Ministry of Education and Science of Ukraine
State Biotechnological University
Institute of Veterinary Medicine and Animal Husbandry
Faculty of Veterinary Medicine
Department of Normal and Pathological Morphology

Album of histology

PART I

CYTOLOGY, HISTOLOGY, EMBRYOLOGY

Student ___ course ____ group

Kharkiv-2022

UDC 619: 611.32
Album of histology for students of the faculty of veterinary medicine. Part I. / Byrka O., Kushch M., Zhigalova O. – K.: RIO KSZVA. – 2022.

Reviewer: O. Bobrytska, DVSc.

Responsible for release: O. Zimmerman, dean of veterinary medicine faculty, associate professor.

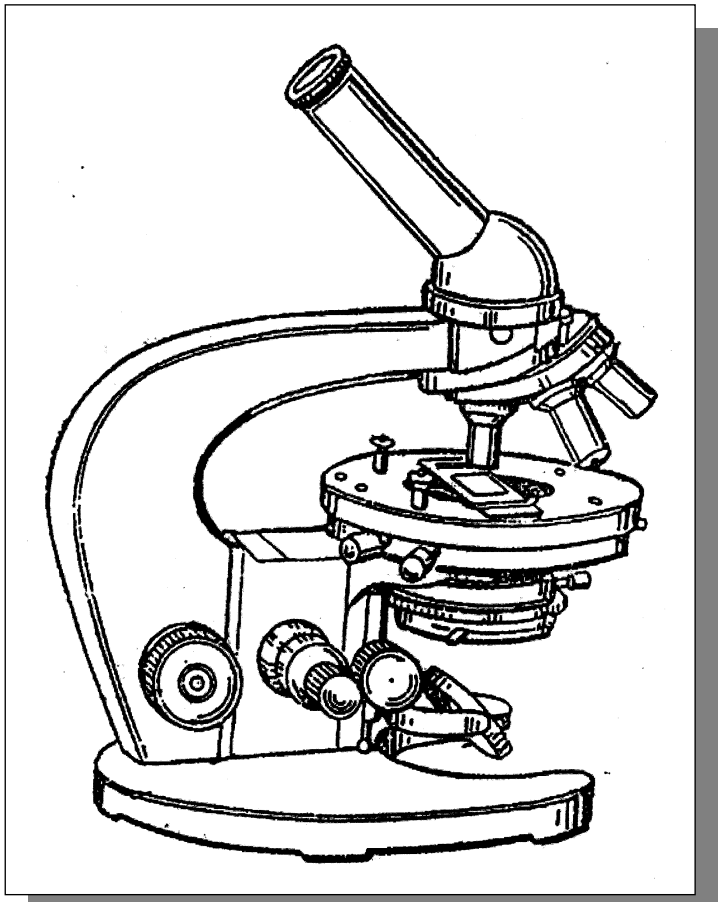
Laboratory class

Date

Topic: Microscopic techniques. Histological preparation techniques. Light microscope organization and instruction how to use it.

Objective: to learn how to use a light biological microscope and to be able to view histological preparations using microscope.
Equipment: light microscopes, histological preparations, handbook, tables.

Light microscope consists of three parts: mechanical, optical and luminating.



I. Mechanical part of microscope comprises:

1. Tube (tubus).
2. Case prism.
3. Nosepiece (revolver system).
4. Stage.
5. Coarse-adjustment knob (makroknob).
6. Fine-adjustment knob (mikroknob).
7. Tube-carrier.
8. Stand.

II. Optical part of microscope (a system of lenses)**:**

9. Objectives are divided into four categories:
   - objectives of a low magnification (8^x or 10^x),
   - middle magnification (20^x),
   - high magnification (40^x, 60^x),
   - very high magnification (100^x).
10. Oculars are also divided into:
   - oculars of a low (5^x or 7^x),
   - middle (10^x),
   - high (15^x) magnification.

III. Luminating part of microscope:

11. Illuminator (mirror, the artificial light).
12. Condenser.
13. Iris diaphragm.
14. Ring for filters, light filters.

The degree of magnification of the microscope is the multiplication of the digits of the objectives and the ocular.

Resolution distance light microscope – the minimum distance between two points in which they differ in the light microscope - 0,2 mkm.

Remember: the microscope provides an **enlarged, inverted and imaginary image** of the object.

A ***histological section*** is a thin slice of tissue (a piece of organ) varying usually from 0.5 to 10 micrometers thick. The histological sections are affixed to glass slide and covered with a coverslip and colored with one or more stains to increase the visibility of various cellular and intracellular components.

Organization of light microscope

While studying microscope organization it is necessary to use it and its drawings in textbooks and handbooks.

Light microscope consists of three parts: mechanical, optical and luminating.

Mechanical part comprises stand, nosepiece, stage, coarse- and fine-adjustment knobs. The support unites all parts of microscope. It comprises stand, tube-carrier and tube (tubus). At the center of stage on its upper surface there is an opening where a preparation is put and clips to hold preparation. Stage also possesses knobs located at side surfaces enabling to move stage.

Nosepiece is connected with a head of tube-carrier. It contains cells for objectives.

Coarse-adjustment knob is located at the side of tube-carrier, whereas fine-adjustment knob – in the stand. The first is used to work at a low power magnification. The second is used to produce a clear image at a high power magnification.

The optical part of microscope comprises objectives and oculars (a system of lenses). Objectives are divided into four categories: objectives of a low magnification (8× or 10×), middle magnification (20×), high magnification (40×) and very high magnification (100×). The latter objective requires using special medium – immersion oil.

Oculars are also divided into oculars of a low (5× or 7×), middle (10×) and high (15×) magnification.

Luminating part of microscope includes mirror, illuminator, diaphragm and ring for filters.

The mirror has concave and flat surfaces. The latter is used at a special illumination.

Illuminator (condenser) is located under the stage and contains lenses to concentrate and direct light on an object. Illumination is regulated by diaphragm. Just beneath the diaphragm there is a ring for light filters. The latter can be uncolored or non-transparent blue, depending on the light source.

How to use a light biological microscope

(Using an optical microscope)

1. Put microscope on the table. Lower condenser and open diaphragm.

2. To prepare a microscope for use, turn the **nosepiece** to bring the **objective with the lowest power** into viewing position.

3. Turn the **coarse-adjustment knob** to lower the tube until the objective is just above the opening in the stage.

4. Look through the **ocular** and adjust **mirror** till a bright circle of light appears.

5. To view a slide, place it on the **stage** with the specimen directly over the opening.

6. Hold the slide in place with clips that are attached to the stage.

7. Look through the ocular and turn the **coarse-adjustment knob** to raise the objective until the specimen comes into focus. (To avoid breaking the slide or the objective, never lower the lens when a slide is on the stage).

8. Turn the **nosepiece** to bring the **objective with higher power** into viewing position.

9. Turn the **fine-adjustment knob** to produce clear image.

10. Turn the nosepiece into initial position and take off the slide.

Preparation of histologic sections

A histological section is a thin slice of tissue varying usually from 0.5 to 10 or more micrometers thick. In preparing such a section, a piece of tissue is either infiltrated with a supporting medium or frozen. Then cut with an instrument called a microtome. Section obtained from tissue infiltrated with plastic can be as thin as 0.5 mcm and show superior details. Excellent preparations as thin as 2–3 mcm can also be made from tissue infiltrated with paraffin-based embedding media.

Sections are affixed to microscope slides and colored with one or more stains to increase the visibility of various cellular and intracellular components.

Steps involved in producing a stained histologic slide using paraffin procedure:

1) ***Removing*** organ sample.

2) ***Cutting*** small pieces. After being removed from an animal, a tissue or an organ is cut into pieces of 1,0×1,0×0,5 cm size.

3) ***Fixation***. These pieces are placed into a fixative, such as buffered formalin or Bouen's, which preserves normal morphology and facilitates further processing.

4) ***Dehydration***. After fixation the specimen is dehydrated by transferring it through a series of alcohols of increasing concentrations to 100% alcohol.

5) ***Clearing.*** Next, it is placed into a substance such as xylene, which is miscible with both 100% alcohol and paraffin. This intermediate step (called clearing) is essential before infiltrating the dehydrated tissue with paraffin because alcohol and paraffin do not mix.

6) ***Embedding*** (Infiltration with paraffin). During infiltration, melted paraffin completely replaces the xylene. This procedure is done in an oven at a temperature just above the melting point of the paraffin mixture.

7) When infiltration is completed, the specimen is transferred to an embedding mold of fresh, melted paraffin, which is allowed to harden. The mold is eventually removed and excess paraffin is trimmed away.

The block of paraffin is then secured to the microtome and oriented appropriately with respect to the knife.

With each revolution of **microtome** handle, specimen move through the blade and a section of desired thickness is produced. Each successive section adheres to the proceeding one, forming continuous ribbon. One or more sections are carefully separated from the ribbon and transferred to the surface of warm water in a waterbath. This softens the paraffin and flattens the section, eliminating wrinkles.

The flatten section is floated onto a slide, which is then placed on a warming table. As a preparation is dried, the section adheres to the surface of slide.

Steps of hematoxylin and eosin staining

1. Removing paraffin with xylene (3 servings) – 3 - 5 minutes in each.
2. Removing xylene with alcohol (100^0, 96^0, 70^0) – 2 - 3 minutes in each.
3. Rehydration of specimen (distilled water) – 1 - 2 minutes.
4. Hematoxylin staining – 2 - 5 minutes.
5. Rehydration (tap water) – 5 - 10 minutes.
6. Hydrochloric acid alcohol (slices differentiation, removing excess hematoxylin) – 3 - 5 seconds.
7. Rehydration (tap water) – 5 - 10 minutes.
8. Eosin staining – 1 - 2 minutes.
9. Dehydration (distilled water) – 1 second (removal of excess eosin).
10. Wipe the wet slide with filter paper.
11. Removing water with alcohol (70^0, 96^0, 100^0) – 3 - 5 seconds in each.
12. Clearing (made transparent) with xylene – 5 - 10 minutes.
13. Covering with mounting medium (coniferous balsam, polystyrene).
14. Topping with a coverslip.

Various stains are available to histologist. **Hematoxylin and eosin (H&E)** is a frequently used combination of stains.

Hematoxylin imparts a purple color to substances and behaves as **basic (cationic) stain.** Substances that become colored by a basic stain are said to be **basophilic**. Methylene blue, toluidine blue, basic fuchsine are basic stains.

Acidic (anionic) stains impart an orange or red color to substances, called **oxyphilic** (acidophilic). Commonly used acid stains are: eosin, orange G, phloxine and aniline blue.

Questions for self-control

1. Name the main stages of histological sections preparation.
2. What a material is placed into fixative for?
3. How dehydration is provided?
4. What tools do we use to make sections?
5. What stains are used in histology?
6. Steps of H&E staining.

Laboratory class

Date

CYTOLOGY

Topic: Cell morphology. Structure of somatic cells.

Objective: to be able to identify compartments of eukaryotic cell and their structural elements on histological slides.
Equipment: light microscopes, histological slides, handbook, tables.

The preparation № 1: **General cells morphology. The liver**. ***Stain:*** **H&E.** Using a low power magnification find out polyhedral cell – hepatocytes. Blood vessels filled with blood cells are situated between the groups of hepatocytes. View hepatocytes with high-power magnification. Find a centrally positioned nucleus, cytoplasm and cell membrane (a distinct boundaries between cells). Keep in mind that cell membrane can be seen only at electron microscope. Find a nucleoli and chromatin within nucleus. **Study** the mentioned above cells compartments and their structural elements on the electron micrograph. ***Draw*** some cells and mark in them: 1. Hepatocytes. 2. Nucleus: a) kariolemma; b) nucleolus; c) chromatin; 3. Cytoplasm.	

Name student _____ Signature of lecturer _____

Laboratory class

Date _____

CYTOLOGY

Topic: Cytoplasmic organelles.

The preparation № 2: **The mitochondria in liver cells of amphibian.**
Stain: *iron hematoxylin.*

At low power magnification find out polyhedral cell – hepatocytes. Intercellular boundaries defined clearly. The cytoplasm with this method is not stain. View these cells with a high power magnification. The nucleus contains nucleolus. Find the mitochondria within the cytoplasm. They appear as threads, sticks or seeds and are stained in black.

In the study of histological preparation:

1) remember: what chemical components determine the ability of cellular structures stained with hematoxylin;

2) explain why a given method of staining are exactly the mitochondria;

3) pay attention to the shape and number of nucleus, sometimes occurs on two nucleus, this is the result amitosis without division of the cytoplasm;

Study the ultramicroscopic structure of mitochondria using electron micrographs. Find out the outer and inner membranes, crystes and matrix there.

Draw a few cells and mark in them:

The preparation № 3: **Golgi complex in nervous cells of cat's craniospinal ganglia.**
Stain: *osmic acid.*

Using a low power magnification view the slide and find out the groups of large spherical cells. They are nervous cells. Their processes are invisible.

Using a high power magnification define the nucleus with nucleolus and Golgi complex elements in the cytoplasm of these cells. The bright large spherical nucleus is positioned centrally. The Golgi complex elements appear as black spirals, threads and hooks and are primarily juxtaposed to the nucleus.

Examine the Golgi complex on electron micrograph. Pay the particular attention to its cisternae.

Draw a few cells and mark in them:

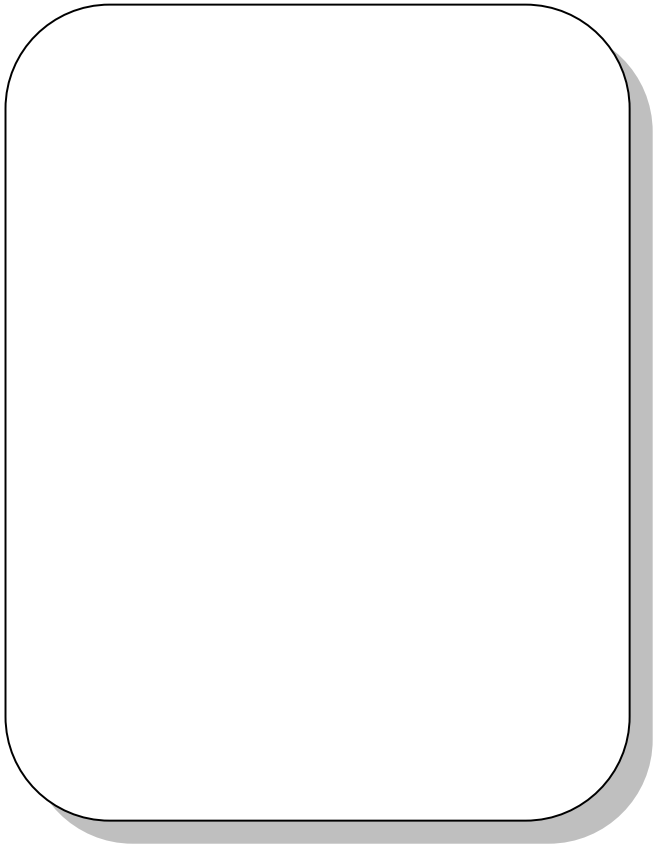
The preparation № 4: **Cell center in equine acaridae's ovum.**
Stain: *iron hematoxylin.*

Using a low power magnification find out the uterus lumen filled with oocytes. They are surrounded by a thick two-laminar coat. There is a big fissure between the coat and oocyte. It is fertilized oocyte or zygote which is at a cleavage stage. Some zygotes are divided into two blastomeres. Using a high power magnification find out centioles within zygote or blastomere cytoplasm. They appear as dark dots, surrounded by light portion of cytoplasm, and are located at the opposite poles oocyte or blastomere. You can see the chromosomes, positioned centrally between centrioles.

Study cytocentrum ultrastructure (longitudinal and transverse section) on the electron micrograph. The transverse section shows that the wall of centriole is composed of nine groups of three tubules.

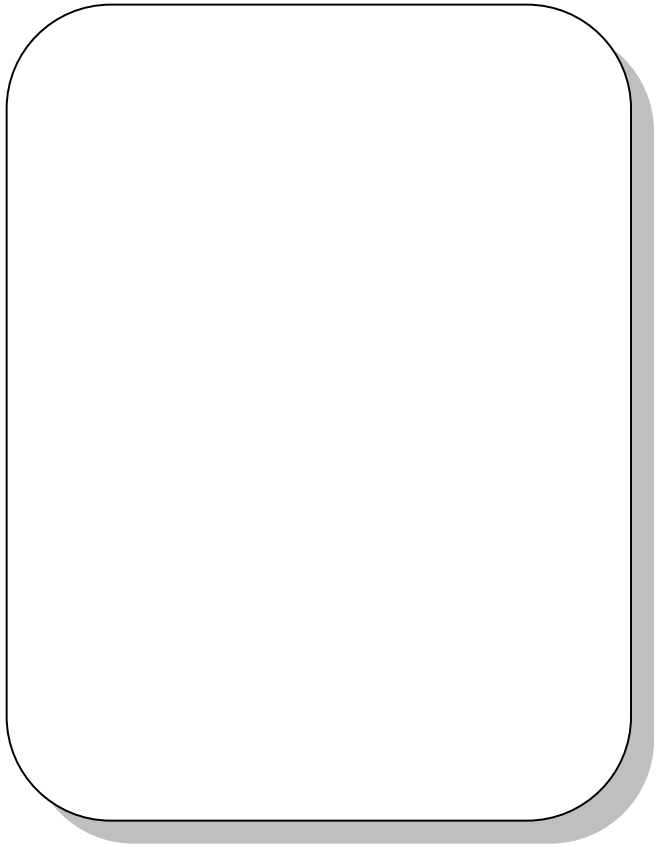
Draw an oocyte and mark in it:

The preparation № 2: **The mitochondria in liver cells of amphibian.**
Stain: *iron hematoxylin.*



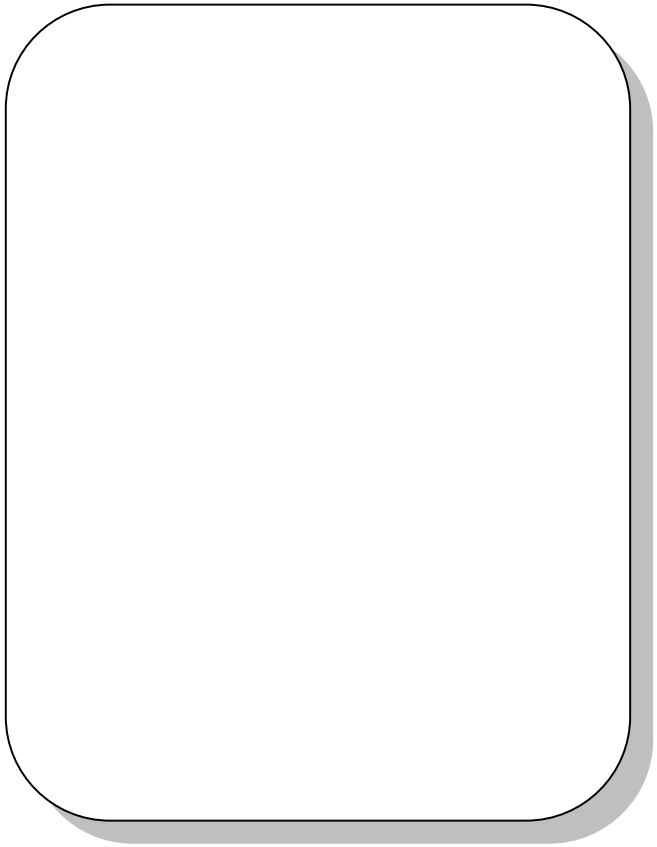
Designations:
1. Nucleus.
2. Nucleolus.
3. Cytoplasm.
4. Mitochondria.

The preparation № 3: **Golgi complex in nervous cells of cat's craniospinal ganglia.**
Stain: *osmic acid.*



Designations:
1. Nucleus.
2. Nucleolus.
3. Cytoplasm.
4 Golgi complex elements.

The preparation № 4: **Cell center in equine acaridae's ovum.**
Stain: *iron hematoxylin.*



Designations:
1. Coat.
2. Fissure.
3. Oocyte (blastomere).
4. Centrioles.
5. Chromosomes.

Name student _____ Signature of lecturer _____

Laboratory class

Date _____

CYTOLOGY

Topic: Inclusions of the cytoplasm.

Objective: to be able to identify compartments of eukaryotic cell and their structural elements on histological slides and electron micrographs.

Equipment: light microscopes, histological slides, handbook, tables.

The preparation № 5: **Glycogen inclusions in hepatocytes.**

Stain: *Best's stain and hematoxylin.*

At a low power magnification find out the rows of polyhedral-shaped pink-red-colored hepatocytes. Blood vessels filled with blood cells are situated between the groups of hepatocytes.

View hepatocytes with a high power magnification. Find a pink-red colored glycogen granules and purple nucleus in them.

Draw few cells and mark in them:

The preparation № 6: **Lipid inclusions in hepatocytes.**

Stain: *osmium acid and safranin stain.*

Using low power magnification find out hepatocytes.

View these cells with high-power magnification and find lipid droplets in cytoplasm. They are black and of different size. The nucleus is pink colored.

Draw some cells and mark in them:

The preparation № 7: **Pigment cells of tadpod's skin.**

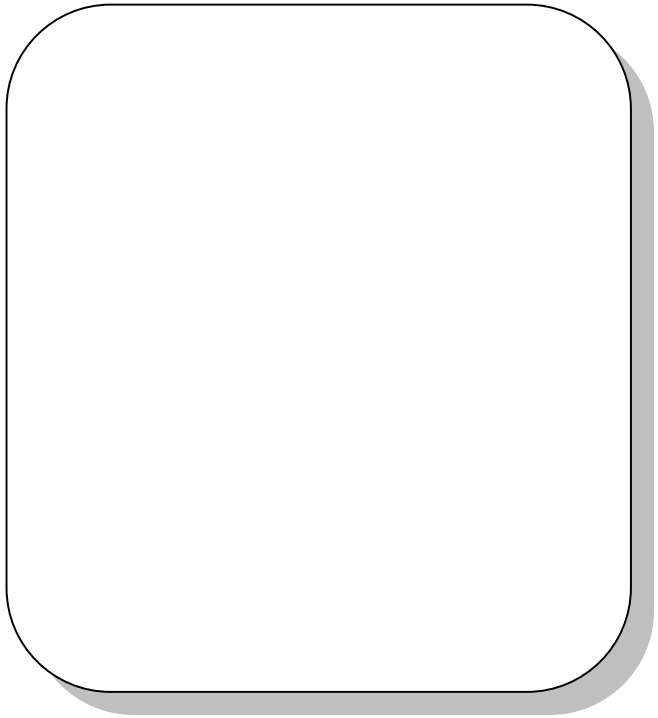
Stain: non-stained.

Using low-power magnification find out pigment cells of green-brown color, that resembles snowflakes.

Using high-power magnification view these cells and find the nucleus, cytoplasmic processes and pigment inclusions within the cytoplasm. The nucleus is positioned centrally. Cytoplasmic processes are branched and of different size. The granules of green-brownish pigment are seen in the cytoplasm.

