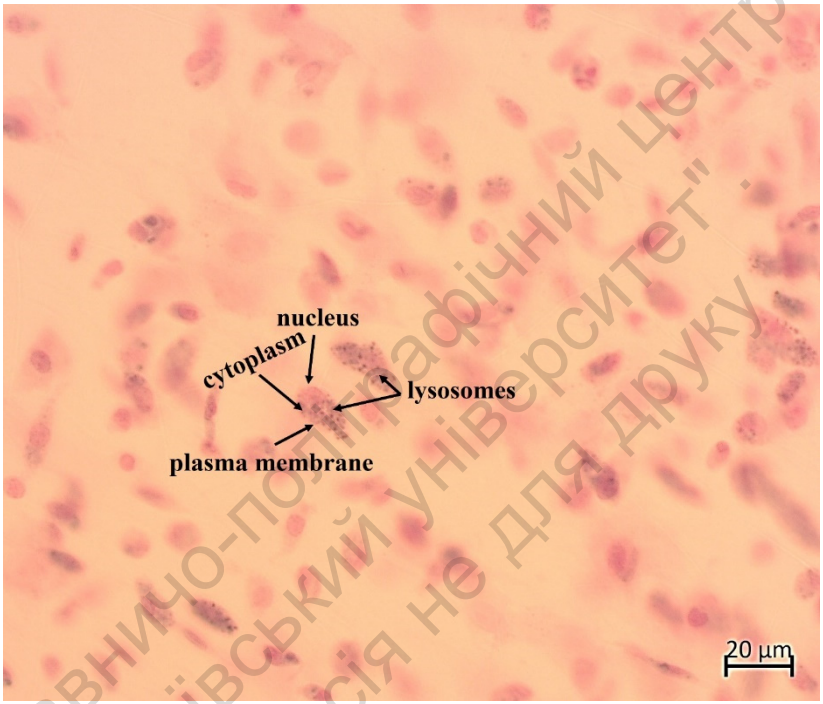


Fig. 21. Light micrograph. Connective tissue. Lysosomes in histiocytes (trypan blue and aluminous carmine staining, ×400)

Practical task 22. Analyse an Electron micrograph of lysosomes and peroxisomes (Fig. 22)

1. Look over a structure of **cytoplasm** in the Electron micrograph.

2. Find primary lysosomes, secondary lysosomes, residual bodies, and peroxisomes.

3. Label lysosome types and peroxisomes in your Workbook.

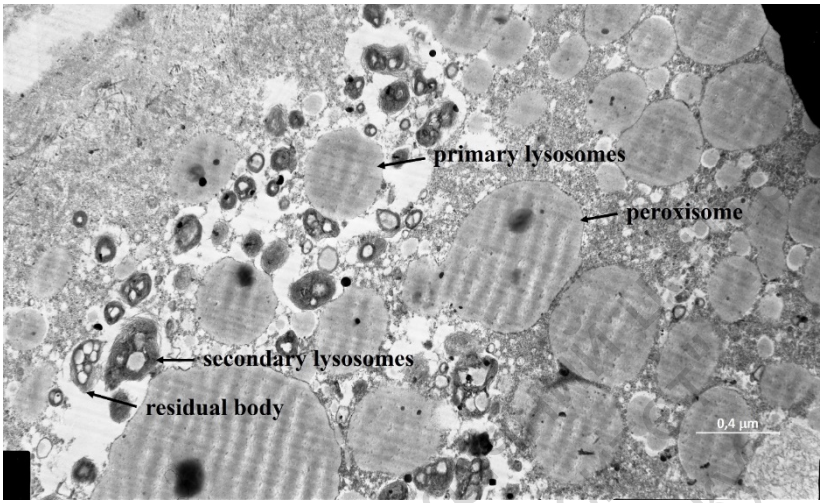


Fig. 22. Electron micrograph. Primary and secondary Lysosomes. Peroxisomes

Control questions

1. Laboratory assistant studies the ultrastructure of hepatocytes from a patient's liver after partial hepatectomy. The cells have pale nuclei with dominant euchromatin and other characteristics that prove a high protein production level. The electronogram laboratory assistant can see cisternae whose membranes are connected to the outer nuclear membrane. This organelle is:

A. mitochondrion
B. rough endoplasmic reticulum
C. Golgi apparatus
D. lysosome
E. peroxisome

2. Ultrastructural analysis of a biopsy sample from the large intestine taken from a 60-year-old patient during an operation shows enterocytes of the epithelium lining the wall of the digestive tube. The cells contain multiple membranes that fill the cytoplasm, looking like

a labyrinth. At the same time, not all of the membranes are smooth. An organelle near the nucleus has ribosomes attached to it. Which organelle is under study?

A. smooth endoplasmic reticulum
B. rough endoplasmic reticulum
C. Golgi apparatus
D. lysosome
E. peroxisome

3. Binding immunoglobulin protein (BiP) is part of the heat shock protein family. It plays a crucial role in reversing or inhibiting the denaturation of cellular proteins in response to stress or high temperatures. It is also induced by agents or conditions that negatively affect the organelle producing these cellular proteins. BiP can be detected in _____.

A. peroxisome
B. lysosome
C. mitochondrion
D. rough endoplasmic reticulum
E. Golgi apparatus

4. The cells that require a lot of energy to function are constantly at risk of reactive oxygen species (ROS) influence. ROS can cause irreversible damage to DNA as it oxidises and modifies some cellular components, preventing them from performing their original functions. Such toxic substances can be neutralised by a single-membrane organelle in the cells due to special enzymes (oxidase, peroxidase, catalase). Which organelle can neutralise toxic substances?

A. ribosome
B. rough endoplasmic reticulum
C. Golgi apparatus
D. lysosome
E. peroxisome

5. It is known that the rough endoplasmic reticulum has its name due to its special structure – it has numerous ribosomes attached to its surface. Ribosomal subunits assemble around mRNA and provide the translation stage of protein synthesis. However, the type and location

of protein produced by ribosomes depends on where these ribosomes are located. All of the following proteins can be synthesised at the rough endoplasmic reticulum ribosomes EXCEPT:

A. transmembrane proteins that pass through the membrane once

B. transmembrane proteins that pass through the membrane two or more times

C. free soluble proteins

D. endoplasmic reticulum lumen-located anchored proteins

E. cytosol-located anchored proteins

6. A laboratory assistant studies samples from the pituitary gland surgically removed from an experimental animal under an electron microscope. The pituitary gland cells demonstrate all signs of protein-hormone-secreting cells. On the electronogram, the laboratory assistant sees cisternae with ribosomes on their membrane connected to the outer nuclear membrane. The organelle under study is _____.

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. Golgi apparatus

D. lysosomes

E. peroxisomes

7. The study of pseudounipolar neurons from sensory ganglions after Nissl staining shows multiple purple cisternae spread around the perikaryon (cell body). They are only absent in the nucleus and axon hillock (the area of cytoplasm where the axon starts). These cisternae belong to a special organelle of neurons – the tigroid (Nissl body) – and produce neuro mediators. Tigroid is the form of _____.

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. mitochondria

D. lysosome

E. peroxisome

8. The study of biological samples from the medulla of the adrenal gland under an electron microscope shows a well-developed labyrinth of membranes spread around the cell's cytoplasm. Not all of these membranes are equal in structure. On the electronogram, we see

cisternae made of membranes with ribosomes attached to them. The function of this organelle is _____.

A. detoxication
B. synthesis of mucus
C. synthesis of protein hormone
D. synthesis of steroid hormone
E. heat production

9. A person who lost consciousness on the street displayed typical symptoms of intoxication, including irregular breathing, pale skin, low body temperature (hypothermia), seizures, and slow breathing. Typically, the liver processes alcohol to mitigate its effects on the body. This is made possible by the highly developed smooth endoplasmic reticulum (sER) and peroxisomes in liver cells, also known as hepatocytes. The function of these cells is:

A. detoxication
B. synthesis of mucus
C. synthesis of protein hormone
D. synthesis of steroid hormone
E. heat production

10. A patient complains to his doctor about constant muscle spasms. One possible explanation is an imbalance in Calcium exchange in the skeletal muscle fibres, which have a specific morphological modification to store this element. Which of these organelles acts as an intracellular Ca^{2+} depot?

A. rough endoplasmic reticulum
B. smooth endoplasmic reticulum
C. Golgi apparatus
D. lysosome
E. peroxisome

11. Many cellular processes rely on ATP as an energy source. For example, ATP serves as a substrate in the Sodium-Potassium pump, which plays a crucial role in maintaining the balance of these molecules across the cell membrane. ATP is the primary energy currency in cells and is synthesised in _____.

A. peroxisomes
B. rough endoplasmic reticulum lumen
C. smooth endoplasmic reticulum lumen

D. mitochondrial matrix
E. mitochondrial intermembrane space

12. Ten essential steps in the citric acid (Krebs) cycle lead to ATP production and are regulated by various enzymes. Which part of mitochondria contains Krebs cycle enzymes?

A. outer mitochondrial membrane
B. inner mitochondrial membrane
C. intermembrane space
D. matrix
E. stroma

13. Their permeability differs Despite the similar outer and inner mitochondrial membrane structures. They are made of a double layer of phospholipids, but their integral proteins vary slightly. Due to this difference, most substances' inner membranes remain almost impenetrable, while the outer ones are much more permeable. The porin proteins can be detected in:

A. inner mitochondrial membrane
B. outer mitochondrial membrane
C. matrix of the mitochondrion
D. peroxisome
E. lysosome

14. The electron transport chain is a sequence of protein complexes and other molecules that facilitate the transfer of electrons between electron donors and acceptors through redox reactions. This process is coupled with the transfer of protons across a membrane. Which part of a mitochondrion contains the electron transport chain?

A. outer mitochondrial membrane
B. inner mitochondrial membrane
C. intermembrane space
D. matrix
E. stroma

15. The mitochondria in a cell are unique. They are believed to have evolved from prokaryotes engulfed by eukaryotic cells and formed an endosymbiotic relationship. This theory suggests that a eukaryotic cell engulfed an aerobic prokaryote, leading to the development of mitochondria. This process allowed the prokaryote to evolve gradually

into a mitochondrion. Which of the following does NOT support the endosymbiotic origin of mitochondria?

A. they have their DNA

B. the number of mitochondria increases through the division of pre-existing mitochondria

C. mitochondrial proteins are primarily coded in nuclear DNA

D. they have some unique lipids that are uncommon in eukaryotes

E. they have their prokaryotic-type ribosomes

16. Brown adipose tissue and white adipose tissue are the two types of adipose tissue found in the human body. While white fat provides insulation, brown fat generates heat and can be found in newborn babies and older adults. Cytochromes determine the colour of brown adipocytes in a specific organelle. Can you tell which organelle is highly developed in brown adipocytes?

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. lysosomes

D. mitochondria

E. Golgi apparatus

17. While studying the energy-producing capabilities of cells, researchers identified an enzyme responsible for generating energy-rich bonds by adding a phosphate group to the ADP molecule, producing ATP – a key energy source in living cells. This enzyme, called ATP synthetase, operates under specific conditions and is typically located within a particular organelle. This organelle is likely to be:

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. mitochondrion

D. Golgi apparatus

E. peroxisome

18. Mitochondria are known to be pretty autonomous organelles that are probably descendants of some bacteria engulfed by the eukaryotic cell. Many features show mitochondria's independence; they even have their protein-synthetizing apparatus, including genetic material

(DNA and RNA) and ribosomes. Which part of a mitochondrion contains mitochondrial DNA?

A. outer mitochondrial membrane
B. inner mitochondrial membrane
C. intermembrane space
D. matrix
E. stroma

19. One of the main features that demonstrate an organelle's autonomy from the nucleus is its ability to produce some proteins independently. To provide this ability, such organelles need DNA as an information carrier, RNA, and ribosomes necessary for transcription and translation. Which animal cell organelle has ribosomes and can make some proteins?

A. peroxisomes
B. lysosome
C. Golgi apparatus
D. mitochondrion
E. smooth endoplasmic reticulum

20. Mitochondria have two distinct membranes with different structures and functions. The outer membrane is highly permeable, whereas the inner membrane is nearly impermeable and contains proteins involved in the electron transport chain and ATP synthesis. The inner mitochondrial membrane contains a specific lipid known as:

A. dolichol
B. ganglioside
C. cholesterol
D. cardiolipin
E. mitofusin

21. Many cellular processes rely on ATP as a source of energy. For example, ATP is utilised as a substrate in the Sodium-Potassium pump, which plays a critical role in maintaining the balance of these molecules across the cellular plasma membrane. ATP is the cell's primary and most readily available energy source and is synthesised in _____.

A. peroxisomes
B. rough endoplasmic reticulum lumen
C. smooth endoplasmic reticulum lumen

D. mitochondrial matrix
E. mitochondrial intermembrane space

22. The citric acid (Krebs) cycle has ten essential steps that lead to ATP production and are regulated by various enzymes. Which part of mitochondria contains Krebs cycle enzymes?

A. outer mitochondrial membrane
B. inner mitochondrial membrane
C. intermembrane space
D. matrix
E. stroma

23. Despite the similar structures of the outer and inner mitochondrial membranes, their permeability is different. They are made of a double layer of phospholipids, but their integral proteins differ slightly. Due to this difference, most substances' inner membranes remain almost impenetrable, while the outer ones are much more permeable. The porin proteins can be detected in:

A. inner mitochondrial membrane
B. outer mitochondrial membrane
C. matrix of the mitochondrion
D. peroxisome
E. lysosome

24. An electron transport chain is a series of protein complexes and other molecules that transfer electrons from electron donors to electron acceptors via redox reactions (both reduction and oxidation co-occurring) and couple this electron transfer with the transfer of protons (H ions) across a membrane. Which part of a mitochondrion contains the electron transport chain?

A. outer mitochondrial membrane
B. inner mitochondrial membrane
C. intermembrane space
D. matrix
E. stroma

25. Unlike other organelles, the cell mitochondria likely evolved from engulfed prokaryotes that once lived as independent organisms. At some point, a eukaryotic cell engulfed an aerobic prokaryote, which

then formed an endosymbiotic relationship with the host eukaryote, gradually developing into a mitochondrion. Which of the following does NOT support the endosymbiotic origin of mitochondria?

A. they have their DNA

B. the number of mitochondria increases through the division of pre-existing mitochondria

C. mitochondrial proteins are primarily coded in nuclear DNA

D. they have some unique lipids that are uncommon in eukaryotes

E. they have their prokaryotic-type ribosomes

26. There are two types of adipose tissue in the human organism. While white fat provides thermo isolation, brown fat, which may be found in newborn babies and older adults, is responsible for thermo-production. The colour of brown adipocytes is defined by the cytochromes present in a specific organelle. Which organelle is highly developed in brown adipocytes?

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. lysosomes

D. mitochondria

E. Golgi apparatus

27. While studying the energy-productive abilities of the cell, scientists discovered an enzyme that creates energy-rich bonds by adding a phosphate group to the ADP molecule, producing ATP – the most common energy substrate in the living cell. This enzyme, due to its function, is called ATP-synthetase. The ATP-synthetase requires specific conditions for its functioning so it can be detected in a particular organelle. This organelle is likely to be:

A. smooth endoplasmic reticulum

B. rough endoplasmic reticulum

C. mitochondrion

D. Golgi apparatus

E. peroxisome

28. Mitochondria are known to be pretty autonomous organelles that are probably descendants of some bacteria engulfed by the eukaryotic cell. Many features show mitochondria's independence; they even

have their protein-synthetizing apparatus, including genetic material (DNA and RNA) and ribosomes. Which part of a mitochondrion contains mitochondrial DNA?

A. Outer Mitochondrial Membrane
B. Inner Mitochondrial Membrane
C. Intermembrane Space
D. Matrix
E. Stroma

Chapter II. CENTRAL COMPARTMENT: NUCLEUS. CELL CYCLE

5. NUCLEUS

The nucleus comprises outer and inner nuclear membranes with perinuclear space between them. It is filled with nuclear juice/ nucleoplasm/karyoplasm, and a nuclear skeleton or karyoskeleton supports its shape. Chromatin is a structured form of DNA that gets compacted with the help of proteins. Based on the level of DNA compactification, there are two types of chromatin.

Chromatin contains DNA and associated nuclear proteins (some well-known histones) necessary for DNA organisation and function. The ratio of DNA to proteins is close to 1:1.3/1.6 and reflects the importance of proteins in the arrangement of DNA molecules. The chromatin package's first level is the DNA molecule coiling around a protein core of 8 histone molecules called ***nucleosomes***. This type of folding reduces the DNA length by approximately seven times. The next level of DNA packaging is associated with shortening the internucleosomal linker region. After that, the loop structure is formed with the participation of framework (non-histone) proteins with subsequent condensation to the chromomeric and chromatid levels.

The state of chromatin is responsible for the manifestation of varying degrees of nuclear basophilia. A microscopic assessment of the nucleus provides information about the cell's state of differentiation and functional activity. The shape of nuclei and their size and structure play an important role in diagnosing various diseases, especially tumours. For example, morphological changes in nuclear organisation, emergence invaginations and evaginations, and abnormal packaging of chromatin are features of neoplastic changes in the cells.

