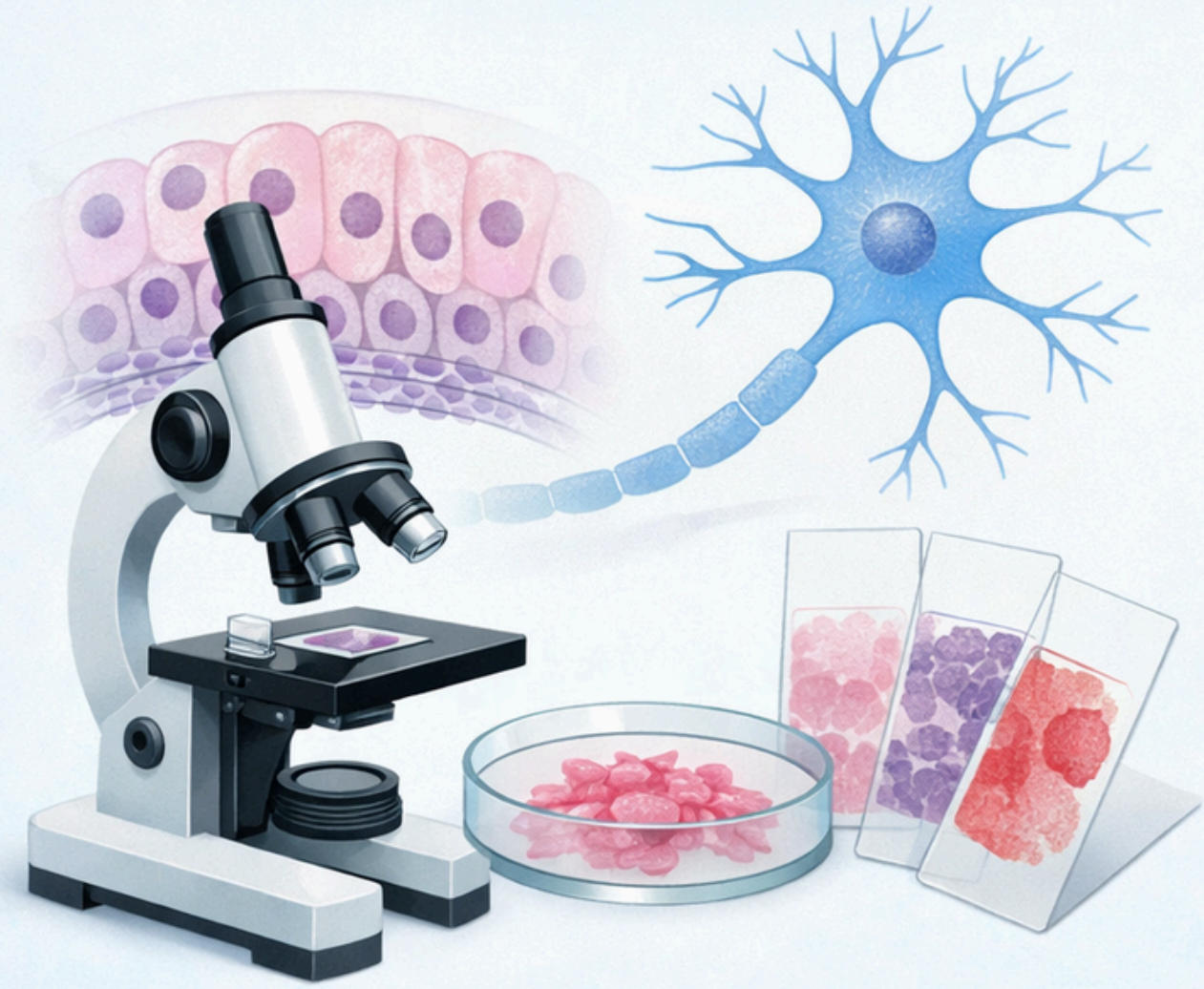
HISTOLOGICAL WORKBOOK. PART I.

Methodological Developments For Laboratory Classes



Erica Dobryanska

Erica Dobryanska

HISTOLOGICAL WORKBOOK. PART I.

Methodological Developments For Laboratory Classes

Monograph

RS Global
Warsaw, Poland
2025

DOI: 10.31435/rsglobal/064

Methodological developments have been recommended to the edition at the meeting of "Department of Basic Medical Disciplines (protocol №3 from 07.11.2019)

Reviewers:
Professor Koval Halyna Mykolayivna, *Doctor of Medical Sciences, Professor, Head of the Department of Microbiology, Virology and Epidemiology of with a course in Infectious Diseases, State Higher Educational Institution "Uzhgorod National University", Uzhgorod, Ukraine.*
ORCID ID: 0000-0003-4391-2950
Assistance Professor Rostoka Larisa Mykhailivna, *Candidate of Medical Sciences, Associate Professor, Head of the Department of Biochemistry and Pharmacology, State Higher Educational Institution "Uzhgorod National University", Uzhgorod, Ukraine*
ORCID ID: 0000-0001-9951-6135.

Erica Dobryanska. (2025). *Histological Workbook. Part I. Methodological Developments for Laboratory Classes.* RS Global Sp. z O.O.

ISBN 978-83-970624-6-7 (eBook)

This text contains the concise thorough presentation of Cytology, Embryology, General and Special Histology, based on modem information of functional morphology of cells, tissues, different organs and systems. This text was created on the basis of the systematized lecture course on Histology, Cytology and Embryology which is delivered at the Histology, Cytology and Embryology Department of State institution for the students of the Faculties of Medicine. Edition is oriented to the effective learning or revision of course of Cytology, Embryology, General and Special Histology and meant for the students in the health professions and advanced undergraduates.

ISBN 978-83-970624-6-7 (eBook)

TABLE OF CONTENTS

MODULE 1

CYTOLOGY AND GENERAL EMBRYOLOGY. GENERAL HISTOLOGY

Criteria for evaluation of knowledge and skills students

Module 1 «Cytology and general embryology. General histology»		
1	2	3
Practical lessons (№№ 1-13, 15-35)	Points	Criteria
	5	The theoretical content of the topic lessons learned perfectly, all the necessary practical skills with lessons mastered the material, provided all program objectives in the album performed better, the quality of their estimated maximum possible score.
	4	The theoretical content of the topic lessons learned in full with no gaps, all the necessary practical skills with lessons mastered the material, provided all program objectives in the album done, the quality of their performance rated a score close to maximum.
	3	The theoretical content of the topic classes mostly learned, without significant gaps, some practical skills learned enough material formed, provided all program objectives in the album done, the quality of any of them have not been evaluated maximum score, some tasks performed correctly.
	2	The theoretical content of the topic lessons learned in part, the gaps have significant character, the necessary practical skills learned material is mainly formed by the majority provided by the tasks performed on the album, some of the assignments contain errors.
	1	The theoretical content of the topic lessons learned in part, the gaps are substantial in nature, some practical skills are not formed, many provided by the tasks in the album are not met, or the quality of their performance rated a score close to the minimum.
	0	The theoretical content of the topic lessons not learned the necessary practical skills are not formed, most of the tasks under the program in the album is not performed or the quality of their performance rated a score close to the minimum, all tasks performed on the album contain gross errors, or generally not enforced (no album).
Concluding lessons of the module 1 and 2 (№№ 8, 20)	10	Answer theoretical questions impeccable (2 points), test tasks performed at 100% (20 tests of 0.3 points = 6 points), diagnosis of histological preparations and electron microphotograph performed flawlessly, the quality of reports describing their estimated maximal score (score 1).
	8, 9	Answer theoretical questions are very accurate (1.6 points), test tasks performed on 80-90% (16-19 tests on 0.3 points = 4.8-5.7 points), diagnosis of histological preparations and electron microphotograph done correctly, the quality of reports describing their estimated score, close to the maximum (by 0.8 points).
	6, 7	Answer theoretical questions basically accurate (1.2 points), test tasks performed on 70-80% (14-16 tests on 0.3 points = 4.2-4.8 points), diagnosis of histological preparations and electron microphotograph done with some errors (by 0.6 points) the quality of any description of the protocols have not been evaluated in very well and above.
	4, 5	Answer theoretical questions are not accurate (0.8 points), test tasks performed on 60-70% (12-14 tests on 0.3 points = 3.6-4.2 points), diagnosis of histological preparations and electron microphotograph carried out with errors (by 0.4), the quality of any describing the protocols have not been evaluated in well and above.
	3	Answer theoretical questions with errors (0.2 points), test tasks performed on 50% (10 tests on 0.3 points = 3.0 points), diagnosis of histological preparations and electron microphotograph carried out with considerable errors (by 0.2 points) or inaccurate, the quality of any of the protocols descriptions have not been evaluated on a satisfactory or higher.

	2	The answer to your question is not theoretical, test tasks performed at least 50% (8 and less tests, up to 2 points), diagnosis of histological preparations and electron microphotograph performed inaccurately, with gross errors, the quality of any description of the protocols have not been evaluated in sufficient and higher.
	0, 1	Theoretical knowledge is missing, test tasks performed at least 40% (6 and less tests), diagnosis of histological preparations and electron microphotograph not done, no reports describe them.
Final module control (practical part)	40	Answers to tests of unmistakable and assessed the maximum possible score (40 x 0.5 points = 20 points), two reports describe the histological preparations and 2 electronic microphotograph meet the standard and the estimated maximum possible score (4 x 5 points = 20 points).
	37-39	Answer to the questions most accurate tests and evaluated close to the maximum score (18-19,5 points), two reports describe the histological preparations and 2 electronic microphotograph close to the standard and total assessed close to the maximum score (4) or / and above (5 points).
	33-36	Answers to the most accurate tests and assessed a high score (16-18 points), two reports describe the histological preparations and 2 electronic microphotograph with irrelevant comments and appreciated mediocre score (3) or / and above (4.5).
	29-32	Answers to the test task is not always accurate and assessed mediocre score (14-16 points), two reports describe the histological preparations and 2 electronic microphotograph with significant inaccuracies and evaluated a low score (2) and / or higher (3,4,5).
	25-28	Answers to tests of inaccurate and evaluated a low score (12-14 points), two reports describe the histological preparations and 2 electronic microphotograph with significant inaccuracies and evaluated a low score (1) or / and above (2,3,4,5).
	11-24	Answers to the test tasks in the most false and evaluated below the passing score (6-12 points), two reports describe the histological preparations and 2 electronic microphotograph with significant inaccuracy (or error) and estimated scores from 0 to 2.
	0-10	Answers to the test tasks in the most erroneous, assessed the lowest score (less than 6) or absent, two reports describing the histological preparations and 2 electronic microphotograph false, assessed the lowest scores (0-1) or absent, or the student refused to perform the task.
Final module control (Theoretical part)	40	Theoretical Answers (4) on test questions perfect, estimated the maximum possible score (4 x 10 points = 40 points), all necessary program sections mastered better, with answers widely used abstract lectures and supplementary material.
	37-39	The theoretical answer to the question best, contain small errors, evaluated close to maximum points (8 and above), provided all program sections discipline mastered very well, with answers applying abstract lectures and supplementary material.
	33-36	The theoretical answer to the questions are good, with some inaccuracies, mostly assessed by high scores (7 and above), provided all program sections discipline mastered equally well, with answers applying abstract lectures.
	29-32	The theoretical answer to the question of errors, evaluated mainly mediocre scores (6 and above), provided the individual program sections discipline mastered uneven, with answers trying to apply abstract lectures.
	25-28	The theoretical answer to the question of substantial errors, mainly low valued points (5 and above), provided all program sections discipline mastered unevenly, with the answer does not apply additional material.
	16-24	The theoretical answer to the question of gross errors, evaluated mainly low scores (4 and above), provided the individual program sections discipline is mastered, the student is not focused on basic concepts or refuses to comply.
	0-15	The theoretical answer to the question incorrect, missing or student refuses to comply, the lowest estimated scores (0-4) provided the program sections discipline is mastered, the student does not have abstracts of lectures.

Before drawing final control module with an admitted students who have fulfilled all the missed lectures, practical classes, protocols drawn up independent work and took at least 57 points for the assessment of current performance, including no less than 6 points after the final control of the content modules 1 and 2. Before drawing final control module with two admitted students who have fulfilled all the missed lectures, practical classes, protocols drawn up independent work and took at least 59 points for the assessment of current performance, including no less than 4 points after the final control of the content modules 3 and 4

Scale of assessments for students

Scores ECTS	statistic
A B C D E	Top 10% students Next 25% of students Next 30% of students Next 25% of students The last 25% of students
FX F	Re-exam Mandatory re-training

Grading scale in Ukraine and its compliance with ECTS:
5 (excellent) - A
4 (good) - B, C
3 (satisfactory) - D, E
2 (Poor) - FX, F

THEMATIC PLAN OF LECTURES

№	Theme	hours
1.	The scope of histology. Cytology.	2
2.	Comparing embryology. General human embryology.	2
3.	Classification of tissues. Epithelial tissue.	2
4.	The blood and lymph.	2
5.	General connective tissue.	2
6.	Sceletal tissues. Bone.	2
7.	Muscular tissues.	2
8.	Nervous tissue.	2
9.	Nervous system.	2
10.	Nervous endings.	2
	In all	20

THEMATIC PLAN OF PRACTICAL TRAINING

№	Theme	hours
1	2	3
Semantic module 1. «FUNDAMENTALS OF CYTOLOGY AND EMBRYOLOGY. HISTOLOGY OF GENERAL AND SPECIAL TISSUES».		
1.	Microscope. Methods of histologic investigation.	2
2.	Cytology. General cellular structure.Cell membrane.	2
3.	Cytology. Cytoplasm structure. Membranes organelles of the cell. Non-membranes organelles of the cell. Inclusion. Cytoskeleton.	
4.	Cytology. Nucleus components. Cell reproduction. Aging and death of cell.	2
5.	The control of mastering of the semantic module 1. «FUNDAMENTALS OF CYTOLOGY».	2
6.	Comparing embryology. Germ cells. Fertilization. Cleavage. Blastula.	
7.	Comparing embryology. Gastrulations. Mesoderm differentiation. Germ layers. Their significance. Formation the axial germ organs and extraembrionic membranes.	2
8.-9.	Human embryology. Gametogenesis. Germ cells. Fertilization.Cleavage. Blastocysta. Human embryology. Implantation and placentation. Gastrulations. The axial germ organs and extra embrionic membrane.	4
10.	The control of mastering of the semantic module 2. «FUNDAMENTALS OF EMBRYOLOGY».	2
11.	An introduction to general histology. Epithelial tissue. Glandular epithelium. Glands.	2
12.	The blood of human and animals. Erythrocytes, platelets. Leucocytes. Hemogramms. Leukocytic formula. Age differents of blood. Lymph.	2
13.	Mesenchyme. Loose connective tissue	2
14.	Dense regular connective tissue. Cartilage.	3
15.	Bone tissue.	2
16.	The control of mastering of the semantic module 3. « HISTOLOGY OF GENERAL TISSUES».	2
17.	Muscle tissue.	2
18.	Nervous tissue. Neuron. Neuroglia.	2
19.	Nervous tissue. Nerve fibres.	2
20.	Nerve endings. Synapse.	2
21.	Spinal ganglion. Nerve.	2
22.	The control of mastering of the semantic module 2 «HISTOLOGY OF SPECIAL TISSUES».	2
23.-24.	THE FINAL CONTROL OF MASTERING OF THE MODULE 1 «FUNDAMENTALS OF CYTOLOGY AND EMBRYOLOGY. HISTOLOGY OF GENERAL AND SPECIAL TISSUES». Theoretical part.	4
	In all	48

Tasks for students' independent work.

№	Theme	hours
1.	Microscope. Methods of histologic investigation.	2
2.	Cytology. General cellular structure. Cell membrane.	2
3.	Cytology. General cellular structure. Cell membrane. Cytoplasm.	4
4.	Cytology. Nucleus components. Cell reproduction. Aging and death of cell.	2
5.	Embryogenesis lantsetnyka and lower vertebrates.	2
6.	General embryology. Embryonic development of vertebrates, the lower and higher vertebrates.	4
7.	Human embryology. Structure and function of amnion, chorion, placenta and umbilical cord.	4
8.	Epithelial tissue.	2
9.	Blood. Lymph.	4
10.	Connective tissue.	4
11.	Cartilage bone tissue.	4
12.	Muscle tissue.	4
13.	Nervous tissue.	4
14.	Preparing for the final control module 1.	6
	In all	48

The distribution of points on the topics and types of control.

	Scores(max)	
	one lessons	In all
1	2	3
Module 1.		
Semantic module 1		
Practical lessons №№ 1-7	5	35
The control of mastering of the semantic module № 8	10	10
Semantic module 2		
Practical lessons №№ 9-18	5	55
The control of mastering of the semantic module № 20	10	10
Students' independent work.		10
THE FINAL CONTROL OF MASTERING OF THE MODULE 1	80	80
In all		200

MODULE 1. TEST ON TOPIC "CYTOLOGY AND EMBRYOLOGY"

Programm questions to the test

1. Main components of the cell. Structure and functions of the cellular membrane.
2. Cell cytoplasm: structure and functions.
3. Cell cytoplasm components. Membrane organells (characteristic features).
4. Cell cytoplasm components. Nonmembrane organells (characteristic features).
5.Cell cytoplasm components. Hyaloplasm and inclusions: their structure and functions.
6. Nuclear components: structure and functions.
7. Components of the nucleus. Karyolemma (structure and functions).
8. Chromosomes: structure, types, karyotype, functions.
9. Nuclear chromatine (chemical structure and significance).
10. Cells reproduction. Characteristic features of different types of reproduction.
11. The cell cycle: periods, their characteristic features.
12. The cell cycle. Interphase: periods, their significance.
13. Cell death.
14. Periods of human embryogeny. Characteristic features of the embriogenesis stages.
15. Germ cells: their differences compared to somatic ones. Spermatozoon: structure and functions. Oocyte: structure and functions.
16. Fertilization: characteristic features.
17. Connection of the embryo with a maternal organism. Implantation. Placenta.
18. Gastrulation. Germ layers. Their significance.
19. Ectoderm and entoderm differentiation.
20. Mesoderm differentiation.
21. Critical periods of the embriogeny. Endo- and exogenic factors, which influence on its development.
22. Tissue. What is this?
23. Tissues classification.
24. Epithelial tissue: origin, localisation, structure and functions.
25. Epithelium morphofunctional classification.
26. Structural peculiarities of different kinds of epithelium.
27. Glandular epithelium. Secretory cycle.
28. Classification of glands.
29. Types of . secretion.
30. Blood components, functions.
31. Chemical components of plasma.
32. Erythrocytes: size, structure and functions. Anisocytosis. Poikilocytosis.
33. Leucocytes: classification, structure and functions. Leucocytosis. Leucopenia.
34. Granulocytes (neutrophils, eosinophils, basophils) and agranulocytes (lymphocytes, monocytes): structure and functions.
35. Hemogram. Clinical significance.
36. Connective tissues: origin, disposition, structure and functions.
37. Connective tissues: classification.
38. Loose connective tissue: structure of cells, functions.
39. Dense connective tissue (regular, irregular): structure and functions.
40. Connective tissue with special properties (mucous, adipous, reticular, pigmental): their structure and functions.
41. The general features of chondroid tissue.
42. Classification and functions of cartilages.
43. Morphofunctional characteristic of cartilages.
44. The structure and functions of perichondrium.
45. Hyaline cartilage: structure and functions.

46. Elastic cartilage: structure and functions.
47. Fibrocartilage structure and functions.
48. Diffferent types of cartilages growth.
49. The general features of bony tissue.
50. Bony tissue: types, particularities of structure and localisation.
51. Morphofunctional characteristic of bone cells (osteoblasts, osteocytes, osteoclasts).
52. Osteon as a morphofunctional unite of compact bone. Structure of the dyaphysis.
53. Growth and regeneration of bones.
54. Morphofunctional and phylogenetic classification of muscle tissue.
55. Smooth muscle: structure, localisation and functions.
56. Sceletal muscle: structure, localisation and functions.
57. Cardiac muscle: structure, localisation and functions.
58. Regeneration of muscular tissue.
59. Nervous tissue: structure and functions.
60. Morphofunctional classification of neurocytes.
61. Neuroglia: classification, structure and functions.
62. Unmyelinated fibres: structure and functions.
63. Myelinated fibres: structure and functions.
64. Classification of nervous endings.
65. Receptors: classification, structure and functions.
66. Synapses: classification, structure and functions.
67. Effectors: classification, structure and functions.
68. Spinal cord: general morphofunctional characteristic. Grey matter (nuclei and their cells compounds). Gliocytes.
69. Peripheral nerve system. Regeneration of nerve after the damage. Simple and complex somatic reflex arc, principal compounds.
70. Morphological and functional peculiarities of spinal node: sensory neurons and neuroglial compounds.
71. Autonomic (vegetative) nerve system: structural peculiarities. Vegetative ganglia: cellular structure and disposition. Reflex arc: special features

THE LIST OF SPECIMENS

1. Plant cells
2. Animal cells
3. Golgi apparatus
4. Mitochondria
5. Lipid inclusions in hepatosutes
6. Glycogen inclusions in hepatosutes
7. Pigmentocytes
8. Mitosis of the plant cells
9. Amitosis
10. Spermatozoa
11. Oocyte of Graafian follicle of the cat ovarium
12. Blastula of amphibian
13. Embryo at the stage of early development
14. Embryo at the stage of latter development. Trunk fold and amniotic fold
15. Ascarides fertilized oocyte.
16. Human placenta. Fetal part.
17. Maternal part of placenta
18. Umbilical cord
19. Amnion

20.Allantois
21. Mesothelium of the peritoneum.
22. Epidermis (fingertip skin).
23. Sebaceous gland of the skin.
24. Blood smear.
25. Loose connective tissue.
26. Tendon.
27. White adipose tissue.
28. Hyaline cartilage.
29. Elastic cartilage.
30. Fibrocartilage.
31. Lamellar bone.
32. Intramembranous bone formation.
33. Intracartilaginous bone formation.
34. Smooth muscle.
35. Sceletal muscle.
36. Cardiac muscle.
37. Chromatophilic substance (Nissl substance).
38. Neurofibrils in neurons.
39. Pseudounipolar neurons.
40. Myelinated fibers.
41. Unmyelinated fibers.
42. Lamellar (Fatter-Pacinian) corpuscle.
43. Spinal cord.
44. Spinal node.
45. Nerve (transverse section in human skin).
46. Intramural nerve plexus
Students should be able to indicate elements in the electron micrographs:
From fig.1 to fig.82.

REFERENCES

1. Luis Carlos Junqueira, Jose Carneiro, Robert O.Kelley: Basic Histology. 7th ed. Stamford, Connecticut, 1992, p.p.
2. Maximov A.A., and Bloom W.: A Textbook of Histology. 7th ed. Philadelphia, London, Saunders Co., 1961, pp.
3. Bloom W. and Fawcett W.: A Textbook of Histology. 9th ed. Philadelphia, London, Toronto, Saunders Co., 1968, pp.
4. Wheater P.R., Burkitt H.Y., Daniels V.Y.: Functional Histology: Text and Colour Atlas. 2nd ed. Edinburg, London, Melburn, New York, 1987, pp.
5. Johanes A.Y., and Rhodin M.D.: An Atlas of Ultrastructure. Philadelphia, London, Saunders Co., 1963, pp.

HISTOLOGY AND ITS METHOD OF STUDY

Histology is the study of the microscopic anatomy of cells an tissues.

Histology is an essential tool of biology and medicine.

Cytology is a science which is about the structure and functions of cells and their derivatives, their reproduction and interactions.

General histology examines the composition of each of the tissue types, including the nature of its cells and extracellular matrix.

Special histology is a science which is about the structure of organ systems.

Embryology is a science which is about the development of an embryo from the fertilization of the ovum to the fetal stage.

The cell theory refers to the idea that cells are the basic unit of structure in every living thing. Development of this theory during the mid 17th century was made possible by advances in microscopy. This theory is one of the foundations of biology. Credit for developing cell theory is usually given to three scientists: Theodor Schwann, Matthias Jakob Schleiden, and Rudolf Virchow.

Cell theory states:

1. that the cell is the basic unit of living systems;
2. that all organisms consist of at least one cell;
3. that cells in multicellular organisms are often specialized;
4. that all cells come from previous cells.

The cell is the structural and functional unit of the organism. Except for cells, in an organism there are their derivative, which have no the cellular structure (intercellular matrix, postcellular structures, symplast, syncytium).

Extracellular matrix (ECM) is produced by cells and excreted to the extracellular space within the tissues, serving as a scaffolding to hold tissues together and helping to determine their characteristics:

1. viscosity (gelatinization);
2. acidity (pH);
3. dynamic (sol ↔gel);
4. potential.

Postcellular structures are derivatives of cells which during a differentiation (more often owing to loss of a nucleus and a part of organelles) have lost the major signs of cells, but have got a number of the properties necessary for execution by them the specialized functions. The postcellular structures at human are erythrocytes, platelets, horny cells of epidermis.

Symplasts are the structures formed as a result of cell fusion with loss of their borders and formation uniform cytoplasmic mass in which there are nucleuses. Symplasts are osteoclasts of bone, an external layer of trophoblast, and fibers of a skeletal muscular tissue.

Syncytium is the structure arising owing to incomplete cytotomia at cell division with preservation of connection between elements of cells by means of cytoplasmic bridges (seminiferous epithelium in the seminiferous tubules of testis).

Overview of methods used in histology

Modern histology has the wide arsenal of various methods of research.

All these methods are connected by the requirement of application of the special device - microscope, and all of they are microscopic methods.

Light microscopy

Conventional light, phase contrast, polarizing, confocal, and fluorescence microscopy are all based on the interaction of photons and tissue components.

With the light microscope, stained preparations are usually examined by transillumination. The microscope is composed of both mechanical and optical parts (fig. 1).

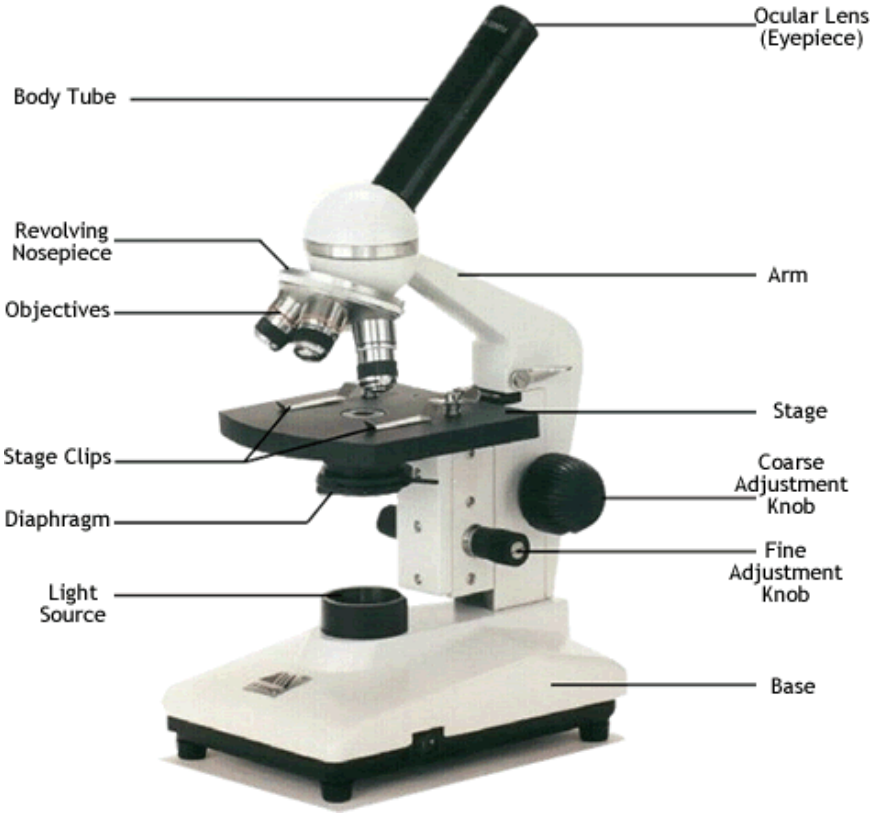


Figure 1. Parts of light microscope.

The optical components consist of three systems of lenses: condenser, objective, and ocular. The condenser collects and focuses the illumination to produce a cone of light that illuminates the object to be observed. The objective lens enlarges and projects the illuminated image of the object in the direction of the ocular lens. The ocular lens (eyepiece) further magnifies this image and projects it onto the viewer's retina or a photographic plate. The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses.

The critical factor in obtaining a crisp, detailed image with the microscope is its resolving power, that is, the smallest distance between two particles at which they can be seen as separate objects. The maximal resolving power of the light microscope is around 0.1 pm; this permits good images magnified 1000-1500 times. Objects smaller than 0.1 pm cannot be distinguished with this instrument

The quality of the image - its clarity and richness of detail - depends on the microscope's resolving power. The magnification is independent of its resolving power and is of value only when accompanied by high resolution. The resolving power of a microscope depends mainly on the quality of its objective lens. The ocular lens only enlarges the image obtained by the objective; it does not improve resolution.

Phase contrast microscopy.Unstained biologic specimens are usually transparent and difficult to view in detail, since all parts of the specimen have almost the same optical density. Phase contrast microscopy, however, uses a lens system that produces visible images from transparent objects.

The principle of phase contrast microscopy is based on the fact that light changes its speed and direction when passing through cellular and extracellular structures with different refractive indices. These changes cause the structures to appear lighter or darker relative to each other. Differential interference optics produces an apparently three- dimensional image of living cells and tissues.

Polarizing microscopy.When normal light passes through a polarizing filter, it exits vibrating in only one direction. If a second filter is placed in the microscope above the first one, with its main axis perpendicular to the first filter, no light passes through, resulting in a dark field effect. If, however, tissue structures containing oriented molecules (such as cellulose, collagen, micro-

tubules, and microfilaments) are located between the two Polaroid filters, their repetitive, oriented molecular structure allows them to rotate the axis of the light emerging from the polarizer. Consequently, they appear as bright structures against a dark background. The ability to rotate the direction of vibration of polarized light is called birefringence and is present in crystalline substances or substances containing oriented molecules.

Confocal microscopy. This type of microscopy uses lasers and computers to produce three-dimensional images of living cells and tissue slices. Because of the way in which the image is produced, the investigator can visually dissect through the specimen, observing structures above or below others. Storing information from each visual plane of the section in a computer allows a three-dimensional image to be reconstructed.

Fluorescence microscopy. When certain fluorescent substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength. In lluorescence microscopy, tissue sections are usually irradiated with ultraviolet light so that the emission is in the visible portion of the spectrum. The fluorescent substances appear as brilliant, shiny particles on a dark background. A microscope with a strong ultraviolet light source is used, and special filters that eliminate ultraviolet light are used after the objective lens to protect the observers eyes.Some naturally fluorescent substances are normal constituents of cells, e.g., vitamin A, vitamin B2, and porphyrins. Other fluorescent compounds that have an affinity for tissues and cells are used as fluorescent stains. Acridine orange is most widely used, because it can combine with DNA and RNA. When observed in the fluorescence microscope, the DNA-acridine orange complex emits a yellowish-green light, and the RNA-acridine orange complex emits a reddish-orange light. It is thus possible to identify and localize nucleic acids in the cells.

Fluorescence spectroscopy is a method of analyzing the light emitted by a fluorescent compound in a microspectrophotometer. It can be used to characterize several compounds present in cells and is of particular importance in the study of catecholamines. The development of fluorescent probes (substances that react specifically with cell components) has permitted highly sensitive assays for various substances within cells.

Electron microscopy. Both transmission and scanning electron microscopy are based on the interaction of electrons and tissue components.

The electron microscope is an imaging system that permits high resolution (0,1 nm). In practice, however, a resolution of 1 nm in tissue sections is considered satisfactory. This by itself permits enlargements to be obtained up to 400 times greater than those achieved with light microscopes.

The electron microscope functions on the principle that a beam of electrons can be deflected by electromagnetic fields in a manner similar to light deflection in glass lenses. Electrons are produced by high-temperature heating of a metallic filament (cathode) in a vacuum. The emitted electrons are then submitted to a potential difference of approximately 60-100 kV or more between the cathode and the anode. The anode is a metallic plate with a small hole in its centre. Electrons are accelerated from the cathode to the anode. Some of these particles pass through the central opening in the anode, forming a constant stream (or beam) of electrons. The beam is deflected by electromagnetic lenses in a way roughly analogous to what occurs in the optical microscope. Thus, the condenser focuses the beam at the object plane and the objective lens forms an image of the object. The image obtained is further enlarged by one or two projecting lenses and is finally seen on a fluorescent screen or is projected onto photographic plates.

Because electron microscopy requires a much thinner section (0.02-0.1 pm), embedding is performed with a hard epoxy plastic. The blocks thus obtained are so hard that glass or diamond knives are usually necessary to section them. Since the electron beam in the microscope cannot penetrate glass, the extremely thin sections are collected on small metal grids. Those portions of the section spanning the holes in the mesh of the grid can be examined in the microscope.

Scanning electron microscopy. A variant of electron microscopy, scanning electron microscopy, and permits pseudo-three-dimensional remembered that the observed product is the end result of a series of processes that considerably distort the image observable in living tissue, mainly through shrinkage. This shrinkage is produced mainly by the heat (60°C) needed for paraffin

embedding; it is virtually eliminated when specimens are embedded in resin. As a consequence of these processes, the spaces frequently seen between cells and other tissue components are artefacts. Furthermore, there is a tendency to think in terms of only two dimensions when examining thin sections, when the structures from which the sections are made actually have three dimensions. In order to understand the architecture of an organ, it is therefore necessary to study sections made in different planes and to reason accordingly.

Another difficulty in the study of microscope preparations is the impossibility of differentially staining all tissue components on only one slide. It is therefore necessary to examine several preparations stained by different methods before a general idea of the composition and structure of any type of tissue can be obtained.

Radioautography. Radioautography permits the localization of radioactive substances in tissues by means of the effect of emitted radiation on photographic emulsions. Silver bromide crystals present in the emulsion act as microdetectors of radioactivity. In radioautography, tissue sections from animals previously treated with radioactive compounds are covered with photographic emulsion and stored in a lightproof box in a refrigerator. After various exposure times the slides are developed photographically and examined. All silver bromide crystals hit by radiation are reduced to small black granules of elemental silver, which reveal the existence of radioactivity in the tissue structures in close proximity to these granules. This procedure can be used in both light and electron microscopy.

By localizing radioactivity in tissue components it is possible obtain data on the sequence of events occurring in tissues. Thus, if a radioactive protein precursor (amino acid) is given to a protein-synthesizing cell, its pathway can be followed in the cell after varying periods of time. Furthermore, the intensity of the process is proportional to the number of granules formed over the tissue components.

Imunohistochemical methods.The immunohistochemical methods are based on the reactions antigen- antibody. Every cell of organism has specific antigen composition which is determined by proteins mostly. It is possible to get by immunization specific antibodies proper to the antigens. Antibodies contact with fluorochromes or enzymes. After processing of the explored histological specimens in the places of localization of the proper antigens the molecules of the marked antibodies, which expose either thanks to luminescence (luminescent microscopy), or on the basis of laying of the products of histochemical reaction (light microscopy), are concentrated. By this method it is possible to identify any cells or substances produced by those or other cells, for example, hormones.

Diagnostic immunohistochemical markers. Immunohistochemistry is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues .This has made it a widely-used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures. Its major disadvantage is that, unlike immunoblotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in immunohistochemistry that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for typing tumors.

Cytospectrophotometry is method of the quantitative measuring of maintenance of different substances in a cell on the basis of study of spectrums of absorption by them light rays.

The method of running cytometry enables to analyse characteristics of cells in suspension which are crossed by focusing laser ray. The proper device is called cytofluorograph. By means this method it is possible to determine sizes and shape of cells, their viability, to divide the cells of initial suspension on subpopulations.

Tissue preparation

Fixation

Chemical fixation with formaldehyde or other chemicals

Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles. The most common fixative for light microscopy is 10% neutral buffered formalin. For electron microscopy, the most commonly used fixative is glutaraldehyde, usually as a 2.5% solution in phosphate buffered saline. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of CH_2 (methylene) linkage, in the case of formaldehyde, or by a C_5HIO cross-links in the case of glutaraldehyde. This process, while preserving the structural integrity of the cells and tissue, can damage the biological functionality of proteins, particularly enzymes, and can also denature them to a certain extent. This can be detrimental to certain histological techniques. Further fixatives are often used for electron microscopy such as osmium tetroxide or uranyl acetate.

Formaldehyde (Formalin)

Properties:

1. The most widely used and routine fixative particularly for paraffin embedded sections.
2. It is a gas with a very pungent odor.
3. The commercially available solution of formaldehyde (formalin) contains 35-40% gas by weight. However, pure stock solution of 40% formalin is unsatisfactory.
4. Formaldehyde is commonly used as 4% solution, giving 10% formalin for fixation. Therefore, it should be diluted 1:10.
5. Formaldehyde is usually buffered to pH 7 with phosphate buffer.
6. It is thought that formaldehydes form cross-links between proteins, creating a gel, thus retaining cellular constituent.
7. It is a forgiving fixative - requires a relatively short fixation time (24 hours) but can be used for long term usage with no deleterious effects on tissue.
8. Prepared by adding 100 ml of 40% formaldehyde to 900 ml distilled water with 4 g sodium phosphatase (monobasic) and 6.5 g sodium phosphate (dibasic anhydrous).

Frozen section fixation

Frozen section is a rapid way to fix and mount histology sections. It is used in surgical removal of tumors, and allows rapid determination of margin (that the tumor has been completely removed). It is done using a refrigeration device called a cryostat. The frozen tissue is sliced using a microtome, and the frozen slices are mounted on a glass slide and stained the same way as other methods. It is a necessary way to fix tissue for certain stain such as antibody linked immunofluorescence staining. It can also be used to determine if a tumour is malignant when it is found incidentally during surgery on a patient.

Processing

The aim of tissue processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5 pm thick for light microscopy and 80- 100 nm thick for electron microscopy.

For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene. Paraffin wax does not provide a sufficiently hard matrix for cutting very thin sections for electron microscopy. Instead,

resins are used. Epoxy resins are the most commonly employed embedding media, but acrylic resins are also used, particularly where immunohistochemistry is required.

Thicker sections (0.35pm to 5pm) of resin-embedded tissue can also be cut for light microscopy. Again, the immiscibility of most epoxy and acrylic resins with water necessitates the use of dehydration, usually with ethanol.

Embedding

After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatine, or wrn) which is then hardened. This is achieved by cooling in the case of paraffin wax and heating (curing) in the case of the epoxy resins. The acrylic resins are polymerised by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned.

Because formalin-fixed, paraffin-embedded (FFPE) tissues may be stored indefinitely at room temperature, and nucleic acids (both DNA and RNA) may be recovered from them decades after fixation, making FFPE tissues an important resource for historical studies in medicine.

Sectioning

For light microscopy, a steel knife mounted in a microtome (fig. 1.2) is used to cut 10-micrometer-thick tissue sections which are mounted on a glass microscope slide. For transmission electron microscopy, a diamond knife mounted in an ultramicrotome is used to cut 50-nanometer-thick tissue sections which are mounted on a 3-millimeter-diameter copper grid. Then the mounted sections are treated with the appropriate stain.

Figure 2. Microtome.

Frozen tissue embedded in a freezing medium is cut on a microtome in a cooled machine called a cryostat.

Staining

Biological tissue has little inherent contrast in either the light or electron microscope. Staining is employed to give both contrasts to the tissue as well as highlighting particular features of interest. Where the underlying mechanistic chemistry of staining is understood, the term histochemistry is used.

Hematoxylin and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Uranyl acetate and lead citrate are commonly used to impart contrast to tissue in the electron microscope.

Special staining: There are hundreds of various other techniques that have been used to selectively stain cells and cellular components. Other compounds used to colour tissue sections include safranin, Congo red, fast green FCF, silver salts, and numerous natural and artificial dyes that were usually originated from the development dyes for the textile industry.

In a chemical classification one divides organic dyes into:

- Azo dyes (the greatest group), e.g. acid and basic azo dyes,
- Nitro and nitroso dyes,
- Chinone dyes, e.g. benzochinones, naphthachinones,
- Di- and triphenylmethan dyes,
- Xanthene dyes with the subdivisions of pyronines and phthaleines,
- Acridine dyes,
- Azine dyes,
- Oxazine dyes,
- Thiazine dyes,
- Vat dyes.

The practical classification of dyes relies on their coloring effects:

- Basic dyes, e.g. methyl green, safranine and fuchsine,
- Acidic dyes, e.g. eosine, acid fuchsine and some anthrachinone dyes,
- Substantive dyes, e.g. benzopurpurine and Congo red,
- Mordant dyes, e.g. haematoxylin and carmine,
- Developing dyes,e.g. aniline black, naphthol red, Echtrot,
- Vat dyes, e.g. indigo and indanthrene dyes,
- Sulfur dyes, e.g. vidal black and pyrogene blue. Other dyes are mainly of industrial interest and not for microscop.

Practical lessons №1

Questions for self-control

1. Which sections include discipline "Cytology, histology, embryology?" 2. What are studying cytology, general histology, embryology and histology special? 3. What disciplines related cytology, histology and embryology? 4. What distinguished periods in the development of cytology, histology and embryology, and what they characterized? 5. Who and when first constructed a light microscope? 6. What research methods are used in cytology, histology and embryology. 7. What are the order of manufacture of permanent histological preparations. 8. What are the most common fixing material. 9. What are the criteria for classification dyes? 10. What parts make up the light microscope?

CELL. CELL MEMBRANE. CELL JNCTIONS. INCLUSIONS

The cell is limited by an active membrane, well-organized structured system of the biopolymers forming a nucleus and cytoplasm, participating in the united aggregate of metabolic and power processes carry ing out maintenance and reproduction of all system as the whole.

The cell is composed of 3 basic parts:

- plasma membrane,
- cytoplasm and
- nucleus.

Cell membrane

Chemically cell membrane consists of lipids, proteins, and oligosaccharides (fig. 3).Under the electron microscope (EM) cell membrane consists of 2 densely stained layers separated by a lighter zone.

The basic structure of membrane is the arrangement of phospholipids' molecules that constitute the basic framework of the membrane. Each molecule consists of: - enlarged polar hydrophilic head and - thin non-polar hydrophobic tail.

Lipids are most stable when organized into a double layer with their non-polar tails directed toward the center of the membrane and their polar heads directed outward.

In addition to molecules of phospholipids the cell membrane contains several proteins.

Integral proteins either completely (integral proteins proper), or partly (semiintegral) are embedded in the lipid bilayer.

Peripheral proteins form a looser association with inner or outer membrane surface.

The carbohydrate layer (glycocalyx) is formed on the external surface of the membrane. It is formed by carbohydrates, which form connections with proteins (glycoproteins) or lipids (glycolipids).

Glycocalyx help establish extracellular microenvironments at the membrane surface that have specific functions in metabolism, cell recognition, and cell association and serve as receptor sites.

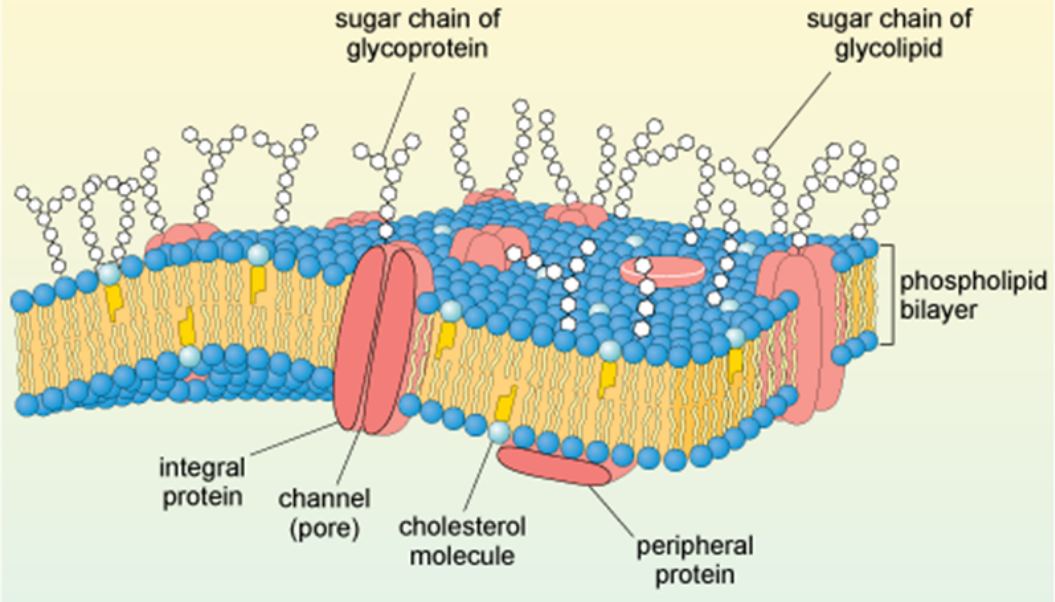


Figure 3. Schematic diagram of the cell membrane

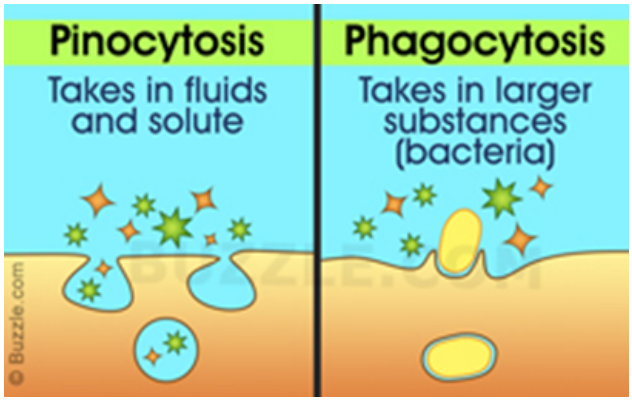
The theory of structure of cell membrane called fluid mosaic- model proposed by S.J. Singer and G. Nicolson.

Functions of the cell membrane:

1. Maintaining the structural integrity of the cell.
2. Regulating of cellular interactions.
3. Recognition of antigens and foreign cells.
4. Interaction between the cytoplasm and the external environment.
5. Movements of the cell (formation of cilia, flagella).
6. Transport of substances into and from the cell.

Endocytosis

Some substances (consisting of small molecules) pass through the passive channels. Larger molecules enter the cell by invagination of a part of the cell membrane, which first surrounds the molecule and then separates to form endocytosis vesicle. Endocytosis is the process of engulfing by cell macromolecules, particulate matter, and other substances from the extracellular space. Endocytosis is divided into 2 categories:



Phagocytosis (cell eating) is the cellular process of engulfing solid particles by the cell membrane to form an internal phagosome. Phagocytosis is involved in the acquisition of nutrients for some cells, and, in the immune system, it is a major mechanism used to remove pathogens and cell debris. Bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytised.

Pinocytosis (cell drinking) is the cellular process of engulfing of fluid and small protein molecules usually smaller than 150 nm in diameter.

Exocytosis is extrusion of materials from the cell (fig.4).

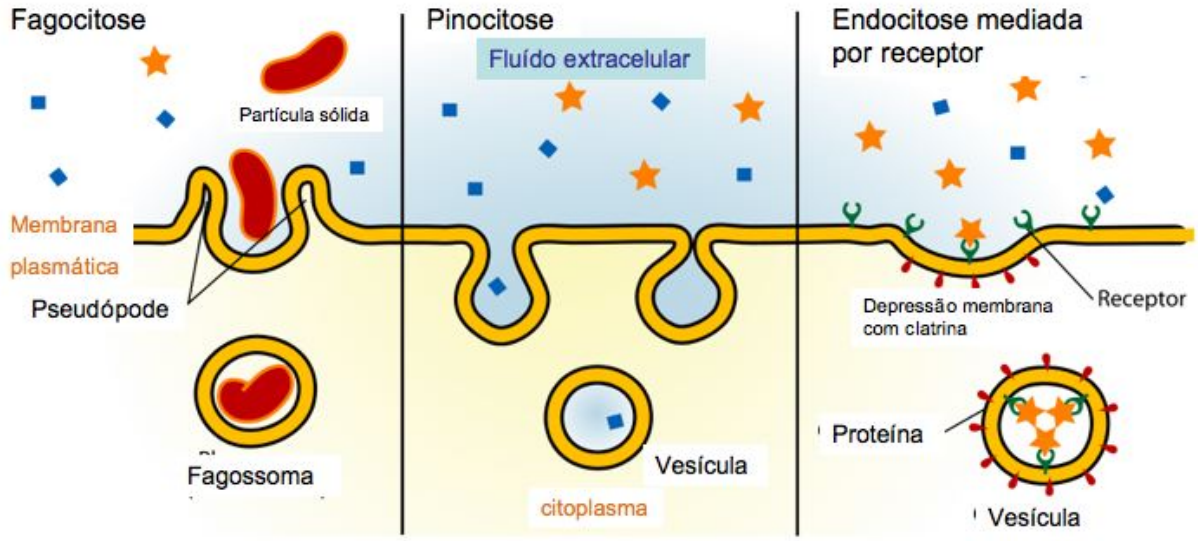


Figure 4.1. Schematic diagram of pinocytosis, phagocytosis, endocytosis.

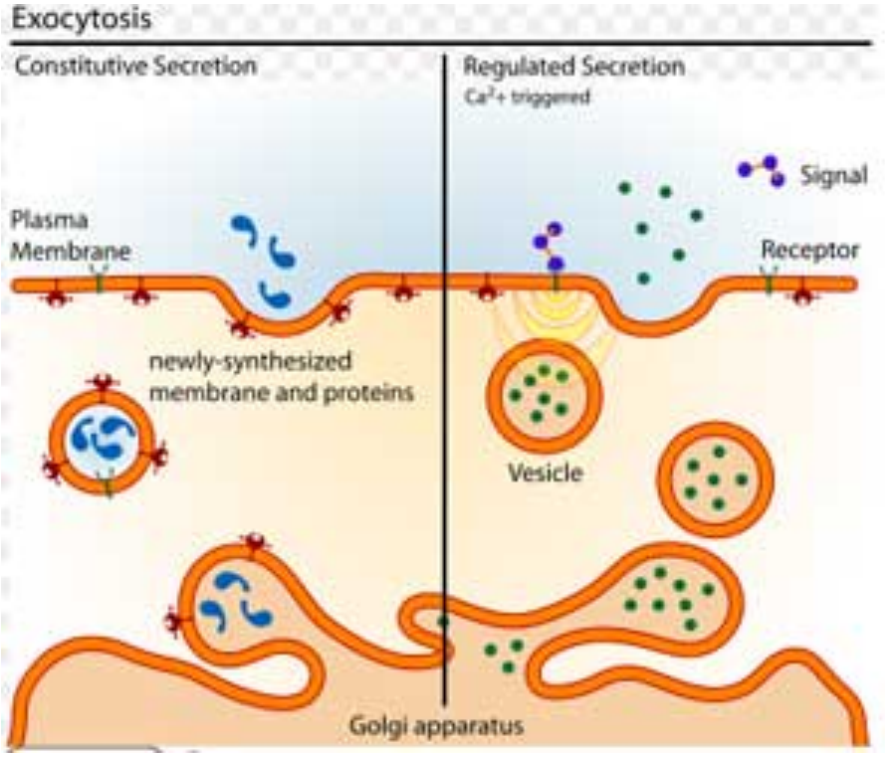
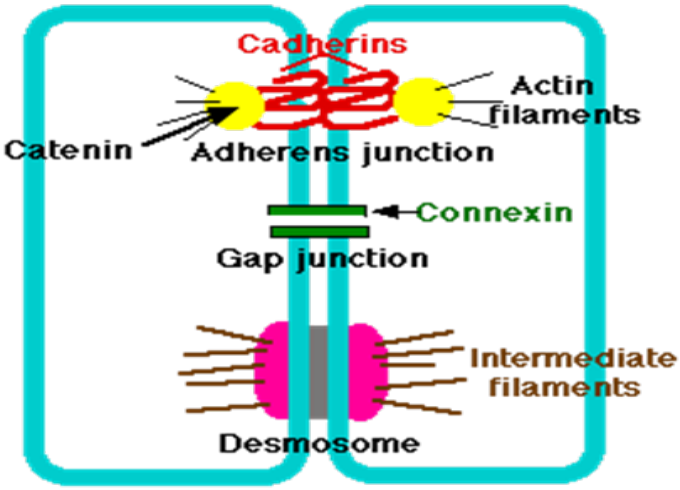


Figure 4.2. Schematic diagram of exocytosis.

Cell junctions

Cell junctions are the types of structures that exist within the tissue of a multicellular organism. They consist of protein complexes and provide contact between neighbouring cells, between a cell and the extracellular matrix. Cell junctions are especially abundant in epithelial tissues.

There are types of cell junctions:
- Adherens junctions and desmosomes (anchoring junctions)
- Desmosomes
- Gap junctions (communicating junction)
- Tight junctions (occluding junctions)



Adherens junctions provide strong mechanical attachments between adjacent cells. They hold cardiac muscle cells tightly together as the heart expands and contracts. They hold epithelial cells together. Adherens junctions are built from (fig.4): cadherins - transmembrane proteins whose extracellular segments bind to each other and whose ntracellular segments bind to catenins. Catenins are connected to actin filaments.

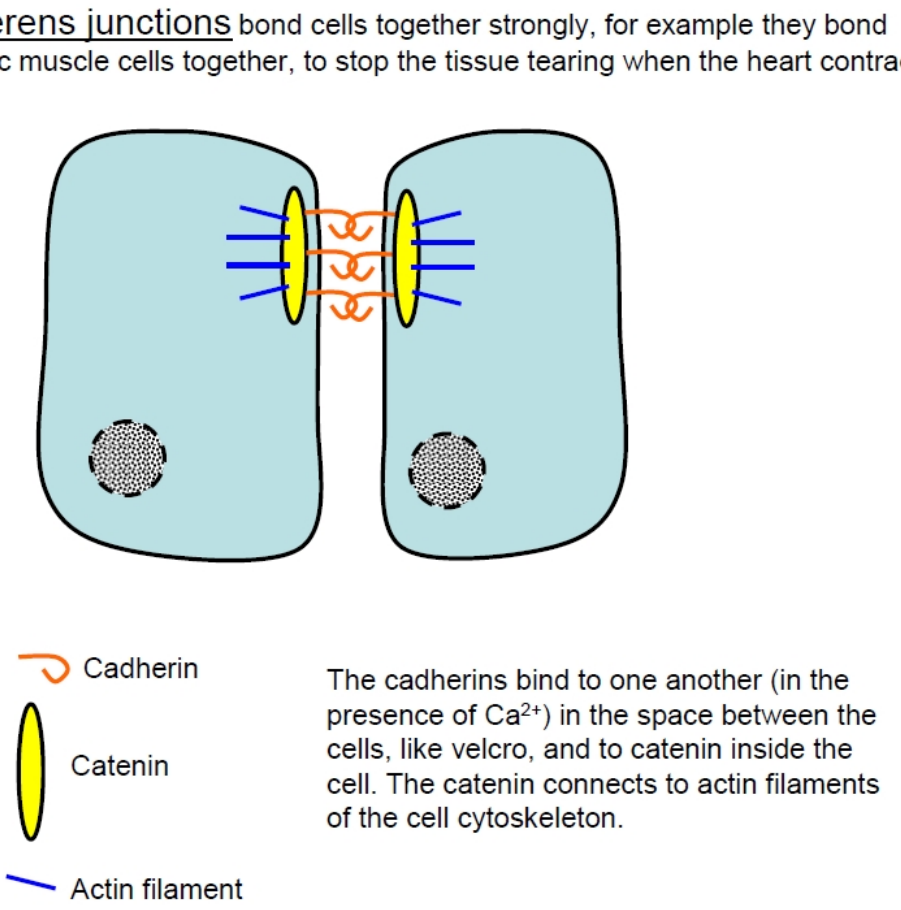


Figure 5. Schematic diagram of adherens junctions.

Desmosomes (fig.6) are molecular complexes of cell adhesion proteins and linking proteins that attach the cell surface adhesion proteins to intracellular intermediate filaments. Desmosomes help to resist shearing forces and are found in simple and stratified squamous epithelium. The intercellular space is very wide (about 30 nm). Desmosomes are also found in muscle tissue where they bind muscles cells to one another.

Hemidesmosomes are the cell-matrix junctions, which mediate adhesion between the basal cells and the basement membrane.

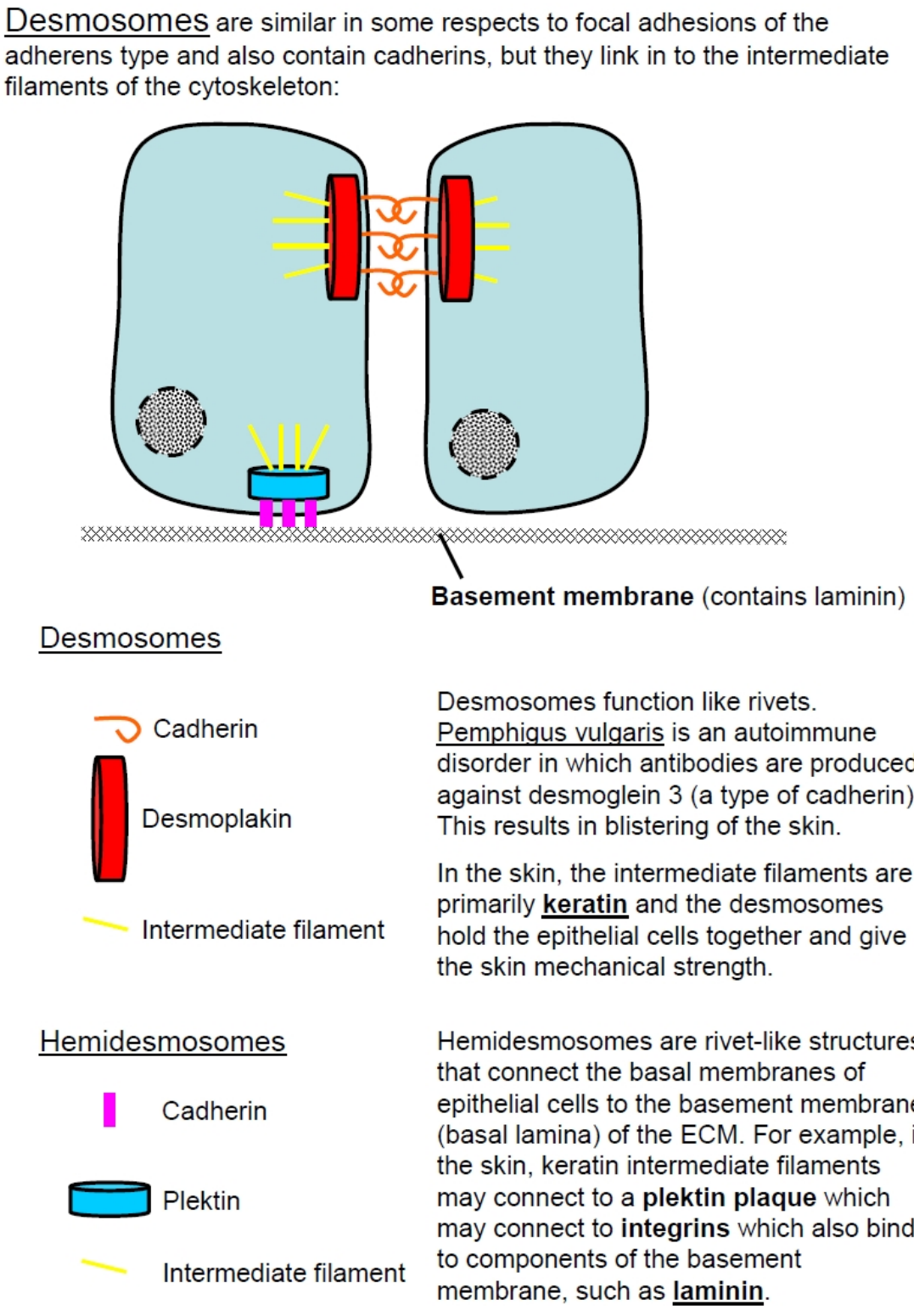


Figure 6. Schematic diagram of desmosome.

Gap junctions (fig. 7) are intercellular channels, which are involved in cell-cell communication. The gap junctions form a pathway for passive diffusion of nutrients, metabolites, ions, and small signalling molecules between adjacent cells. Structurally, each gap junctional channel is composed of pair of connexons (hemichannels), which leave a narrow intercellular gap between the neighbouring cell membranes. Each connexon is composed of 6 connexin proteins lining the transmembrane channel.