Draw a pigment cell and mark in it:

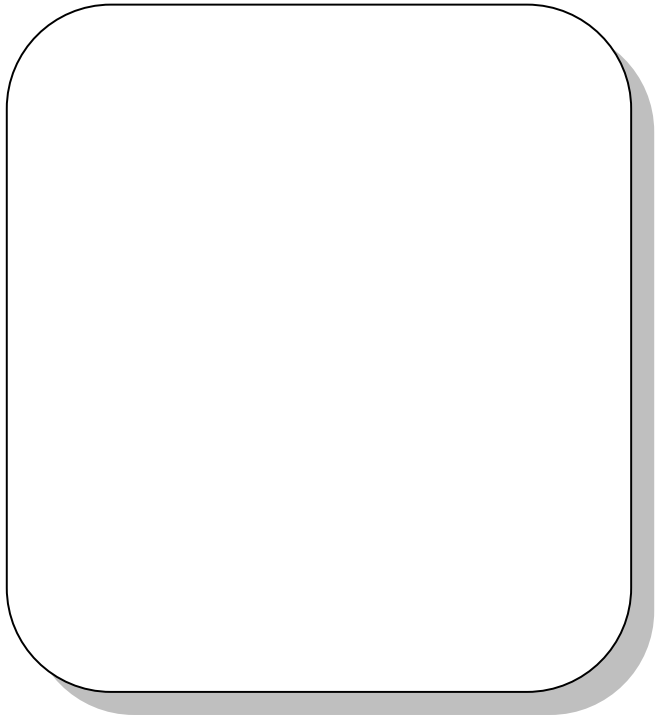
The preparation № 5: **Glycogen inclusions in skin in hepatocytes.**
Stain: *Best's stain and hematoxylin.*



Designations:

1. Nucleus.
2. Cytoplasm.
3. Glycogen.
4. Vacuoles of fat inclusions.

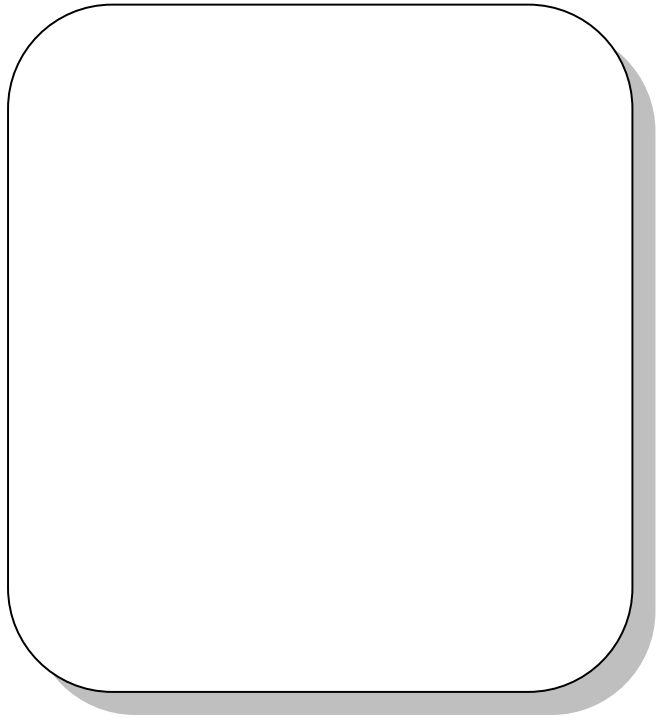
The preparation № **6: Lipid inclusions in hepatocytes.**
Stain: *osmium acid and safranin stain.*



Designations:

1. Nucleus.
2. Cytoplasm.
3. Lipid droplet.

The preparation № 7: **Pigment cells of tadpod's skin.**
Stain: non-stained.



Designations:

1. Melanocytes.
2. Nucleus.
3. Cytoplasm.
4. Cytoplasmic processes.
5. Pigment inclusions – melanin granules.

Name student _____

Signature of lecturer _____

Laboratory class

Date _____

CYTOLOGY

Topic: Cell division.

Objective: to be able to identify structural elements of cell nucleus, all stages of mitosis and amitosis on histological slides.
Equipment: light microscopes, histological slides, handbook, tables.

The preparation № 8: **Mitosis in onion root cells.**
Stain: *iron hematoxylin.*

Most eucaryotic cells is reproduced by mitosis. In this process, a cell divides and forms two identical daughter cells. Each daughter cell receives a set of chromosomes identical to those of the original cell.

Using a low power magnification view the onion root and find a reproductive zone, where the mitotic cell division occurs (it is just above the root cover). Turn to the high power magnification and find an interphase cell within reproductive zone. It is cuboidal or columnar-shaped cell surrounded by well-expressed membrane with big nucleus, containing one or two nucleoli and chromatin granules. Find also cells at different phases of mitosis: prophase, metaphase, anaphase and telophase.

Draw an interphase cell and mark in it: membrane; nucleus; nucleolus; cytoplasm.

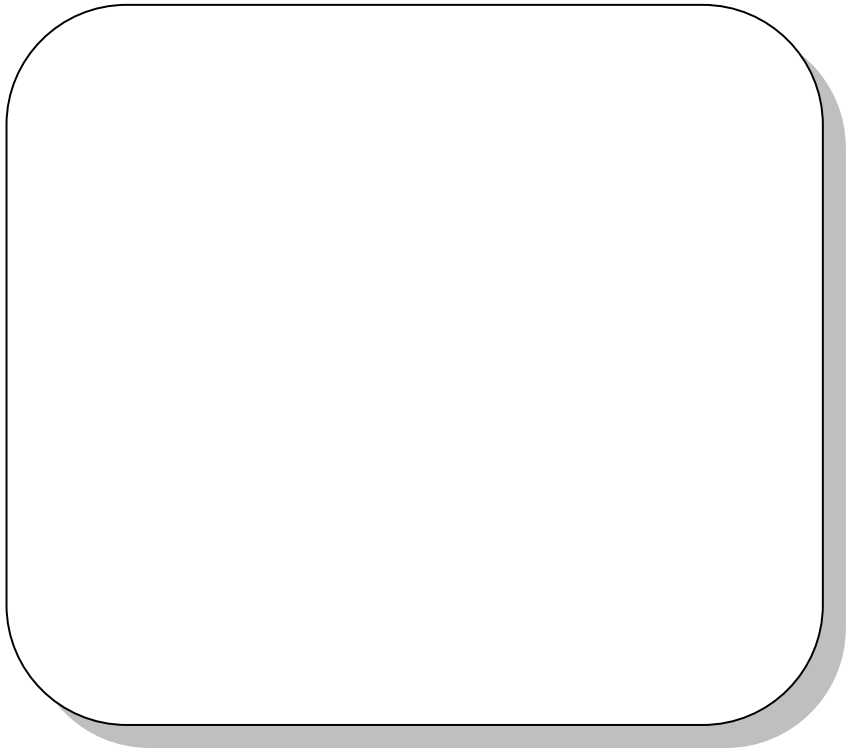
Draw also a cell at different phase of mitosis:

- prophase (stage of loose ball),
- metaphase (equatorial plate or mother star),
- anaphase (late stage) and
- telophase (daughter cells).

The preparation № 9: **Amitosis in urinary bladder epitheliocytes (imprint).**
Stain: H&E

Using a low power magnification find a group of epithelial cells and study them using a high power magnification. Pay attention that the boundaries between epitheliocytes are almost invisible due to pale stained cytoplasm. You can observe them while lowering condensor and closing diaphragm. Find cells at amitosis, paying attention to their nucleus shape. When amitosis starts the nucleus elongates and an isthmus appears (eight-like nucleus). Then the isthmus disappears and cell become two-nucleated. After that the division of cytoplasm occurs. If the division of cytoplasm is postponed or it does not occur at all, the nuclei divide again and multinucleated cells appear. View such cells and ***draw*****:** a cell with elongated nucleus, a cell with eight-like nucleus, two-nucleated cell and multinucleated cell.

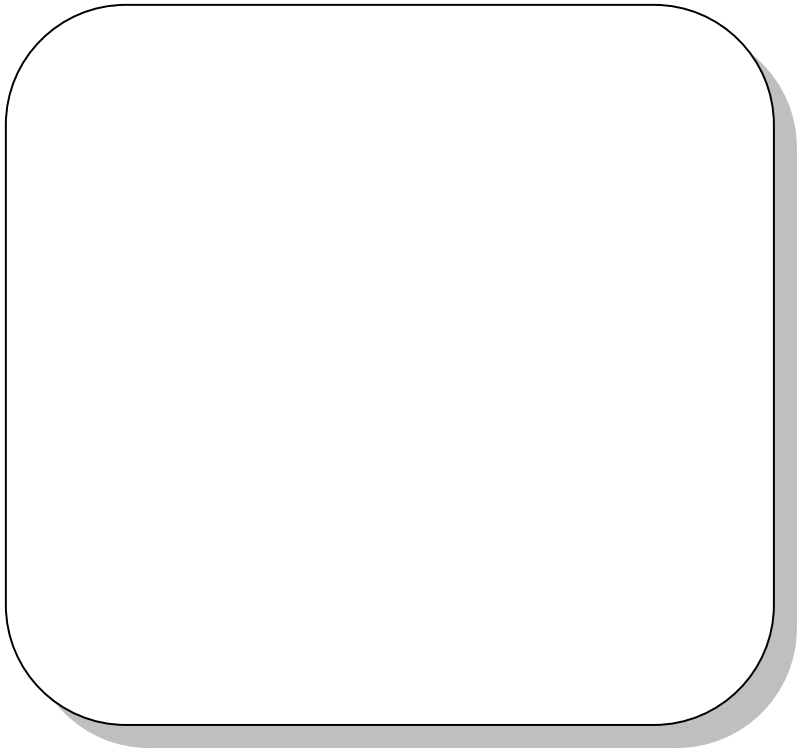
The preparation № 8: **Mitosis in onion root cells.**
Stain: *iron hematoxylin.*



Designations:
1. Interphase cell.
2. Prophase.
3. Metaphase.
4. Anaphase.
5. Telophase.
6. Chromosomes.

Name student _____

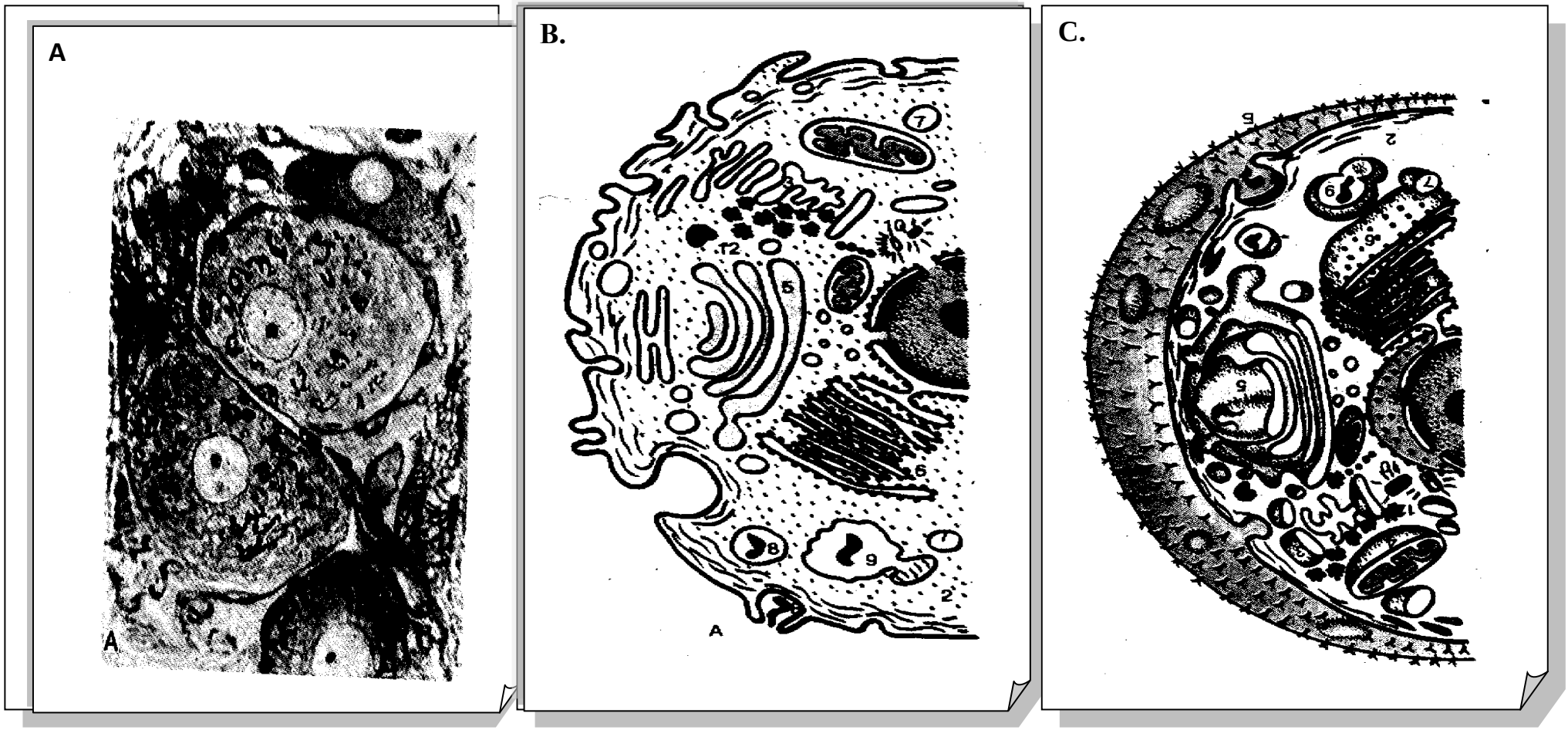
The preparation № 9: **Amitosis in urinary bladder epitheliocytes (imprint).**
Stain: H&E



Designations:
1. Interphase cells.
2. Cell with elongated nucleus.
3. Cell with eight-like nucleus.
4. Two-nucleated cell.

Signature of lecturer _____

Schematic representation of somatic cells in light (A) and electron microscopy: transmission (B) and scanning (C).

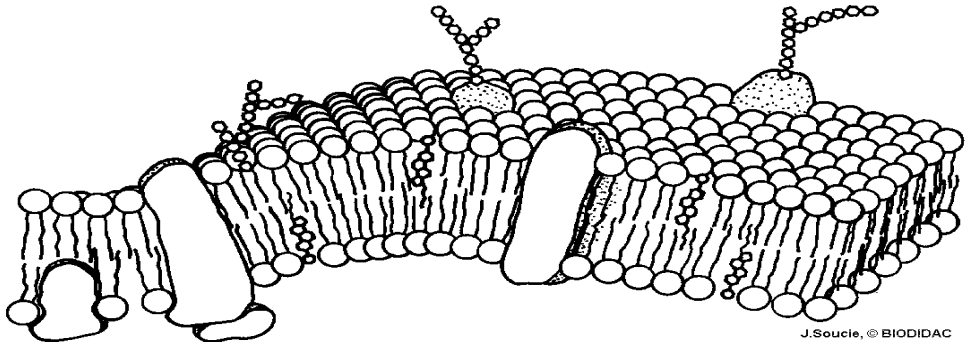


Designations:

1. Nucleus (core)
2. Nucleolus
3. Heterochromatin
4. Karioplazma
5. Kariolemma
6. Nuclear pores
7. Cytoplasma
8. Plasmolemma
9. Mitochondria
10. Golgi complex
11. Lysosome
12. Smooth endoplasmic reticulum
13. Rough endoplasmic reticulum
14. Ribosomes
15. Centrosome

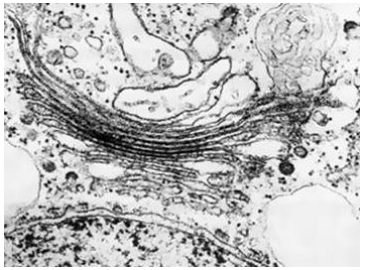
Homework
"Cytology"

Schematic diagram of the cell membrane



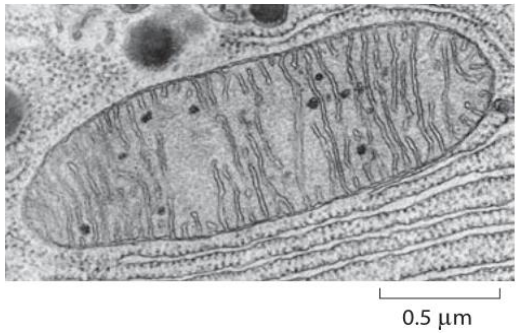
1 - polar heads of lipid molecule,
2 - non-polar tails of lipid molecule,
3 - integral proteins,
4 - peripheral proteins,
5 - surface proteins,
6 - sugar chain of glycolipid,
7 - sugar chain of glycoprotein.

Three-dimensional model of the Golgi apparatus.



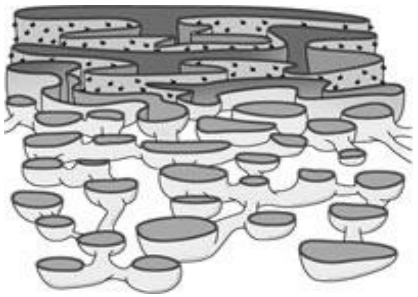
1 - cis (forming) face,
2 - trans (maturing) face,
3 –cisternae,
4 - lumen,
5 - newly forming vesicle,
6 - secretory vesicle.

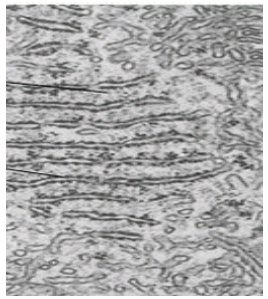
Schematic diagram of mitochondrion.



1 - outer membrane, 2 - inner membrane, 3 - cristae, 4 - matrix space.

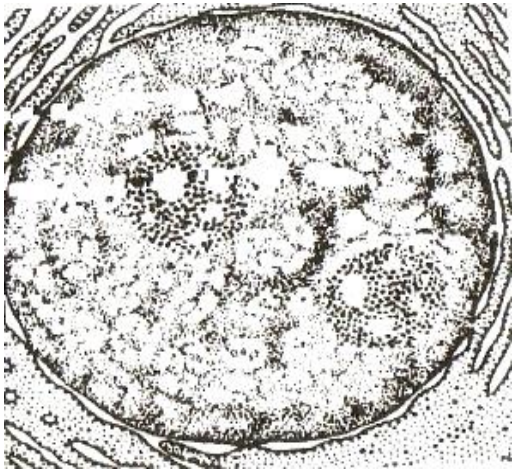
Schematic diagram of endoplasmic reticulum.





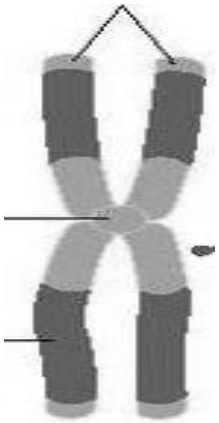
1 - rough endoplasmic reticulum,
2 - smooth endoplasmic reticulum,
3 - lumen of smooth reticulum,
4 – ribosomes.

Schematic diagram of structure of a nucleus.

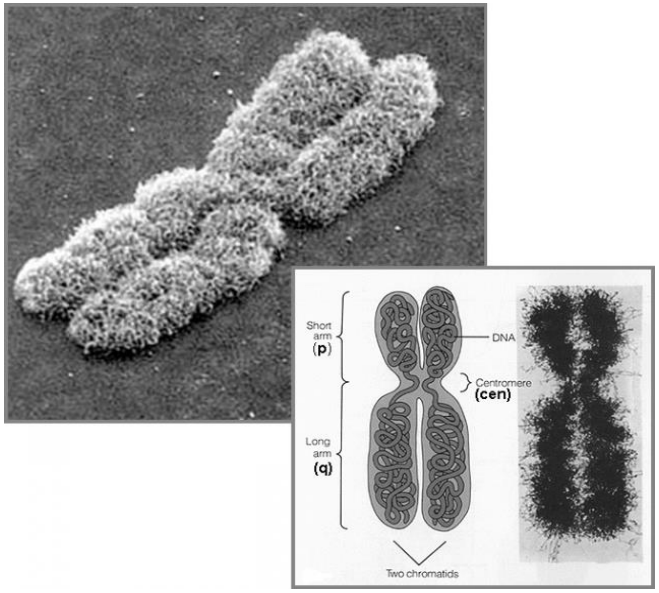


1 - nuclear envelope,
2 - nuclear pores,
3 - ribosomes,
4 - nuclear fibrous lamina,
5 - heterochromatin,
6 - euchromatin,
7 - nucleolus.

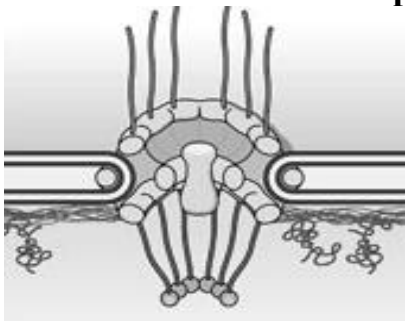
Structure of chromosome.



1 - telomeres;
2 - centromere;
3 - arms.

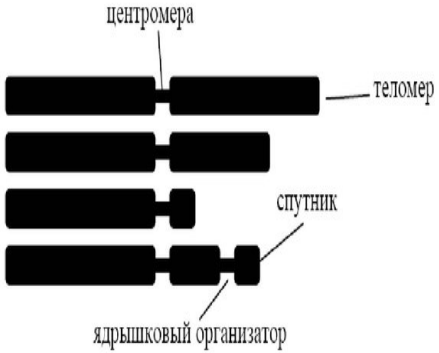


Schematic cross-section of the nuclear pore complex.



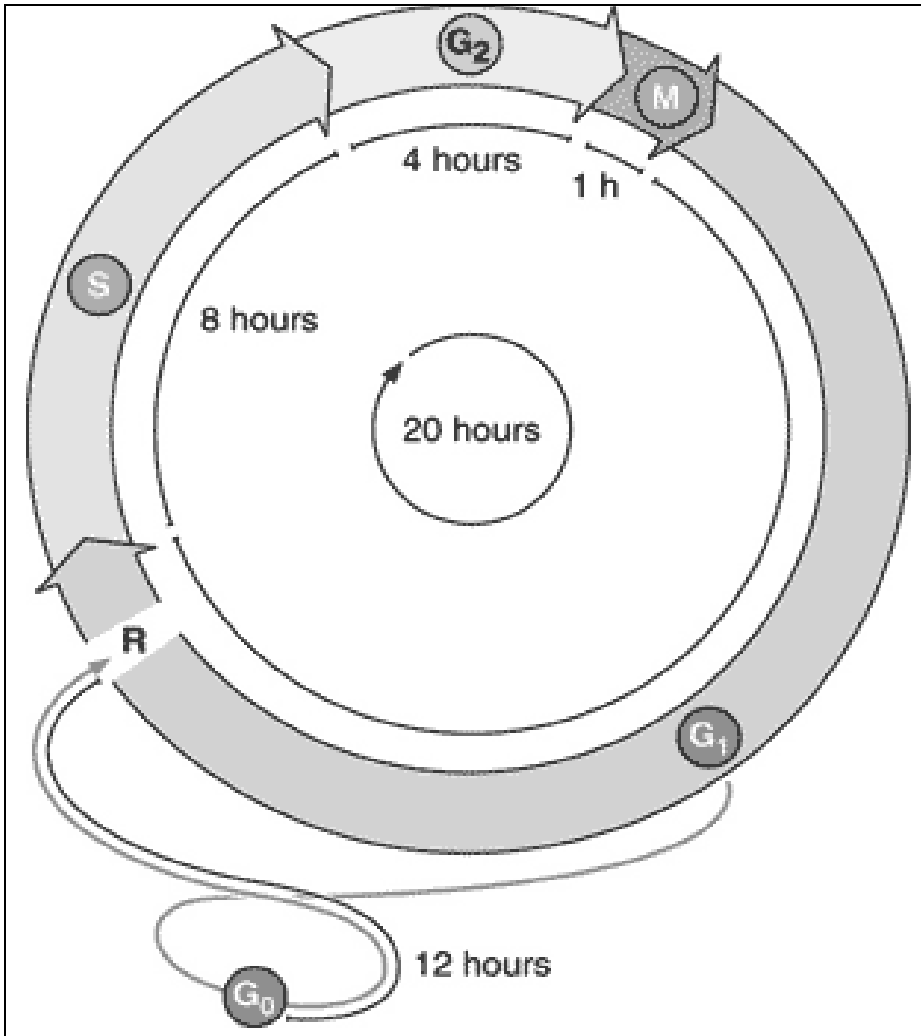
1 - cytoplasm,
2 - nucleus,
3 - cytoplasmic ring,
4 - internuclear ring,
5 - central granule,
6 - nuclear envelope,
7 - nuclear lamina,
8 - chromatin.