Based on the level of DNA compactification, there are two types of chromatin.

Euchromatin is bright, less condensed, and active (involved in protein synthesis).

Heterochromatin is dark, condensed, and inactive (not involved in protein synthesis). If heterochromatin can decondense and become active euchromatin again, it is called facultative. If it is forever condensed and may never become active, it is called constitutive (Barr body is a compacted second X-chromosome in females).

With the standard method of staining the cell with eosin and haematoxylin, the nucleus acquires a purple colour because it contains a high concentration of acids (DNA and RNA), so it is stained with basic haematoxylin. Cell activity can be determined by the intensity of nuclear staining – in cells that are growing or actively functioning and synthesising proteins, the nucleus will be light because euchromatin will prevail in it, and nucleoli will also be clearly visible. With an increase in synthetic activity, the number of nuclear pores will increase because they provide transport from the nucleus (subunits of ribosomes assembled in the nucleolus; mRNA synthesised as a result of transcription) and from the cytoplasm (histones, nucleotides). In inactive, old cells, the nucleus is dark because it is dominated by heterochromatin.

The nucleolus is the specific region of the nucleus with DNA encoding ribosomal RNA. It has obvious microscopic hallmarks that attract attention during the microscopy of cells. The nucleolus is the site of ribosomal RNA synthesis and reflects the functional activity of cells. The nucleolus is made of three parts according to different stages of ribosomal subunit production. It contains a DNA site (loose fibrillar centre) (granular component), transcribed ribosomal RNA **(dense fibrillar component**) and assembled ribosomal subunits (granular component). Synthetically active cells have euchromatic nuclei with obvious nucleolus. Moreover, the nucleolus contains some regulatory cell-cycle proteins.

The nuclear envelope consists of the double membrane and nuclear cytoskeleton associated with the nuclear inner membrane. Between the inner and the outer membrane is a perinuclear space. Nuclear membrane **pore complexes** provide transport to and from the nucleus.

Thus, they regulate the connection of the nucleus with the cytoplasm. Eight pore proteins (***nucleoporins***) form cytoplasmic and nuclear rings and centrally located **pore** channels that exchange proteins, ribosomes, nucleotides, etc., between the nucleus and cytoplasm. Substances should have a signal sequence called the nuclear localisation signal and bind to receptor importin, which passes them through the nuclear pore complex from the cytosol to the nucleus. The opposite direction of transport from the nucleus toward cytosol is mediated by receptor exportin, which binds to the nuclear export sequence in the molecule's structure. GTP molecule is used as a source of energy.

The network of the ***nuclear lamina*** (the nuclear skeleton) is involved in stabilising the nuclear membrane and the attachment of chromatin to it. ***Lamins*** (belong to intermediate filaments), together with lamin-associated proteins, are important players in the nuclear envelope. Gene mutation encodes Lamin A causes alteration of the nuclear lamina, formation of the irregularly shaped nucleus with invaginations and evaginations, and altered function. Persons with such mutation have accelerated ageing, early hair loss, marked thinness, joint alterations and impaired motor activity.

Lamins are involved in the disassembling of the nuclear membrane during mitosis. Like most intermediate filaments, their phosphorylation causes their disintegration, followed by nuclear envelope fragmentation into small vesicles.

The main functions of the nucleus include:

1. Storage of information:
   a) reparation;
   b) replication;
   c) recombination;
   d) distribution during division.
2. Implementation of information:
   a) synthesis of mRNA (transcription), tRNA, and rRNA;
   b) production of ribosomal subunits from proteins and rRNA.

Laboratory tasks

Purpose: ***Master the nucleus organisation: light microscopic morphological appearance of inner content: chromatin. According to the cell's functional activity, the condition is synthetically active and synthetically inactive. The nucleolus is a part of the nucleus, as are its morphological peculiarities and structure.***

Practical task 23. Analyse the light micrograph of the Rabbit liver histological specimen (Fig. 23)

1. Look over the Rabbit liver histological specimen using an objective with 40× lens.

2. Find liver cells, their plasma membrane, cytoplasm, nucleus, nucleolus, and nuclear membrane. Focus on the **navy-blue** heterochromatin granules, light-stained euchromatin, and thin lamina of the nuclear membrane envelope.

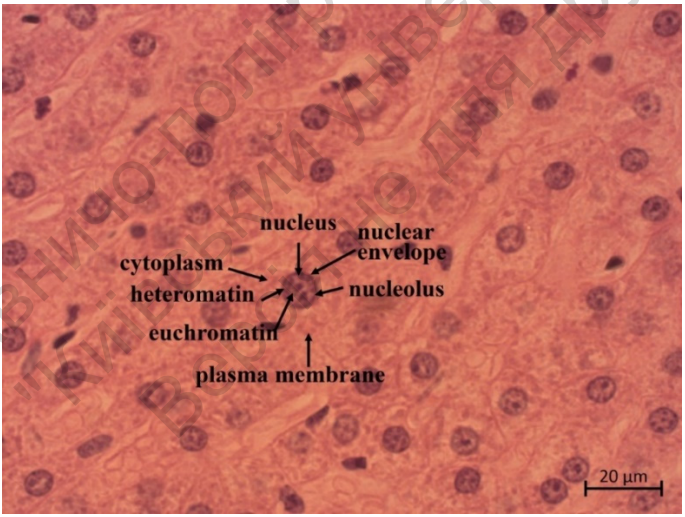


Fig. 23. Light micrograph. Rabbit liver. Nucleus structure
(hematoxylin and eosin staining, ×630)

3. Draw interphase liver cells in your Workbook, and label the parts of cells in which the nucleus contains a nucleolus, functionally active euchromatin and functionally inactive heterochromatin.

Practical task 24. Analyse an Electron micrograph of the Nucleus (Fig. 24)

1. Look over the structure of the **nucleus** in the Electron micrograph.

2. Find the outer nuclear membrane, inner nuclear membrane, perinuclear space, nuclear pore, wall surface heterochromatin, heterochromatin, euchromatin, nucleoplasm, and nucleolus.

3. Label the nuclear part in your Workbook.

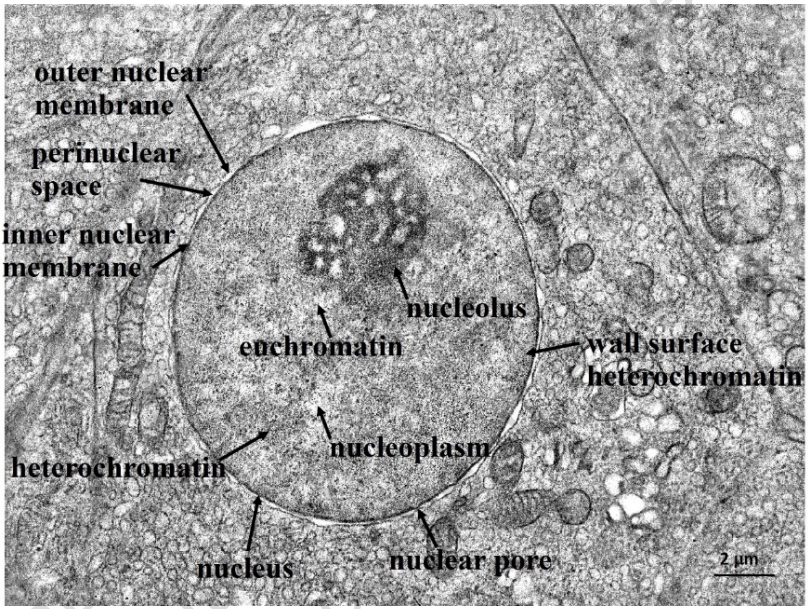


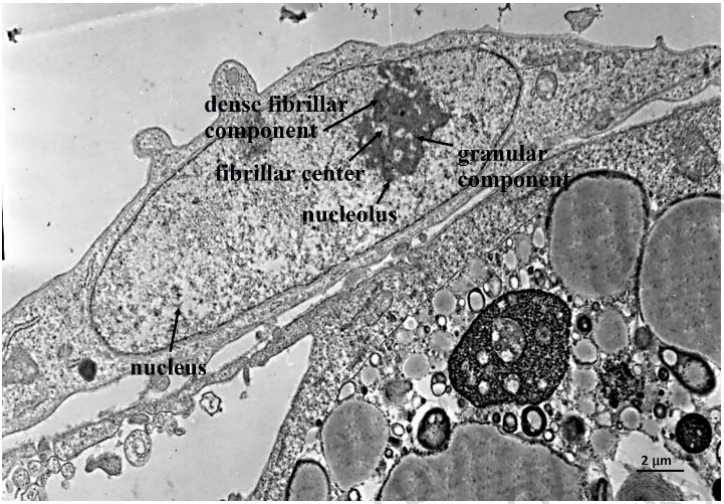
Fig. 24. Electron micrograph. Nucleus

Practical task 25. Analyse an Electron micrograph of the Nucleolus (Fig. 25)

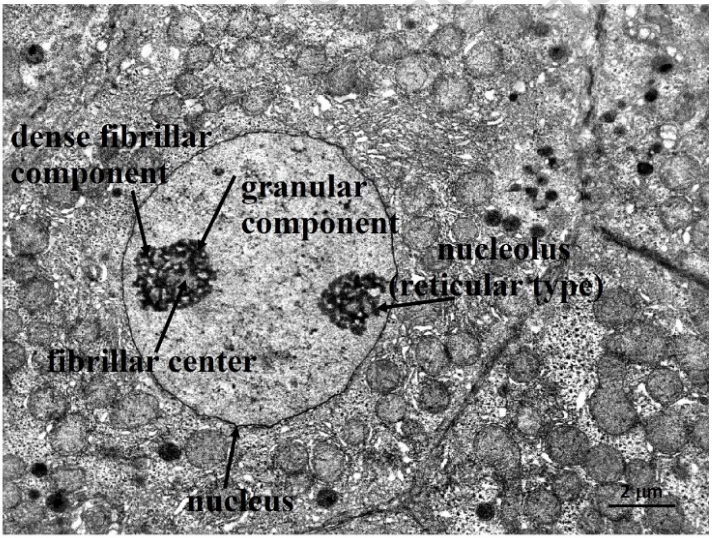
1. Look over two types of **nucleolus, Compact (A) and Reticular (B)**, in the Electron micrograph.

2. Find the outer fibrillar centre, dense fibrillar, and granular components.

3. Label nucleolus structural components in your Workbook.



A



B

Fig. 25. Electron micrograph. Nucleolus.
A) Compact type of nucleolus.
B) Reticular type of nucleolus

Control questions

1. A 29-year-old patient sought medical attention at the hospital due to persistent nosebleeds. Subsequent blood tests revealed a low platelet count. A red bone marrow biopsy was recommended to analyse the blood stem cells responsible for this process to further assess the patient's blood production capacity. What statement about these cells is true?

A. They have a high nuclear index
B. They have a low nuclear index
C. Their nucleus is dark due to dominating euchromatin
D. All statements are true
E. All statements are false

2. A baby was taken to the hospital by its parents as it was showing the characteristic signs of premature ageing. After a series of clinical check-ups, progeria was detected. It is a disease connected with the disordered genes coding nuclear lamina proteins. Several nuclear lamina proteins can be received after alternative splicing of the transcribed gene. Transcription is the process of:

A. DNA repair
B. Doubling of all DNA molecules
C. RNA synthesis
D. DNA synthesis
E. Polypeptide synthesis

3. During the crime scene investigation at the location of the murder, forensic scientists discovered multiple blood droplets. A quick analysis using a light microscope revealed the presence of Barr bodies in the neutrophils of the blood, indicating that the blood belonged to a female. What is the form of the Barr body in the interphase cell?

A. Euchromatin
B. Facultative heterochromatin
C. Constitutive heterochromatin
D. Nucleoplasm
E. Nuclear juice

4. A 52 y.o. A man working open-air for many years has addressed the doctor because of rash and itchy skin that an allergy couldn't explain. After histological analysis, the doctor diagnosed skin cancer. It is known that the excessive influence of sunlight on the skin may lead to cancer due to damage to DNA structure and arrangement. Choose an INCORRECT property of DNA molecules:

A. Can form double-stranded structures
B. Their nucleotides contain deoxyribose
C. Can act as an enzyme
D. Contain cytosine
E. Contain uracil instead of thymine

5. A 45 y.o. The man was taken to hospital with chest pain and fatigue. Medical examination and various laboratory tests helped to diagnose dilated cardiomyopathy. This disease may be caused by mutations that lead to nuclear envelope disorders. The nuclear envelope is considered as an extension of:

A. Mitochondrion
B. Peroxisome
C. Golgi apparatus
D. SER
E. RER

6. The nucleus is the site of multiple essential processes related to protein production and regulation of cell metabolism. Most of these processes are energy-consuming, so the ATP produced by mitochondria in the cytoplasm must enter the nucleus through nuclear pores. How many globular subunits does the nuclear pore contain in each of the three rings?

A. 3
B. 6
C. 8
D. 12
E. 24

7. One of the most dramatic diseases connected with proteins that form nuclear lamina is progeria – pre-mature ageing. It is caused by a mutation in the gene coding several lamin proteins. The nuclear lamina in human cells is composed of all the following proteins EXCEPT:

A. Lamin A
B. Lamin B1

C. Lamin B2
D. Lamin C
E. Lamin D

8. The cell study from the autopsy material of a 75-year-old deceased male patient demonstrates a structure surrounded by two membranes filled with pale zones of euchromatin and dark zones of heterochromatin. What structure is under the electron microscope study?

A. Mitochondria
B. Nucleus
C. Nucleolus
D. Rough endoplasmic reticulum
E. Chloroplast

9. Hutchinson–Gilford syndrome leads to pre-mature ageing and is caused by a single gene mutation. This gene can make several proteins necessary for supporting the shape of the nucleus. An abnormal form of lamin A protein is produced when it gets mutated. The other lamin protein, Lamin C, is not damaged by mutation even though the same gene codes it because it is made from another version of mRNA. Different mature mRNAs can be formed from one hnRNA template through:

A. Termination
B. Differential capping
C. Initiation
D. Polyadenylation
E. Alternative splicing

10. A 15-year-old female patient comes to the dietologist because of nearly total fat loss beneath the skin. After a medical check-up and several laboratory studies, the doctor diagnoses laminopathic lipodystrophy. This rare genetic disease is associated with proteins of the nuclear skeleton called lamin proteins. Where are lamin proteins located?

A. In perinuclear space
B. Attached to the outer nuclear membrane
C. Attached to the inner nuclear membrane
D. In nucleolus
E. Attached to ribosomes

6. CELL CYCLE. CELL DEATH

The period between two cell divisions or between cell division and death is called ***the cell cycle***. It includes the division itself (mitosis/meiosis) and interphase.

6.1. Interphase

Interphase includes:

6.1.1. G1/growth /post-mitotic/pre-synthetic phase that comes right after division and provides the growth of the cell to its original size. RNA and protein synthesis are the main processes that help with the cell's growth. After growing to a standard size, some cells stop dividing for a period of time (prolonged G1 phase). Other cells become highly differentiated and lose their ability to divide – they stay in the so-called G0 phase (nerve cells). The cells immediately start dividing again, prepare for replication, and move to the next cell cycle phase.

6.1.2. The Synthetic phase is dedicated to doubling DNA – replication.

6.1.3. G2/post-synthetic/pre-mitotic phase when tubulin synthesis for the division spindle is happening.

6.1.4. Mitosis is typical for all somatic cells. It includes four phases.

Prophase:

- chromatin condenses into chromosomes;
- the nucleolemma breaks up into separate vesicles;
- the nucleus is destroyed;
- a division spindle begins to grow from the cell poles.

Metaphase:

- chromosomes are located at the equator of the cell, forming the metaphase plate;
- the division spindle attaches to the kinetochores.

Anaphase:

- sister chromatids diverge to the poles.

Telophase:

- chromosomes decondense into chromatin;
- the karyolemma is assembled from the vesicles;
- a nucleolus is formed again;
- the division spindle disintegrates.

6.2. Apoptosis

Apoptosis comes from the Greek origin of *Apo*, meaning "from", and Ptōsis, meaning "falling"; hence describing apoptosis as "falling off". Apoptosis may be viewed as self-sacrifice by the cell to prevent collateral damage to the surrounding area. **Apoptosis** describes the standard and controlled part of cell death, which plays a crucial role in the growth or development of an organism.

The human body needs to get rid of cells safely, and there are many reasons why they do that. These could be due to:

- Cells being senescent (old).
- Cells having finished their function.
- Necessity to replace cells with brand new, mature and fully functioning ones.
- Cells that are infected and need to be removed/eaten.
- Embryogenesis where, for example, the hands are webbed; hence, cells need to be removed.
- Cells cannot carry on healthily, such as if the cell has been damaged beyond repair and has decided to initiate cell death. This is useful in preventing cancer growth.
- Immunologically, during T-cell development, cells that attack self-antigen are destroyed.

Apoptosis has two pathways: ***intrinsic and extrinsic***. ***The intrinsic pathway*** is also described as **the mitochondrial pathway, and the extrinsic pathway is the death receptor pathway**.