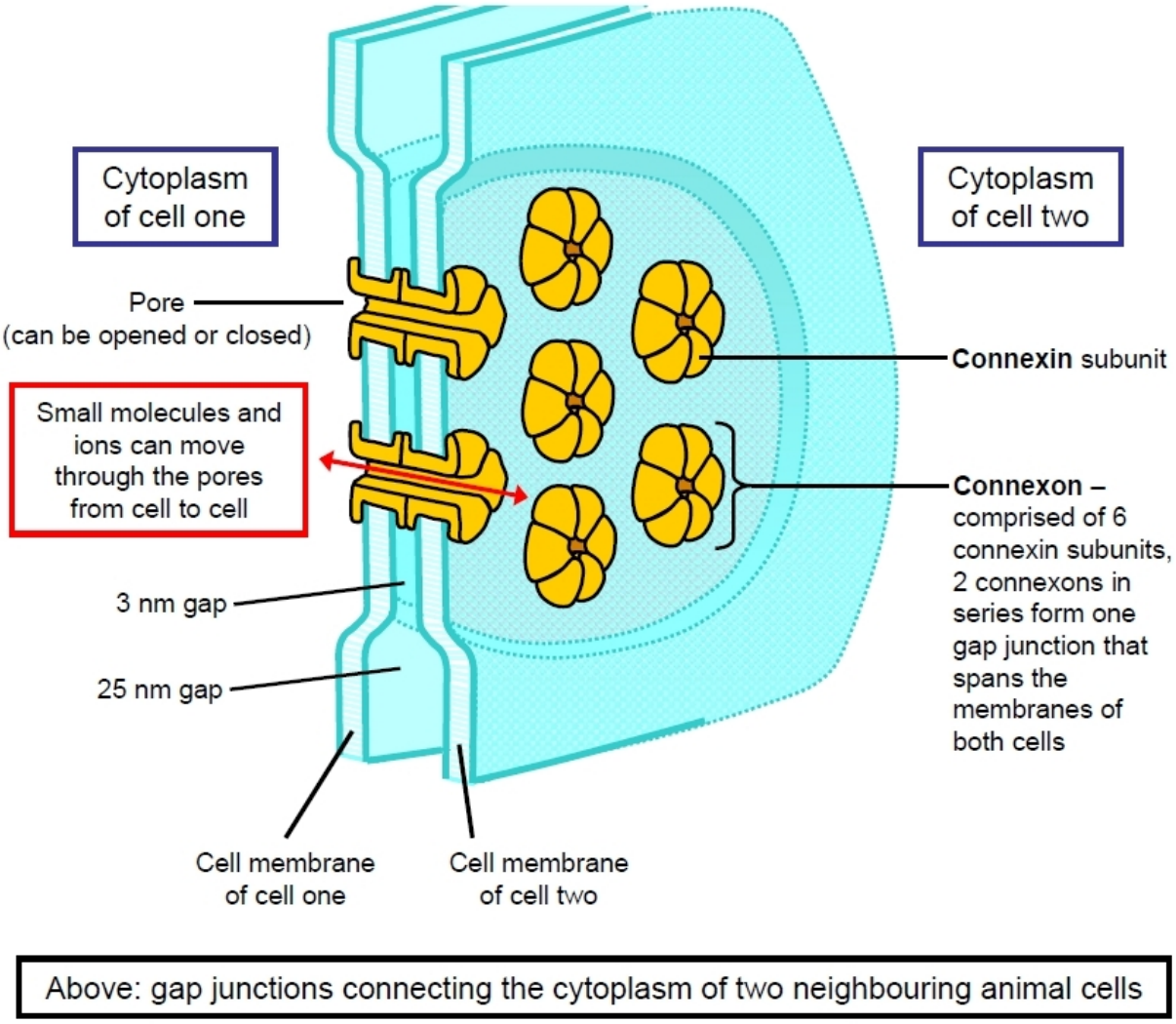


Figure 7. Schematic diagram of gap junctions.

Tight junctions, or zonula occludens (fig. 8) are the closely associated areas of two cells whose membranes join together. These junctions act as barrier that prevents the movement of molecules into intercellular spaces.

Tight junctions are composed of a branching network of sealing strands, each strand acting independently from the others. Each strand is formed from a row of transmembrane proteins embedded in both plasma membranes, with extracellular domains joining one another directly.

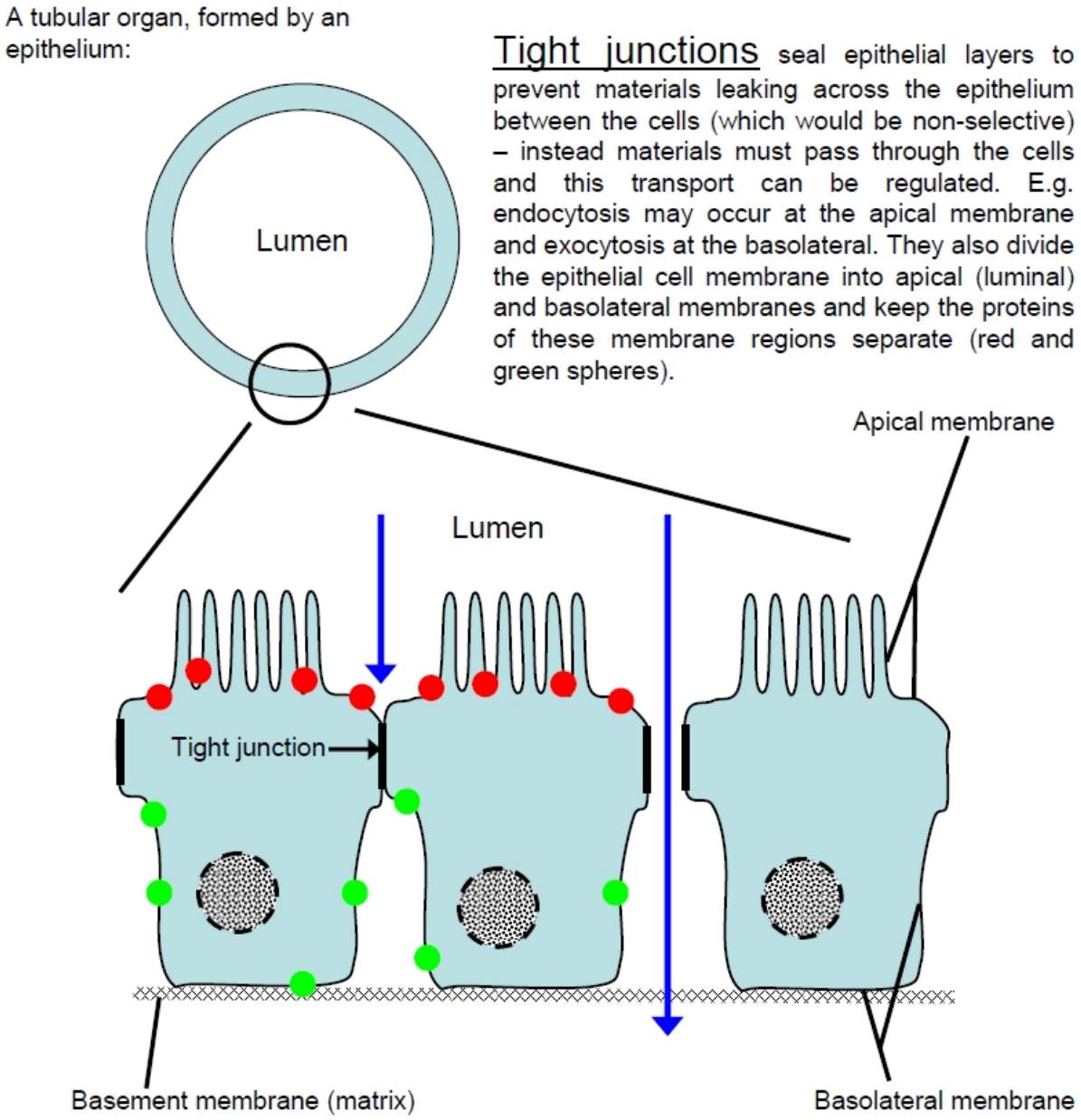


Figure 8. Schematic diagrams of tight junctions.

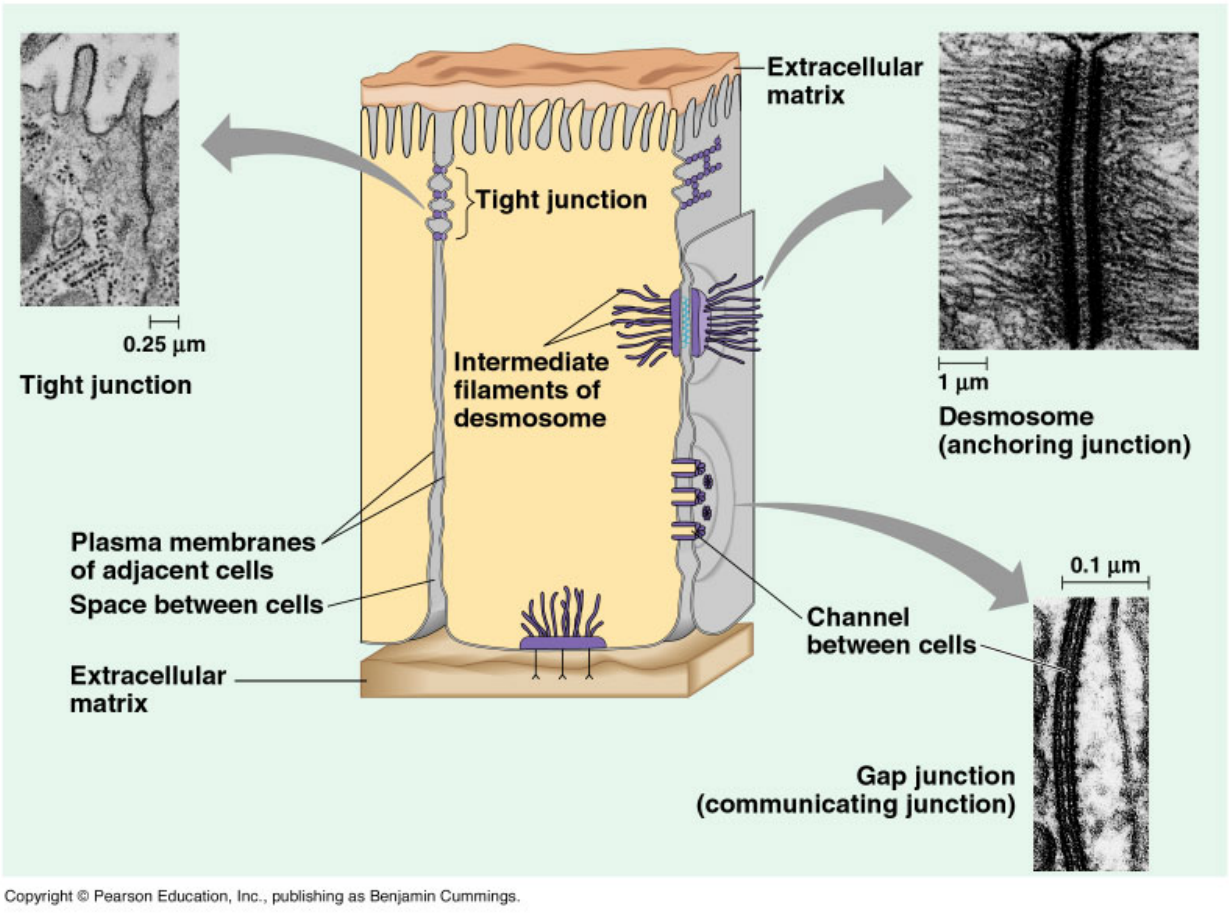


Figure 9. Schematic diagrams of junctions.

Cytoskeleton

Cells possess a cytoskeleton that provides a structural framework, facilitates intracellular transport, supports cell junctions and transmits signals about cell contact and adhesion, and permits motility. The three structural elements of the cytoskeleton are *microfilaments, intermediate filaments,* and *microtubules.* All are dynamic structures assembled from protein subunits and disassembled as cellular activities and external influences on the cell change.

Microfilaments are 6 to 8 nm in diameter and consist of globular actin molecules polymerized into long filaments (Figure 10.1.). Microfilaments form tracks for the movement of myosin and serve as intracellular "muscles" for maintenance of cell shape, movement, and contractility. Microfilament networks, along with actin-binding and actin-bundling proteins, are found in association with adhesive cell junctions, as a "web" beneath cell membranes, especially the apical membrane, and as the structural "core" of microvilli, filopodia, and lamelipodia. Actin interacts with the other two components of the cytoskeleton.

- in skeletal muscle they integrate with thick (16 nm) myosin filaments;
- in most cells microfilaments are present as a thin sheath just beneath the plasmolemma. These filaments appear to be associated with membrane activity such as endocytosis, exocytosis, and cell migratory activity (pseudopodial processes).

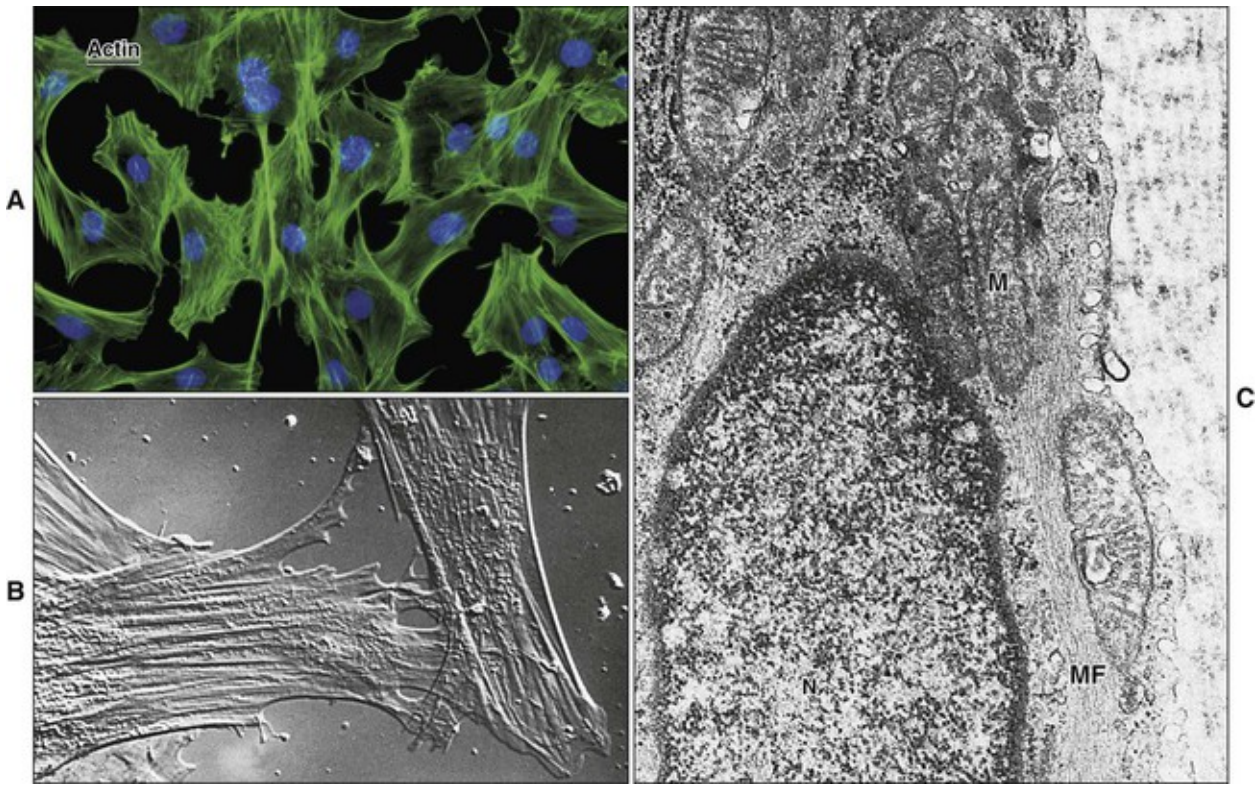


Figure 10.1. Microfilaments. ***A****, Cultured osteogenic cells labeled with an antibody to actin, the main protein constituting microfilaments, using the fluorescence technique (nuclei are stained using DAPI [4,6-diamino-2-phenylindole] and appear blue).* ***B****, Nomarski differential interference contrast image of microfilament bundles in the cytoplasm of cultured fibroblasts from the pig periodontal ligament. Nomarski images are produced by using the interference conditions generated by optical-path-length differences of two beams of coherent light. The microfilament bundles appear as elongated, raised lines.* ***C****, Electron micrograph of microfilaments in the cytoplasm of a fibroblast.* MF, *Microfilaments;* M, *mitochondria;* N, *nucleus. (****A****, Courtesy of P. Tambasco de Oliveira;* ***B****, courtesy of J. Aubin.)*

Intermediate filaments are approximately 10 nm in diameter and have a diverse protein composition. They are not contractile but are important in the maintenance of cell shape and contact between adjacent cells and the extracellular matrix. In cells of mesenchymal origin, such as fibroblasts and osteoblasts, intermediate filaments are polymers of the protein *vimentin* (Figure 10). In epithelial cells, intermediate filaments consist of cytokeratins. The filaments form bundles, called *tonofilaments,* which anchor onto desmosomes (Figure 10.2., *B* and *C*). Cytokeratins are a multigene family of proteins made up of basic and more acidic proteins. Cytokeratins occur as linked acidic and basic pairs with differing combinations in different types of epithelia. Their expression patterns have been used to determine the relationship between cell types and as an indication of the origin of various tumors.

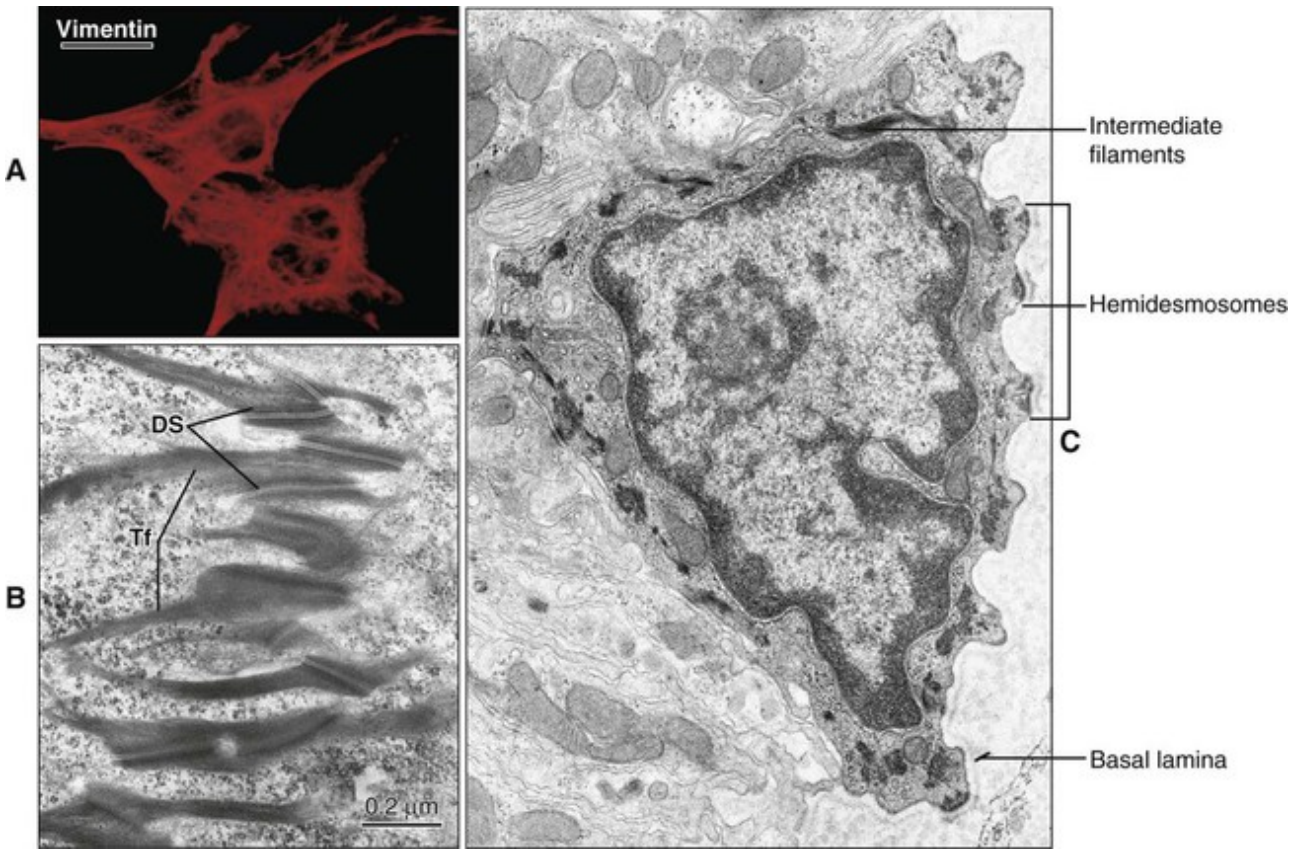


Figure 10.2. ***A,*** *Intermediate filaments in cultured osteogenic cells stained for vimentin by the immunofluorescence method.* ***B*** *and* ***C,*** *Electron micrographs of intermediate filaments; these form discrete bundles, called tonofilaments* (Tf), *that insert into the desmosomal plaques* (DS) *or distribute around the periphery of a cell.* ***C,*** *Basal cell of a salivary gland excretory duct; hemidesmosomes form attachments to the basal lamina surrounding the duct.*

Microtubules are tubular or cylindrical structures with an average diameter of 25 nm (Figure 11). Microtubules are composed of the protein *tubulin* arranged in rings stacked end to end, making up the tubules. Microtubules provide internal support for the cell; are the basis of motility for certain organelles, such as cilia; act as guide paths and part of the motor mechanism for the movement of secretory vesicles and other organelles; and serve to position certain organelles within the cell.

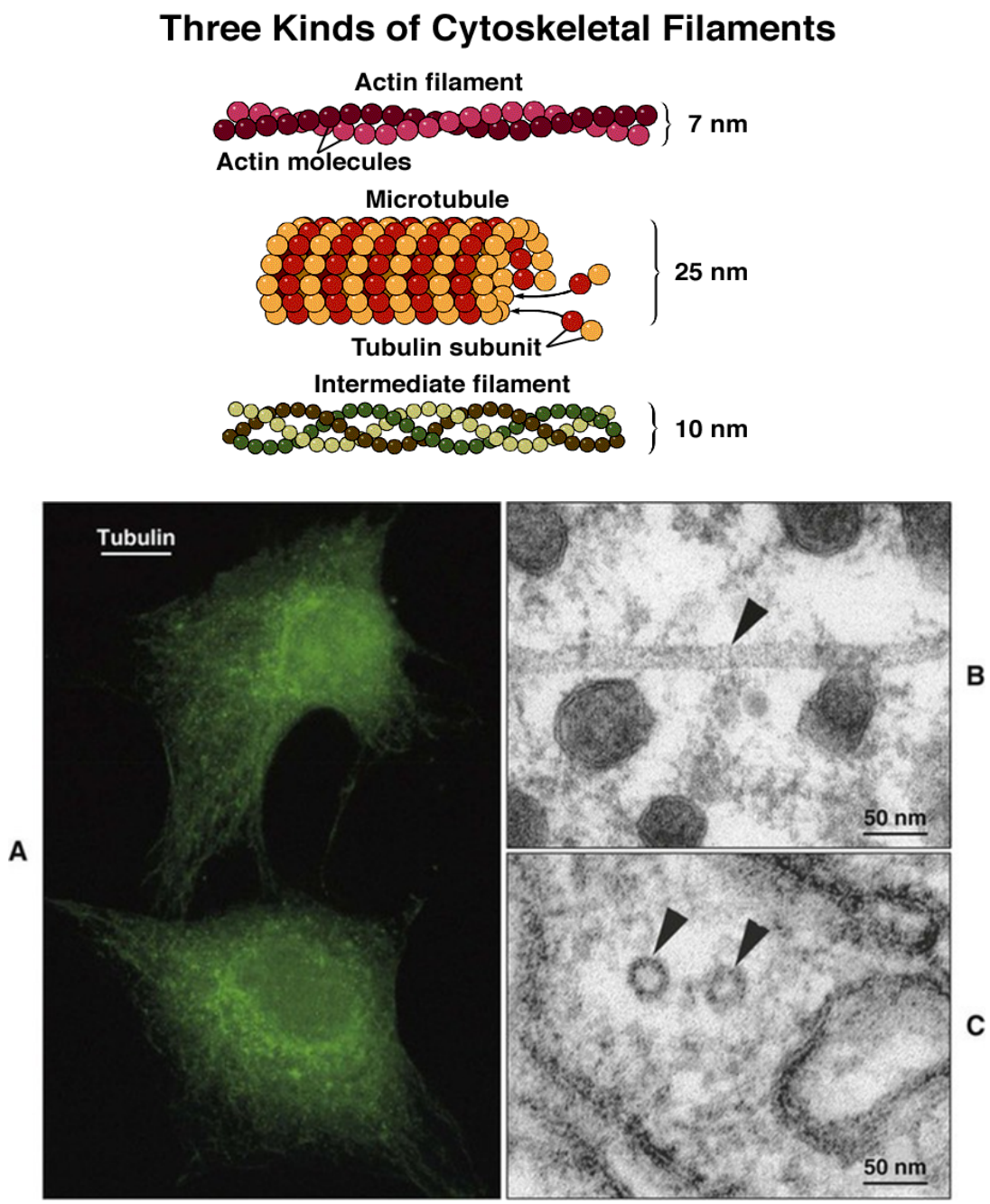


Figure 11. Microtubules. ***A,*** *Fluorescent micrograph of cultured osteogenic cells labeled with an antibody to tubulin, the main protein of microtubules. Under the fixation conditions used here, the microtubules are seen as unpolymerized tubulin (*dotted *immunofluorescence pattern).* ***B*** *and* ***C,*** *Electron micrographs of portion of a longitudinally oriented (****B****) microtubule and cross-sectioned (****C****) microtubule.*

The cells contain a class of intermediate-sized filaments with diameter of 8-12 nm. Intermediate filaments occur in the cells of many tissues:

- vimentin filaments are characteristic of cells of mesenchymal origin.
- desmin is found in smooth muscle and in the Z disks of skeletal and cardiac muscle;
- cytokeratins are found in most epithelia.

Cytoplasmic inclusions

Inclusions are temporary components of the cytoplasm, mainly composed of accumulated metabolites or deposits of varied nature. Cytoplasmic inclusions are subdivided in

- trophic,
- secretory,

- excretory,
- pigment.

The trophic inclusions are lipid droplets in adipose tissue, adrenal cortex cells, and liver cells; carbohydrate accumulations in several cells in the form of glycogen.

The secretory inclusions are protein secretory granules in glandular cells.

The excretory inclusions are similar with secretory, they contain the metabolic substances.

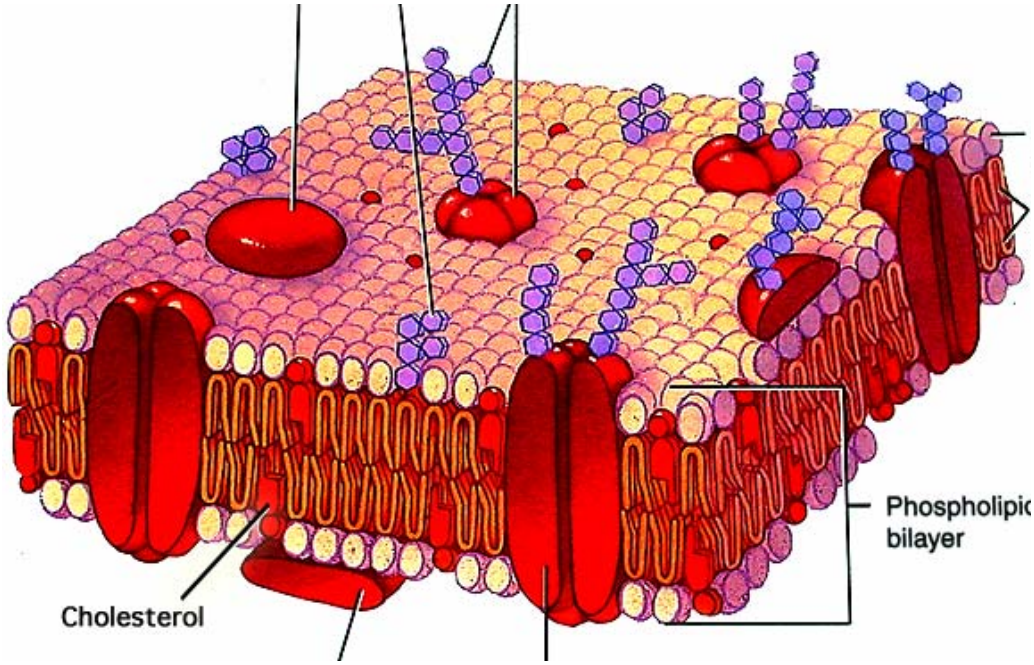
The pigment inclusions are often found in the cells. They may synthesize by the cell: lipofuscin, a yellow-brown substance in neurons and cardiac muscle; melanin in the epidermis of the skin, in the pigment layer of the retina. The pigment inclusions may come from outside the body (e.g., carotene).

Practical lessons №2

Questions for self-control

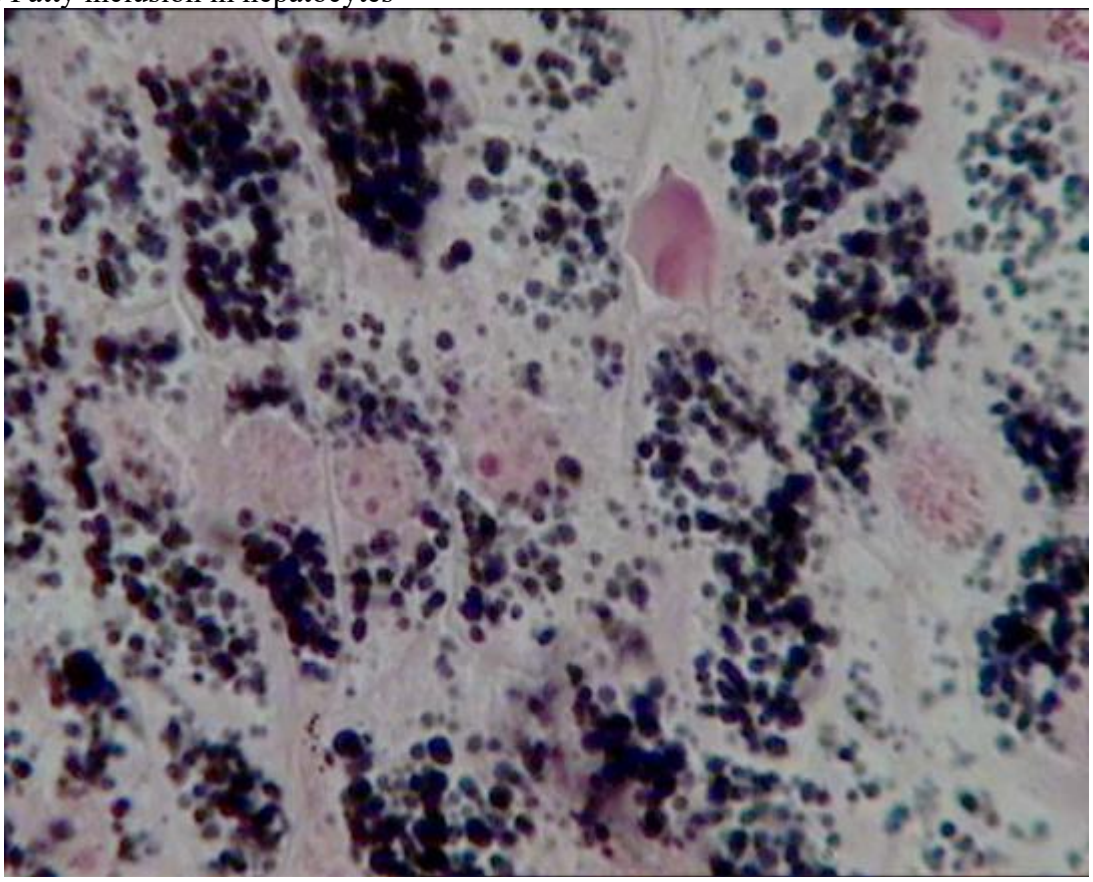
1. That cytology studies? 2. Who and when first saw the cells? 3. Who is the creator of cell theory? 4. When was established cell theory? 5. The main provisions of cell theory and its justification. 6. Determination of the cell. 7. What are cells? 8. called substance that forms a cage? 9. The chemical composition of protoplasm. 10. Physical and chemical properties of protoplasm. 11. What forms may be cells and how they caused? 12. What size can have cells? 13. What formed the cell membrane? 14. The functions of the cell membrane. 15. How to divide the cell membrane?

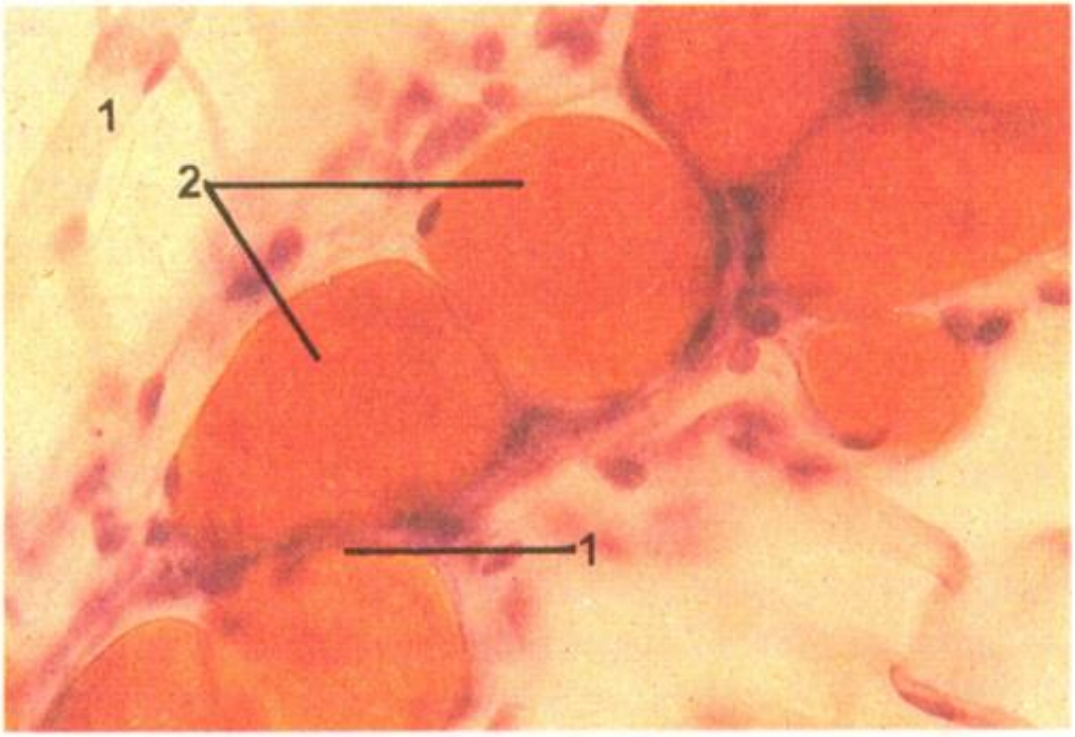
1. Designate the basic structures plasmolemma:



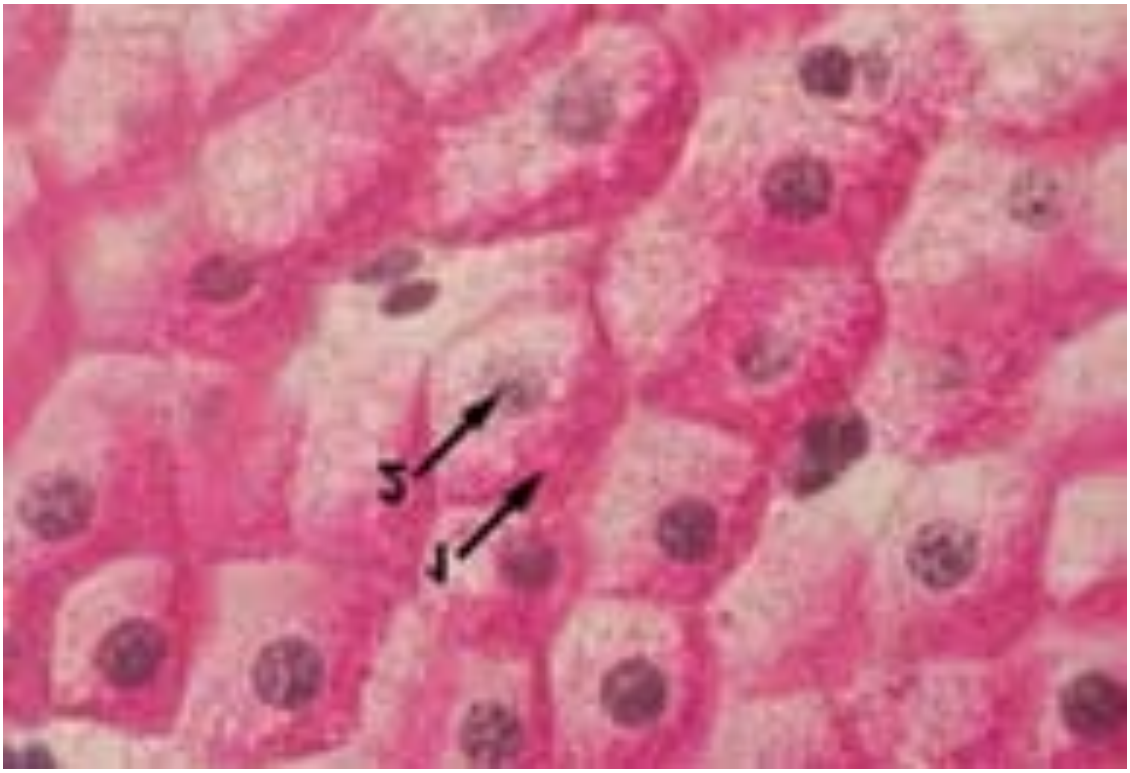
2. Sketch of histopreparaty:

a) Fatty inclusion in hepatocytes

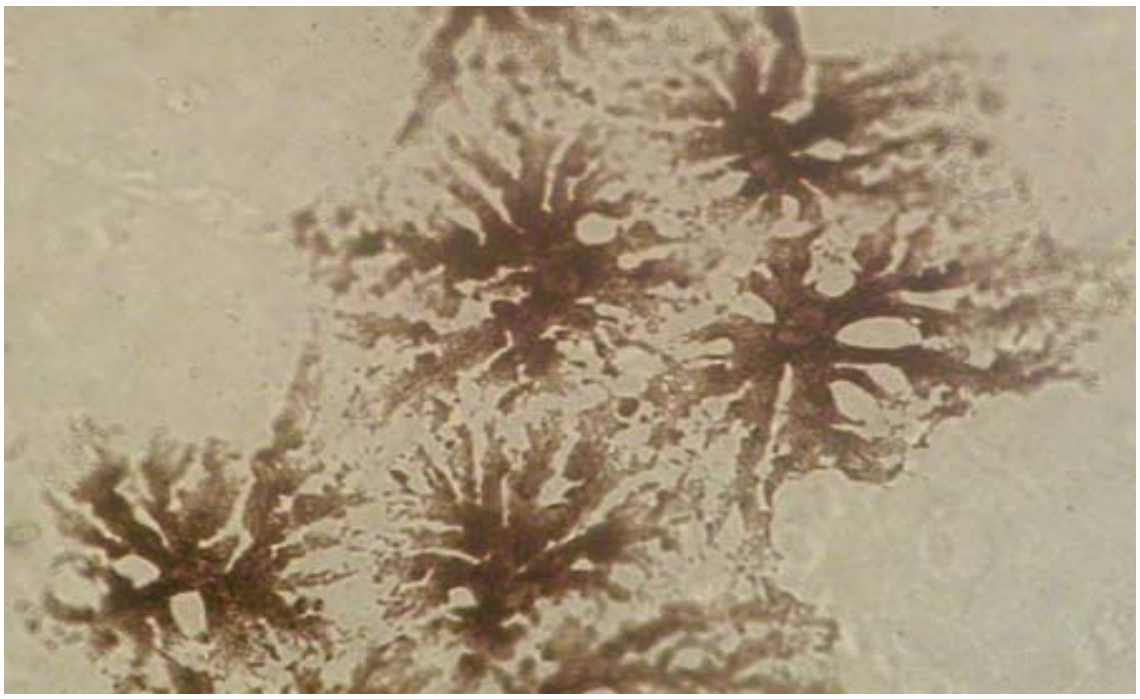




b) glycogen inclusion in hepatocytes



c) pigmented melanocytes inclusion in the iris of the eye



The teacher's signature:

CYTOPLASM. ORGANELLES

Cytoplasm

The cytoplasm is the part of the cell lying between the cell membrane and nucleus. It consists of matrix, in which several components such as organelles and inclusions are embedded.

Organelles

The organelles are the specialized subunits within a cell that have specific functions.

Classifications of the organelles

1. On the basis of their structure the organelles are subdivided on membranous (mitochondria, endoplasmic reticulum, Golgi complex, lysosomes, peroxisomes) and nonmembranous (ribosomes, microtubules, microfilaments, intermediate filaments, centrioles, cilia and flagella).

2. On the basis of their functions the organelles are subdivided on general and special.

General organelles such as ribosomes, mitochondria, endoplasmic reticulum, Golgi complex, lysosomes, peroxisomes, and centrioles are in any cell.

Special organelles are in specialized cells (neurofibrils and Nissl bodies - in neurons, myofibrils - in muscle cells, cilia in cells of respiratory epithelium, flagella - in human spermatozoa).

Membranous organelles

Mitochondria

Mitochondria are spherical or cylindrical organelles, which are composed of an outer membrane and inner membrane (fig.12).

The inner membrane projects folds, termed cristae, into the interior of the mitochondrion. The space located between the two membranes, is termed the intermembrane space. The other space, the matrix, space, is enclosed by the inner membrane. Matrix contains mitochondrial DNA, ribosomes, tRNA, and the enzyme system that generate ATP by means of the citric acid cycle, oxidative phosphorylation, and P-oxidation of fatty acids.

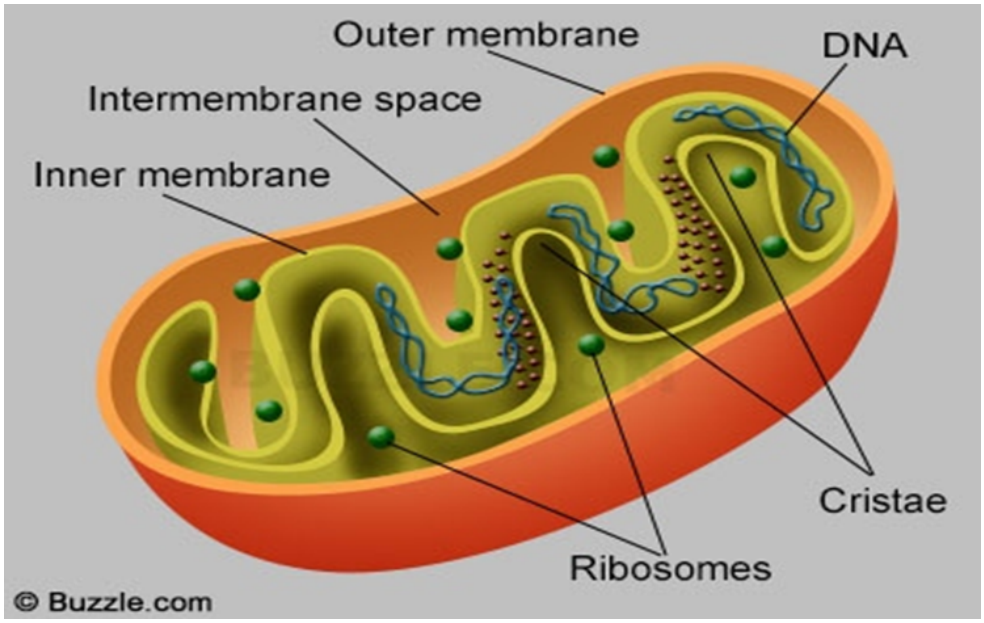


Figure 12. Schematic diagram of mitochondrion.

The end result of these reactions, which take place in the mitochondrial matrix, is the production of CO_2, water, and heat and the accumulation of energy in high-energy compounds such as ATP.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is the site of the lipid and carbohydrate synthesis, protein segregation from the cytoplasm. With light microscope endoplasmic reticulum appears as deep blue staining particles usually concentrated in the basal part of the cells. With electron microscope it appears as a rich network of membrane bound flattened tubules and sacs.

There are two types of endoplasmic reticulum: rough and smooth.

Rough (granular) endoplasmic reticulum (RER) is prominent in cells specialized for the protein secretion, such as pancreatic acinar cells (digestive enzymes), fibroblasts (collagen), plasma cells (immunoglobulin). The rough endoplasmic reticulum consists of tubules and flattened cisterns. The width of its components can be from 20 to 1000 nm. On the cytoplasmic surface of the endoplasmic reticulum there are polyribosomes, giving them granular appearance.

The principal function of the rough endoplasmic reticulum is to synthesis and segregate proteins destined for export or intracellular use.

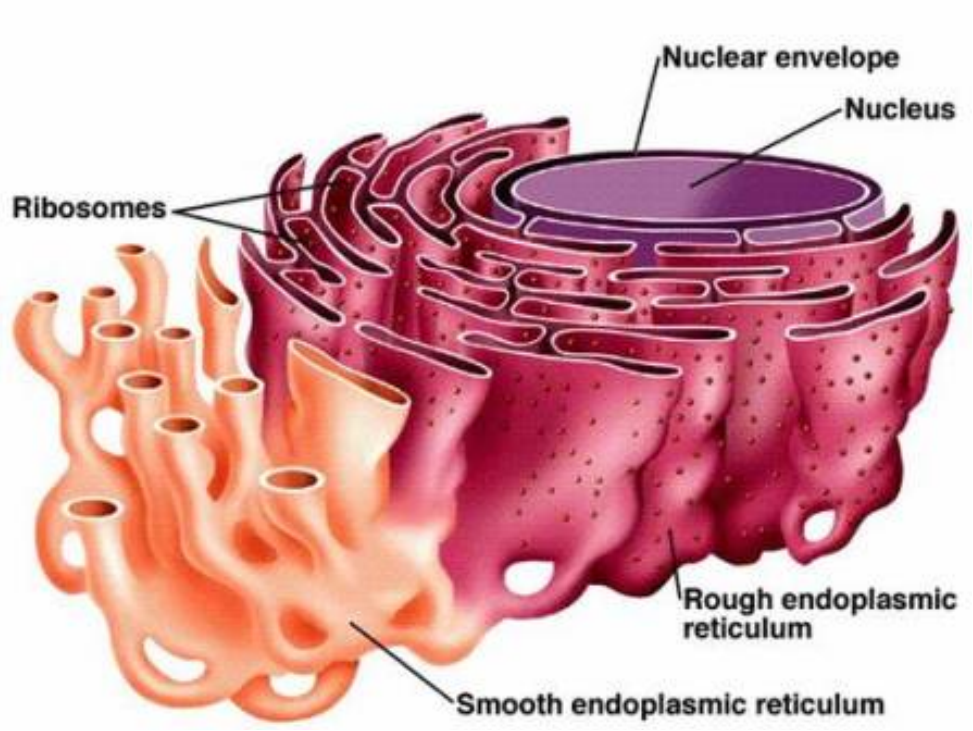


Figure 13. Schematic diagram of endoplasmic reticulum.

Smooth (agranular) endoplasmic reticulum (SER) is the membranous network within the cell. These are devoid of ribosome granules. Its cisterns are more tubular and more likely to appear as a profusion of interconnected channels of variable size s and shape. Its components can have a width of 50 to 100 nm.

They are concerned with steroid and carbohydrates synthesis, lipid metabolism. detoxication processes, depositing calcium.

Golgi apparatus

Golgi apparatus is composed of a series of stacked, flattened, membrane-limited sacs or cisternae and tubular extensions. Small vesicles are seen in association with the cisternae (fig.14). The Golgi apparatus is polarized both morphologically and functionally.

Through transport vesicles that fuse with the Golgi cis face, the complex receives several types of molecules produced in the rough endoplasmic reticulum (RER). After Golgi processing, these molecules are released from the Golgi trans face in larger vesicles to constitute secretory vesicles, lysosomes, or other cytoplasmic components.Proteins, which are synthesized in endoplasmic reticulum, migrate to Golgi apparatus, where they are stored and condensed into granular for secretion. Lysosomes may also be produced in the Golgi apparatus.

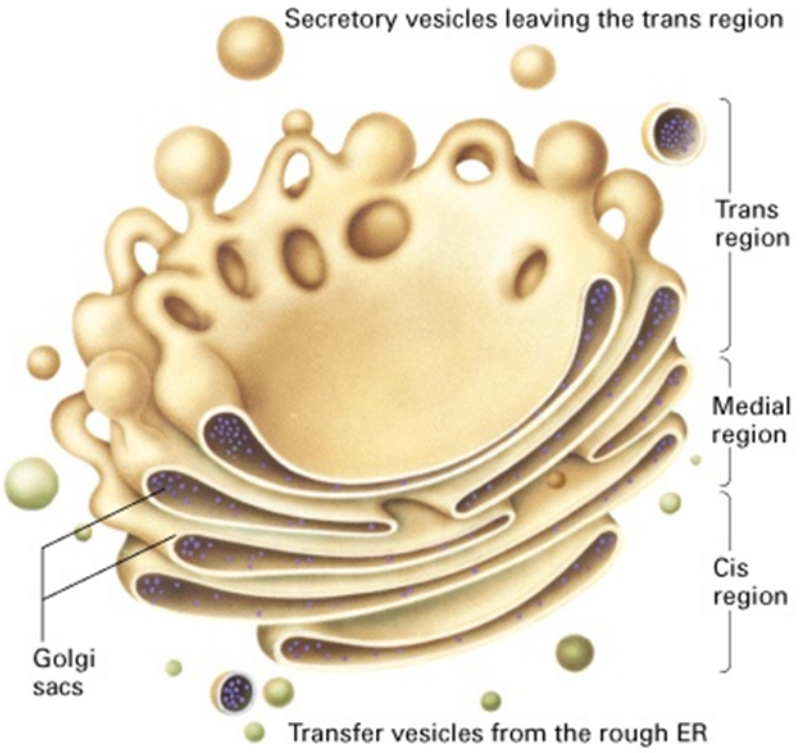


Figure 14. The model of the Golgi apparatus.

Lysosomes

The lysosomes are sites of intracellular digestion and turnover of cellular components (fig.15). Lysosomes are membrane-limited spherical vesicles that contain a large variety of hydrolytic enzymes (more than 40). Lysosomes are present in almost all cells, but they are particularly abundant in cells with phagocytic activity (macrophages, neutrophilic leukocytes).

Lysosomes that have not entered into a digestive event are called as primary lysosomes.

Secondary lysosomes are those in which digestion occurs.

Secondary lysosomes result from the fusion of endocytosis material with primary lysosomes to form phagosome. Secondary lysosome is also known as a phagolysosome.

Following digestion of the contents of the secondary lysosome, nutrients diffuse through the lysosomal membrane and enter the cytoplasm.

Undigestable compounds are retained within the vacuoles, which are now called residual bodies.

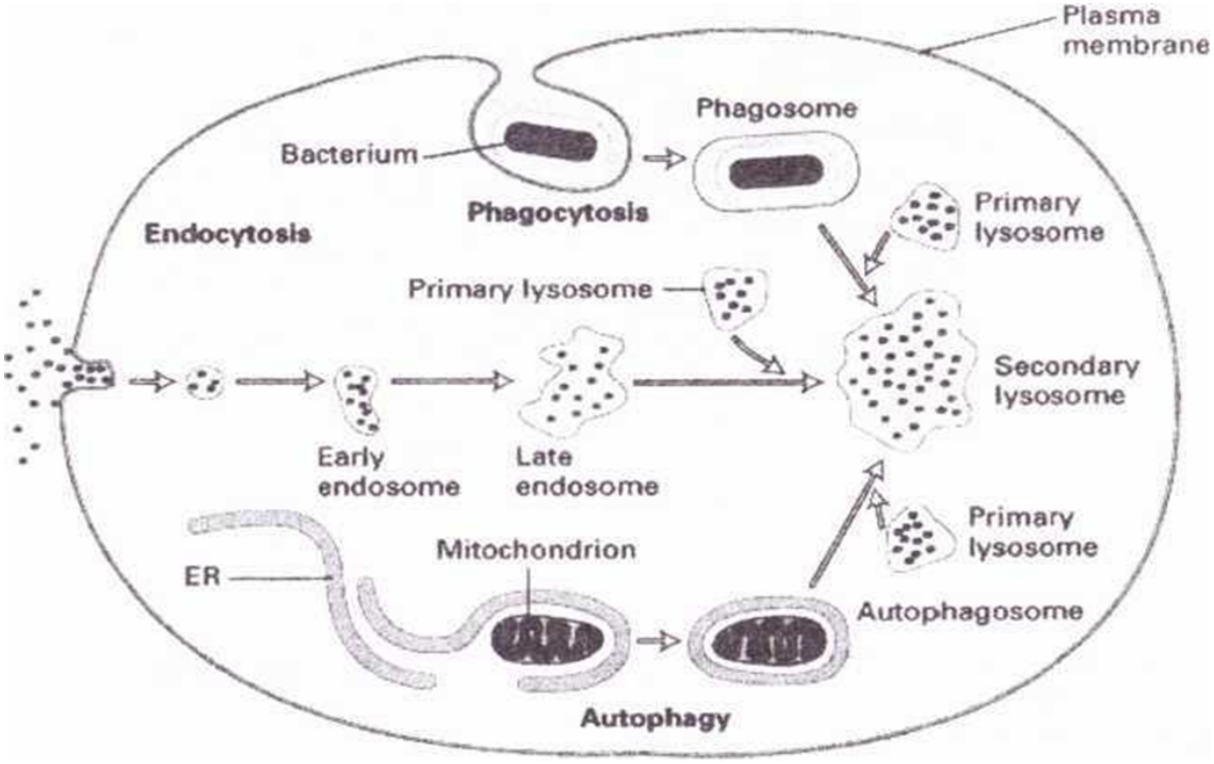


Figure 15. Schematic diagrams of the lysosomes.

Another function of the lysosomes concerns the turnover of cytoplasmic organelles. Primary lysosomes fuse with this structure and initiate the lysis of the enclosed cytoplasm. The resulting secondary lysosomes are known as autophagosomes.

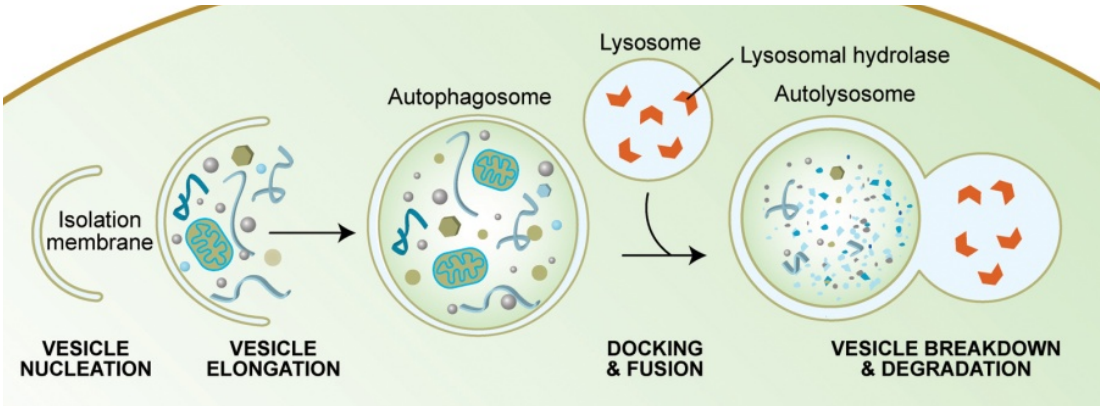


Figure 16. Schematic diagrams of the autolysosomes.

Peroxisomes

Peroxisomes are small (0,5 jam diameter) spherical membrane- limited organelles that contain oxidative enzymes, particularly catalase and other peroxidative enzymes. The oxidative enzymes react with other substances to form hydrogen peroxide (H_2O_2). Hydrogen peroxide is toxic substance. Catalase decomposes hydrogen peroxide to water and oxygen ($2H_2O_2$ —$+2H_2O$ $+O_2$). Peroxisomes protect the cell from the effects of hydrogen peroxide, which could cause damage to many important cellular constituents.

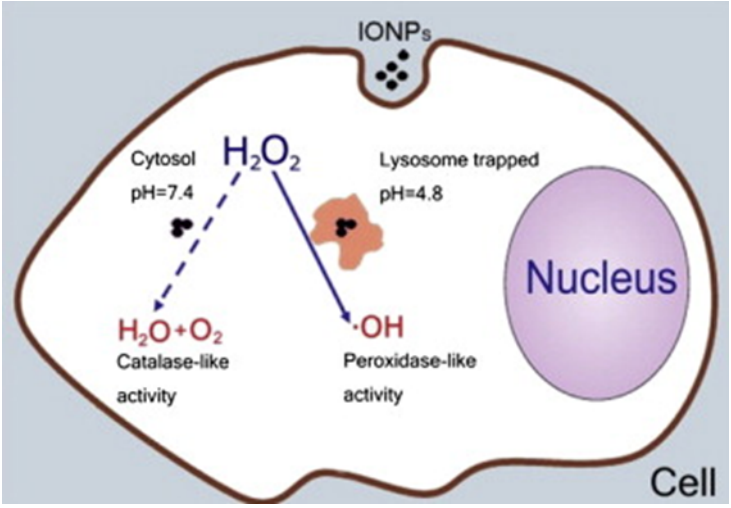


Figure 17. Schematic diagrams of the peroxisomes.

Nonmembranous organelles

Ribosomes

Ribosomes are present in relation to rough endoplasmic reticulum. They may also lie free in the cytoplasm. They may be present singly (monosomes) or in groups (polysomes). "Free" ribosomes synthesize proteins that will remain in the cell as cytoplasmic structural or functional elements. Polysomes of the rough endoplasmic reticulum synthesize proteins for export from the cell and integral proteins of the plasma membrane.

Each ribosome consists of proteins and ribonucleic acid (RNA). Ribosome consists of 2 subunits of different size (fig.18). Ribosomes play an essential role in protein synthesis.

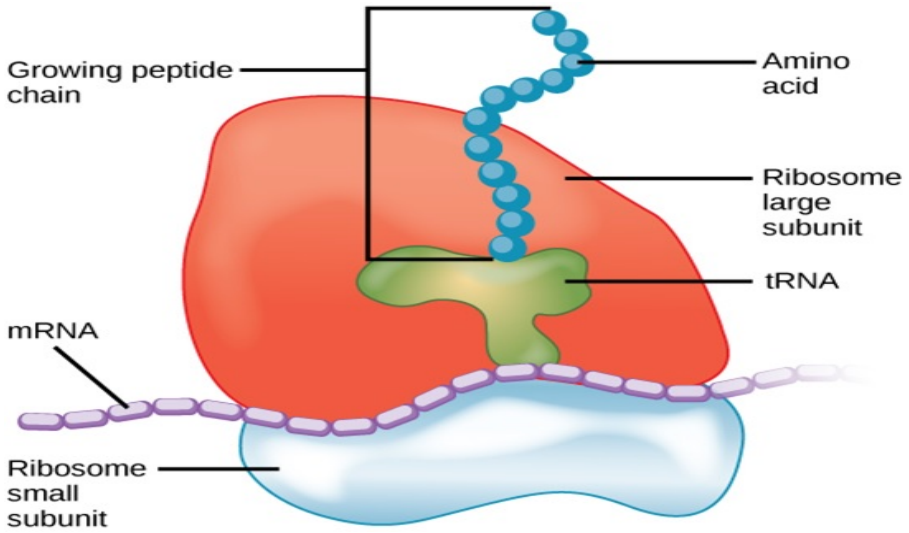


Figure 18. Schematic diagram of ribosome.

The cytoskeleton

The cytoplasmic matrix contains a complex network of microtubules, microfilaments, and intermediate filaments.

These structural proteins not only provide for the form and shaping of cell but also play an important role in cytoplasmic and cellular movements.

Microtubules are thin elongated elements of cell cytoplasm; they are circular in cross section (fig.19) with diameter of 24 nm.