Types of metaphase chromosomes.



1. metacentric chromosome;
2. submetacentric chromosome;
3. acrocentric chromosome;
4. satellite chromosome;
5. centromere;
6. telomeres;
7. satellite;
8. nucleolus organizer.

Schematic diagram of cell cycle. Cell life cycle.



Cell cycle divided into **two stages:**

In cell biology, **mitosis** (/maɪˈtoʊsɪs/) is a part of the cell cycle when replicated chromosomes are separated into two new nuclei. Cell division gives rise to genetically identical cells in which the number of chromosomes is maintained.

I. **Mitosis,** consisting of **four phases:**

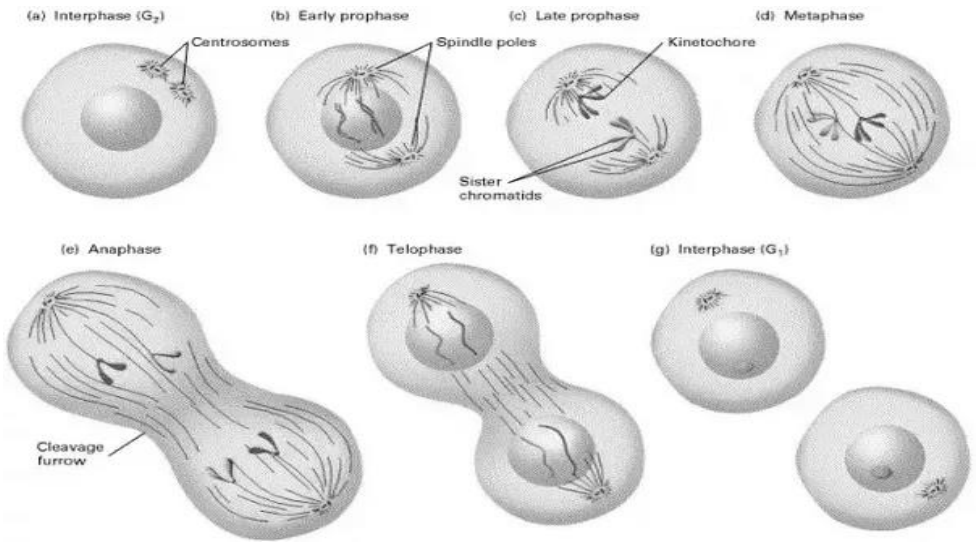
1.prophase,
2.metaphase,
3.anaphase,
4.telophase.

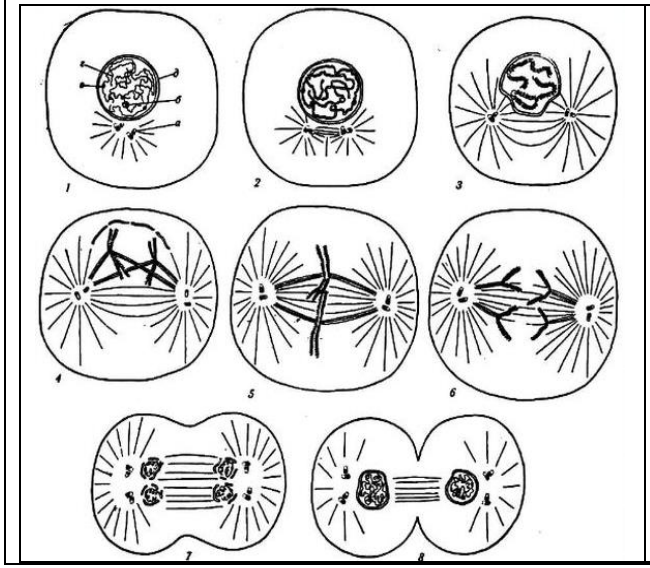
Interphase is the phase of the cell cycle in which a typical cell spends most of its life. During this phase, the cell copies its DNA in preparation for mitosis.[1] Interphase is the 'daily living' or metabolic phase of the cell, in which the cell obtains nutrients and metabolizes them, grows, reads its DNA, and conducts other "normal" cell functions

II. Interphase is divided into **three phases:**

-G1 (presynthetic),
-S (DNA synthesis),
-G2 (post-DNA duplication).

Schematic diagram of phases of mitosis.





I - interphase:
1 - centrosomes,
2 - chromatin,
3 - nucleolus;
II - prophase:
4 - aster;
III – metaphase:
5 - spindle,
6 - metaphase plate;
IV -anaphase:
7 - daughter chromosomes,
V - telophase:
8 - nucleolus forming.

Questions for self-control

1. Name the main parts of light microscope.
2. Name the main stages of histological sections preparation.
3. Enumerate the composing parts of a cell.
4. Ultrastructure and functions of cell membrane (plasmolemma).
5. What a cytoplasm is composed of?
6. Criteria of organelles classification.
7. What membranous organelles do you know?
8. Ultrastructure and functions of mitochondria.
9. Enumerate specialized organelles.
10. Ultrastructure and functions of Goldgi apparatus.
11. Enumerate microscopic organelles.
12. Ultrastructure, classification and function of endoplasmic reticulum.
13. What is a cytocentrum composed of?
14. Functions of cytocentrum.
15. Structure of centrioles.
16. Classification of inclusions.
17. Enumerate ultramicroscopic organelles.
18. Structure of lyzosomes and peroxisomes.
19. What organelles form cytosceleton? Describe their organization.
20. Structure and function of ribosomes.
21. Enumerate nucleus function.
22. Composing parts of nucleus.
23. Organization and function of nucleus membrane.
24. Chromatin, its chemical composition. Chromosomes.
25. Organization and function of nucleolus.
26. How are cells reproduced?
27. Give definition of a cell cycle. What is it composed of?
28. Periods of interphase. What events occur during these periods?
29. Enumerate phases of mitosis. What events occur in each of them?
30. Amitosis and its types.

Laboratory class

Date _____

EMBRYOLOGY

Topic: Organization of germ cells.

Objective: to be able to identify the sperm cells of mammals and birds, oocyte of mammals and amphibians as well as their structural components on histological slides.

Equipment: light microscopes, microscopic preparations of mammalian and avian sperm cells, histological slides of mammalian oocyte, handbook, tables.

The preparation №10: **Mammalian oocyte (egg) (section of cat ovary).**

Stain: H&E.

Using a low power magnification view ovary and find out round follicles in its peripheral zone. They contain oocytes at the growth stage. Find out big mature follicle among them. It is filled by follicular fluid. The inner surface of its wall possesses an ovary hill with an oocyte.

View the oocyte at a high power magnification. Find out a cytoplasm and an eccentrically positioned nucleus. The latter contains large nucleolus and granules of chromatin. Pay attention to the cortical (peripheral) layer of cytoplasm. Inclusions are absent in it and it stains pink. Cortical layer of cytoplasm is bounded by primary membrane (plasmolemma). Secondary membrane is located above the primary one and is composed of some layers of follicular cells and their derivatives. They form corona radiata and zone pellucida just above the primary membrane.

Draw an oocyte and mark in it:

The preparation №11: **Amphibians oocyte (egg) (section of frog ovary).**

Stain: H&E.

At low magnification to find large cells incorrectly rounded shape with a large bright nucleus shifted to one of its poles (**pole animal**).

At the nucleus a large number of nucleoli. Oocytes are at different stages of growth. In the ovum even at low magnification to look at the possible inclusion of yolk in the form of granules of different sizes, focusing on **pole vegetative** cells.

At the opposite pole animal can see the **inclusions of melanin.** Outside, surrounded by secondary membrane formed layer of flat follicular cells and unstructured substance.

The preparation №12: **Sperm cells.**
A - Guinea pig's sperm cells.
B - Cock's sperm cells.
Stain: *iron hematoxylin*

A.–Using a low power magnification find out sperm cells at preparation. They possess flagella. Study them using a high power magnification and pay attention to the shape of their head (oval or pear-like) and flagellum (tail).

B.–Using a low power magnification find out sperm cells on the preparation and view them at a high power magnification. Pay special attention to the shape of their head. In contrast to that of mammalian's sperm cell its head is elongated and is continued with the tail without any distinct boundary.

Draw some sperm cells and mark: Study electron micrograph of sperm cell and find head, neck and tail there. Determine nucleus and acrosome within the head, proximal centriole and cranial part of distal centriole within the neck. View the tail, determine its parts, axoneme within it and mitochondria in its transitional part.

The preparation № 10: **Mammalian oocyte (egg) (section of cat ovary).**

Stain: H&E.

Designations:
1. Nucleus.
2. Nucleolus.
3. Cytoplasm.
4. Cortical layer of cytoplasm.
5. Primary membrane.
6. Secondary membrane:
   a) zona pellucida;
   b) corona radiata.

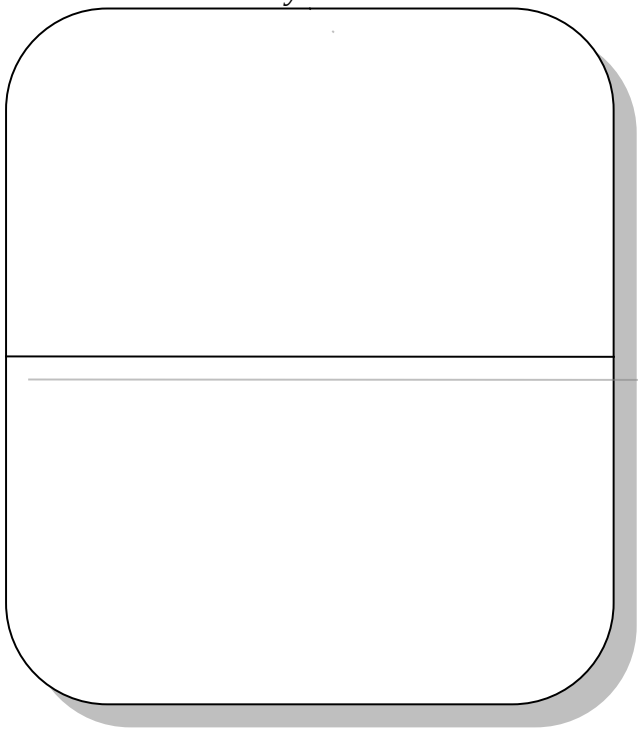
The preparation № 11: **Amphibians oocyte (egg) (section of frog ovary).**

Stain: H&E.

Designations:
1. Pole animal.
2. Pole vegetative.
3. Nucleus.
4. Nucleoli.
5. Cytoplasm.
6. Yolk plates.
7. Primary membrane.
8. Secondary membrane.
9. Inclusions of melanin.

The preparation № 12: **Sperm cells.**
A - Guinea pig's sperm cells.

B - Cock's sperm cells.
Stain: *iron hematoxylin*.



Designations:
1. Head.
2. Acrosome.
3. Neck.
4. Tail.

Name student _____

Signature of lecturer _____

Laboratory class

Date _____

EMBRYOLOGY

Topic: Gametogenesis. Fertilization. Cleavage. Blastula.

Objective: to be able to identify the process of fertilization, types of zygote cleavage and types of blastula on histological slides.

Equipment: light microscopes, histological slides of fertilization, total equal and unequal cleavages and blastula, handbook, tables.

The preparation № 13: **Fertilization of equine's Ascaridia oocyte.**

Stain: *iron hematoxylin.*

Using a low power magnification find out separate oocytes. Their cytoplasm is spongious and in most cases lies closely to the membrane. Two blue-violet densily-stained bodies are visible within cytoplasm. Pay attention to their location. Some of the oocytes contain only one body, located peripherally and other one – centrally. In other oocytes both of the bodies are positioned centrally. Move an oocyte with peripherally located body to the center of microscope field of view and study it using a high power magnification. Peripherally positioned cone-shaped body is a sperm cell penetrated oocyte. Centrally positioned body is a nucleus of oocyte. ***Draw*** this oocyte and mark in it.

After that move an oocyte with closely located bodies to the viewing position and study it at a high power magnification. Pay attention that both of the bodies are round in shape and contain granules or threads of chromatin. One of them is a male pronucleus whereas another one is a female pronucleus.

Draw this oocyte and mark:

The preparation № 14: **Cleavage of acarida's oocyte (zygote).**

Stain: *iron hematoxylin*

An equal cleavage is characteristic of acarida's oocyte. Using a low power magnification of microscope find out a transverse section of acarida's uterus, filled in with oocytes at different stages of cleavage. The membrane of oocyte does not cleave. A purple-stained polar body lies adjacent to the embryo and an oocyte membrane. Using a high power magnification find out and ***draw*** oocytes at different stages of cleavage.

1. Fertilized oocyte (zygote). It contains centrally positioned chromosomes and centrioles at opposite poles (mitosis metaphase).

Mark at the figure: 1 – oocyte's membrane; 2 – zygote; 3 – chromosomes; 4 – centrioles; 5 – polar bodies.

2. An oocyte at telophase of mitosis. Zygote is elongated with centrally positioned isthmus (8-like). Chromosomes near the isthmus are seen to move to different poles. The latter contain visible centrioles.

Mark at the figure: 1 – oocyte's membrane; 2 – zygote; 3 – isthmus; 4 – chromosomes; 5 – centrioles; 6 – polar bodies.

3. 2-blastomeres stage. Zygote is split into two equal-sized blastomers. Their cytoplasm contains nuclei and duplicated centrioles near one of the poles.

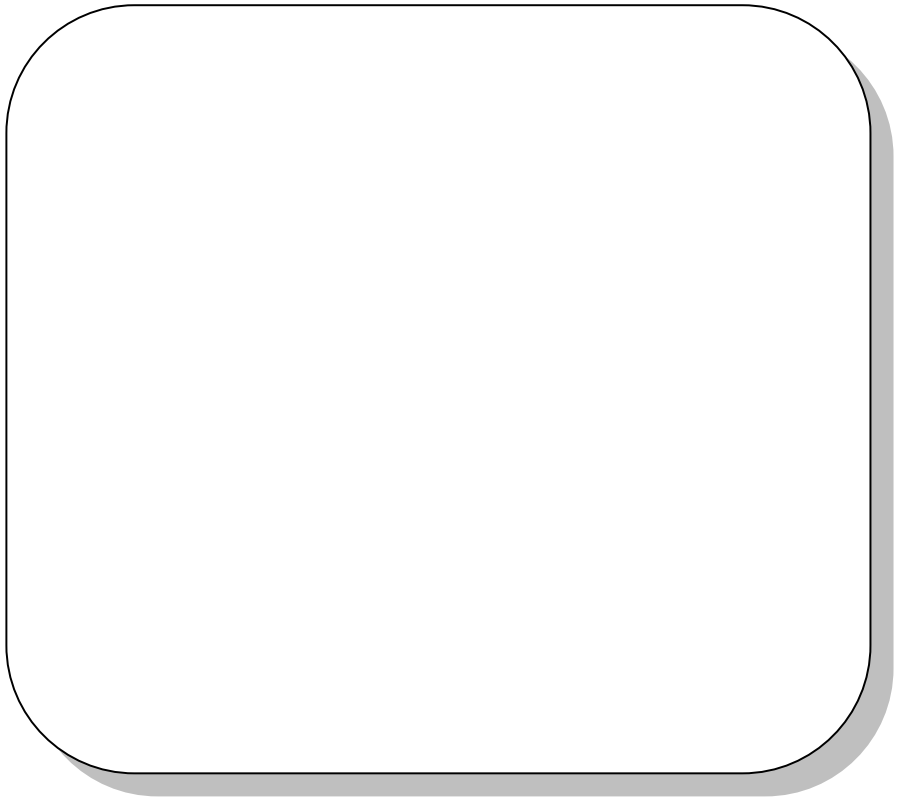
Mark at the figure: 1 – oocyte's membrane; 2 – elastomers; 3 – nucleus; 4 – centrioles; 5 – polar bodies.

4. 4-blastomeres stage. 4 equal-sized elastomers are visible at the preparation.

Make marks similar to that at previous figure.

The preparation № 13: **Fertilization of equine's Ascaridia oocyte.**

Stain: *iron hematoxylin***.**

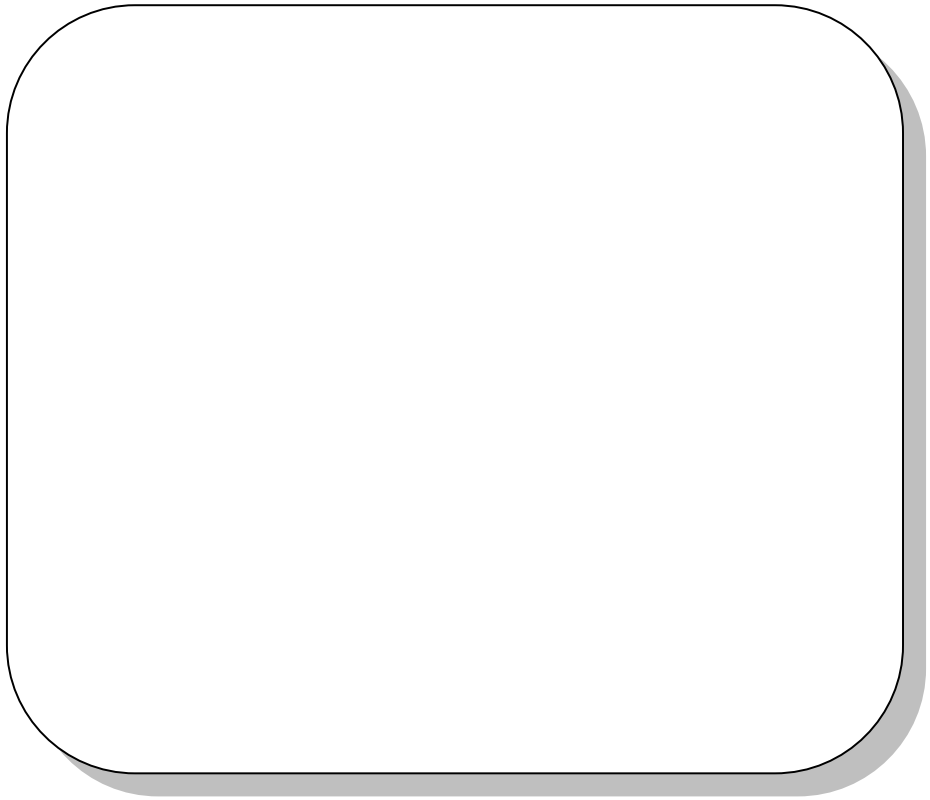


Designations:

1. Membrane.
2. Cytoplasm.
3. Oocyte's nucleus.
4. Sperm cell.
5. Oocyte I order.
6. Chromosomes.
7. The first polar bodie.
8. Oocyte II order.
9. The second polar bodie.
10. Mature ovum.
11. Female pronucleus.
12. Male pronucleus.

The preparation № 14: **Cleavage of acarida's oocyte (zygote).**

Stain: *iron hematoxylin***.**



Designations:

1. Membrane.
2. 2-blastomeres stage.
3. 4-blastomeres stage.
4. Chromosomes.

The preparation № 15: **Cleavage of frog's oocyte (zygote).**

Stain: *pikrofuxin and hematoxylin.*

Total equal cleavage is characteristic of frog's oocyte. View an oocyte using a low power magnification. It is split on unequal-sized blastomeres (4 or more). Small blastomeres (micromeres) are located at the animal pole whereas big ones (macromeres) are located at the vegetative pole. Orient a slide to locate the animal pole to the top of viewing field. View blastomeres and determine the directions of cleavage furrows.

Draw a slide and mark in it:

The preparation № 16: **Frog's blastula.**

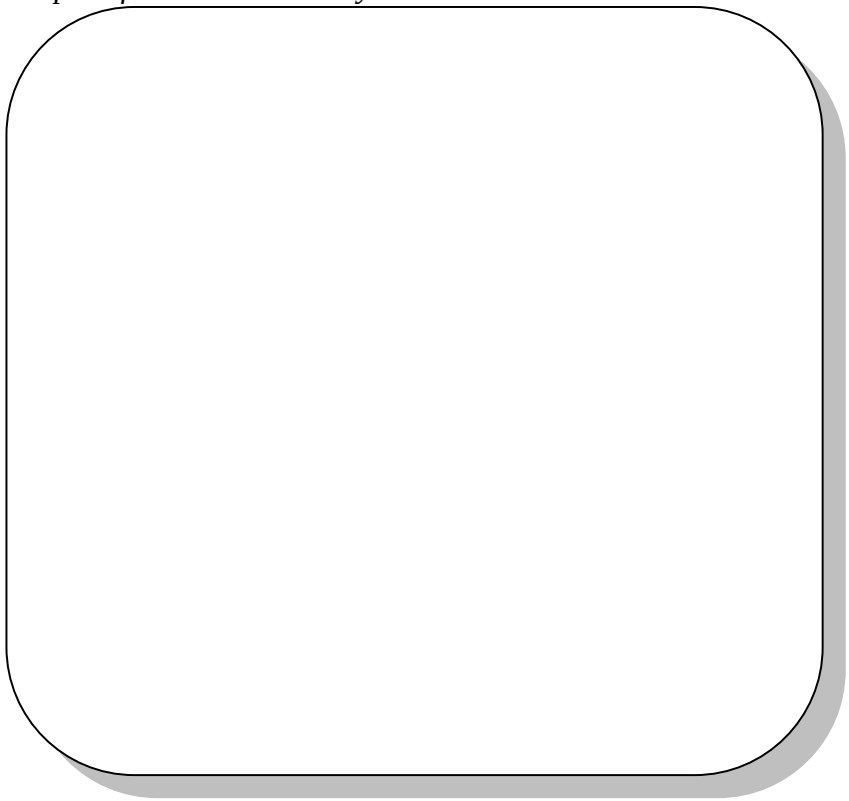
Meridian section.