6.2.1. The intrinsic apoptotic pathway (also referred to as the mitochondrial pathway) responds to triggers from inside the cell, like irreparable DNA damage, radiation, high calcium concentration, hypoxia, proteins being misfolded and oxidative stress. MOMP (mitochondrial outer membrane permeabilisation) is the climactic deciding factor in the intrinsic pathway. The Bcl-2 family sensors activate the Bax and Bak (two cellular proteins), which act on the

mitochondria by creating pores to release Cytochrome-C and other pro-apoptotic proteins (they also deactivate proteins that inhibit apoptosis). Through a series of steps, the caspase enzymes activate and commit the cell to apoptosis. Caspase enzymes cleave vital proteins within the cell, such as cytoskeleton/organelles/nuclear membrane. The cell demonstrates bleb formation, which starts to break away from the cell. These blebs are then eaten by macrophages and are recycled.

6.2.2. The extrinsic apoptotic pathway begins outside the cell when conditions in the extracellular environment determine that a cell must die. If a cell recognises it is damaged, it may signal for destruction by altering its membrane, flipping the inner leaflet membrane out to expose phospholipids (specifically, phosphatidylserine) to the outer leaflet, and ultimately triggering a response from patrolling lymphocytes. Upon noticing the altered membrane, the lymphocytes will initiate the extrinsic apoptosis pathway for that cell.

The extrinsic pathway is initiated when macrophages recognise that a cell has completed its task, if the cell is senescent or if the cell has a pathological condition. The macrophage then initiates cell death for that particular cell. TNF-alpha, a signalling protein, binds to the death receptor named TNF receptor 1. Through a series of steps, a multi-complex protein is created, which is called the death-inducing signalling complex (DISC). DISC activates the caspase cascade, which causes safe and controlled cell degradation through blebbing.

Apoptotic failure in cancer. When cell cycle regulators detect unrepairable DNA, signals are released to call for cell death.

Cancer cells usually employ mechanisms that evade these signals, resulting in immortal cell lines full of mutated DNA that is no longer constrained by cell cycle checkpoints.

6.2.3. Morphology of the apoptotic cell. Characteristics of apoptosis are cell shrinkage, an intact membrane, chromatin condensation (pyknosis), apoptotic bodies, and lack of inflammatory response. Potentially dangerous cytoplasmic enzymes are carefully packaged and blebbed off the membrane in vacuoles called apoptotic bodies. Macrophages later phagocytose these bodies without causing local damage. The key to controlled apoptosis is caspases. Under the electron microscope, apoptosis shows typical ultrastructural features. A first series of alterations occur at the nuclear level with chromatin

condensation and segregation into sharply delineated dense masses and nuclear pyknosis. Then, the cytoplasm is affected by condensation and vacuolisation.

Eventually, the entire cell shows convolution of its membrane with an irregular cell outline and shape, shrinkage, abnormal packaging of intact organelles in cytoplasm, nuclear budding and fragmentation in the presence of an intact nuclear envelope. The last step in the apoptotic process is cell fragmentation into membrane-bounded apoptotic bodies with well-preserved organelles.

6.3. Necrosis

Necrosis typically occurs when extensive damage to the cell membrane and internal structures pushes the cell past reversible injury. It is usually the result of acute external factors: ischemia, physical agents (mechanical trauma, temperature, radiation), chemical agents (toxins), immunological injury, etc. Necrotic cell death results in a specific presentation, such as damage to the plasma membrane, cellular swelling, and unregulated nuclear degradation.

A significant role in necrosis is played by ATP imbalance. Ischemia, for example, typically ends in necrotic cell death. With the decreased delivery of oxygen to the cell, oxidative phosphorylation performed by the mitochondria becomes compromised. This results in a reduced amount of ATP available in the cytoplasm. Insufficient ATP turns off the transporter in cells with a Na^+/K^+ (sodium-potassium) pump. Sodium increases within the cell as it can no longer be pumped out. This results in cellular swelling as the increased intracellular sodium pulls water in. Cellular swelling, blebbing, and loss of microvilli are typical presentations of necrotic cell death. If ATP is restored in time, these presentations are reversible. If ATP is not restored before further cell damage, the loss of ATP progresses to have extensive effects on the cell.

6.3.1. Role of Calcium in necrosis. Without the restoration of ATP, the Ca^+ pump ceases to function, causing an influx of calcium ions. Free calcium levels are usually kept low inside the cytoplasm as calcium activates many cellular processes. The increased free calcium in the cell begins interacting with cellular enzymes that cause further membrane and nuclear damage. Phospholipase, protease, endonuclease,

and ATPase are all activated by increased cytoplasmic Calcium. ***Phospholipase*** damages the plasma membrane by hydrolysing phospholipids, resulting in whorled patterns called myelin figures. ***Protease*** results in proteolysis, breaking down proteins inside the cell and disrupting cellular activity. ***Endonuclease*** cleaves the phosphodiester bond in a polynucleotide chain, collapsing nuclear chromatin into clumps. ***ATPase*** breaks down any remaining ATP, quickening the loss of energy and leading to further damage.

Free cytoplasmic calcium increases mitochondrial permeability by opening a transitional pore. Exposure to mitochondrial contents artificially releases cytochrome C (Cyt C), an essential factor in the electron transport chain, apoptosis, and cell death. Cyt C begins a cascade of cell injury by direct activation of caspases. This would be one way that necrosis and apoptosis begin to overlap, as caspase activation is a primary mechanism of apoptotic death. In necrotic cell death, the release of caspases is coincidental to cellular injury. In apoptosis, caspase activation is purposeful and initiated through regulating factors.

6.3.2. Morphology of the necrotic cell. The cellular characteristics of necrotic cell death do not always follow the pattern in ischemic injury, but it serves as a good example. Necrosis results in cell lysis and exposure of cytoplasmic contents in an uncontrolled setting as the plasma membrane is damaged, regardless of the type of damage that led it to this stage. A release of cellular content into the surrounding area generally elicits an inflammatory response not seen in apoptosis. During necrosis, the nucleus passes several changes: pyknosis, when the genetic material condenses dramatically inside it; karyorrhexis, when the nucleus fragments; karyolysis, when it gets paler and paler due to resorption.

The alterations in cytoplasm can be split into early and late changes. The early phase of necrosis is characterised by homogenous pink cytoplasm in the case of eosin and hematoxylin staining. Later, eosinophilia increases, so the staining is getting more intense. Degradation of cytoplasmic proteins leads to the appearance of ghost-like cells that lose adherence to the basement membrane and other neighbouring cells, which can be found free in lumens or tubules. The late phase of necrosis may be characterised by cell rupture, which results in loss of integrity and release of cell components.

Laboratory tasks

Purpose: ***Master the morphological appearance of cells at different stages of the cell cycle. The main traits of mitosis stages are prophase, metaphase, anaphase and telophase. Microscopic features of programmed cell death: apoptosis.***

Practical task 26. Analyse the light micrograph of the Red Bone Marrow smear (Fig. 26)

1. Look over the Red Bone Marrow smear using an objective with 40× lens. Focus on the nucleus of nuclear cells.

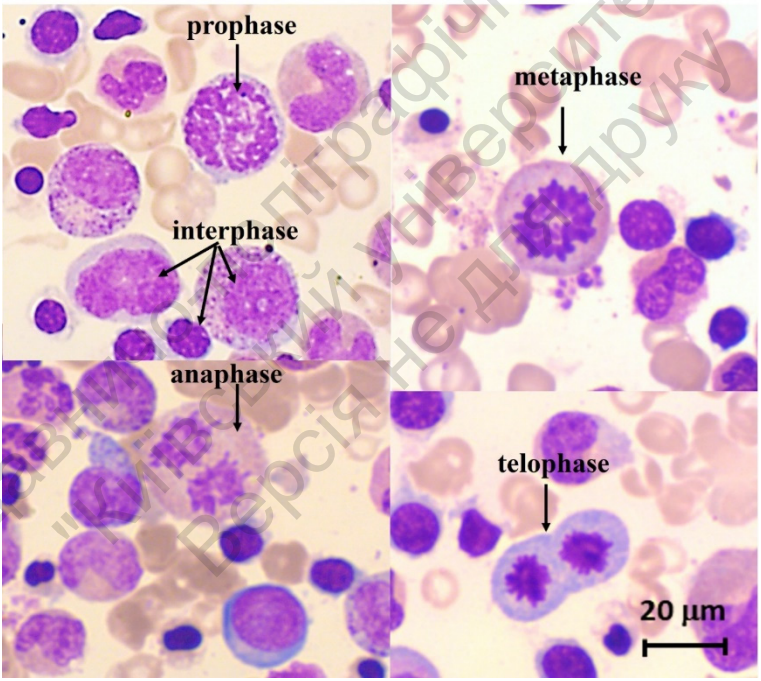


Fig. 26. Light micrograph. Red bone marrow. Nucleus structure
(Romanovsky-Giemsa staining, ×630)

2. Find nuclear cells in interphase. Analyse the organisation interphase nucleus with **navy-blue granules** of inactive **heterochromatin** and light-stained active **euchromatin**. Find cells in mitosis. Focus on cell organisation in **prophase**, **metaphase**, **anaphase** and **telophase**.

3. Draw red bone marrow nuclear cells at different cell cycle stages in your Workbook and label the stages.

Practical task 27. Analyse the light micrograph of Human skin histological specimen (Fig. 27)

1. Look over the Human skin histological specimen using an objective with 40× lens.

2. Find the sebaceous gland region. Focus on differences in cell nuclei. Find nuclei typical for interphase cells with euchromatin, heterochromatin and nuclear envelope. After that, find pyknotic nuclei which have condensed chromatin. These are apoptotic cells.

3. Label the indicated cells in your Work Book by drawing sebaceous gland cells with an unchanged interphase and pyknotic nucleus.

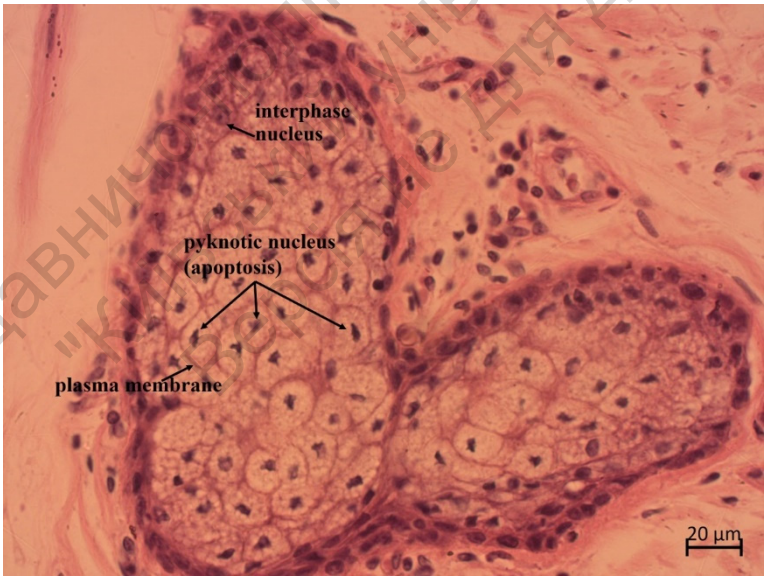


Fig. 27. Light micrograph. Human skin. Sebaceous gland. Apoptosis (hematoxylin and eosin staining, ×400)

Control questions

1. The typical laboratory analysis of biopsy samples involves examining somatic cells under a light microscope to study their morphology. Cells are usually observed during a specific phase of their cell cycle, which is the most prolonged phase and offers the highest probability of capturing them in this active state. This longest part of the cell cycle is known as _____.

A. Interphase
B. mitosis
C. cytokinesis
D. binary fission
E. karyokinesis

2. During interphase, the structuring of genetic material in human cells differs from that during cell division. Somatic cells of the body reproduce through mitosis, involving various changes in nuclear structures, including DNA. This results in differences in the presence of specific structures during interphase. Which of these structures are present during the interphase?

A. mitotic spindle
B. nuclear envelope
C. nuclear reticulum
D. X-shaped chromosomes
E. V-shaped chromosomes

3. One of the main characteristics and dangers of cancer development is the unstoppable division of cancer cells that leads to the growth of the tumour. Cancer can be seen as disrupting normal restriction point function, as cells continually and inappropriately reenter the cell cycle. At what cell cycle period do cells pass the restriction point R?

A. G1-period
B. S-period
C. G2-period
D. G0-period
E. S0-period

4. Stem cells of our body can divide and substitute those cells with limited life spans and periodically die (epithelial, blood cells, etc). In any case, stem cells can't start a new division immediately after the previous one is finished, as specific processes must take place in the cell before division. Some of them must happen right before the start of mitosis. The part of interphase during which a cell produces tubulins for future mitotic spindle and finishes preparation to division is called _____.

A. G1-period
B. S-period
C. G2-period
D. G0-period
E. S0-period

5. After partial hepatectomy (surgical removal of a portion of the liver) in a 30-year-old patient, some hepatocytes in the remaining part of the liver that did not divide for some time restored their ability for mitosis. This ability increases the liver's regenerative potential. After an intensive, regenerative process, the cells stopped division again. Cells that have temporarily stopped divisions but retain the ability to return to the division process are in the _____.

A. elongated G1-period
B. elongated S-period
C. elongated G2-period
D. G1-period
E. G2-period

6. The analysis of a red bone marrow smear from the biopsy of a 25-year-old volunteer donor for his younger brother revealed multiple hematopoietic cells at different cell cycle stages. The specific stage of the cell cycle when the nucleus divides into two nuclei is called the _____.

A. prophase
B. interphase
C. cytokinesis
D. mitosis
E. anaphase

7. Colchicine is a medication that inhibits the assembly of the mitotic spindle, which allows for the study of chromosomes in a cell's metaphase. The mitotic spindle's primary function is to facilitate the separation of chromatids to opposite poles of the cell. During cell division, the fibres of the mitotic spindle attach to duplicated chromatids, aiding in chromatid separation. This attachment occurs at a proteinous structure associated with duplicated chromatids in eukaryotic cells, called _____.

A. glucose
B. kinetochore
C. cytoplasm
D. fission
E. ATP

8. Cell division is a complicated process that includes a long preparation and several stages that rearrange chromatids between the daughter cells. The last phase of mitosis is telophase, which forms new nuclei around these chromatids. The previous process taking place in telophase is cytokinesis, which describes _____.

A. the splitting of a cell during mitosis
B. the duplication of genetic material during mitosis
C. the condensing of chromatin into chromosomes during mitosis
D. the separation of chromosomes during mitosis
E. the splitting of a cell nucleus

9. One of the nucleus's roles in a cell's life is participation in protein production. Not only does it transcribe information to mRNA, but it also produces subunits of ribosomes for future translation. The function of ribosomal subunit production belongs to nucleoli. During cell division, this production stops, and DNA from nucleoli condenses to chromosomes, and they disappear. At what stage of mitosis do the nucleoli disappear?

A. prophase
B. metaphase
C. anaphase
D. telophase
E. cytokinesis

10. Mitosis in human organisms ensures that one chromatid from the 46 replicated chromosomes passes into each new cell. Before this separation starts, all the chromosomes are arranged in the equatorial zone of the cytoplasm in the mother cell, making the so-called "equatorial plate." At what stage of mitosis do the chromosomes line up in the equatorial plate?

A. prophase
B. metaphase
C. anaphase
D. telophase
E. cytokinesis

Chapter III. EMBRYOLOGY

7. GAMETOGENESIS. CHARACTERISTICS OF THE GAMETES

Embryology ("embryon" – embryo, "logos" – science or study) is the science of the development of the organism from the formation of gametes and fertilisation to the birth. Teratology is the study of abnormalities in embryo development.

Ontogenesis (ontogeny, "onto" – being, existence, "genesis" – origin) is the organism's individual development of an organism from fertilisation to maturity (death).

Periods of human life: ***pre-embryonic*** (progenesis, prezygotic), ***embryonic*** (prenatal) and ***post-embryonic*** (postnatal). The ***pre-embryonic (progenesis, prezygotic)*** period is a period of formation and maturation of germ cells (gametogenesis). The ***embryonic (prenatal)*** period lasts from zygote formation to birth, including the germinal (initial), embryonic, and fetal stages. The ***post-embryonic*** period begins with birth and ends with death.

7.1. Gametogenesis (progenesis)

In the second week of embryogenesis, germ cells appear in the region of Hansen's node and migrate to the yolk sac. During the fourth week of embryogenesis, gametes migrate from the yolk sac to the developing gonads and increase in number through mitotic divisions. Gametogenesis then begins, including meiosis – to reduce the number of chromosomes to the haploid number of 23.

Meiosis consists of two divisions. During meiosis I male (primary spermatocytes) and female (primary oocytes) germ cells replicate their DNA, homologous chromosomes align in pairs (synapsis) and then interchange of chromatid segments (crossing over), duplicated chromosomes form the metaphase plate, and separate (centromeres do not split), the cell divides. Two secondary gametocytes (23 duplicated chromosomes, 2N) are formed. During meiosis II – 23, duplicated chromosomes align at the metaphase plate, separate and form

23 single chromosomes (centromeres split), four haploid gametes (23 single chromosomes, 1N) are formed in spermatogenesis, and one haploid gamete and three polar bodies are formed in oogenesis. Gametes contain 22 autosomes (non-sex) and one sex chromosome. The sex chromosome contains two X (XX) for a female and one X and one Y (XY) for a male. During the first week of embryo development, the female cell randomly inactivates one of the X chromosomes. Light microscopy can observe the Barr body (the inactivated X chromosome) near the nuclear membrane.