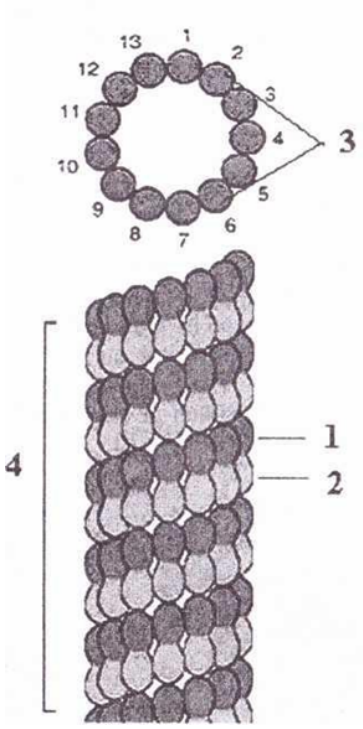


Figure 19. Schematic representation of microtubule. 1 - a-tubuiin molecules, 2- p-tubulin molecules, 3 - subunits, 4 - microtubule

The subunit of a microtubule composed of a- arid P-tubulin molecules. Under appropriate conditions tubulin subunits polymerize to form microtubules. A total of 13 subunits are present in one complete turn of the spiral.

Microtubules provide the basis of several complex of cytoplasm components, including centrioles, basal bodies, cilia, and flagella.

Centrioles are cylindrical structures which composed of highly organized microtubules (fig.20). Centrioles lie at right angles to each other. Each centriole is composed of 9 triplets of microtubules (9x3 + 0). Centrioles play important role in the formation of the mitotic spindles of dividing cells.

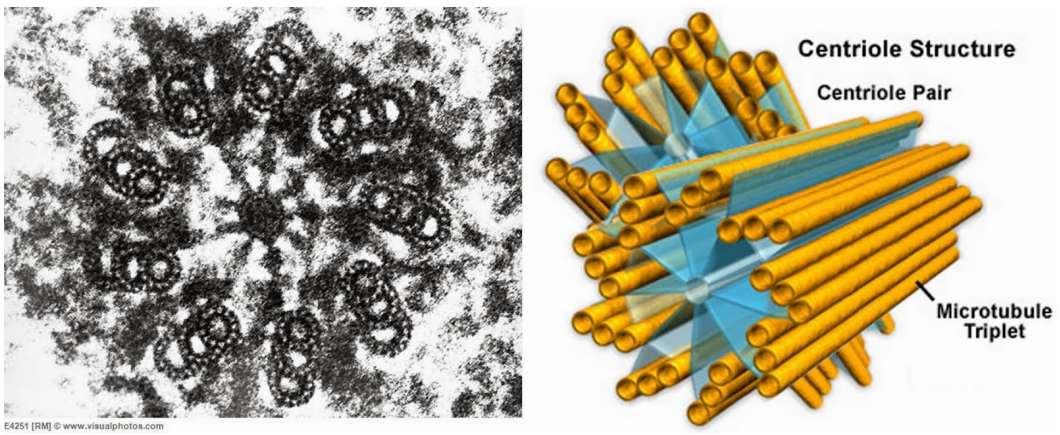


Figure 20. Schematic diagram of cell centre (I) and centriole (II).

Cilia (fig. 21) and flagella are motile processes with a highly organized microtubular core; they extend from the surface of some cell types.

Ciliated cells usually possess a large number of cilia that range 2 to 10 nm in length. Flagellated cells normally have only 1 flagellum, which ranges in length from 100 to 200 nm. The core of these structures consists of 9 pairs of microtubules surrounding 2 central tubules (doublets) (9 x-2 + 2).

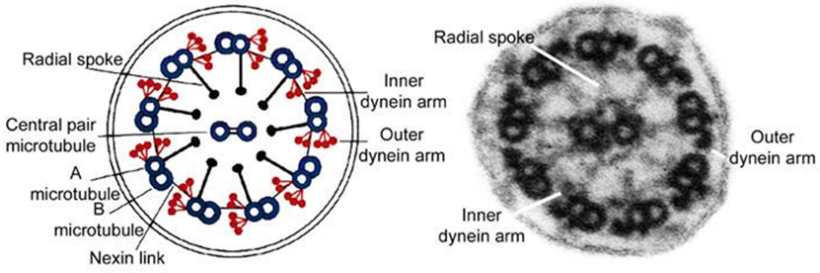
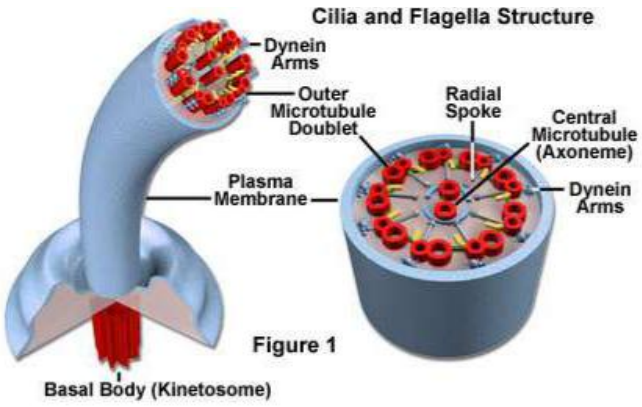


Figure 21. Schematic diagram of flagella.

At the base of each cilium or flagellum is a basal body. This body is identical to a centriole (9 x-3 + 0).



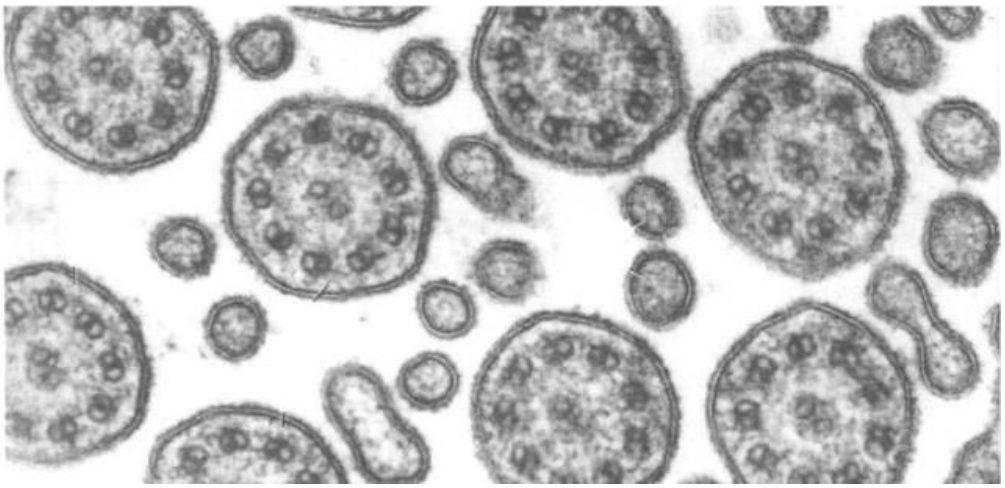


Figure 22. Schematic diagram of cilium . 1 - axoneme (9 x 2 + 2), 2 - basal body (9x-3 + 0).

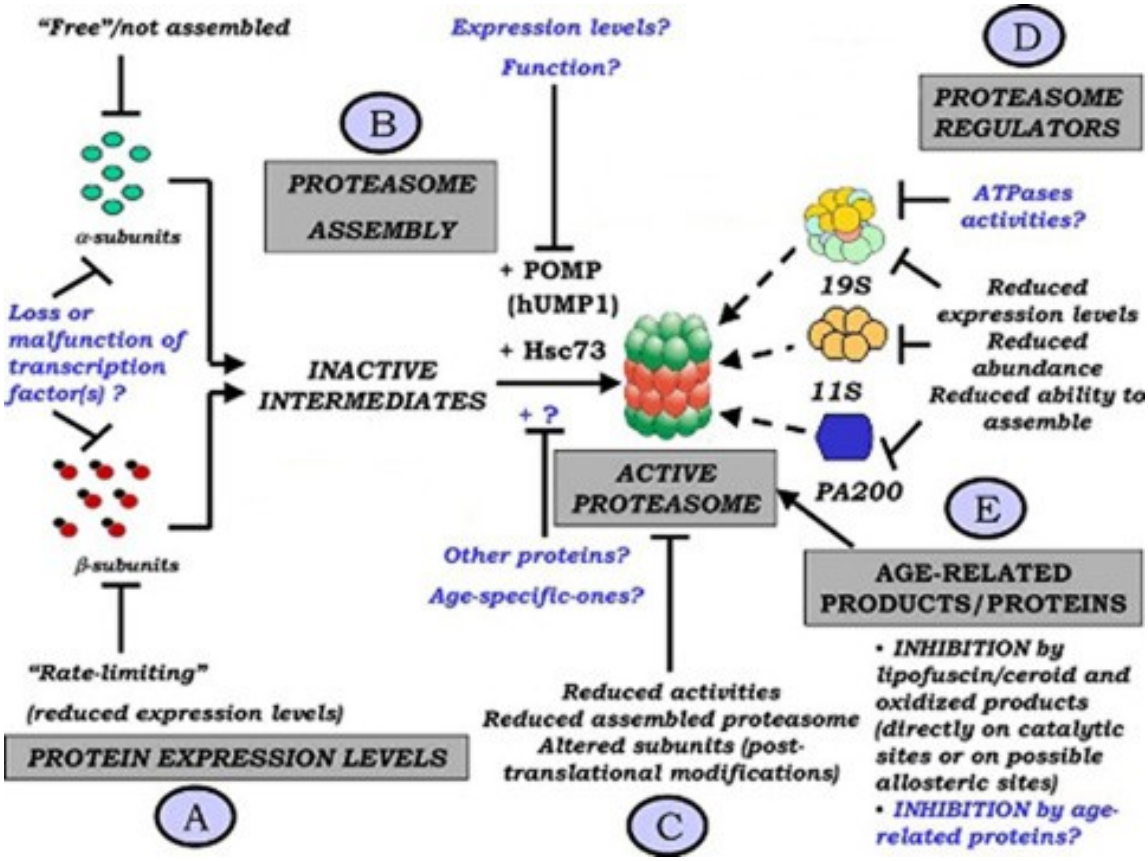
Proteasom

Proteasome - submicroscopic membrane organelle no purpose, which serves as a splitting of protein molecules with primary defective or damaged structure. Proteasome - a large poliprotease complex. Every cell in the human body contains about 30 thousand. Proteasomes. The molecular weight of this organelle is about two million Daltons. Each proteasome consists of tubulary and one or two regulatory units; located on the last one or two ends organelles. Tubular part contains four

rings located in series, each of which consists of seven subunits that surround the central channel proteasome. Regulatory parts recognize and attach proteins intended for destruction. They provide the unwinding of protein molecules and pushing them into the central channel tubulary part where proteases cut them into pieces of different lengths. These fragments are then split other enzymes to amino acids used for the synthesis of new proteins.

In the mechanism of recognition proteins to be split in the proteasome, the main role of ubiquitin process, ie joining the protein molecules of the protein ubiquitin.

Numerous studies have shown that ubiquitin proteins and their subsequent destruction in the proteasome provides the normal course of many processes, regulation of intracellular metabolism, immune surveillance, the release of abnormal protein molecules, cell division and intercellular communication, development and growth of the body, circadian rhythms. Violation of the same mechanisms and ubiquitin slowing or blocking the destruction of proteins in the proteasome is a manifestation of some genetic abnormalities (eggs cystic fibrosis), neurodegenerative disorders (Parkinson's disease, Alzheimer's disease), many viral diseases, carcinogenesis.



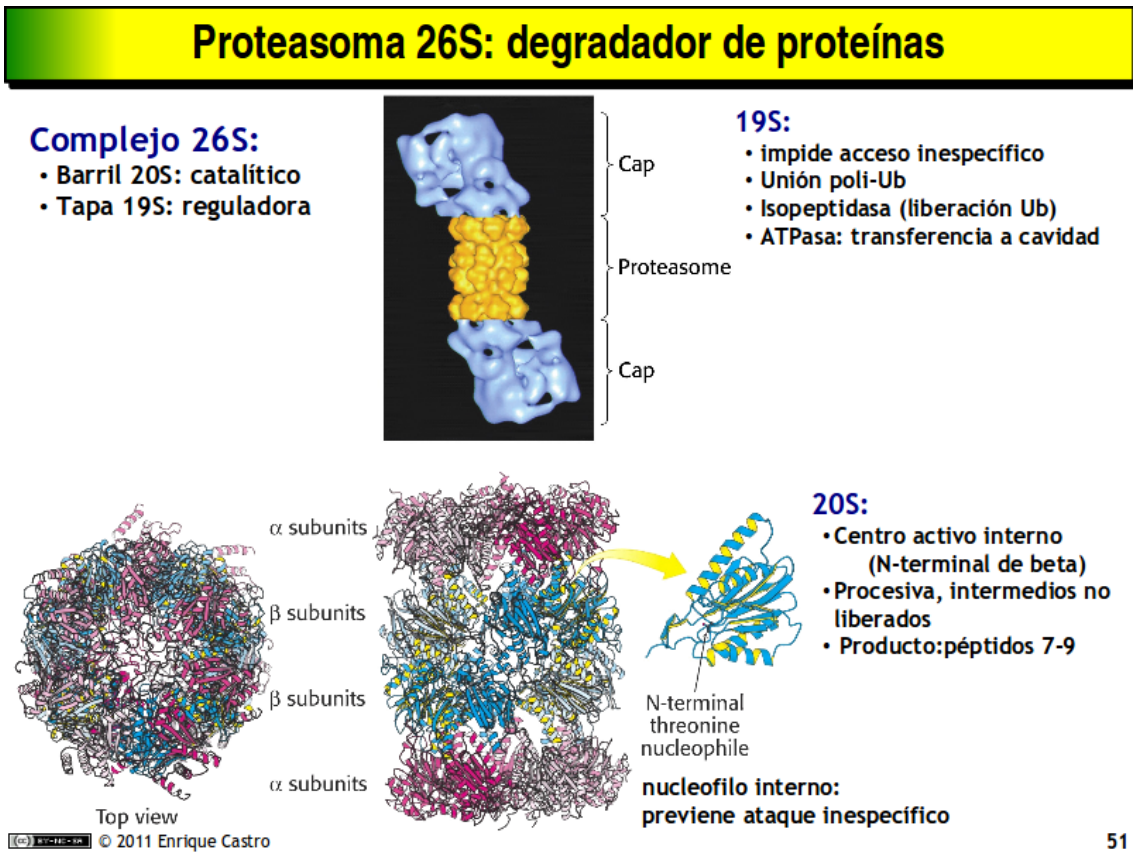


Figure 23. Schematic diagram of proteasome.

Abnormalities in microtubules and filaments. The proteins are proteins that stabilize microtubules. They are abundant in neurons in the central nervous system. The proteins interact with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. When the proteins are defective, the microtubules disintegrate, collapsing the neuron's transport system. This may result first in malfunctions in biochemical communication between neurons and later in the death of the cells. It can result in dementias, such as Alzheimer's disease - incurable, degenerative, and terminal disease.

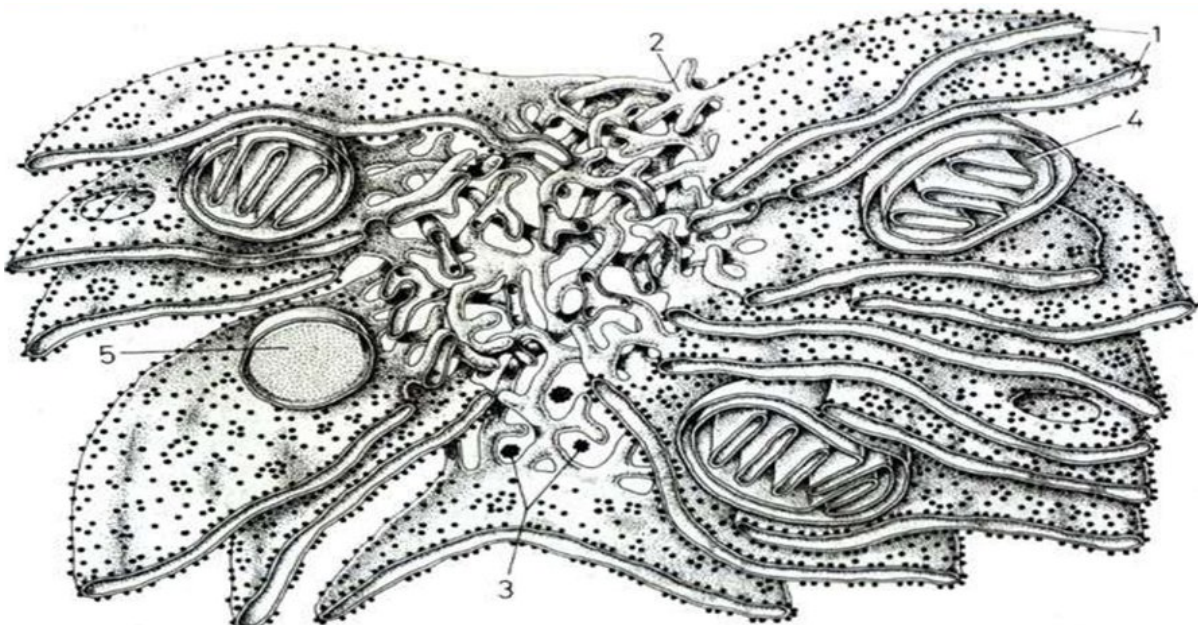
Lysosomal storage diseases are a group of inherited metabolic disorders that result from defects in lysosomal function. Lysosomal disorders originate from an abnormal accumulation of substances inside the lysosome. When the lysosome doesn't function normally, excess products destined for breakdown and recycling are stored in the cell. Lysosomal storage diseases affect mostly children and they often die at a young age. The symptoms of lysosomal storage disease can include developmental delay, movement disorders, seizures, dementia, deafness and/or blindness. Some people with lysosomal storage disease have enlarged livers (hepatomegaly) and enlarged spleens (splenomegaly), pulmonary and cardiac problems, and bones that grow abnormally.

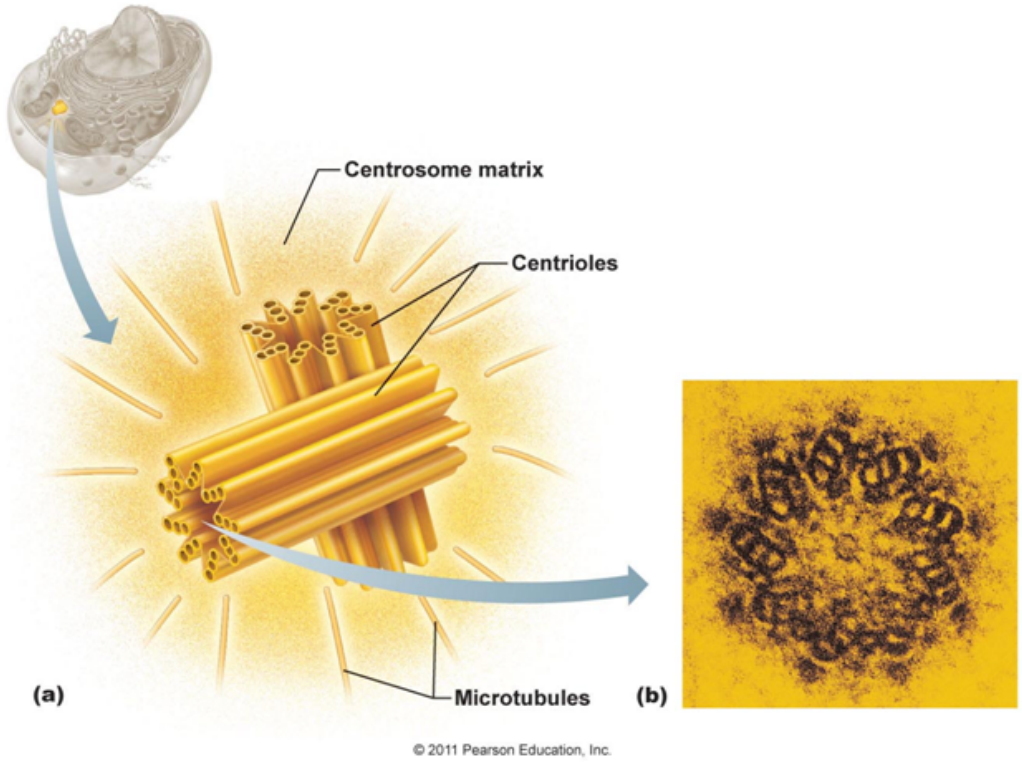
Mitochondrial diseases are a group of disorders caused by dysfunctional mitochondria. Mitochondrial diseases are often caused by mutations to mitochondrial DNA that affect mitochondria function. Symptoms include poor growth, loss of muscle coordination, muscle weakness, visual problems, hearing problems, learning disabilities, mental retardation, heart disease, liver disease, kidney disease, gastrointestinal disorders, respiratory disorders, neurological problems, autonomic dysfunction, and dementia.

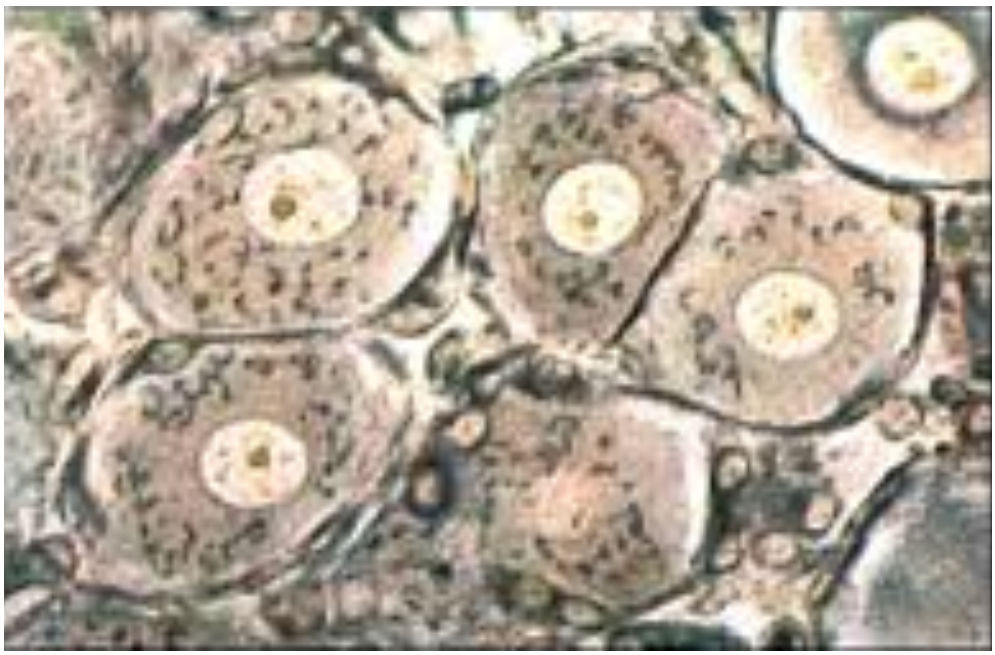
Practical lessons № 3

Questions for self-control

1. What are the criteria for classification organelles? 2. The structure and function of mitochondria, endoplasmic reticulum and Golgi complex. 3. Structure, function and classification lysosomes. 4. Structure and function of peroxisomes. 5. What nemembranni organelles. 6. Structure and function of microtubules, microfilaments and cell center. 7. Name the organelles special purpose. 8. Structure and function of cilia, microvilli, microfibrils. 8. What is cytoplasmic inclusion? 9. Classification inclusions.







The teacher's signature:

NUCLEUS. NUCLEAR ENVELOPE. CHROMATIN. CELL DIVISION

NUCLEUS. The nucleus is an essential component of the cells; it takes a deep base stain.The nucleus is usually spherical; but oval in columnar cells; flattene in squamous cells; rod shaped in smooth muscle cells.

The nuclei are central in most cells; basal in mucous cells; peripheral in skeletal muscle fibers.

Most cells have one nucleus. Occasionally binucleate cells are found in urinary bladder epithelium, liver and cardiac muscle. Skeletal muscle fibers are multinucleated.

The functions of the nucleus

1. Keeping of the genetic information (in the molecules of DNA).
2. Realization of the genetic information.
3. Reproduction and transfer of the genetic information (during the cell division).

The structure of the nucleus

The interphase nucleus has 4 parts: nuclear envelope, chrotnadn, nucleolus, and nuclear matrix (fig.24).

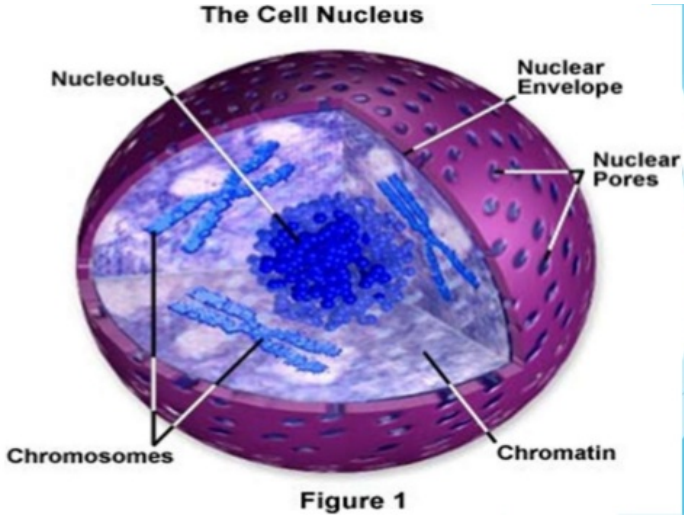


Figure 24. Schematic diagram of structure of a nucleus.

Nuclear envelope

The nucleus is surrounded by 2 parallel unit membranes separated by a narrow space called perinuclear cisternal space. Together, the paired membranes and intervening space make up the nuclear envelope.

The outer layer of the nucleus membrane is continuous with the endoplasmic reticulum.Closely associated with the inner membrane of the nuclear envelope is a protein structure called fibrous lamina. The fibrous lamina forms part of the nuclear matrix. Around the nuclear envelope, at sites where inner and outer membranes fuse, there are circular gaps, the nuclear pores.The nuclear pore function is bidirectional nucleocytoplasmic transport.The nuclear pore complex (NPC) (fig.25) consists of an assembly of eight spokes arranged around a central channel.

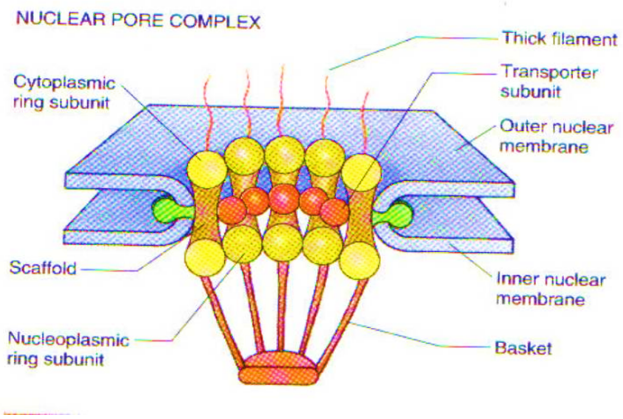


Figure 25. Schematic cross-section of the nuclear pore complex.

The spokes are connected to rings at the nuclear and cytoplasmic surfaces, and the spoke-ring assembly is anchored within the nuclear envelope at sites of fosion between the inner and outer nuclear membranes. Protein filaments extend from both the cytoplasmic and nuclear rings, forming a distinct basketlike structure on the nuclear side. The central channel is approximately 40 nm in diameter, which is wide enough to accommodate the largest particles able to cross the nuclear envelope. It contains a structure called the central transporter, through which the active transport of macromolecules is thought to occur. The nuclear pore complex provides a channel for transport of substances between the nucleus and the cytoplasm. There are roughly 3000 NPCs situated in the nuclear envelope. Smaller molecules that are less than 9 nm in diameter, like ions and metabolites, may freely diffuse through the NPC between the nucleus and the cytoplasm. Larger molecules, between 9 and 28 nm in diameter, must be actively transported through the NPC in a controlled process that is selective and energy dependent.

Chromatin is little blue staining particles within the nucleus. Chromatin (fig.26) is composed mainly of coiled strands of deoxyribonucleic acid (DNA) bound to basic proteins (histones). The basic structural unit of chromatin is the nucleosome. It consists of a core of 8 histone molecules. Approximately 2 loops of DNA are wrapped around the core octamer. A long strand of nucleosomes is coiled to produce a unit chromatin fibril about 30 nm in diameter.

In dividing cells chromatin is condensed and organized into discrete bodies called chromosomes. 2 types of chromatin can be distinguished with both the light and electron microscopes.

Heterochromatin is coiled segments of chromosomes and stain deep blue. It forms the chromatin particles of interphase nucleus, and is inert and inactive.

Euchromatin is uncoiled segments of chromosomes and stain poorly or not at all. it is active and directs the cell activities in the production of protein.

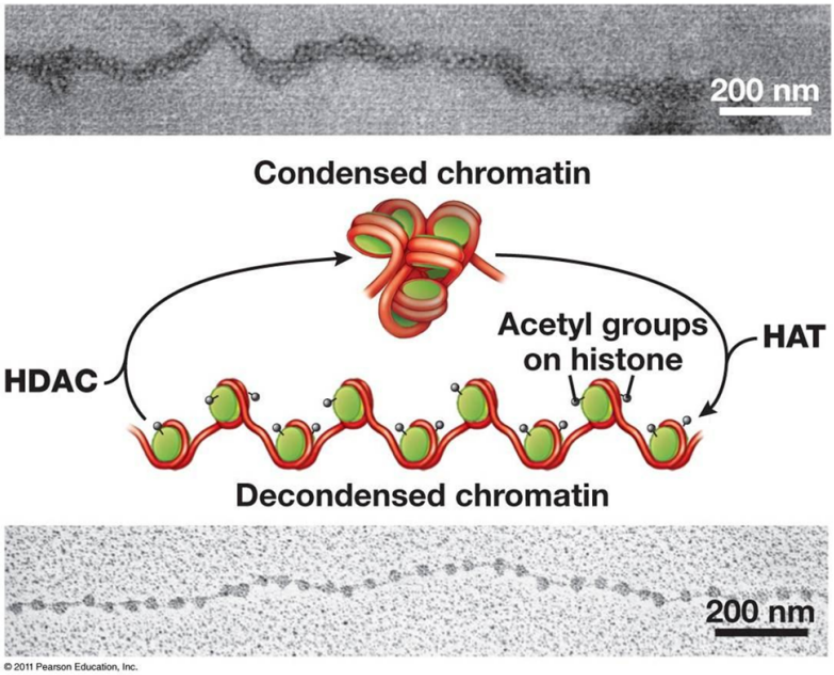


Figure 26. Orders of chromatin packing.

Chromosomes are little rod-like bodies in the nucleus, which take a deep basic stain (fig.27).Each chromosome is formed by 2 chromatids that are joined together at a point called the centromere. Each chromatid has 2 arms, one on either side of the centromere. Each chromosome differs from one another in total length and in the relative length of the two arms.

The chromosome pattern of an individual is known as Chromosomes control the heredity and activities of the cell.

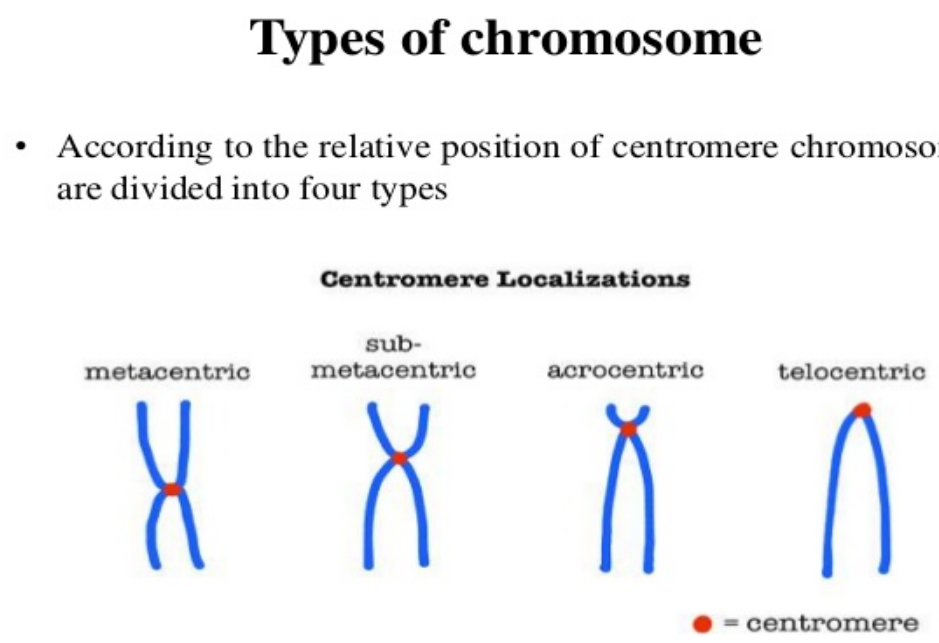


Figure 27. Structure of chromosome.

Nucleolus.The nucleolus is a spherical structure, usually basophilic when stained with hematoxylin and eosin (flg.28).

The nucleolus consists of:

1) densely packed ribonucleoprotein fibers, the pars fibrosa, which is composed of primary transcripts of rRNA genes, and is situated mainly in the central part of nucleolus;
2) the pars granulosa, consisting of granules (maturing ribosomes);
3) nucleolar organizer DNA.
4) Proteins, synthesized in the cytoplasm, become associated with rRNA in the nucleolus; ribosome subunits then migrate into the cytoplasm.

Nuclear matrix

The nuclear matrix is the component that fills the space between the chromatin and the nucleoli in the nucleus. It is composed mainly of proteins (some of which have enzymatic activity), metabolites, and ions. The fibrous lamina of the nuclear envelope is a part of the nuclear matrix.

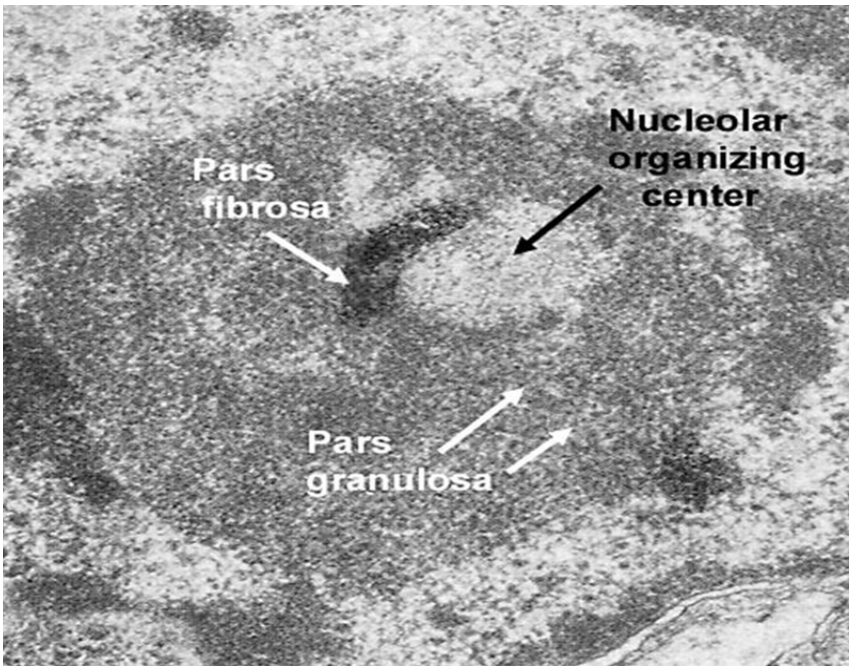


Figure 28. Electron micrograph of a nucleolus.

Cell division

The cell division or mitosis can be observed with the light microscope. During this process, the parent cell divides, and each of the daughter cells receives a chromosomal karyotype identical to that of the parent cell.

Mitosis

Mitosis (fig.29) is characterised by a series of changes in which there is equal division of nuclear material (kuryokinesis), followed by the division of the cell (cytokinesis).Phases of mitosis are prophase, metaphase, anaphase, telophase

Prophase

The nuclear membrane and the nucleolus disappear. The chromatin granules resolve into chromosomes. As a prelude to mitosis each chromosome is duplicated, each of which is called a chromatid The paired chromatids lie side by side and are united at one point only termed the centromere. The centrioles divide, move in the opposite direction and finally take up positions at opposite poles of the cell. The centrioles are connected by microtubules forming mitotic spindle.

Metaphase

The chromosomes migrate to the equatorial plane of the cell, where each divides longitudinally to form two chromatids (metaphase plate).

Anaphase

The centromere divides and the two chromatids become completely separated from each other. The spindle tubules pull the separated group of chromatids to opposite poles. Two groups of chromatids, now chromosomes are identical.

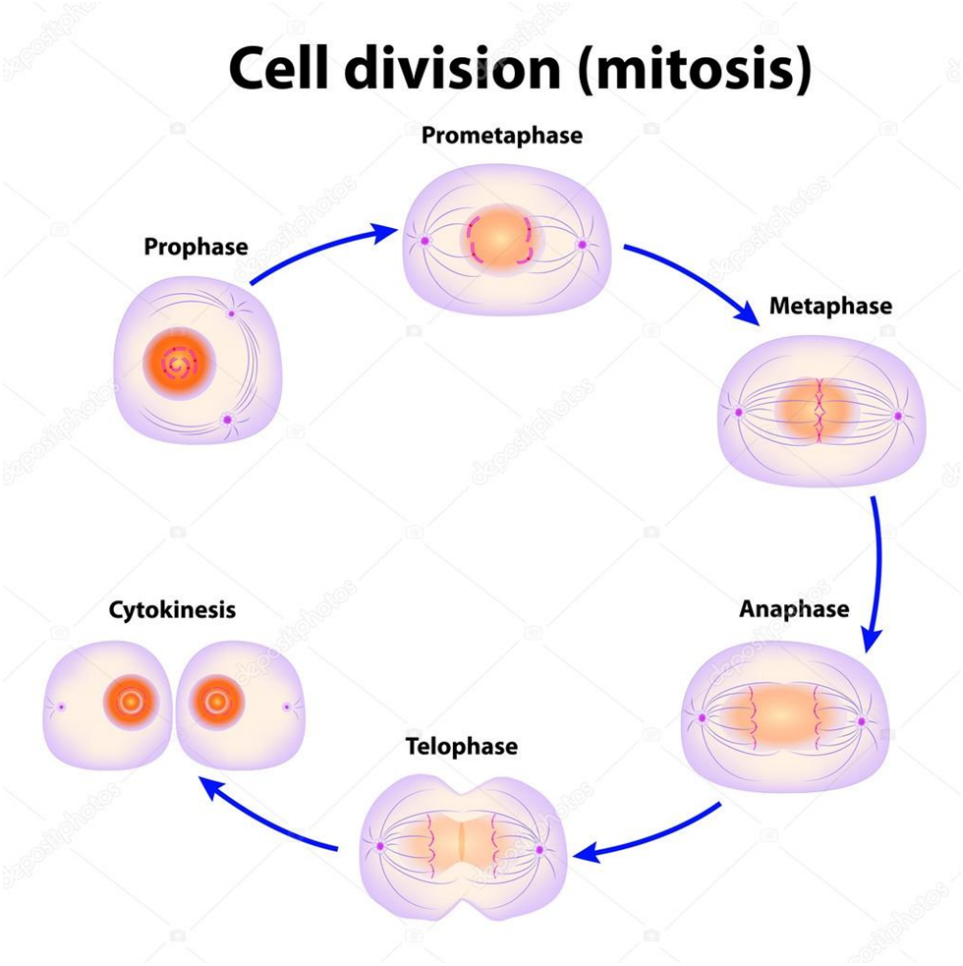


Figure 29. Schematic diagram of phases of mitosis.

Telophase

Constriction develops at the equator of the elongated cell. It deepens and divides the cell into two daughter cells. In each daughter cell, the chromosomes lose their identity, become uncoiled to varying extent and are seen as chromatin particles. The nucleoli and the nuclear membrane have reformed.

Endomitosis is a process by which chromosomes replicate without the division of the cell nucleus.

Polyploidy is the state of having greater than the diploid content of DNA, has been recognized in a variety cells (liver cells, megakaryocytes of red bone marrow). Polyploid cells are larger than diploid ones; not surprising in view of the increased amount of DNA in their nucleus.

Meiosis

Meiosis occurs only in sex cells during maturation, and comprises of first and second meiotic divisions.

The first meiotic division

1. Prophase is divided into 4 substages:

-leptoten: chromosomes become prominent and stain deeply with basic dyes;

- zygotene:23 pairs of genetically homologous chromosomes become attracted to each other and lie side by side; this Is called pairing (conjugation) of chromosomes;

- pachytene (tetrad formation and coiling): each chromosome splits into two chromatids except at centromere, and forms tetrads, the chromatids become partially coiled around each other;

-diplotene (interchange of segments): homologous chromosomes move apart except at chiasmata Chiasmata are points of contact, where exchange of genetic material takes place between two chromatids. At the chiasmata parts of two chromatids (one from each homologous pair) break and then join diagonally. This is called crossover, which results in redistribution of genetic material. Finally the chromosomes (with two chromatids) uncoil and slip apart (diakinesis);

2. Metaphase: chromosomes with paired chromatids arrange themselves at the equator;
3. Anaphase: centromeres do not divide and each member of homologous chromosomes, consisting of a pair of chromatids, is dragged to opposite poles of the spindle;
4. Telophase: cell divides into two containing one half (haploid) chromosomes.

The second meiotic division follows closely after the first meiotic division with practically no resting stage. This is exactly like mitosis but the phases are much shortened. Four cells are derived from the two cells of 1st meiotic division. Each has haploid number of chromosomes and each has its own particular share of the genetic material.

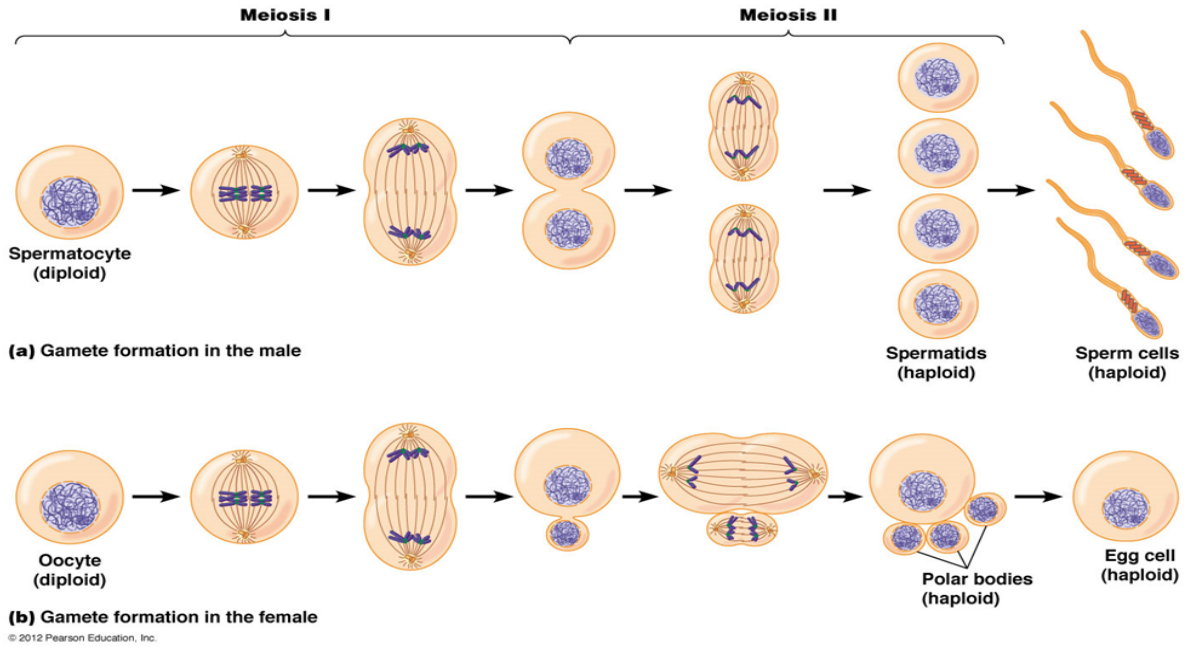


Figure 30. Schematic diagram of phases of meiosis

Cell cycle

The alternation between mitosis and interphase is known as the It can be divided into two stages: mitosis, consisting of four phases already described (prophase, metaphase, anaphase, and telophase), and interphase.

Interphase is divided into three phases: G_1 (presynthetic), S (DNA synthesis), and G_2 (post-DNA duplication).

It is during the G_1; phase that RNA and protein synthesis occur and the cell volume, previously reduced to one-half by mitosis, is restored to its normal size.

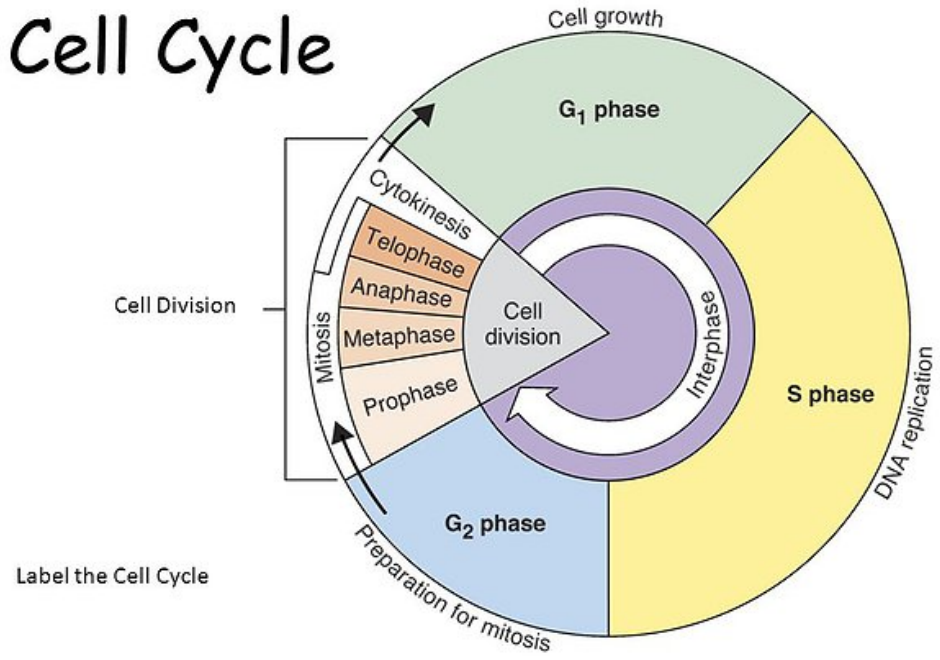


Figure 31. Schematic diagram of cell cycle.

In cells that are not continuously dividing, the cell cycle activities may be temporarily or permanently suspended. Cells in such a stage of development are referred to as being in a G_0 phase:

1. neurons;
2. hepatocytes;
3. epithelial and blood stem cells.

Synthesis and replication of DNA and centrioles take place in the S phase.

Processes that occur during the G2 phase are the production and accumulation of energy to be used during mitosis and the synthesis of tubulin to be assembled in microtubules during mitosis.

Cell death

Cell death may occur as a result of acute cell injury or an internally encoded suicide program. Cell death may result from accidental cell injury (necrosis) or mechanisms that cause cells to self-destruct (apoptosis).

Necrosis, or accidental cell death. Necrosis is a pathologic process. It begins with impairment of the cell's ability to maintain homeostasis. Necrosis occurs when cells are exposed to an unfavourable physical or chemical environment (e.g., hypothermia, hypoxia, radiation, low pH, cell trauma) that causes acute cellular injury and damage of the plasma membrane. Under physiologic conditions, damage to the plasma membrane may also be initiated by viruses, substances such as complement, or proteins called perforins.

As a result of cell injury, damage to the cell membrane leads to an influx of water and extracellular ions. Intracellular organelles, such as the mitochondria, rER, and nucleus, undergo irreversible changes that are caused by cell swelling and cell membrane rupture (cell lysis). As a result of the ultimate breakdown of the plasma membrane, the cytoplasmic contents, including lysosomal enzymes, are released into the extracellular space. Therefore, necrotic cell death is often associated with extensive surrounding tissue damage and an intense inflammatory response.

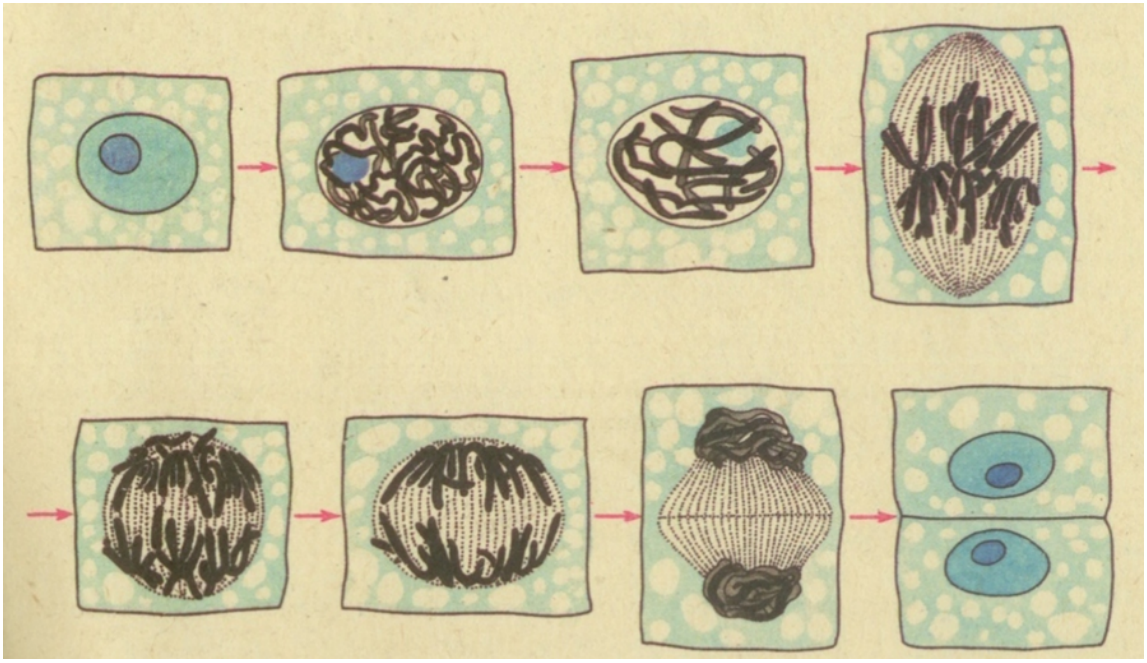
Apoptosis also referred to as programmed cell death. In Greek, apoptosis translates to the "dropping off" of petals or leaves from plants or trees. Apoptosis represents as a physiologic process. Apoptosis is a mode of cell death occurs under normal physiologic conditions. During apoptosis, cells that are no longer needed are eliminated from the organism. This process may occur during normal embryonic development or other normal physiologic processes, such as follicular atresia in the ovaries. Cells can initiate their own death through activation of an internally encoded suicide program. Apoptosis is characterized by controlled autodigestion, which maintains cell membrane integrity; thus, the cell "dies with dignity" without spilling its contents and damaging its neighbours. Cells undergoing apoptosis show characteristic morphologic and biochemical features (flg.3.9) such as DNA fragmentation decrease in cell volume, membrane blebbing without loss of membrane integrity, and formation of apoptotic bodies, causing cell breakage. Apoptotic bodies are later removed by phagocytic cells without inflammatory reactions.

Practical lessons №4

Cytology. Nucleus components. Cell reproduction. Aging and death of cell.

Questions for self-control

1.Nuclear components: structure and functions.
2. Components of the nucleus. Karyolemma (structure and functions).
3. Chromosomes: structure, types, karyotype, functions.
4. Nuclear chromatine (chemical structure and significance).
5. Cells reproduction. Characteristic features of different types of reproduction.
6. The cell cycle: periods, their characteristic features.
7. The cell cycle. Interphase: periods, their significance.
8. Cell death.



The teacher's signature:

BASES OF GENERAL EMBRYOLOGY

Embryology is a science which is about the formation, early growth, and development of an embryo from the fertilization of the ovum to the fetus stage.

Embryogenesis is the process by which the embryo is formed and develops, until it develops into a fetus.

Structure of the spermatozoon

The human male sexual cell - spermatozoon is 60 pm long, actively motile.

Spermatozoon is divided into 3 main parts (fig.32):

- head,
- neck (or connecting piece), and
- tail or flagellum.

Flattened head of spermatozoon includes:

a small dense nucleus with a haploid set of the chromosomes surrounded anteriorly by an acrosome, which contains enzymes as hyaluronidase, corona penetrating enzyme used for penetrating the female egg; acrosome is derivative of Golgi complex.

The neck (connecting piece) is narrow, contains proximal centriole.

The tail consists of middle piece, principal piece and end piece.

The middle piece contains a distal centriole, a central axoneme with many mitochondria spiralled around it (helical mitochondrial sheath), used for ATP production for sperm motility. Axoneme consists of parallel microtubules in a characteristic “9x2+2” pattern, which is surrounded by the longitudinal dense fibers. The principal piece constitutes most of the tail and consists of the axoneme surrounded by a sheath of fibers.

The end piece consists of the axoneme only and is the narrowest part of the sperm.

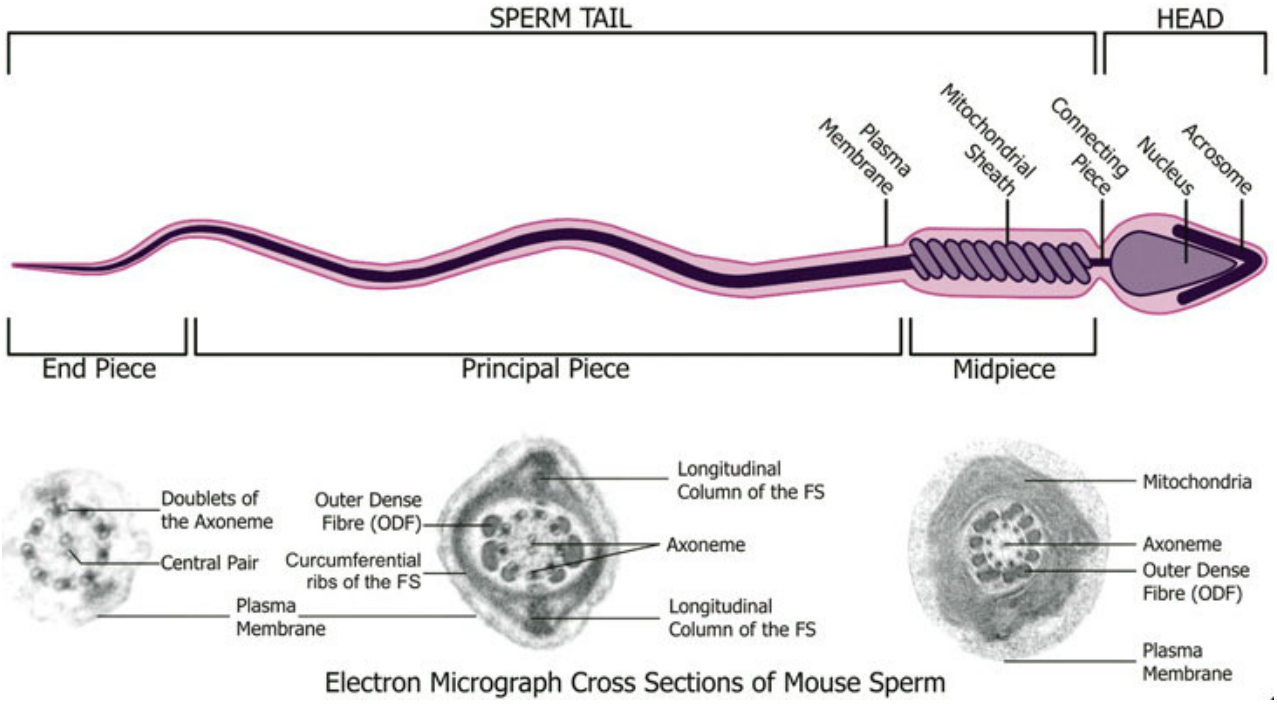


Figure 32. Structure of a spermatozoon.

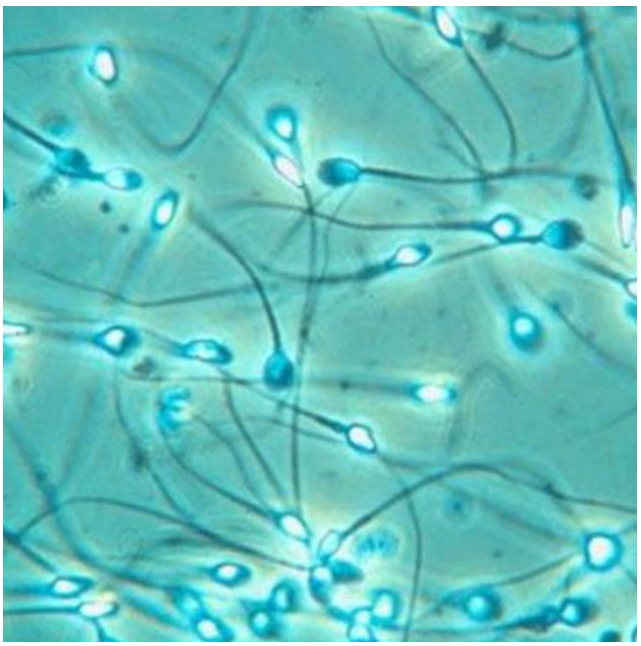


Figure 33. Structure of a spermatozoon.

Structure of the ovum

The ovum (oocyte) is generally spherical, nonmotile gamete with yolky cytoplasm and enclosed in one or more egg envelopes (fig.34).

Size of ovum varies in different animals and depends upon the amount of yolk from 10 micrometers to a few centimetres. Largest sized egg is of ostrich and is about 170 x 135 mm.

The nucleus of an ovum is large, has haploid set of chromosomes.

The cytoplasm contains yolk inclusions, is differentiated into outer, smaller and transparent exoplasm or egg cortex and inner, larger and opaque endoplasm or ooplasm.

Egg cortex is with some cytoskeletal structures like microtubules and microfilaments, and cortical granules of mucopolysaccharides.

Endoplasm is with cell organelles, informosomes tRNAs, histones, enzymes etc. Yolk granules contain proteins, phospholipids and carbohydrates used for a feed of a germ.

The side of ovum with nucleus is called animal pole, while the opposite side is called vegetal pole.

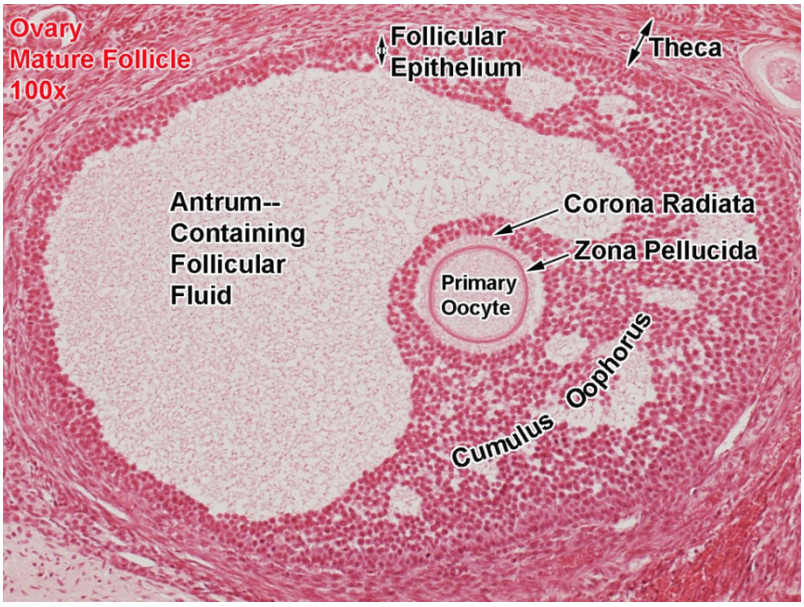


Figure 34. Structure of oocyte.

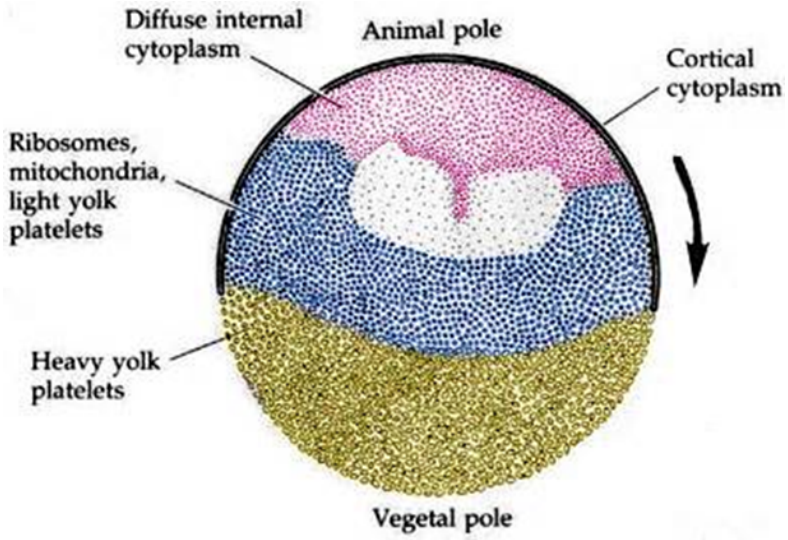


Figure 35. Structure of oocyte.