Stain: *pikrofuksyn and hematoxylin.*

Study preparation using a low power magnification. Frog's blastula is called amphiblastula (multylayer blastula). Orient a preparation in a right way. Its animal part, containing micromeres should be positioned at the frontal part of the view. Determine roof (animal part), bottom (vegetative part), and edge zone, located between the roof and bottom. Pay attention to the wall of the blastula (blastoderm). It is formed by some layers of blastomeres. Determine parts of the blastula, where its wall is composed of macro- and micromeres. Find out the cavity of blastula (blastocoel) and pay attention to its location.

Draw blastula and mark in it:

The preparation № 15: **Cleavage of frog's oocyte (zygote).**
Stain: *pikrofuxin and hematoxylin.*

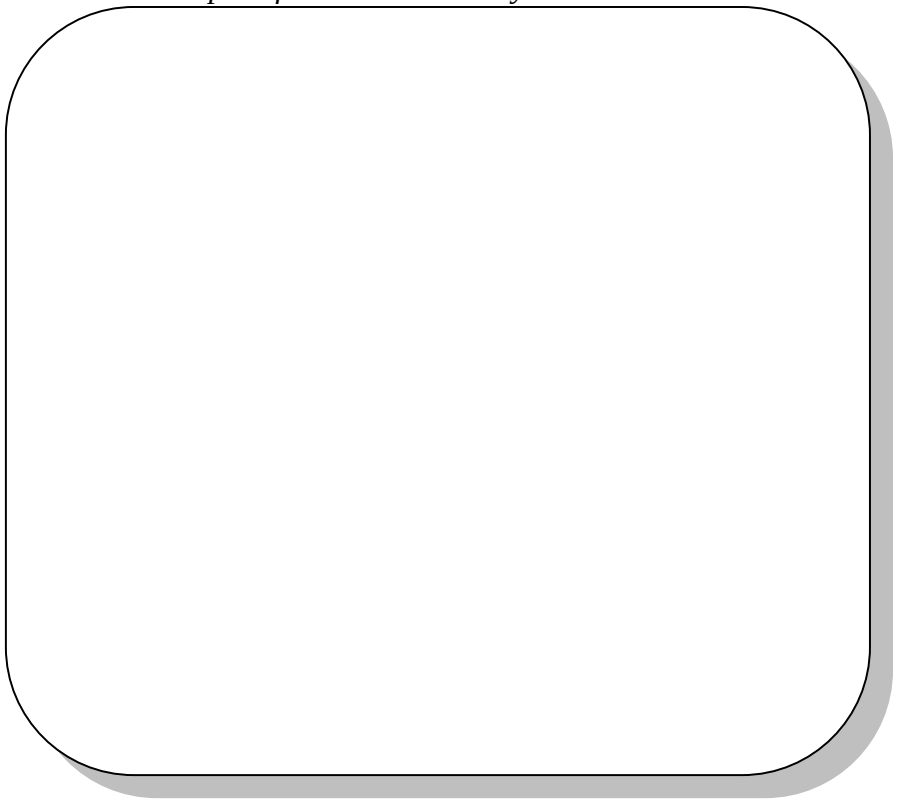


Designations:
1. Pole animal.
2 Pole vegetative
3. Meridian cleavage furrow**.**
4. Width cleavage furrow.
5. Micromeres.
6 Macromeres.
7. Inclusions of melanin.

`

Name student_____

The preparation № 16: **Frog's blastula.**
Stain: *pikrofuxin and hematoxylin.*



Designations:
1. Blastoderm:
   a) roof;
   b) bottom;
   c) edge zone.
2. Blastocoel.
3. Micromeres.
4. Macromeres.
5. Inclusions of melanin.

Signature of lecturer_____

Laboratory class

Date

EMBRYOLOGY

Topic: Gastrulation. Gastrula.

Objective: to be able to identify the process of the formation of germ layers and axial organs during gastrulation in chick embryo.
Equipment: light microscopes, histological slides of gastrulation, handbook, tables.

The preparation № 17: **Chick embryo of 2 - day incubation. Total preparation.**

Stain: *hematoxylin.*

Using a low power magnification or magnifying glass orient the preparation (germinal disk), which corresponds to the anterior part of the embryo, in such way, when its widen part is situated in the anterior part of the view. Histological preparation is the embryonic disc at the center of which is the embryo, which is visible from the dorsal surface.

Identify the head end of the embryo. At the front end to look at the **eye 2 bubbles** (in lateral position seen only one) and **brain bubbles** that are going on in the **neural tube**.

On both sides of the neural tube are the primary segments – **somites** (segmented part mesoderm) in the form of small plates. Their number is 12 - 14 pairs on the second day of incubation.

In the anterior part of the first pair of somites, a **heart** is found in the form of a sac and **yolk veins**. Behind, in the last pairs e somites, neural tube continues in the **residue of the primary streak.**

Outside the embryo to look at the **rudimentary shells embryonic disc** and found **blood islands**.

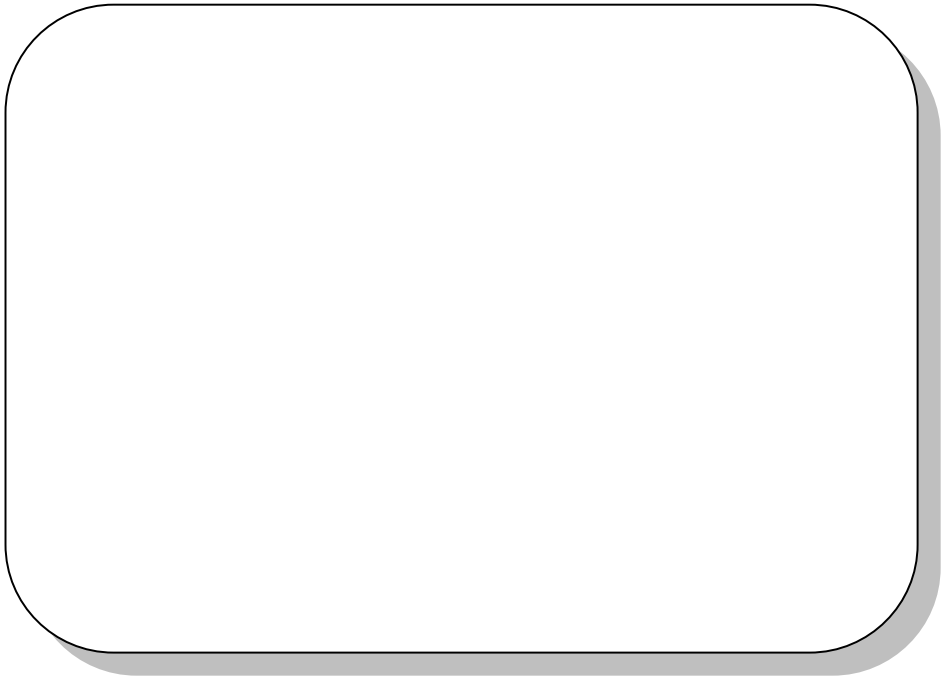
The preparation № 18: **Somites, notochord and neural tube in chick embryo. Transverse section of the embryo (2 - day incubation).**

Stain: *hematoxylin.*

Using a low-power magnification orient the widen part of preparation into the center of microscope view. Find out neural tube, resembling an oval with cleft-like cavity inside, there. Find out ectoderm, which forms the frontal surface of the embryo and lies just above the neural tube. Notochord lies beneath the neural tube. Back surface of the embryo is bounded by endoderm. View mesoderm at both sides of neural tube. It is represented by somites, segmental legs and splanchnotome. The latter comprises parietal layer (adjacent to ectoderm), visceral layer (adjacent to entoderm) and coelom (secondary cavity of the body), lying between them. Find out extraembryonic ectoderm, mesoderm (splanchnotom) and endoderm at the peripheral part of the preparation.

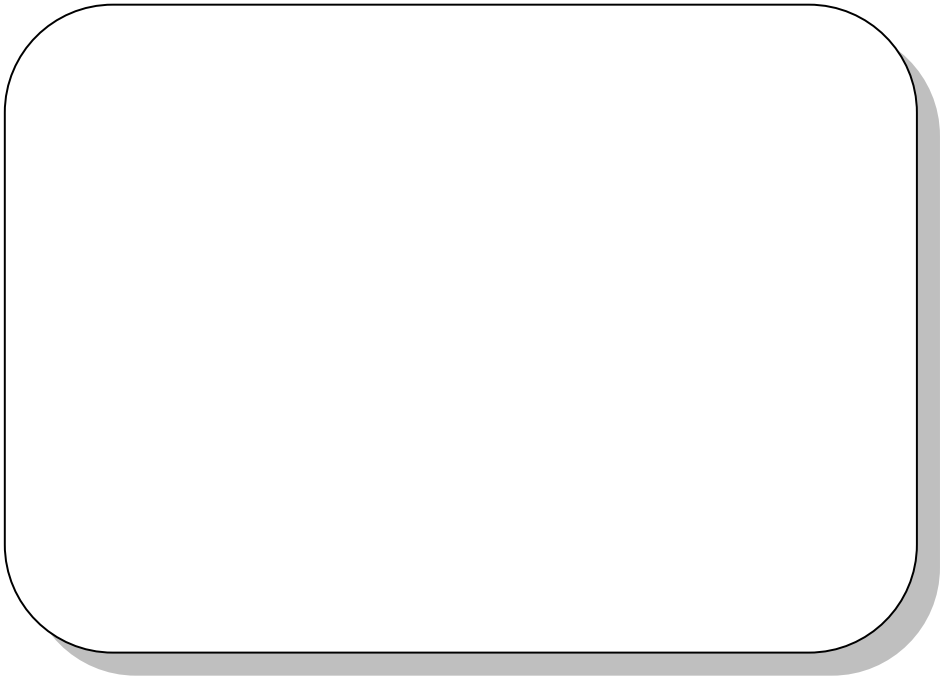
Draw an embryo and mark:

The preparation №17: **Chick embryo of 2 - day incubation. Total preparation.**
Stain: *hematoxylin.*



Designations:
1. Brain bubbles.
2. Eye bubbles.
3. Neural tube.
4. Somites.
5. The residue of the primary streak.
6. Heart.
    a) yolk veins.
7. Blood islands.
8. Germinal dysk.

The preparation № 18: **Somites, notochord and neural tube in chick embryo. Transverse section of the embryo (2 - day incubation).** ***Stain:*** *hematoxylin.*



Designations:
1. Ectoderm.
2. Endoderm.
3. Mesoderm.
    a) somites;
    b) nefrohonadotomes - segmental legs;
    c) parietal layer mesoderm - splanchnotome;
    d) visceral layer mesoderm - splanchnotome.
4. Neural tube.
5. Notochord.
6. Coelom.
7. Primary blood vessels.

Extraembryonic organs (fetal membranes). Placenta.

Objective: to know the origin of extraembryonic organs formation and their significance in the avian and mammalian embryo development. To know the components of placenta and its significance in the development of embryo in placental mammals.

Equipment: schemes of placenta structure, light microscopes, histological slides, handbook, tables.

The preparation № 19: **Body's and amniotic's folds cross-section of the chicken embryo (3 - day incubation).**

Stain: *hematoxylin.*

At low power magnification embryo find and compare it with the preparation № 18. Its size increased significantly, was the thickness of the wall of the neural tube, in the notochord appeared located near are two (some preparation have one) **big blood vessels** - aortic tab.

Somite differentiated on dermatomes, sklerotomy myotomes and who in the future partially or completely lose segmentation. **Dermatomes** defined on either side of the neural tube during ectoderm in a dense cellular bands that give rise to connective tissue on the skin - the dermis. **Sklerotoms** and **myotomes** each other not are separate and form a cell mass below the dermatomes. Later they will beginning under the axial skeleton and skeletal muscles. In the area of nefrohonadotomes (before the split sheets splanhnotomes) defined cross sections of the primary kidney tubules. Changed form the embryo, where there are signs of separation of embryonic and outside the embryonic parts by forming **body's folds** by ectoderm and mesoderm parietal layer. These folds are directed downwards and raise over embryo yolk, in the future, when combined, they form the ventral wall of his body. Lateral them ectoderm and mesoderm parietal layer mesoderm form **amniotic folds** that will grow towards each other and will close on the body of the embryo and form the **amnion** - due to the inner surface and **serous membrane** - due to the outer surface. Growth body's folds causes the formation of intestinal groove.

These layers form the yolk sac in the wall of which is determined by blood vessels.

***The preparation № 20*: The placenta of a cow.**

Stain: H&E.

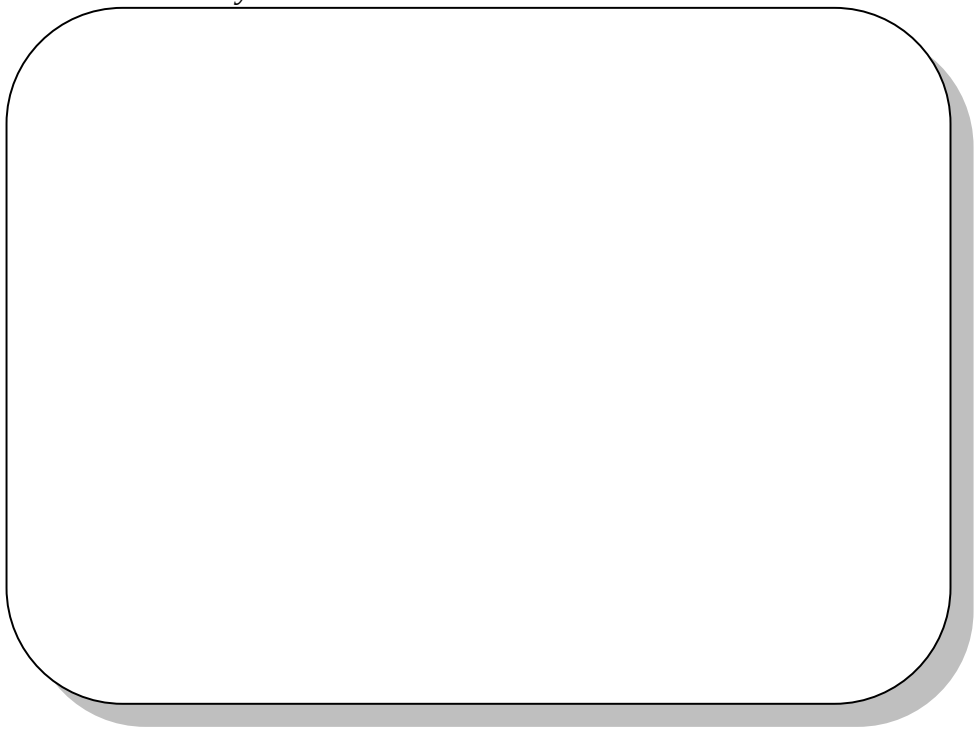
At low magnification to identify elements chorion and endometrium. **Chorionic** delicate tissue with different structure and basophilia. In preparation to determine **chorion** and **villi** or their fragments embedded in the **endometrium**. Endometrium is oxyphilic. Examine closely the preparation and determine a part chorionic **embryonic connective tissue** fibrous structure of the **blood vessels**, the **epithelium** formed cell layer that covers the outside and chorion villi and in contact with the tissues of the endometrium. In **endometrium** to identify **epithelium** and **uterine glands**, which are immersed chorionic villi and **connective tissue with blood vessels**.

In the presence of the preparation longitudinal sections chorionic villus noted that the **gland epithelium partially damaged** and epithelium villi in contact directly with the connective tissue of the endometrium basis.

Types of mammal placenta, depending on the distribution of chorionic villi and their contact with uterus mucosa membrane.

The preparation № 19: **Body's and amniotic's folds of the chicken embryo (3 - day incubation).**

Stain: *hematoxylin.*



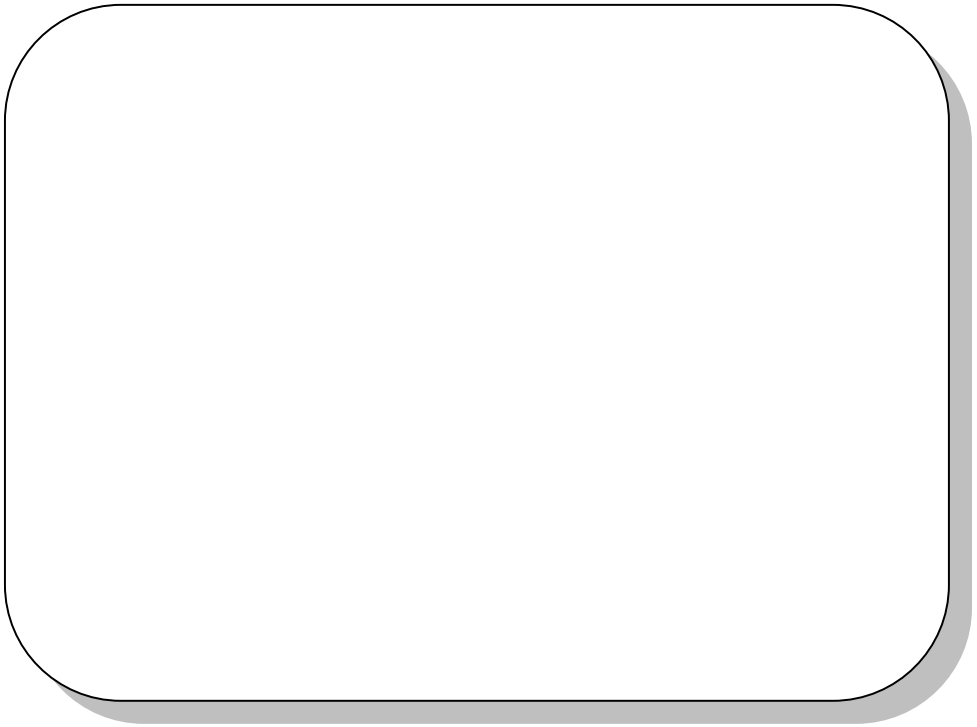
Designations:

1. Ectoderm.
2. Endoderm.
3. Parietal layer mesoderm.
4. Visceral layer mesoderm.
5. Neural tube.
6. Notochord.
7. Tab aorta.
8. Dermatomes.
9. Primary kidney tubules.
10. Mesenchyme.
11. Intestinal groove.
12. Blood vessels in the wall of yolk sac.
13. Coelom.
14. Body's folds.
15. Amniotic's folds.

Name student _____

***The preparation № 20*: The placenta of a cow.**

Stain: H&E.



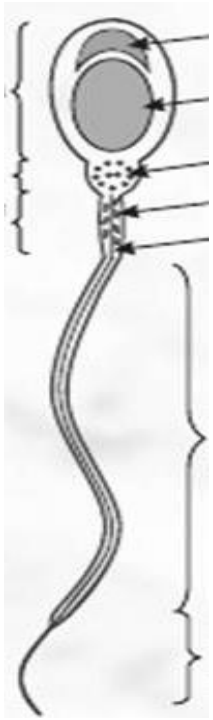
Designations:

1. Chorionic plate.
2. Chorionic villi:
   a) blood vessels.
3. Endometrium:
   a) epithelium of the uterine glands;
   b) connective tissue base.

Signature of lecturer _____

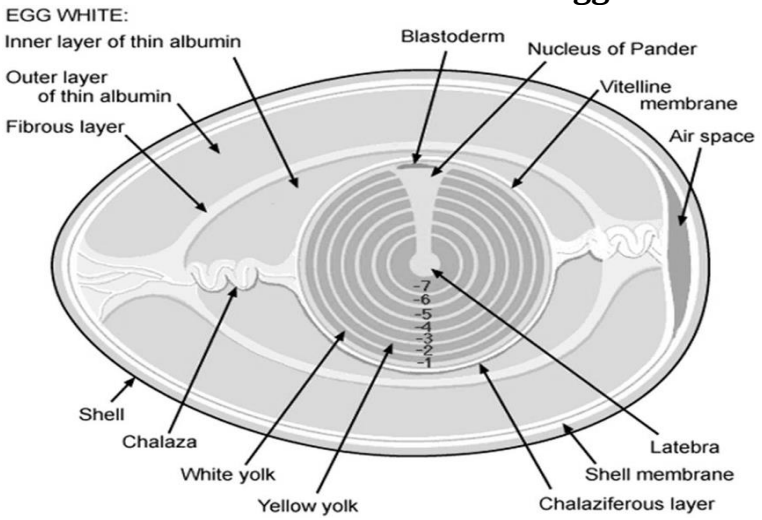
Homework
"EMBRYOLOGY"

Structure of a spermatozoon

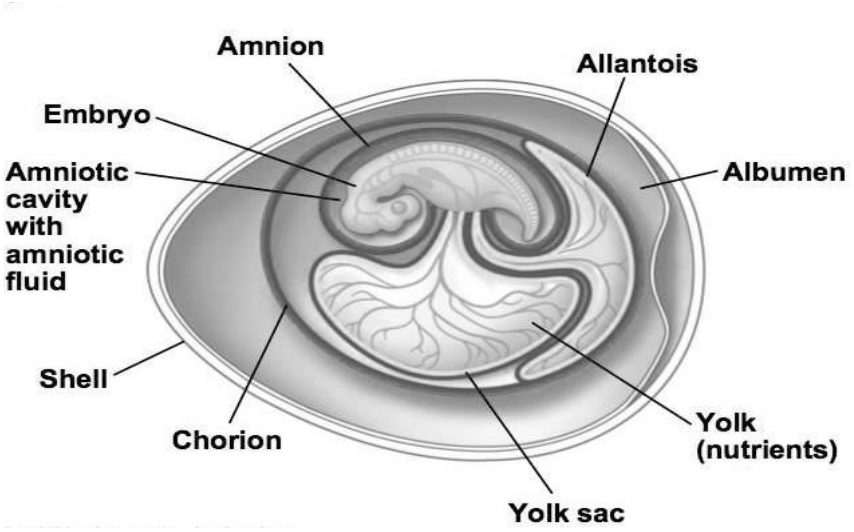


I - head,
II - neck (connecting piece),
III - tail: 1 - middle piece, 2 - principal piece, 3 - end piece.
4 - nucleus,
5 - acrosome,
6 - proximal centriole,
7 - distal centriole,
8 - mitochondrial sheath,
9 - axoneme.