7.2. Spermatogenesis

Spermatogenesis begins at puberty and is represented by 4 phases: reproduction (spermatogonia divide by mitosis), growth (cell size increases four or more times), maturation (meiosis) and formation. The primary spermatocytes then enter ***the maturation period.*** It has two consecutive divisions: meiosis I (reduction division) and meiosis II (equational division). In meiosis I, prophase consists of 6 stages:

The preleptotene stage. At this stage, pronounced accumulations of heterochromatin appear in the nucleus of cells, which are called prochromosomes. They are associated with the nucleolus and the nuclear envelope. DNA duplication occurs (the spermatocytes' ploidy becomes equal to 2n4c). The clone of spermatocytes moves from the lower basal layer to the upper adluminal layer.

The leptotene stage. At this stage, the chromosomes begin to condense and become visible. Each chromosome consists of two chromatids. DNA duplication has already occurred (spermatocytes' ploidy is 2n4c). The mitochondria swell and branch as they divide.

The z*ygotene stage*. Homologous chromosomes approach each other, located one along the other. This process is called conjugation or synapsis. Before conjugation, each of the homologous chromosomes doubles.

The pachytene stage. Chromosomes become much thicker and shorter at this stage. Synapsis is completed, and crossing-over (the exchange of sections of homologous chromosomes with the formation of crossovers – chiasms) takes place.

The diplotene stage. Each of the homologous chromosomes splits into two chromatids, resulting in the formation of tetrads.

The diakinesis stage. In this stage, the chromatids' maximum shortening and thickening occur due to their spiralisation. The chromosomes are separated from each other.

In *the metaphase of meiosis I*, duplicated homologous chromosomes are located on both sides of the equator, and the distribution of paternal and maternal chromosomes is random. This also determines the genetic individuality of organisms. In *anaphase*, the doubled homologous chromosomes move to the poles, and in *telophase,* cytotomy occurs, and two secondary spermatocytes (1n2c) are formed.

The second division of meiosis (*meiosis II*) is known as the equational division. It follows immediately after meiosis I and proceeds in the same way as usual mitosis. During anaphase II of meiosis, the chromatids move to the poles, and as a result of telophase, spermatids are formed containing instead of chromosomes chromatids. Spermatids contain a haploid number of chromosomes, represented by one chromatid (1n1c).

All cells formed during spermatogenesis (spermatogonia, primary and secondary spermatocytes, spermatids) remain connected to each other by cytoplasmic bridges in cell associations (clones). The final separation of the cells occurs during the formation phase. The maintenance of cytoplasmic bridges between cells is of great biological importance. The entire diploid genome and the products of its activity are necessary for the complete differentiation of spermatozoa. Firstly, because the original diploid genome may contain defective, lethal alleles of genes, the cell that has received them will die if it is not provided with the products of the normal allele located in the nuclei of the cells that have received them. Secondly, some male germ cells receive the X-sex chromosome and others the Y-sex chromosome. Each of them contains many important genes necessary for the development of spermatozoa. Therefore, thanks to cytoplasmic bridges, developing male germ cells receive the products of the activity of the entire genome.

The formation phase is the longest phase of spermatogenesis. During this phase, the spermatozoa are formed from the spermatids. This phase is often named spermiogenesis. It lasts longer than any of the other phases (about 50 days). Spermiogenesis begins with the formation of an acroblast from the Golgi apparatus and then an

acrosome, which contains enzymes for penetration through the pellucid zone of the oocyte. The centrosome, consisting of two centrioles, moves to the opposite pole. The proximal centriole is adjacent to the nucleus and remains unchanged. It participates in the cleavage of the zygote because the oocyte does not contain a centrosome. The distal centriole splits into two parts. One part forms the flagellum, which becomes the axial thread of the tail. The second part plays the role of the basal body. Elements of the cytoskeleton are formed: segmented columns, dense fibres, and longitudinal columns with ribs. The cytoplasm of the spermatozoon is greatly reduced, and the nucleus becomes elongated, compact and hyperbasic. In the final stages of formation, the spermatozoa are separated from the common cytoplasm that binds them together and become free. The cytoplasm remaining after separation (residual bodies) is phagocytised by the epithelial cells of the convoluted seminiferous tubules of the testes (Sertoli cells).

7.3. Oogenesis

The initial cells in ovogenesis are the primary germ cells (gonocytes) that develop during the early embryonic period in the female gonad (ovary). These cells form part of the epithelium of the indifferent gonad. This epithelium then grows in the form of strands into the mesenchyme of the primary kidney (mesonephros), then splits into separate islets (Pflüger's tubes). These islets contain germ cells surrounded by epitheliocytes (hereinafter called follicular cells). The gonocytes become ovogonia. These small cells enter the *reproductive phase* and divide intensively by mitosis. As a result, their number reaches 2–7 million by the end of embryonic development. Cell division is not accompanied by cytotomy, so, as a result, as in spermatogenesis, a true syncytium (clone of cells) is formed. By the time of birth, the phase of reproduction is over. From the end of the 3rd month of embryogenesis until the birth of a girl, some oogonia become primary oocytes, while others continue to divide. From the 8th to the 14th week of embryogenesis, the cytoplasmic bridges between the oogonia are destroyed, the syncytium disintegrates, and the oogonia and oocytes are surrounded by flattened cells of the coelomic epithelium called follicular cells. Primordial follicles are

formed. After birth, the reproduction of oogonia stops, and they all become primary oocytes, which are blocked at the diplotene stage of the first meiotic division.

Next, the primary oocytes enter into a long *growth phase*. This period is divided into two parts:

1) a period of small (slow) growth lasts from birth to puberty;

2) a period of large (rapid) growth, which occurs cyclically throughout each menstrual cycle. During the period of rapid growth, the preparation for meiosis takes place. Thus, the growth period can last from 12 to 50 years. The third phase of ovogenesis – *maturation* – begins before ovulation. The first meiotic division occurs, forming a second oocyte and a first polar body. The second oocyte enters the second meiotic division but is blocked in metaphase. Meiosis II follows and is blocked in the metaphase of the second division of meiosis.

During fertilisation, a spermatozoon enters the secondary oocyte, and the oocyte is called a penetrated oocyte. Penetration of sperm stimulates the oocyte to complete the second meiotic division. An ovotida and a polar body are formed. The end result of oogenesis is one ovotida and three polar bodies.

Thus, during oogenesis, there is no stage of a mature ovum, but a stage of ovotida, which is formed when the spermatozoon penetrates the secondary oocyte. The mammalian ovotida is characterised by the presence of two separate haploid sets of chromosomes in the form of male and female pronuclei. Combining these sets into a single diploid leads to the formation of a zygote. If fertilisation does not occur, the germ cell degenerates at the secondary oocyte stage.

Unlike spermatogenesis, the cells produced by two divisions of meiosis are asymmetric. A primary oocyte gives rise to a large secondary oocyte and a very small polar body (which can divide into two). The cytosol and organelles are transferred to the secondary oocyte, leaving the polar bodies with a relatively small amount of cytoplasm. A secondary oocyte forms an ootida and a third polar body. Thus, two divisions produce one ovum and three polar bodies, which soon die and are phagocytosed by follicular cells. The ovum loses its centrioles. Thus, the ovum or ootida, as such, does not actually exist: the second oocyte in the process of fertilisation immediately turns into a zygote, i.e. a single-cell embryo, and the ovotida stage drops out.

7.4. Characteristics of the gametes

The sperm cells. The spermatozoon consists of a head and a tail (flagellum) and is 60–70 μm long. The length of the head is only 4 μm. The *tail* is structurally divided into connecting pieces, midpieces, principal pieces, and terminal (end) pieces. The annulus demarcates the midpiece and principal piece.

In histological specimens, spermatozoa have elongated pear-shaped basophilic nuclei. The nuclei are characterised by a dense chromatin arrangement containing protamines (small basic proteins). The DNA in the chromatin is packed in parallel so that the nucleus has a crystal-like structure, and its volume is reduced to a minimum. There are two opinions on the existence of nucleosomal organisation of chromatin: some authors note its absence, and others admit its existence, but in a special version. The sperm nucleus is haploid and contains 22 autosomes and 1 sex chromosome, which can be either an X or a Y chromosome. The number of spermatozoa with an X or Y chromosome is approximately the same. The nuclear membrane of spermatozoa is completely devoid of a nuclear pore. In the anterior part of the spermatozoon head, under the plasmalemma, there is an *acrosome* – a derivative of the Golgi apparatus and an analogue of the lysosome. Its membrane is adjacent to the plasmalemma at the front and the nuclear envelope at the back. The acrosome contains 10–12 different enzymes that break down the components of the zona pellucida: hyaluronidase, proteases, glycosidases, lipases, neuraminidase, phosphatases, etc.

The cytoplasm of the spermatozoon is reduced to a minimum and covers the nuclei with a very thin layer. At the same time, the cytoskeleton is quite well developed, which ensures that the shape of the head and flagellum is maintained.

The spermatozoon's connecting piece (neck) contains the proximal centriole, which is adjacent to the nucleus and is located in the deepening of the nuclear membrane. Here is the distal centriole. The distal centriole gives rise to the axial filament or axoneme. It has the structure of a flagellum and consists of nine peripheral microtubule doublets and a central pair of microtubule doublets. Outside and opposite each axoneme's doublet, there is one so-called segmented column (outer fibril) in the connecting region. Therefore, the total number of segmented columns is nine.

In the midpiece of the tail, segmented columns continue into nine dense fibres. The mitochondria sheath surrounds the axoneme and dense fibres.

The number of microtubules is greatly reduced in the distal part of the tail. A plasma membrane covers the tail. The plasma membrane is capable of forming and conducting an electrical impulse. It is initiated by acetylcholine, which is produced in the flagellum itself and realises its effect through acetylcholine receptors located in the plasmalemma. The movement of the tail allows the spermatozoa to move at a speed of 1–5 mm per minute.

The egg cells. The female sex cell (*ovum, ovotida*) is also haploid. During ovulation, a second oocyte emerges from the ovary with incomplete meiosis blocked in metaphase II. This block of the chromosomal apparatus of the ovulated oocytes is quite stable. It is only removed by fertilisation. The ovum is large and round in shape. Its diameter is about 130 μm. The nucleus of the ovum contains 23 chromosomes, one of which is the sex X chromosome. The cytoplasm (ooplasm) contains mitochondria, Golgi apparatus, well-developed granular and agranular endoplasmic reticulum, and numerous inclusions: trophic (vitellin granules) and pigment. Vitellin in mammals is synthesised inside the oocyte (endogenous yolk – little or none in humans), consists of lipids, amino acids, proteins, mineral salts and pigments – lipochromes, which are soluble in lipids and give yellow colour to yolk granules.

The egg cell is surrounded by plasmalemma. Beneath this is a thick layer of cytoplasm (2–3 μm) with cortical granules. The cortical granules are derived from lysosomes and contain various enzymes, including ovoperoxidase. The ovum has a well-developed and peculiarly organised cytoskeleton. Its components are associated with the plasmalemma and cause a constant modification of the cell surface (microvilli can appear and disappear, and the localisation of receptors can be changed). Cytoskeleton elements can also be found in the cortical layer. They are associated with *morphogens,* which are either special proteins or mRNA for their synthesis. After fertilisation of the egg, morphogens, under the influence of the cytoskeleton, change their location in the ooplasm of the cortical layer, causing ooplasmic segregation and ootypic differentiation. The cortical layer thus plays an important role in the organisation of the egg cell and in fertilisation.

A zona pellucida and a layer of follicular cells surround the oocyte. There is a small perivitelline space between the zona pellucida and the oocyte plasmalemma, which increases significantly after fertilisation as the contents of the cortical granules are released into this space. Follicular cells contact the oocyte plasmalemma through holes in the zona pellucida.

The classification of egg cells. In vertebrates, the egg cells are classified according to the presence of yolk, its quantity and distribution. According to the presence of yolk: alecithal (without yolk) and lecithal (with yolk). Depending on the amount of yolk, the lecithal cells are divided into oligolecithal (small amount of yolk), mesolecithal (moderate amount of yolk) and polylecital (multi-yolk). According to the distribution of yolk in the ooplasm: isolecithal and telolecithal.

In isolecithal eggs, the yolk is evenly distributed. In the telolecithal eggs, the yolk is located at one pole, called vegetative, and at the other pole, called animal, where the organelles and the nucleus are concentrated.

The eggs of mammals, including humans, are oligo isolecithal; they contain a very small amount of yolk evenly distributed throughout the ooplasm.

Transmission of hereditary information. The nucleus of germ cells performs three main functions: to store and transmit genetic information, implement hereditary information, and control the synthetic processes in the cytoplasm. In addition, some DNA is also found in the cytoplasm and in the mitochondria. Trigger proteins are substances produced in the cytoplasm. They can regulate the activity of the cell`s genome in the nucleus.

Laboratory tasks

Purpose: ***Master the microscopic structure of gametes.***

Practical task 28. Analyse the light microscope features of spermatozoa (Fig. 28)

1. Look at the sperm smear using an objective with an 40× lens. Concentrate on the area where the smear is thin enough to see individual spermatozoa.

2. View an individual spermatozoon. You can see the head and the tail of the spermatozoon.

3. Draw several spermatozoa. Accurately convey their shape, location, and the head and tail relationship.

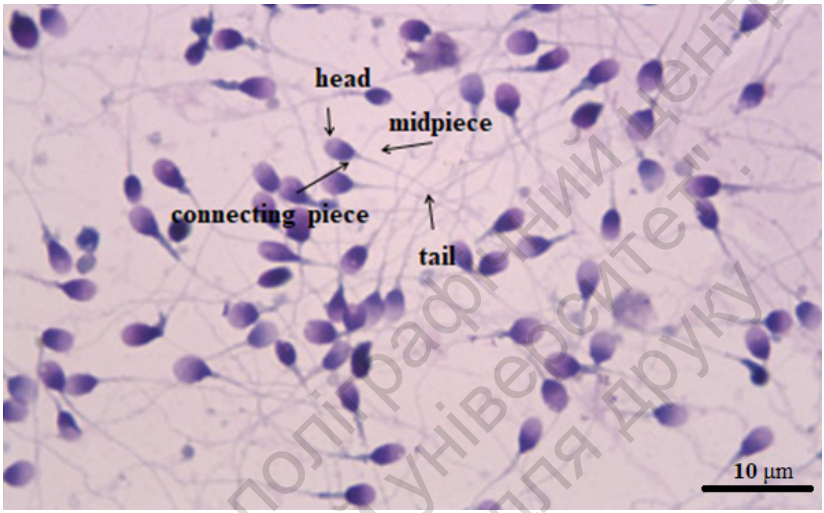


Fig. 28. Light micrograph. Spermatozoa
(Heidenhain's iron hematoxylin stain)

Practical task 29. Analyse the oocyte's microscopic features on the cat ovary's histological specimen (Fig. 29)

1. Look over the cat ovary histological specimen using an objective with an 10× lens. Locate oocytes in the tissue.

2. Switch to the objective with 40× lenses.

3. Focus on the one oocyte. Find the nucleus, cytoplasm, Zona pellucida, and Corona radiate (follicular cells).

4. Draw the oocyte in your Work Book and label the nucleus, cytoplasm, Zona pellucida, and Corona radiate (follicular cells).

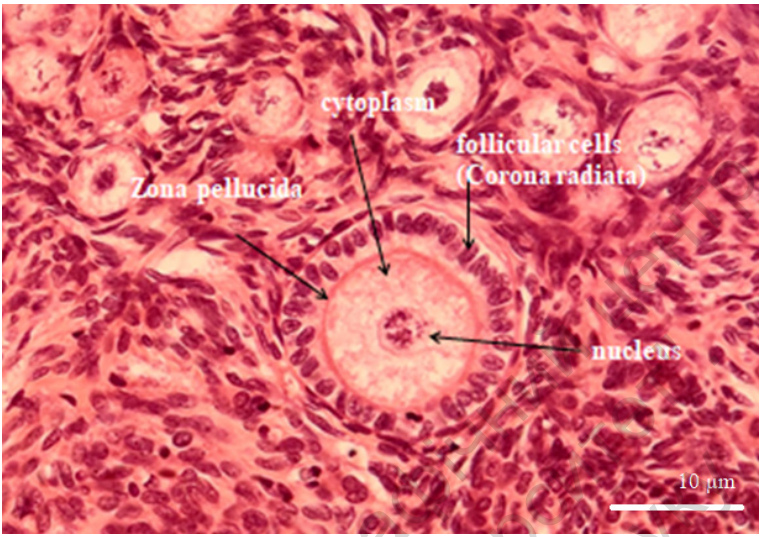


Fig. 29. Light micrograph. Ovary of the cat
(hematoxylin and eosin stain)

Control questions

1. Which of the listed stages are the stages of intrauterine development?
   A. Pre-embryonic, embryonic and post-embryonic
   B. Progenesis, embryonic and post-embryonic
   C. Germinal, embryonic and fetal
   D. Prezygotic, prenatal and postnatal
   E. Germinal, embryonic and postnatal

2. In the early stages of embryonic development, primordial germ cells gather in the yolk sac after migrating from the region of the Hansen's nodule of the epiblast. At which time do the primordial germ cells appear in the area of Hansen's nodule?
   A. On 4th week of embryogenesis
   B. On 2nd week of embryogenesis
   C. On 3rd week of embryogenesis
   D. On 6th week of embryogenesis
   E. On 1st week of embryogenesis

3. During this process, cells replicate their DNA, homologous chromosomes align in pairs, and then interchange chromatid segments, duplicated chromosomes form the metaphase plate, separate, and the cell divides. What is the name of this process?