Classification of the oocytes

Classifications of the oocytes are based on amount and distribution of a yolk (lecithos) in the cytoplasm.

Oocyte classification by amount of the yolk (fig.36):

- microlecithal. oligolecithal (very little yolk):
  - primary (amphioxus, sea urchin);
  - secondary (secondary yolk reduction) (placental mammals);
- mesolecithal (medium amount of yolk) (frog);
- macrolecithal, polylecithal (with large amount of yolk) (bird).

Oocyte classification by distribution of the yolk (fig.37):

- telolecithal (y olk is distributed in gradient, concentrated at one pole of the egg, usually vegetal) (bird);
- isolecithal (yolk is evenly distributed throughout egg cytoplasm of microlecithal oocytes) (human);
-centrolecithal (the placement of the yolk in the centre of the cytoplasm of the oocyte).

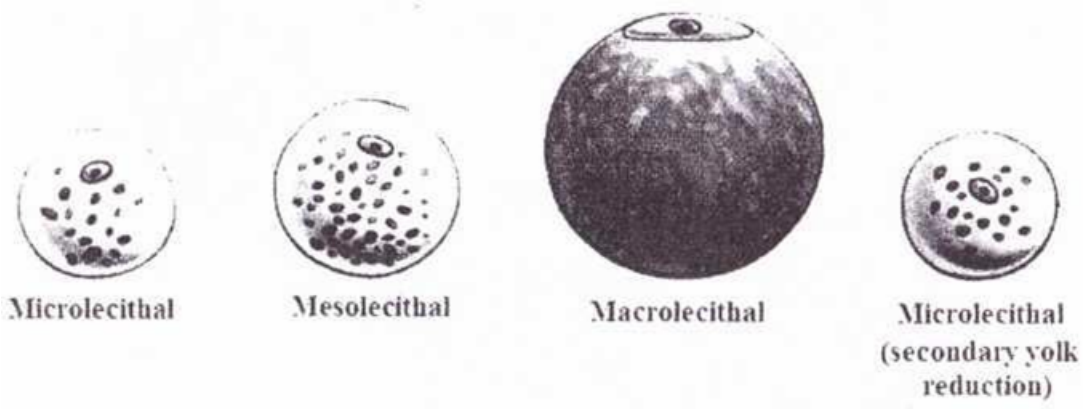
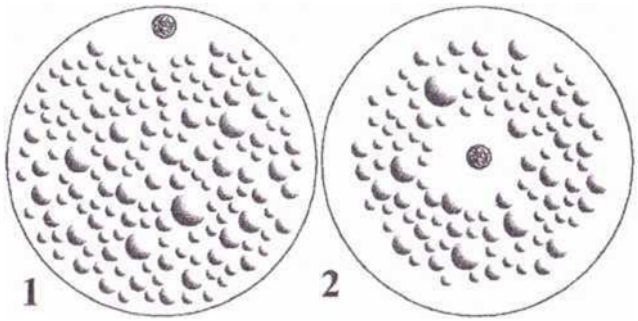


Figure 36. Types of the oocytes by amount of the yolk.



Classification Of Eggs Based Upon Amount Of Yolk

Egg Type	Representative Organisms
a) Alecithal - Almost without yolk.	Placental mammals
b) Microlecithal - Small amount of yolk.	Echinoderms, amphioxus, marsupials
c) Mesolecithal - Moderate amount of yolk.	Lung-fishes, frogs, and toads.
d) Macrolecithal - Very large amounts of yolk.	Sharks, bony fishes, reptiles, birds, insects.

Figure. 37. Types of the oocytes by distribution of the yolk. - telolecithal, 2 – centrolecithal.

Fertilisation

The fertilisation is the fusion of male and female sexual cells

qualitatively new cell — a zygote (impregnated oocyte, or a monocelled germ) (fig.4.5). Fertilization of the ovum occurs in the ampulla of the uterine tube.

During fertilisation distinguish three phases:

1) chemotaxis;
2) sperm activation/acrosomal reaction;
3) sperm/egg adhesion.

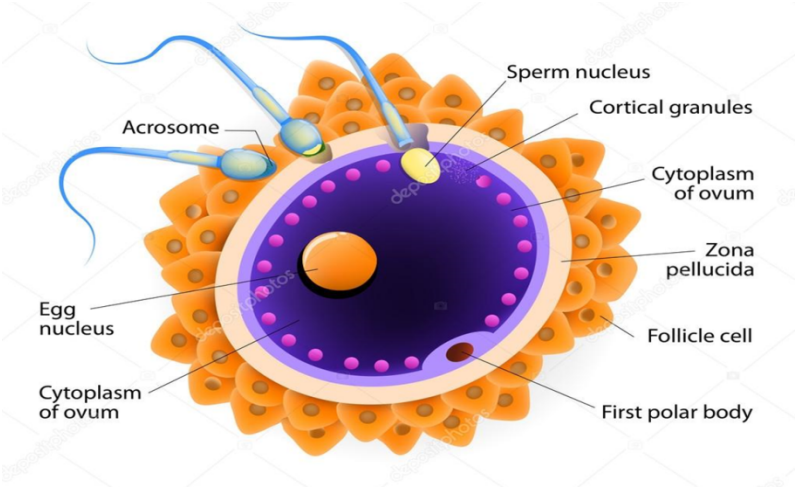


Figure. 38. Structure of the ovum.

1. Chemotaxis is provided by the specific factors, secreted by sexual cells (hamons). During the time the sperm spend in the female reproductive tract, while swimming towards the oocyte, they acquire the capacity to fertilise it - a process called capacitation.

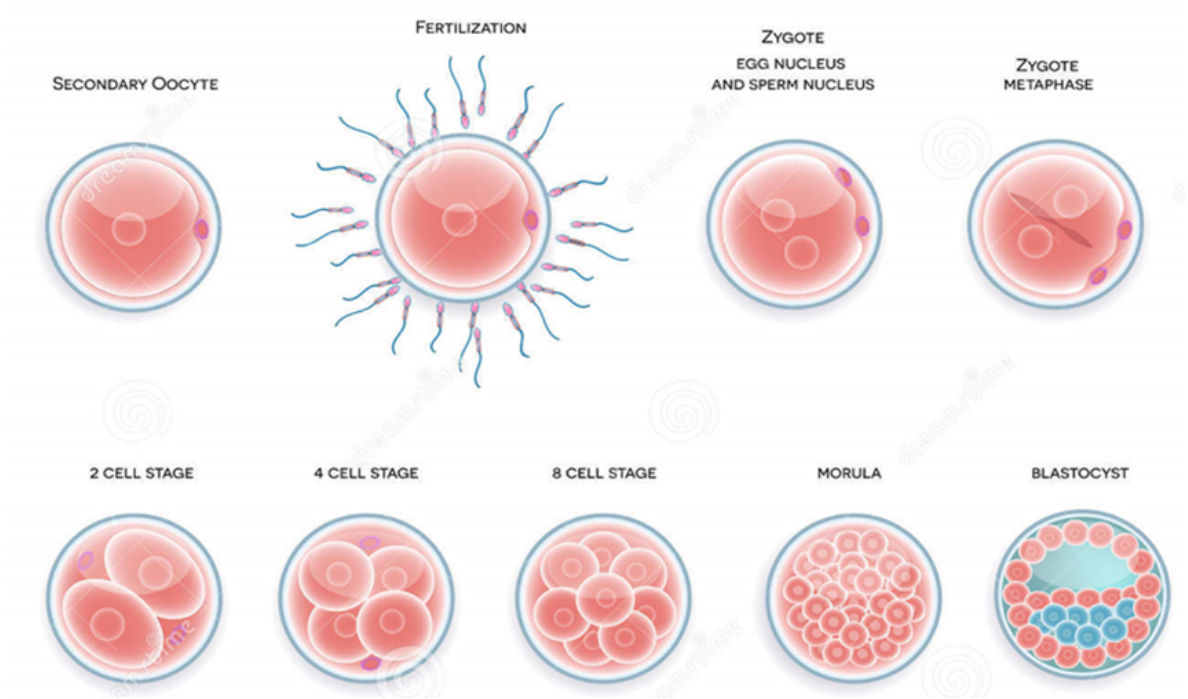


Figure 39. Fertilisation and beginning of cleavage.

When the sperms reach the corona cells of the oocyte they become hyperactivated - they start beating their tails.

The sperms disperse the cumulus oophorus and when they reach the oocyte, they first bind to the zona pellucida. A chemical is released here by the sperms in a process called the acrosomal reaction in which the acrosome is removed.

The acrosomal enzymes dissolve the zona pellucida. At this time, the oocyte transforms the zona to an impenetrable barrier, thus preventing other sperm from entering it (monospermia).

The contact of the sperm with the oocyte surface results in the cortical reaction. The cortical reaction is exocytosis of the egg's cortical granules. When the fertilizing sperm contacts the egg plasma membrane, it causes calcium to be released from storage sites in the egg, raising the intracellular free calcium concentration. This triggers fusion of the cortical granule membranes with the egg plasma membrane, liberating the contents of the granules into the extracellular space. Fusion begins near the site of sperm contact, and then as the wave of calcium release sweeps around the egg, a wave of cortical granule fusion results.

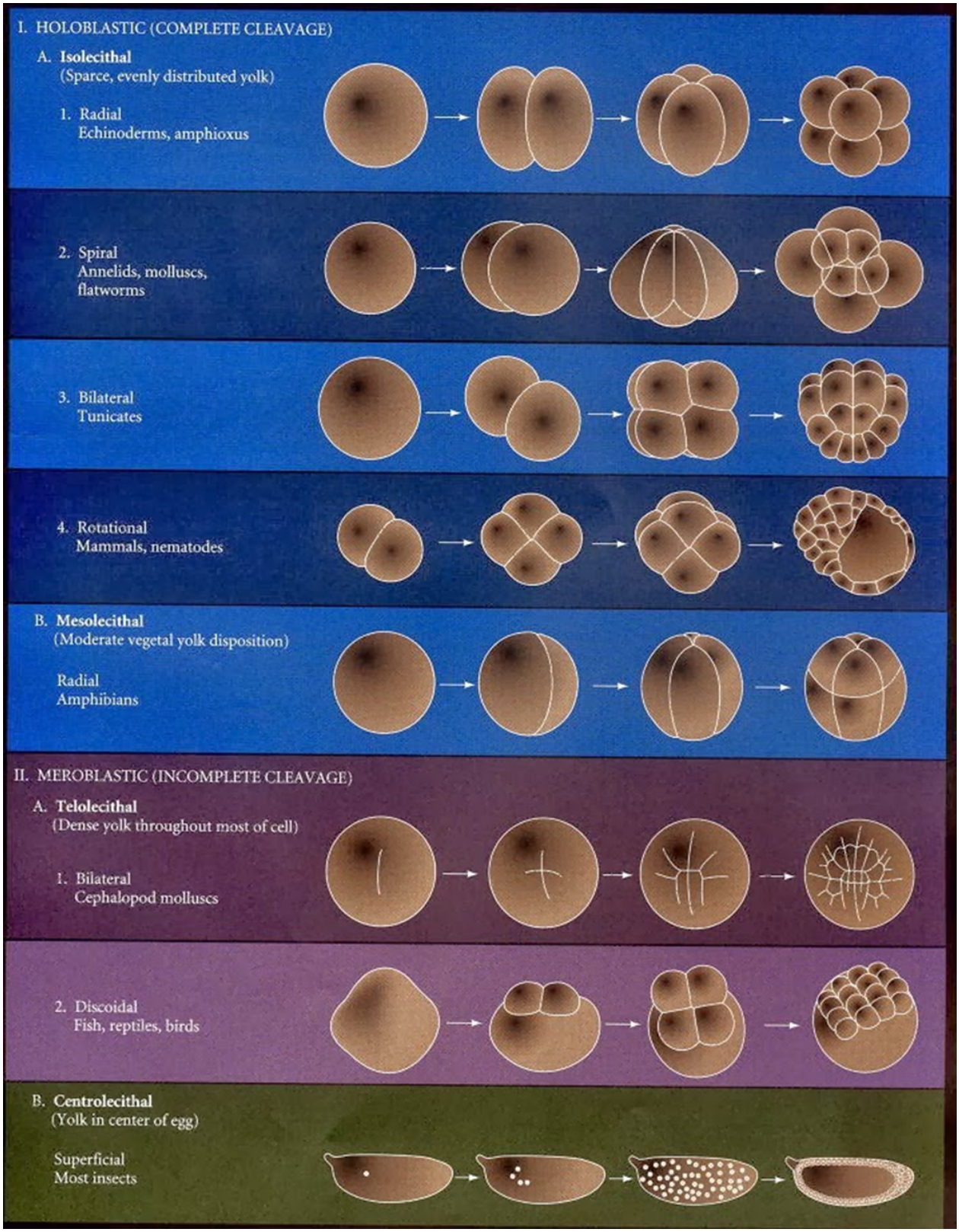
The genetic material of the sperm (the male pronucleus) and the genetic material of the egg (the female pronucleus) then fuse - to form an embryo.

The results of fertilization are:

- restoration of diploid set of chromosomes;
- determination of the sex of new individual;
- beginning of the cleavage.

Cleavage

The cleavage is mitotic division of diploid cell (zygote) with increase the number of cells without increase of their total volume. These cells (blastomeres) become smaller with each cleavage division.



Cleaving cells have a modified cell cycle, in which two phases, G| and G2, are completely omitted. The cells cycle rapidly between M and S phases.

As a result of cleavage the multicellular germ, which looks like a mulberry or a dense congestion of cells (morula) (fig.39), and then as a bubble with a small cavity (blastula) is formed.

Blastula has a wall - blastoderm and a cavity, filled with a liquid

- a product of secretion blastomeres. In blastoderm distinguish the roof formed due to the shattered material of animal pole, a bottom — from a material of a vegetal pole and the regional zones located between them (fig.40).

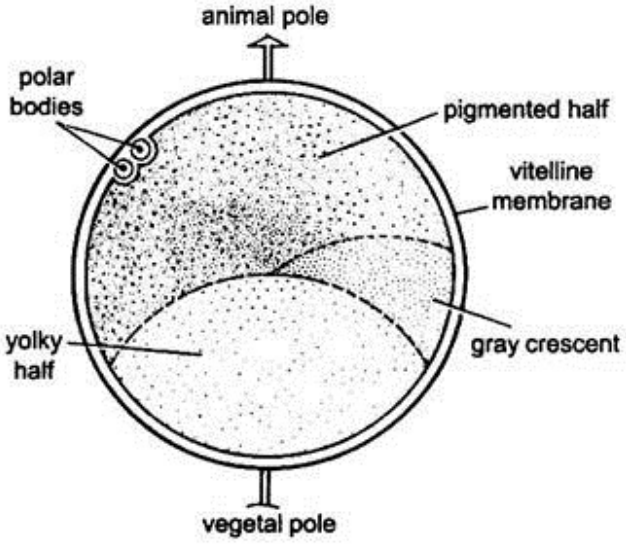


Figure 40. Fertilisation and cleavage.

The pole of the egg with the highest concentration of yolk is referred to as the vegetal pole while the opposite is referred to as the animal pole (fig.40).

Gastrulation

The gastrulation is complex process of chemical and morphogenetic changes, accompanying with reproduction, growth, directed moving and differentiation of cells therefore germ layers are formed: external (ectoderm), middle (mesoderm) and internal (endoderm) — sources of tissues and organs, complexes of axial organs.

Types of gastrulation

Four basic types of gastrulation are distinguished: delamination, immigration, invagination, and epiboly. The result of gastrulation is formation of the gastrula.

Delamination (fig. 41.1) is the process in which the cells split, converting the one-layered wall of the embryo to a two-layered one - external ectoderm and internal endoderm.

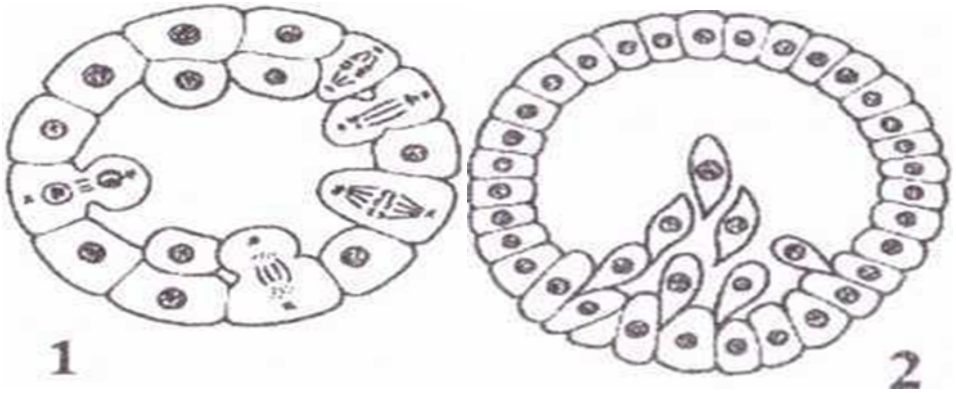


Figure 41.1.

In immigration, or settlement, (fig. 41. 2) certain cells move to the interior of the embryo and settle under the superficial layer, forming a middle layer - mesoderm; immigration may be unipolar (settlement from one place) or multipolar (from various places).

Invagination, or intrusion, (fig.41. 3) is the process by which the wall of a one-layered embryo gradually turns inward and forms external layer - ectoderm an internal layer - endoderm.

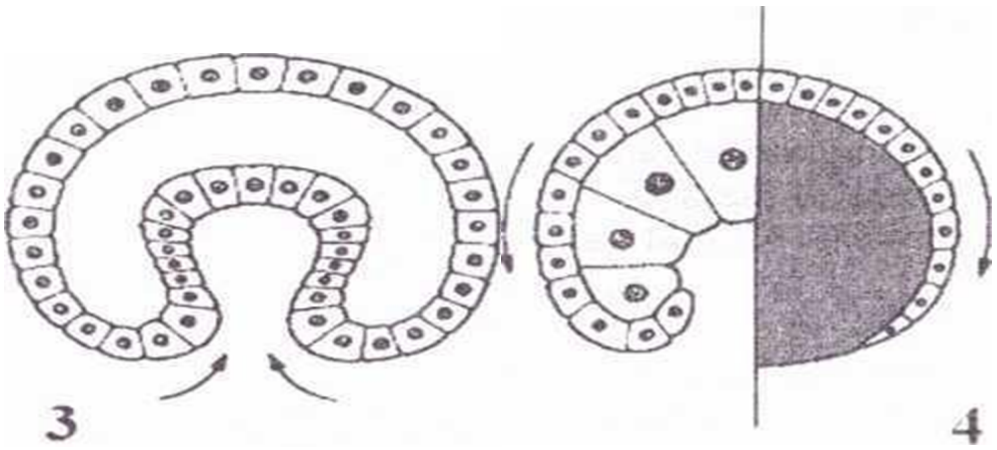


Figure 41.2.

In epiboly, or overgrowth, (fig.41. 4) relatively large cells (macromeres) rich in yolk are overgrown by the small ones (micromeres) and find themselves inside, forming an internal layer.

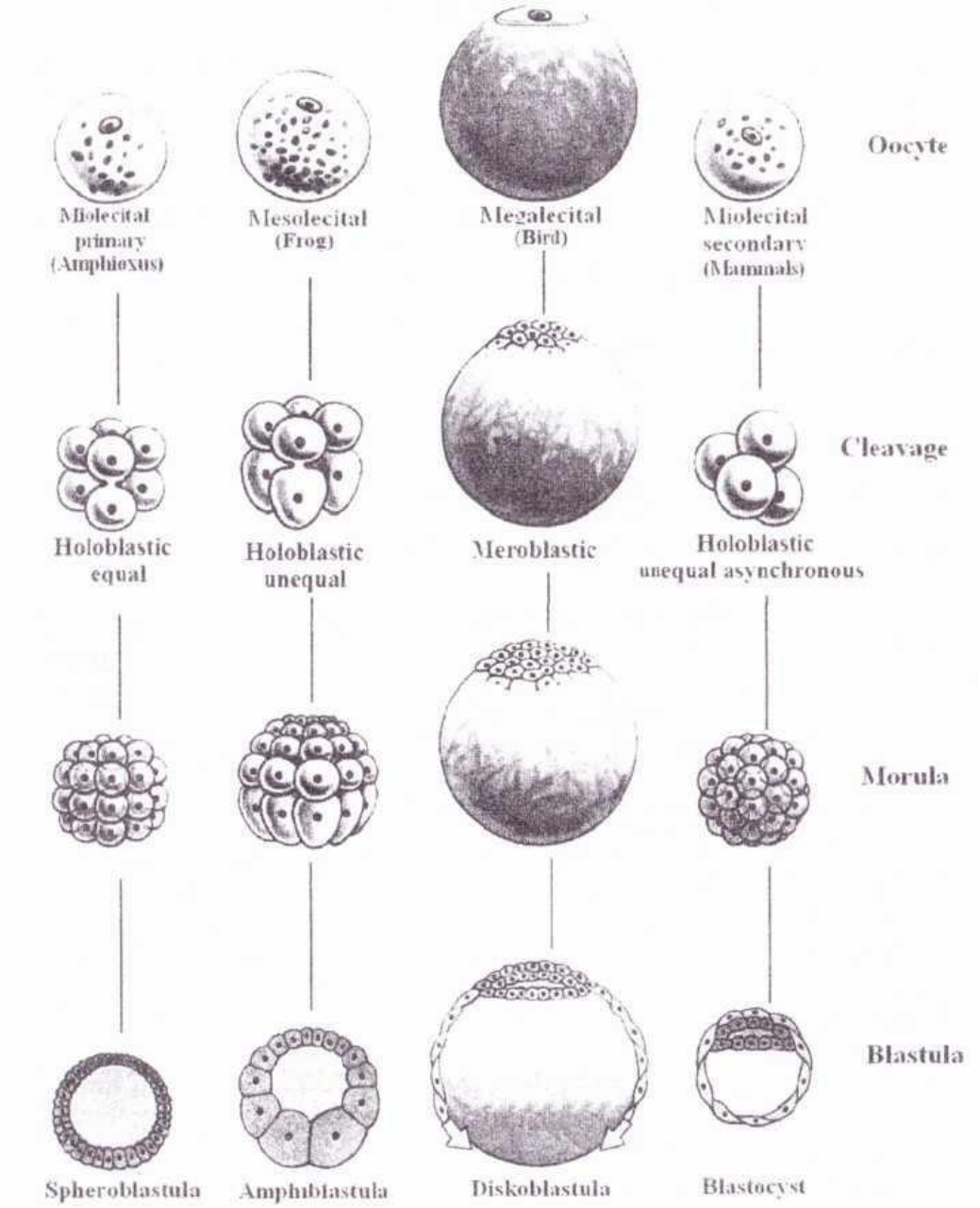


Figure 42. Schematic diagrams of the types of cleavage, blastulae, and gastrulae.

Differentiation of the embryonic layers

Differentiation of the ectoderm

Ectoderm is subdivided into:

- extraembryonic being by a source of formation of amnion and
- intraembryonic.

Intraembryonic ectoderm gives rise to:

- neural tube (brain and spinal cord);
- prechordal plate (epithelium of oral cavity, oesophagus, respiratory tract);
- placodes (the internal ear);
- neural crests (neurons of the sensory spinal and autonomic ganglia; cells of the adrenal medulla; pigment cells of the skin);
- skin ectoderm (epidermis and its derivatives, epithelium of cornea, enamel of teeth, epithelium of vagina and the anal canal of the rectum).

At a differentiation of the ectoderm germ parts - skin ectoderm, neuroectoderm, placodes, notochord, and extraembryonic ectoderm, being by a source of formation amnion, are formed. The smaller part of ectoderm, located above a notochord (neuroectoderm), gives rise to a differentiation of a neural tube and neural crests.

Neural tube gives rise to the brain and spinal cord.

Neural crests give rise to the neurons of the sensory spinal ganglia and V, VII, VIII, IX, X cranial nerves; the neurons of sympathetic ganglia; the cells of the adrenal medulla; chromaffin tissue; the pigment cells of the skin.

The most part of intraembryonic ectoderm is formed skin ectoderm, giving rise of the stratified squamous epithelium of skin (epidermis) and its derivatives, epithelium of cornea, epithelium of organs of a mouth, enamel of teeth, epithelium of the anal canal of the rectum, epithelium of vagina.

Placodes give rise to epithelial structures of the internal ear.

Most of notochord disappears, but parts of it persist in the region of each intervertebral disc as the nucleus pulposus.

Differentiation of endoderm

The differentiation of endoderm results to formation in a body of an embryo of an intestinal tube and to formation extra-embryonic endoderm.

From endoderm of an intestinal tube develops simple epithelium of a stomach, intestines and those glands, epithelium of a liver and a pancreas.

Extra-embryonic endoderm gives rise to epithelium of yolk sac and allantois.

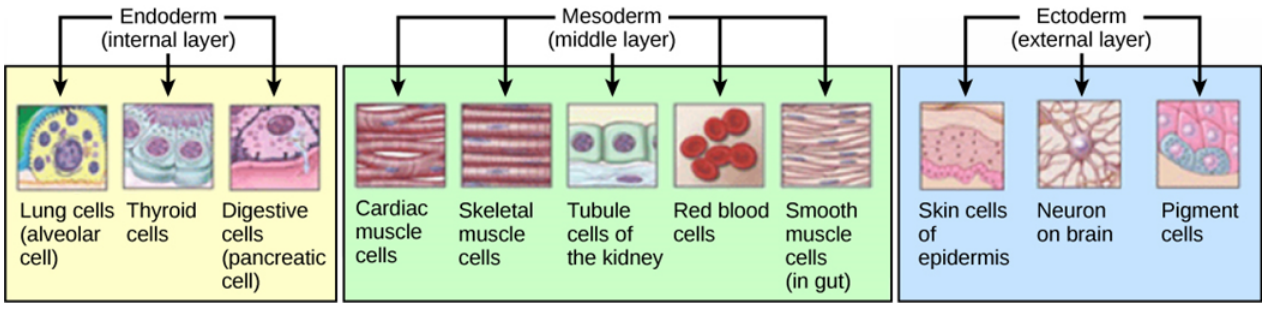


Figure 43. Differentiation of the embryonic layers.

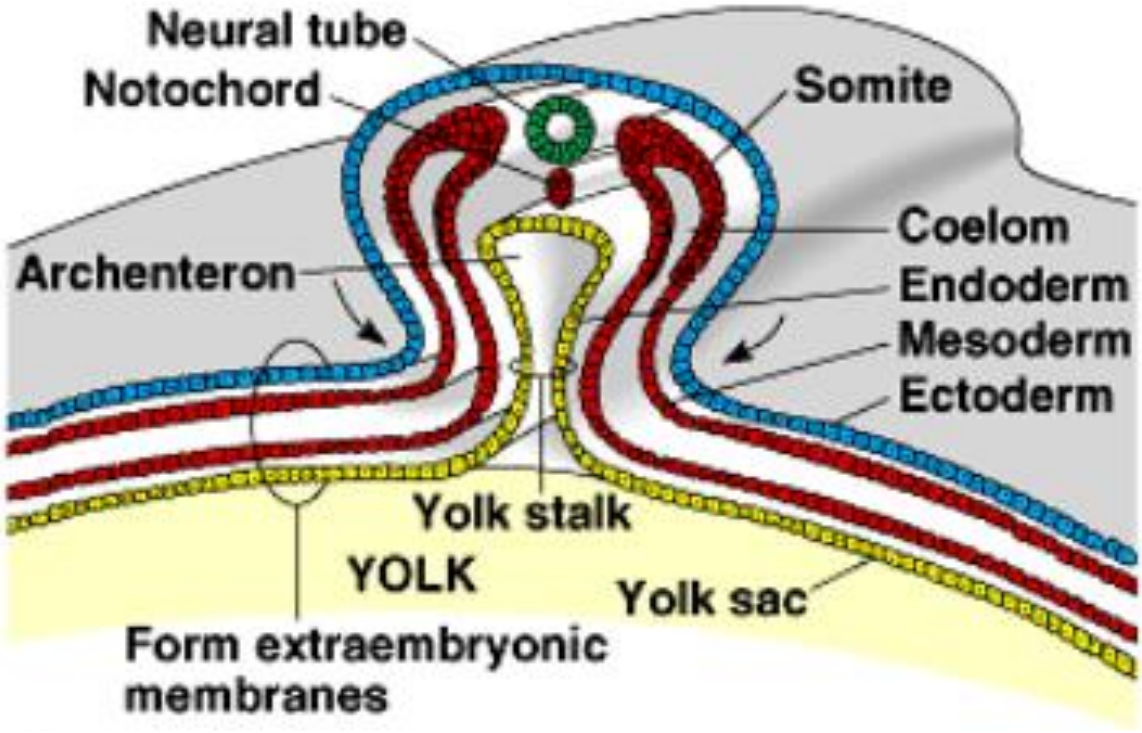


Figure 44. Differentiation of the embryonic layers.

Differentiation of mesoderm

The intra-embryonic mesoderm is subdivided into 3 parts.

1.The paraxial mesoderm - the cells, on either side of the notochord.

The paraxial mesoderm is segmented into cubical masses called somites.

Somites are differentiated on 3 parts:

- myotome, giving rise a skeletal muscular tissue,
- sclerotome, being a source of development bone and cartilage tissues, and also
- dermatome, forming dermis of a skin.

Process of segmentation of the paraxial mesoderm and formation of the somites begins in a head part of an embryo and is quickly distributed in caudal direction.

More laterally, the mesoderm forms a thinner layer called the lateral mesoderm It is not segmented, and split on two parts - visceral or splanchnopleuric (giving rise of heart, adrenal cortex, stroma of testes and ovary, connective and smooth muscle tissues of internal organs and blood vessels) and parietal, or somatopleuric (giving rise of serous membranes).

Between these two, there is a longitudinal strip of mesoderm called

intermediate mesoderm (giving rise of the organs of urogenital system).

Mesenchyme

The mesenchyme is the undifferentiated connective tissue found in the early embryo between the embryonic layers and axial organs. Most mesenchyme is derived from mesoderm.

Mesenchyme consists of small stellate (star) shape cells containing large oval nuclei with prominent nucleoli. Processes of mesenchymal cells extend and contact those of other cells to form a three dimensional cellular network (fig.4.11). A semi-fluid ground substance fills the extracellular spaces. Fibers are present, but are very fine and sparse.

Mesenchymal cells are multipotential cells that can be transformed into other types.

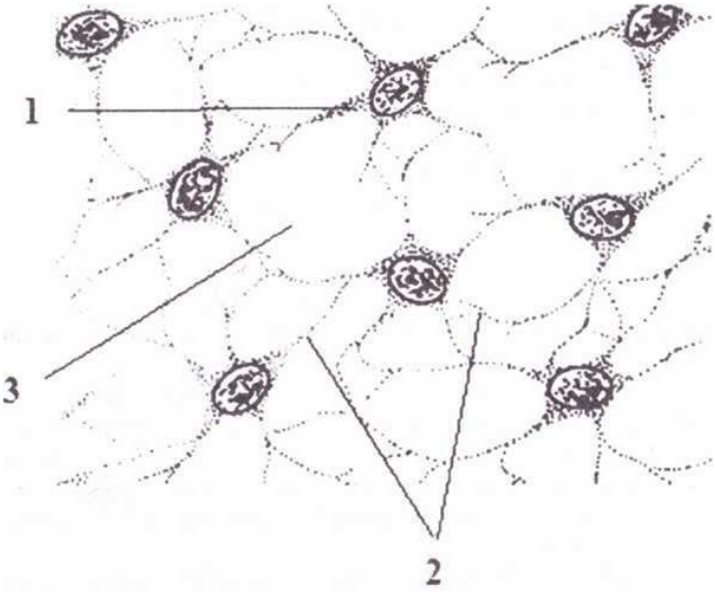


Figure 45. Mesenchyme.
1 - nuclei of mesenchymal cells, 2 - processes of mesenchymal cells, 3 - matrix.

Mesenchyme gives rise to all the connective tissues of the body, as well as to some other tissue:
- connective tissues;
- walls of blood vessels;
- cells of blood and lymph;
- smooth muscle tissue;
- microglia of nerve tissue.

Fetal organs

The fetal organs develop in process of embryo development outside of its body; carry out the different functions providing growth and development of the germ.

These are
- yolk sac,
- amnion,
- allantois
- umbilical chord,
- chorion,
- and placenta.

Yolk sac

The yolk sac is a membranous sac attached to an embryo, providing early nourishment in the form of yolk, the main function is the hematopoiesis that begins in the mesenchyme at 3-d weeks of embryogenesis.

The yolk sac develops from extraembryonic endoderm and extra embryonic mesoderm (fig.46).

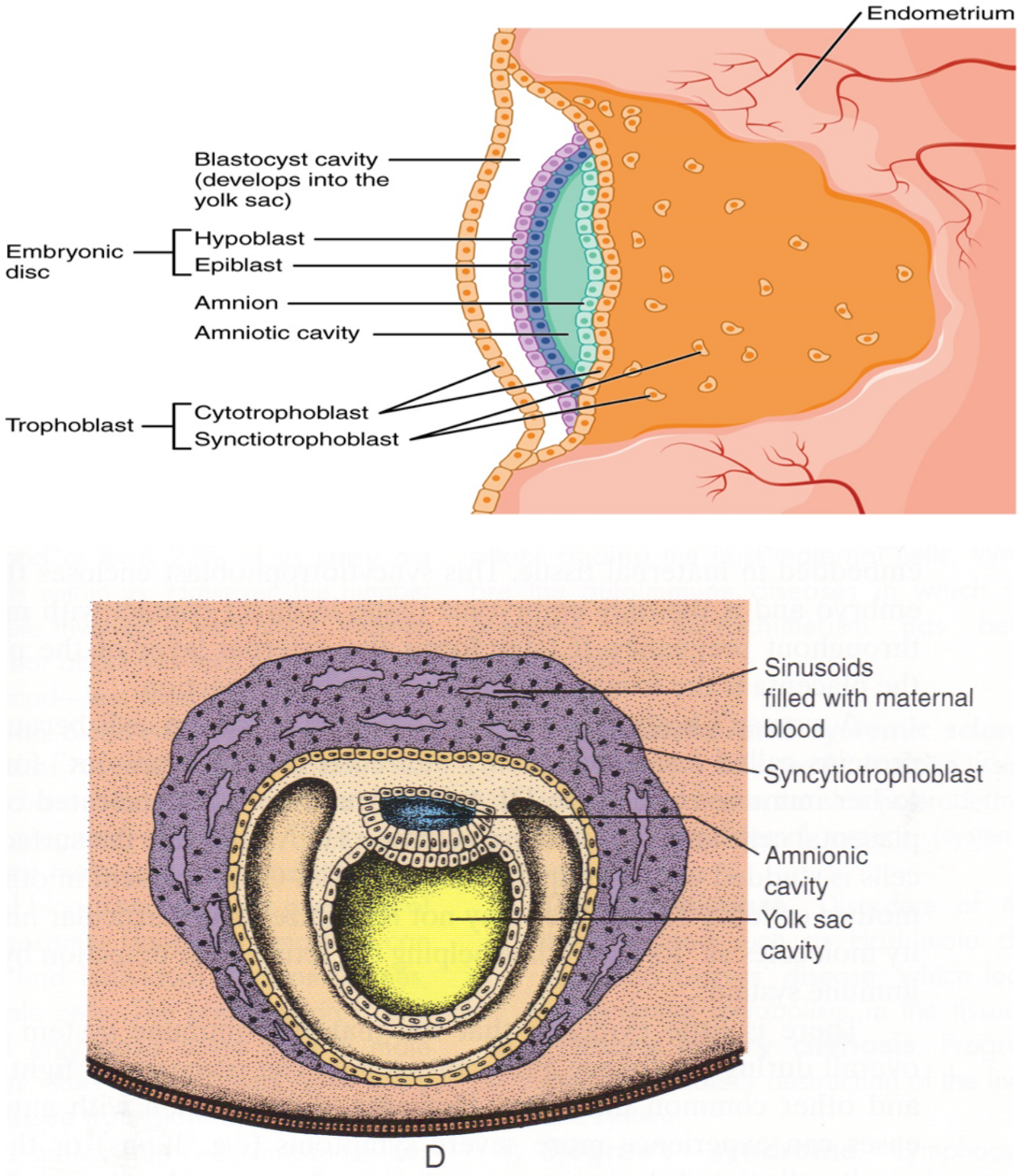


Figure 46. Structure of yolk sac and amnion.

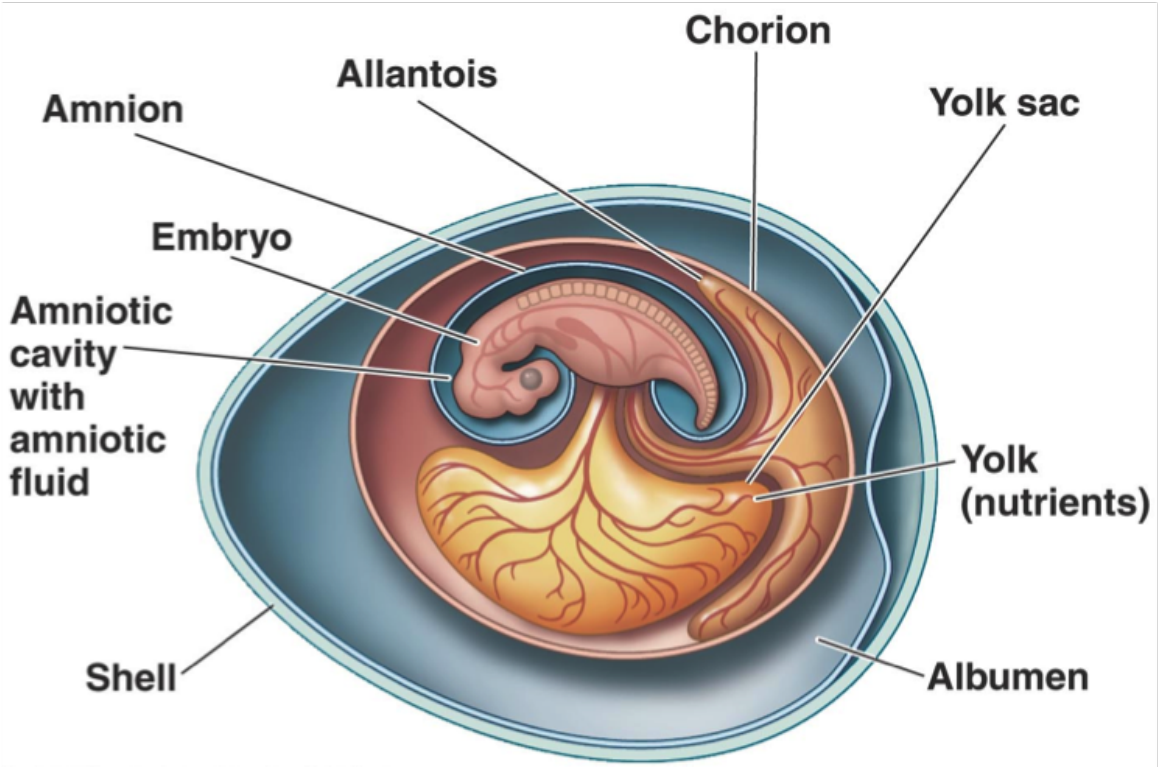


Figure 47. Structure of embryonic and extraembryonic organs.

Yolk sac carries out trophic and hematopoietic functions (primitive blood cells begin to form first in the yolk sac).

Amnion

The amnion is a membrane building the amniotic sac that surrounds and protects an embryo. The primary function of this organ is the protection of the embryo for its development. It stems from parts of the extraembryonic mesoderm on the outer side and the extraembryonic ectoderm on the inner side (fig.46-47). Amnion contains amniotic fluid in which there is a fetus.

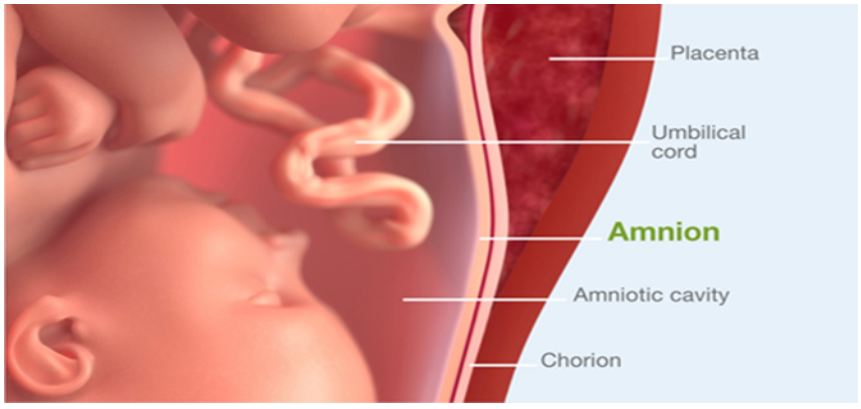


Figure 48. Structure of embryonic and extraembryonic organs.

Allantois

The allantois is a small endodermal diverticulum arises from the yolk sac near the caudal end of the embryo. This diverticulum of extraembryonic entoderma grows into the extraembryonic mesoderm (fig.48). Allantois helps the embryo exchange gases and handles liquid waste in the early stages of embryogenesis, for 21 days through the allantois located in the amniotic leg germinate blood vessels from the embryo body in the secondary villi of the chorion.

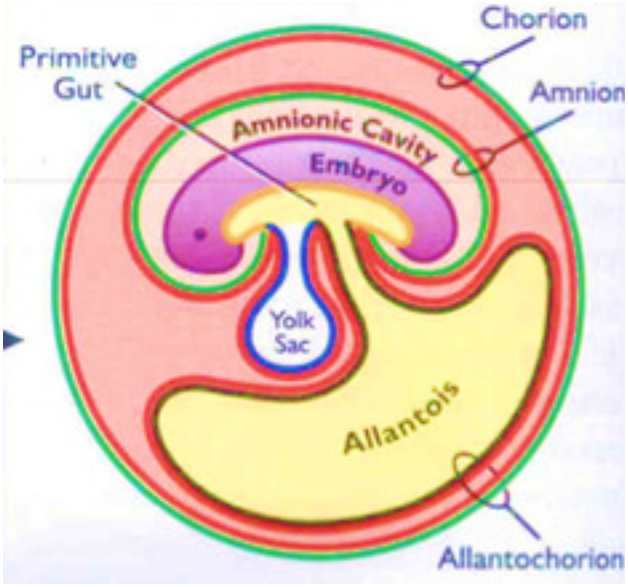


Figure 49. Structure of embryonic and extraembryonic organs.

Chorion

Chorion is a villous envelope of the fetus. it is divided into smooth chorion and villous. The chorion is one of the membranes that exist during pregnancy between the developing fetus and mother. It is formed by extraembryonic mesoderm and two layers of trophoblast (syncytiotrophoblast and cytotrophoblast) and surrounds the embryo and other membranes. The chorionic villi emerge from the chorion, invade the endometrium, and allow transfer of nutrients from maternal blood to fetal blood.

On 6-7 days of embryogenesis: The chorionic villi are formed as elongated projections from the surface of the trophoblast. The primary villi consist of only cytotrophoblast and syncytyotrophoblast covering(fig.50.1).

On 14-15 days of embryogenesis. Secondary villi (fig.50.2) form when mesoderm pushes into primary villi. Secondary' villi cover all of chorionic sac

On 21 days of embryogenesis. Tertiary villi (fig.50.3) form when mesenchymal cells in villi differentiate into blood vessels. Embryonic blood begins to flow through capillaries of chorionic villi. Diffusion of nutrients/'wastes between maternal and embryonic circulations through walls of villi begins.

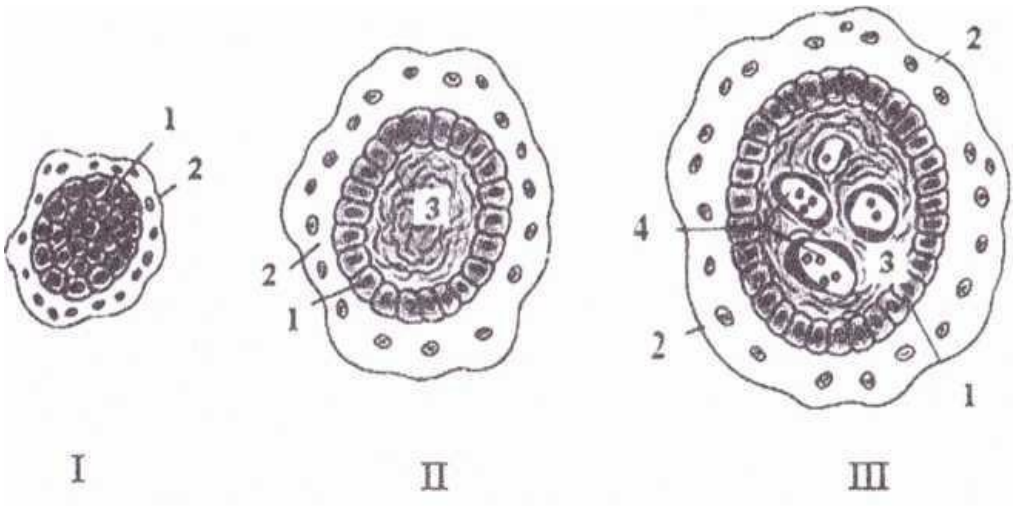


Figure 50. Formation of the chorionic villi. I - primary villus, II - secondary villus, III - tertiary villus, 1 - cytotrophoblast, 2 - syncytiotrophoblast 3 - mesodermal core, 4 - capillaries of the villus (fetal capillaries)

Placenta

The placenta is an organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply.

Functions of placenta

- Nutrition, gas exchange, waste elimination.
- Endocrine (placenta secretes hormone that are important during pregnancy human chorionic gonadotropin, human placental lactogen, estrogen, progesterone, relaxin).
- Protective

Classifications of the placentae

The placentae of all eutherian (placental) mammals provide common structural and functional features, but there are differences among species in gross and microscopic structure of the placentae.Distinguish four types of placentae (fig.51):

- epithelioehoreal,
- desmocltoreal,
- endotheliochoreal,
- haemochoreal

In epitheliochoreal placentae chorionic villi, growing into apertures of uterine glands, contact with epithelium of these glands (camel, horse, pig).

In desmochoreal placentae chorion partly destroys epithelium of uterine glands and villi of chorion grow into connecting tissue of lamina propria, for example at ruminant artiodactyl mammal (cow, sheep).

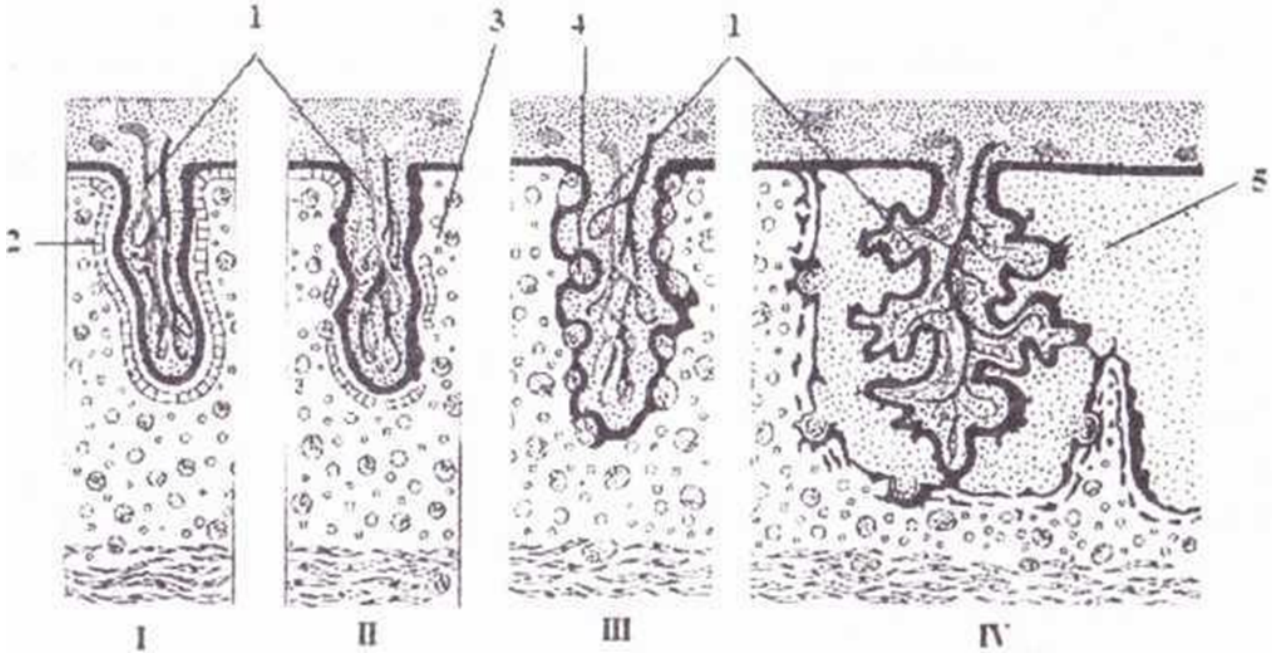


Figure 51 . Types of placentae. I - epitheliochoreal, II - desmochoreal, III - endotheliochoreal, IV - haemochoreal placenta; 1 - chorionic villi, 2 - epithelium of uterine glands, 3 - connective tissue of lamina propria of endometrium, 3 - blood vessels of endometrium. 4 - parent blood (from K). //. Aphanasyeva, H.A.Uryna u dp., 1999)

In **endotheliochoreal placenta** villi of chorion destroy epithelium and connective tissue and contact with the endothelium of blood vessels (cats, dogs).

Haemochoreal placenta destroys a wall of uterus vessels and villi of chorion contact directly to parent blood (Humans, rodents).

Classification based on a character of trophic.

In a placenta of type I chorion absorbs from mother tissues proteins and splits them up to amino acids, synthesis of embryo proteins occurs in a liver of an embryo.

In placentas of type II chorion acquires from parent tissues mainly amino-acids and synthesizes embryo-specific proteins; the embryo receives thus ready proteins which uses for construction of own tissues.

Synthesis of proteins of embryo at animals having 2-nd type of placenta occurs mainly in chorion and consequently with a birth the level of synthetic processes sharply decreases. Naturally, those germs after a birth rather long time metabolize only parent milk also are unable to eat independently.

Classification based on placental shape

Examination of placentae from different species reveals striking differences in their shape and the area of contact between fetal and maternal tissue:

-In a placenta of diffuse type almost the entire surface of the chorion is involved in formation of the placenta (horses and pigs).

- In a placenta of cotyledonary type multiple, discrete areas of attachment called cotyledons are formed by interaction of patches of allantochorion with endometrium (ruminants).

-Placenta of zonary type takes the form of a complete or incomplete band of tissue surrounding the fetus (carnivores like dogs and cats, seals, bears, and elephants).

- Discoid type: single placenta is formed and is discoid in shape (primates and rodents).

Human placenta is of haemochoreal, discoid, of type II.

In humans, the placenta has a circular shape and measures about 15 to 20 cm in diameter, 2-2,5 cm in thickness, weighing 500 to 600 g.

It has a dark reddish-blue or maroon color. It connects to the fetus by an umbilical cord.

Functions of humans placenta

1. **Nutrition**, gas exchange, waste elimination

Perfusion of the intervillous spaces of the placenta with maternal blood allows the transfer of nutrients and oxygen from the mother to the fetus and the transfer of waste products and carbon dioxide back from the fetus to the mother.

2. **Endocrine**

Placenta secretes hormone (secreted by syncytiotrophoblast of chorionic villi) that is important during pregnancy:

1) **human chorionic gonadotropin** (hCG) suppresses the maternal immunologic response so that placenta is not rejected.
2) **human placental lactogen** (hPL) promotes mammary gland growth in preparation for lactation in the mother. It also regulates maternal glucose, protein, fat levels so that this is always available to the fetus.
3) **estrogen** contributes to the woman's mammary gland development in preparation for lactation and stimulates uterine growth to accommodate growing fetus.
4) **progesterone** is necessary to maintain endometrial lining of the uterus during pregnancy. This hormone prevents preterm labor by reducing myometrial contraction.
5) **relaxin** is produced by decidua cells; softens the cervix and pelvic ligaments in preparation for childbirth.
6) It secretes neurokinin B

3.Protective

1) IgG antibodies can pass through the human placenta, there by providing protection to the fetus.
2) Cloaking from immune system of mother (immune tolerance in pregnancy).
3) It secretes interleukin 2.
4) Placenta forms a "protective barrier" against infectious agents. Nevertheless, there are some infections that can cross this barrier:

- The rubella virus may be responsible for a miscarriage during pregnancy (before the first month), for embryopathies or for fetopathies.

- Toxoplasmosis (caused by a protozoic parasite) is harmless for the mother, but can cause severe anomalies in the fetus.

- Listeriosis (traced back to a gram-positive Listeria monocytogenes) can be responsible for intrauterine death or neonatal sepsis.

- The cytomegalovirus is generally can be responsible for miscarriages, microcephaly and growth retardation.

- With herpes simplex genitalis a risk of neonatal contamination exists through infection in the birth canal.

- HIV infection can also be transmitted from an infected mother to her offspring.

In addition, the placenta also presents an incomplete barrier against certain injurious effects of drugs: antibiotics and corticoids can pass through the placental barrier.

The same is true for certain medications the teratogenic effects of which are today well documented. Thalidomide, mainly responsible for phocomelia as well as Roaccutane (retinoic acid, commonly used for treating acne), are highly teratogenic.

The consumption of barbiturates, drugs and alcohol during pregnancy are also to be avoided.

Structure of placenta

The placenta consists of **fetal part** and **maternal part** (fig.5.13).

The **fetal part, the chorion**, consists of:

- **branching chorionic plate**, portion of the chorion in the region of its uterine attachment, it consists of the mesoderm and is covered by

- **amniotic membrane**,

-chorionic villi which are bathed with maternal blood from the lacunae of the decidua basalis.

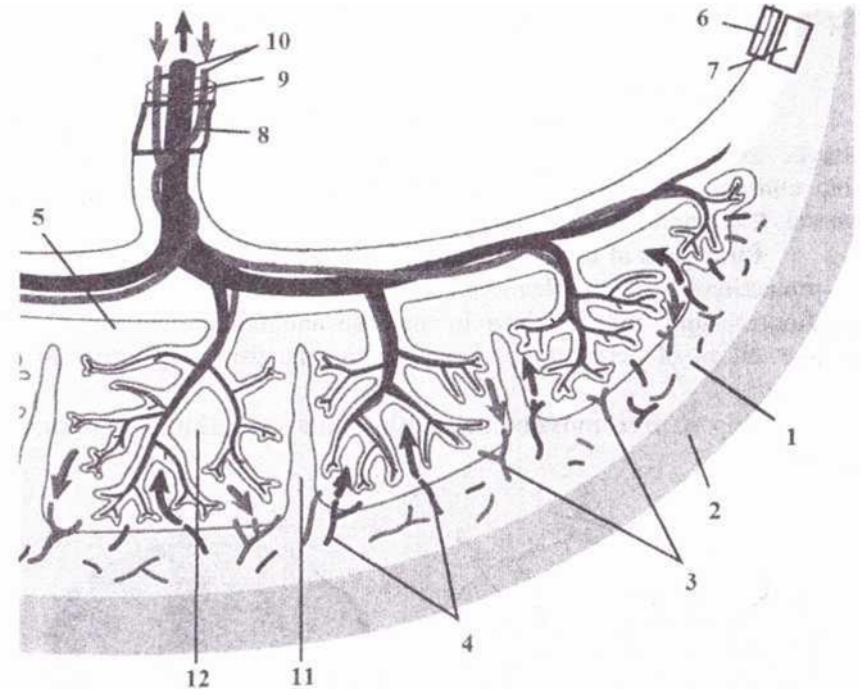


Figure 52. Schematic diagram of the placenta. 1 - decidua, 2 - myometrium, 3 - endometrial veins, 4 - endometrial arteries, 5 - villus paremchyma, 6 - amnion, 7 - chorion, 8 - umbilical cord, 9 - umbilical, 10 - umbilical areteries, 11 - connective tissue septa, 12 - lacune with maternal blood

The **maternal part of the placenta** consists of:

- modified basal lamina of endometrium (decidua basalis),

- lacunae filled with maternal blood and

-connective tissue septae, separating cotyledons from each other.

Cells from the connective tissue stroma of the decidua basalis form the decidual cells. These cells are large; they produce prolactin and other biologically active substances.

Structural and functional unit of the placenta is cotyledon, formed of chorionic villus both its secondary and tertiary branchings. Cotyledons are compartments of placenta, which are separated by the septae. The total of cotyledons in a placenta reaches 30-50 (fig.53).

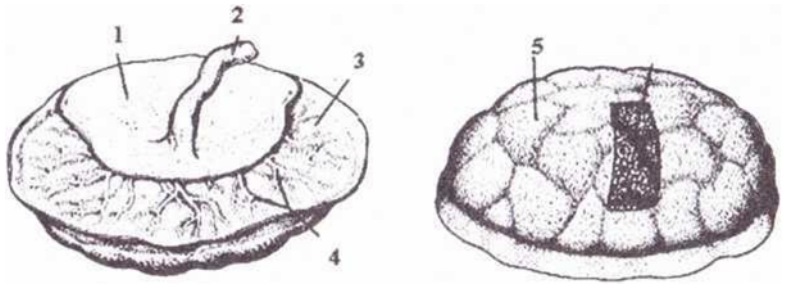


Figure 53. Schematic diagram of the cotyledon. 1 - amnion, 2 - umbilical cord, 3 - chorionic plate, 4 - chorionic vessels, 5 - cotyledon.

1. Classification based on layers between fetal and maternal blood(placental barrier)

Just prior to formation of the placenta, there are a total of 6-7 layers of tissue separating maternal and fetal blood:

- fetal vessels endothelium,
- basal membrane of fetal blood vessels
- extraembryonic mesenchyme,
- basal membrane of cytotrophoblast
- cytotrophoblast,
- Symplastotrophoblast
- Fibrinoid Langhansa

Since the 3rd month of pregnancy cytotrophoblast begins to disappear, only the simplastotrophoblast remains. This leads to a thinning of the placental barrier and increase its permeability. However through the placental barrier from blood of mother alcohol, narcotics, medicinal substances, nicotine, and also many hormones will easily penetrate into blood of the embryo.

Umbilical cord

The umbilical cord is elastic tube of approximately 55-60 cm in length, connecting germ (fetus) with a placenta. It is covered by amnion and consists of mucous connecting tissue (Warton's jelly) and blood vessels (two umbilical arteries and one vein) (fig.53).

Functions of the umbilical cord:

- protection of the umbilical vessels from compression, providing thus continuous supply of an embryo by nutrients and oxygen;
- prevention of penetration of harmful agents from a placenta to an embryo;
- ensuring of free movements of the embryo within the amniotic cavity.

Practical lessons № 5-6

Questions for self-control

1.Periods of embryogeny. Characteristic features of the embriogenesis stages.

2. Germ cells: their differences compared to somatic ones. Spermatozoon: structure and functions. Oocyte: structure and functions.