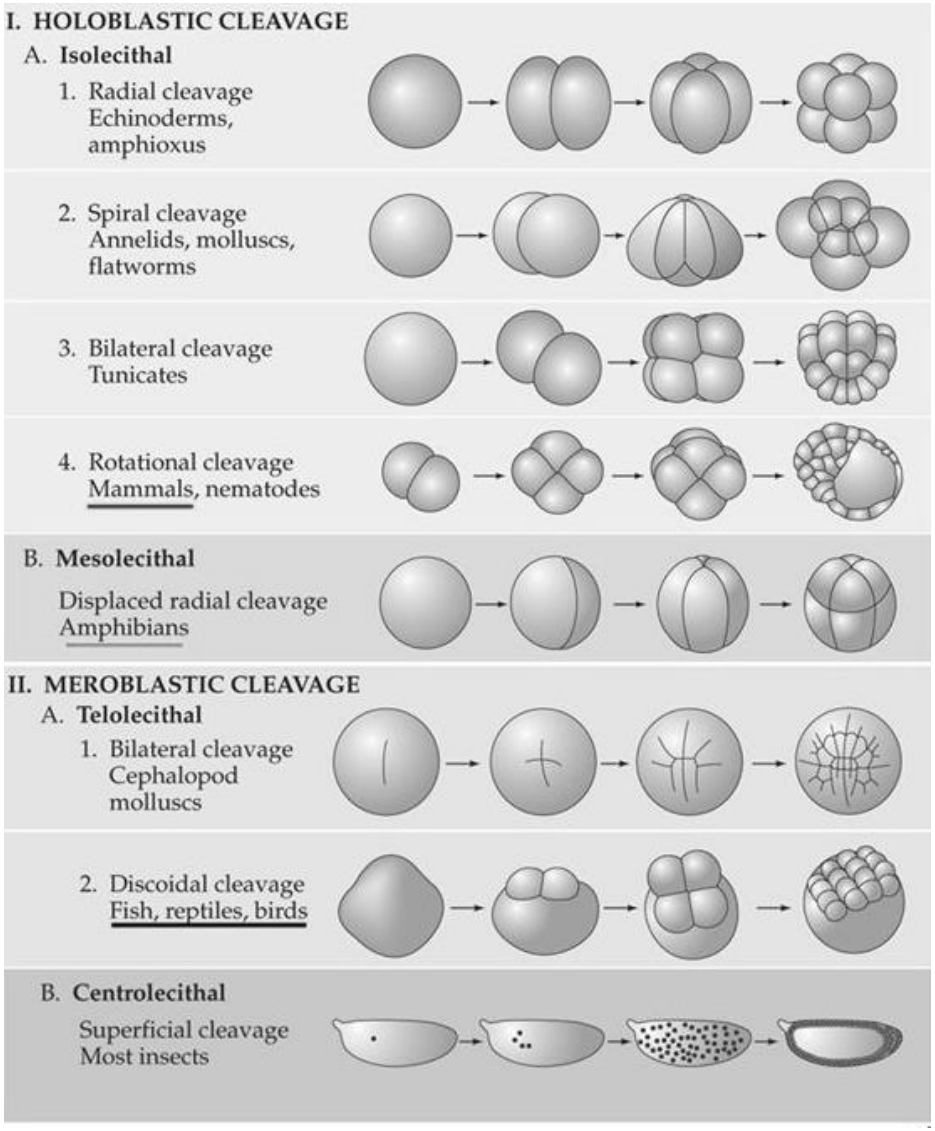
Structure of the fertilized hen's egg.



Extraembryonic organs (fetal membranes).



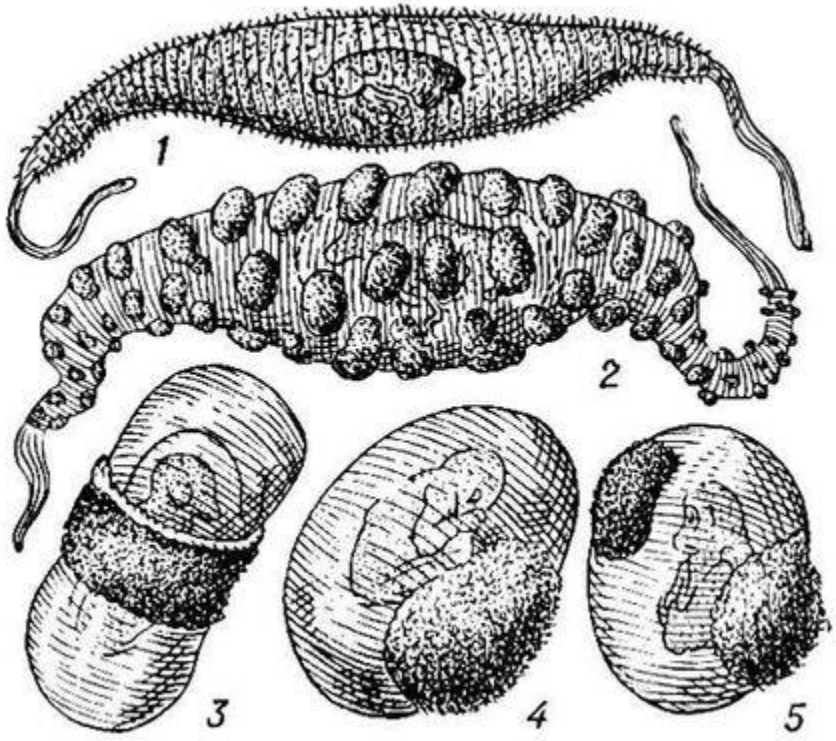
Schematic diagrams of the types of cleavage.



Questions for self-control

1. What is a sperm cell composed of?
2. Enumerate composing parts of sperm cell's tail.
3. What does a sperm cell's head contain?
4. What organelles are in the tail of sperm cell?
5. Organization of axonema.
6. Peculiarity of bird's sperm cell organization.
7. Enumerate composing parts of bird's egg.
8. What does an oocyte cytoplasm contain?
9. What organelle is absent in an oocyte?
10. What a secondary membrane of an oocyte is formed by? What functions does it perform?
11. Classification of oocytes depending on yolk amount and its distribution in cytoplasm.
12. What types of zygote cleavage do you know?
13. What is the result of zygote cleavage?
14. Types of blastula and their differences.
15. What parts are determined in blastula?
16. What is a blastocoel?
17. What is the wall of blastula formed of?
18. What type of cleavage is characteristic of oligolecytal oocytes (zygote), mesolecytal and polilecytal ones?
19. What is gastrulation?
20. Enumerate germ layers and axial organs.
21. What ways of gastrulation do you know?
22. How does gastrulation occur in birds?
23. Organization of embryonic disc in chick.
24. Due to what processes the primary streak and primary node are formed?
25. What is the neurula and how does it occur?
26. What parts has mesoderm?
27. Differentiation of germ layers and axial organs.
28. What are the origins of extraembryonic organs of chick embryo?
29. Enumerate fetal membranes in birds and mammals.
30. The origin of fetal membranes development.
31. Functions of fetal membranes.
32. What is placenta? Its role in embryo development.
33. Parts of placenta. What are they formed by?
34. Criteria of placenta classification.
35. What type of placenta is typical for ruminants, horse, pig, dog and primates?

Types of placentas on the location of the villi on the chorion.



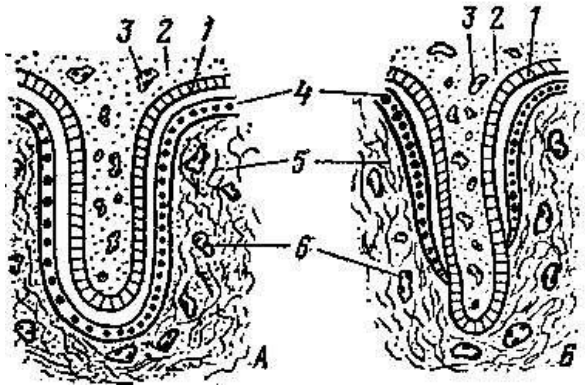
1. **Diffuse** (horse, pig, camel, dolphin).

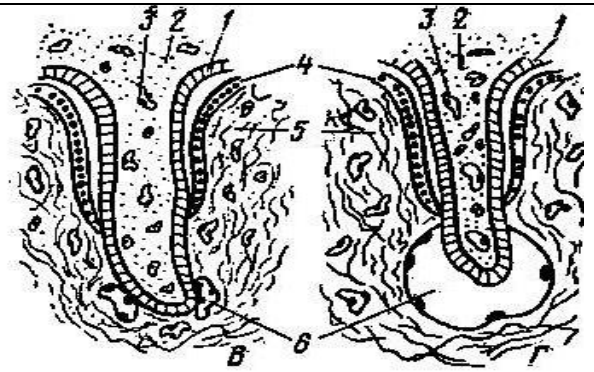
2. **Cotyledon** (cow, sheep, goat).

3. **Belt** (meat-eating animals - dog, cat, wolf).

4,5. **Discoidal** (rodents (rabbit, mouse); primates (monkey, man).

Types of placentas on the contact of fetal and maternal parts.





А. - epitheliochoreal placenta;
Б. - desmochoreal placenta;
В. - endotheliochoreal placenta;
Г. - haemochoreal placenta.

1. epithelium of chorionic villi;
2. connective tissue of the chorionic villus;
3. blood vessels of the chorionic villus;
4. the epithelium of the mucous membrane of the uterus;
5. connective tissue of the mucous membrane of the uterus;
6. blood vessels of the uterine mucosa.

Laboratory class

Date _____

GENERAL HISTOLOGY

Topic: **Epithelial tissue.**

Objective: to be able to identify different types of surface epithelia on histological preparations.
Equipment: light microscopes, histological slides of different types of epithelia, handbook, tables.

The preparation № 21: **Simple squamous epithelium of rat's omentum (mesothelium). Total preparation.**

Silver stain.

Using a low power magnification find out a gray-brown-stained piece of omentum. Orient slide to the center of your view and study it at a high-power magnification. Find out big cells with black contours and define a pale nucleus inside them. Some cells may have two nuclei. All cells lie close to each other and form a sheet.

In some places between the cells are small openings (space) - **"hatches"** in which fabric is absorption of fluid from body cavities.

Draw some cells and mark:

The preparation № 22: **Simple columnar (cylindrical) epithelium of dog's small intestine.**

Stain: H&E**.**

Using a low power magnification find out the transverse section of small intestine. **Intestinal villi** are protrusions of the lamina propria of the mucous membrane, basically they have loose connective tissue and are covered with epithelium. Dips of the epithelial layer in lamina propria form **crypts.**

Using a high-power magnification study the structure of wall. A single layer of columnar-shaped epithelial cells, lying on the basal membrane, forms the mucous membrane. The basal pole of cells contains nucleus. Basal membrane are separated by the loose connective tissue rich in blood capillaries. The epithelial layer consisting **three types of cells.** The most common are **1. - columnar enterocytes with border.** Their oval nuclei, shifted to the basal pole. Border at the apical pole, formed by special organelles - ***microvilli***. The border is an oxyphilic tape at the apical pole of epithelial cells.

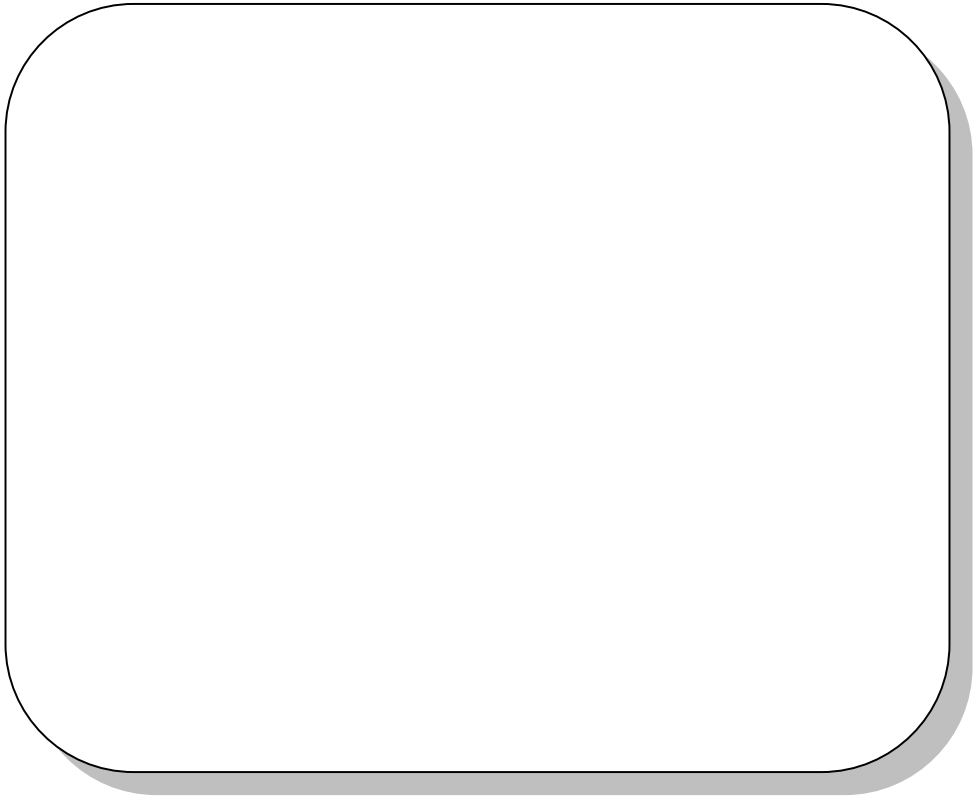
2. - **goblet cells** - have the shape of a glass. Their nucleus was displaced to the basal pole as a result of accumulation in the cytoplasm of large amounts of mucosal secretion. When stained with hematoxylin and eosin, their cytoplasm is slightly basophilic or nonstain.

3. - In crypts, **enterocytes without border.** They are prismatic, with hyperchromic nuclei. Cambial cells. They are divided by mitosis.

Draw a simple columnar (cylindrical) epithelium and mark in it:

The preparation № 21: **Simple squamous epithelium of rat's omentum (mesothelium). Total preparation.**

Silver stain.

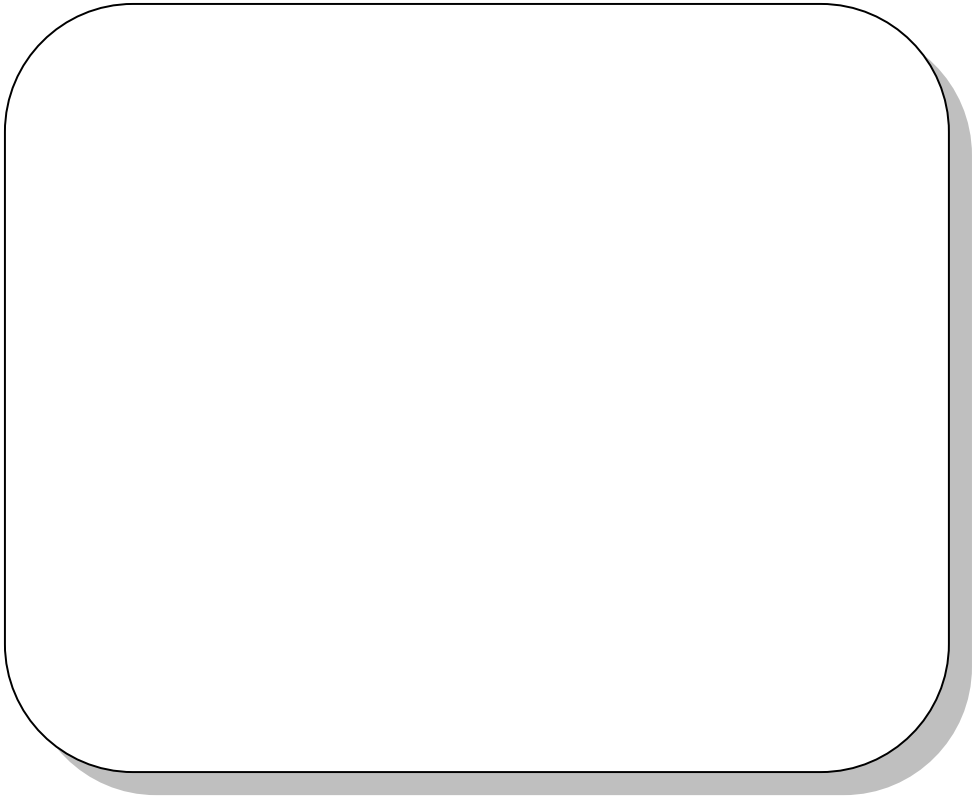


Designations:

1. Epithelial cells.
2. Nucleus.
3. Cytoplasm.
4. Cell's border.
5. "Hatches"**.**

The preparation № 22: **Simple columnar (cylindrical) epithelium of dog's small intestine.**

Stain*:* H&E.



Designations:

1. Intestinal villi.
2. Crypts.
3. Basal membrane.
4. Loose connective tissue.
5. Columnar epithelial cell with border.
6. Columnar epithelial cell without border.
7. Goblet cells.
8. Mitotic figures.

The preparation № 23: **Simple columnar (cylindrical) ciliated epithelium of cat's trachea.**
Stain*:* H&E.

Using a low power magnification find out the transverse section of trachea. Using a high power magnification study the structure of wall. A single layer of columnar-shaped epithelial cells which contain special organelles - **cilia**, lying on the basal membrane, forms the mucous membrane. The basal pole of cells contains nucleus. Basal membrane are separated by the loose connective tissue rich in blood capillaries. The epithelial layer consisting **three types of cells.** The most common are:

1 - columnar ciliated epithelial cells, which at the apical pole with special organelles — cilia. The nuclei of these cells oval, located in the central part of the cytoplasm.

2 - goblet cells - have the shape of a glass. Their nucleus was displaced to the basal pole as a result of accumulation in the cytoplasm of large amounts of mucosal secretion. When stained with hematoxylin and eosin, their cytoplasm is slightly basophilic or nonstain.

3 - in crypts, **cambial cells.** They are prismatic, with hyperchromic nuclei. Cambial cells. They are divided by mitosis.

These cells are pinched between the prismatic and goblet cells, and their apical pole reaches the free surface of the epithelial layer.

Draw a simple columnar (cylindrical) ciliated epithelium and mark in it:

The preparation № 24: **Stratified squamous non-keratinizing epithelium and glandular epithelium of dog's esophagus.**
Stain*:* H&E.

Using a low power magnification orient the esophageal mucosa in a way the epithelial sheet is directed to the top of your view. The connective tissue of mucous membrane underlies the epithelium.

Using a high power magnification view the epithelium. Find the **basal cell layer**, which is formed by the columnar epithelial cells, lying on the basal membrane. Their nuclei are located near the apical pole of cells. The **pickle cell layer** consisting of several cell rows is located above the basal cell layer. Its epithelial cells possess polygonal or processed shape and centrally positioned nuclei. Several rows of **flattened cells** that constitute the surface cell layer are located above the pickle cell layer.

Explain: why the nuclei of epithelial cells are colored differently? why is the cytoplasm of the basal layer cells basophilic?

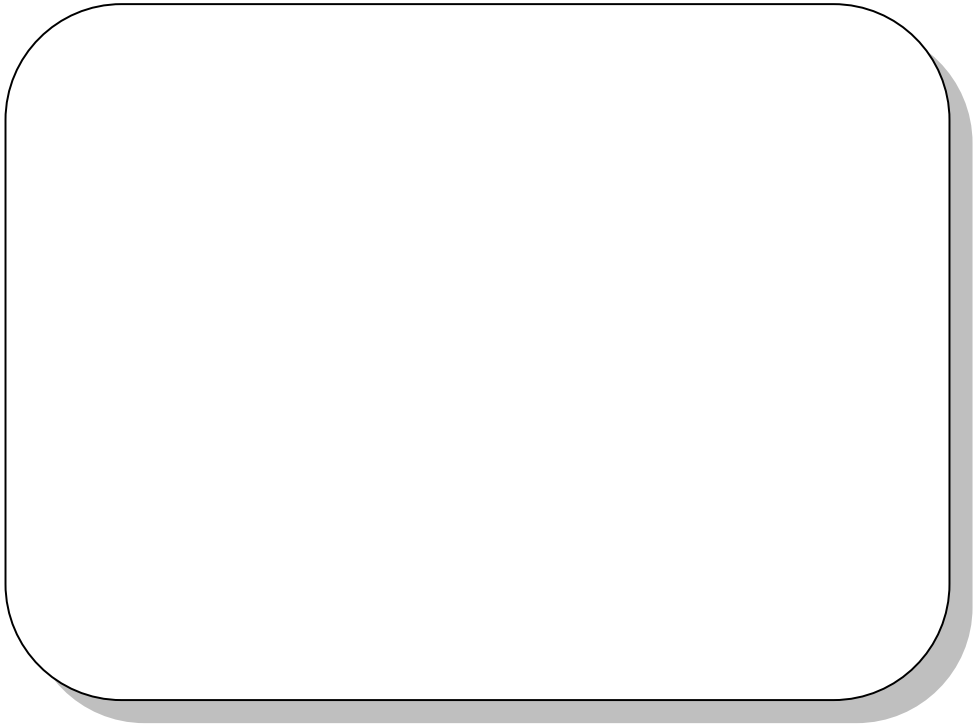
Using a high power magnification find out the **folding branched tubular-alveolar mucous glands** in loose connective tissue mucosa. They consist of ***secretory departments*** and ***excretory ducts.***

Secretory departments are formed by glandular cells - glandulocytes, whose cytoplasm is basophilic and the flattened nucleus is displaced to the basal membrane.

Excretory ducts have the form of tubes, cut at different levels, the wall of which is formed by low prismatic or cubic cells with hyperchromic nuclei.

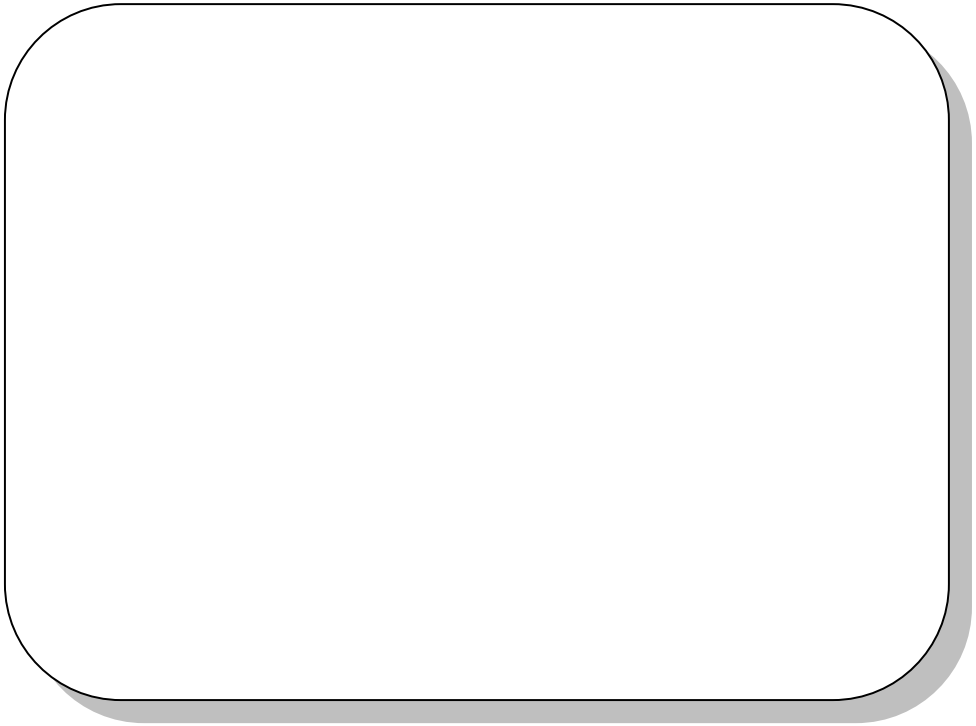
Draw a fragment of esophagus and mark in it:

The preparation № 23: **Simple columnar (cylindrical) ciliated epithelium of cat's trachea.**
Stain: H&E.



Designations:
1. Basal membrane.
2. Columnar ciliated epithelial cells.
3. Goblet cells.
4. Cambial cells.
5. Loose connective tissue.

The preparation № 24: **Stratified squamous non-keratinizing epithelium and glandular epithelium of dog's esophagus.**
Stain: H&E.



Designations:
1. Basal membrane.
2. Basal cell layer epithelium.
3. Pickle cell layer epithelium.
4. Surface cell layer epithelium.
5. Loose connective tissue.
6. Secretory departments of the mucous glands.
7. Excretory ducts of the mucous glands.