A. meiosis II
B. meiosis I
C. mitosis
D. amitosis
E. none of them

4. The study involves examining samples of both male and female gametes to determine the set of chromosomes found in a normal gamete. Which set of chromosomes will be found in a normal gamete?

A. 22 single chromosomes
B. 23 autosomes and one sex chromosome
C. 23 autosomes
D. 22 autosomes
E. 22 autosomes and one sex chromosome

5. At what time does the primary formation of spermatocytes begin?

A. On 4th week of embryogenesis
B. On 2nd week of embryogenesis
C. At birth
D. During the fetal period
E. At puberty

6. The correct order of spermatogenesis is _____.

A. spermatogonia → primary spermatocyte → secondary spermatocyte → spermatids → spermatozoa
B. primary spermatocyte → secondary spermatocyte → spermatogonia → spermatozoa → spermatids
C. spermatids → spermatogonia → primary spermatocyte → secondary spermatocyte → spermatozoa
D. spermatogonia → spermatids → spermatozoa → primary spermatocyte → secondary spermatocyte
E. spermatozoa → spermatogonia → primary spermatocyte → secondary spermatocyte → spermatids

7. In the longitudinal section of a spermatozoon, mitochondria can be observed in the electronogram. Which part of spermatozoon is represented?

A. head
B. neck
C. middle piece
D. tail
E. cortex

8. Oogenesis (in humans) starts _____.

A. before birth
B. in the first ten days after birth
C. in the first year after birth
D. at the beginning of puberty
E. at the end of puberty

9. During oogenesis, the process of meiotic division results in the formation of both large and small cells; the large cell is a matured oocyte. The small cell is _____.

A. pole body
B. polar body
C. animal pole
D. vegetal pole
E. micromere

10. The specialised secretory vesicles found in egg cells are known as _____.

A. acrosomes
B. secretory granules
C. vitelline envelope
D. female pronuclei
E. cortical granules

8. FERTILISATION. CLEAVAGE

8.1. Fertilisation

Fertilisation is the process of fusion of male and female germ cells (***pronuclei***) to form a single-celled embryo – a ***zygote***.

Fertilisation is preceded by insemination – the introduction of male germ cells in the female reproductive tract (vagina). The insemination is polyspermal (a man`s ejaculate contains between 48 and 200 million sperm cells per ejaculate). However, only about 200–300 of these can reach the ampulla or infundibulum of the fallopian tubes (fertilisation occurs). If the number of spermatozoa in the ejaculate is low (oligozoospermia) due to insufficient lytic activity, fertilisation does not occur.

The cervical canal mucus and sperm prostaglandins play an important role in sperm migration in the female genital tract. It is known that the cervical canal is filled with mucus, the consistency which becomes liquid at the time of ovulation. At the same time, the mucus acquires an alkaline reaction, which contributes to the survival of the spermatozoa. The prostaglandins in the seminal fluid cause contractions of the myometrium, leading to a partial extrusion of the mucous plug from the cervical canal and its protrusion into the lumen of the vagina. The spermatozoa are embedded in the mucous plug, which, with subsequent relaxation of the uterus, is pulled back into the cervical canal with the spermatozoa. The further movement of the spermatozoa in the cervical canal is facilitated by a peculiar rearrangement of the glycoproteins of the cervical mucus, in which micelles form parallel passages to the cervical canal. After the spermatozoa enter the cervical canal, a kind of selection of spermatozoa takes place. Leukocytes phagocytose defective cells. Some of the sperm are deposited in the cervical canal and released later, increasing the chance of fertilisation, for example, if ovulation is delayed.

Before fertilisation, the sperm must gain the ability to disintegrate the oocyte`s barriers. These barriers of an oocyte are corona radiata (formed by follicular cells), zona pellucida (formed by glycoproteins, ZP1, ZP2, and ZP3), and the vitelline membrane of the oocyte. This ability is achieved by two processes: capacitation and acrosome reaction.

Before fertilisation, the spermatozoa are activated by the influence of mucous secretion of the fallopian tubes. At the same time, the female body factors (pH, mucus, progesterone, chemoattractants, etc.) act on the spermatozoon to promote their ability to migrate and fertilise. Viable, motile spermatozoa cannot fertilise an egg if they do not undergo final maturation in the female genital tract. Capacitation is the process by which a spermatozoon acquires the ability to fertilise an egg. Only after capacitation can spermatozoa bind to the zona pellucida and then carry out an acrosomal reaction, penetrate the egg cell and fertilise it. For effective capacitation, the spermatozoa should be in the female genital tract for about seven hours.

During capacitation, significant changes occur in the protein components of the sperm plasmalemma: some substances are removed, while other proteins are significantly modified. The phosphorylation of tyrosine residues of membrane and cytoplasmic proteins is enhanced, thereby increasing the motor activity of spermatozoa and their ability to undergo the acrosomal reaction. The process of tyrosine residue phosphorylation is initiated by progesterone, which enters the fallopian tubes with the follicular fluid. The sequence of events during capacitation is: progesterone → formation of superoxide anion (O_2) and release of cholesterol from the plasma membrane → activation of adenylate cyclase → increase in cAMP content → activation of protein kinase A → phosphorylation of tyrosine residues → capacitation. Initially, only a small proportion of the spermatozoa undergoes capacitation simultaneously; the rest enter it individually. Thus, the first portion of activated spermatozoa is replaced by another, and so on, maintaining a constant high ability to fertilise. The changes that occur during capacitation play an important role in the subsequent acrosomal reaction.

Chemotaxis plays an important role in the convergence of gametes. The sperm tail membrane contains special receptors that are responsible for this process. These are associated with G proteins. These proteins activate adenylate cyclase, which, through the cascade, increases the concentration of calcium ions in the cytoplasm of the sperm tail. As a result, the mobility and speed of movement of the spermatozoon tail is increased. Excitation of the tail plasmalemma

receptors is caused by chemoattractants – substances secreted by the egg cells and follicular cells of the corona radiata. They are also present in the follicular fluid of the bursting follicle and enter the fallopian tube with the ovulated cell. Progesterone also increases the motor activity of sperm cells by increasing the concentration of calcium in the sperm.

The ejaculate of a healthy young man contains approximately 200 million spermatozoa, which retain the ability to fertilise for 2 days. About 2 hours after insemination, the spermatozoa reach the egg cell and surround it. Due to the synchronous movement of spermatozoa flagella, the egg cell begins to perform rotational movements. After contact with the follicular cells of the corona radiata, an acrosomal reaction occurs.

Acrosomal reaction. The acrosomal reaction releases enzymes from the acrosomes of the spermatozoa. The morphological manifestation of the acrosomal reaction is the fusion of the acrosomal membrane with the sperm plasmalemma in the anterior part of the head. A major role in the acrosomal reaction is played by the rapid entry of calcium ions into the head of the sperm, which triggers the synthesis of cyclic nucleotides and increases the activity of ATPase. This leads to an increase in intracellular pH and the insertion of the acrosomal reaction. It initiates the interaction of the ZP3 glycoprotein of the zona pellucida with the receptor enzyme (β1, 4-galactosyltransferase I) in the plasmolemma of the sperm head. The acrosome enzymes (hyaluronidase and acrosin) break down the bonds between corona radiata cells, allowing the spermatozoa to penetrate. Egg cell denudation (partial or complete release of the egg cell from the follicular cells) in the fallopian tubes also plays an important role. With complete denudation, the sperm interact immediately with the zona pellucida.

The zona pellucida is a more significant barrier in the spermatozoa way. Initially, sperm cells bind to specific receptors. The most well-known receptor proteins for spermatozoa are the glycoproteins ZP1, ZP2 and ZP3. Spermatozoa attachment to the zona pellucida is species-specific. After attachment to the zona pellucida, enzymes associated with the inner acrosomal membrane dissolve the small area

of the zona pellucida to which the spermatozoon is attached. Active movements of the tail allow the spermatozoon to migrate through the zona pellucida for 5–10 minutes.

The oolemma contains a receptor system for interaction with complementary sperm receptors. After interaction and fusion of the plasma membranes of sperm and oocyte, the sperm head is introduced into the ooplasm.

Polyspermy block. Although many sperm cells attach themselves to the egg cell at the same time, only one of them will contribute its genome to the egg cell. If the nuclei of two sperm penetrate each other (dyspermia), a triploid embryo with 69 chromosomes is formed. During the cleavage of such zygotes, additional spindles are formed, leading to abnormal divergence of chromosomes, the appearance of numerical anomalies and cessation of further embryo development.

There are several mechanisms that are responsible for the prevention of polyspermy.

1. Simultaneously with the beginning of the interaction of two gametes in the egg a cortical reaction occurs, triggered by a rapid increase in the concentration of calcium ions in the egg cell. However, cortical granules quickly move under the cytolemma, and their contents are released into the perivitelline space under the zona pellucida. This forms a fertilisation coat. This coat is irresistible to sperm. This envelope is also important during cleavage.

2. Cortical granules contain enzymes, including various hydrolases. These enzymes cleave the ZP2 receptors and modify the ZP3 receptors of the zona pellucida, which, therefore, lose their ability to bind other sperm. This prevents the development of polyspermy. At the same time, the cortical granule enzymes block the acrosomal reaction in other sperm. All these changes in the zona pellucida result in a late blockade of polyspermy.

3. Simultaneously with the modification of ZP, the cortical granules enzymes change the molecular organisation of the oolemma, which acquires new properties, including a positive charge (+20 mV), repelling positively charged sperm (an early block of polyspermy, lasting 0.1 seconds).

Sincarion. The male and female pronuclei swell due to the replacement of protamines in chromatin by histones. The pronuclei approach each other, lose their nuclear membranes and fuse. The process of fusion of the pronuclei is called synkaryon. Their genomes are mixed, and the diploid set of chromosomes is restored. The result is a one-cell embryo – a *zygote*.

The sperm contributes to the egg cell: its haploid set of chromosomes, the cleavage signal protein, the proximal centriole (the ovum does not contain centrioles), and some mitochondria. Fertilisation activates the egg cell, increasing the concentration of Ca^{2+} in the cytoplasm, which serves as a signal for the second meiotic division. A fertilised second oocyte completes meiosis by forming a haploid mature egg cell and a second polar body. It is located next to the first one between the zona pellucida and the plasmalemma. Sometimes, this body is not eliminated and may even be incorporated into the zygote genome. This leads to the formation of a tumour called an ovarian teratoma.

8.2. Cleavage

After a short rest period, the zygote enters a new period of embryogenesis – cleavage.

During cleavage, the unicellular zygote transforms into a multicellular embryo. The embryo, which is up to 2 weeks of development, is called *conceptus*. The cleavage is a series of mitotic divisions. The characteristic feature of cleavage is the absence of the G1 period, resulting in the cells not having time to increase in size. Therefore, with each division, the cells become smaller. In addition, the total volume of the embryo decreases by 20–40 % during cleavage. The cells in the cleavage stage are called *blastomeres*.

In mammals, including humans, cleavage is holoblastic (the entire material of the zygote is divided), unequal (blastomeres of different sizes are formed), asynchronous (blastomeres do not divide simultaneously: after the stage of two blastomeres, the stage of three blastomeres begins, as one of them starts dividing later than the second, etc.). Asynchrony and unequal cleavage divisions do not

appear immediately but from the second division onwards. The first two blastomeres are approximately the same size.

As a result of cleavage, blastomeres of different sizes are formed: large dark blastomeres and small light blastomeres. The light blastomeres divide more quickly, and, as a result, they surround the large dark blastomeres from the outside and occupy an inner position. The light blastomeres form the trophoblast, which will later serve as a source for developing the chorionic epithelium. The dark blastomeres (embryoblast) give rise to the body and the embryo's provisional organs, except the chorion.

The compacted embryo is called a morula at the 16–32 cell stage. It is formed on the 3^{rd} day of embryogenesis. The morula cells are connected by gaps and tight junctions (compactification of the embryo). Until the blastocyst stage, the embryo is surrounded by a zona pellucida. Its functions: barrier; during cleavage, the blastomeres are compactly arranged in a limited three-dimensional space; prevents the embryo from adhering to the epithelium of the fallopian tube (this prevents ectopic (tubal) pregnancy); prevents embryonic adhesion in multiple pregnancies.

By the 4^{th} day of development, the trophoblast cells begin to secrete fluid, which accumulates inside the morula, forming a cavity and displacing the embryoblast to one of the poles. The blastocyst is formed. It consists of the blastoderm (trophoblast), the blastocoel (the inner cavity) and the embryoblast (the inner cell mass). The fertilisation envelope is destroyed and shed.

In humans, cleavage occurs during the first week of embryogenesis. During this time, the embryo enters the uterine cavity and begins to implant.

Implantation is the process by which the embryo penetrates the mucous membrane of the uterus (endometrium) and establishes close connections with its blood vessels. Implantation consists of two phases: *adhesion* (adherence to the endometrium) and *invasion*. Before implantation, the trophoblast is divided into two layers: cellular trophoblast, or cytotrophoblast (inner layer) and symplastotrophoblast (plasmodiotrophoblast, syncytiotrophoblast, syntrophoblast) – outer layer. Symplastotrophoblast acquires adhesive properties that allow it

to adhere to the endometrium. The most important cell adhesion molecules (CAMs) during implantation are cadherins, integrins, and, to a lesser extent, selectins, as well as the proteins trophinin, tascin, and biscin. The expression of CAMs is controlled by progesterone, and the conceptus modulates their qualitative composition.

Implantation usually occurs in the endometrium of the posterior wall of the uterus. It can also occur in any other part of the endometrium. It all depends on the adhesive properties of the trophoblast (closely related to the length of the fallopian tubes).

Before implantation, the endometrium of the uterus undergoes significant changes. Under the influence of corpus luteum progesterone, it acquires properties favourable to successful implantation and enters the secretory phase of the menstrual cycle. The endometrial epithelium and the uterine glands are hypertrophied. The synthesis and secretion of mucus (include glycoproteins, glycogen, and lipids) increase sharply. In the early stages of the development of the concept, all these substances are the source of its nutrition. The blood supply to the endometrium increases greatly. Glycogen accumulates in the connective tissue cells of the lamina propria.

The symplastotrophoblast, which begins to form primary villi during implantation, synthesises and secretes enzymes that lyse endometrial tissue. This leads to invasion (penetration into the mucous membrane).

After the trophoblast destroys the endometrium blood vessel walls, blood flows out of them and washes the embryo. The edges of the mucous membranes over the embryo then fuse together. In humans, implantation is deep and interstitial because the embryo penetrates deep into the endometrium and destroys its blood vessels.

Implantation lasts about 40 hours. During implantation, the concept of food type changes. For a short time after fertilisation, the nutrition is *autotrophic*, then histotrophic (secret of mucous cells covering the epithelium of the oviduct, uterus, uterine glands and products of tissue breakdown in the initial phases of implantation). After the destruction of blood vessels, the type of nutrition is *hemotrophic* (nutrients for the fetus come from the mother's blood).