3. Fertilization: characteristic features.

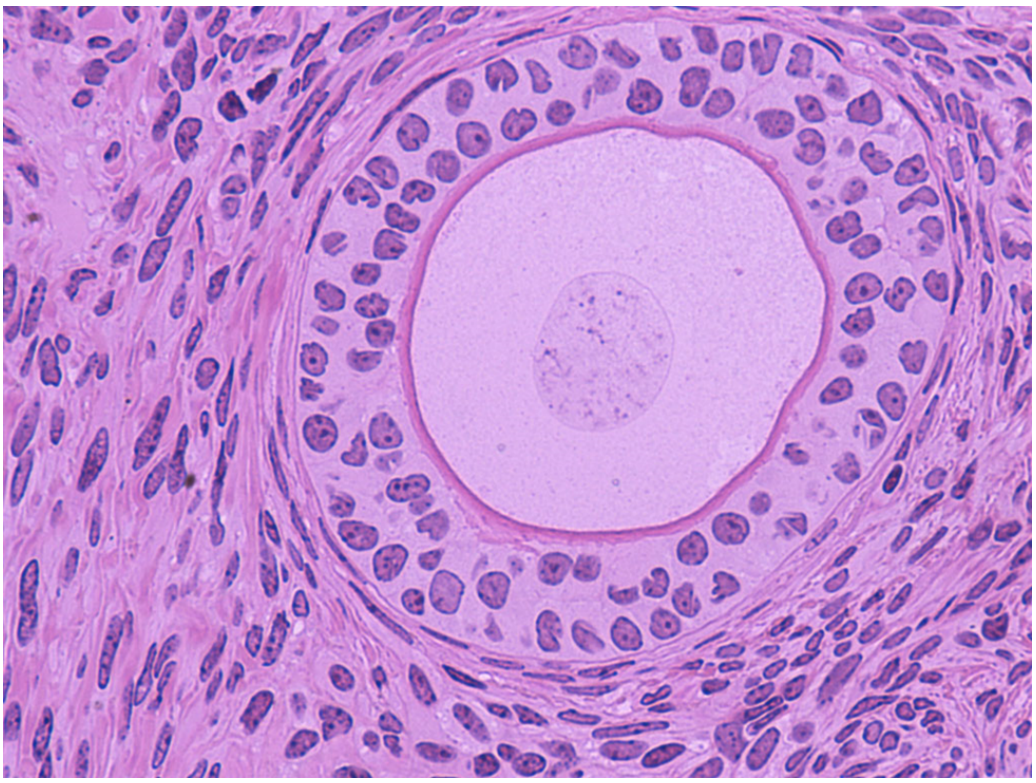
4. Connection of the embryo with a maternal organism. Implantation. Placenta.

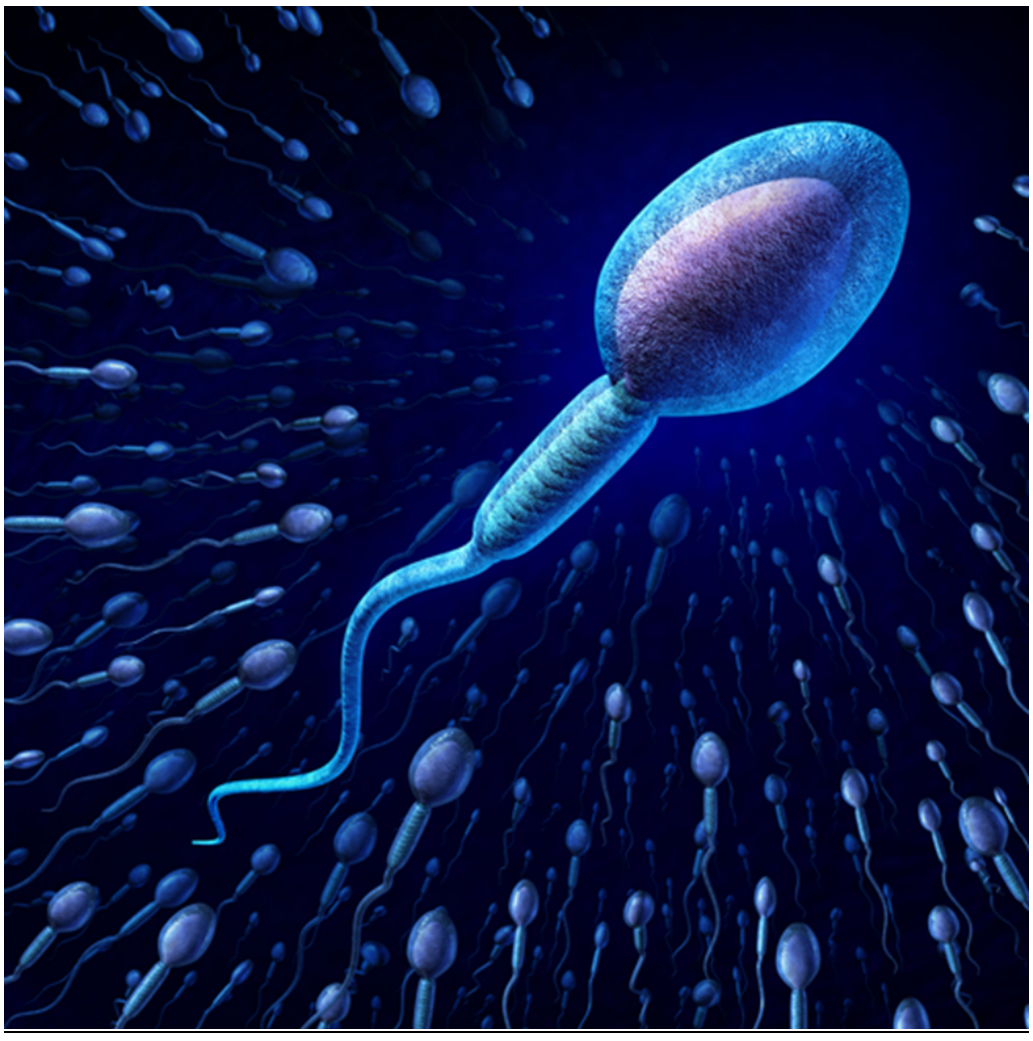
5. Gastrulation. Germ layers. Their significance.

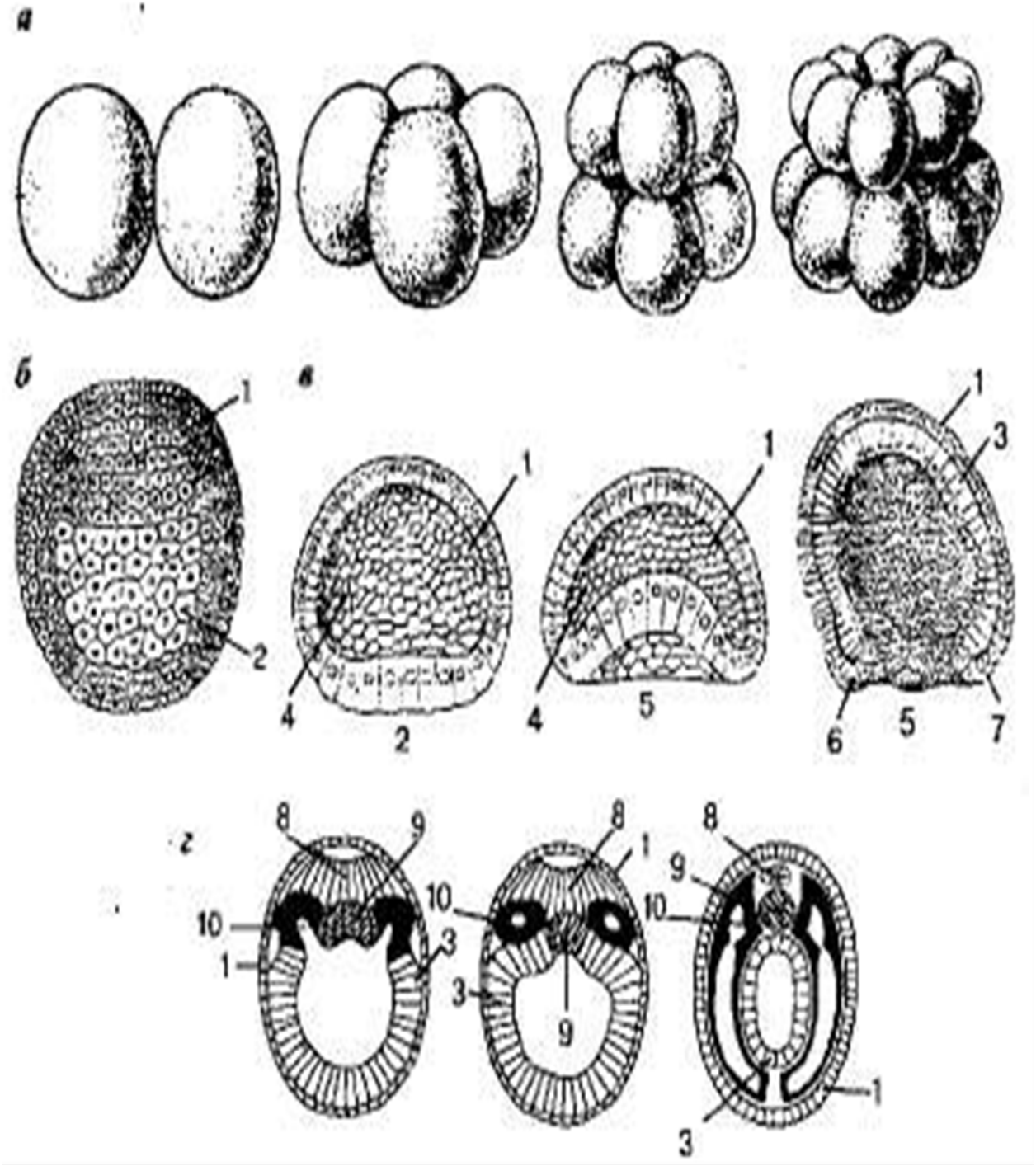
6. Ectoderm and entoderm differentiation.

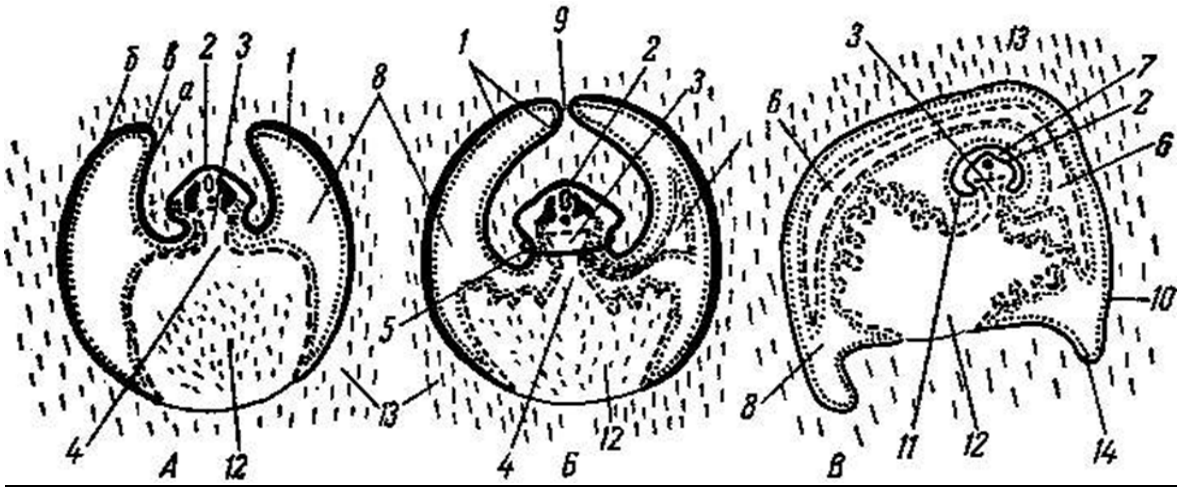
7. Mesoderm differentiation.

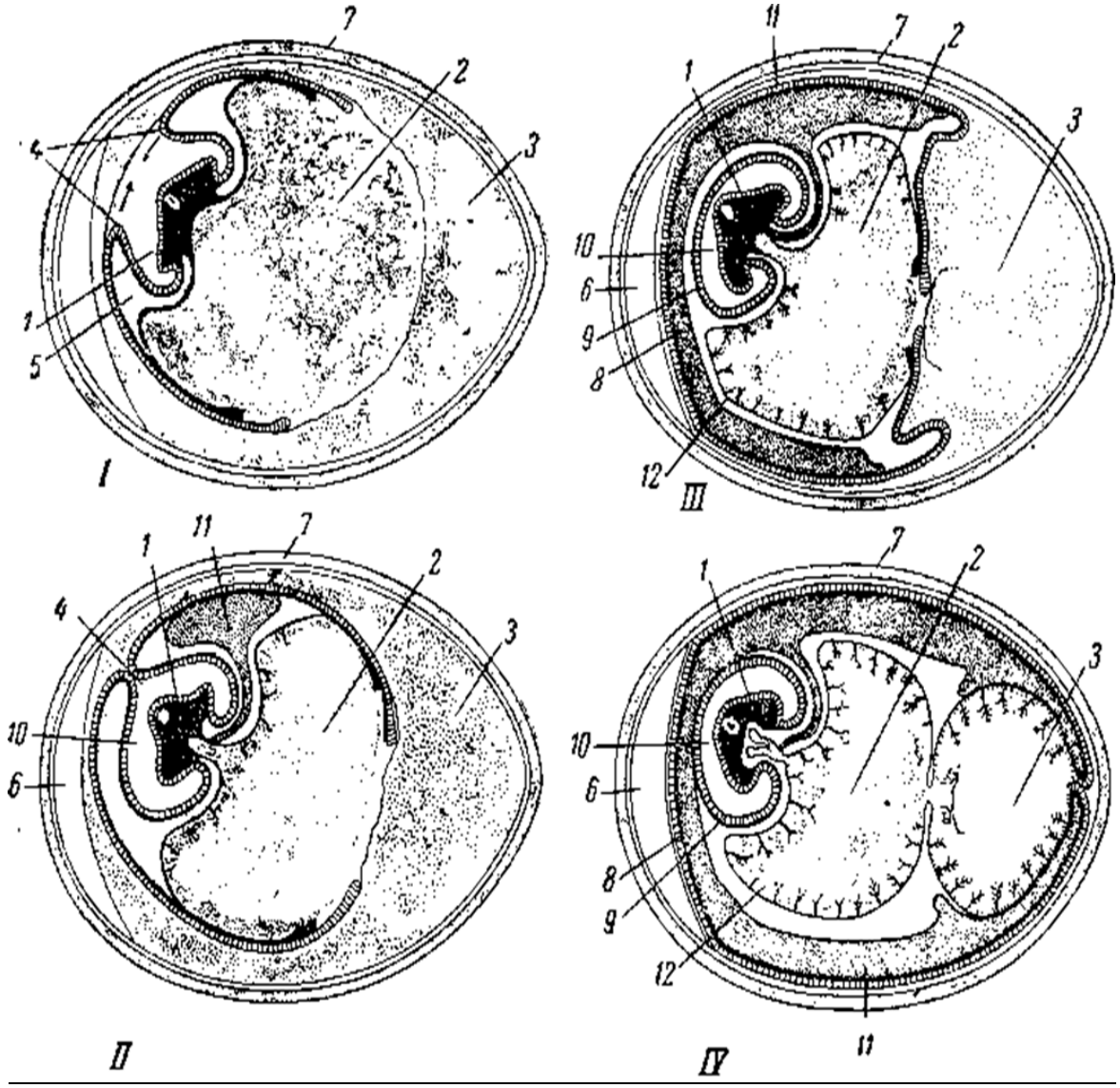
8. Critical periods of the embriogeny. Endo- and exogenic factors, which influence on its development.

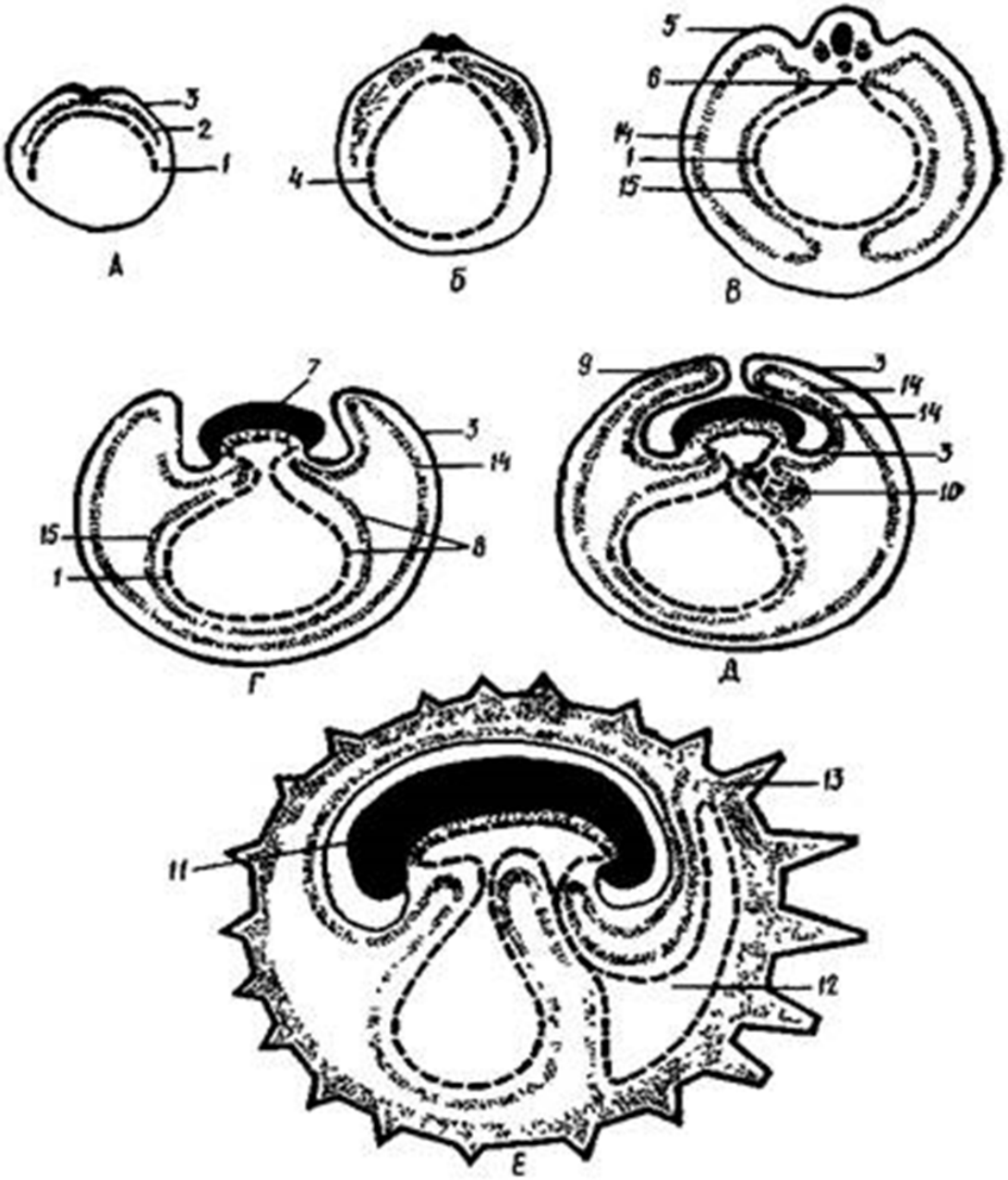





а
б
1
2
в
1
4
2
1
4
5
1
3
6
5
7
г
8
9
10
1
10
3
8
1
3
9
8
9
10
1
3


А
Б
В


I
II
III
IV



The teacher's signature:

HUMAN EMBRYOLOGY

In the development of an individual organism which is termed the ontogeny, distinguish two main periods: prenatal and postnatal development.

Prenatal development is the period from the time of fertilisation until birth.

Postnatal development is the period beginning immediately after the birth of a child and extending for about six weeks.

Human prenatal development can be divided into three stages:

- initial (1-st week of development),

- **embryonic** (2-8 week of development),

-**fetal** (since 9-th week of development till a birth of the child).

Duration of human prenatal development is nine months (38 - 40 weeks or 266-280 days).

The initial stage begins with fertilization. The fertilized egg (a zygote) moves toward the uterus. Cell division of a zygote begins approximately 24 to 36 hours after fertilization. Cell division continues at a rapid rate and the cells then develop into a blastocyst. **Blastocyst is attached** to the uterine wall (**implantation**).

The embryonic stage (embryogenesis) begins after implantation and continues until cell differentiation into the various body systems has been mostly completed. Structures, including the placenta and umbilical cord, important to the support of the embryo, develop.

Embryogenesis consists of the main stages:

- progenesis – it is formation of germ cells (spermatozoon and oocytes)
- fertilization and zygote formation;
- cleavage and blastocyst formation;
- implantation;
- gastrulation (formation of the germ layers);
- histogenesis (formation of different tissues from undifferentiated cells, which are constituents of three primary germ layers);
- organogenesis (period of human development during which the embryo is becoming a fully functional organism capable of independent survivia);
- systemogenesis (formation of the the functional systems).

Fusion of a spermatozoon from oocyte resulting in formation of one-cell embryo – zygote

The biological significance of fertilization are the next:

• 1. The restorations of the full chromosomal set.
• 2. Determination of the embryo sex.
• 3. Initiation of a cleavage.

Fertilization in most of the cases occurs within the ampullary region of the uterine tube on days 14-15 of ovarian-menstrual cycle (after the ovulation – oocyte release from the ovary) and insemination with sperm. Fertilization is accounted as the first day of embryogenesis and includes the following steps:

* Capacitation
* Acrosomal reaction
* Penetration of corona radiata and zona pellucida
* Fusion of the oocyte and sperm cell membranes
* Cortical and zona pellucida reactions
* Resumption of 2^{nd} meiotic division
* Metabolic activation of the egg
* Restoration of diploid number of chromosomes,

In female this process takes place in the ampullar portion of the Fallopian (uterine) tube. Some specific processes occur in the course of fertilization, which includes **distant and contact stages.**

Distant stage of fertilization means special interaction of ovum (in uterine tube) and spermatozoa, which enter female vagina.

Contact stage begins after their meeting in uterine tube.Only 1% of sperm deposited in the vagina enter the cervix, where they may survive for many hours. Movement of sperm from the cervix to the oviduct is accomplished primarily by their own propulsion, although they may be assisted by movements of fluids created by uterine cilia. The trip from cervix to oviduct requires a minimum of 2 to 7 hours, and after reaching the isthmus, sperm become less motile and cease their migration. At ovulation, sperm again become motile, perhaps because of hemoattractants produced by cumulus cells surrounding the egg, and swim to the ampulla where fertilization usually occurs. Spermatozoa are not able to fertilize the oocyte immediately upon arrival in the female genital tract but must undergo **capacitation** and the **acrosome reaction** to acquire this capability.

Capacitation is a period of conditioning in the female reproductive tract that in the human lasts approximately 7 hours. Much of this conditioning, which occurs in the uterine tube, entails epithelial interactions between the sperm and mucosal surface of the tube. During this time a glycoprotein coat and seminal plasma proteins are removed from the plasma membrane that overlies the acrosomal region of the spermatozoa. Only capacitated sperm can pass through the corona cells and undergo the acrosome reaction.

So, distant stage of fertilization includes next processes: capacitation and taxis of spermatozoa. This stage begins with special activation of spermatozoa and continues with their active movement forward the ovum). Spermatozoa in vagina are not active. In the female genital way they have to be activated.

Capacitation means physiological changes or special activation of spermatozoa, which occurs with them in female sex way. It is the process by which enzymatic secretions of the uterus and uterine tube of the female genital tract strips glycoproteins from sperm cell membrane. Cholesterol of sperm plasma membrane is removed during capacitation, which results in the increased fluidity of the membrane that is required for the fusion of acrosomal membrane with the sperm cell membrane. Only after such changes they begin to move toward. Capacitation lasts about 7 hours.

Taxis – active passage of spermatozoa.

Chemotaxis (chemotropism) – movement toward to the oocyte, which produces special chemical substances – hormones.

Reotaxis – is the sperm ability for advance against the current of fluid secreting by the epithelium, which lines the cervix, uterus and uterine tube (against the mucus flow in the uterine tube).

Stigmotaxis – against the peristaltic contractions of Fallopian tube.

Distant interaction of germ cells is regulated by prostaglandins, fertilizing proteins (androhormones, hynohormones), progesterone and pH.

Seminal fluid of semen contains prostaglandins, which have pharmacodynamic action on the smooth muscle of the uterus and uterine tube. **Prostaglandins** stimulate uterine contractions, rhythmic contractions of uterine tube muscles and smooth muscles at all. Thanks to these peristaltic contractions the ovum is transported along the Fallopian tube to the uterine space.

Oocyte and spermatozoa are moving from the opposite sites of female genital tract. Spermatozoa are much rapid, however, to be counted for by their intrinsic motility.

Prostaglandins assist in spermatozoa movement through the uterus and tubes to the site of fertilization in ampullar portion of the Fallopian (uterine) tube.

Fertilizing proteins promote chemotaxis. The chemical substances, which are produced by the ovum and sperms, are known as gamones.

The oocyte secretes 2 types of **hynohormones**: the first one stimulates spermatozoa in opposite to second one, which agglutinates them.

Androhormones are produced by spermatozoa. Androhormones of firs type blocks the movement of male germ cells and androhormoneII lyses the oocyte membrane.

Progesterone is secreted by the corpus luteum of the ovary after ovulation and stimulates the secretion of nutrient-rich fluid by mucosal epithelium of female reproductive tract (glandular epithelium of uterine tube and cervix, and uterine glands - crypts).

PH of surrounding environment has great influence on the process of fertiluization. The matter is, the average speed of spermatozoa in female body is at about 2-3 mm per minute but it varies very much with different pH.

Sperms move slowly in the acidic environment of the vagina, but move rapidly in the alkaline medium of the uterus.

It was found that few motile spermatozoa may appear in the ampulla of the uterine tube 5 minutes after their deposition near the external uterine os, however, it took up to 45 minutes to other spermatozoa to complete the journey. Only about 200-500 sperms usually reach the site of fertilization. Most of them degenerate and are resorbed by the female genital tract.

When spermatozoa get in contact with oocyte **(contact stage of fertilization),** they are binding to it. Corona radiata and zona pellucida of oocyte have an important role in the recognition of homologous sperms and blocking polyspermy. Glucosyltransferase receptors of the sperm cell membrane bind to zona pellucida receptors, ZP-3 molecules. The last ones have two regions:

1) the sperm receptors that recognise intergral proteins of their plasmalemma;

2) the other region of ZP-3 molecule binds to receptor proteins located in the head of the sperm, triggering the acrosomal reaction. Acrosome membrane fuses with corona radiata of oocyte and acrosomal enzymes are released from acrosome. The effusion of enzymes results in digestion of intercellular junctions. 200 – 500 spermatozoa reach the site of fertilization and introduce in the tunica granulosa. First of all **denudation** – dispersion of the corona radiata cells – occurs. During 12 hours due to the contraction of spermatozoa tails the oocyte rolls to the uterus with the speed 4 times in a minute and loss follicular cells.

Then **penetration** of zona pellucida begins, most important enzymes are hyaluronidase and trypsin-like protease, acrosin, which lyses zona pellucida, permitting the flagella movement of the sperm to propel the sperm toward the oocyte. Penetration is accomplished by limited proteolysis of the zona pellucida in front of the advancing sperm. Additional sperms may be found attached to zona pellucida. Several sperms may penetrate zona pellucida, but only one sperm completes the fertilization process **(monospermy).**

At last, the oolemma (the proper cell membrane of oocyte) is solved and spermatozoon enters ooplasm. The sperm binds to receptors of oocyte plasma membrane. Cell membranes of gametes fuse and break down at the area of fusion. The sperm nucleus with 23 chromosomes, neck with centriol and mitochondria of middle piece enter cytoplasm of oocyte. The sperm's plasma membrane remains behind.

The contact of the sperm with the oocyte surface results in the ***cortical reaction*****.** The cortical reaction is exocytosis of the egg's cortical granules.

The fusion of germ cells is responsible for several postfusion reactions: **fast block of polyspermy, cortical reaction and zona reaction**.

The fast block of polyspermy includes changes of membrane resting potential of oolemma that prevent contact between oocyte and another sperms. It consists of large and long-lasting (few minutes) depolarization of the oolemma.

Cortical reaction is slow component, after oolemma polarity changes $Ca^{++}$is released from ooplasmic stores and promotes effusion of the oocyte cortical granules content outside and its reaction with remnant of zona pellucida and oolemma. As a result of this cell size decreases thus forming **perivitelline space**. Enzymes within the cortical granules (proteases) act to hydrolyze ZP-3 molecules in zona pellucida, the sperm receptors, thus preventing additional sperms from reaching the oocyte. These enzymes degrade the glycoprotein oocyte receptors for sperm binding and make it impermeable to other sperms (monospermy).The content of cortical granules, which are released into **perivitelline space**, also causes changes if the plasma membrane.

These enzymes form the perivitelline barrier by cross-linking proteins on the surface of zona pellucida. This event promotes final and permanent block to polyspermy. So, **tunica of fertilization** appears.

There are such mechanisms of cortical reaction:

1. Modification of cell membrane potential (minus to plus).
2. Releasing of the Ca++ from the depot to hyaloplasm.
3. Cortical granules exocytosis.
4. Storage of the water between the ovolemma and zona pellucida in perivitellin space.
5. Hardening of zona pellucida with formation of fertilization tunica.

Sperm nucleus enters the secondary oocyte and the ovum completes its second meiotic division. This result in two haploid cells formation: the ovum and polar body. Polar body with 23 s-chromosomes is extruded into the perivitelline space between the oolemma and tunica of fertilization. Chromosomes, which remain in ovum reconstitute into the **female pronucleus**.

Within the cytoplasm of the oocyte, the nucleus of the sperm enlarges to form the **male pronucleus**, it turns around; at that time 2 nuclei may be seen in the cell: so called male and female **pronuclei**. Since that time till the moment of their fusion such unicellular organism is named "**synkarion**". Two pronuclei soon meet approximately the center of the ovum. They fuse, forming a zygote with the diploid chromosomes number. After conjugation mitosis begins. Zygote is genetically unique because it contains new combination of chromosomes that is different from that in the cells of parents.

Chromosomal sex of embryo and child is determined at the time of fertilization. It is clear that one chromosome of each of the 23 pairs is derived from the mother and the other from the father. Fertilization by the X-(gynecospermium) or Y-bearing (androspermium) sperms will result in origin of female embryo in the first case and male in the second one.

Cleavage of human zygote begins 30 hours after fertilization in uterine tube (tubal period) and lasts during next 6 days (till 7^{th}). Since 5-6 day embryo enters the uterine space (uterine period). As a result multicellular organism is developing. His name is "blastula" which consists of blastomers. During all this period of time blastula has a constant size and is surrounded by tunica of fertilization.

Cleavage process directly depends on the type of oocyte, his yolk inclusions volume and disposition. Human oocyte is olygolecital II isolecital that is why **human zygote has full (complete) subequal asynchronic cleavage**. It means all the zygote is dividing into blastomers (fully) and all the blastomers, which appear during the cleavage, are almost equal in size. The differences between blastomers may be seen just after the first division: one of the blastomers is a little bit smaller and lighter than the other one. The blastomeres are oval and lie parallel to each other. The nucleus of each cell becomes invisible about 1^{st} hour before the next division. The division of two-cell stage is dichotomus, but the blastomeres do not divide synchronously. At this stage, as at subsequent stages, the larger light cell divides first. The larger cell divides 40 h after fertilization and three-cell stage is formed. So, different blastomers are dividing at different time (asynchronically), their amount changes in such way: 2, 3, 5 …

After the nine-cell stage the blastomeres change their shape and tightly align themselves against each other to form a compact ball of cells. This phenomenon is called compaction. It is mediated by cell surface adhesion glycoproteins (ovomorulin). Compaction permits greater cell-to-cell interaction. When there are 12 to 32 blastomeres, the developing conceptus is called **morula.** The spherical morula exists about 3 days after fertilization been rolling in the uterine tube.

About 72 hours after fertilization human morula contains 12 blastomeres.

The 12-cell stage consists of: 11 small light peripheral cells, which surround a larger (dark) centrally placed one.

Smaller cells are more numerous and divide more rapidly. They form outer cell mass , are called **trophoblasts.**

Larger cells divide slower and they few in number. Internal cells of the morula are called inner cell mass, are called **embryoblasts. Cleavage means enlargement of total cell number with simultaneous reduction of their size resulting from the abbreviated mitotic cycle**

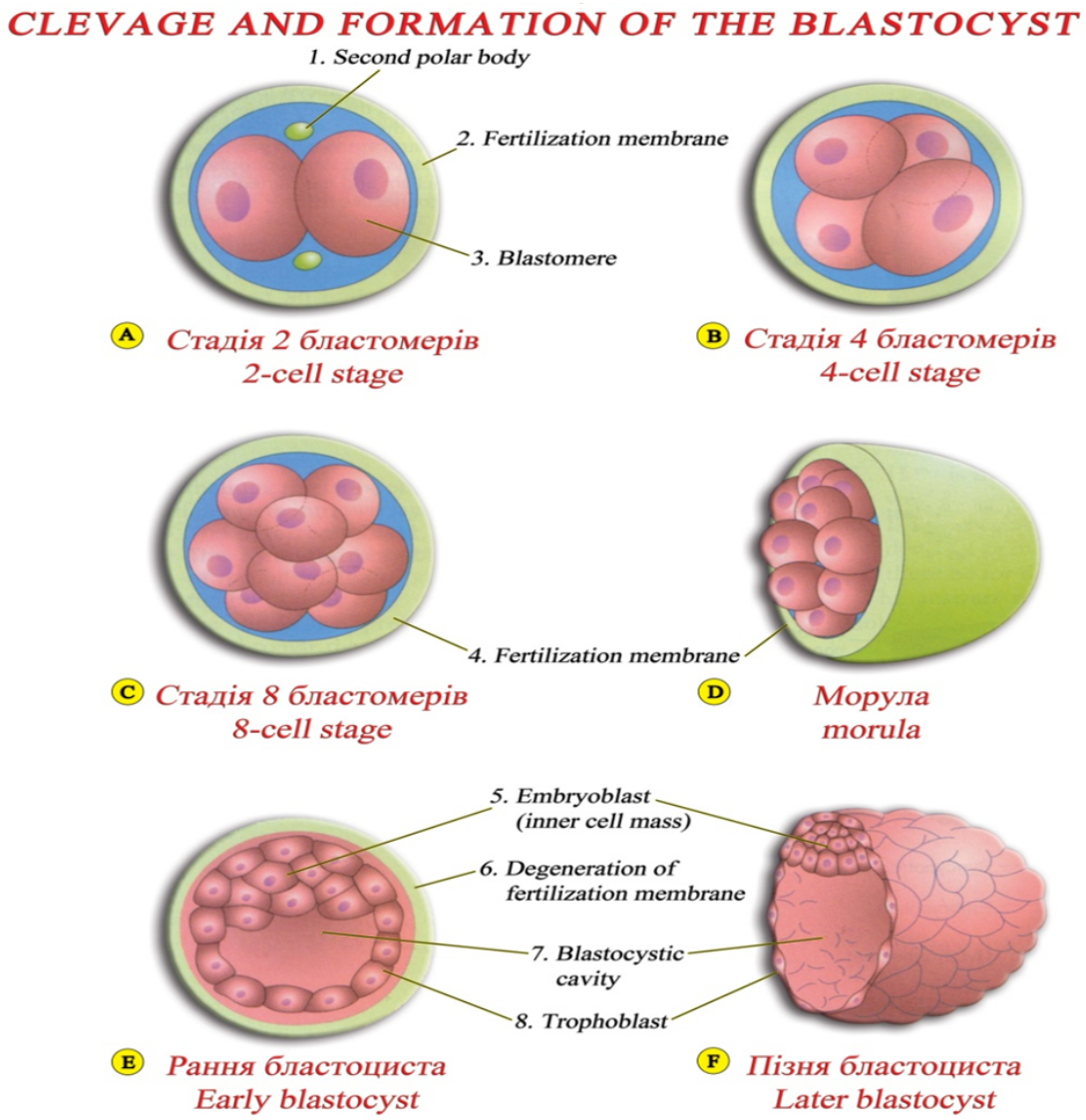


Figure 54. Human cleavage.

The morula is surrounded by tunica of fertilization, which is of great importance in keeping the blastomeres in a restrained and compacted cluster. Morula remains enclosed by abovementioned tunica through which it is nourished by diffusion of oxygen and low molecular metabolites from uterine tube secretions (**histiotrophic nutrition**).

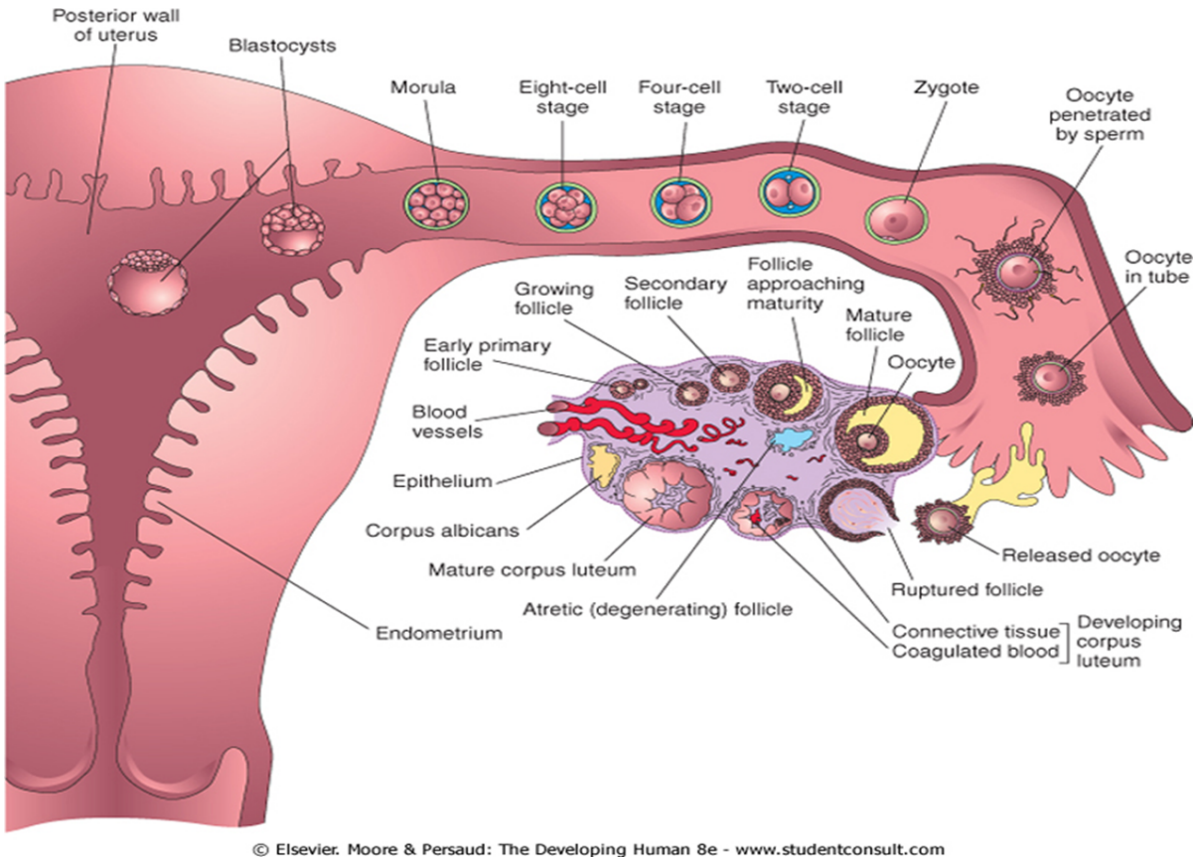


Figure 55. Human cleavage and implantation.

During the 6th day after fertilization, the blastocyst attaches to the endometrial epithelium with its embryonic pole. This triggers the differentiation of the trophoblast into an **inner cytotrophoblast and an outer syncytiotrophoblast**. By the end of the first week, the blastocyst is superficially implanted. At about seven days a flattened layer of cuboidal cells, called hypoblast (endoderm), appears on the surface of the embryoblast. In 5-5, 5 days blastocyte gets in a uterus. During about 2 days (with 5 for 7 day) the germ passes a stage of free blastocyst.

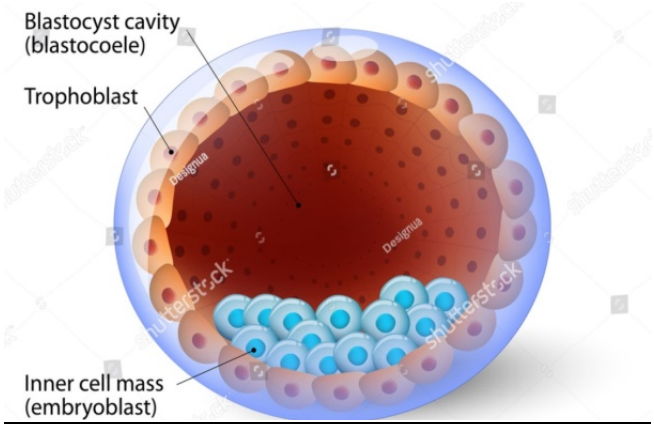


Figure 56. Structure of free blastocyst.

During 2-3-4th days **blastula** consists of blastomers (till 32), which are tightly connected one to each other and it looks like a raspberry. It is **morula**. It rolls along the uterine tube to uterine cavity and reach it at the 5th day. At that time blastula consists of larger dark blastomers (**embryoblast**), which are surrounded with smaller slightly flattened and light ones – **trophoblast** and contains the space inside (blastocel).

The blastocyst cavity (blastocel) appears between the trophoblast and the inner cell mass and separates them except of one side where they remain in contact. Such type of blastula is called **"blastocyst"**, it appears as **a result of full subequal asynchronic cleavage.** The wall of blastocyst (trophoblast) is known as **blastoderm.** It has **abembryonic pole** (over embryoblast) and opposite **vegetative pole.** In the blastocyst stage zona pellucida becomes thinner and disappears. The trophoblastic cells, which have the capacity to invide the mucosa, to come into direct contact with the endometrium.

Implantation

The blastocyst usually implants on the posterior uterine wall. The implantation begins on the day 7 post-fertilization and and continues during about 40 hours.

Two stages of implantation distinguish: **adhesion** and **invasion** (penetration).

At the first stage (day 7 post-fertilisation) the trophoblast of the blastocyst attaches to the endometrial epithelium (fig.57).

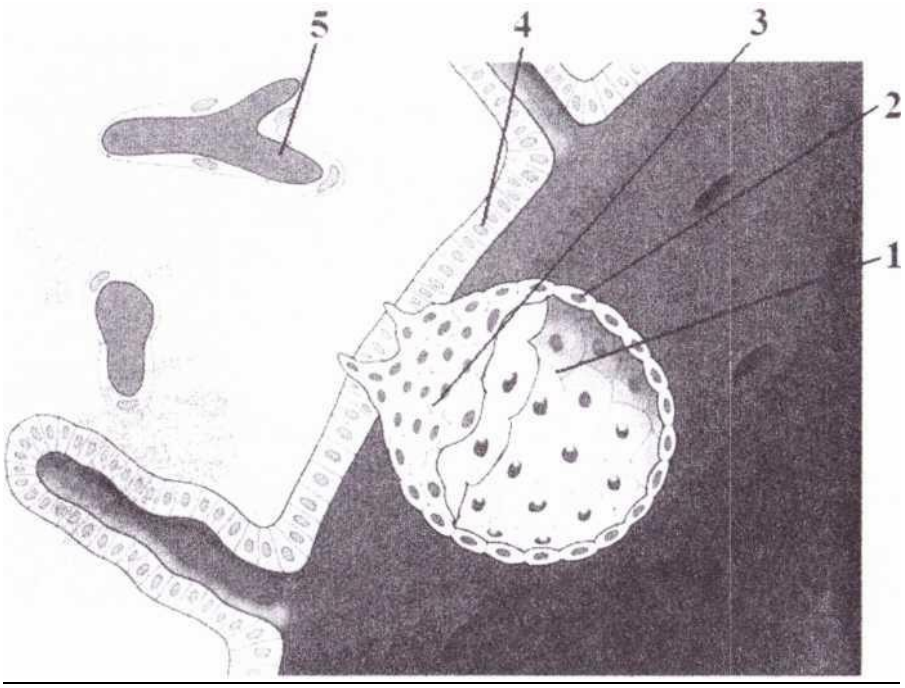


Figure 57. Implantation. Phase of adhesion. Day 6 post-fertilisation. 1 - blastocyst, 2 — trophoblast, 3 - inner cell mass, 4 - epithelium of endometrium, 5 - uterine vessels.

During implantation, the trophoblast forms two layers - an outer syncytiotrophoblast and an inner cytotrophoblast. The inner cell mass becomes organised into a two-layered plate of cells called the embryonic disc, with the amniotic cavity above and the yolk sac below. These layers are ectoderm and endoderm (fig.58).

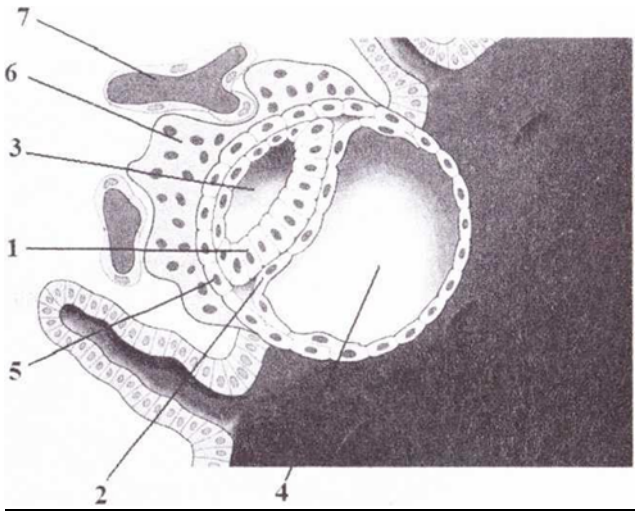


Figure 58. Implantation. 1 - ectoderm, 2 - endoderm, 3 - amniotic cavity, 4 - yolk cavity, 5 - cytotrophoblast, 6 - syncitiotrophoblast, 7 - uterine vessels

During the second stage syncytiotrophoblast, producing enzymes destroys the endometrium of the uterus (fig.57). Thus formed villi of trophoblast, invading in the uterus, consistently destroy its epithelium, then the connecting tissue and walls of vessels, and trophoblast enters direct contact to blood of mother vessels.

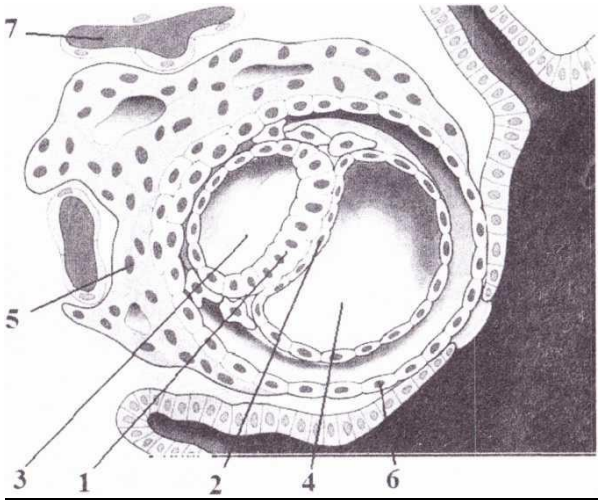


Figure 59. Implantation. Phase of invasion. Day 9 post-fertilisation. 1-ectoderm, 2 -endoderm, 3 - amniotic cavity, 4 - yolk cavity, 5 - syncitiotrophoblast, 6 -cytotrophoblast, 7 -uterine vessels.

At the beginning of the second week the blastocyst is embedded in the endometrial stroma. The endometrial cells around the early conceptus enlarge and accumulate glycogen and lipids. These cellular changes, together with the vascular and glandular alterations in the endometrium, are called the decidual reaction.

Three different regions of the decidua are identified according to the implantation site. The decidua basalis is the portion of the endometrium that underlies the implantation site. The decidua basalis torms a compact layer, called the **decidual (basal) plate**. The **decidua capsularis** is a thin portion of the endometrium that overlies the conceptus. The **decidua parietalis** (vera) includes the remaining endometrium of the uterus and the cervix (fig.58).

Nutrition of the conceptus is initially **histiotrophic** - the uptake of oviductal and uterine secretions by the trophoblast.

Later, it switches to **haemotrophic nutrition** - exchange between the maternal and fetal circulations within the placenta.

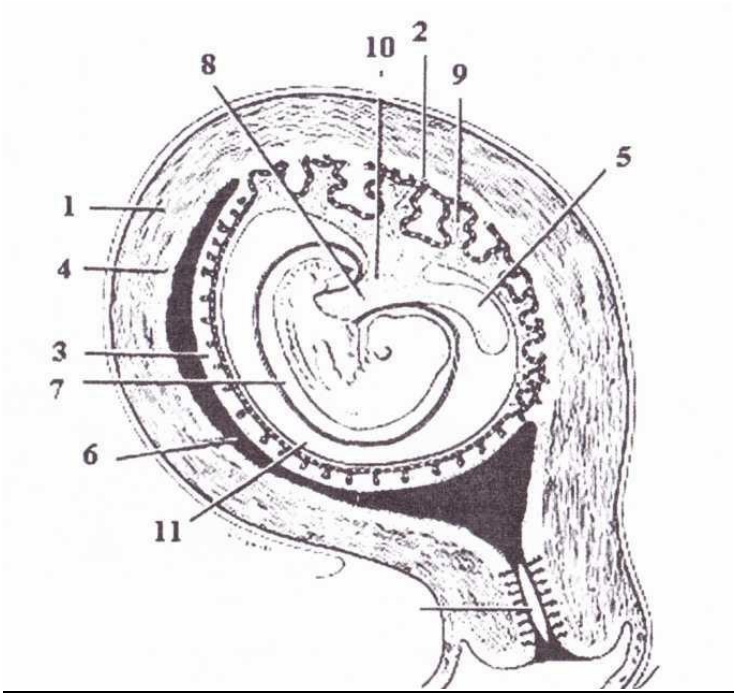


Figure 60. The regions of the decidua. 1 - myomertrium, 2 - decidua basalis, 3- decidua capsularis, 4 - decidua parietalis, 5 - yolk sac, 6 - uterine cavity, 7 - amniotic cavity, 8 - umbilical cord, 9 - chorionic villi, 10 - allantois, 11 - chorionic cavity.

Gastrulation is complex process of formation of three germ layers of the embryo: ectoderm, mesoderm, and endoderm.

Human gastrulation occurs in two phases.

The ***first phase*** of the gastrulation precedes implantation or goes during it (day 7 post-fertilisation). This phase occurs by ***delamination,*** thus the inner cell mass (embryoblast) differentiate into two layers: the ***hypoblast,*** consisting of small cuboidal cells, and the ***epiblast,*** consisting of high columnar cells.

The two-layered plate that will differentiate into the embryo is called the ***embryonic disc (germ shield).*** The epiblast forms the **floor of the amniotic** cavity and is peripherally continuous with a thin epithelial layer of the ***amnion.*** Flattened cells probably originating from the hypoblast form an ***exocoelomic membrane (Hauser's membrane).*** This membrane and the hypoblast form the lining of the ***exocoelomic cavity (primitive yolk sac).*** The hypoblast adjacent to the epiblast is the ***roof of the yolk sac.***

Cells derived from the yolk sac form the extraembryonic mesoderm (**extraembryonic** ***mesenchyme)*** and fill the space between the trophoblast externally and the amnion and the exocoelomic membrane internally . Large cavities within the extraembryonic mesoderm become confluent and form the extraembryonic coelom. The extraembryonic coelom splits the extraembryonic mesoderm into two layers: the extraembryonic somatic mesoderm, lining the trophoblast and amnion, and the extraembryonic splanchnic mesoderm, covering the yolk sac. The extraembryonic somatic mesoderm and two layers of trophoblast constitute the chorion. The extraembryonic somatic mesoderm and the extraembryonic part of the ectoderm constitute the amnion. The extraembryonic coelom expands to form a large chorionic cavity, within which the embryo and the attached amniotic and yolk sac are suspended by the **connecting stalk.**

BILAMINAR EMBRYONIC DISC FORMATION(SECOND WEEK OF DEVELOPMENT)

Embryoblast differentiates into bilaminar embryonic disc*(germ shield)*:

• Epiblast

• Hypoblast (local thickening of which forms prechordal plate, indicating future cranial region of the embryo)

Trophoblast differentiates into:

• Cytotrophoblast

• Syncytiotrophoblast, which erodes blood vessels of endometrium and establish haematotrophic circulation

Cavities formation:

• Amniotic cavity(sac)

• Umbilical vesicle (yolk sac)

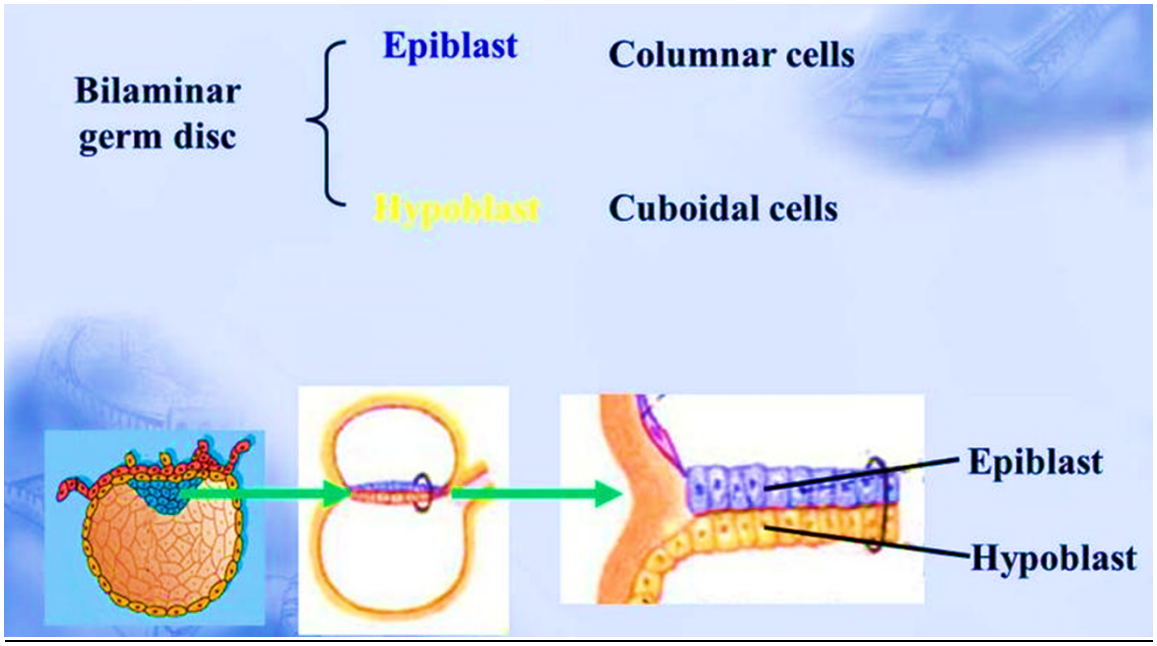


Figure 61. Structure of germ shield.

The **second phase** of the gastrulation begins **on 14-15 day** and continues about day 17 post-fertilisation. The second phase of gastrulation occurs by way **of *immigration***. The germ gets a three-layer structure.

The second phase of the gastrulation begins with the formation of the **primitive streak**. The ***primitive streak*** is a linear band of thickened epiblast that first appears at the caudal end of the embryo and grows cranially. At the cranial end its cells proliferate to form the ***primitive (Hensen's) node***. With the appearance of the primitive streak it is possible to distinguish cranial (primitiv e node) and caudal ends of the embryo. The cells that proliferate in the region of the *primitive streak* pass sideways, pushing themselves between epiblast and hypoblast. These ceils form **intraembryonic mesoderm.**

The cells that enter through **the *primitive (Hensen's) node*** will become the midline cellular cord known as the *notochordal process.* The notochordal process transforms into the notochord will eventually become the nucleus pulposis of each intervertebral disk.

The notochordal process grows cranially until it reaches the *prechordal plate*, the future site of the mouth, in this area the ectoderm is attached directly to the endoderm without intervening mesoderm. This area is known as the *oropharyngeal membrane,* and it will break down to become the mouth. At the other end of the primitive streak the ectoderm is also fused directly to the endoderm; this is known as the *cloacal membrane*, or primordial anus.

The **spaces between the germ layers are filled with *mesenchyme.***

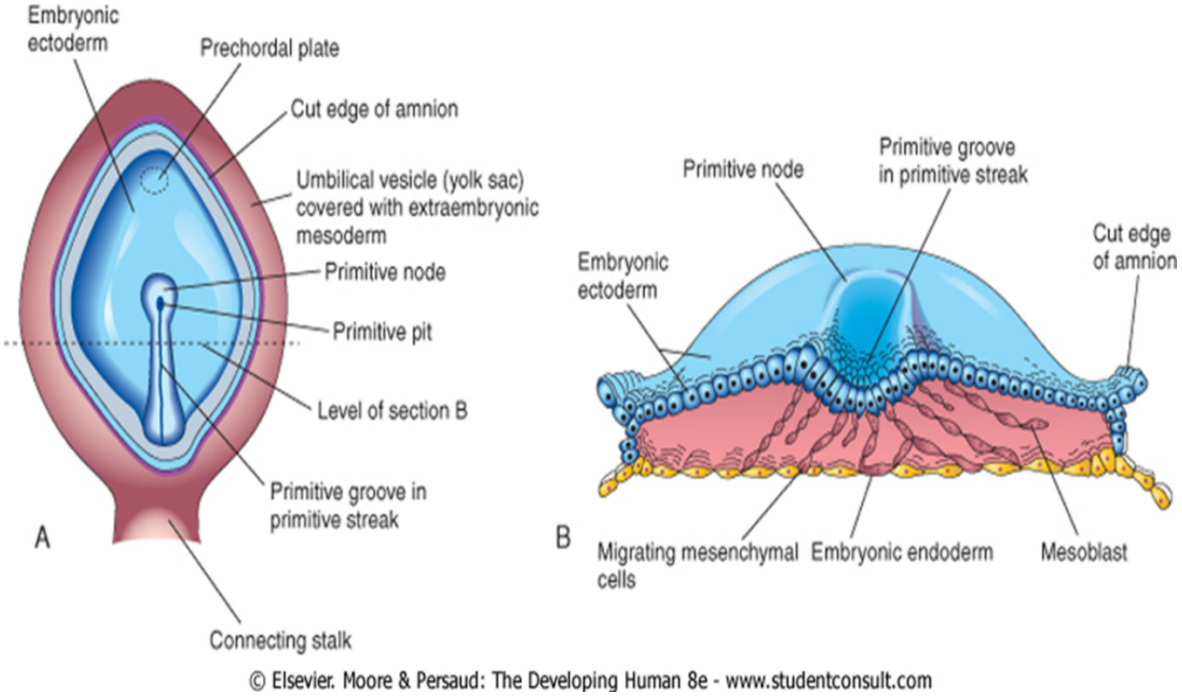


Figure 62. The second phase of the gastrulation.

Mesenchyme

The ***mesenchyme*** is the undifferentiated connective tissue found in the early embryo between the embryonic layers and axial organs. Most mesenchyme is derived from mesoderm.Mesenchyme consists of small stellate (star) shape cells containing large oval nuclei with prominent nucleoli. Processes of mesenchymal cells extend and contact those of other cells to form a three dimensional cellular network . A semi-fluid ground substance fills the extracellular spaces. Fibers are present, but are very fine and sparse.

Mesenchymal cells are multipotential cells that can be transformed into other types.

Mesenchyme gives rise to all the connective tissues of the body, as well as to some other tissue:

- connective tissues;
-walls of blood vessels;
- cells of blood and lymph;
-smooth muscle tissue;
-microglia of nerve tissue.

Folding of the embryo

The folding of the flat trilaminar embryonic disc into a cylindric embryo establishes the general body form.Folding occurs by differential growth of tissues. Neural ectoderm grows faster than the surrounding skin ectoderm and consequently folds to form a neural tube. Similarly, skin ectoderm grows faster than the underlying mesoderm and endoderm, and this differential growth causes folding of the trialminar disc and gives shape to the embryo.

Folding in the medial plane produces the head and tail folds, and results in the incorporation of part of the yolk sac into the embryo.

These are not three separate folds but occur simultaneously and merge into one another. **The notochord, neural tube and somites stiffen the dorsal axis of the embryo.**

Folding of the embryo in the horizontal plane produces the lateral folds and the formation of the lateral and ventral body walls. Part of the yolk sac is incorporated into the embryo as the midgut.