Name student _____

Signature of lecturer _____

Laboratory class

Date _____

Topic: Connective tissue.

Mesenchyme. Blood of mammals.

Objective: to be able to identify mesenchyme and blood cells of mammals on histological preparations.
Equipment: light microscopes, histological slides of mesenchyme, smears of mammalian blood, tables.

The preparation № 25: **Mesenchyme of chick embryo.**

Stain: H&E.

At the transverse section of chick embryo using low a power magnification find out sites of prospective organ derivations and pale areas containing a low differentiated mesenchyme between them. Orient such area to the center of the field of view and study it at a high power magnification. Find out spindle-shaped or stellate mesenchymal cells. Large nucleus is located centrally and predominantly posses oval shape. Adjacent mesenchymal cells contact by the processes. The extracellular matrix is located between cells.

Draw some mesenchymal cells and mark:

The preparation № 26: **Blood smear of livestock (or other domestic mammal).** ***Romanovsky-Giemsa stain.***

Using a low power magnification find out an area with uniform cells layer. The great majority of cells consist of erythrocytes stained in light pink. A few leukocytes with purple nuclei are visible among them.

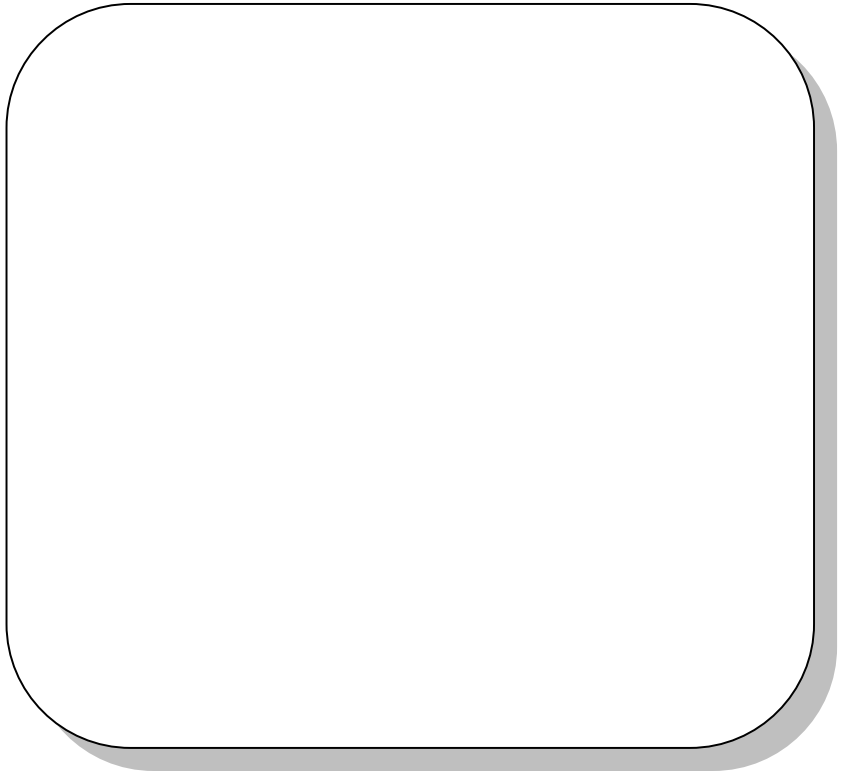
View cells at a high-power magnification. **Erythrocytes** are small disc-like anucleate cells with pale central part. Find out ***granulocytes*** (neutrophils, eosinophils and basophils) among leucocytes. **Nuetrophils** possess dark-staining monolobed or segmented nucleus and pale gray cytoplasm, containing violet, dustlike specific granules, difficult to resolve with a light microscope. **Eosinophils** are found rarely. Their nucleus is similar to that of nuetrophils but is less dense and has fewer lobes. Their cytoplasm stains pale blue or gray and contains large specific granules staining various shades of orange, pink or red with eosin. **Basophils** are not often found in blood smear. They possess irregular, bilobed or highly segmented nucleus. The granules of basophils are fairly large, round to oval and stain reddish purple to dark purple. View basophils at demonstrative preparations.

Agranulocytes include lymphocytes and monocytes. **Lymphocytes** are often found in a blood smear. They possess large nucleus, which occupies almost entire cytoplasm. The latter appears as a thin basophilic rim, surrounding nucleus. Find out small, medium and large lymphocytes. **Monocytes** are the largest leukocytes with horseshoe-shaped, kidney-shaped oval or irregular nucleus. Their cytoplasm is generally pale gray-blue and may contain dustlike, azurophilic granules. It often contains vacuoles that give it a foamy appearance. **Platelets** are membrane-bound fragments of cytoplasm from large cells called megakaryocytes. They are small, stain pale blue and have purple central granules that may be apparent. They occur singly or in clusters in smears.

To differentiate cells use figures in textbooks and atlases.

Draw blood cells and mark:

The preparation № 25: **Mesenchyme of chick embryo.**
Stain: H&E.

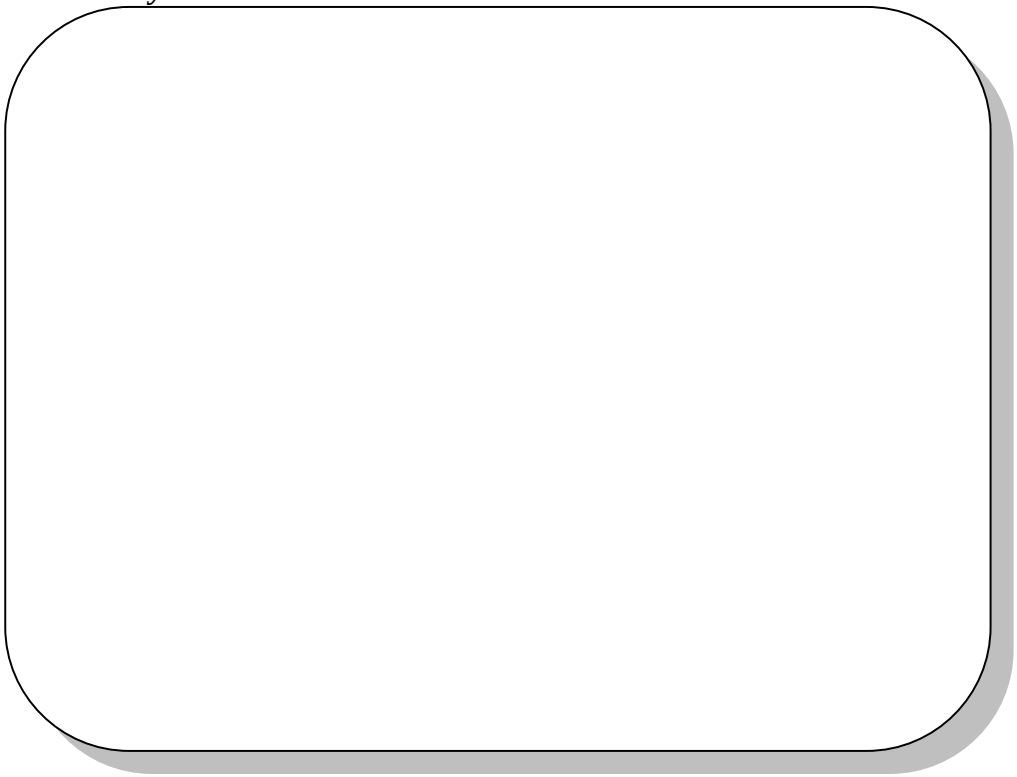


Designations:

1. Mesenchymal cells.
2. Nucleus.
3. Cytoplasm.
4. Processes.
5. Extracellular matrix.
6. Primary blood vessels.
7. Blood cells.

Name student _____

The preparation 26: **Blood smear of livestock (or other domestic mammal).**
Romanovsky-Giemsa stain.



Designations:

1. Erythrocyte.
2. Neutrophils:
   a) young;
   b) sticks nuclei;
   c) segmented nuclei.
3. Eosinophil.
4. Basophil.
5. Lymphocytes:
   a) small – 8 mkm;
   b) average – 8-11 mkm;
   c) large – more 11 mkm.
6. Monocyte.
7. Platelets.

Signature of lecturer _____

Laboratory class

Date _____

Topic: Connective tissue.
Blood of birds. Reticular tissue.

Objective: to be able to identify blood cells of birds and reticular tissue on histological preparations.
Equipment: light microscopes, histological slides of smear of birds blood and reticular tissue, tables.

The preparation № 27: **Blood smear of hen (or other domestic birds).**
Romanovsky-Giemsa stain.

Most of bird's blood cells are similar to those in mammals. Only some of them differ.

Erythrocytes of birds are oval-shaped nucleated cells. Nucleus is oval and stains dark blue. Instead of platelets blood of birds contains true cells – **thrombocytes**. Like erythrocytes they are oval, but smaller in size. Cytoplasm of thrombocytes is basophilic and contains oval to round nucleus. Cytoplasm of avian **nuetrophils** possesses rod-like structures, staining pink-red with eosin, instead of granules.

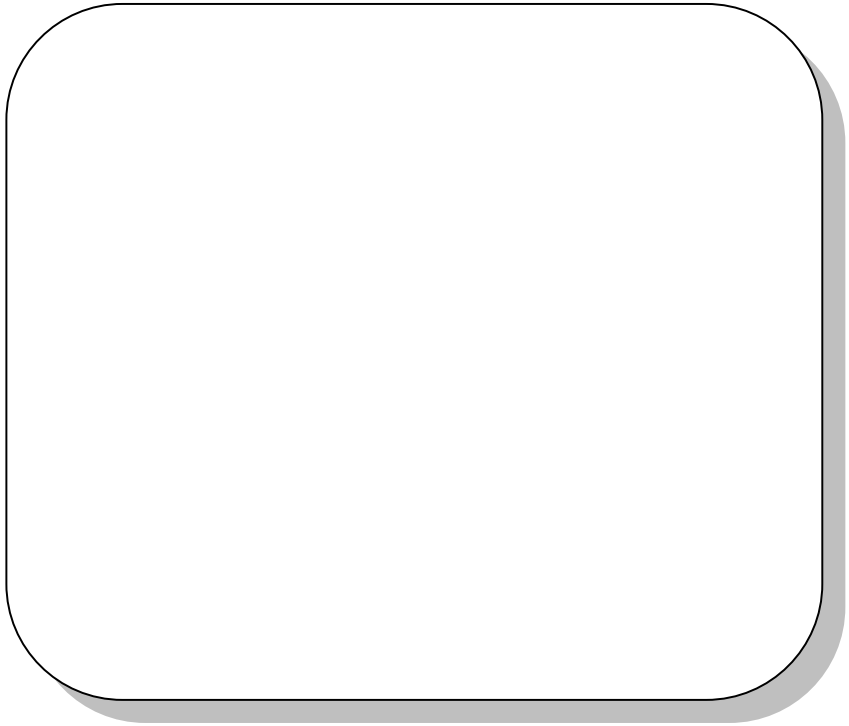
View blood cells using a high-power magnification, ***draw*** them and mark:

The preparation № 28: **Reticular tissue of cat's lymph node.**
Stain: H&E.

At the periphery of the lymph node reticular tissue, which form its base contains a lot of lymphocytes disguising the tissue. Using a low power magnification find out pale areas, containing a low amount of lymphocytes at the central part of the lymph node. Orient such area at the center of viewing position and study it at a high power magnification. Find out reticular cells. Their cytoplasm forms processes. Adjacent reticular cells contact with each other by processes and form reticular structures (syncytium). Nuclei of reticular cells are round or oval and positioned at the center of the cell. View lymphocytes within the loops of syncytium. They are round cells with large nucleus, which occupies almost the whole cytoplasm. Between reticular cells there is an extracellular matrix. Their fibers can be defined by using special staining.

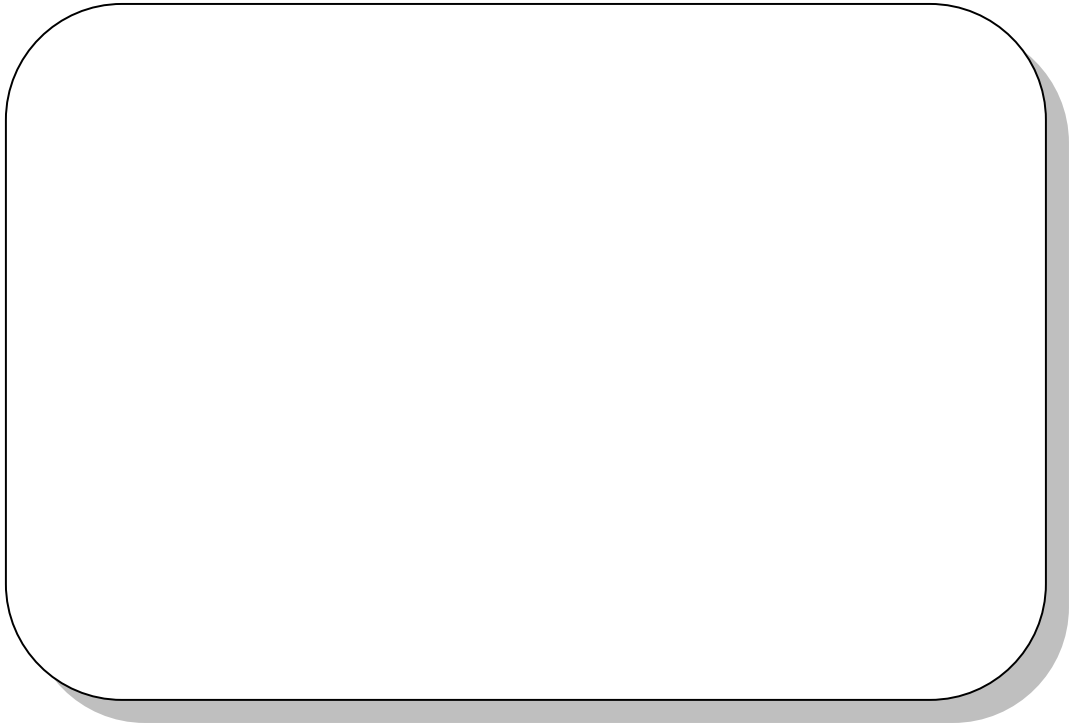
Draw slide and mark:

The preparation № 27: **Blood smear of hen (or other domestic birds).**
Romanovsky-Giemsa stain.



Designations:
1. Erythrocytes.
2. Neutrophils.
3. Thrombocytes.

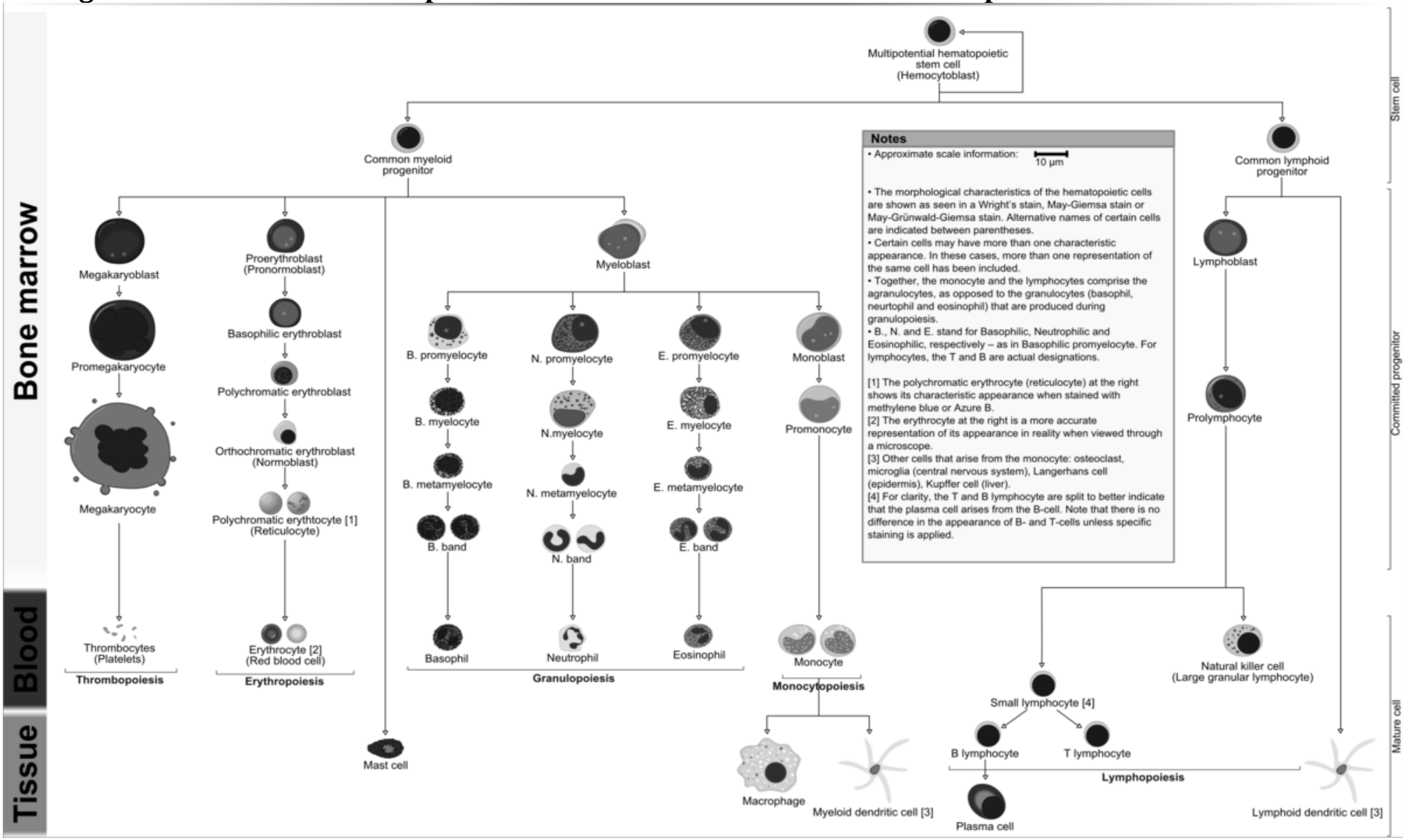
The preparation № 28: **Reticular tissue of cat's lymph node.**
Stain: H&E.



Designations:
1. Reticular cells.
2. Nucleus.
3. Cytoplasm.
4. Processes.
5. Extracellular matrix.
6. Lymphocytes.
7. Macrophages.

Name student_____ Signature of lecturer_____

Diagram that shows the development of different blood cells from haematopoietic stem cell to mature cells



Laboratory class

Date _____

Topic: Connective tissue.

Loose connective tissue. Dense connective tissue.

The preparation № 29: **Loose connective tissue of rat's hypodermis. Film preparation.**
Stain: *iron hematoxylin.*

View a preparation at a low power magnification. Pay attention to the content of an extracecellular substance. It is higher than the amount of cells. An extracellular substance contains a large amount of ground substance appearing like empty spaces and relatively few fibers (all three types are present). Find out collagen and elastic fibers within extracellular matrix. The first are thick and wavy. They do not branch and anastomose with each other and form bundles, oriented in different directions. The second are thin. They do not form bundles but can branch and connect each other. An extracellular matrix contains nuclei of cells, which should be examined at a high power magnification. Among cells differentiate fibroblasts, histiocytes, adventitial cells (pericytes), mast cells (tissue basophils), plasmocytes and lymphocytes. Use drawings in textbooks, handbooks and atlases for cell differentiation. ***Draw*** a preparation and mark:

Designations:

1. Collagen fiber.
2. Elastic fiber.
3. Extracellular matrix.
4. Fibroblast.
5. Histiocyte.
6. Mast cell.
7. Adipocytes.
8. Adventitial cells.
9. Blood capillaries.

The preparation № 30: **Longitudinal section of calves' tendon.**

Stain: H&E.

Tendon is built of **dense collagen regular connective tissue.**

Using a low power magnification one can see bundles of parallel collagen fibers. View a tendon at a high power magnification. Find out a primary bundle (single collagen fiber). Nuclei of fibroblasts are located between fibers.

Primary bundles are united to form secondary bundles, surrounded by loose connective tissue layers, called endotenonium. Nuclei of loose connective tissue cells are stained purple.

Secondary bundles are united into tertiary bundles, surrounded by loose connective tissue layers – perytenonium. Find out blood vessels within endo- and perytenonium.

Draw a tendon and mark:

The preparation № 31: **Longitudinal section of bulls' ligament.**

Stain: *pikrofuxin and hematoxylin.*

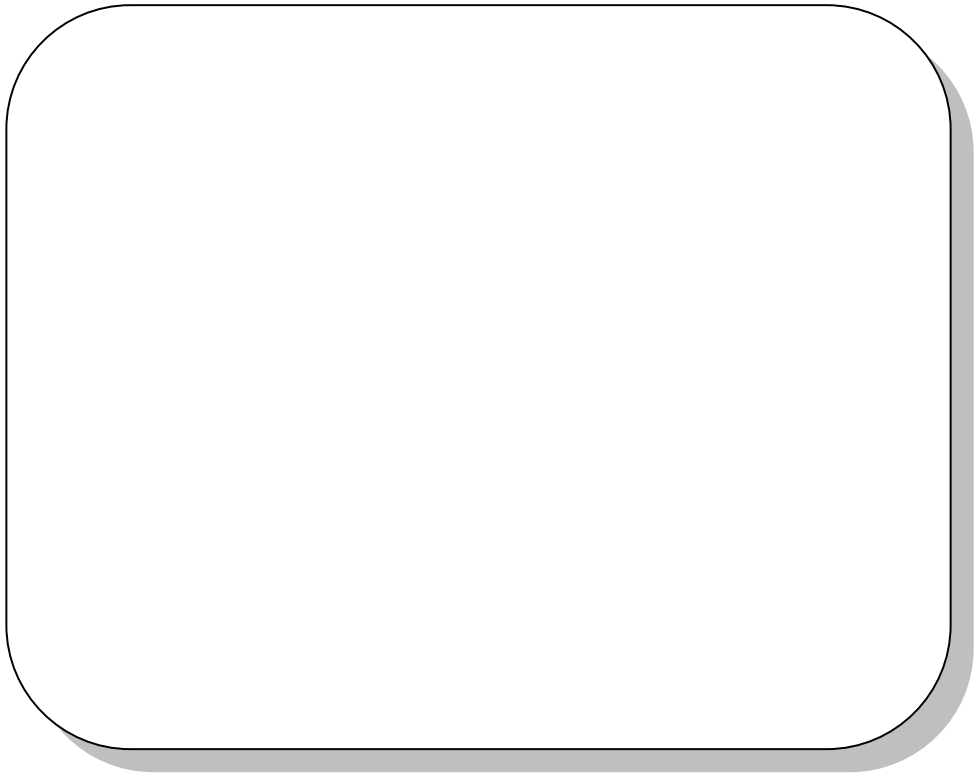
Ligament is built of **dense elastic connective tissue.**

Using a low power magnification one can see bundles of parallel thick elastic fibers stained in yellow. View a ligament at a high power magnification. Find out a thin collagen fibers painted in pink. Nuclei of fibroblasts are located between fibers. Nuclei of loose connective tissue cells are stained purple. Find out blood vessels within loose connective tissue layers.