Laboratory tasks

Purpose: ***Master the microscopic structure of the zygote, cleavage and blastocyst.***

Practical task 30. Analyse the light microscope image of the zygote, cleavage (2-cell stage, 4-cell stage) and blastocyst (Fig. 30)

1. Look at the zygote, cleavage (2-cell stage, 4-cell stage) and blastocyst on the light microscope image.

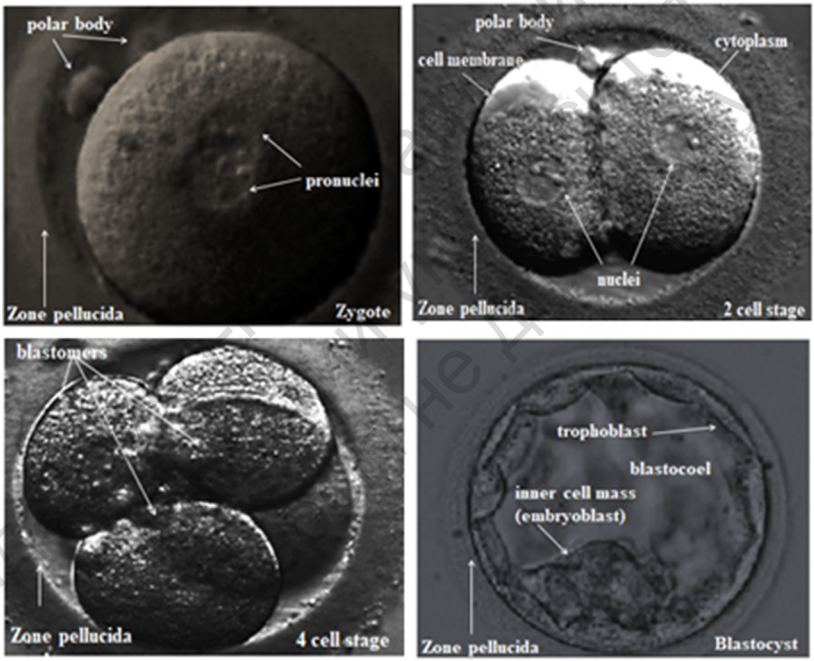


Fig. 30. Light micrograph. Zygote. Cleavage. Blastocyst

2. ***The zygote:*** locate the cell membrane, cytoplasm, pronuclei, polar bodies, zone pellucida. ***2-Cell stage:*** locate zone pellucida, polar bodies, blastomeres, their cell membrane, cytoplasm and nucleus. ***4-Cell stage*****:** locate the zone pellucida, polar body, blastomeres, their

cell membrane, cytoplasm and nucleus. ***The blastocyst*:** locate the zone pellucida, trophoblast, embryoblast (inner cell mass), and blastocoel.

3. In your Workbook, label the following: ***zygote*** – cell membrane, cytoplasm, pronuclei, polar bodies, zone pellucida; ***2-cell stage*** – zone pellucida, polar body, blastomeres, their cell membrane, cytoplasm and nucleus; ***4-cell stage*** – zone pellucida and blastomeres; ***blastocyst*** – zone pellucida, trophoblast, embryoblast (inner cell mass) and blastocoel.

Control questions

1. When a sperm enters an oocyte during fertilisation, what stage of oogenesis is the human oocyte in?
   A. primary oocyte
   B. secondary oocyte
   C. mature ovum
   D. zygote
   E. ovary
2. Name the series of structural changes in sperm that occur in the female reproductive tract _____.
   A. maturation
   B. spermiogenesis
   C. spermiation
   D. capacitation
   E. activation
3. Which structure is the derivative of the fertilisation envelope?
   A. egg cell's plasma membrane
   B. sperm cell's plasma membrane
   C. corona radiata
   D. zona pellucida
   E. yolk
4. Name the process by which blocking of polyspermy is achieved _____.
   A. cortical reaction
   B. acrosomal reaction

C. metabolic activation
D. formation of polar bodies
E. phagocytic degradation of sperm cells

5. Select the name of the one-cell (first) stage of embryo development _____.
A. blastomere
B. blastocyst
C. germ disk
D. zygote
E. implant

6. Name the type of ligand that corresponds to the specific binding of spermatozoa and initiates the acrosome reaction _____.
A. ZP I
B. ZP II
C. ZP III
D. ZP IV
E. ZP V

7. What is the name of a series of mitotic divisions in a newly formed embryo?
A. fertilisation
B. cleavage
C. blastulation
D. gastrulation
E. neurulation

8. In what term does cleavage occur in humans?
A. after the implantation and under the fertilisation envelope
B. after the implantation and out of the fertilisation envelope
C. before the implantation and under the fertilisation envelope
D. before the implantation and out of the fertilisation envelope
E. during the implantation and under the fertilisation envelope

9. The histological specimen of a human embryo is being examined through a microscope. The specimen shows an early embryo composed of tightly packed blastomeres lacking a visible internal cavity. What is the name of this stage?
A. coeloblastula
B. amphiblastula

C. stereoblastula
D. morula
E. discoblastula

10. What type of blastula is inherent in the human?
A. periblastula
B. blastocyst
C. coeloblastula
D. discoblastula
E. stereoblastula

9. GASTRULATION. ORGANOGENESIS

9.1. Morphogenetic processes in embryogenesis

In biology, morphogenesis (from the Greek 'morphê', form, and 'genesis', formation) is the process of formation and development of organs, systems, and body parts of organisms, both in the single organism (ontogenesis) and the historical, evolutionary development (phylogeny).

The main morphogenetic processes in embryogenesis are cell reproduction, determination, differentiation, induction, integration, cell recognition, adhesion, cell movements, and apoptosis.

Cell proliferation. The proliferation of cells during embryogenesis leads to an increase in the number of cell populations. Methods of cell proliferation during this period must ensure equal distribution of chromosomes between daughter cells. Segregation and mitosis are used to achieve this.

Determination (from the Latin "*determinatio*": to limit, to conclude, to determine) is the process of genetic determination of the further path of cell development, formation of qualitative uniqueness of parts of the body in the early stages of its development and determination of the path of further development of parts of the embryo. The potential of cells to differentiate into different cell types is very high at the beginning of embryonic development. Gradually, the expression of some genes and the repression of others occurs, narrowing the possibilities for differentiation.

Differentiation (from the Latin *differentia* – 'difference') is the process of the implementation of a genetically determined programme for the formation of a specialised phenotype of cells, reflecting their ability to perform certain specialised functions. Differentiation results in biochemical, morphological and functional changes. Biochemically, it involves synthesising specific proteins and, morphologically, developing specialised organelles and inclusions that ensure the performance of particular functions not characteristic of other cell types. The differentiation of a cell involves the selective activation of genes. A shift in the ratio of the nucleus to the cytoplasm towards a predominance of the cytoplasm over the nucleus is an essential morphological indicator of cell differentiation. At all stages of

ontogenesis, differentiation takes place. The differentiation of cells is due to their determination.

Induction (from the Latin *inductio* – 'excitation, guidance') is an interaction between parts of the embryo in which one part (the inductor) is in contact with another part (the reactive system), which determines its developmental direction. Induction involves the production of morphogenesis-regulating factors by the inducer substrate, which leads to the differentiation process's activation (or inhibition). The receptors of the inducible structures perceive these factors.

Induction is the influence of some structures on other neighbouring structures. Inducers can be the most common factors, such as nutrients or oxygen, pH levels, specific salt concentrations, hormones, neurotransmitters, and many unidentified chemicals in the later stages of development.

The ability to respond to different types of inductive influences is called the competence of the embryonic material.

Induction is the influence of the microenvironment that leads to the expression of genes competent to respond to these factors.

Integration (from the Latin *ntegratio* – 'unification') combines individual structures into more complex organised systems, characterised by strong interconnections, interactions and interdependencies. During embryogenesis, cells can form long-term associations – cell aggregations.

Cell recognition is the specific interaction of cells with each other or non-cellular structures. It is provided by particular cytoreceptors located in the cell membrane. As a result of recognition, morphogenetic processes such as cell adhesion, cessation of cell migration, formation of cell ensembles and interaction of cells with each other develop.

Cell adhesion is the ability of cells to attach components of the intercellular substance selectively. Adhesion is both a cell recognition process and an implementation mechanism. Adhesion occurs through the interaction of glycoproteins of cells in contact with the plasmalemma that recognise each other, or glycoproteins of the plasmalemma of the cell and the extracellular matrix. Glycoproteins are adhesion molecules. Their disappearance from the plasma membrane and adhesive contact disruption allow cells to move. Directed cell migration is ensured by recognising adhesion molecules

in the plasma membrane of other cells or the intercellular substance. Cell adhesion during histogenesis is thought to ensure the onset, progression and termination of cell migration and the formation of their communities.

Cells attach to components of the intercellular substance through focal adhesive contacts, and cells attach to each other through intercellular contacts.

The adhesion molecules are specific to each tissue type. Adhesion ensures the exchange of information between cells using signalling molecules and gap junctions.

Cell movements are determined by many factors of cell vital activity: different metabolism levels, uneven mitotic activity, cell recognition, intercellular contacts of various strengths, induction, etc. Cell movements are significant during gastrulation (see below).

Apoptosis, a genetically programmed cell death, is an integral part of many stages of embryogenesis.

9.2. Gastrulation

Gastrulation is the process by which germ layers are formed. There are several ways of gastrulation:

- invagination – invagination (infolding) of the cell sheet into embryo:
- immigration (ingression) – migration of cells into the embryo;
- delamination – splitting or migration of one sheet into two sheets (epiblast and hypoblast);
- epiboly – the expansion of one cell sheet over other cells.

In humans and other mammals, gastrulation proceeds in two phases and ends with the formation of germ layers containing the rudiments of various tissues. It can rightfully be called mixed gastrulation.

The first gastrulation phase begins on the 7th day of embryogenesis and runs concurrently with implantation. This phase is carried out by delamination – splitting the embryoblast into two sheets: the epiblast, or primary ectoderm, and the hypoblast, or primary endoderm. These sheets form a two-layered germinal disc. The cells of the epiblast are cylindrical, whereas the cells of the hypoblast are cubic or flat. The epiblast serves as a source of development for the entire embryo, as well as the amniotic ectoderm, while the morphogenetic potential of

the hypoblast is sharply limited: its cells migrate along the inner surface of the trophoblast and participate in the formation of the yolk sac wall, which is tightly adjacent to the trophoblast, and allantois. At this point, the gastrulation process is temporarily halted, and provisional organs, such as the yolk sac, amnion, and chorion, can form, ensuring the embryo's further development. Their formation takes about one week (see Class 6, Part 2).

The second phase of gastrulation begins on days 14–15 of embryogenesis. Outside is the chorion. It consists of two layers: the trophoblast and the extra-embryonic mesenchyme. The trophoblast, in turn, is divided into two sheets: the outer symplastotrophoblast and the inner cytotrophoblast. The chorion forms secondary villi. The cavity of the embryo is filled with extra-embryonic mesenchyme. It contains two vesicles: the amnion, composed of extraembryonic ectoderm and extraembryonic mesenchyme, and the yolk sac, formed by extraembryonic endoderm and extraembryonic mesenchyme. The sacs are juxtaposed and attached to the chorion by an amniotic stalk formed by an extraembryonic mesenchyme. The embryo's body is formed by the cells at the bottom of the amniotic sac and the cells at the roof of the yolk sac, and it is called the germinal disc. It consists of primary ectoderm (epiblast) and primary endoderm (hypoblast). The hypoblast is only formally the embryo's body, as it moves sideways during development to allow germinal endoderm cells to migrate from the epiblast and become the extraembryonic endoderm of the yolk sac. The posterior wall of the yolk sac grows into the amniotic stalk and forms the fourth provisional organ, the *allantois*.

The second phase of gastrulation is carried out by cell migration and their partial invagination. The most important processes take place in the epiblast. Epiblast cells proliferate intensively and move from the anterior to the posterior end of the embryo body. They move in two streams from the two edges of the epiblast. Some of the cells turn towards the centre of the epiblast earlier than others, while the rest reach the posterior end of the germ shield. At this point, the two streams of cells meet and start moving in opposite directions towards the anterior end of the embryo. As a result of these migrations, a cluster

of cells called the *primary streak* forms in the centre of the epiblast. In front of the primary streak, *Hensen's (primitive knot) node* is formed.

The primary streak is the site through which cell material migrates under the epiblast to form the mesoderm and germinal endoderm, and the primitive knot is the site where cells migrate under the epiblast, forming the *chordal process (chord*). These cells are located anterior to the Hensen's node in the epiblast.

As a result of this process a third germ layer, the mesoderm, is formed between the primary streak and the hypoblast. The embryo becomes three-layered. The central cells of the epiblast located in front of the Hensen's node form a notochord after their migration under the epiblast. Some of the cellular material of the primary streak migrates to the hypoblast and is integrated into it, occupying a central position. The intestinal endoderm is formed from this material, and the primary endoderm is displaced to the periphery, forming the yolk sac wall. Cells from all germ layers, but mainly from the mesoderm, are displaced and fill the entire space between the germ layers – the secondary mesenchyme. After separating the embryonic material from the extraembryonic organs, the secondary mesenchyme is subdivided into embryonic and extraembryonic mesenchyme.

After the formation of the three germ layers (ectoderm, endoderm and mesoderm), their differentiation begins. This process occurs during the 3rd week of embryogenesis.

Ectoderm. It is initially called the *primary ectoderm (epiblastoma*) because it contains skin ectoderm, neuroectoderm, chordal process, intestinal endoderm, and *mesenchyme*. During the second phase of gastrulation, mesoderm, chordal process, and intestinal endoderm materials are evicted from the epiblast.

The neural plate is formed in the ectoderm, which first turns into a neural groove, then gradually separates from the rest of the ectoderm, sinks under it, and closes to form the neural tube. At the same time, the part of the ectoderm between the neuroectoderm and the skin ectoderm gives rise to *ganglionic plates (neural crest)* that lie on the sides of the neural tube. The formation process of the neural tube and the neural crest is called *neurulation*.

The neural tube is the source of the development of the brain, spinal cord, posterior pituitary gland, cranial nerves, motor roots of the spinal nerves, retina and optic nerve. The ganglionic plates give rise to the nervous tissue of the spinal (posterior roots), cranial and autonomic nerve ganglia and nerves, and the adrenal medulla. Part of the neural crest turns into *neuromesenchyme* (*ectomesenchyme*), which gives rise to the connective tissues of the head, the bones of the skull and visceral arches, the dentin of the teeth, and the outer membranes of the eye are formed.

Some of the ectoderm cells migrate to the primary endoderm even at the time of the mesoderm formation and become embedded between its cells. These cells form the intestinal endoderm, and the entire primary endoderm becomes the extraembryonic endoderm of the yolk sac.

The primary ectoderm becomes the *secondary* or *skin ectoderm* after all the above-mentioned rudiments have been expelled from it. It serves as a source for the development of multilayered epithelium: the epidermis of the skin and its derivatives (hair, glands, nails); epithelium of the oral cavity and anal rectum; stratified epithelium of the lower part of the vagina; tooth enamel; epithelium of the anterior and intermediate lobes of the pituitary gland; anterior corneal epithelium, conjunctival epithelium of the eye; lens; epithelium of the inner ear.

Mesoderm. Initially, the mesoderm is a loose cluster of cells (presomitic mesoderm), and then the mesoderm is divided into *dorsal* and *ventral* mesoderm. The dorsal mesoderm is divided into segments – *somites* – along the length of the embryo. Segmentation of the dorsal mesoderm begins at the anterior end and spreads rapidly caudally. The number of somites increases over time. Each somite differentiates into 3 parts: outer – *dermatome*, middle – *myotome*, inner – *sclerotome*. First, the sclerotome, then the myotome, and then the dermatome are separated. The dermatome gives rise to the *dermatomal mesenchyme*, which gives rise to the skin's dermis. The myotome serves as a source for the formation of skeletal (locomotor) striated muscle tissue and non-locomotor (not involved in the formation of the musculoskeletal system) striated muscle tissue of the tongue, cheeks, lips, facial muscles, pharynx, soft palate, part of the oesophagus, anal region

rectum, vagina. The sclerotome gives rise to the *sclerotome mesenchyme*, which forms bone and cartilage tissue.

Between the dorsal and ventral mesoderm is the intermediate mesoderm, or *nephrotome.* It is segmented in the anterior parts of the embryo, while it does not undergo segmentation in the posterior parts. From the segmented sections of the nephrotome, the pronephros and the primary kidney develop sequentially, and in the male body, the efferent tubules of the epididymis develop. The non-segmented part of the nephrotome is called the nephrogenic tissue. It is a source for forming the epithelium of all parts of the final kidney nephron.

The ventral mesoderm (*splanchnotome*) does not undergo segmentation. It is divided into two sheets – the visceral and parental sheets. Between them is the secondary cavity of the body – the caelom. From the sheets of the splanchnotome develop mesothelium of the serous membranes, striated cardiac muscle tissue, adrenal cortex, and epithelium of the gonads. Cells are evicted from the visceral layer of the splanchnotome to form the splanchnotome mesenchyme, from which connective and smooth muscle tissues of internal organs and vessels are formed.

Endoderm. As a result of the formation of body folds, the embryo's body rises above the provisional organs and separates from them. The embryo folds into a tube. At the same time, this leads to forming an intestinal tube from the intestinal endoderm, which is separated from the extraembryonic endoderm of the yolk sac. The intestinal tube is the source for forming the epithelium of the stomach, intestine, liver, gallbladder and pancreas.

Axial complex. The formation of the axial complex is based on three important processes: neurulation, differentiation of the mesoderm, formation of the body folds with separation of the embryo from the extraembryonic organs and formation of the intestinal tube.

The axial complex consists of: skin ectoderm; neural tube and ganglionic plates; somites, consisting of dermatomes, myotomes and sclerotomes; nephrotomes; splanchnotomes; chordal process; intestinal tube; mesenchyme. Each of them is a source for developing one or more types of tissues.