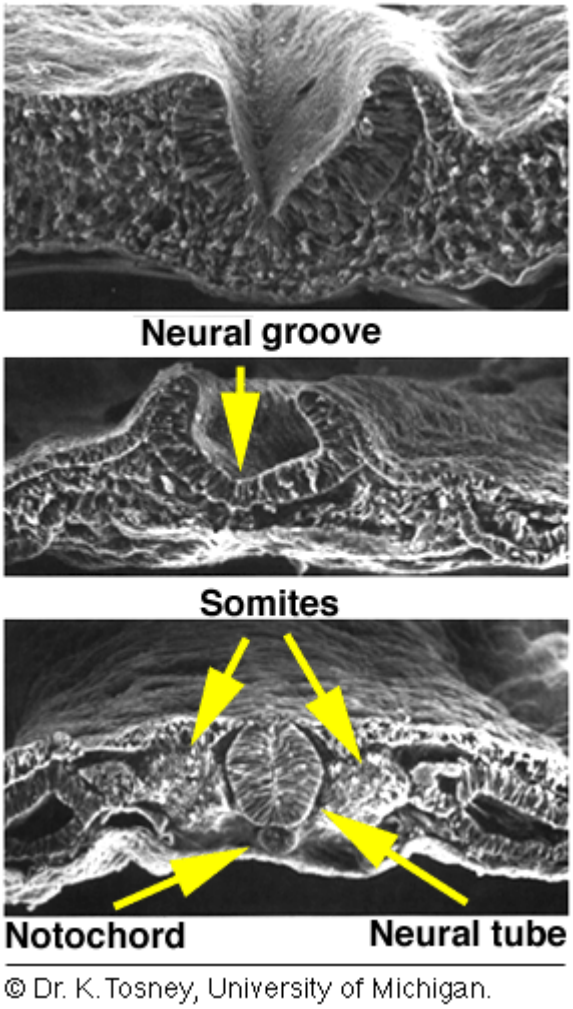
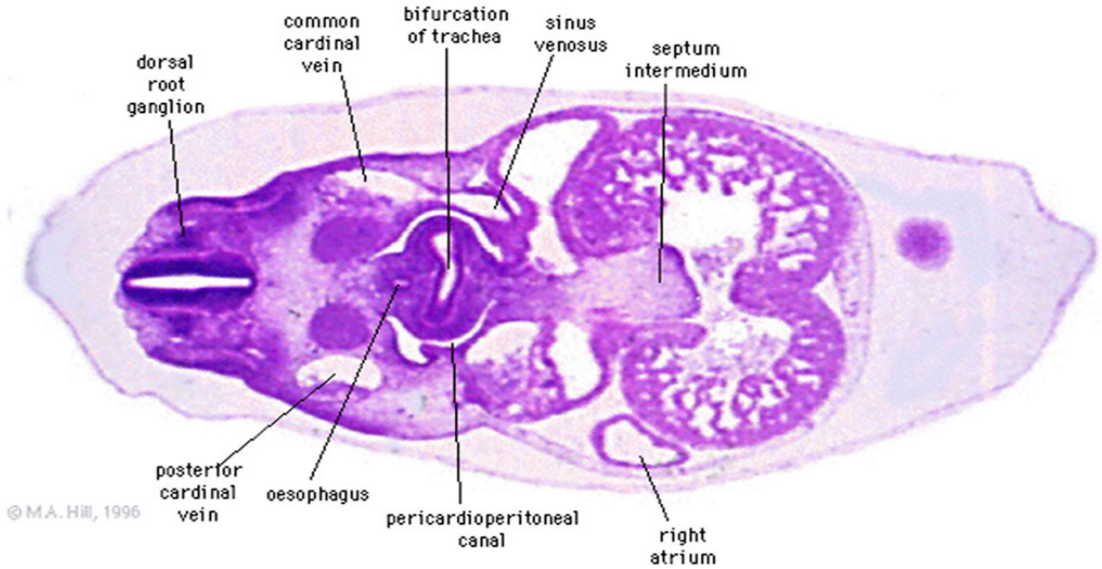


Figure 63. Folding of the embryo.



Differentiation of the germ layers

Derivatives of the ectoderm. Ectoderm gives rise to:
- the central nervous system;
- the peripheral nervous system;
- the sensory epithelium of the ear, nose and eye;

- the epidermis, hair and nails; and
- the subcutaneous, mammary;
- pituitary gland;
- the enamel of teeth.

Neural crest cells give rise to the cells of ganglia andensheating cells of the peripheral nervous system, pigment cells of the dermis, muscles, connective tissue and bone of the branchial arches, suprarenal medulla and meninges.

Derivatives of the endoderm

Cranio-caudal and lateral folding of the embryo causes the incorporation of the part of the yolk sac into the body cavity and the formation of a tube-like gut. This layer gives rise to the epithelial lining of the:

- gastrointestinal system,
- repiratory system,
- tympanic cavity and auditory tube, and
- the parenchyme of the tonsils, thyroid, parathyroid, thymus, liver and pancreas.

Derivatives of the mesoderm

Initially, the thin sheath of mesodermal layer proliferates and forms the paraxial mesoderm medially and lateral plate laterally. They are connected by the intermediate mesoderm. The lateral plate divides into two layers: somatic (parietal) and **splanchnic (visceral) mesoderm**.

The **paraxial mesoderm** breaks into segmented blocks, the **somites** (42- 44 pairs). The epithelial cells forming the somites loose their epithelial shape and migrate in the direction of the notochord and the spinal cord to form the **sclerotome** (future vertebral column). The dorsal wall of the somite differentiates into the **myotome** (furute skeletal muscles) and the **dermatome** (future dermis).

The **intermediate mesoderm** forms **nephrotomes** cranially and nephrogenic cord caudaily, both developing into the excretory units of kidneys, gonads, ducts and accessory glands.

Splanchnic (visceral) mesoderm is divided into two sheets **visceral and parietal.** These layers limit the secondary body cavity of the embryo called the **whole**. From visceral and parietal layers of the splanchnotome develop visceral and parietal pleural, peritoneal and pericardial leaves (serous membranes).

Extra-embryonik human organs are amnion, yolk sac, allantois, chorion, placenta, umbilical cord. The structure and function of these organs are described in the previous section.

The system mother - fetus

The system mother - fetus arises during pregnancy and includes two subsystems - an organism of mother and an organism of a fetus, and also a placenta being a link between them.

Interaction between an organism of mother and an organism of a fetus is provided by neurohumoral mechanisms.

Organisms of mother and fetus are dynamic system of homological organs. Damage of any organ of mother conducts to disturbance of development of the same organ of a fetus.

The critical periods of development

Individual development proceeds from the formation of germ cells (sperm and egg) through fertilization, embryonic and fetal development, infancy, early childhood, and adolescence. Specific events during each of these broad developmental stages may create sensitivity to environmental influences. Damage from environmental exposures may occur and manifest itself immediately or may not appear until subsequent stages of development or after development is complete.

Such periods in human ontogenesis are:

1) development of sexual cells (oogenesis and spermatogenesis);
2) fertilization;
3) implantation (7-8 days);
4) development of axial organs, differentiation of germ layers, and formation of a placenta (3-8 weeks);
5) stage of strengthened growth of a brain (15-20 weeks);
6) formation of the main functional systems of an organism and a differentiation of the sexual organs (20-24 weeks);
7) birth;
8) period till 1 year;
9) puberty (11 - 16 years).

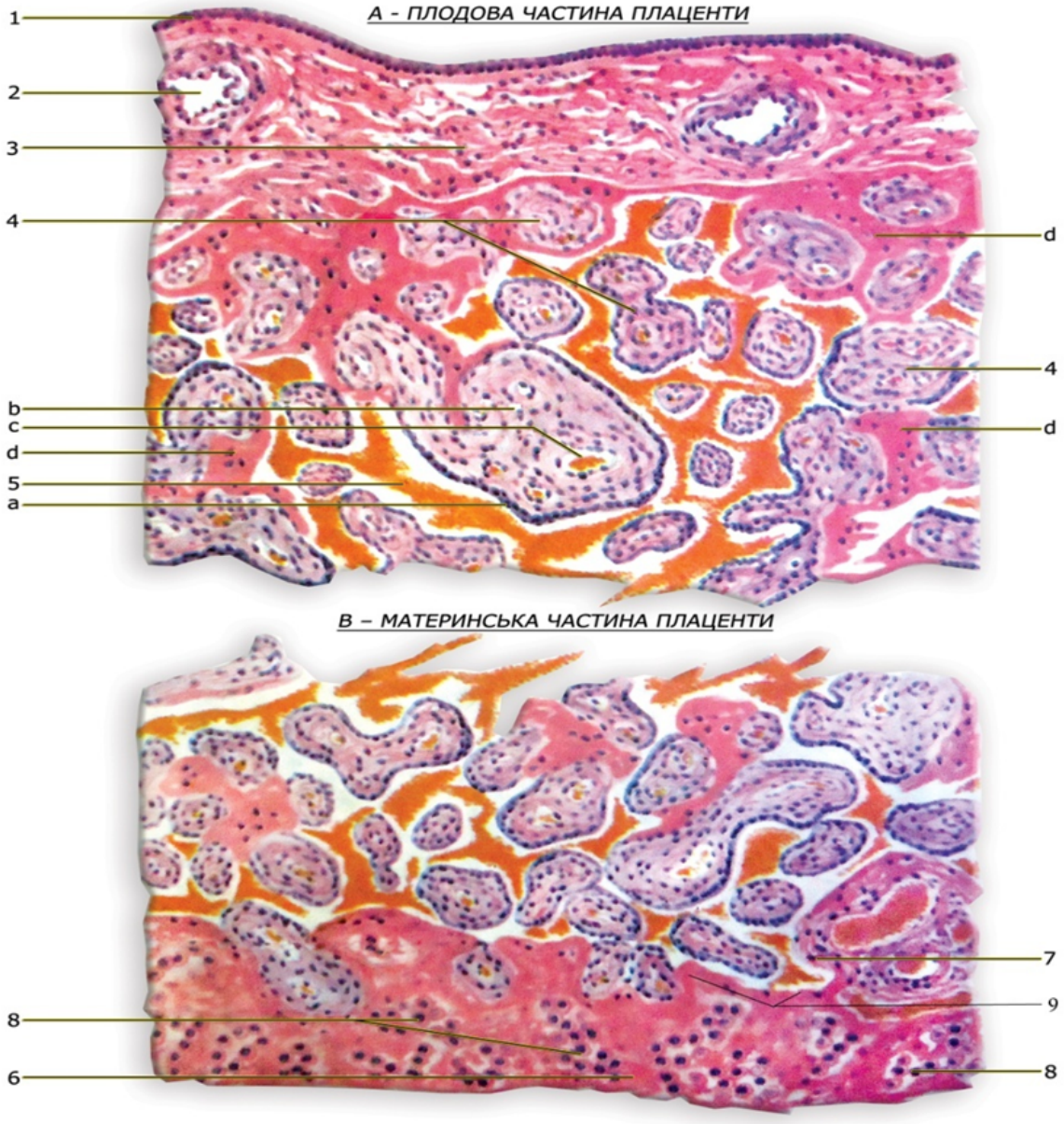
Many factors such as chemical substances, including many medicinal, an irradiation (for example, X-ray in diagnostic dozes), hypoxia, starvation, drugs, nicotine, viruses, etc can be damaging in the critical periods.

Practical lessons № 7-8

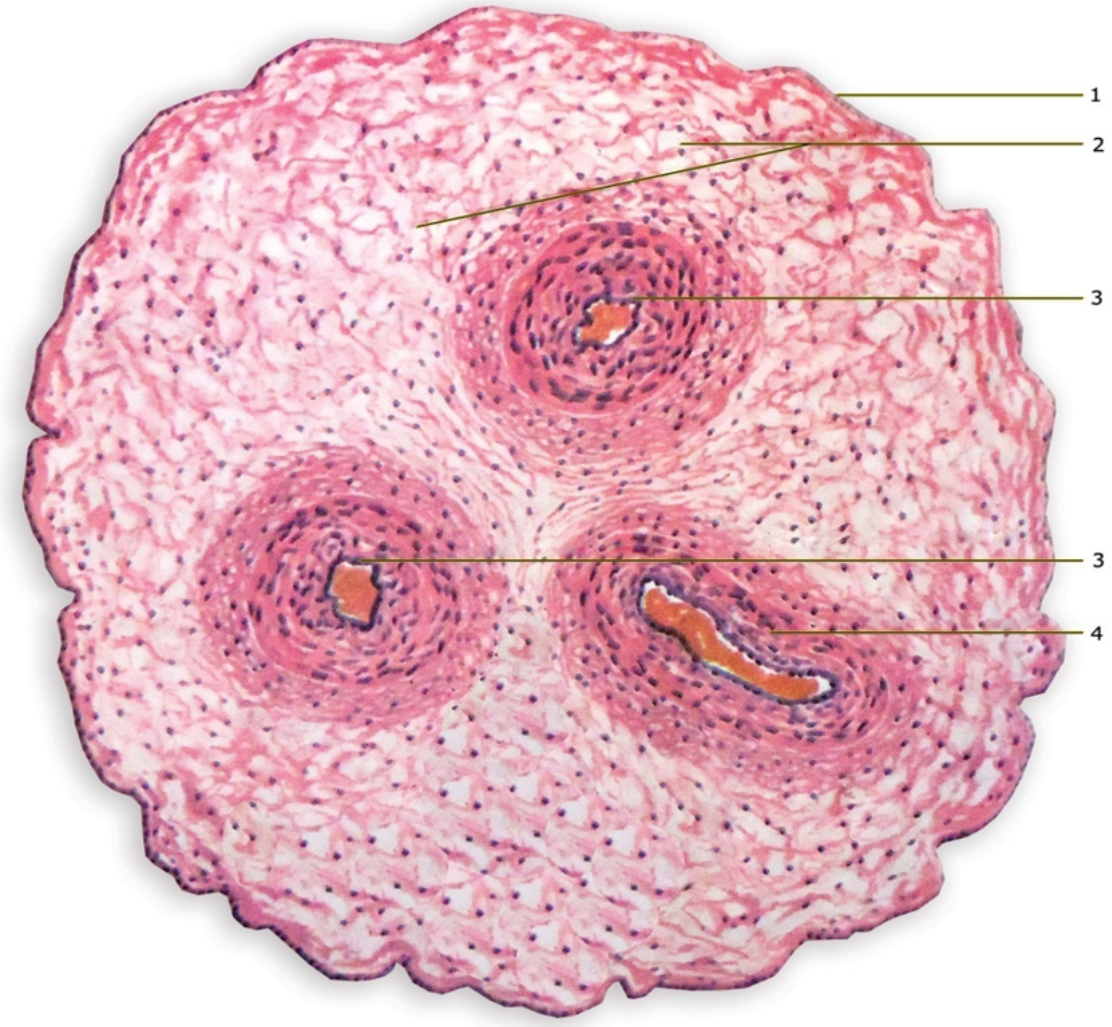
Questions for self-control

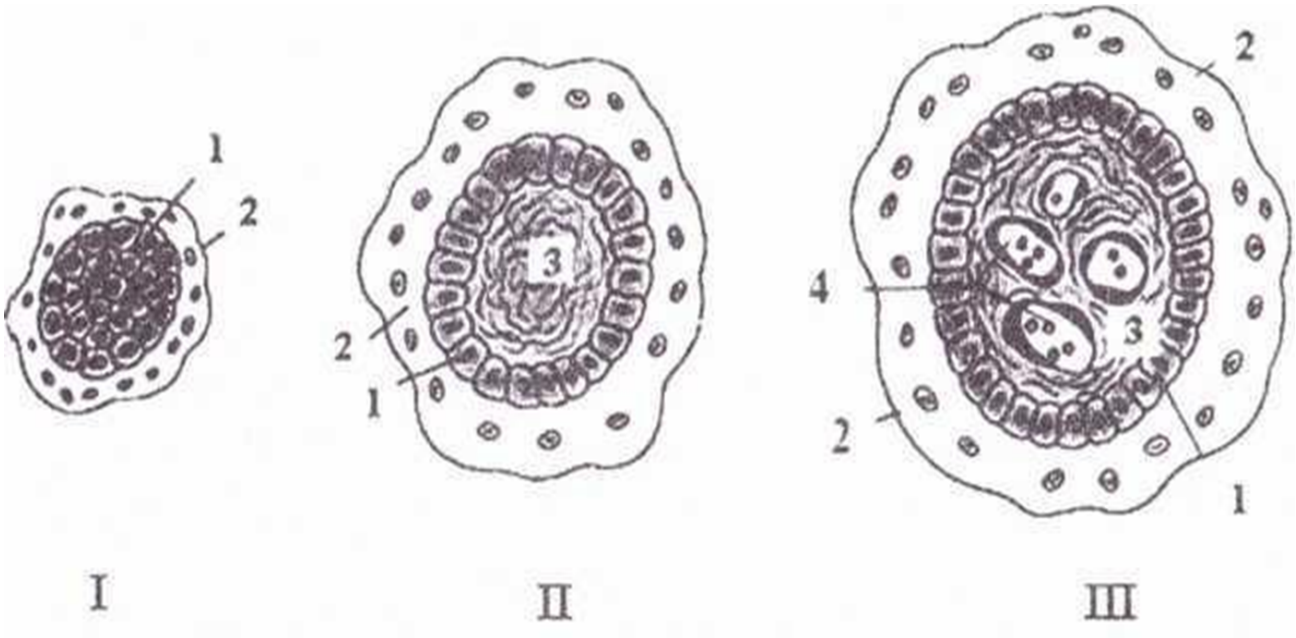
1. Periods of embryogeny. Characteristic features of the embriogenesis stages.
2. Germ cells: their differences compared to somatic ones. Spermatozoon: structure and functions. Oocyte: structure and functions.
3. Fertilization: characteristic features.
4. Connection of the embryo with a maternal organism. Implantation. Placenta.
5. Gastrulation. Germ layers. Their significance.
6. Ectoderm and entoderm differentiation.
7. Mesoderm differentiation.
8. Critical periods of the embriogeny. Endo- and exogenic factors, which influence on its development.

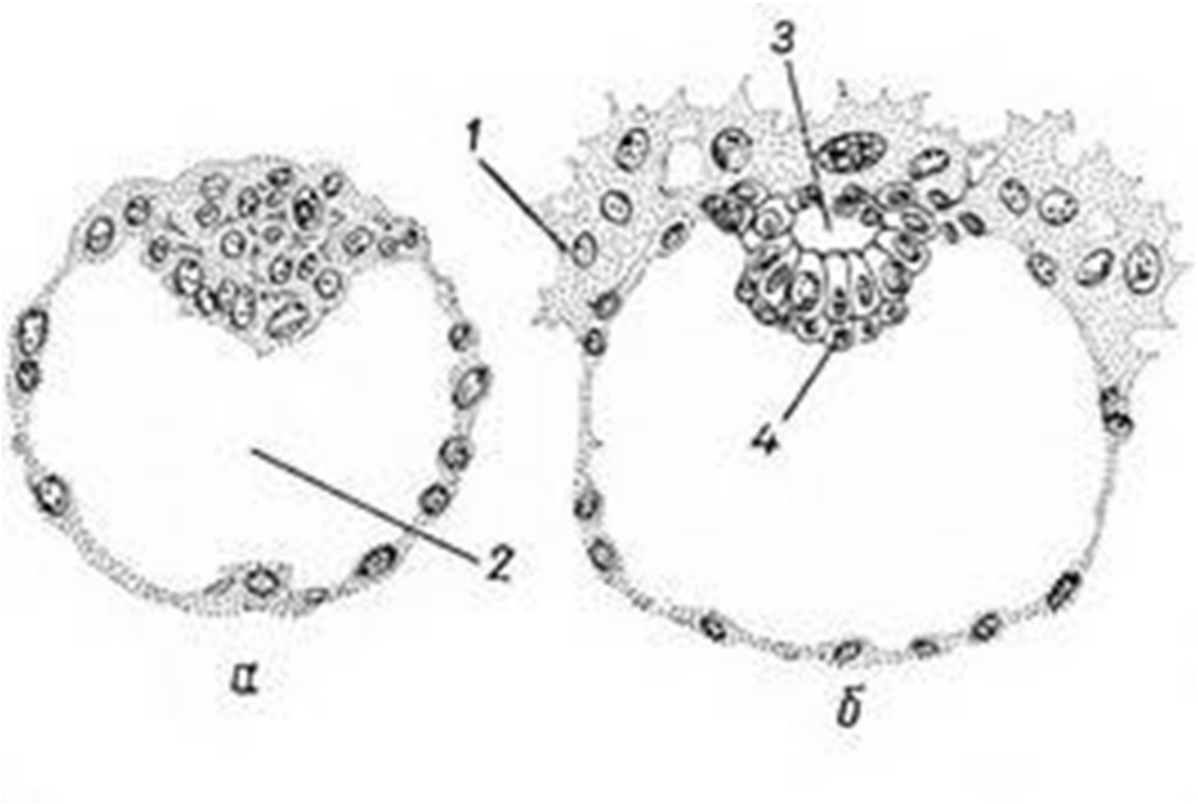
FETAL AND MATERNAL PART OF PLACENTA

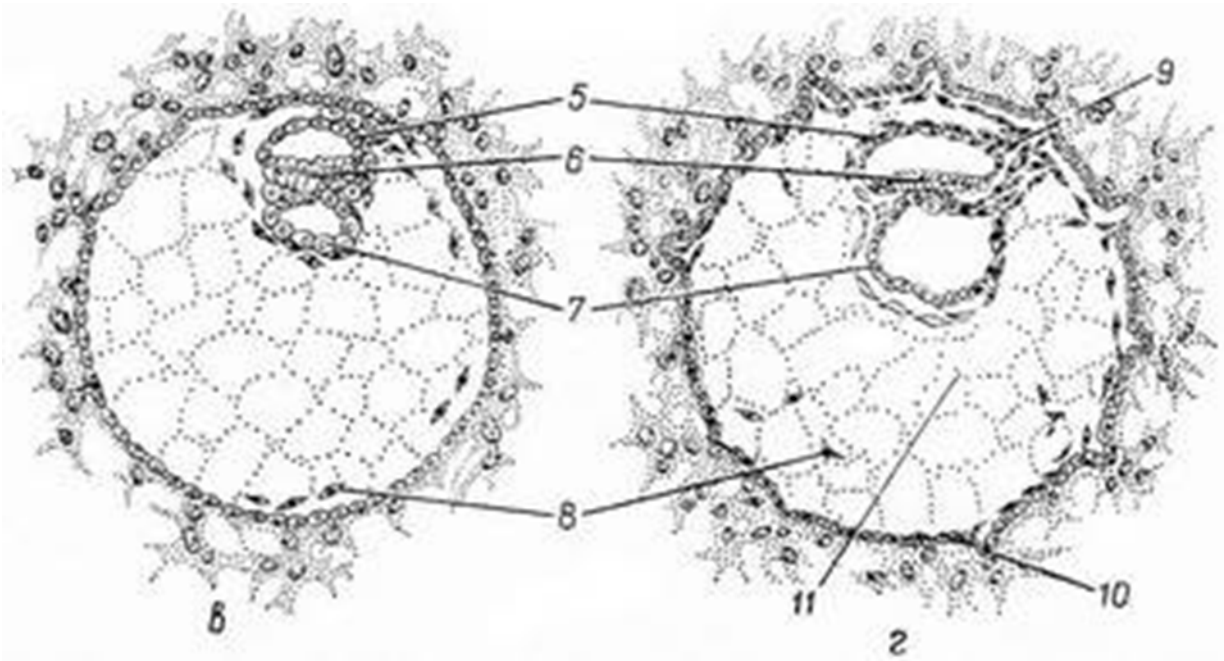


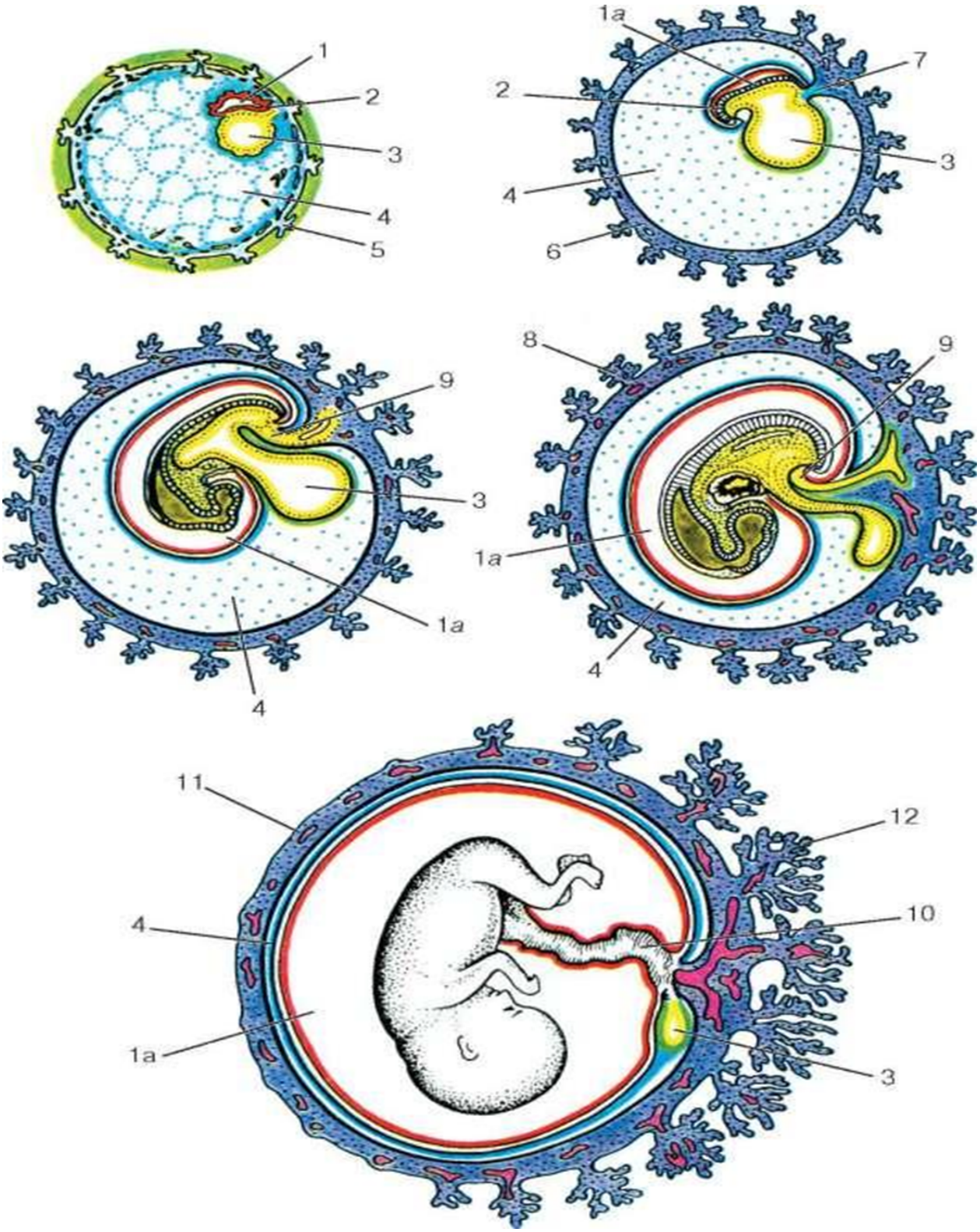
UMBILICAL CORD






1
2
3
4
а
б


5
6
7
8
9
10
11
в
г



The teacher's signature:

GENERAL PRINCIPLES OF ORGANIZATION AND CLASSIFICATIONS OF THE TISSUES EPITHELIAL TISSUES (EPITHELIA). GLANDULAR EPITHELIA. GLANDS

The tissue is the system of the cells and the extracellular matrix, which specialized on the execution of the definite functions.

The human body is composed of four basic types of tissue:

1. epithelial tissue characterized by a virtual lack of intercellular substances between adjacent cells; forms skin and lines interior surfaces of body (gut, peritoneum, etc.);epithelial cells can also be arranged in masses with a secretory function.

2. connective (tissues of the internal environment of the organism) tissue cells usually are widely separated by an abundant intercellular matrix; includes blood, cartilage, bone, tendons, adipose (fat); 2 Major Divisions = CT Proper and CT Supportive.

3. muscle tissue - elongate cells separated by fine, vascular (many blood vessels and capillaries) connective tissue. Three types occur: skeletal, cardiac, and smooth - each is specialized for particular functions.

4. nerve tissue -cells are grouped into masses or bundles, many with long processes; specialized for sensory reception and transmission of impulse functions.

Structural and functional elements of tissues are:

1. Cells are the main elements of all tissues which are determining their basic properties.
2. Intercellular substance is the cumulative product of activity of cells of the tissue.
3. Postcellular structures is the derivatives of cells which during a differentiation have lost the major signs, characteristic for cells (more often owing to loss of a nucleus and part of organelles), but have got a number of the properties necessary for performance by them specialized functions (erythrocytes and platelets, dead cells of the epidermis, hair and nails).
4. **Symplasts** are the structures formed as a result of cell fusion with loss of their borders and formation uniform cytoplasm mass in which there are nuclei (osteoclasts, an external layer of trophoblast - symplastotrophoblast, fibers of a skeletal muscular tissue).
5. **Syncytium** is the structure arising as a result of incomplete cytotomia at cell division. The connections between elements of cells are preserved by means of cytoplasmic bridges (spermatogenic epithelium of convoluted seminiferous tubules of testis).

Differentiation is process during which cells of the tissue pass a number of stages of development, gradually getting structural and functional properties of mature elements.

Differon is set of all cells making the given line of a differentiation - from the least differentiated (stem) up to the most mature differentiated. Many tissues contain several cellular differons which cooperate with each other.

Stem cells are the least differentiated cells of the given tissue being a source of development of its other cells.

Epithelial tissues (epithelia)

Epithelial tissues are widespread throughout the body. They form the covering of all body surfaces, line body cavities and hollow organs, and are the major tissue in glands.

Functions of epithelium

1. Protection, protect underlying tissues from mechanical injury, harmful chemicals, invading bacteria and from excessive loss of water.
2. Absorption: certain epithelial cells lining the intestine absorb nutrients from the digestion of food.
3. Secretion, in glands, epithelial tissue is specialised to secrete specific chemical substances such as enzymes, hormones and lubricating fluids.
4. Excretion, epithelial tissues in the kidney excrete waste products from the body and reabsorb needed materials from the urine. Sweat is also excreted from the body by epithelial cells in the sweat glands.
5. Sensation: sensory stimuli are detected by specialized epithelial cells; specialized epithelial tissue containing sensory nerve endings is found in the skin, eyes, ears and nose and on the tongue.
6. Diffusion, simple epithelium promotes the diffusion of gases, liquids and nutrients; because they form such a thin lining, they are ideal for the diffusion of gases (e.g. walls of capillaries and lungs).

7. Contraction e.g., myoepithelial cells have ability to contract.

8. Cleaning: ciliated epithelium assists in removing dust particles and foreign bodies which have entered the air passages.

EPITHELIAL TISSUES - General Features

1) Cells are closely packed together.
2) Intercellular substance is reduced to a minimum.
3) Cells rest on the basal lamina.
4) Polarity of epitheliocytes (in the epitheliocytes there are apical and basal poles).
5) All epithelia have no blood vessels. They derive their nutrition from the blood vessels of underlying connective tissue.
6) Availability of intercellular junctions.
7) High ability to regeneration.

Morphofunctional classification of the epithelia

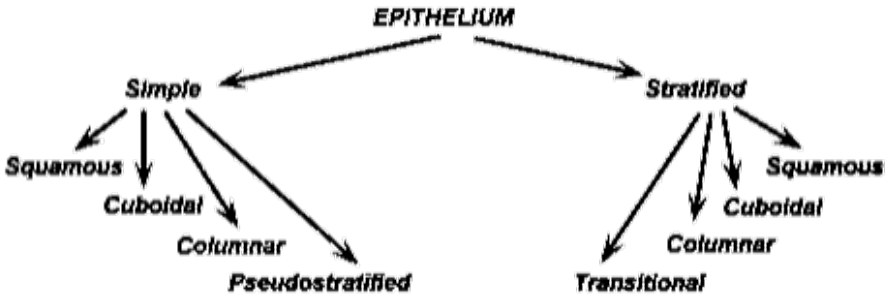
Epithelia are classified according to the structure and functions into 2 main groups:

- covering (integumentary) epithelia,
- glandular epithelia.

Covering epithelia are tissues whose cells are organized in the layers that cover the external surface or line the cavities of the body.

Covering epithelia are classified according to the number of cell layers and morphology of the cells in the surface layer:

1- Simple epithelia consist of only one layer of cells.
2- Stratified epithelia contain more than one layer.



Histogenetic classification of the epithelia

Histogenetic type of epithelium	Embryonic sources	Examples
1. Epidermal	Ectoderm	Epithelia of the • skin • nasal cavity • mouth cavity • anal canal • cornea
2. Enterodermal	Endoderm	Epithelia of the • alimentary systems •respiratory systems
3. Celoneph rodermal	Celom, nephrotome	Epithelia of the • nephron • genital ducts • ovary • testis • prostate gland • renal cortex • mesothelium
4. Angiodermal	Mesenchymame	• Endothelium
5. Ependymoglial	Neuroectoderm	• Ependymal cells that line the cavities of the CNS

Basal lamina. Basement membrane

The basal lamina connects the epithelium and subjacent connective tissue. With electron microscope the basal lamina consists of 2layers: inner lamina lucida (thin amorphous layer of glycoprotein) and outer lamina densa (thick network of collagen fibrils) (fig.64). Outside the basal lamina is associated with the reticular lamina; it consists of delicate reticular fibres.

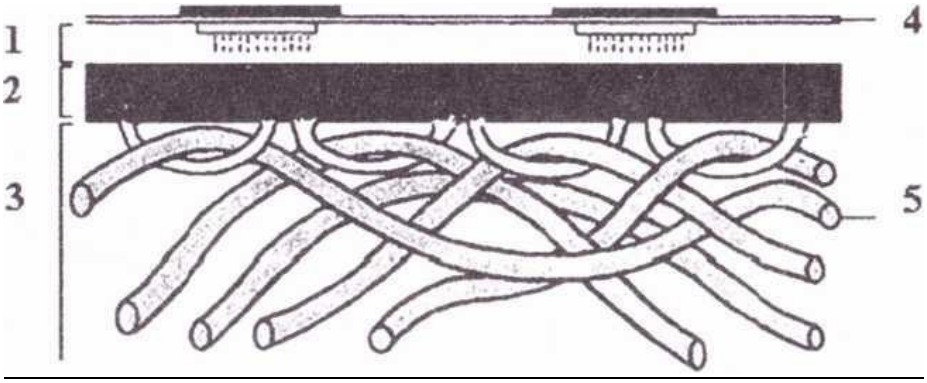


Figure 64. Schematic diagram of the basement membrane. 1 - lamina lucida, 2 - lamina densa, 3 - reticular lamina, 4 - plasmolemma of the basal surface of the epitheliocytes, 5 - collagen fibers (from EbiKoe B.J1., 2007)

The combination of basal lamina and the layer of reticular fibres appear as a single membrane under the optical microscope termed the basement membrane.

Simple squamous epithelium:

- It is composed of one layer of flat cells (having one flat nucleus).
- It is found in the alveoli of the lungs, in the kidney glomeruli, in the lining of the heart, blood vessels and lymphatic vessels and in the lining of the ventral body cavities.
- Because this epithelium is the thinnest of all, it is well adapted for diffusion (for example gas exchange between alveoli and blood in the lung or exchange of waste and nutrients between blood and surrounding tissues), filtration (of plasma in the kidney glomeruli to produce urine), secretion (of a lubricating substance in the lining of the body cavities).

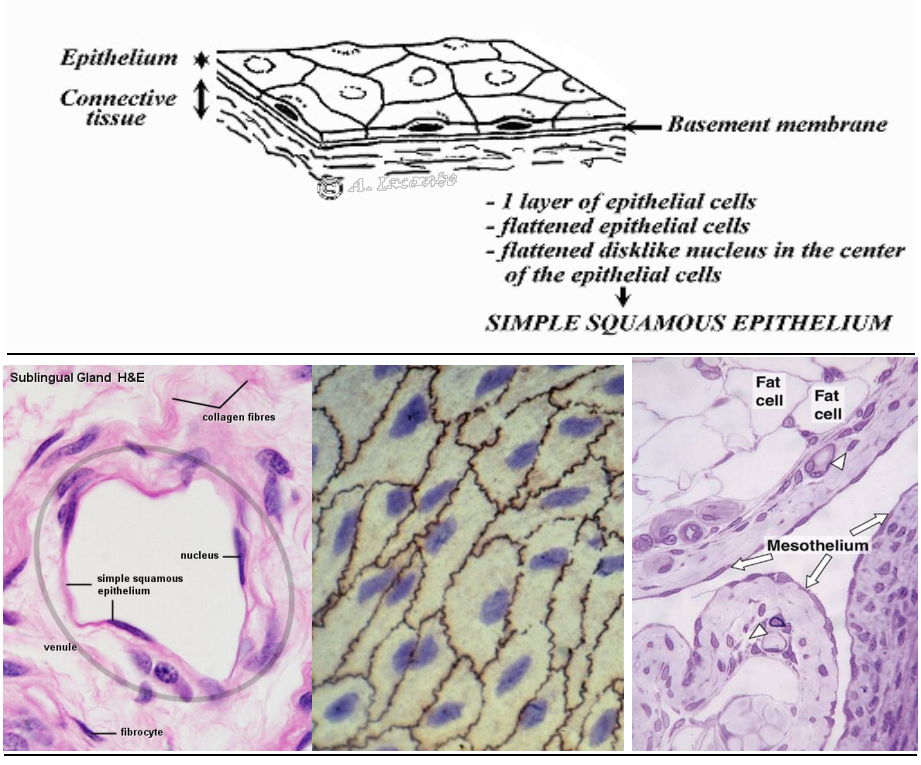


Figure 65. Simple squamous epithelium; endothelium, mesothelium.

Simple cuboidal epithelium:

- It is composed of one layer of cuboidal cells (having one round nucleus).
- It is found in small glands, kidney tubules and ovary surface.
- It is adapted for secretion and absorption of substances (for example to give urine its final composition, it moves substances in and out of the kidney tubule).

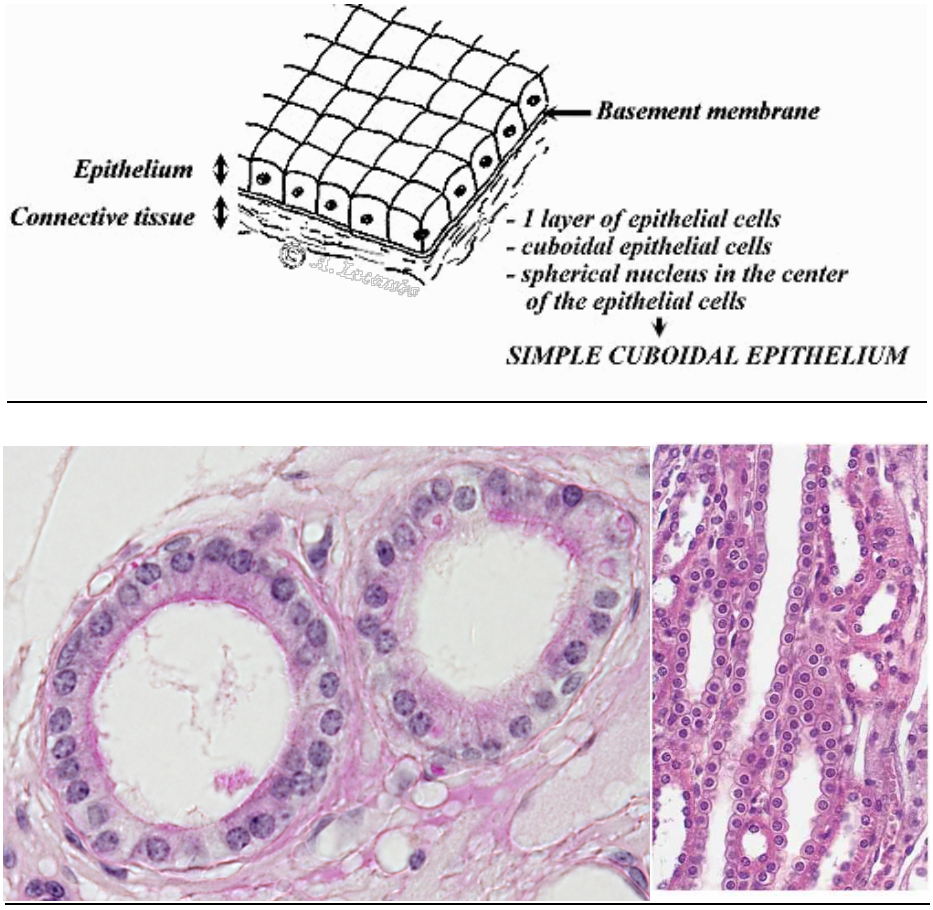


Figure 66. Simple cuboidal epithelium.

Simple columnar epithelium:

- It is composed of one layer of columnar cells (having one oval nucleus). They can be ciliated or non-ciliated.
- The non-ciliated, simple columnar epithelium contains microvilli on the apical surface of its cells. Microvilli increase the surface area of the epithelium, and thus, the non-ciliated type is found mainly lining the digestive tract and is involved in absorption of digested food products and in secretion of mucus, enzymes and other substances. It is also found lining the ducts of some glands.
- The ciliated simple columnar epithelium contains, of course, cilia on its apical surface. It is found in the small bronchi, the uterine tubes and part of the uterus. It is involved in the secretion of mucus and other substances and in moving mucus or female reproductive cells.

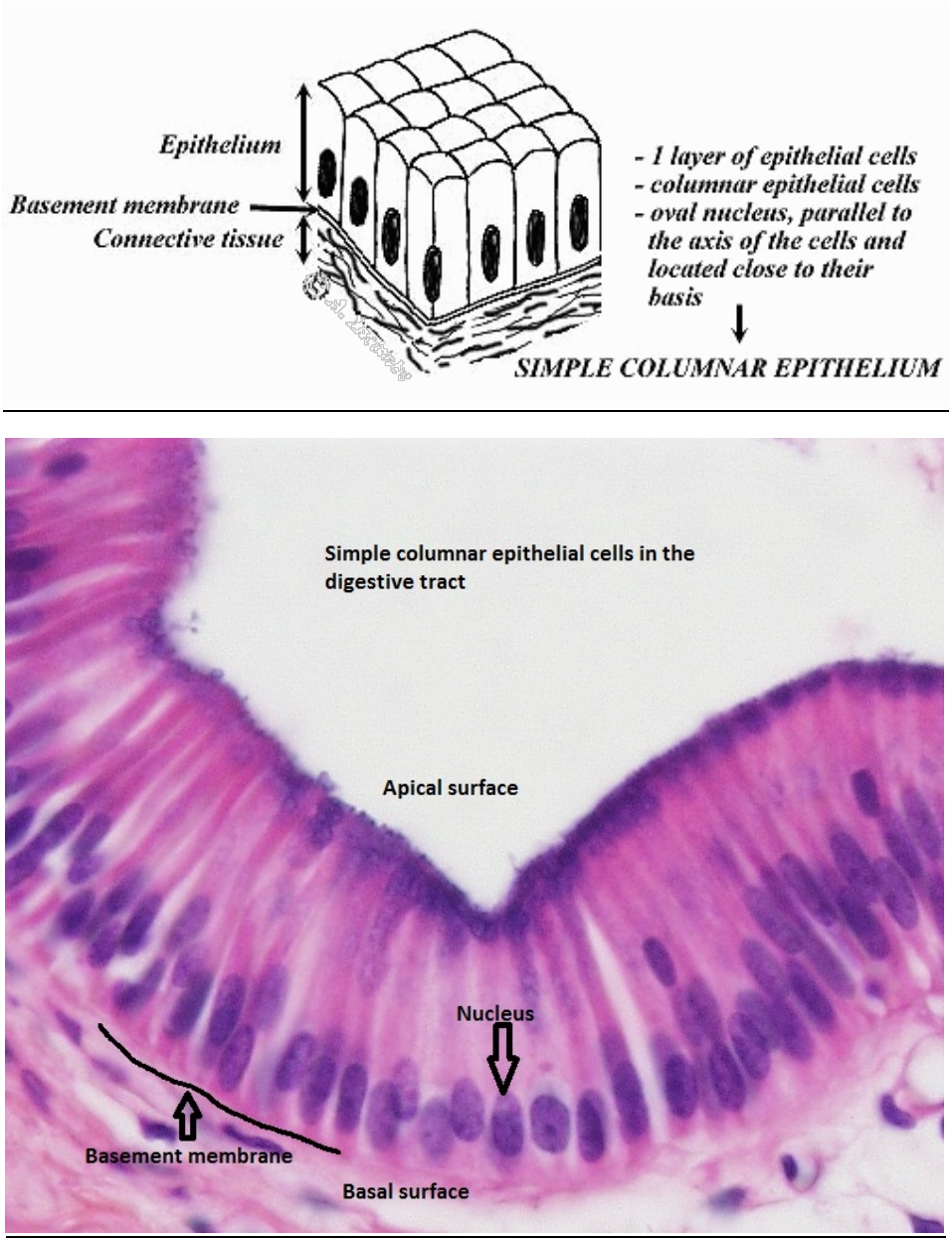
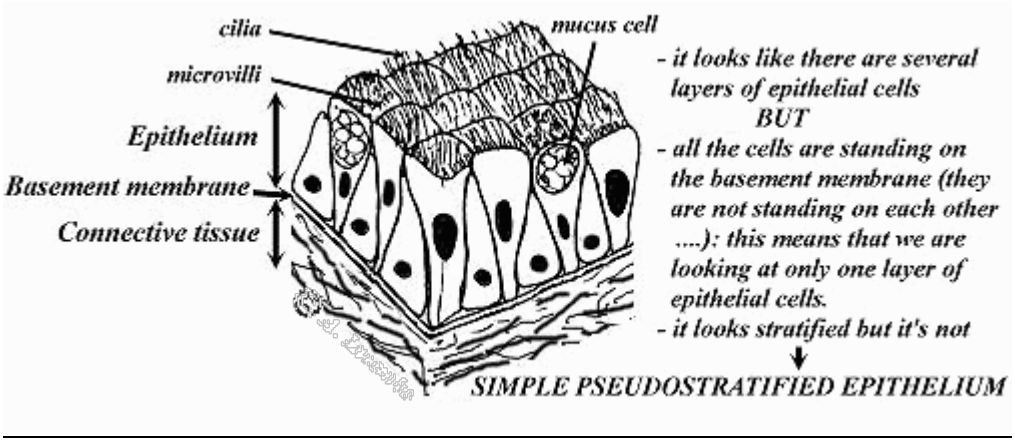


Figure 67. Simple columnar epithelium.

Simple pseudostratified epithelium:

- This epithelium has only one layer of cells: all its cells rest on the basement membrane, but it appears stratified because the cells are of different heights and their nuclei are at different levels. They can be ciliated or non-ciliated.
- The non-ciliated pseudostratified epithelium is found lining part of the male urethra and ducts of large glands.
- The ciliated pseudostratified epithelium contains cilia on its apical surface. It is found in the trachea, primary bronchi and in most of the upper respiratory tract and is involved in secretion and propulsion of mucus.



The ciliated pseudostratified epithelium contains 5 type of cells:

1. **ciliated columnar cells** constitute the most abundant type; they have about 300 cilia on its apical surface;
2. **mucous goblet cells** have mucous droplets on their apical portion;
3. **brush cells** have numerous microvilli on their apical surface. The basal surface of these cells is in contact with afferent nerve endings. Thus, the brush cells are regarded as a receptor cells;
4. **basal (short) cells** are small rounded cells that lie on the basal lamina; they differentiate into the other cell types;
5. **small granule cells** have numerous granules; these cells constitute a population of cells of the diffuse neuroendocrine system.

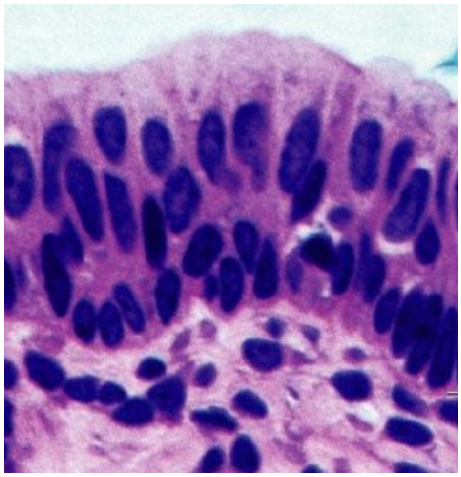


Figure 68. Simple ciliated pseudostratified epithelium.

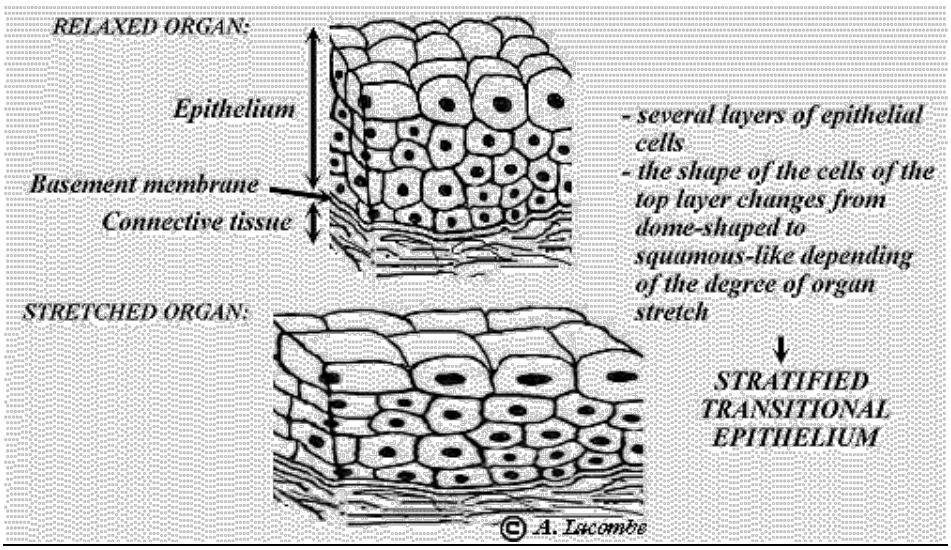
Stratified transitional epithelium:

- This epithelium has several layers of epithelial cells. It is found lining surfaces of organs subjected to stretch, such as the bladder, the ureters and part of the urethra.
- Its allows for distension of the urinary organ.
- Because the shape of the cells at the surface layer is transitory (changes depending on the degree of stretching of the organ), this epithelium is called transitional. It will look like a stratified squamous epithelium if it is stretched or a stratified cuboidal epithelium if it is unstretched.

Transitional epithelium is made up of three types of cell **layers**:

1. basal,
2. intermediate, and
3. superficial.

These cells contain a prominent Golgi apparatus and an array of membrane-bound vesicles. These function in the packaging and transport of proteins, such as keratin, to the superficial cell **layer**.



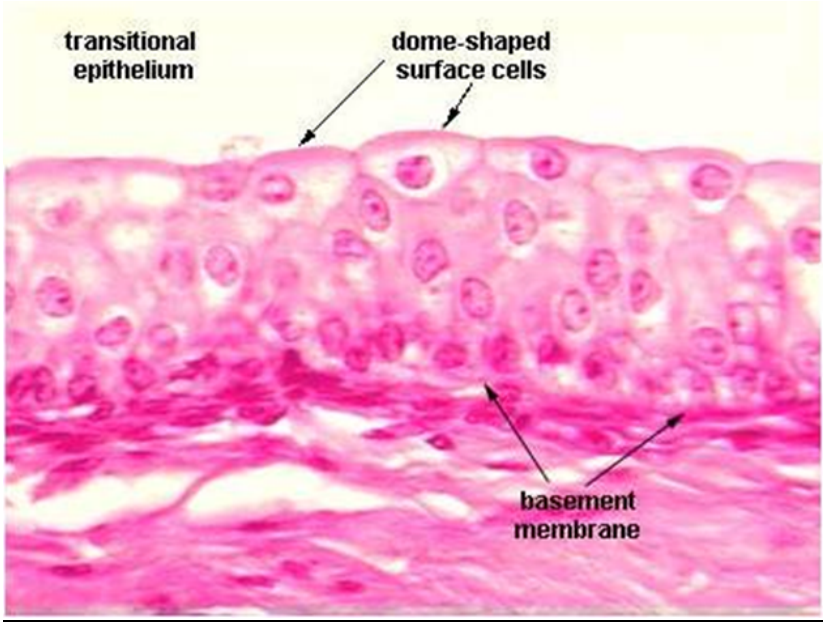
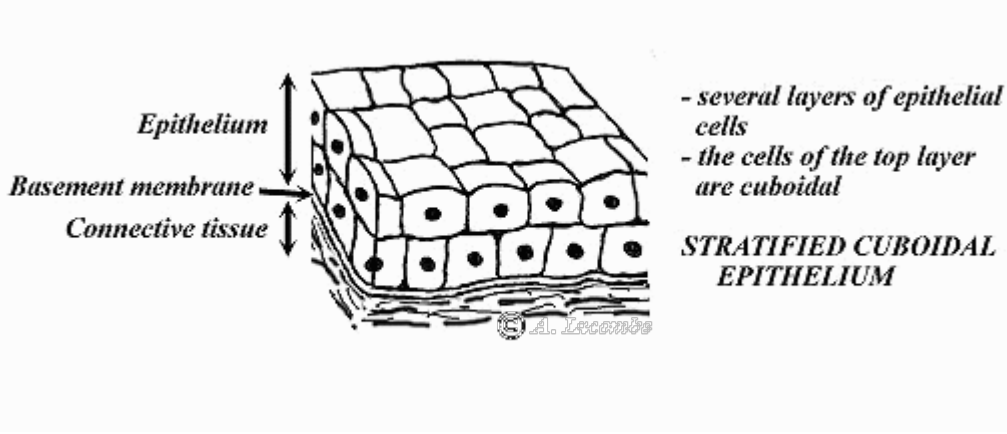


Figure 69. Stratified transitional epithelium.

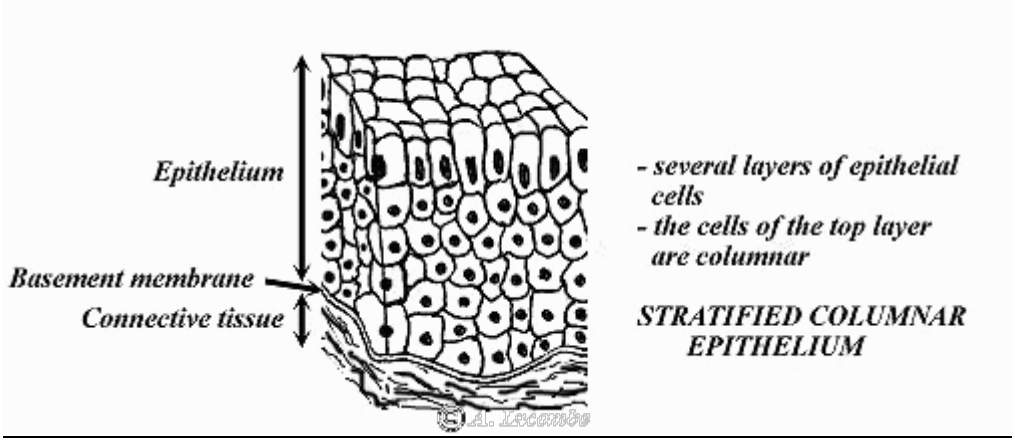
Stratified cuboidal epithelium:

- This epithelium has several layers of epithelial cells, but the surface layer of this epithelium is composed of cuboidal cells.
- It is found in the largest ducts of sweat glands, mammary glands, salivary glands and in parts of the male urethra.
- Its role is protection.



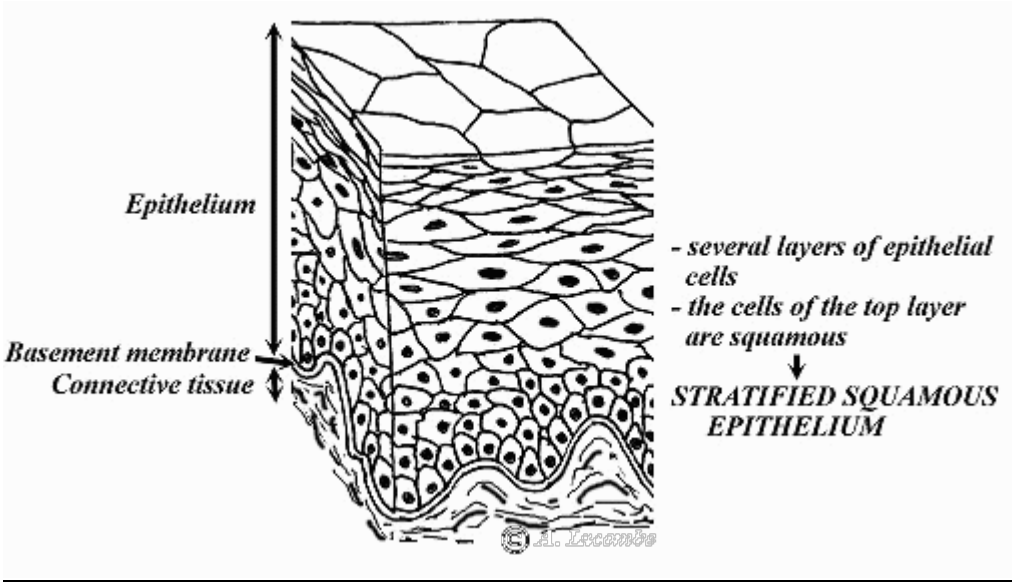
Stratified columnar epithelium:

- This epithelium has several layers of epithelial cells, but the surface layer of this epithelium is composed of columnar cells.
- Very rare: it lines part of the urethra, large ducts of some glands, portion of the conjunctiva of the eye.
- Its roles are protection and secretion.



Stratified squamous epithelium:

- This epithelium has several layers of epithelial cells, but the surface layer of this epithelium is composed of flat cells. It is the thickest of all the epithelia and its function is protection.
- The cells of the surface layer may or may not contain keratin, a tough protective protein which prevents water loss, is resistant to friction and repels bacteria.
- Keratinized, stratified squamous epithelium forms the epidermis of the skin.
- Non-keratinized stratified squamous epithelium lines wet surfaces subjected to abrasion, such as the lining of the mouth, oesophagus, tongue, part of the epiglottis and vagina.



Stratified squamous non-keratinised epithelium consists of 3 layers:

1. Stratum hasale contains a single layer of columnar or cuboidal cells resting on the basal lamina.
2. Stratum spinosum consists of polygonal cells.
3. Surface layer consists of squamous cells.

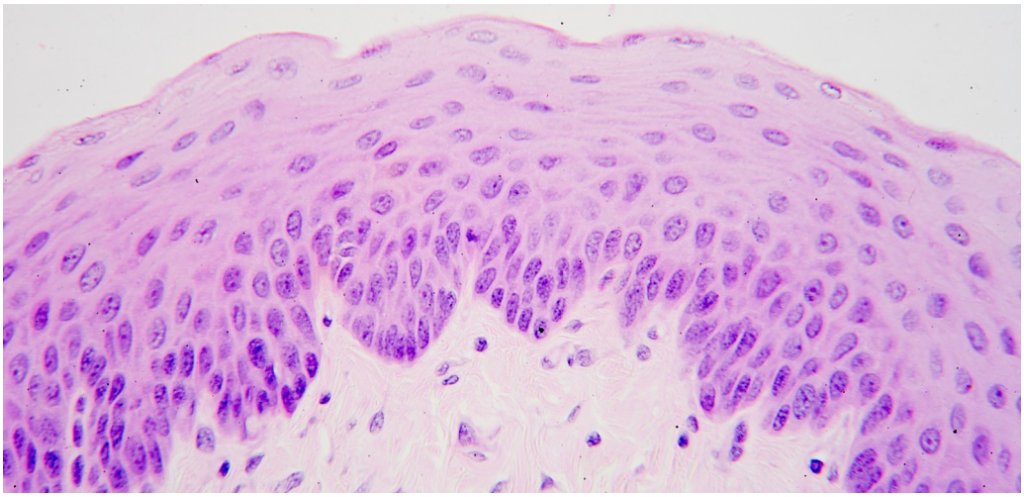


Figure 70. Stratified squamous non-keratinised epithelium

Stratified squamous keratinised epithelium consists of 5layers:

1. Stratum hasale (stratum germinativum) consists of a single layer of basophilic columnar or cuboidal cells resting on the basal lamina.
2. Stratum spinosum consists of polygonal cells with a central nucleus and a cytoplasm whose processes are filled with bundles of filaments.
3. Stratum granulosum is characterized by 3 to 5 layers of flattened polygonal cells with the cytoplasm filled with coarse basophilic granules called keratohyalin granules.
4. Stratum lucidum is thin layer of flattened eosinophilic cells. The ceils are dying or already dead and contain droplets of eleidin.
5. Stratum corneum consists of 15-20 layers of flattened cornified plates or dead cells consisting keratin (scleroprotein).

It is found in: epidermis of skin.