Loose connective tissue as part of tendons and ligaments accomplishes trophic, protective, regenerative functions.

Draw a ligament and mark:

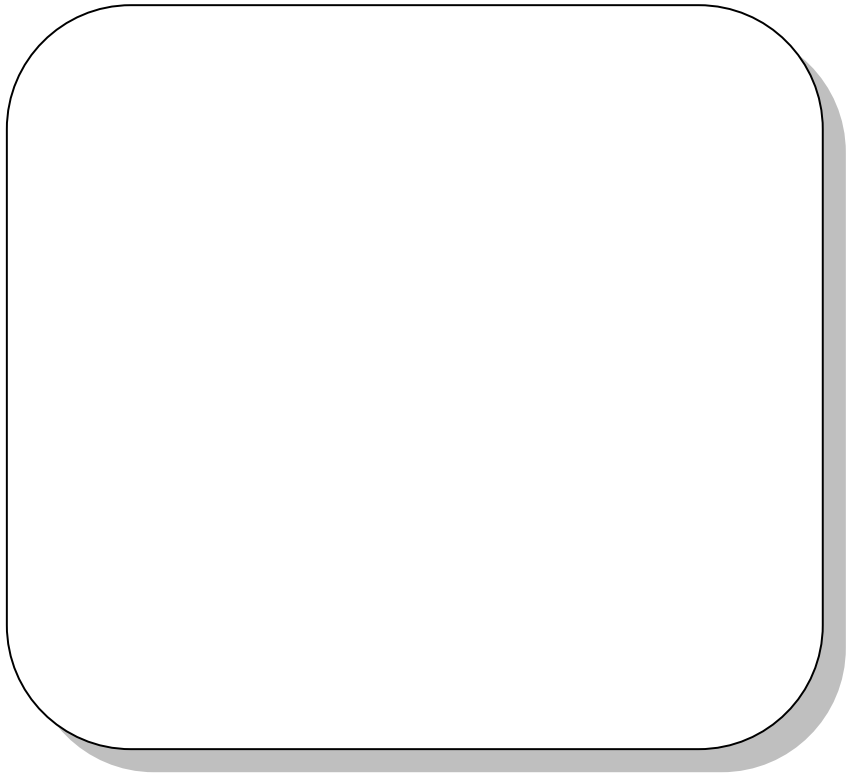
The preparation № 30: **Dense collagen connective tissue with cut tendons calf.**
Stain: H&E**.**



Designations:
1. Primary bundle (a collagen fiber).
2. Secondary bundle of collagen fibers.
3. Fibrocytes' nuclei.
4. Endotenonium.
5. Tertiary bundle of collagen fibers.
6. Perytenonium.
7. Blood vessels.

Name student _____

The preparation № 31: **Longitudinal section of bulls' ligament.**
Stain: *pikrofuxin and hematoxylin.*



Designations:
1. Elastic fibers.
2. Collagen fibers.
3. Fibrocytes' nuclei.
4. Loose connective tissue layers.

Signature of lecturer _____

Laboratory class

Date _____

Topic: Connective tissue. Cartilage.

Objective: to be able to identify different types of cartilage and bone at histological preparations.

Equipment: light microscopes, histological slides of different types of cartilage and bone, handbook, tables.

The preparation № 32: **Section of hyaline cartilage of rabbit's rib.** ***Stain:*** H&E.

Hyaline cartilage of rabbit's rib is formed by hyaline cartilage tissue. View a cartilage using a low power magnification. At the periphery of cartilage there is a loose connective tissue and bundles of muscle fibers. Pay attention to the accumulations of adipocytes in a loose connective tissue. They appear as rings and form accumulations resembling reticulum. A loose connective tissue also contains large blood vessels. The outer surface of cartilage is covered by perichondrium, which appears as a red strip and contains cells' nuclei. The cartilage is invested by a perichondrium. View the perichondrium and cartilage at a high power magnification. The outer portion of perichondrium is a dense irregular connective tissue whereas the inner layer is chondrogenic, containing cells with the capacity to form chondroblasts. Find out the bundles of collagen fibers (stained in red), cells' nuclei and blood vessels in perichondrium. In cartilage beneath perichondrium find out chondroblasts. They are flatterned or eliptical near the surface of the cartilage and gradually become spherical deeper in the tissue and are surrounded by basophilic matrix. Chondrocytes are located deeper in the cartilage and confined to small spaces (lacunae) within the matrix. Chondrocytes lie separately in lacunae or form small clusters (isogenous groups). The latter are the result of mitosis of chondrocytes. Each isogenous group can contain 2 to 4 chondrocytes. Pay attention to the staining of matrix, which lacks fibers. The surface layers of cartilage is oxyphilic (stains pink), but deeper portions are poor basophilic (pale blue). Isogenous groups are surrounded by basophilic matrix.

Draw hyaline cartilage and mark:

Designations:

1. Perichondrium.
2. Cartilage.
3. Chondroblasts.
4. Chondrocytes.
5. Isogenous group.
6. Matrix.

The preparation № 33: **Elastic cartilage of porcine pinna.**
Stain: hematoxylin and orsein.

A pinna (external ear) is formed by elastic cartilage. Using a low power magnification find out a perichondrium and a cartilage and view them at a high power magnification. Morphological features of elastic cartilage are identical with those of hyaline cartilage except for the presence of elastic fibers. They are stained dark pink by orsein. Isogenous groups may be observed more frequently. They are not surrounded by basophilic extracellular substance.

Draw an elastic cartilage and mark in it:

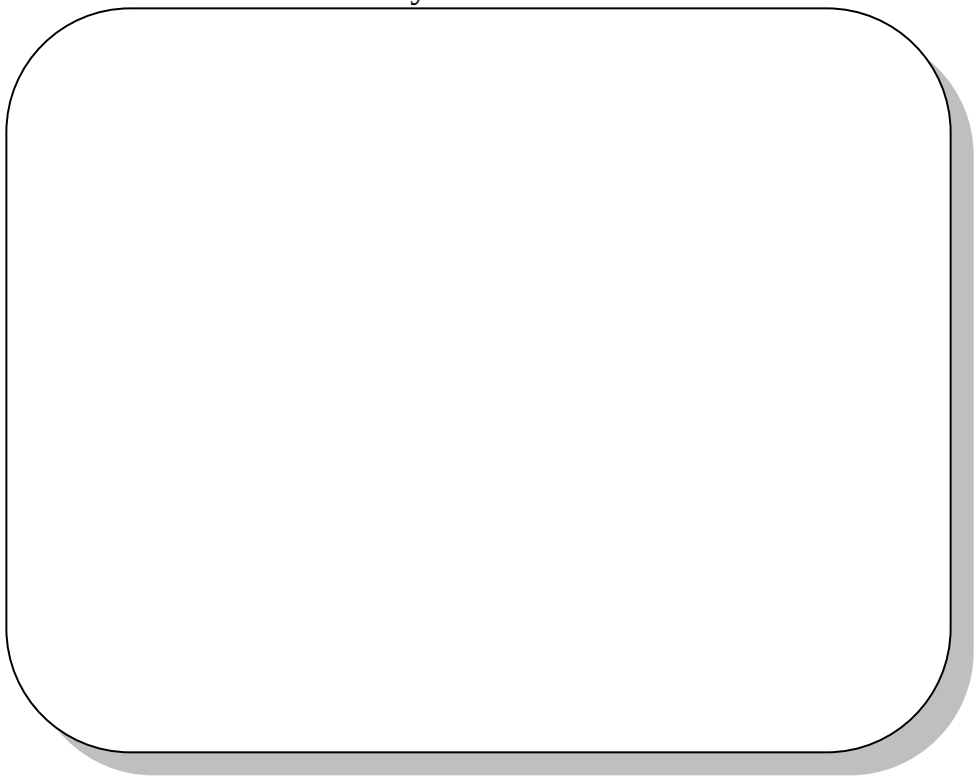
The preparation № 34: **Fibrocartilage of calf intervertebrate disk.**
Stain: H&E.

Fibrocartilage is an intermediate form between dense collagenous tissue and cartilage. It occurs at intervertebrate disks and at sites where ligaments and tendons are attached to the hyaline cartilage. Using a low power magnification find out a site of fibrocartilage. It is made by large collagenous fascicles (bundles) in orderly array that are separated by isolated portion of cartilage. The chondrocytes reside between the fascicles.

View fibrocartilage at a high power magnification. ***Draw*** a cartilage and mark:

The preparation № 33: **Elastic cartilage of porcine pinna.**

Stain: hematoxylin and orsein.



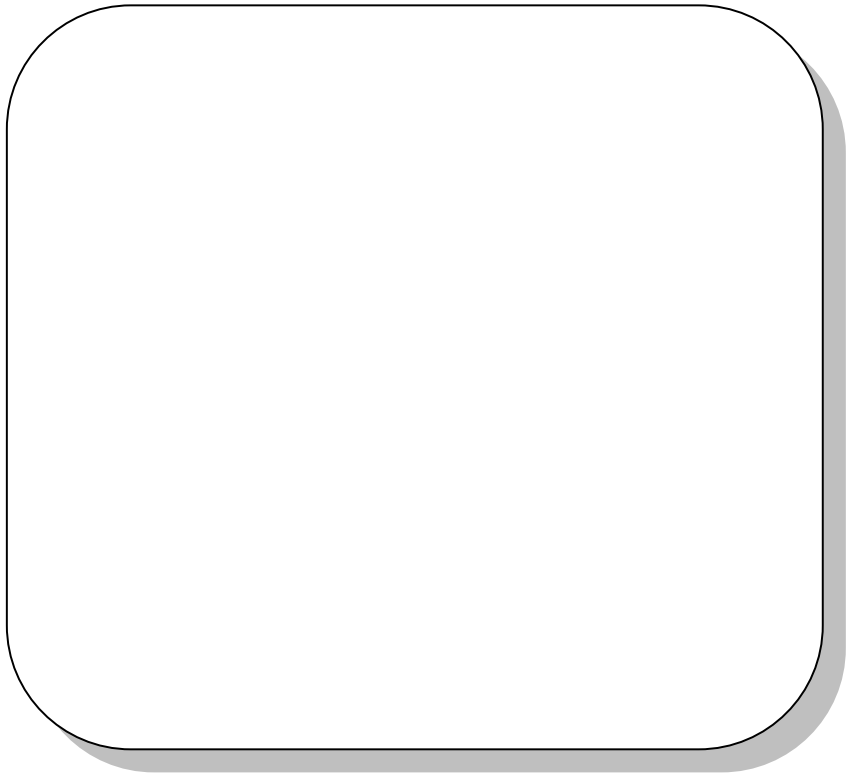
Designations:

1. Perichondrium.
2. Cartilage.
3. Chorndroblasts.
4. Chondrocytes.
5. Isogenous groups.
6. Extracellular substance.
7. Elastic fibers.

Name student _____

The preparation № 34: **Fibrocartilage of calf intervertebrate disk.**

Stain: H&E.



Designations:

1. Chondrocytes.
2. Isogenous group.
3. Extracellular substance.
4. Collagen fibers.

Signature of lecturer _____

Topic: Connective tissue. Bone.

The preparation № 35: **Diaphysis of a human long bone.**
Transverse section.
Schmorl stain.

A compact bone makes bone diaphysis. At a low power magnification find out periosteum, the outer, convex surface of circumferential lamellae and the inner, concave surface of endosteal lamellae, facing the marrow cavity. The surface formed by the endosteal lamellae is often more irregular than the surface formed by the circumferential lamellae. The space between these two sets of lamellae is filled by Haversian systems and interstitial lamellae. Only few of the Haversian systems are "textbook" circular. Osteocyte lacunae are visible between the lamellae. Canaliculi become visible at a high magnification.

Draw compact bone and mark in them:
Designations:

1. Periosteum.
2. Common external bone plates.
3. Common internal bone plates.
4. Osteons.
5. Osteon canal.
6. Intermediate plates.
7. Osteoblasts.
8. Osteocytes.

Name student _____ Signature of lecturer _____

Laboratory class

Date _____

Topic: Muscle tissue.

Objective: to be able to identify smooth and striated (skeletal and cardiac) muscle tissue on histological preparations.
Equipment: light microscopes, histological slides of different types of muscle tissue, handbook, tables.

The preparation № 36: **Striated skeletal muscle tissue of rabbit's tongue.**

Stain: *iron hematoxylin.*

View a slide at a low power magnification. Outside tongue is surrouded by mucosa membrane, covered by stratified squamose epithelium. The base of tongue is formed by striated skeletal muscles. Find out bundles of muscle fibers, cut longitudinally and transversally there. They are pale violet. Transversally cut bundles of muscle fibers are round and located beneath mucosa membrane. Bundles are separated by thick layers of loose connective tissue, especially expressed at the central part of section. Find out nuclei of loose connective tissue cells, blood vessels and adipocytes there. The latter resemble ring. View bundles of myocyte at a high power magnification, starting with those, sectioned longitudinally. They are cylindrical structures with many nuclei, oriented along the fibers. Pay attention on the fibers striation. Light strips are I-disks of myofibrils whereas dark strips are A-disks. Muscle fibers are separated by layers of loose connective tissue (endomysium). Find out nuclei of its cells there. Bundles of muscle fibers are united by abundant layers of loose connective tissue (perymisium). The latter, as mentioned above, contains a lot of adipocytes and blood vessels. At transverse section muscle fibers are round, oval or polygonal in shape. Pay especial attention to the nuclei location. They lie at the peripheral zone of sarcoplasm just beneath the sarcolemma. Find out endomysium between separate muscle fibers and perimisium, surrounded their bundles.

Draw some bundles of muscle fibers cut londitudinally and transversally and figure out:

The preparation № 37: **Myocardium of equine heart.**

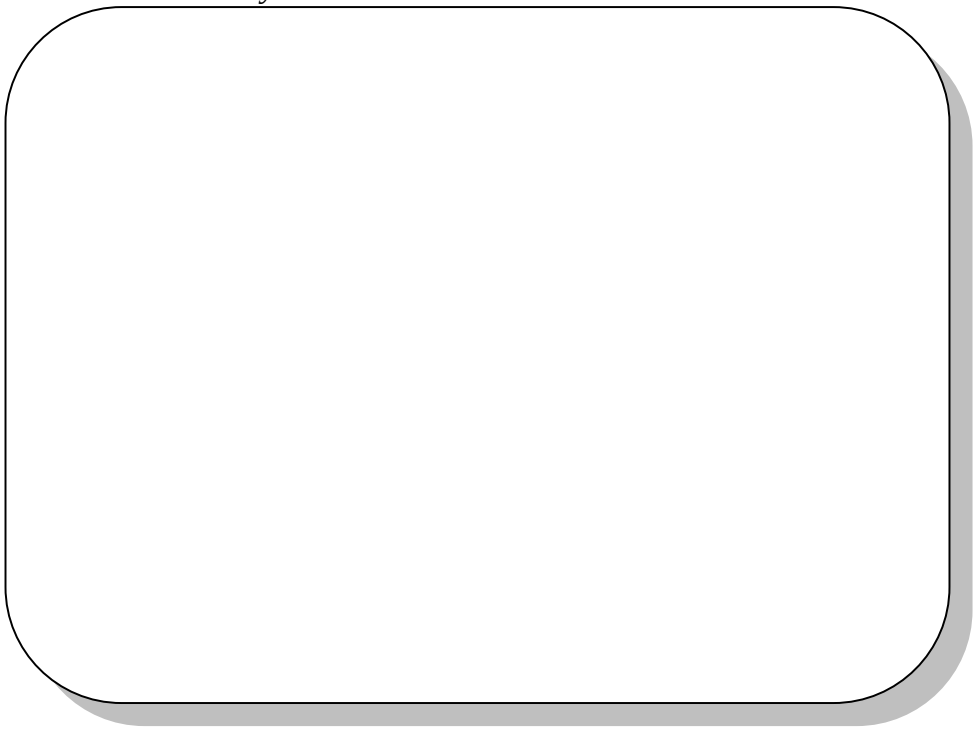
Stain: *iron hematoxylin.*

Myocardium is made of striated cardiac muscle tissue. It is divided into working (typical) and conductive (atypical). The preparation contains only a longitudinal section of working striated cardiac muscle. Using a low power magnification view cardiac muscle fibers. They are arranged in parallel and separated by fine layers of loose connective tissue (endomysium) with cells' nuclei and blood vessels. Using high power magnification find out intercalated discs, which are specialized connections between one cardiac muscle cell and another. They appear as thick dark strips, oriented transversally in cardiac muscle fibers. The latter are made by cardiac muscle cells, arranged in chain. Cardiac muscle cells are structural and functional units of striated cardiac muscle tissue. They are mononucleate. The oval nucleus is located centrally. Cardiac myocyte is striated like skeletal muscle. Find out anastomoses between cardiac myocytes.

Draw a slide and mark:

The preparation № 36: **Striated skeletal muscle tissue of rabbit's tongue.**

Stain: *iron hematoxylin.*



Designations:

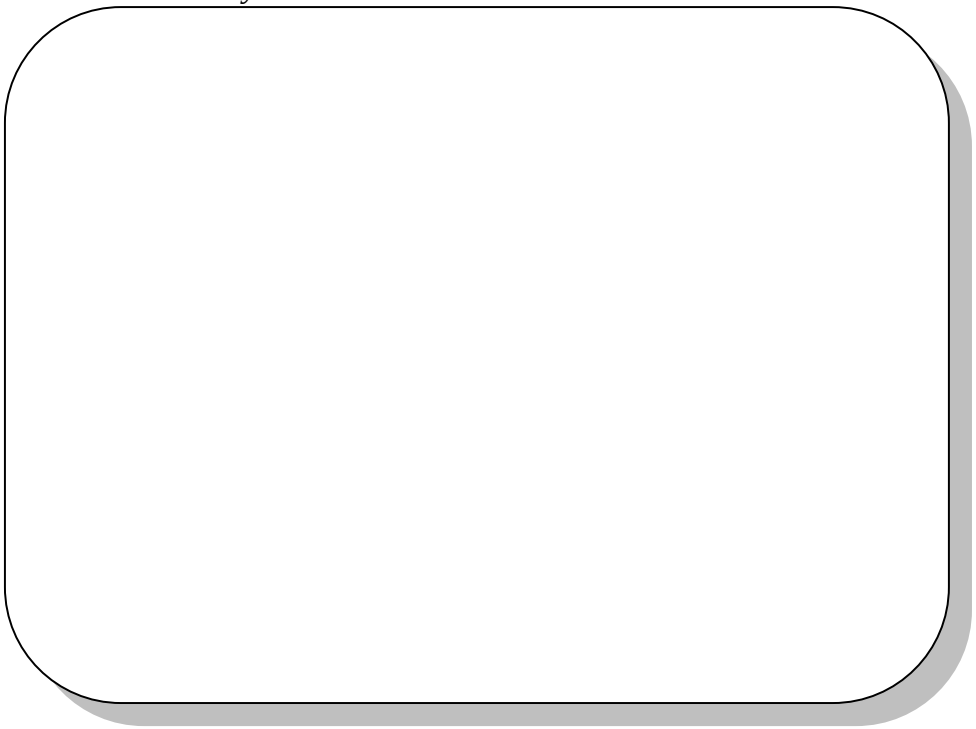
I – Longitudinal section.

II – Cross section.

1. Muscle fiber.
2. Sarcolemma.
3. Sarcoplasm.
4. Nuclei.
5. Myofibrils.
6. A-discs.
7. I-discs.
8. Cross strips.
9. Endomysium.
10. Perimisium.
11. Adipocytes.

The preparation № 37: **Myocardium of equine heart.**

Stain: *iron hematoxylin.*



Designations:

1. Muscle fibers.
2. Intercalated discs.
3. Cardiac muscle cell.
4. Anastomoses of fibers.
5. Nucleus.
6. Sarcoplasm.
7. Myofibrils.
8. A-discs.
9. I-discs.
10. Cross strips.
11. The layers of loose connective tissue.

The preparation № 38: **Smooth (non-striated) muscle tissue of urinary bladder wall**.

Stain: H&E.

Using a low power magnification view a slide and find out a muscle membrane, built by smooth muscle tissue. Indicate bundles of myocytes, cut transversally and longitudinally there. The first are round-shaped.

Using a high power magnification examine bundles of myocytes, beginning with their longitudinal section. They appear as long spindle-like cells with rod-like nuclei. These are myocytes. Find out fine layers of loose connective tissue – endomisium between separate myocytes. Groups of myocytes form a bundle surrounded by more extended layer of loose connective tissue. Find out nuclei of loose connective tissue cells and blood vessels there. Myocytes cut transversally are round to oval with centrally located nuclei. In some cells nuclei are not visible, because they were not at section site. Find out an endomisium between myocytes. Study a loose connective tissue, which surrounds bundles of myocytes.

Draw some bundles of myocytes cut transversally and longitudinally and figure out:

Designations:

I – Longitudinal section.
II – Cross section.

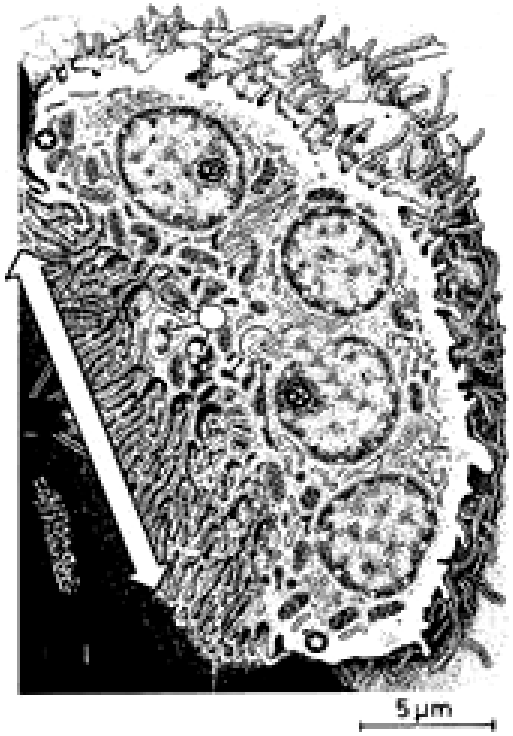
1. Myocyte.
2. Nucleus.
3. Sarcolemma.
4. Sarcoplasm.
5. Endomysium.

Name student _____

Signature of lecturer _____

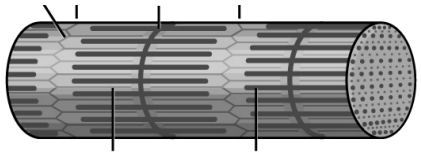
Homework
„Connective tissue. Muscle tissue.”

Schematic diagram of osteoclast.

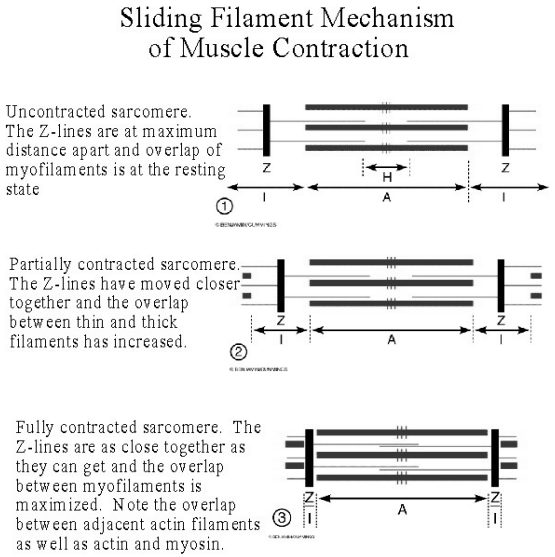


1 - nuclei,
2 - Golgi complex,
3 - lysosomes,
4 - area of destruction of bone.