Mesenchyme. The mesenchyme forms very early. The *primary mesenchyme (extra-embryonic mesoderm)* migrates from the epiblast during the 2nd week of embryogenesis and participates in the formation of provisional organs. The secondary mesenchyme forms the internal environment and the smooth muscle tissue. All three germ layers give rise to the secondary mesenchyme, but the mesoderm is the main source. The dermatome mesenchyme is formed from the dermatome of the mesoderm. The dermatome mesenchyme serves as a source for developing the skin's connective tissue. The sclerotome forms sclerotome mesenchyme – the source of bone and cartilage tissue. Finally, the splanchnotome mesenchyme is formed from the splanchnotome. The splanchnotome mesenchyme is the source for developing several internal environment tissues and smooth muscle tissue. Mesenchyme that develops from parts of the mesoderm is called mesodermal mesenchyme. Part of the mesenchyme is formed from the outer germ layer – the ectoderm, or neuroectoderm (neural crest). This mesenchyme is called ectomesenchyme or neuromesenchyme. The source of ectomesenchyme is the endoderm of the anterior part of the intestinal tube. Mesenchymal cells migrate between the three germ layers, occupying all the space between them.

The mesenchyme comprises process cells connected to each other by intercellular contacts, forming a functional (false) syncytium. Between the cells is the intercellular substance. Thin mesenchymal fibrils and tissue fluid form it.

The functions of the mesenchyme in the embryo are varied. It plays the role of embryonic connective tissue: its cells synthesise the primary (primitive) intercellular substance; mesenchyme performs trophic, supportive, regulatory, barrier-protective and morphogenetic functions. At the same time, the mesenchyme is an important embryonic germ (often called the fourth germ layer): numerous tissues are formed from it (connective tissues, blood and lymph, smooth muscle tissue, etc.).

The process of tissue formation in embryogenesis is called histogenesis. Histogenesis includes cell division, cell growth, apoptosis, cell migration, cell adhesion, determination, differentiation, induction, and segregation.

9.3. Organogenesis

Organogenesis is the process of the formation of various organs in the body from the germ layers. A synchronised series of morphological and functional alterations and transformations occur during organogenesis. The embryo is divided into relatively independently developing local systems that give rise to organs. Many mechanisms of histogenesis and organogenesis are common. Cell proliferation, migration, differentiation and death, extracellular matrix modifications, and germ-layer interactions include organogenesis. Cellular processes lead to morphological transformations – cell sheet bending (formation of the central nervous system, digestive organs, etc.), epithelial layer thickening (development of some organs of the oral cavity and sense), and cell cavitation (formation of hollow organs). Organogenesis is also impossible without the participation of nervous, endocrine and immune regulation.

Organogenesis (tissue differentiation into organs) occurs between the 6th and 8th week of embryogenesis. At this time, the vesicles of the central nervous system undergo the development. The brain and spinal cord form from the neural tube. The anterior part of the neural tube first turns into three cerebral vesicles – he forebrain (prosencephalon), the midbrain (mesencephalon), and the hindbrain (rhombencephalon). Then, five cerebral vesicles form – the forebrain separates into the telencephalon and the diencephalon; the midbrain forms the midbrain; the hindbrain separates into the metencephalon and the myelencephalon. The diencephalon gives rise to the thalamus, hypothalamus, optic cups, and neurohypophysis. The telencephalon develops into the cerebrum. The metencephalon develops into the pons and the cerebellum. The myelencephalon gives rise to the medulla oblongata in the adult brain.

By the 4th week of embryogenesis, the heart has established its four chambers and blood vessels are formed. The formation of the main membranes of the heart and blood vessels, the complication of their structure, the differentiation of cardiomyocytes and the formation of the heart's conduction system occur during the second month of embryogenesis. At the same time, the main endocrine organs are being formed. In the 4th to 6th week, all gastrointestinal tract organs are formed. The liver and pancreas begin to form at the end of the third

week of embryogenesis and then undergo further development. A secondary kidney is formed in the 8th week of embryogenesis, and then its further development occurs. By the 8th week, the differentiation of the gonads is completed. Organs of the immune system begin to form later than other organs. The lymph nodes first appear by the end of the eighth week; the spleen is formed by the end of the fifth week. The thymus is formed by the end of the first month of embryogenesis but is not populated with lymphocytes until the end of the second month.

During weeks six to eight of development, the main organ systems of the embryo are formed. The ninth week marks the beginning of the fetal period, which lasts until birth. The growth and differentiation of anatomical structures take place.

Laboratory tasks

Purpose: ***Master the microscopic structure of the embryo structure in the different stages of its development.***

Practical task 31. Analyse the microscopic structure of the primitive streak of the chicken embryo, whole mount (Fig. 31)

1. Examine the histological specimen of the primitive streak using an 10× objective.
2. Locate the dark zone (area opaca), light zone (area pellucida), embryonic disc, primitive streak, primitive pit, and Hensen's node.
3. Draw the primitive streak in your Workbook and label the structures shown.

Practical task 32. Analyse the microscopic structure of the primitive streak of the chicken embryo, cross-section (Fig. 32)

1. Examine the histological specimen of the primitive streak using a 10× objective. If necessary, switch to an objective with a 20× lens.
2. Locate the ectoderm, entoderm and mesoderm.
3. Draw the primitive streak in your Workbook and label the structures shown.

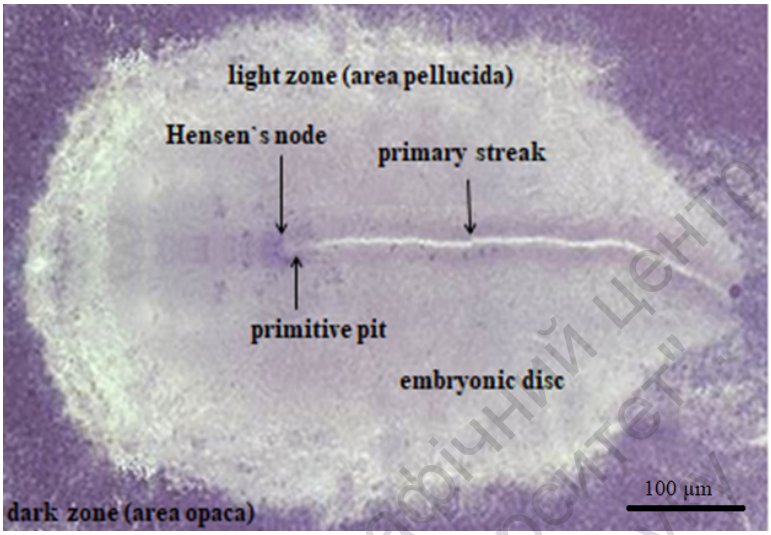


Fig. 31. The primitive streak of the chicken embryo, whole mount (hematoxylin stain)

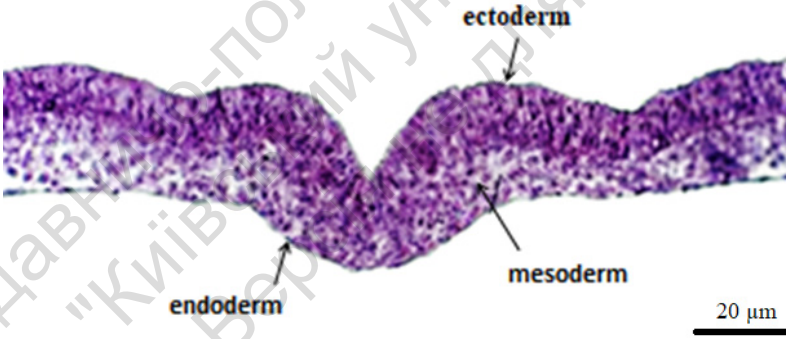


Fig. 32. The primitive streak of the chicken embryo, cross-section (hematoxylin stain)

Practical task 33. Analyze the microscopic structure of the frog embryo, stage of middle neurula (Fig. 33)

1. Examine the histological specimen of the neurulation in frog embryo, showing middle stage, using an objective with a 20× lens.

2. Locate the neural groove, neural plate, notochord, cavity of gut, ectoderm, endoderm and mesoderm.

3. Draw the frog embryo in your Work Book and label the structures shown.

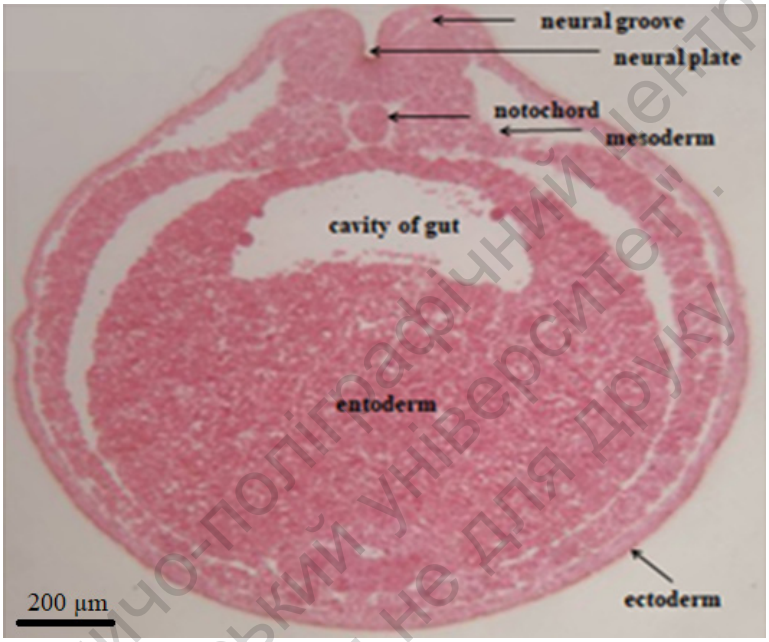


Fig. 33. Neurulation in a frog embryo, middle neurula stage
(hematoxylin and eosine stain)

Practical task 34. Analyse the microscopic structure of the chicken embryo, 34 hours of incubation, whole mount (Fig. 34)

1. Examine the histological specimen of the chicken embryo, 34 hours of incubation, whole mount, using a 10× objective. Switch to an objective with a 20× lens.

2. Locate the eye vesicles, cerebral vesicles (prosencephalon, mesencephalon and rhombencephalon), notochord, neural tube, somites, and heart.

3. Draw the chicken embryo and 34 hours of incubation in your Work Book and label the structures shown.

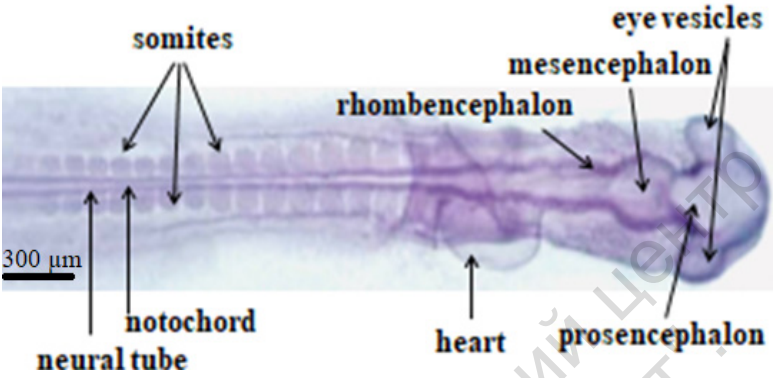


Fig. 34. The chicken embryo, 34 hours of incubation, whole mount
(hematoxylin stain)

Practical task 35. Analyse the microscopic structure of the chicken embryo, 46 hours of incubation, cross-section (Fig. 35)

1. Examine the histological specimen of the chicken embryo, 46 hours of incubation, using a 20× objective.

2. Locate the notochord, neural tube, ectoderm, mesoderm, endoderm, somites, coelom, splanchnotom, and nephrotome.

3. Draw the chicken embryo and 46 hours of incubation in your Work Book and label the structures shown.

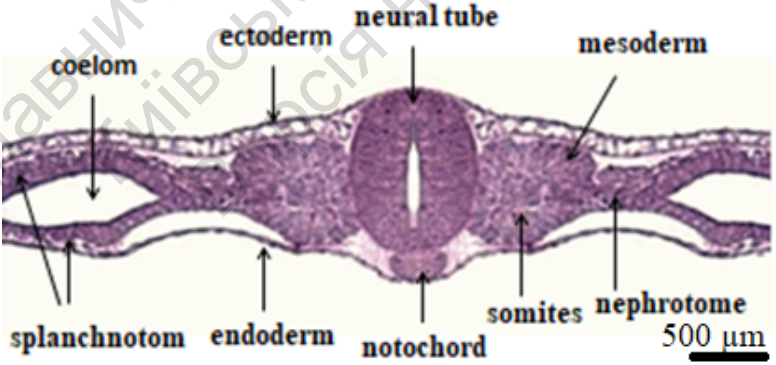


Fig. 35. The somites, chord and neural tube of the chicken embryo, 46 hours of incubation, cross-section
(hematoxylin stain)

Practical task 36. Analyze the microscopic structure of the chicken embryo, 72 hours of incubation (Fig. 36)

1. Examine the histological specimen of the chicken embryo, 72 hours of incubation using an 4× objective.

2. Find the head and heart regions. Locate the diencephalon, optic vesicle and lens in the head region and the heart, foregut, aorta and spinal cord in the heart region.

3. Draw the chicken embryo, 72 hours of incubation in your Work Book and label the structures shown.

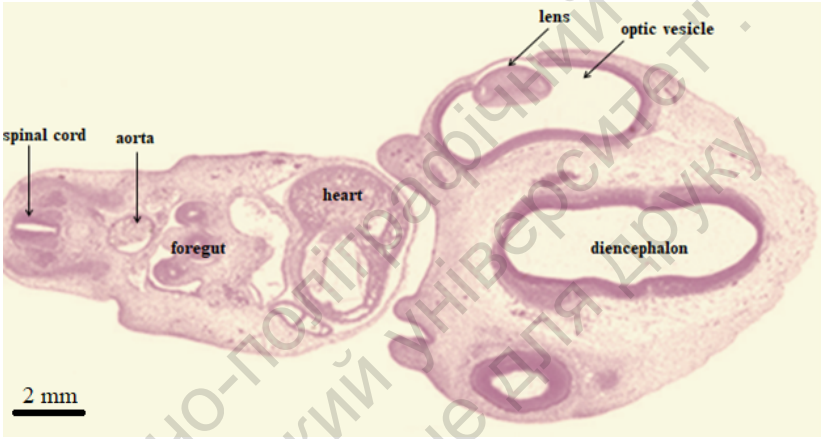


Fig. 36. The chicken embryo, 72 hours of incubation, cross section (hematoxylin stain)

Control questions

1. Name the process of gastrulation, in which a sheet of cells folds into an embryo _____.

A. invagination
B. involution
C. ingression
D. delamination
E. epiboly

2. Indicate from which part of the human embryo is formed during gastrulation embryonic primary tissues ________.

A. trophoblast
B. epiblast
C. hypoblast
D. umbilical vesicle
E. notochord process

3. Specify the name of the primary cavity of the embryo ________.

A. blastocoel
B. coelom
C. archenteron
D. gastrocoel
E. blastopore

4. Indicate which germ leaf gives rise to chordae ________.

A. embryonic mesoderm
B. extra-embryonic mesoderm
C. embryonic ectoderm
D. extra-embryonic ectoderm
E. embryonic endoderm

5. Choose which structural components the somite consists of ________.

A. dermatome, sclerotome, splanchnotome
B. sclerotome, splanchnotome, paraxial mesoderm
C. myotome, sclerotome, nephrotome
D. sclerorome, myotome, splanchnotome
E. dermatome, myotome, sclerotome

6. During embryonal development, the embryo passes through several stages. Please choose the correct order for them.

A. cleavage → fertilization → gastrulation → neurulation → organogenesis
B. fertilization → cleavage → neurulation →gastrulation → organogenesis
C. fertilization → cleavage → gastrulation → neurulation → organogenesis
D. fertilization → cleavage → gastrulation → organogenesis → neurulation
E. fertilization → gastrulation → neurulation → cleavage → organogenesis

7. Choose between which structure the neural crest is formed _____.

A. ectoderm and neural tube
B. neural tube and notochord
C. notochord and endoderm
D. somit and notochord
E. neural tube and endoderm

8. Indicate whether all germ leaves are involved in the formation of the digestive tract, except:

A. ectoderm and mesoderm
B. ectoderm only
C. mesoderm only
D. ectoderm and endoderm
E. all three germ layers participate in the formation of the digestive tract

9. Choose which tissue can give a signal that changes the behaviour of the cell _____.

A. responder
B. target tissue
C. competent tissue
D. inducer
E. signal tissue

10. Indicate which cell types are not derived from the neural crest _____.

A. melanocytes
B. neurocytes of the spinal cord
C. Schwann cells
D. odontoblasts
E. sympathoadrenal cells

10. EXTRAEMBRYONIC ORGANS

10.1. Extraembryonic *(provisional)* organs

Extraembryonic (provisional) organs are temporary organs of the embryo and fetus that ensure its normal development. The sources of their development are the extraembryonic parts of the germ layers. Extraembryonic organs perform barrier, homeostatic, trophic and respiratory functions. These organs are the placenta, umbilical cord and four extraembryonic membranes (yolk sac, amnion, chorion and allantois). Some of them (allantois, yolk sac) exist briefly and then undergo reduction; others (placenta, chorion, amnion, umbilical cord) exist and perform their functions until birth.