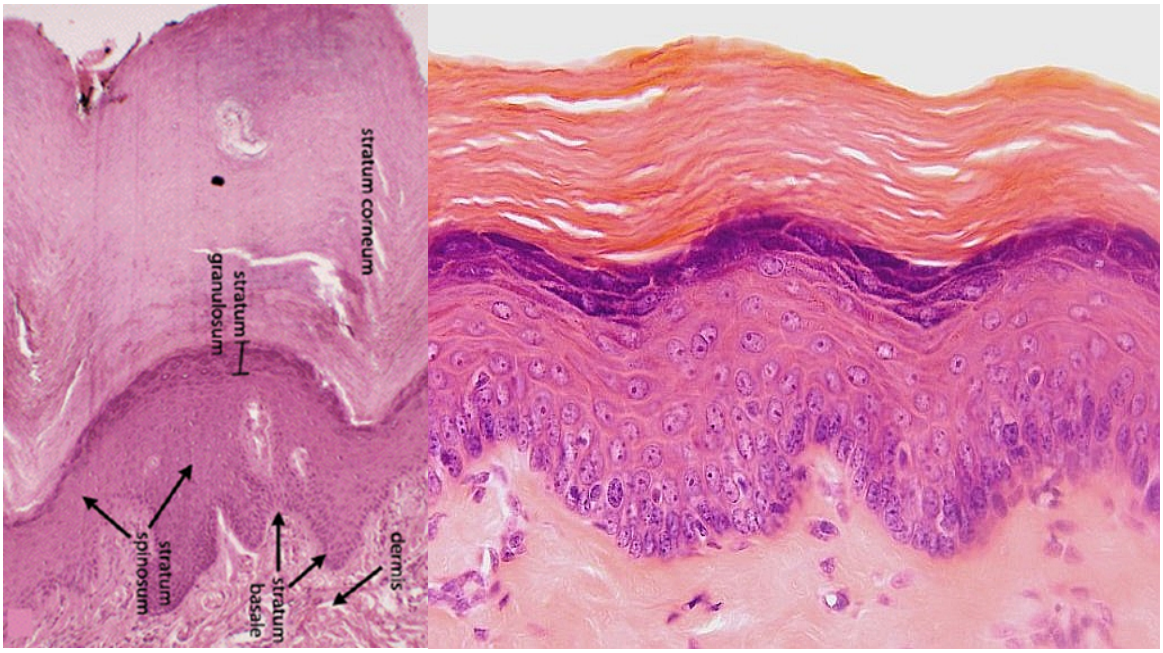


Figure 71. Stratified squamous keratinised epithelium

Glandular epithelia
The cells of glandular epithelia are specialized to produce a fluid secretion.
Glands are classified according to their mechanism of secretion.

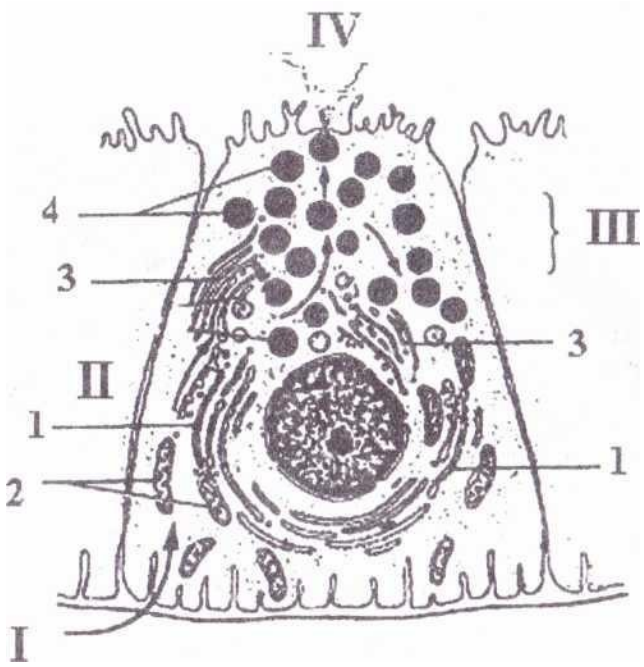


Figure 72. Structural organization of the glandular cell during secretory cycle. I - phase of absorption of initial substances, II- phase of synthesis of a secretion, III - phase of accumulation of the synthesized product, IV - phase of removing of a secretion, 1 - rER, 2 - sER, 3 - Golgi complex, 4 - secretory granules (from EHIKOG B.JI., 2007)

1. **Exocrine glands** secrete their products via ducts onto the apical (or epithelial) surface (fig.72.1).

2. **Endocrine glands** release their secretion directly into blood or lymph vessels. These glands have no ducts (ductless glands) (fig.71 II).

3. **Paracrine glands** are similar to endocrine glands but secretions reach target cells by diffusion through the extracellular space to affect neighbouring cells.

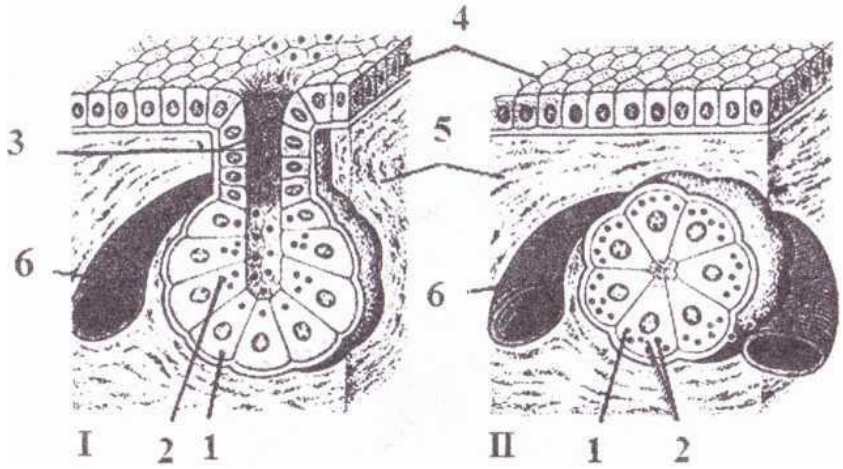


Figure 73. Structure of the exocrine and endocrine glands. I - exocrine gland, II - endocrine gland; 1 - secretory portion, 2 - secretory granules, 3 - duct of the exocrine gland, 4 - covering epithelium, 5 — connective tissue, 6 — blood vessel (from IO. I f A(jiaiiachea, H.A JOpima u dp., 1999)

Morphology of secretory cycle.

Process of secretion in glandular cells proceeds cyclically and includes 4 phases:

I. Phase of absorption of initial substances: substrates for synthesis of secretion come from blood through plasmolemma of basal pole of a glandular cell.

II. Phase of synthesis of a secretion, processes of a transcription and translation in rough endoplasmic reticulum (RER) and Golgi complex (for protein secretions), smooth endoplasmic reticulum (SER) (for steroid substances).

III. Phase of accumulation of the synthesized product', increase of the maintenance of secretory granules in cytoplasm of glandular cells.

IV. Phase of extrusion of a secretion: exocytosis of contents of secretory granules.

Morphological characteristic of the exocrine glands

Exocrine glands consist of two parts (fig.73):

I. The secretory portion, which contains the cells responsible for the secretory process;

II. The duct system, which transport the secretion to the exterior of the gland.

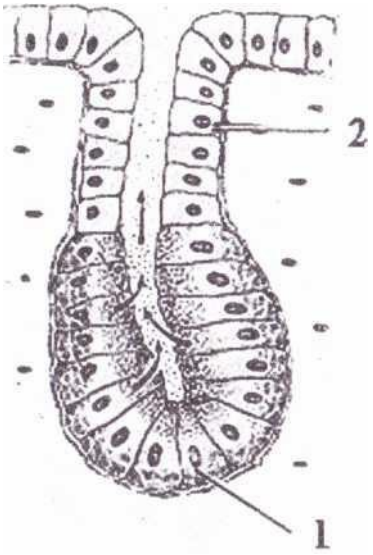


Figure 74. Structure of the exocrine gland. 1 - secretory portion, 2 - duct (from Junqueira L.C., Carneiro J., 2005)

Exocrine glands are classified based on six different morphological criteria.

1. Number of secretory cells

- Unicellular glands (goblet cells) (fig.74) are mucus-secreting cells. They are found in the epithelium of trachea, bronchi, small and large intestine.
- Multicellular glands consist of many cells.

2. Location of the secretory cells in relation to the epithelium

- Intraepithelialglands are goblet cells; described above.
- Extraepithelial glands are all large exocrine glands.

3. Nature of secretion

- Mucous glands contain mucous-secreting cells (e.g., lingual glands);
- Serous glands-, the secretory portions contain only serous cells (e.g., parotid gland, pancreas);
- Mixed glands (serous-mucous), the secretory portions contain both mucous and serous cells and the secretion is mixed (e.g., submandibular, sublingual salivary glands).

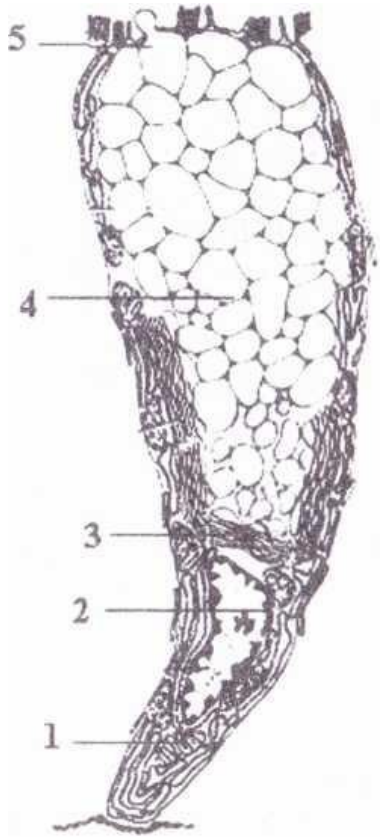


Figure 75. Diagram of uniceliuiar mucous gland. 1 - rER, 2 - nucleus, 3 - Golgi complex, 4 - accumulation of glycoprotein granules, 5 - exocytosis (from Junqueira L.C., Carneiro J., 2005)

4. Mechanism of secretion (fig.75)

- **Merocrine**: No part of the cell is lost, only the secretory product is expelled by the process of exocytosis. It is the most common mode of secretion and is seen in serous, mucous, and mixed glands.
- **Apocrine:** Part of the apical cytoplasm of the cell is lost. Secretion is discharged within free, unbroken, membrane-bound vesicles (apocrine sweat glands and mammary gland).
- **Holocrine:** The entire secretory cell is lost (discharged within the lumen of the duct) (sebaceous glands).

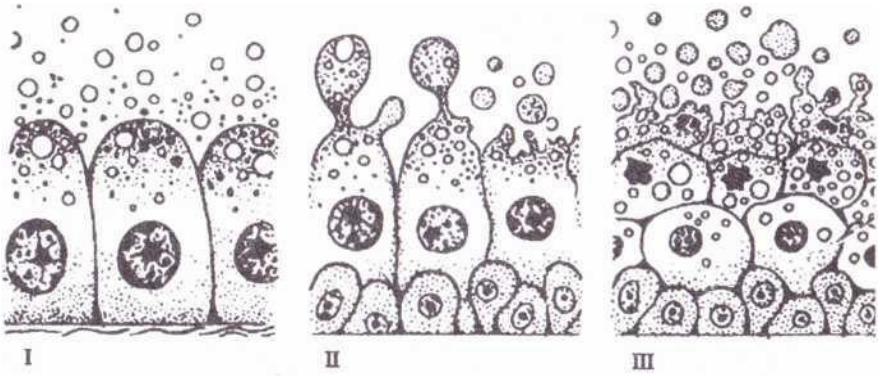


Figure 76. Types of glandular secretion. I - merocrine, II - apocrine, III - holocrine (from 10. KAcpaiiacbee, H.AJOpuna u dp., 1999)

5. Shape of secretory portions (fig. 76)

-**Tubular**: An elongated group of secretory cells with a lumen shaped like a tube.
- Acinar (or alveolar): sac-like group of secretory cells arranged about a small lumen.
-**Tubulo-acinar**: lumen of secretory units has both of the above listed shapes.

6. Arrangement (branched or not) of duct system (fig.76)

- **Simple glands:** Glands of this type have an unbranched duct into which the cells secrete. Each secretory portion empties separately on an epithelial surface.
- **Branched glands**: Several secretory units empty into an unbranched excretory duct.
- **Compound glands**: These glands have a highly branched duct system. Secretory portions empty into an elaborate branched duct system, which, in turn, drain into larger ducts.

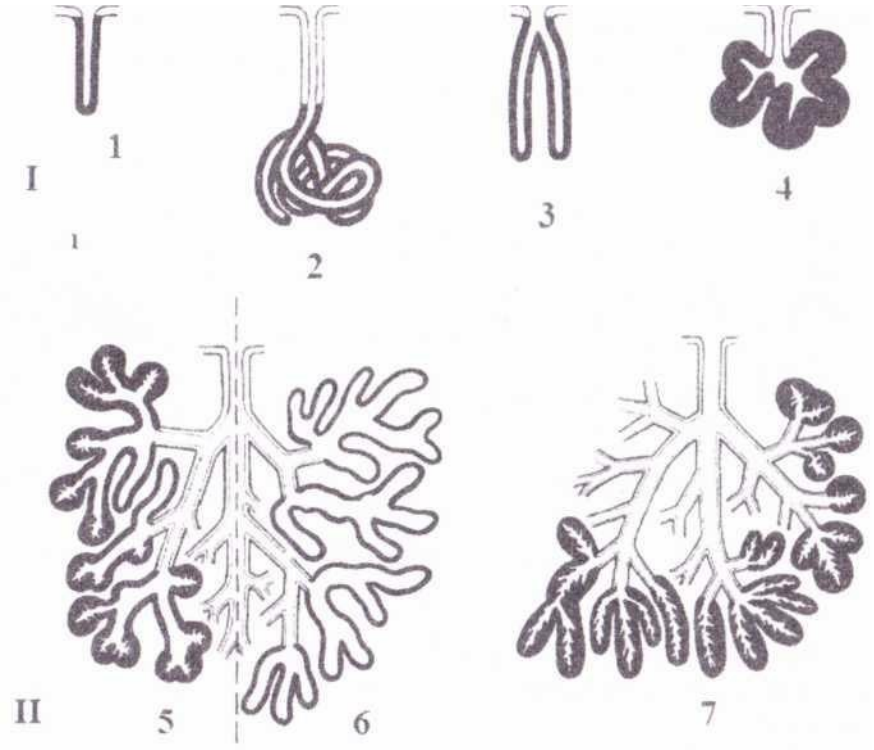


Figure 77. Principal types of exocrine glands. I - simple, II - compound; 1 - simple tubular, 2 - simple coiled tubular, 3 - simple branched tubular, 4 - simple branched acinar, 5 - compound tubuloacinar, 6 - compound tubular, 7 - compound acinar.

Practical lessons № 9

Questions for self-control

1. Tissue. What is this?
2. Tissues classification.
3. Epithelial tissue: origin, localisation, structure and functions.
4. Basal lamina. Basement membrane
5. Epithelium morphofunctional classification.
6. Simple squamous epithelium.
7. Simple cuboidal epithelium.
8. Simple columnar epithelium.
9. Pseudostratified epithelium.
10. Stratified squamous nonkeratinized epithelium.
11. Stratified squamous keratinized epithelium.
12. Transitional epithelium.
13. Glandular epithelium. Secretory cycle.
14. Classification of glands.
15. Types of secretion.

Simple squamous epithelium.

Simple cuboidal epithelium.

Simple columnar epithelium.

Pseudostratified epithelium.

Stratified squamous nonkeratinized epithelium.

Stratified squamous keratinized epithelium.

Transitional epithelium.

The teacher's signature:

BLOOD AND LYMPH

Blood is a specialized type of connective tissue in which the fluid intercellular substance is called plasma.

Quantity of the blood of an adult man is 5 - 6 litres, of an adult woman- 4-5 litres, about 7% of the body weight.

The functions of blood

1. Transportation of dissolved gases (oxygen and carbonic dioxide), nutrients, hormones, and metabolic wastes, medicines.
2. Regulation of the pH and ion composition of interstitial fluids.
3. Restriction of fluid losses at injury sites.
4. Defense against toxins and pathogens. Blood transports white blood cells, specialized cells that migrate into peripheral tissues to fight infections or remove debris. Blood also delivers antibodies, special proteins that attack invading organisms or foreign compounds.
5. Stabilization of body temperature. Blood absorbs the heat generated by active skeletal muscles and redistributes it to other tissues.

Components of the blood

I. **Formed (cellular) elements** constitute 40-45% of the total volume of blood:
1. Erythrocytes;
2. Leukocytes;
3. Platelets.

II. Plasma constitutes 55-60% of the total volume of blood.

Plasma

The components of the plasma are: 90% - water,
9% - organic compounds,
1% - inorganic salts.

The main components of organic substances are proteins.

Three primary classes of plasma proteins are: **albumins, globulins, and fibrinogen**. These three classes make up over 99% of the plasma proteins. The remainder consists of circulating enzymes, hormones, and prohormones.

Albumins constitute **60 percent** of the plasma proteins. Albumins are the main component and have a fundamental role in maintaining the osmotic pressure of the blood. Albumins are also important in the transport of fatty acids, thyroid hormones, some steroid hormones, and other substances.

Globulins account for approximately **35 percent** of the proteins in plasma. Examples of important plasma globulins include antibodies and transport globulins. Antibodies, also called immunoglobulins, attack foreign proteins and pathogens. Transport globulins bind small ions, hormones, or compounds that might otherwise be lost at the kidneys or that have very low solubility in water.

Fibrinogen functions in clotting. Under certain conditions, fibrinogen molecules interact, forming large, insoluble strands of fibrin. These fibers provide the basic framework for a blood clot.

Origins of the plasma proteins

The liver synthesizes and releases more than 90 percent of the plasma proteins, including all albumins and fibrinogen, most globulins, and various prohormones. Antibodies are produced by plasma cells that are derived from lymphocytes.

Staining of blood cells

Blood cells are studied in the smears. The smears are prepared by spreading a drop of blood in a thin layer on a microslide (fig.78).

Blood smears are stained with special mixtures of red (Eosin) and blue (Azur II) dyes by Romanowsky- Giemsa.

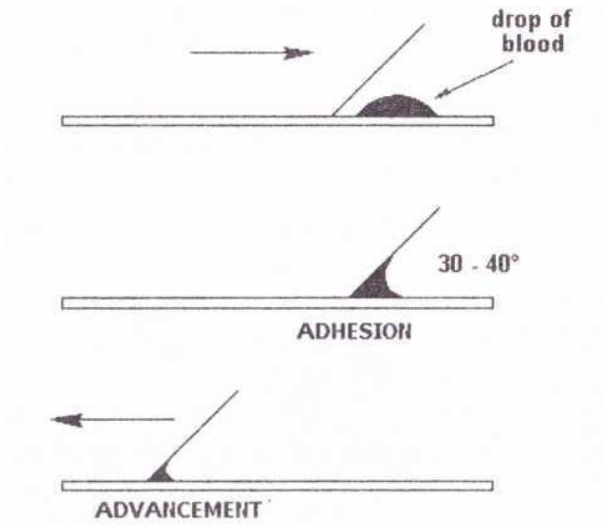


Figure 78. Making the smear.

Form elements of blood are divided into two groups:

1. Form elements that perform their functions in the blood are erythrocytes and platelets;

2. Form elements that perform their function in the loose connective tissue of different organs are granulocytes and agranulocytes.

1. Form elements that perform their functions in the blood.

Erythrocytes (red blood cells) are postcellular structures which have no nuclei and organells. These cells give whole blood its deep red colour. The erythrocyte is highly adapted for its principal function - oxygen and carbon dioxide transport. Erythrocyte consists of only an outer plasma membrane enclosing the iron- containine; respiratory protein hemoglobin (Hb), which binds and transports oxygen, and carbon dioxide, and the limited number of enzymes necessary for maintenance of gaseous transport function.

Each Hb molecule has a complex quaternary shape. The Hb molecule has two alpha chains and two beta chains of polypeptides. Each individual chain is a globular protein subunit. Each Hb chain contains a single molecule of heme, a pigment complex.

Erythrocytes are circular biconcave discs; biconcave shape increases the surface area (fig.79).

The normal concentration of erythrocytes in blood is 3,7 - 4,9x10^{I2}/L in women and 3,9-5,5xl0^{I2}/L in men.

The **hematocrit** is the percentage of whole blood occupied by cellular elements. The normal hematocrit in adult males is 40 -54; in adult females is 37- 47.

The gender difference of erythrocytes reflects the fact that androgens (male hormones) stimulate red blood cell production, whereas estrogens (female hormones) do not.

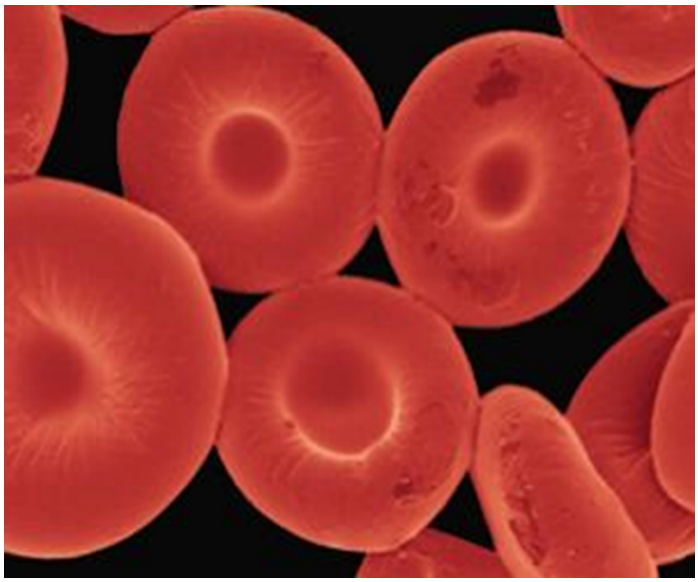


Figure 79. Dyskocytes.

Red blood cells in humans are largely form biconcave disks called dyskocyte. Normally dyskocyte accounted for 80% of red blood cells. There are other forms of erythrocytes - planocytes (having a flat surface) spherocyte (spherical) ehinocyte (with spikes) and others. **This diversity of normal physiological termed poikilocytosis (from the Greek "poykilos" - diverse, "cytos" - cell). When the number of red blood cells modificated forms over 20%, the same phenomenon called pathological poikilocytosis.**

The form erythrocytes support beta-sialohlikoproteyin in erythrocyte membranes and special frame is constructed from protein spektryn, which is adjacent to the inside plasmolemma and associated another protein – ankeryn.

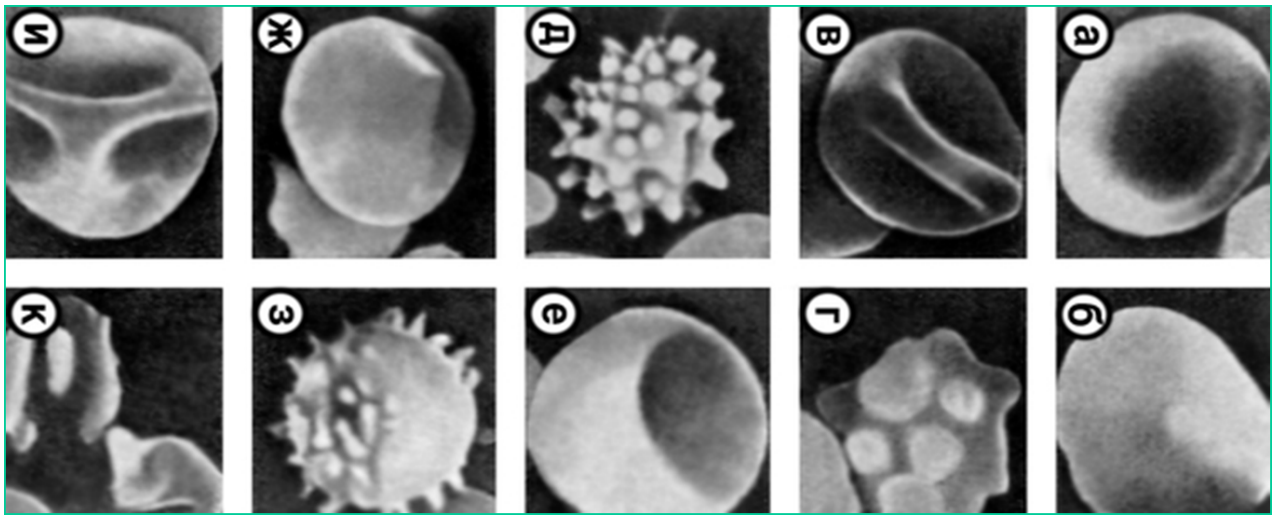


Figure 80. Various forms of red blood cells, which are under scanning electron microscopy.

Human erythrocyte has 7,5 micrometers (pm) in diameter, 2,6 pm thick at the rim, and 0,8 pm thick in the centre. Erythrocytes with diameter than 9 pm are called macrocytes and those diameters less than 6 pm are called microcytes. Change of sizes of erythrocytes of macro- and mikrotsytiv than 25%, is called **anisocytosis.**

Some normal hemoglobin types are; Hemoglobin A (Hb A), which is 95-98% of hemoglobin found in adults, Hemoglobin A2 (Hb A2), which is 2-3% of hemoglobin found in adults, and Hemoglobin F (Hb F), which is found in adults up to 2.5% and is the primary hemoglobin that is produced by the fetus during pregnancy.

Haemoglobin is a large protein molecule folded around four iron atoms and it has a quaternary structure. A quaternary structure is where two or more polypeptide chains join together due to chemical bonds which could be ionic, covalent or hydrogen bonds.

In the case of haemoglobin there are four polypeptide chains. Each one of these polypeptide chains contains a haem group which is able to bind to one oxygen molecule. Therefore four oxygen molecules can be transported by each haemoglobin molecule. In every red blood cell there are approximately 270 million haemoglobin molecules and so each red blood cell can carry about 1080 million oxygen molecules.

Oxygen + Haemoglobin ⇌ **Oxyhaemoglobin HbO_2**

Dioxide+ Haemoglobin ⇌ **Dioxidehaemoglobin $HbCO_2$**

Carbon monoxide+ Haemoglobin→ Methemoglobin **HbCO**

The major functions of Hb are to transport oxygen (O_2) from the lungs to peripheral tissues and carbon dioxide (CO_2) from the tissues to the lungs.

Iron must be in its reduced (ferrous, Fe2+) state for Hb to bind O_2. **Oxidized or "met" Hb (ferric, Fe3+) cannot bind O_2.**

Interaction between the heme Fe and CN- or CO formed **methemoglobin** is a hemoglobin in the form of metalloprotein, in which the iron in the heme group is in the Fe3+ (ferric) state, not the

Fe2+ (ferrous) of normal hemoglobin. Methemoglobin cannot bind oxygen, which means it cannot carry oxygen to tissues. Methemoglobin cannot carry oxygen because oxidized ferric iron cannot bind it. An increase in methemoglobin level results in decreased delivery of oxygen to the tissues. Individuals with methemoglobin levels less than 25% are generally asymptomatic. If the methemoglobin level increases to more than 30% of total hemoglobin, cyanosis (bluish discoloration of skin and mucous membranes) and symptoms of hypoxia (dyspnea, headache, vertigo, change in mental status) occur. Levels of methemoglobin greater than 50% can lead to coma and death (carbon monoxide poisoning).

Young forms of erythrocytes are called reticulocytes. They are not fully saturated hemoglobin, it is inherent polichromatofiliya. In its cytoplasm containing reticulocytes network structure.In the normal amount of reticulocytes is 1-5% of the total number of red blood cells. The increase in the number of diagnostic feature is enhanced hematopoiesis. **Lifetime** Human erythrocytes survive in the circulation for about **120 days.** Worn-out erythrocytes are removed from the circulation mainly by macrophages of the spleen and bone marrow.

Platelets are only about 20% of the diameter of red blood cells. The normal platelet count is **150,000-350,000 per microliter of blood**, but since platelets are so small **(2-4 pm in diameter**), irregular, discshaped non- nucleated membrane-bound cell **fragments of giant polyploid cells of the bone marrow - megakaryocytes,** they make up just a tiny fraction of the blood volume. Morphologically, platelets distinguish between **hyalomer** (cytoplasmic analogue) and **granulomer** (different types of granules). External platelets **covered with a plasmolemma containing "antennae"** that help platelet adhesion. The principal function of platelets is to prevent bleeding. Platelets are produced in the bone marrow, the same as the red cells and most of the white blood cells. Platelets are produced from very large bone marrow cells called megakaryocytes. As megakaryocytes develop into giant cells, they undergo a process of fragmentation that results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin . Platelets are actually not true cells but merely circulating fragments of cells. But even though platelets are merely cell fragments, they contain many structures that are critical to stop bleeding. They contain proteins on their surface that allow them to stick to breaks in the blood vessel wall and also to stick to each other. They contain granules that can secrete other proteins required for creating a firm plug to seal blood vessel breaks. Also platelets contain proteins similar to muscle proteins that allow them to change shape when they become sticky.

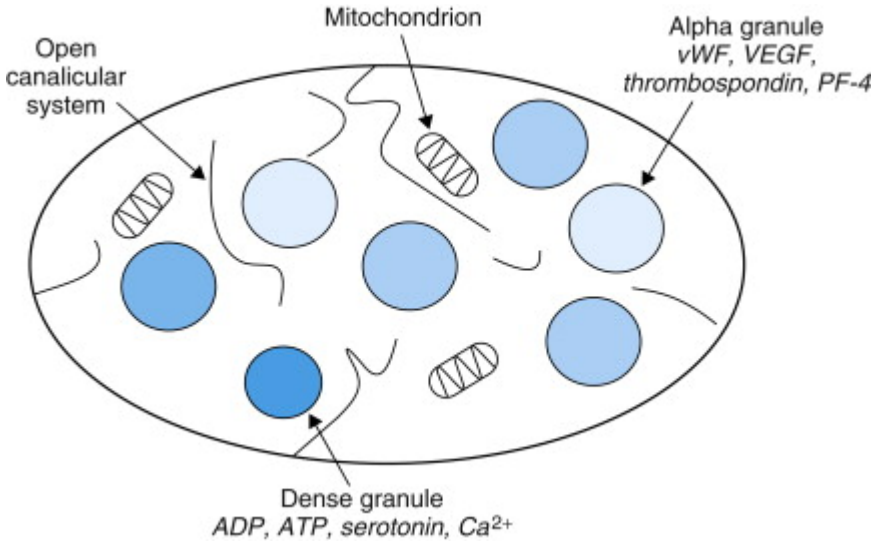


Figure 81. Overview of platelet structure.

An overview of platelet structure can be found in Figure 80. Platelets are anucleate. A network of interconnected channels, the open canalicular system, extends from the inside of the platelet to the outside environment and may function to allow the rapid release of the constituents of platelet

granules. Mitochondria produce ATP and may also participate in the regulation of the platelet activation response.Platelets contain three types of granules, which release their contents upon activation. Platelets contain lysosomes, although the significance of this organelle is not clear. The most numerous type of granule is the alpha granule, which contains proteins that provide the surface of platelet adhesion. For example, vWF, fibrinogen and vitronectin are matrix proteins contained within the alpha granule that may contribute to thrombus formation and stabilization. Platelet alpha granules also contain factors that promote new blood vessel formation and inflammation. Alpha granules contain both factors that promote new blood vessel growth (angiogenic factors), and factors that inhibit new blood vessel growth and stabilize established vessels (angiogenesis inhibitors). Angiogenic factors in alpha granules include vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and platelet derived growth factor (PDGF). Some of the angiogenesis inhibitors include angiostatin, thrombospondin, and endostatin. Regulators of inflammation, or cytokines, are also present in alpha granules. Examples include platelet factor 4 (PF4), CCL5 (RANTES), and interleukin-8 (IL-8). Alpha granule and dense granule release typically are coordinated. Contents of the dense granule include ATP, ADP, serotonin, and calcium. These factors contribute to platelet recruitment and thrombus stabilization at a site of injury.

Platelet is divided into four zones based on structure and function.

- **Peripheral zone** consists of cell membrane covered by a glycocalyx. The membranes of the platelet are rich in glycoproteins, which serve as receptors in platelet function.

- **Structural zone** is beneath the peripheral zone and is the framework of the platelet, the cytoskeleton. This zone consists of microtubules, actin filaments, myosin. They are circumferentially arranged and responsible for maintaining the platelet's dick shape as well as the contractile system that, upon activation, allows shape change, pseudopod extension.

- **Organelle zone** consists of the granules and cellular components, such as lysosomes, peroxisomes, mitochondria. The alpha granules contain adhesive proteins, such as fibrinogen, coagulation factors, plasminogen, etc. The delta (dense) granules contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), serotonin, and calcium. The lambda granules are similar to lysosomes and contain proteolytic enzymes.

- **Membrane zone** includes two types of membrane channels. The platelet has a surface-connected system of channels called the open canalicular system. Through the open canalicular system, plasma substances enter the interior of the platelet and platelet products exit. The dense tubular system originates from rough endoplasmic reticulum, serves as a storage site for calcium ions.

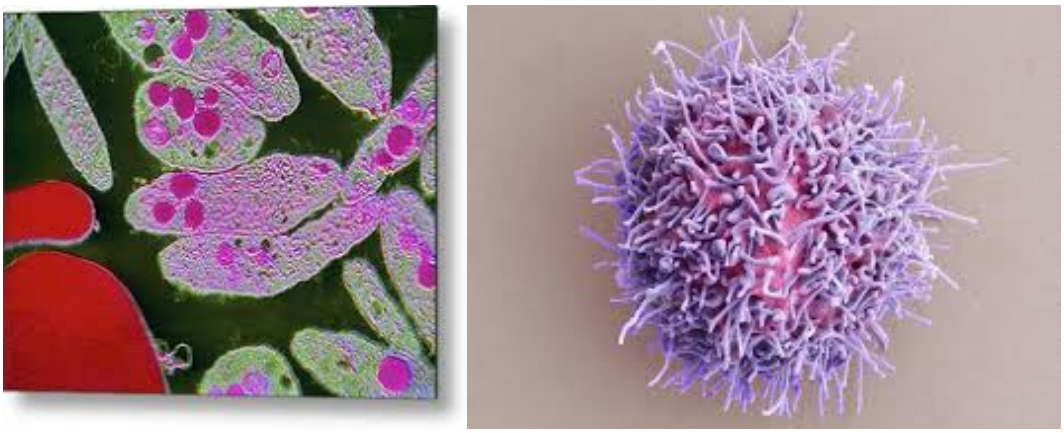


Figure 82. Platelets.

Lifetime

The average lifetime of a platelet is normally **just 5 to 9 days.**

Function

The function of platelets is the maintenance of hemostasis by the formation of thrombi, when damage to the endothelium of blood vessels occurs.

2. Form elements that perform their function in the loose connective tissue.

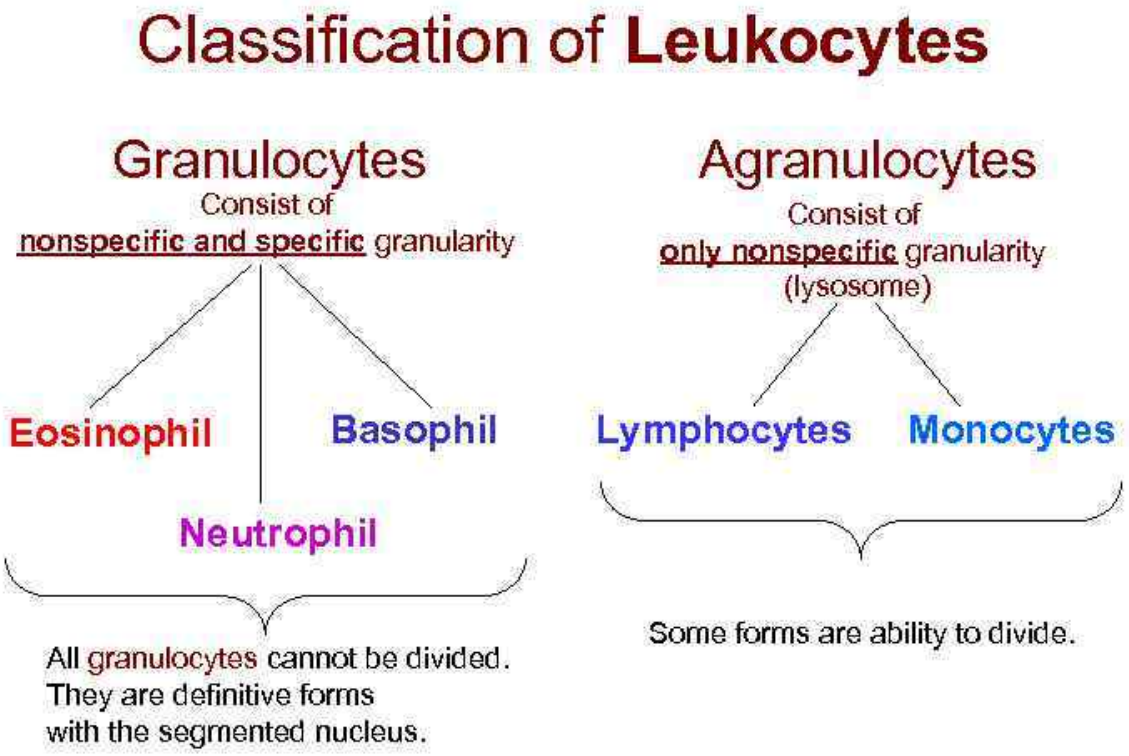
Leukocytes

Leukocytes (white blood cells) include a variety of cells specialized for immune defense against foreign material or organisms.

The normal concentration of leukocytes in blood is 6-9x10^9/L. Thenumber of leukocytes in the blood varies according to age, sex, and physiologic conditions. An increase in the number of leukocytes over the upper limits is called **leukocytosis**, and a decrease below the lower limit is called **leukopenia.**

On the basis of the presence and type of the granules in their cytoplasm and the shape of the nucleus, white blood cells are classified into two groups: **granulocytes and agranulocytes.**

The term granulocyte is due to the presence of granules in the cytoplasm of these cells. In the different types of granulocytes, the granules are different and help us to distinguish them. In fact, these **granules have a different affinity towards neutral, acid or basic stains and give the cytoplasm different colours.** So, granulocytes distinguish themselves in neutrophils, eosinophils (or acidophil) and basophils.



Granulocytes contain specific granules in cytoplasm; their nuclei have two or more lobes (fig.81)

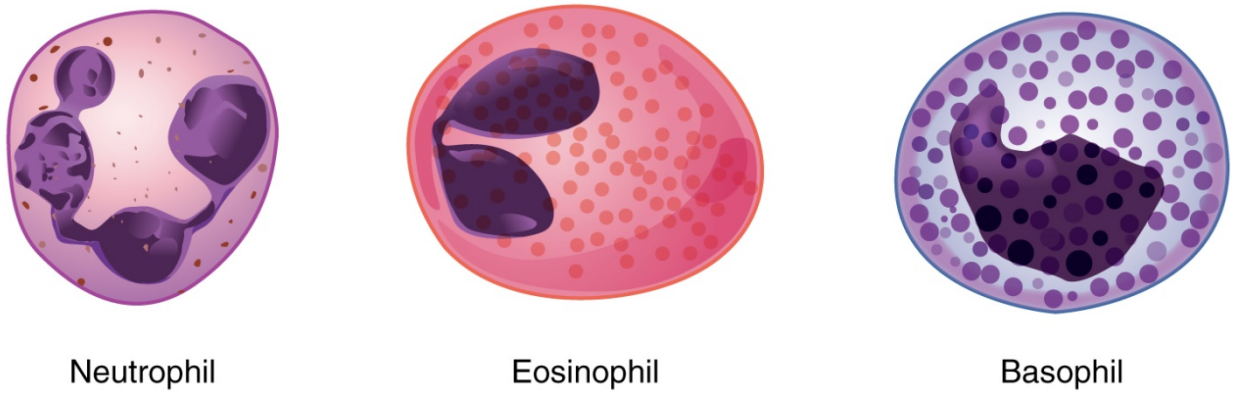


Figure 83. Granular Leukocytes.

Agranulocytes have no specific granules and contain non-lobated nuclei

Granulocytes

Neutrophils

Neutrophils constitute 65 - 75 % of circulating leukocytes. Neutrophils granulocytes have an average diameter of 10-12 p.m. in peripheral blood smears. Nuclei of neutrophils are lobated, **having 2-5 lobes.** The lobes are connected by thin threads of chromatin (fig. 84).

On a **degree of a maturity distinguish neutrophils with the various shapes of the nuclei** (fig.84):

- **young** (metamyelocytes) have kidney-shaped nuclei, constitute 0,5%,
- **band** cells have nonsegmented horseshoe-shaped nuclei, constitute 1-6%,
- **mature** have segmented nuclei, constitute 47-72%.

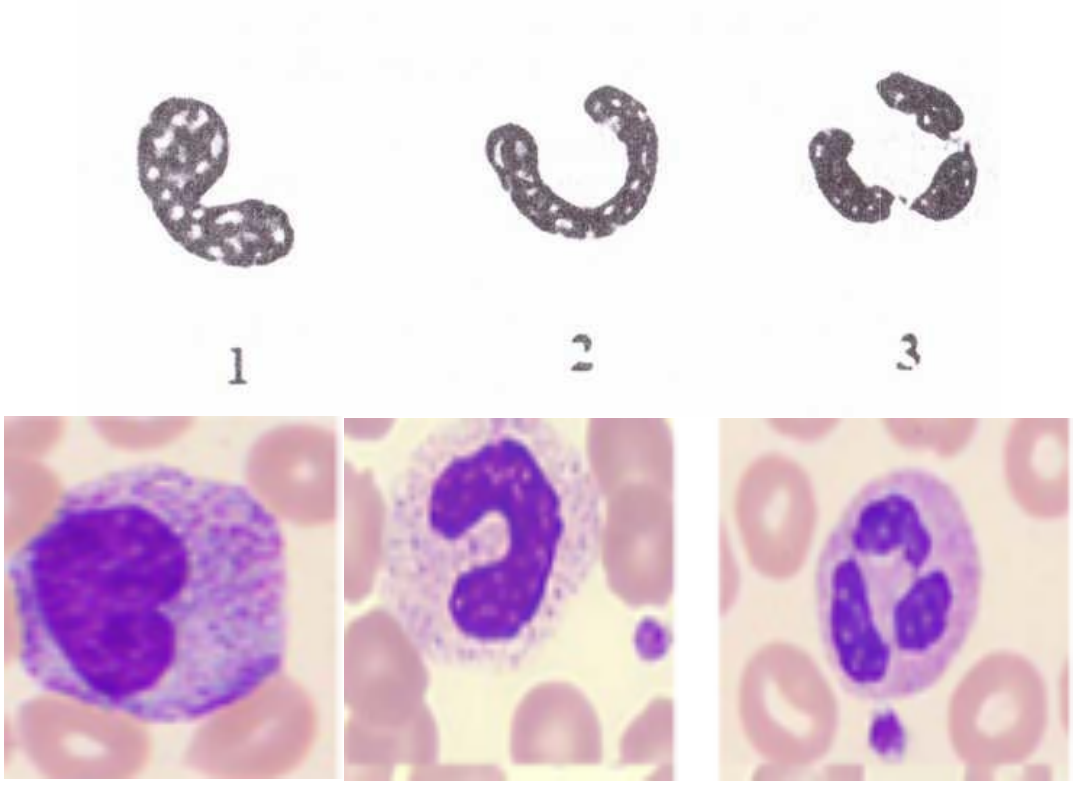


Figure 84. Types of neutrophils. 1 - young (metamyelocytes), 2 - band cell, 3 – segmented.

In female, the inactive X chromosome appears as a drumsticklike appendage on the one of the lobe of the nucleus (Barr body) (fig.85).

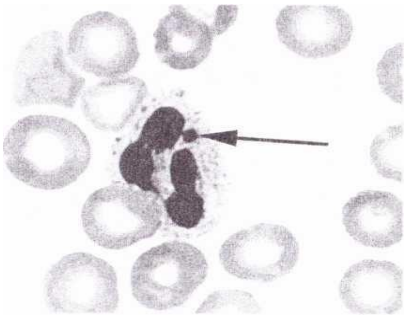


Figure 85. Barr body of neutrophil nucleus.

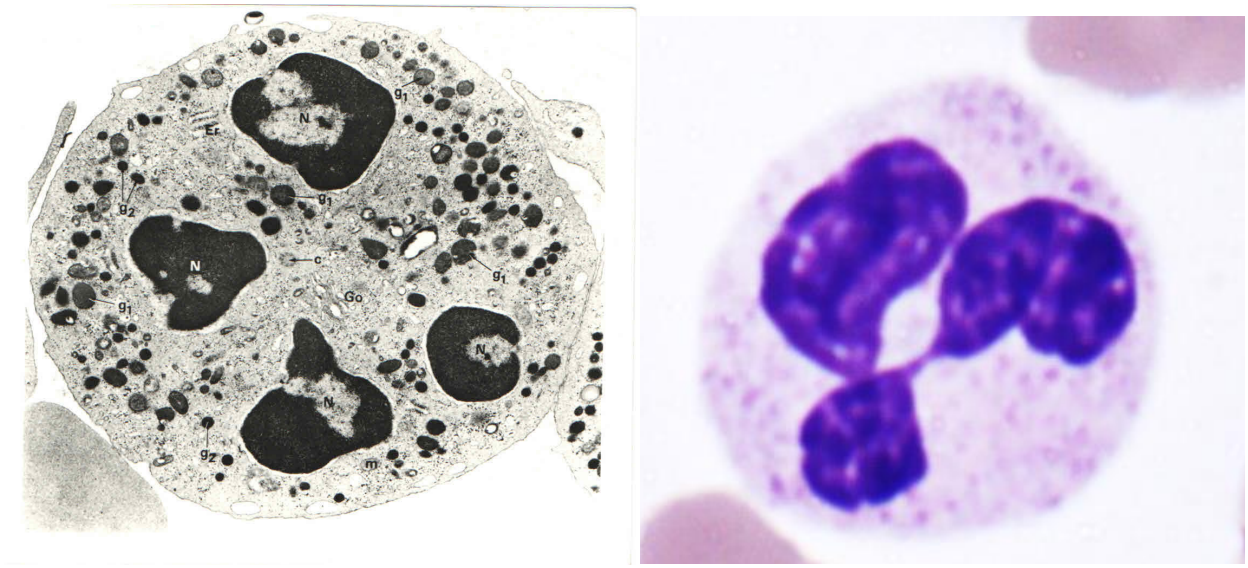


Figure 86. Neutrophils.

Cytoplasm is light pink in colorand contains two types of granules:

- **primary** azurophilic granules, which are primary **lysosomes** and contain the enzymes (acid phosphatase, elastase, collagenase, cationic antibacterial proteins).

- **secondary specific neutrophilic granules**, which are small 0,2-0,5 pm., contain various enzymes (alkaline phosphatase, collagenase, lactoferrin, lysozyme).

Lifetime

Neutrophils are short-lived cells w'ith a half-life of 6-7 hours in blood and of 1 -4 days in spleen and other tissues.

Function

Neutrophils are phagocytes, capable of ingesting microorganisms or particles. **They are called microphages (once in life they phagocytize and then die).** Neutrophils constitute a defense against invasion by microorganisms, especially bacteria.Chemotaxis is the process which is responsible for the migration of the neutrophils towards the infection or inflammation site. The receptors present at the surface of the cell, helps the neutrophils to detect the chemical gradients of interleukin-8 (IL-8), interferon gamma (IFN- gamma), and Ca (fragment released from complement component C5). These molecules are responsible for directing the path of migration.

Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines.

Neutrophils have three strategies for directly attacking micro- organisms:

- phagocytosis,

- release of granule proteins and

- generation of neutrophil extracellular traps (NETs) (NETs provide a high local concentration of antimicrobial components and bind, kill microbes independent of phagocytic uptake; in addition, NETs may serve as a magical barrier that prevents further spread of pathogens).

Eosinophils

Eosinophils constitute 2 - 5% of circulating leukocytes. Eosinophil granulocytes have an average diameter of 10-12 pm in peripheral blood smears. Eosinophils also have lobated nuclei (2-4 lobes).

On a **degree of a maturity distinguish neutrophils with the various shapes of the nuclei**:

- young (metamyelocytes) have kidney-shaped nuclei,

- **band** cells have nonsegmented horseshoe-shaped nuclei,

- **mature** have segmented nuclei more often 2 lobs.

Cytoplasm may more often have a basophilic color and contains two types of granules:

- **primary** azurophilic granules, which are primary lysosomes and contain the enzymes

- **specific eosinophilic granules** are large (0,7-1,5 pm.) and elongated; contain **crystalline** cores oriented parallel to the long axis of the granules.

Specific eosinophilic granules contain the major basic protein, cationic protein, eosinophil-derived neurotoxin and peroxidase.

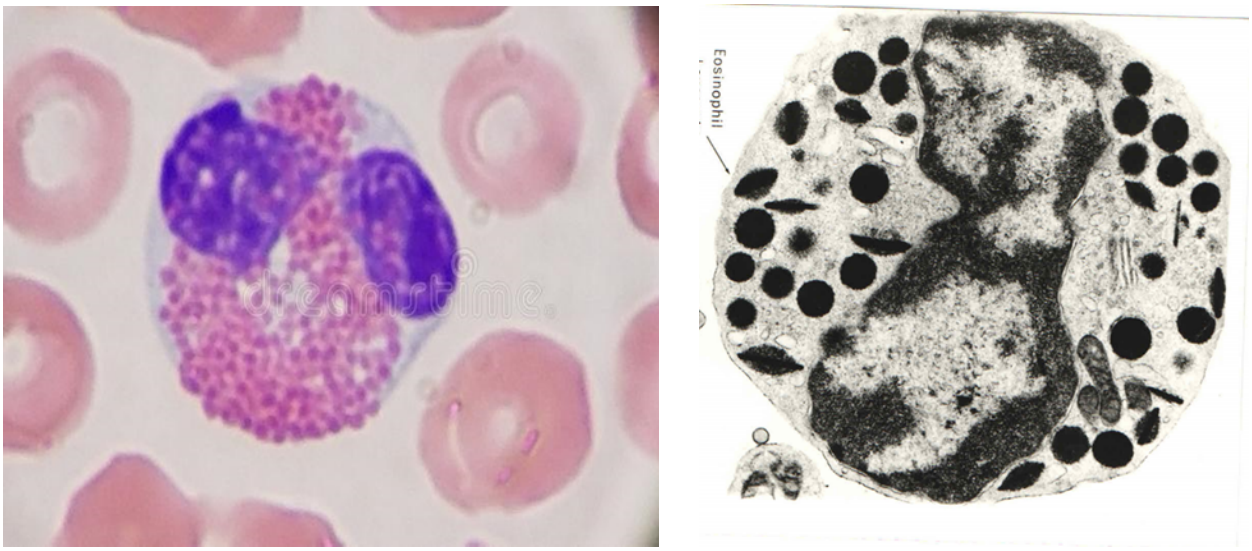


Figure 87. Eosinophils.

Lifetime

Eosinophils circulate for 4-5 hours in the blood, then migrate into the tissues (skin, mucosa of digestive, respiratory and reproductive tracts) and function 8 - 12 days.

Functions

1. Participation in immune (allergic) reaction.
2. Destruction of parasites (e.g. enteric nematodes) by the toxic basic protein.
3. Limited ability to participate in phagocytosis.
4. Diapedesis (eosinophils can migrate from the blood stream into the body tissues, in a process called diapedesis; this allows the leukocytes to fight localized infections in the tissues directly).

Basophils

Basophils constitute 0 - 1% of circulating leukocytes.

Basophils have an average diameter of 12-15 pm in peripheral blood smears.

They have lobated (2-3 lobes) or S-shaped nuclei.

Cytoplasm have a basophilic color and contains two types of granules:

- **primary azurophilic granules**, which are primary lysosomes and contain the enzymes.
- **specific basophilic granules** (1,2-1.8pm.) which stain metachromatically with the basic dye of the usual blood stains.

Metachromasia is a property of cells to be painted in other colour distinguished from colour of dye.

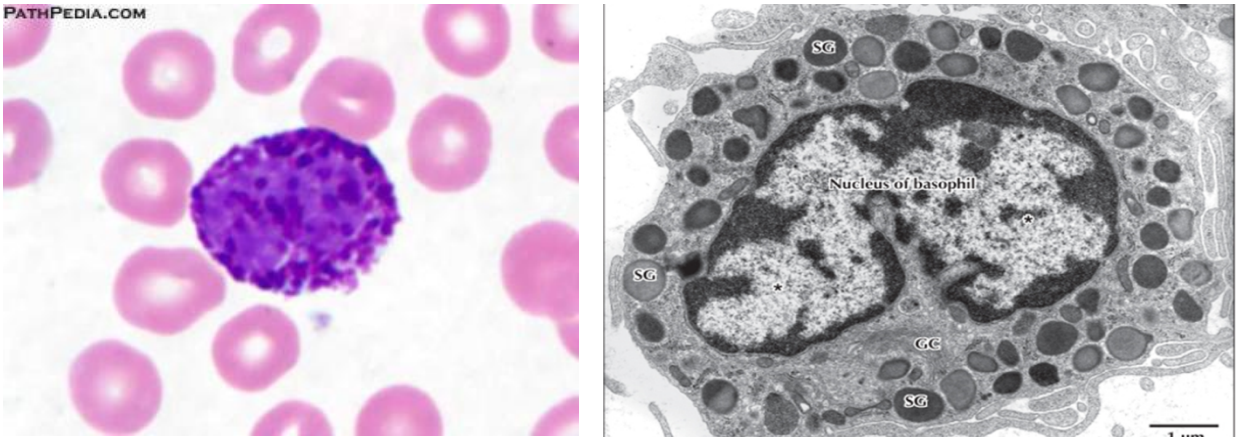


Figure 88. Basophils.

Basophilic specific granules contain:

- **heparin** (anticoagulant) and
- **histamine** (dilatation of blood vessels and increase of their permeability).

Lifetime

Basophils circulate for a few hours in the blood, then migrate into the tissues and function for a few days.

Functions

Basophils appear in many specific kinds of inflammatory reactions.

Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues.

They can be found in unusually high numbers at sites of ectoparasite infection.

They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions.

Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy.

1 Agranulocytes

Monocytes

Monocytes constitute 6 - 8 % of circulating leukocytes.

Monocytes have an average diameter of 12-20 um in peripheral blood

smears. Cytoplasm have a basophilic color, nuclei are oval, horseshoe- or kidney-shaped, and excentric in position.Cytoplasm is basophilic,contains fine azurophilic granules (lysosomes).

Lifetime

Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body.

Function

In the tissues monocytes mature into tissue resident macrophages **(macrophages repeatedly phagocytize and convert the antigen from the corpuscular form to the molecular).**

Monocytes or macrophages have three main functions in the immune system:

1. phagocytosis,
2. antigen presentation,
3. cytokine production.

Phagocytosis is the process of uptake of microbes and particles followed by digestion and destruction of this material.

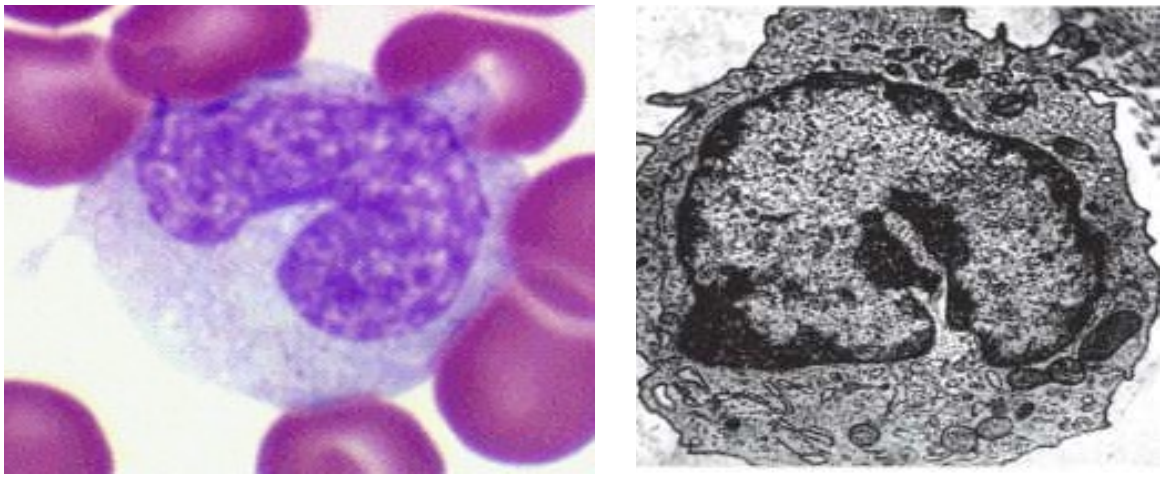


Figure 89. Monocyte.

Microbial fragments that remain after such digestion can serve as antigen. The fragments can be incorporated into MHC molecules and then traffic to the cell surface of macrophages. This process is called antigen presentation and it leads to activation of T lymphocytes, which then provide a specific immune response against the antigen.

Other microbial products can directly activate monocytes and this leads to production of cytokines (tumor necrosis factor (TNF), interleukin- 1 (IL-1) and interleukin-12 (IL-12)).

A majority of macrophages are stationed at strategic points where microbial invasion or accumulation of dust is likely to occur.

Each type of macrophage, determined by its location, has a specific name:

1.alveolar macrophages are located in pulmonary alveolus; Histiocytes are located in connective tissue; Kupffer cells in liver; microglia in neural tissue; osteoclasts in bone tissue etc.

Lymphocytes

Lymphocytes constitute 20-35 % of circulating leukocytes.

Lymphocytes can be classified into several groups according to their sizes:

- small lymphocytes have diameter - 6-8 pm,
- medium-sized lymphocytes - 8-9 pm, and
-large lymphocytes - 10-18 pm.

The small lymphocytes are predominant in the blood. Lymphocytes have are large, spherical nuclei often indented on one side; very dense and dark blue due to heavy chromatin mass. Cytoplasm as a thin rim around the nucleus is slightly basophilic.

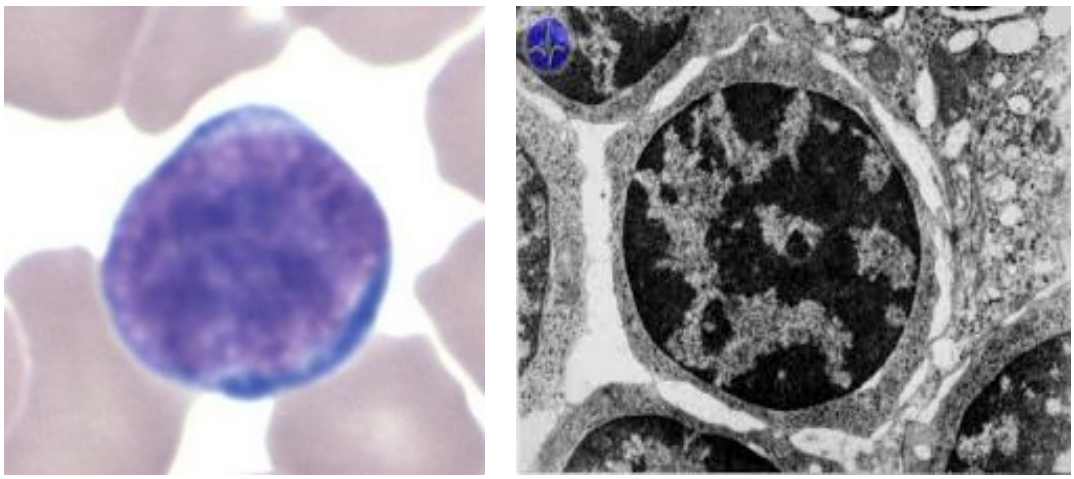


Figure 90. Small lymphocytes.

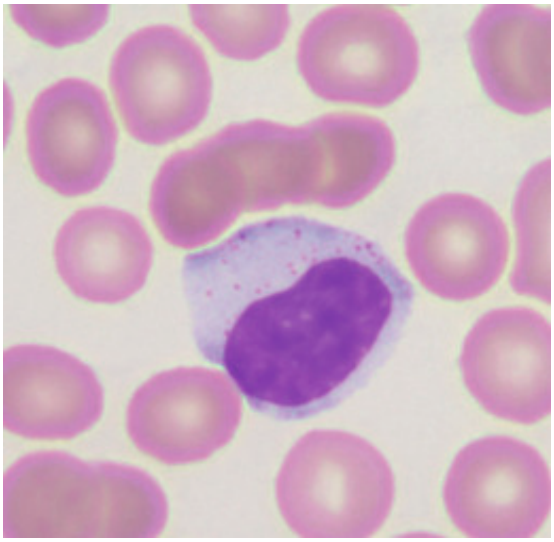


Figure 91. Large lymphocytes.

Large granular lymphocytes (LGLs) have abundant, pale blue cytoplasm with distinct medium to large azurophilic cytoplasmic granules. LGLs represent up to 15% of circulating lymphocytes, or less than 0.6×10^9/L in normal adults, located in the immune system.