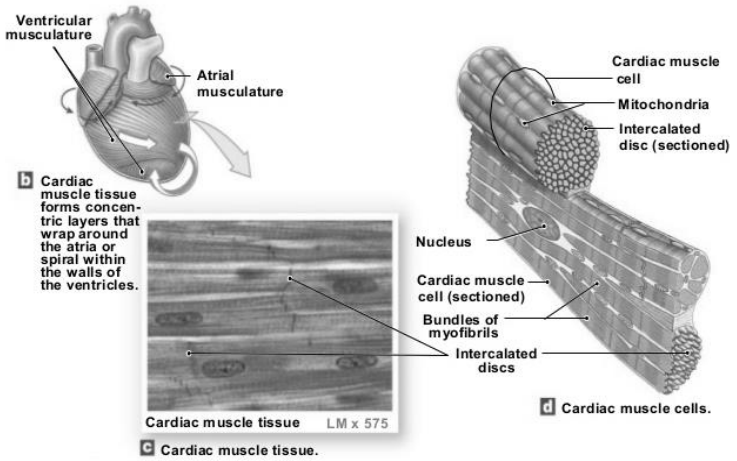
Diagram of contractile system of skeletal muscle fiber.

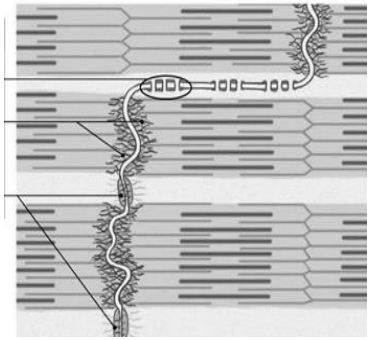


M - M-line;
Z - Z-iine;
A - anisotropic or A-band;
I - isotropic or I-band;
Sarcomere.



Schematic diagram of intercalated disk of cardiac muscle cell.





1 - gap junction,
2 - fascia adherens,
3 - desmosome.

Laboratory class

Date : _____

Topic: Nervous tissue.

Objective: to be able to identify neuronal cells and nerve fibers.
Equipment: light microscopes, histological slides of neuronal cells, histological slides of nerve fibers, handbook, tables.

The preparation № 39: **Spinal cord of dog.**

Stain: silver impregnation.

Using a low power magnification find out a gray matter of spinal cord. It is located centrally and resembles butterfly's wings. Find out brown stained neurons in the gray matter. View neurons at a high power magnification. Define perykaryon with pale nucleus and processes in a neuron. Type of processes cannot be defined at this preparation. Processes in some neurons are not visible or they are very short. Keep in mind, neurons of spinal cord are multypolar cells. During slide preparation they were not included into slide.

Draw a neuron and mark:

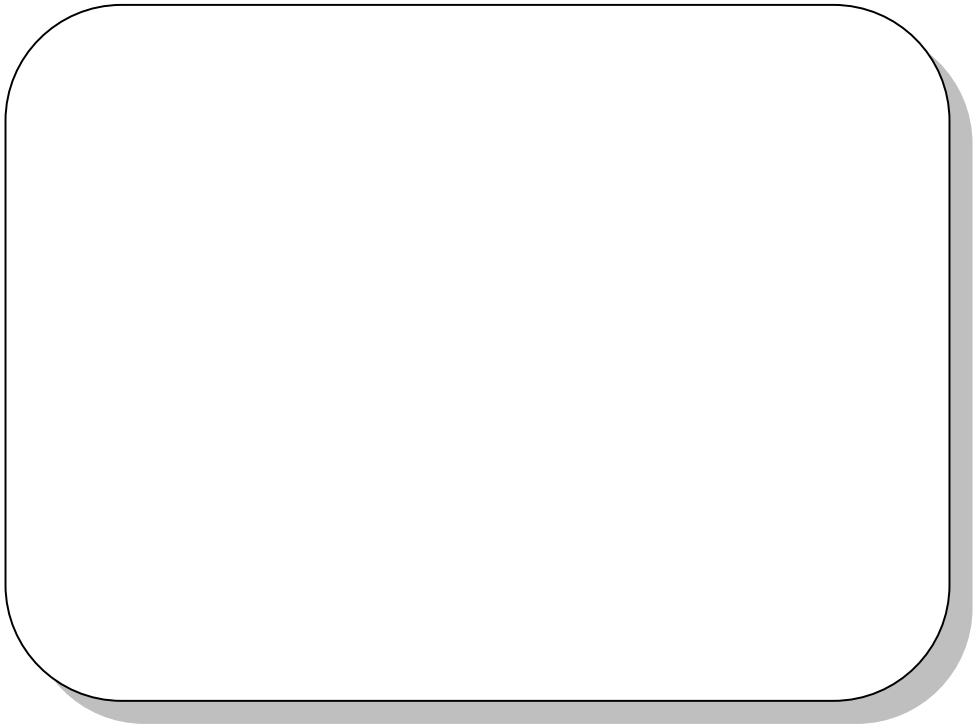
The preparation № 40: **Myelinated nerve fibers of nervus ischiaticus in frog.**

Stain: osmium acid and boric carmine stain.

At a low power magnification find out separated nerve fibers of gray-black color. View separate fiber at a high power magnification. It contains a continuous pale axial cylinder (axon) positioned centrally. Axon is incased in tough inelastic membrane (neurilemma) formed by Schwann cells (neurolemmal cells). The latter lay down myelin coating on axon. Myelin sheath is interrupted at regular intervals – node of Ranvier. The piece of nerve fiber between two nodes are called internodal segment, which corresponds to one neurolemmal cell. Thus, nodes represent sites, where two neurolemmal cells contact each other. Find out clefts in myelin coat. Outside from myelin layer there is a pale layer of neurilemma, which lacks myelin but contains nuclei of neurolemmal cells.

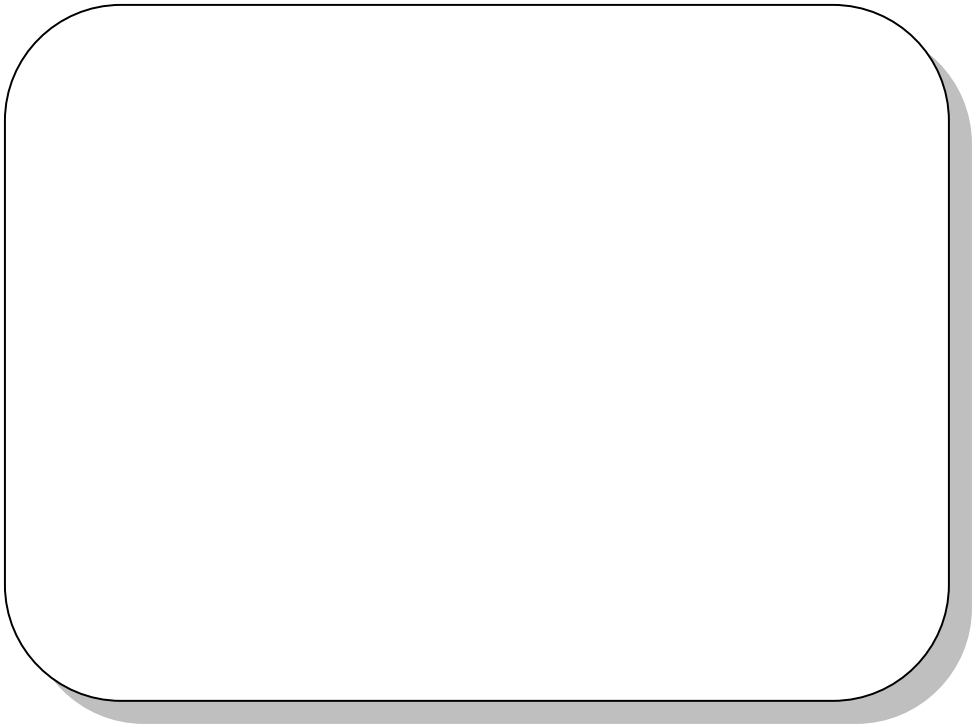
Draw some fibers and mark:

The preparation № 39: **Spinal cord of dog.**
Stain: silver impregnation.



Designations:
1. Perykarion.
2. Nucleus.
3. Nucleolus.
4. Neyrofibryls.
3. Processes.

The preparation № 40: **Myelinated nerve fibers of nervus ischiaticus in frog.**
Stain: osmium acid and boric carmine stain.



Designations:
1. Axis cylinder.
2. Myelin sheath.
3. Node of Ranvier.
4. Nuerilemma (internodal segment).
5. Nucleus od nuerolemmal cell (notches myelin).

The preparation № 41: **Unmyelinated nerve fibers of bovine spleen nerve.**

Stain: H&E.

Using a low power magnification view bundles of pink nerve fibers. Find out separated fibers and view them at a high power magnification. Pay attention to that separate fiber appears as a pale-pink ribbon and contains a chain of nuclei. Keep in mind you can see only the membrane of nerve fiber, which is made of nuerolemmal cells, arranged in chain. Their contacts (end to end) are invisible. Axial cylinders are deepened into neurolemmal cells cytoplasm.

Draw some fibers and mark:

The preparation № 42: **Myelinated nerve fibers. Transverse section.**

Stain: osmium acid stain.

Using a low power magnification find out a fascicle of nerve fibers round in shape and stained in brown-black color. View the fascicle at a high power magnification. Find out separate fibers, appearing as pale circles of different diameters, surrounded by dark strips. Pale circles are axial cylinders whereas dark strips represent myelinated sheath of nerve fiber. Nerve fibers are separated by a layer of loose connective tissue – endoneurium. Each fascicle is enclosed by a layer of loose connective tissue – perineurium.

Draw a fascicle of fibers and mark:

The preparation № 41: **Unmyelinated nerve fibers of bovine spleen nerve.**

Stain: H&E.

Designations:
1. Cytoplasm of neurolemmal cells.
2. Nuclei of neurolemmal cells.

The preparation № 42: **Myelinated nerve fibers. Transverse section.**

Stain: osmium acid stain.

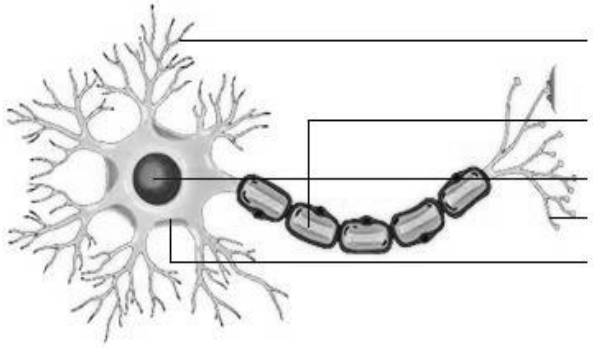
Designations:
1. Myelinated nerve fibers.
2. Unmyelinated nerve fibers.
3. Endonevrium.
4. Perinevrium.

Name student _____

Signature of lecturer _____

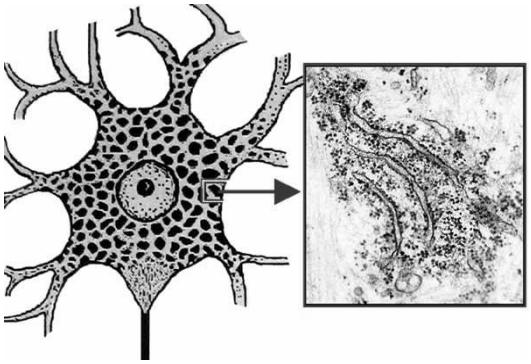
Homework
„Nervous tissue”

Structure of the neuron.

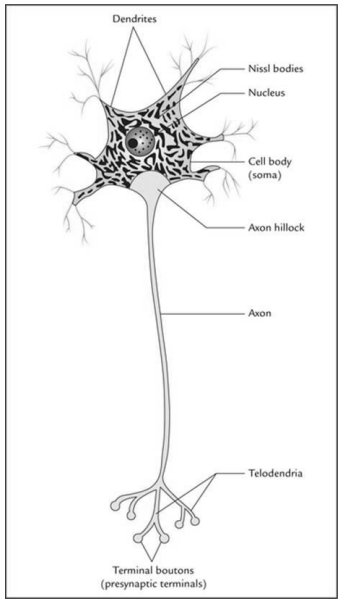


1 - nucleus,
2 - cell body,
3 - axon, myelin sheath,
4 - nerve ending,
5 - dendrites.

Nissl bodies in the neurons.



1 - nucleus,
2 - Nissl bodies.

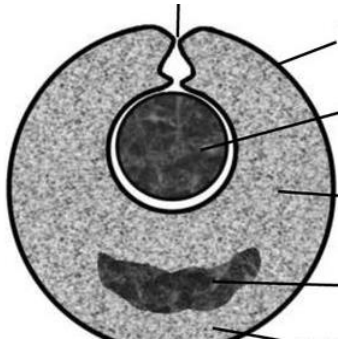


Main types of neurons.

I - bipolar,
II - pseudounipolar,
III - multipolar,

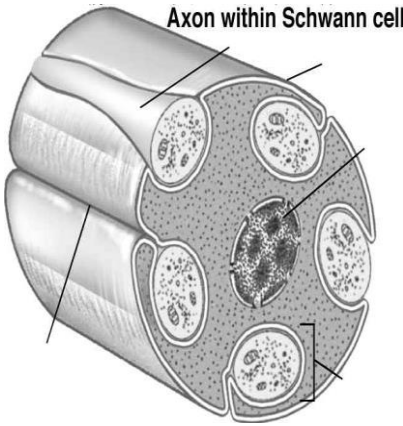
1 - axon,
2 - dendrite (dendrites).

Schematic diagram of the myelinated nerve fiber. Cross section.



1 - axial cylinder (axon),
2 - lemocyte (Schwann cell),
3 - plasmatic membrane of lemocyte,
4 - nucleus of Schwann cell,
5 - cytoplasm of Schwann cell,
6 - mesaxon.

Schematic diagram of the unmyelinated nerve fiber.



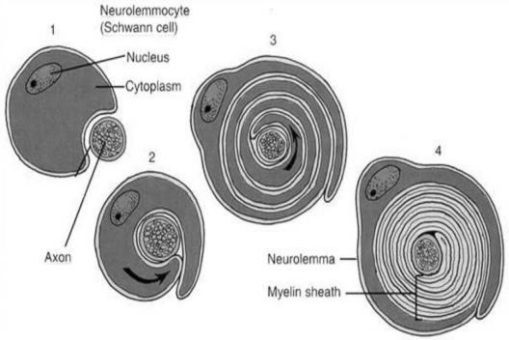
1 - cytoplasm of Schwann cell,

2 - nucleus of Schwann cell,

3 - cytolemma of Schwann cell,

4 - axial cylinders (axons),
5 - mesaxon.

Consecutive phases of myelin formation in nerve fibers.



1 - invagination of a single axon into Schwann cell,

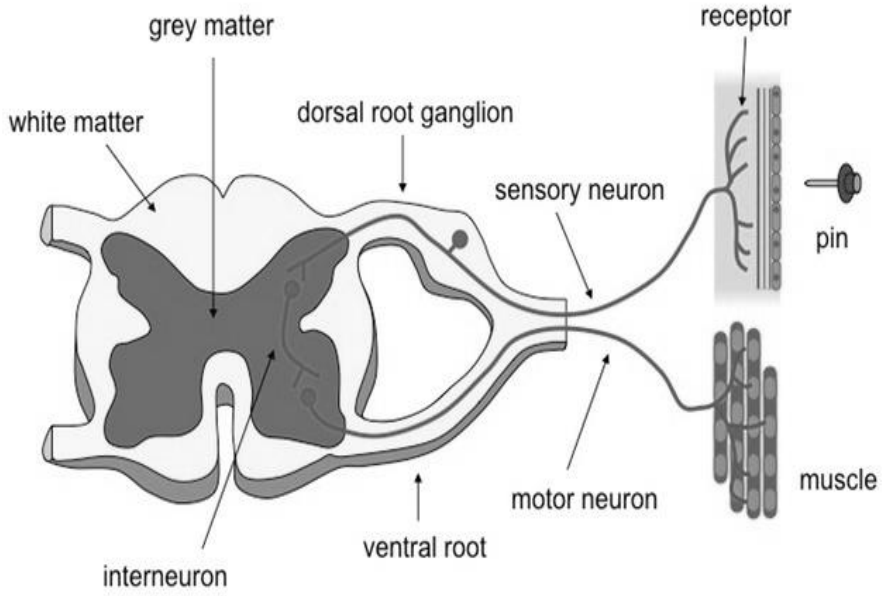
2 - formation of a mesaxon,

3 - rotation of the mesaxon around the axon,

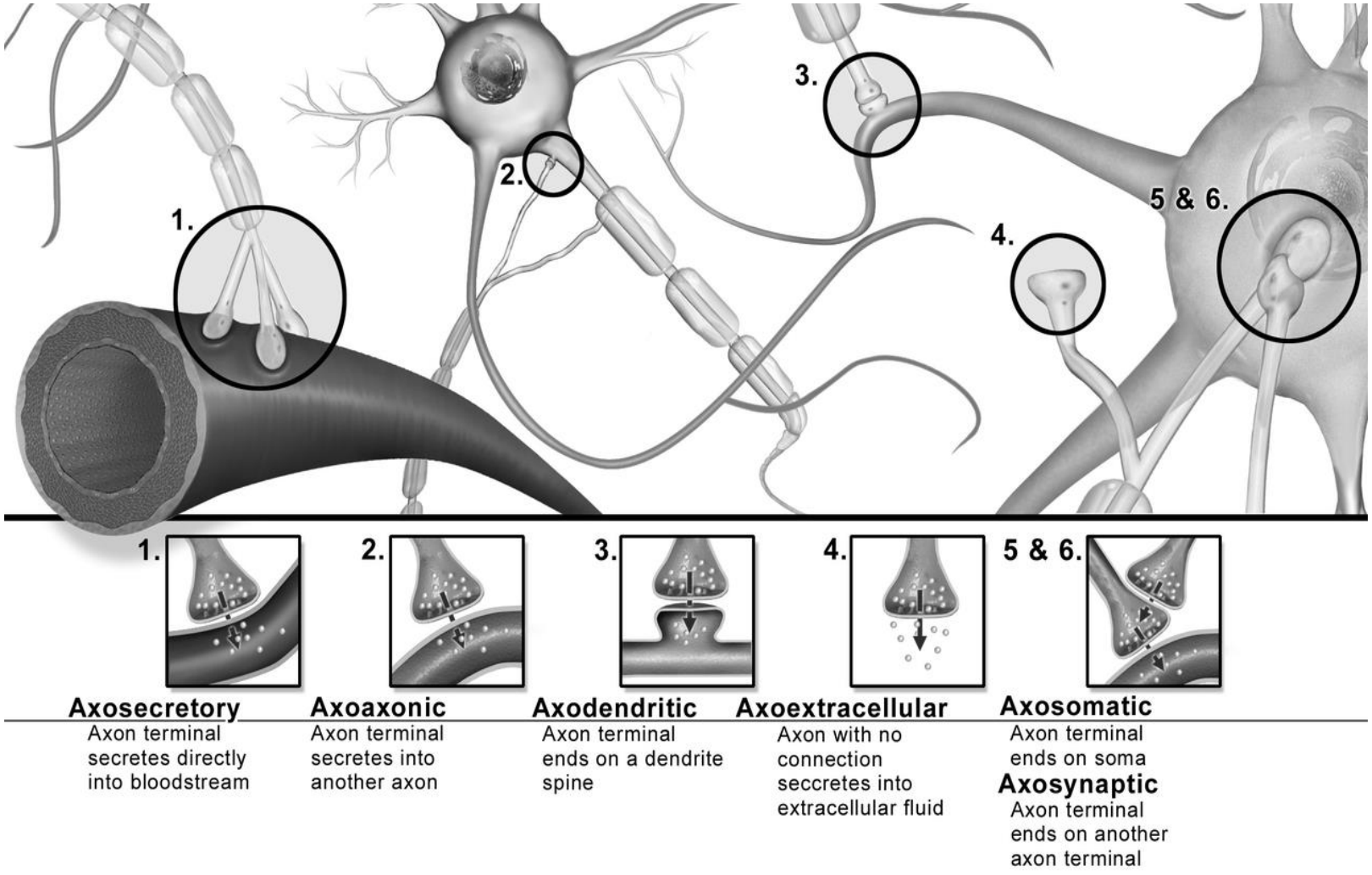
4 - enveloping the axon in concentric layers of Schwann cell cytoplasm and plasma membrane,

5 – mesaxon.

Reflex arc diagram.



Types of synapses.



Questions for self-control

1. What are the functions of epithelial tissue?
2. Characteristics of epithelial tissue.
3. Classification of epithelial tissue.
4. Surface epithelium.
5. Glandular epithelium.
6. Classification of surface epithelium.
7. Glands and criteria of their classification.
8. Secretion.
9. Regeneration of glandular epithelium.
10. Enumerate blood cells in mammals and birds.
11. Structural and functional characteristic of erythrocytes.
12. Classification of leucocytes and their characteristic.
13. Classification and function of granulocytes.
14. What cells do granulocytes include?
15. Structure, functions and classification of lymphocytes.
16. Structural and functional peculiarities of monocytes.
17. Leucocyte formula.
18. Structural and functional characteristics of platelets and thrombocytes.
19. Hematopoiesis.
20. Organization and functions of mesenchyme.
21. Organization and functions of reticular tissue.
22. Connective tissue cells.
23. What does the extracellular matrix of connective tissue consist of?
24. Organization of collagen and elastic fibers.
25. What varieties of dense connective tissue do you know?
26. Organization of a tendon.
27. Enumerate cells of cartilage.
28. Organization of extracellular matrix of cartilage.
29. Hyaline cartilage.
30. Elastic cartilage.
31. Fibrouse cartilage.
32. Features of bone.
33. Bone.
34. Extracellular matrix of bone.
35. Types of bone. Peculiarities of their organization.
36. Organization of diaphysis of long bone.
37. Classification of muscle tissue
38. Smooth muscle tissue. Organization of myocyte.
39. Ultramicroscopic organization of myofilamet.
40. Striated skeletal muscle tissue. Organization of muscle fiber.
41. Ultramicroscopic organization of myofibrils.
42. Mechanism of muscle fibers contraction.
43. Striated working cardiac muscle tissue.
44. Organization of cardiac myocytes.
45. What is the nervous tissue composed of?
46. Organization of neuron.
47. Classification of neurons.
48. Chromatophilic substance of neurons. What is it made of?
49. Synapses.
50. Organization of chemical synapse.
51. Neuroglia.
52. Macroglia.
53. Microglia.
54. What is the nerve fiber consist of?
55. Classification of nerve fibers.
56. Organization of myelinated nerve fiber.
57. Organization of unmyelinated nerve fiber.
58. What is nerve ending?
59. Classification of nerve endings.
60. Sensory nerve endings
61. Effector (motor) nerve endings.