The amnion and the yolk sac are initially formed as two closely adjacent vesicles: the amniotic sac and yolk sac. The amniotic sac is the first to form. It is formed by the stratification of epiblast cells and the formation of small cavities, which merge to form the amniotic cavity.

The amnion is formed from the primary extra-embryonic ectoderm and the primary mesenchyme. The cells of amnion are divided into two types: the cells facing the hypoblast (the bottom of the amniotic sac) are the main ones in the embryo (they give rise to the germ layers and all the embryonic rudiments); the cells facing the trophoblast form the wall of the amnion (extra-embryonic amniotic ectoderm). In the beginning, these cells are absent in the upper part of the amniotic cavity, and its wall is formed by trophoblast cells. As a result of the growth and migration of the second group of cells, they completely cover the trophoblast and form the epiblast lining of the amniotic cavity. The amnion consists of amniotic epithelium and two layers of connective tissue (compact and spongy). The amnion secretes fluid and forms an aqueous membrane around the embryo, protecting the embryo from the external environment. The amnion also ensures the reabsorption of amniotic fluid, stimulates embryogenesis and the activity of the gastrointestinal tract of the fetus and produces prostaglandins that stimulate labour.

The yolk sac. The source of the yolk sac development is the extraembryonic endoderm and the extraembryonic ectoderm. The yolk sac is formed due to the multiplication of hypoblast cells

(extraembryonic endoderm) and their invasion from the inside of the cytotrophoblast. The cells that fill the embryo cavity are evicted from the epiblast. They also grow between the trophoblast and the yolk sac's extraembryonic endoderm and underlie the amniotic sac's extraembryonic ectoderm. These cells form the primary extra-embryonic mesenchyme (primary mesoderm). The yolk is fully formed by the 11th day of embryogenesis. After forming the body fold, the yolk sac separates from the intestinal tube, but remains connected to it by the yolk stalk, then displaced into the space between the chorion and the amnion and incorporated into the umbilical cord. After the 7-8 weeks of embryogenesis, development reverses. During the early stages of embryogenesis, the yolk sac provides nutrition to the embryo by breaking down the yolk masses and absorption of nutrients from the uterine vessels before the chorion formation. In addition to nutrients, the yolk sac also absorbs oxygen from the blood vessels of the uterus. The yolk sac is the place where the primary germ cells are deposited. From the 3rd to the 7–8th week of embryogenesis, the yolk sac performs the haematopoietic function (extra-embryonic period of haematopoiesis). The first blood vessels are formed in the yolk sac (primary angiogenesis).

The chorion is formed from the extraembryonic mesenchyme and the trophoblast. Its development has three periods: the previous period, the villous period (primary, secondary, tertiary villi) and the cotyledons formation period. The chorion provides trophic, respiratory, regulatory and homeostatic functions. It participates in the formation of the fetal part of the placenta.

Placenta. The sources of placental development are the chorionic membrane (transformed trophoblast of the blastocyst) and decidual membrane (pregnancy-transformed functional layer of the endometrium). The chorionic membrane is an embryonic part of the placenta, and the decidual membrane is a maternal part. The placenta connects two organisms into a single system. The functions of the placenta are trophic, respiratory, excretory, barrier, and endocrine (progesterone, estrogens, chorionic gonadotropin, somatomammotropin, etc.).

Umbilical cord. The main source of the umbilical cord development is the mesenchyme of the amniotic stalk and the yolk sac (stalk). The

umbilical cord includes the allantois and its vessels. The umbilical cord contains two umbilical arteries and one umbilical vein. On the outside, the umbilical cord is covered by the amniotic membrane. The main functions of the umbilical cord are to connect to the placenta and to carry blood to and from the embryo.

The allantois formation follows gastrulation. It is the protrusion of the ventral wall of the hindgut endoderm into the amniotic sac. The allantois consists of two layers: extraembryonic endoderm and extraembryonic mesenchyme. It is covered on the outside by the amniotic extra-embryonic mesenchyme. The distal part of the allantois grows rapidly and turns into a sac connected to the intestine. The allantois is present until the 2nd month of embryogenesis. In humans, it does not reach large sizes. During the umbilical cord formation, the allantois is incorporated into the cord and then undergoes reduction. The allantois participate in the formation of the vascular network of the placenta. The proximal part of the allantois is used to form part of the transitional epithelium of the urinary bladder.

10.2. Twins (multiple pregnancy)

About 1 per cent of pregnancies are multiple pregnancies, and usually only 1 foetus is developed. Multiple pregnancies in which there are 3 or more fetuses are extremely rare. There are two main types of multiple pregnancy – ***monozygotic*** and ***dizygotic*** or ***polyzygotic***, when identical or fraternal twins are born, respectively.

A single zygote gives rise to ***monozygotic (identical) twins***. This occurs when the blastomeres separate during fragmentation into two groups. All cells in the early embryo are considered to be totipotent. This means that each of them has the capacity to form a normal embryo. Blastomeres can separate between the two-cell stage of the embryo and the morula stage, and the result is the formation of two blastocysts which are implanted separately. In this case, each embryo will form its own placenta, just as in the case of dizygotic twins. In the majority of cases, identical twins are formed at the stage of the blastocyst. The inner cell mass (embryoblast) splits into two parts. Each part is a separate embryo. The embryos develop in separate amniotic sacs, but share a common placenta. Conjoined twins can occur if the inner cell mass does not separate completely.

The most common type of twin is ***dizygotic (fraternal)***. It is characterised by the fertilisation of two eggs by two sperm. Two zygotes are formed. Each of the dizygotic twins is in its own amniotic sac, they have separate chorions, and their placentas may be fused together or separated. Dizygotic twins may be of the same or different sexes and have different phenotypes, just as siblings born at different times. Vascular anastamoses are sometimes formed when the placenta and chorionic plate fuse.

10.3. Critical periods in the development of an embryo

During ontogenesis, particularly in embryogenesis, there are periods of *increased sensitivity* of the developing germ cells (in progenesis) and of the embryo (in embryogenesis). Each stage of development of the embryo as a whole and its individual organs begins with a relatively short period of qualitative reorganization, accompanied by the determination, proliferation and differentiation of cells. These periods include spermatogenesis and oogenesis (meiosis), fertilization, implantation (gastrulation), differentiation of germ layers and formation of organs, placentation (final maturation and formation of the placenta), formation of many functional systems, and birth.

The brain has a special place in the development of human organs and systems. In the early stages, the brain acts as the primary orchestrator of the differentiation of the surrounding tissues and the rudiments of the organs (in particular the sensory organs). Later, brain is characterised by intensive cell proliferation. This requires optimal trophic conditions.

The embryo is most vulnerable to various harmful influences (*teratogens*) during these short periods of increased sensitivity (***critical periods***). An agent that disrupts embryonic development, resulting in birth defects, spontaneous abortion, and delayed physical and mental development is a teratogen.

Different chemicals, including many drugs, radiation (including diagnostic doses of X-rays), hypoxia, fasting, drugs, nicotine, viruses, etc. can be harmful exogenous factors during critical periods. Chemicals and drugs that penetrate the placental barrier are particularly dangerous for the fetus in the first 3 months of

pregnancy, as they are not metabolized and accumulate in high concentrations in its tissues and organs. Drugs interfere with the development of the brain. Starvation and viruses cause malformations and even intrauterine death.

In human ontogenesis, a number of *critical periods of development* can be distinguished (in progenesis, in embryogenesis and in postnatal life). These include the following: development of germ cells (oogenesis and spermatogenesis); fertilization; implantation (6–10 days of embryogenesis); development of axial organ rudiments and formation of the placenta (3–8 weeks of development); brain development (15–20 weeks); formation of the main systems of the body (20–24 weeks); birth; neonatal period (up to 1 year); puberty (11–16 years).

About half of all aborted embryos are thought to be chromosomally abnormal. So, this procedure ensures the elimination of embryos with genetic defects and reduces the chance of congenital abnormalities developing. The number of abnormally formed embryos that die before implantation is not known, as the pregnancy is not diagnosed at this stage.

10.4. Methods for the diagnosis of abnormalities in human development

Non-invasive and *invasive* methods are used to detect developmental abnormalities. A number of developmental abnormalities of the foetus and its organs can be detected by ultrasound in pregnant women. Malformations of the central nervous system and chromosomal abnormalities can be detected by measuring alpha-fetoprotein in the mother's blood serum. The development of the foetus can also be monitored by laparoscopy (*fetoscopy*), in which the laparoscope is passed through the abdomen and into the uterine.

Amniocentesis is a technique where a sample of amniotic fluid is taken through the abdominal wall of the mother. A chromosome analysis of the cells in the amniotic fluid and other examinations are then carried out.

Also, there are other ways in which foetal abnormalities can be diagnosed. But the main task of medical embryology is to prevent their development.

It is possible to avoid inheriting a number of unfavourable characteristics through assisted reproduction technologies using gametes from apparently healthy donors. The development of genetic engineering allows local damage to the genetic apparatus of the cell to be corrected. For example, there is a method in which a biopsy is taken from the testicles of a man who has a genetic disease. Introducing normal DNA into the spermatogonia, followed by transplanting the spermatogonia into a pre-irradiated testicle (to destroy the genetically defective germ cells) and allowing the transplanted spermatogonia to multiply, results in newly formed sperm being free of the genetic defect.

The method of sperm cryopreservation is used to preserve male germ cells associated with the risk of radiation, injury, etc. and allows long-term preservation of sperm fertility.

Assisted reproductive technologies (ART) are available for the treatment of infertility in both men and women. Female gametes are retrieved by laparoscopy. Using a special needle, the ovarian membrane is punctured in the area of the ovarian follicle and the oocyte is aspirated and fertilised by sperm. Oocytes can be fertilized in a petri dish *(in vitro fertilisation, IVF)* or, if there is a low sperm count, a single sperm can be injected into each oocyte (*intra cytoplasmic sperm injection, ICSI*).

The embryo can be transferred into the uterus or fallopian tube at the 2- to 8-cell stage, but the most commonly used stage is the 5 to 7-day embryo to ensure that the blastocyst stage is healthy.

In assisted reproductive technologies, *preimplantation genetic diagnosis (PGD)* is used to reduce the risk of passing on inherited diseases. Polar bodies from oocytes, blastomeres (at the cleavage stage) or trophectoderm cells (at the blastocyst stage) from preimplantation embryos can be used in preimplantation genetic diagnosis. These cells are genetically analysed. Only those embryos found to be free of the desired genetic defect are transferred. Haemophilia A, muscular dystrophy, sickle cell anemia, Tay-Sachs disease, cystic fibrosis and Down's syndrome are among the more than 100 diseases that can be detected.

Laboratory tasks

Purpose: ***Master the microscopic structure of embryonic and extraembryonic (provisional) organs.***

Practical task 37. Analyze the microscopic structure of the chicken embryo, 54 hours of incubation, cross section (Fig. 37)

1. Examine the histological specimen of the chicken embryo, 54 hours of incubation, using a 10× objective.
2. Locate the embryonic structures (notochord, neural tube, ectoderm, mesoderm, endoderm, coelom, splanchnotom, aorta) and extraembryonic organs (amnion and chorion).
3. Draw the chicken embryo and 54 hours of incubation in your Work Book and label the structures shown.

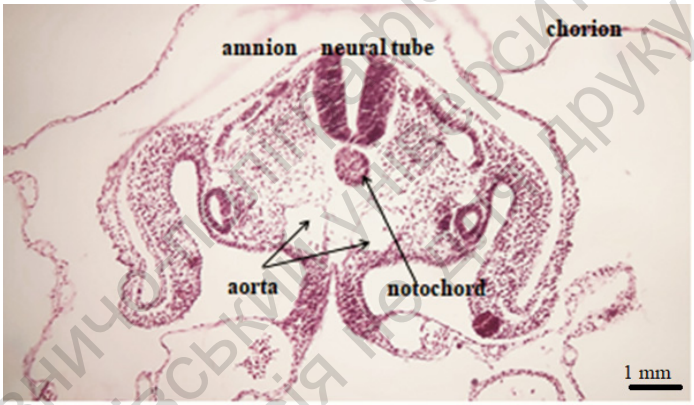


Fig. 37. The chicken body and amniotic folds, 50 hours of incubation, cross-section
(hematoxylin stain)

Practical task 38. Analyze the microscopic structure of the umbilical cord (Fig. 38)

1. Examine the histological specimen of the pig umbilical cord an 4× objective.
2. Locate the structures of the umbilical cord: the amniotic membrane, two umbilical arteries and one umbilical vein, Wharton's Jelly (mucous connective tissue).
3. Draw the umbilical cord in your Work Book and label the structures shown.

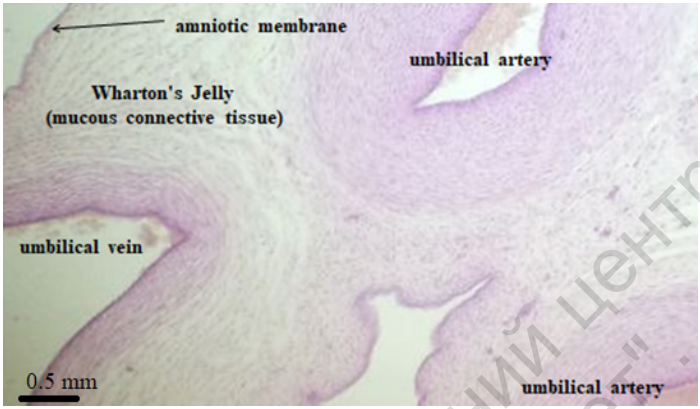


Fig. 38. The pig umbilical cord
(hematoxylin and eosin stain)

Practical task 39. Analyze the microscopic structure of the chorionic villi (Fig. 39)

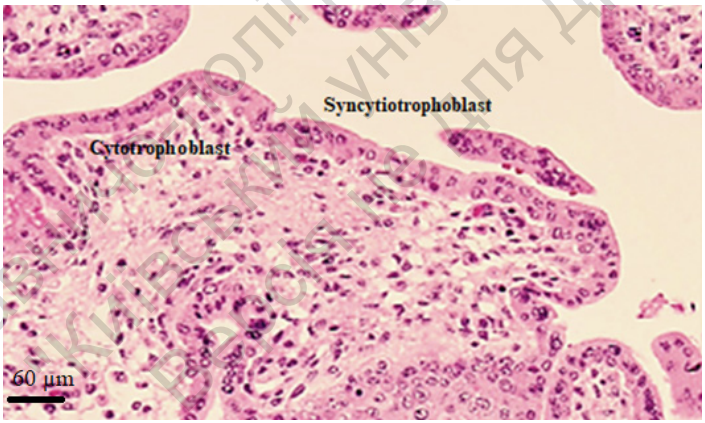


Fig. 39. The pig placenta
(hematoxylin and eosin stain)

1. Examine the histological specimen of the pig placenta with a 10× objective
2. Locate the villi of the pig placenta.
3. Draw the chorionic villi of the pig placenta in your Work Book and label the structures shown.

Control questions

1. Name the process of gastrulation, in which a sheet of cells folds into an embryo _____.

A. invagination
B. involution
C. ingression
D. delamination
E. epiboly

2. Indicate from which part of the human embryo is formed during gastrulation embryonic primary tissues _____.

A. trophoblast
B. epiblast
C. hypoblast
D. umbilical vesicle
E. notochord process

3. Specify the name of the primary cavity of the embryo _____.

A. blastocoel
B. coelom
C. archenteron
D. gastrocoel
E. blastopore

4. Indicate which germ leaf gives rise to chordae _____.

A. embryonic mesoderm
B. extra-embryonic mesoderm
C. embryonic ectoderm
D. extra-embryonic ectoderm
E. embryonic endoderm

5. Choose which structural components the somite consists of _____.

A. dermatome, sclerotome, splanchnotome
B. sclerotome, splanchnotome, paraxial mesoderm
C. myotome, sclerotome, nephrotome
D. sclerorome, myotome, splanchnotome
E. dermatome, myotome, sclerotome

6. During embryonal development, the embryo passes through several stages. Please choose the correct order for them.

A. cleavage → fertilization → gastrulation → neurulation → organogenesis

B. fertilization → cleavage → neurulation →gastrulation → organogenesis

C. fertilization → cleavage → gastrulation → neurulation → organogenesis

D. fertilization → cleavage → gastrulation → organogenesis → neurulation

E. fertilization → gastrulation → neurulation → cleavage → organogenesis

7. Choose between which structure the neural crest is formed _____.

A. ectoderm and neural tube

B. neural tube and notochord

C. notochord and endoderm

D. somit and notochord

E. neural tube and endoderm

8. Indicate whether all germ leaves are involved in the formation of the digestive tract, except:

A. ectoderm and mesoderm

B. ectoderm only

C. mesoderm only

D. ectoderm and endoderm

E. all three germ layers participate in the formation of the digestive tract

9. Choose which tissue can give a signal that changes the behaviour of the cell _____.

A. responder

B. target tissue

C. competent tissue

D. inducer

E. signal tissue

10. Indicate which cell types are not derived from the neural crest _____.

A. melanocytes
B. neurocytes of the spinal cord
C. Schwann cells
D. odontoblasts
E. sympathoadrenal cells

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