Lymphocytes can be classified into several groups according tofunctional significance.

The three major types of lymphocyte are:

- T cells**,**

- B cells and

- natural killer (NK) cells.

Characteristic of T lymphocytes (T cells)

T cells represent 60-80% of blood lymphocytes. They have a long lifespan and are involved in cell-mediated immunity.

These cells originate in the bone marrow and migrate to the thymus, where they differentiate into immunocompetent cells. Initially, lymphocytes are genetically programmed to recognize a single antigen out of virtually an infinite number of possible antigens. This process is termed antigen-independent proliferation and differentiation. Then immunocompetent cells migrate to the blood, lymph, and special T- regions of peripheral (secondary) lymphoid organs where they undergo antigen-dependent activation and differentiation into effector lymphocytes (cytotoxic cells, helpers, and suppressors) and memory cells. T memory cells react rapidly to the reintroduction of the same antigens.

Characteristic of B lymphocytes (B cells)

B- cells are named so because they were first recognized in the bursa of Fabricius in birds. They have variable lifespan and are involved in humoral immunity by production and secretion of circulating antibodies (immunoglobulins).

B cells represent 20-30 % of blood lymphocytes derive from bursa- equivalent organs (red bone marrow and GALT in mammals) where they undergo antigen-independent proliferation and differentiation. Then these cells migrate to the blood, lymph, and special B-regions of peripheral (secondary) lymphoid structures where they proliferate and differentiate into the effector lymphocytes (antibody- secreting plasma cells) and memory cells which react very rapidly to reintroduction of the same antigen. This process is called antigen- dependent activation and differentiation.

Characteristic of natural killer (NK) cells

NK cells constitute about 5-10% of circulating lymphocytes. NK cells develop from the same precursor cells as T and B cells. These cells genetically are programmed to recognize transformed cells (tumor cells or infected with a virus). Following recognition of antigens, NK cells release proteins (perforins and fragmentins) that open holes in foreign cell membranes, with consequent self-destruction (a process known as apoptosis) or cell lysis.

Lifetime

Lymphocytes vary in life span; some live only a few days, while others survive in the **circulating blood for many years.**

Hemogramm.

Hemogramm reflects contents of separate formed elements per 1 liter of blood:

Erythrocytes - 3, 9-5, 5xl0^{12}/L in women and 4. 1 -6x 10^{12}/L in men.

Leukocytes - 6 - 9x 10^{9}/L.

Platelets - 150-450x10^{9}/L.

Leukocytic formula of peripheral blood reflects relative contents of different leukocytes as regards total quantity of leukocytes accepted for **100%.**

Leukocytic formula.

Basophils	Eosinophils	Neutrophils			Lymphocytes	Monocytes
		65 - 75%:				
		young	band cells	Segmented cells		
0 - 1%	1 - 5%	0 - 0,5%	1 - 6%	47 - 72%	19 - 37%	3 - 8%

The shift of the leukocyte formula to the left indicates the presence of an inflammatory process in the body - a decrease in segmented neutrophils and an increase in young and band cells.

The shift of the leukocyte formula to the right indicates a disturbance of the process of hematopoiesis in the red bone marrow - this is an increase in segmented nuclear neutrophils and a decrease or absence of young and band cells.

Lymph

Lymph is a part of the interstitial fluid, the fluid which is in the interstices of all body tissues. Interstitial fluid becomes lymph when it enters a lymph capillary. The lymph then travels to at least one lymph node before emptying ultimately into the right or the left subclavian vein, where it mixes back with blood.

Lymph is a clear to yellowish watery fluid. Lymph contains the same proteins as in plasma of blood, but in smaller amounts.

Volume of lymph in the human body is 1-2 liters.

Functions of the lymph

- Return of protein and fluid from the tissues to the circulation.
- Absorption and transport of fat from the small intestine.
- Immunological - circulation of immune cells such as lymphocytes and dendritic cells, removal of bacteria.

Lymph consists of:

- plasma and
- formed elements.

Concentration of formed elements in the lymph is 2-20x10^9/L.

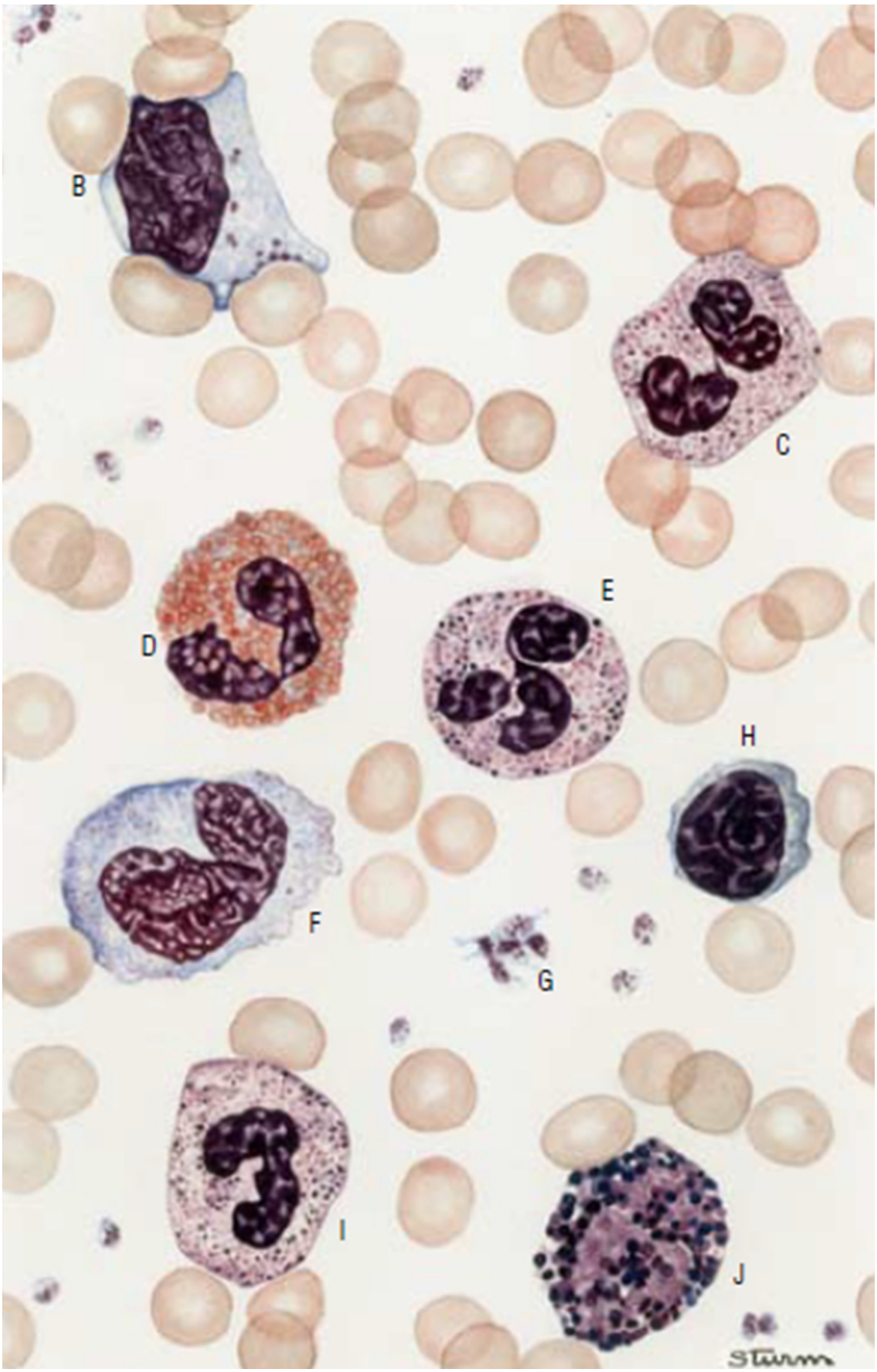
Formed elements of lymph

Lymphocytes constitute 90%;
Monocytes constitute 5%;
Eosinophils constitute2%;
Neutrophils constitute 1%;
Other cells constitute 2%.

Practical lessons № 10

Questions for self-control

1. Blood components, functions.
2. Chemical components of plasma.
3. Erythrocytes: size, structure and functions. Anisocytosis. Poikilocytosis.
4. Leucocytes: classification, structure and functions. Leucocytosis. Leucopenia.
5. Granulocytes: neutrophils, structure and functions.
6. Granulocytes: eosinophils , structure and functions.
7. Granulocytes: basophils , structure and functions.
8. Agranulocytes:lymphocytes, structure and functions.
9. Agranulocytes: monocytes, structure and functions.
10. Blood platelets (thrombocytes).
11. The difference between microphages and macrophages.



The teacher's signature:

CONNECTIVE TISSUES. LOOSE AND DENSE CONNECTIVE TISSUES. CONNECTIVE TISSUES WITH SPECIAL PROPERTIES

The connective tissues are the complex of derivatives of the mesenchymal origin, consisting of cellular differons and the extracellular substance, participating in maintenance of a homeostasis of the internal environment of an organism.

Functions of connective tissue

- Metabolic functions. All the metabolites from the blood pass from capillary beds and diffuse through the adjacent connective tissue to cells and tissues. The adipose tissue serves as an energy store and also provides thermal insulation.

- Regulative. Connective tissues regulate the activity of other tissues by means of biologically active substances and contact interactions.

- Defensive functions. Various components of the connective tissue play roles in the defense or protection of the body (plasma cells, lymphocytes, neutrophils, eosinophils, basophils, mast cells). Macrophages are important in tissue repair as well as defense against bacterial invasion. The fibroblasts of connective tissue proliferate in respoase to in jury of organs and migrate to and deposit abundant new collagen fibers, resulting in the formation of fibrous scar tissue.

- Structural support The connective tissues serve several functions, of which the most prominent function is structural support to enable maintenance of anatomical form of organs and organ systems. Examples include the connective tissue capsules surrounding organs. The loose connective tissue acts to fill the spaces between organs. The tendons and the elastic ligaments are examples of specialized orderly forms of connective tissue.

Classification of the connective tissues

1. Fiber connective tissue (connective tissue proper)
1.1 Loose (areolar) connective tissue
1.2 Dense connective tissue
1.2.1 Regular
1.2.2 Irregular
2. Connective tissues with special properties
2.1 Adipose tissue
2.2 Reticular tissue
2.3 Mucous tissue
2.4 Pigment tissue
3. Supporting connective tissue
3.1 Cartilage
3.2 Bone

General principle of organization of connective tissue

Connective tissue consists of:

1. connective tissue cells,
2. extracellular matrix:
   - ground substance,
   - protein fibers (collagen, reticular, elastic).

Types of connective tissue proper (**fiber connective tissue**). The loose connective tissue is characterized by **low maintenance of fibers in intercellular substance**, great volume of the ground substance, numerous cellular compositions.

This tissue is the more abundant in organism.

Loose connective tissue is found out in all organs - it forms their stroma, fills in spaces between function elements of other tissues, accompanies with nerves and pots, is part of a skin and mucosa.

Loose connective tissue has a delicate consistency; it is flexible, well vascularised.

Loose connective tissue consists of cells and extracellular matrix.

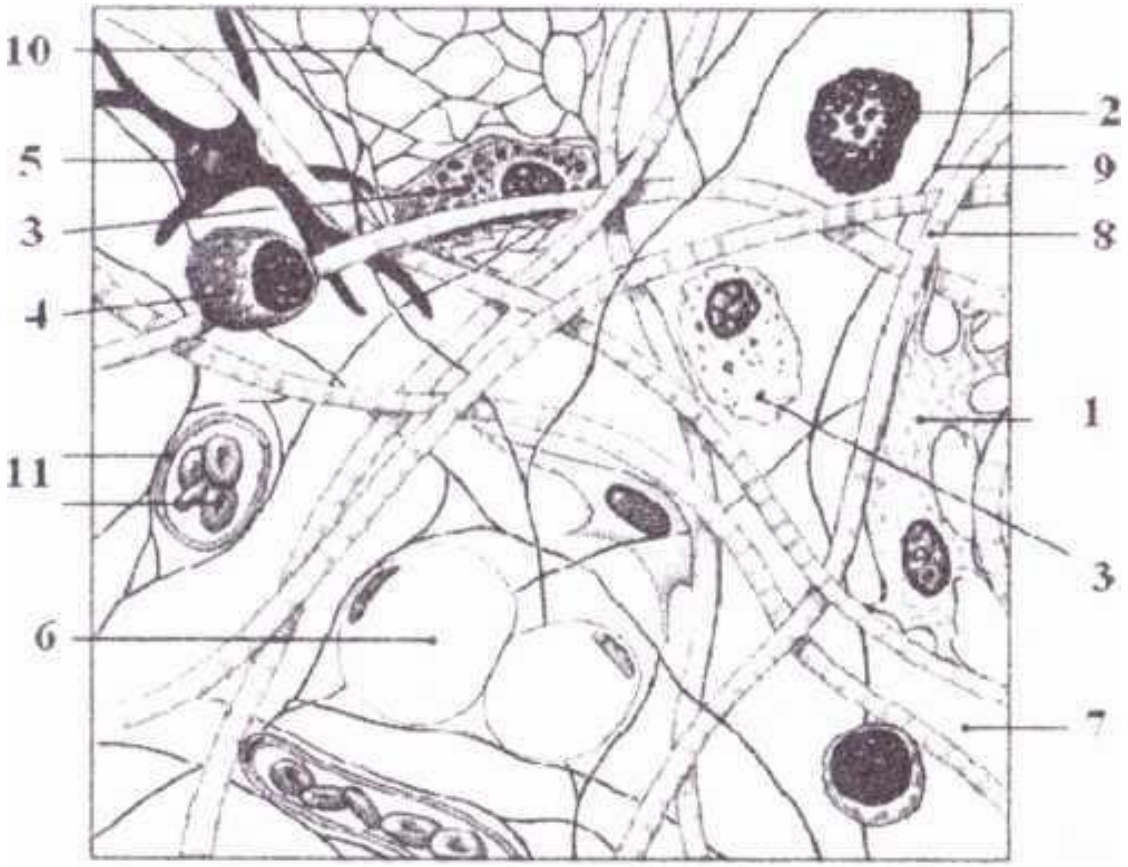


Figure 92. Schematic diagrams of loose connective tissue. 1 - fibroblast, 2 - mast cell, 3 - macrophage, 4 - leukocyte, 5 - pigment cell, 6 - adipocyte, 7 - extracellular matrix, 8 - collagen fibers, 9 - elastic fibers, 10 - reticular fibers, 11 - blood vessel (from Ross M.H., 2003)

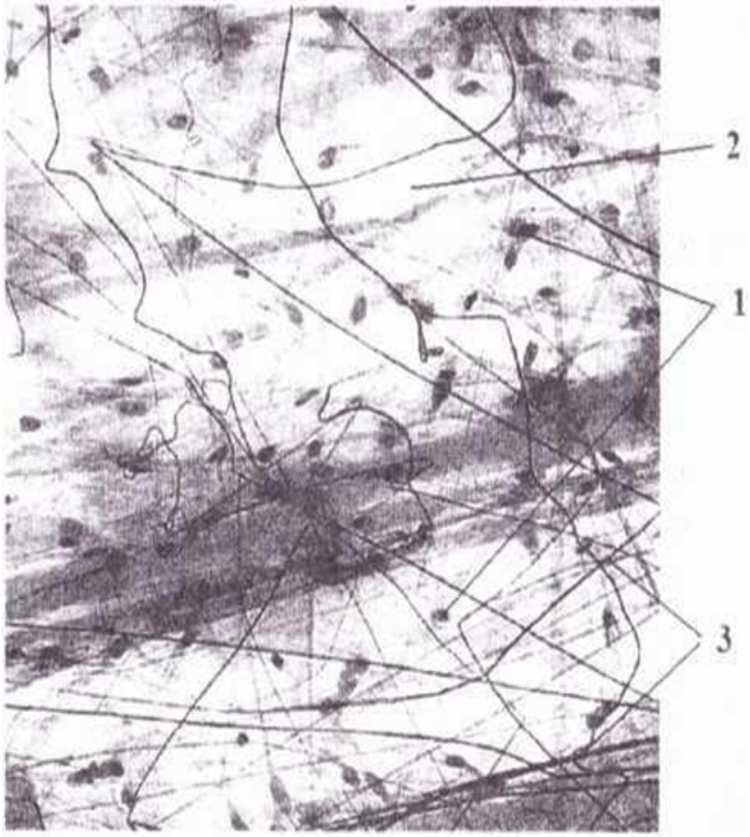


Figure 93. Photomicrograph of loose connective tissue. 1 - cells, 2 - extracellular matrix, 3 - fibers.

The cells of loose connective tissue are fibroblasts, fibrocytes. macrophages, mast cells, plasma cells, fat cell (adipocytes), pigment cells, leukocytes, pericytes, and adventitional cells.

Collagen, elastic, reticular fibers appear in this tissue, although the portion of reticular fibers is small.

Connective tissue cells

I. Fibroblast differon includes fibroblasts and fibrocytes (fig.94).

1. **Fibroblasts** are the dominant cells of the connective tissue. It is responsible for the synthesis of fibers and components of intercellular matrix.

The fibroblast is elongated cell with an ovoid nucleus. The cytoplasm is rich in rough endoplasmic reticulum, Golgi complex is well developed.

2. **The fibrocyte** is spindle-shaped. It is more mature, small cell with dark elongated nucleus. The cytoplasm is rich in rough endoplasmic reticulum, Golgi complex is badly developed. Badly secreted fibers and components of intercellular matrix.

3. **Myofibroblasts** are large cells with ruffled membranes and highly active endoplasmic reticulum. They are distinguishable from fibroblasts at the level of the EM by their high level of exocytotic vesicles and their fibers. At the level of the light microscope they can be distinguished by the presence of smooth muscle actin staining. Myofibroblasts possess bundles of microfilaments which terminate at the cell surface in a specialized adhesion complex, termed the fibronexus or mature local adhesion. This complex bridges the myofibroblast's internal microfilaments with extracellular fibronectin domains thus functioning as a contractile mechanism that enables these cells to generate force to the surrounding extracellular matrix. Myofibroblasts migrate to and are highly responsive to chemokines released at the site of injury. In the case of ischemia or heart failure, the injury is wide spread, thus the myofibroblasts can be found in large areas of the heart. Myofibroblasts produce and secrete a number of cytokines themselves which help to maintain the inflammatory response to injury.

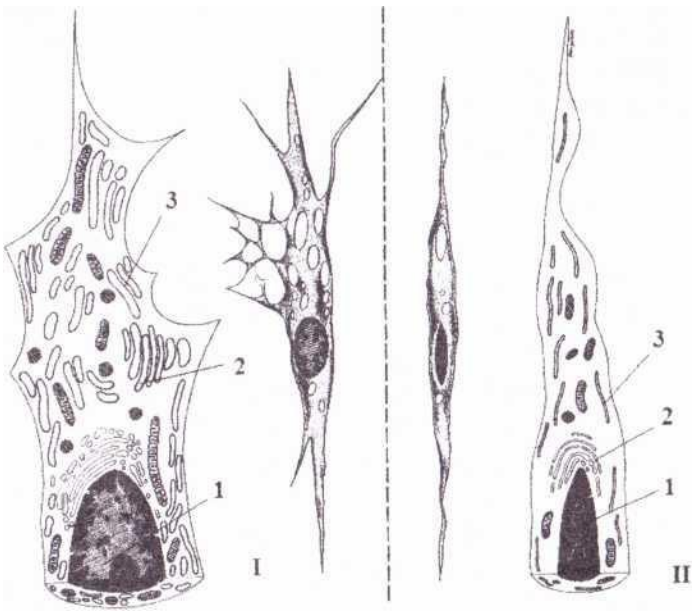


Figure 94. Diagram of the fibroblast and fibrocyte. I - fibroblast, II - fibrocyte, 1 - nucleus, 2 - Golgi complex, 3 - rough endoplasmic reticulum (from Junqueira L.C., Cameiro J., 2005)

II. Macrophages (histiocytes) are characterized by their phagocytic capacity. Macrophages derive mainly from precursor cells from the bone marrow that divide, producing monocytes that circulate in the blood. In a second step, these cells migrate into the connective tissue and are called macrophages.

Macrophages, which are distributed throughout the body, are present in most organs and constitute the mononuclear phagocyte system. In certain regions, macrophages have special names, e.g., Kupffer cells in the liver, microglial cells in the central nervous system, and osteoclasts in bone tissue.

The macrophages are large irregular cells with processes. They have a well-developed Golgi complex, many lysosomes, and a prominent rough endoplasmic reticulum (fig.95).

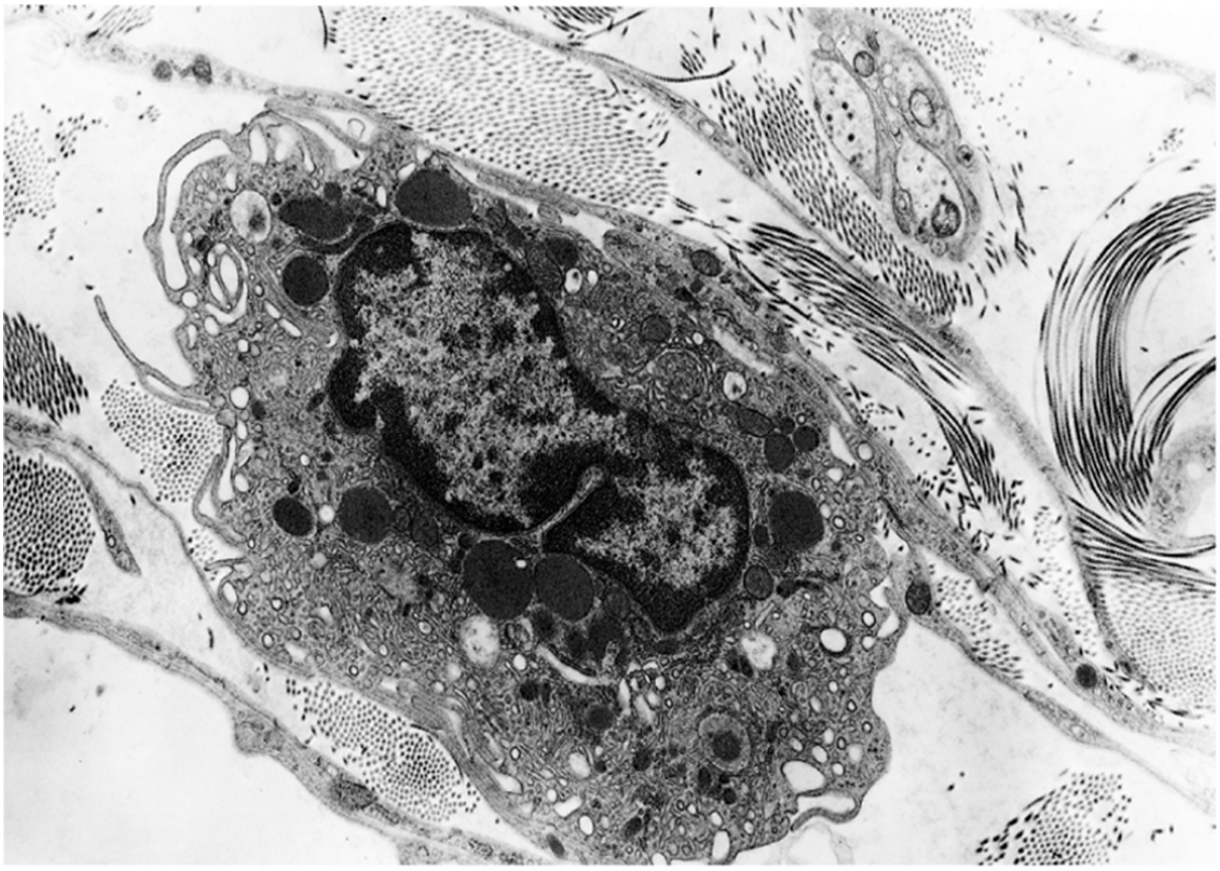


Figure 95. Histiocytes.

III. Mast cells are oval to round connective tissue cells, whose cytoplasm is filled with basophilic granules. The small and spherical nucleus is centrally situated (fig. 96). Mast cell granules are stained metachromatically (purple after toluidine blue staining) because they contain glycosaminoglycans, histamine, neutral proteases, and eosinophil chemotactic factor of anaphylaxis (ECF-A).Metachromasia is a property of cells to be stained in other colour distinguished from colour of dye.

The principal function of mast cells is the storage of chemical mediators of the inflammatory response.

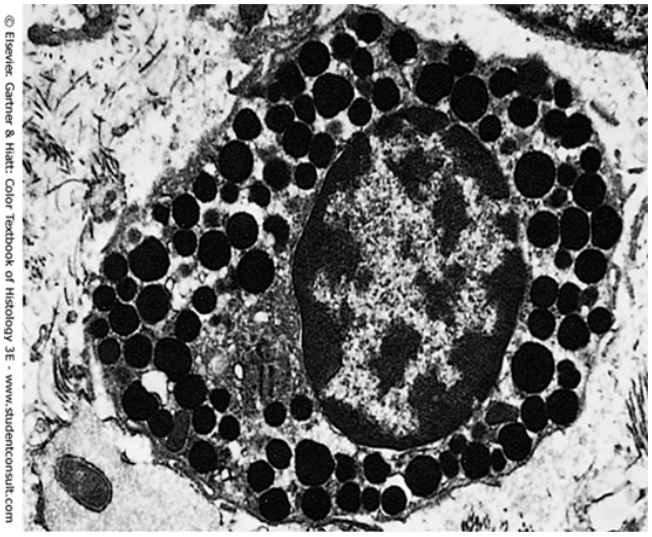


Figure 96. Mast cells.

Granules contain:

- **heparin** (anticoagulant) and
- **histamine** (dilatation of blood vessels and increase of their permeability).
- in animals**, serotonin.**

Types of the mast cells

There are two populations of mast cells in connective tissues.

One type is called the **connective tissue mast cell** (dermis and stroma of different organs), in which the proteoglycan in the granules is mainly heparin, a substance with anticoagulant activity.

In the second type, termed **mucosal mast cells** (lamina propria of mucosa); the granules contain chondroitin sulfate instead of heparin.

Mast cells originate from stem cells in the bone marrow.

The surface of mast cells contains specific receptors for IgE, a type of immunoglobulin produced by plasma cells.

IV. Plasma cells (fig. 97) are large with an eccentric round nucleus. Nucleus contains the chromatin clumped in a characteristic **''clock face"** pattern. **Cytoplasm is basophilic**, which filled **with rough endoplasmic reticulum**. Rough endoplasmic reticulum is concentrically located around of a nucleus. Well developed Golgi complex displaces the nucleus to one side of the cell (**perinuclear halo).**

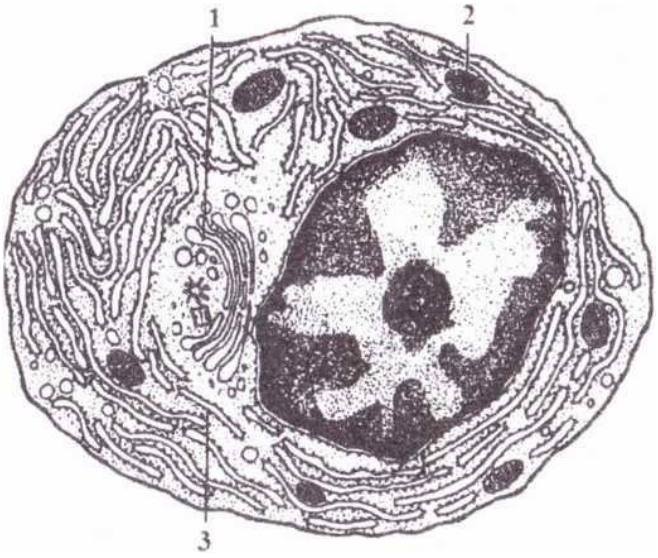


Figure 97. Schematic diagram of plasma cell. 1 - Golgi complex, 2 - mitochondria, 3 - rough endoplasmic reticulum (from EbiKOft B.JT., 200)

Remember that plasma cells are the altered B-lymphocytes which produce antibodies.

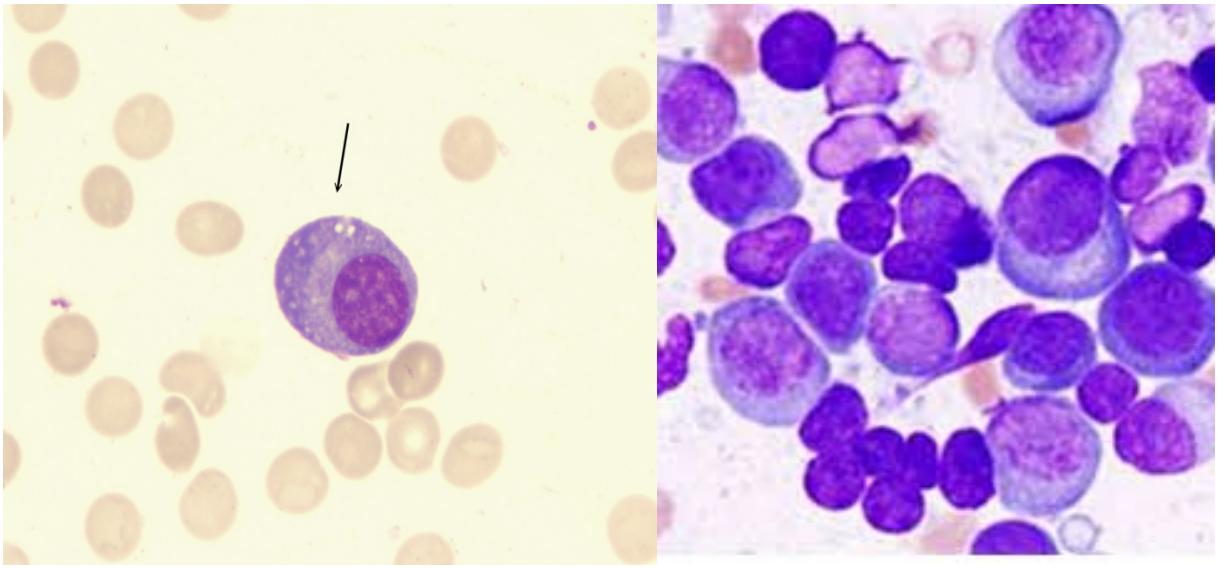


Figure 98. Plasma cells.

Plasma cell is a final stage of development of B-lymphocyte.
Function: maintenance of humoral immunity by synthesis of antibodies.

V. Fat cells (adipocytes) are round with peripheral nuclei. Cytoplasm forms a thin peripheral layer around the central droplet of fat. Adipocytes also are called ring cells (fig. 99).

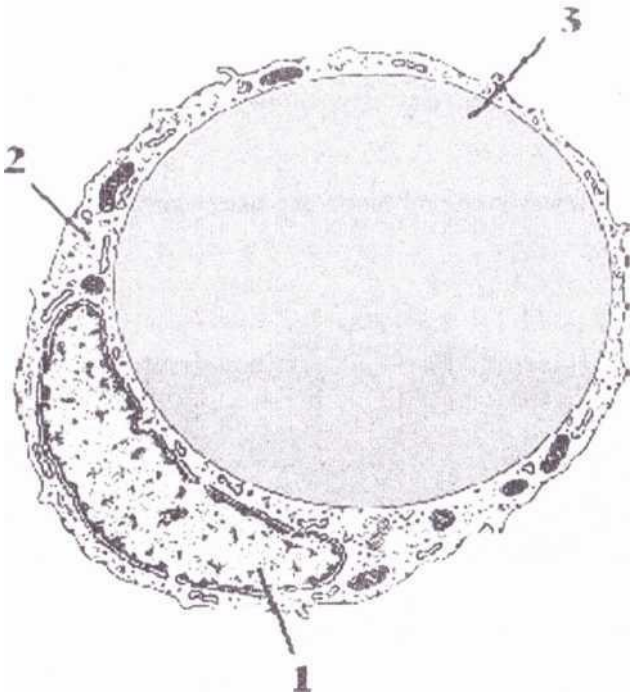


Figure 99. Schematic diagram of the adipose cell. 1 - nucleus, 2 - cytoplasm, 3 - fat droplet (from T. L. Lentz, 1971)

Functions:
- storage of neutral fats (food material);
- producing of heat.

VI. Pigment cells (melanocytes) are stellate with long branching processes and small round nucleus, cytoplasm contains melanin granules.

VII. Adventitional cells are the cells accompanying blood vessels. During a differentiation can turn in fibroblasts, myofibroblasts and adipose cell.

Pericytes surround blood capillaries and are the part of their wall (fig.100).

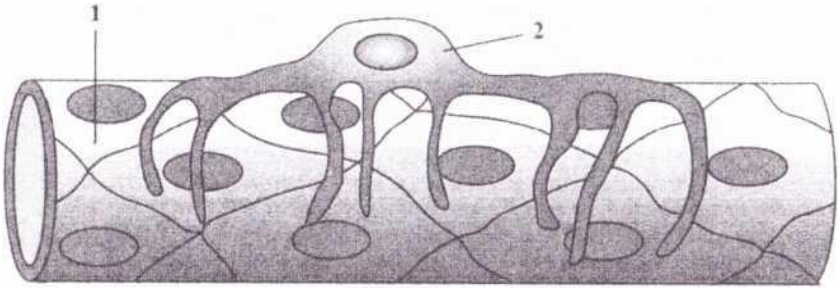


Figure 100. Schematic diagram of the pericyte. 1 - capillary, 2 - pericyte

Leukocytes are frequently found in connective tissue. They migrate across capillary and venule walls from the blood. There is a continuous movement of leukocytes from blood to connective tissue, and this process (diapedesis) increases greatly during inflammation.

Ground substance

The amorphous intercellular ground substance is colour less, transparent, and homogeneous; it varies from soft jelly to semisolid in consistency. It fills the space between cells and fibers of the connective tissue. The ground substance is formed mainly by two classes of components: glycosaminoglycans and glycoproteins.

Fibers

Connective tissue fibers are long, slender protein polymers that are present in variable proportions in the different types of connective tissue.

There are three main types of connective tissue fibers: collagen, reticular, and elastic.

Collagen and reticular fibers are composed of the protein collagen, and the elastic fibers are composed of the protein elastin.

Collagen fibers are the most numerous fibers in connective tissue.

With the light microscope collagen fibers are **seen in bundles.** The bundles may be straight or wavy. The bundles often **branch**, or anastomose with adjacent bundles, but the individual fibers do not branch.

Collagen **fibril**s are thin, elongated structures with diameter 20 - 90 nm. They have **transverse striation** with a periodicity of **64 nm** (fig.101). The transverse striations of the collagen fibrils are determined by the overlapping arrangement of the subunits **tropocollagen molecules**.

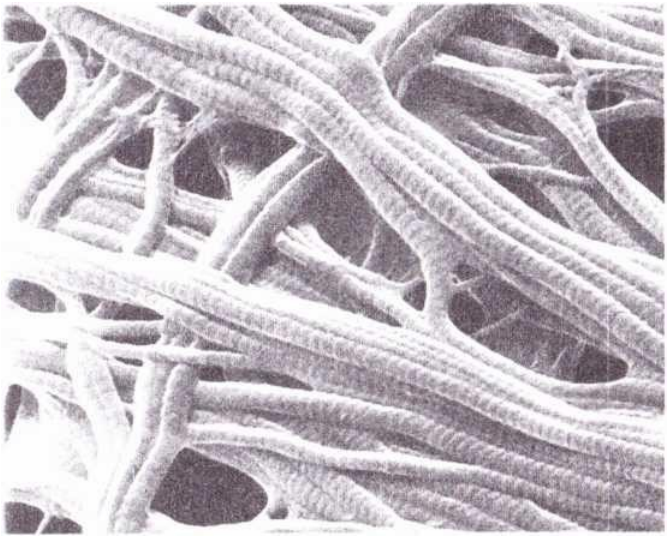


Figure 101. Electron micrograph of collagen fibers.

Formation of collagen fibers. Collagen is synthesized with fibroblasts.

Intracellular stage includes y synthesis of polypeptide alpha chains on polyribosomes of rough endoplasmatic reticulum from amino acids (glycin, prolin, and hydroxyprolin);

-3 alpha chains wrap around each other to form a triple helix except at the terminals where the polypeptide chains remain uncoiled; the resultant molecule is soluble procollagen monomer excreting from a cell.

Extracellular stage

- **procollagen** is converted into tropocollagen by cleavage of terminal propeptides by specific procollagen peptidases;

- **tropocollagen** then spontaneously self-assembles into multimolecular aggregates, which are aligned end-to-end to form banded fibrils; crosslinks are formed between specific amino acids, which stabilize the collagen fibril and provide tensile strength.

Localization of collagen fibers:tendon,aponeurosis, intervertebra1 disks.

Reticular fibers are thin, with a diameter 0, 5-2, 0 jam. They form an extensive network in certain organs. They are not visible in haematoxylin- eosin specimens but can be stained black by impregnation with silver salts.

Chief distribution of reticular fibers: smooth muscle, endoneurium, and the framework of hematopoietic organs (spleen, lymph nodes, red bone marrow).

Elastic fibers have diameter 0,2-10,0 jm, branch and anastimose with each other, shaping three-dimensional networks; they do not form bundles.

Elastin is the main protein component of elastic fibers.

Elastic fibers can be demonstrated by staining with orcein. Elastic fibers can be stretched and return to their original length when tension is released.

Localization of elastic fibers: lungs, fibrocartilage, skin, and wall of aorta.

Dense connective tissue

Dense connective tissue consists of same components found in loose connective tissue but there are fewer cells and clear predominant collagen fibers.

The main property dense connective tissue - very high mechanical strength - it is caused by presence of potent bundles of collagen fibers. Orientation of these fibers corresponds to a direction of action of forces, which cause deformation.

Dense irregular connective tissue has the collagen fibers that are arranged in bundles without a definite orientation. The collagen fibers form a three-dimensional network in this tissue and provide resistance to stress from all directions (fig.102). This type of tissue forms the dermis.

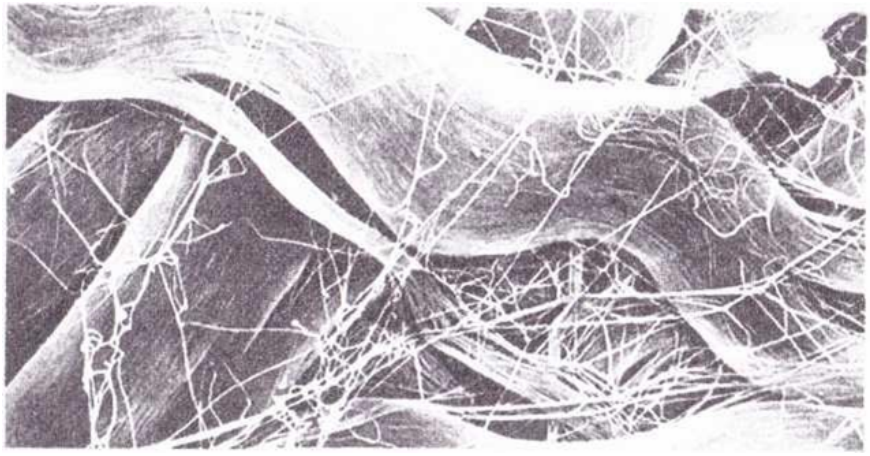


Figure 102. Electron micrograph of dense irregular connective tissue.

Dense regular connective tissue

The collagen bundles of dense regular connective tissue are arranged according to a definite pattern.

Tendons are the most common example of dense regular connective tissue. Tendons are cord-like structures that attach muscle to bone. Tendons (fig. 103) consist of parallel bundles of collagen fibers separated by a small quantity of amorphous intercellular substance. Rows of fibroblasts (tendinocytes) are situated between these bundles. Their flbrocytes contain elongated nuclei parallel to the fibers.

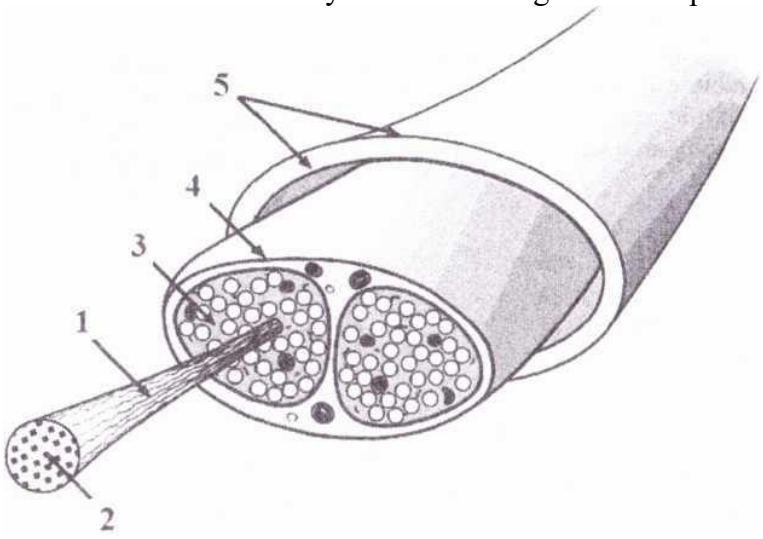


Figure 103. Dense regular connective tissue of tendon. 1 - collagen fibres. 2 - tendinocytes, 3 - endotenon, 4 - epitenon, 5 - synovial sheath

The collagen bundles which are situated between the tendinocytes are called **primary bundles.** Primary bundles aggregate into larger bundles (**secondary bundles)** that are enveloped by loose connective tissue containing blood vessels and nerves (**endotenon)**. The substance of tendon is surrounded by a thin connective tissue capsule, the epitenon. Some tendons are also surrounded by a specialized **synovial sheath.**

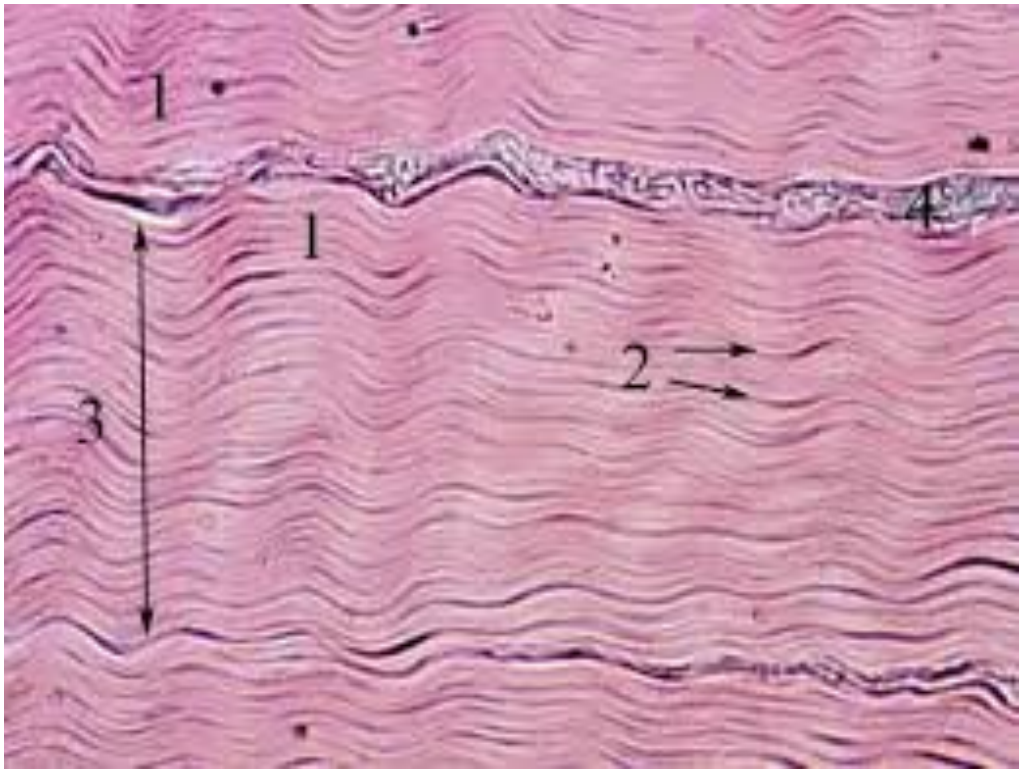


Figure 104. Tendon. 1 - primary bundles, 2 - tendinocytes nuclei, 3 - secondary bundles, 4 - endotendineum.

Connective tissue with special properties

Adipose tissue

There are two types of the adipose tissue:

- white (unilocular, or yellow),

-brown (multilocular).

White (unilocular) adipose tissue (fig.105) contains cells, each contain only one large fat droplet (ring cells). White adipose tissue is found in subcutaneous, omentum and mesentery regions. Unilocular adipose tissue subdivided into lobules by a partition of connective tissue.

Figure 105. White (unilocular) adipose tissue. 1 -adipose cell, 2 - cytoplasm, 3 – nuclei

Functions of adipose tissue:

-trophic,

- supporting, protective and plastic - adipose tissue surrounds organs and fills in spaces between them; softening impacts, it protects them from mechanical traumas; it substitutes a tissue of some organs after their involution (thymus, mammary gland, bone marrow);

- energy storage: food that is excess to requirements is converted into fat and stored within adipose tissue;

- heat-insulating - adipose tissue has properties heat-isolator due to what it interferes with excessive heat waste by an organism;

-heat-forming - the part of the energy formed owing to oxidation of power- intensive lipid molecules turns to heat;

-depot of liposoluble vitamins (A, D, E, K) and serves large depots of steroid hormones;

- endocrine - synthesizes estrogens and hormone, which regulate consumption of food -leptin.

Brown (multilocular) adipose tissue is a specialized form of adipose tissue in hibernating and newborn mammals. It is greatly reduced in adulthood.

Brown adipose tissue is mainly found in subscapular, interscapular, and mediastinal areas. Cells ot the brown (multilocular) adipose tissue have several fat droplets and many mitochondria, are rich in heme-containing cytochromes (fig.106).

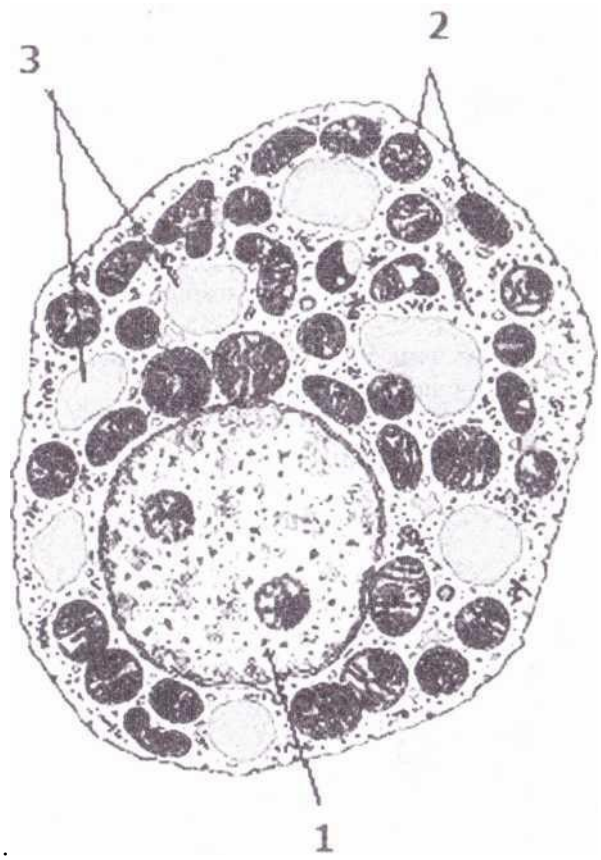


Figure 106. Schematic diagram of brown adipose tissue cell.
1 - nucleus, 2 - mitochondria, 3 - fat droplets (from T. L. Lentz, 1971)

This specialized tissue can generate heat by “uncoupling" the respiratory chain of oxidative phosphorylation within mitochondria.

The function of this tissue in humans appears to be of importance mainly in the first months of postnatal life, when it **produces heat and thus protects the newborn against cold.**

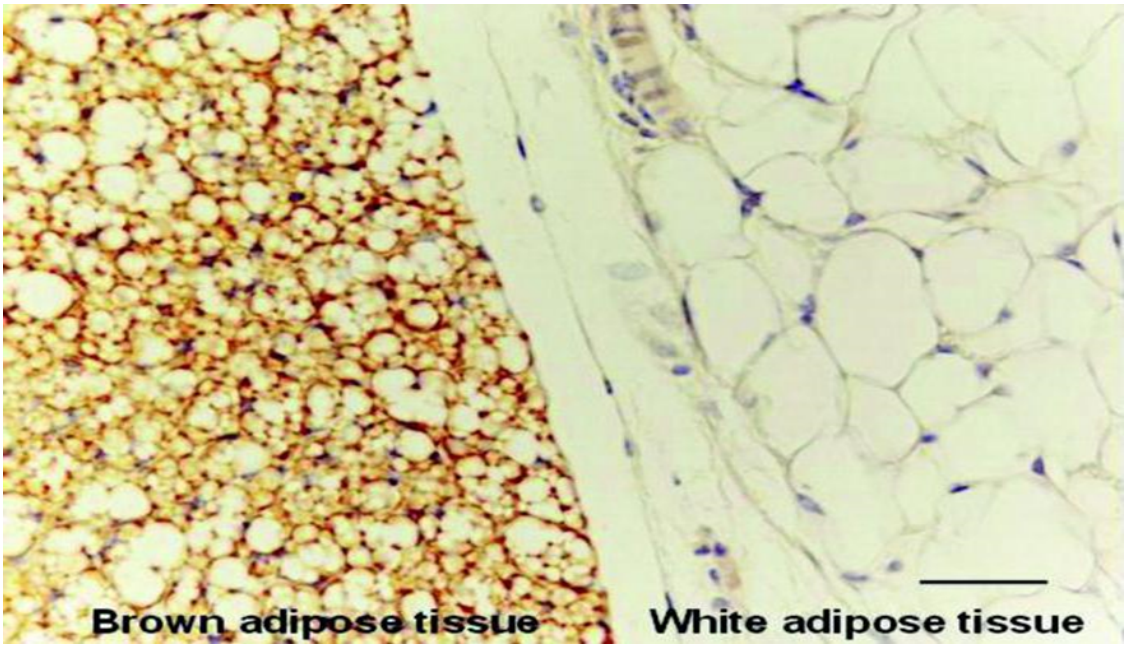


Figure 107. White and brown adipose tissue.

Reticular tissue

The reticular tissue is a specialized loose connective tissue that provides the architectural framework of the myeloid (bone marrow) and lymphoid (lymph nodes, spleen) hematopoietic organs.

Reticular tissue consists of reticular cells and branched reticular fibers (fig.108). Cells and fibers form supporting network for hematopoietic cells.

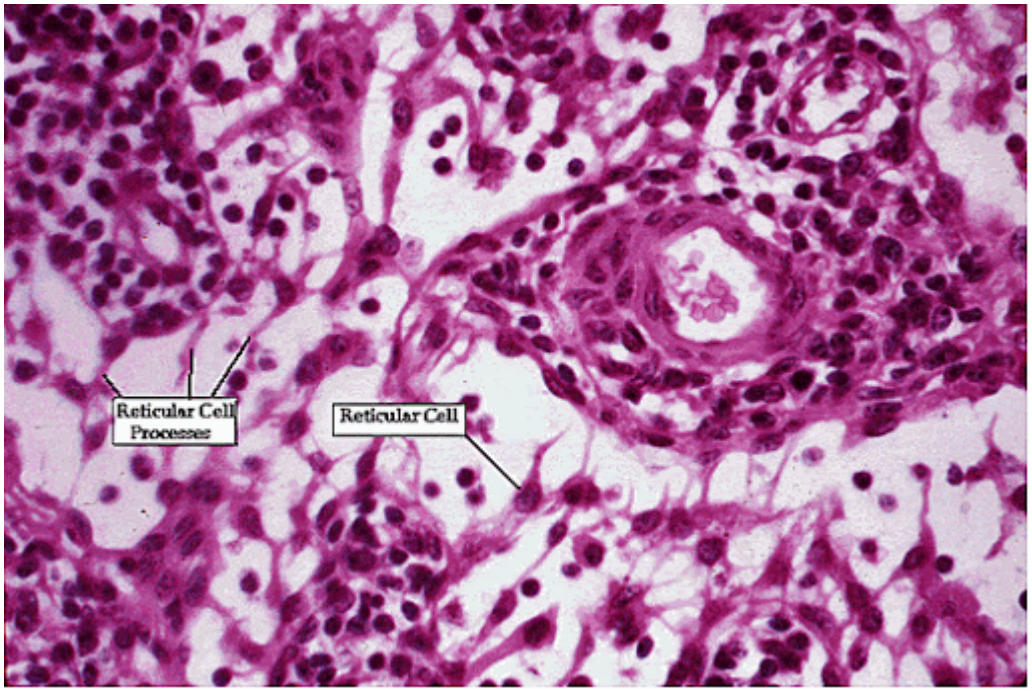


Figure 108. Reticular tissue: reticular cells, reticular fibers.

Mucous tissue

The mucous tissue has an abundance of ground substance **composed of hyaluronic acid**. It is a .jelly like tissue containing very few fibers. The cells in this tissue are mainly fibroblasts (fig.109).

Mucous tissue is the principal component of Wharton's jelly of the umbilical cord.

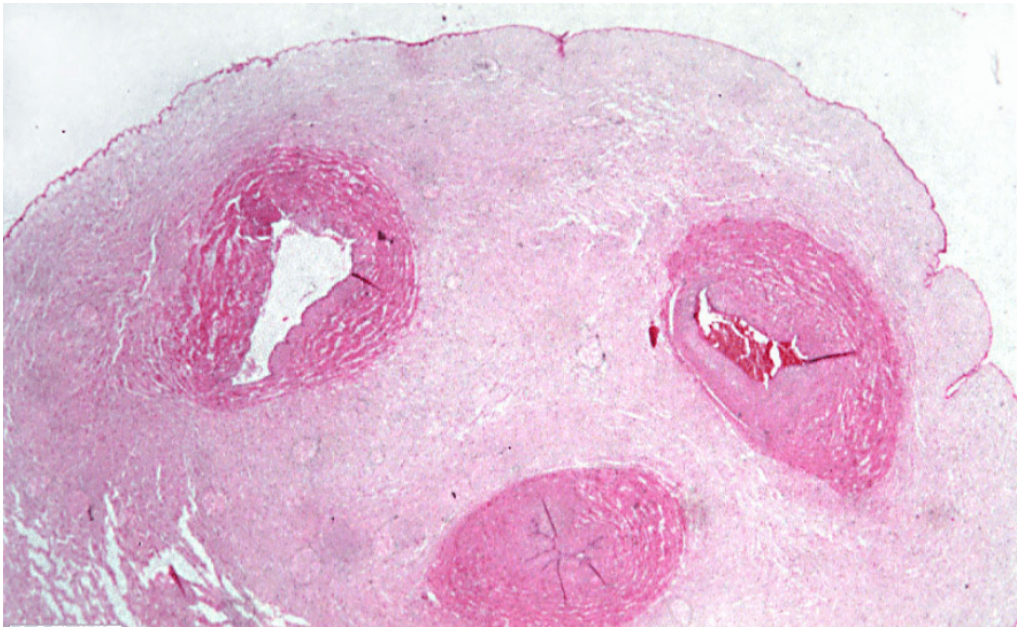


Figure 109. Wharton's jelly.

Pigment tissue

The pigment connective tissue reminds loose connective tissue, however contains numerous pigment cells (melanocytes) (fig.110).

Function of melanin of pigment cells is to protect the organism against the damaging effects of nonionizing ultraviolet irradiation.

Localization, in humans, melanin is the primary determinant of skin colour. It is also found in hair, the pigmented tissue underlying the iris of the eye, and the stria vascularis of the inner ear. In the brain, tissues with melanin include the medulla and zona reticularis of the adrenal gland, and pigment- bearing neurons within areas of the brainstem, such as the locus coeruleus and the substantia nigra.

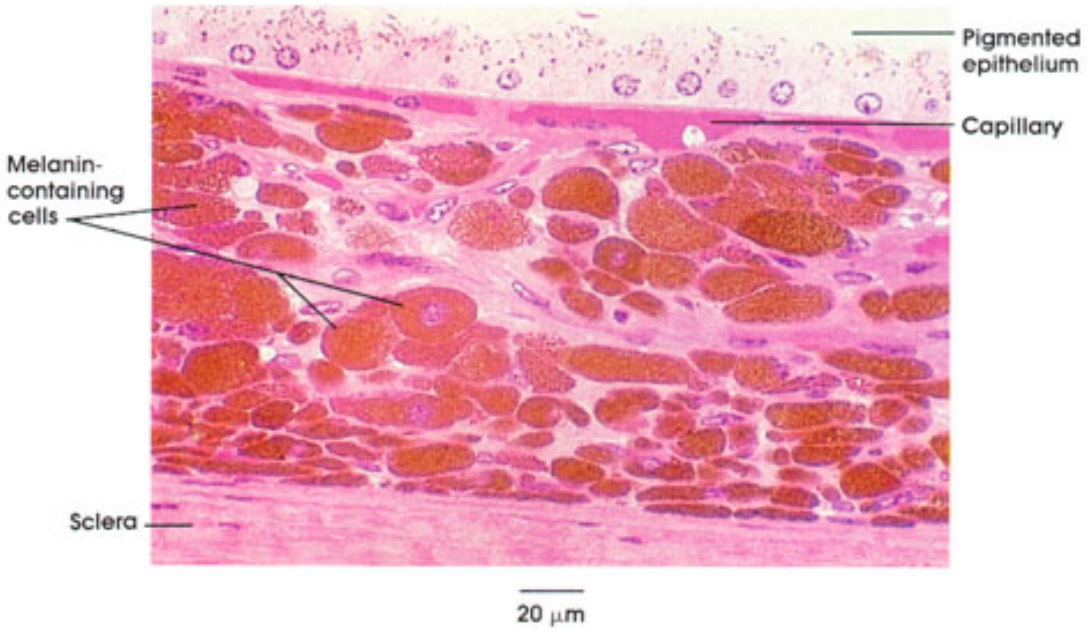


Figure 110. Pigment connective tissue.

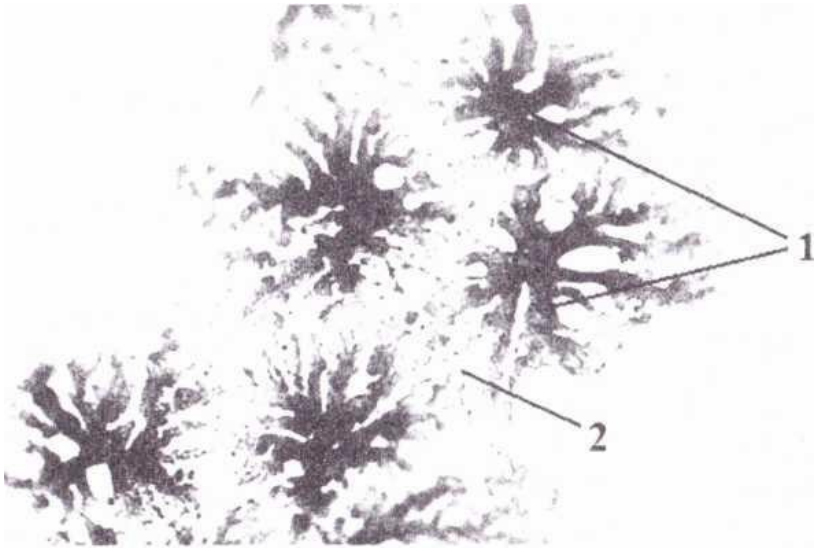


Figure 111. Pigment tissue. 1 - pigment cell, 2 - ground substance, 3 – fibers

Types of melanin

Eumelanin is found in hair, areola, and skin. In humans, it is more abundant in people with dark skin. There are two different types of eumelanin: black and brown. Black eumelanin is mostly in non-Europeans and aged Europeans, while brown eumelanin is in mostly young Europeans. A small amount of black eumelanin in the absence of other pigments causes grey hair. A small amount of brown eumelanin in the absence of other pigments causes yellow (blond) color hair.

Pheomelanin is also found in hair and skin and is both in lighter and darker skinned humans. Pheomelanin imparts a pink to red hue and, thus, is found in particularly large quantities in red hair. Pheomelanin is concentrated in the lips, areola, nipples, glans of the penis, and vagina. Pheomelanin also may become carcinogenic when exposed to the ultraviolet rays of the sun.

Neuromelanin is the dark pigment present in pigment bearing neurons of four deep brain nuclei: the substantia nigra, the locus coeruleus ("blue spot"), the dorsal motor nucleus of the vagus nerve (cranial nerve X), and the median raphe nucleus of the pons.

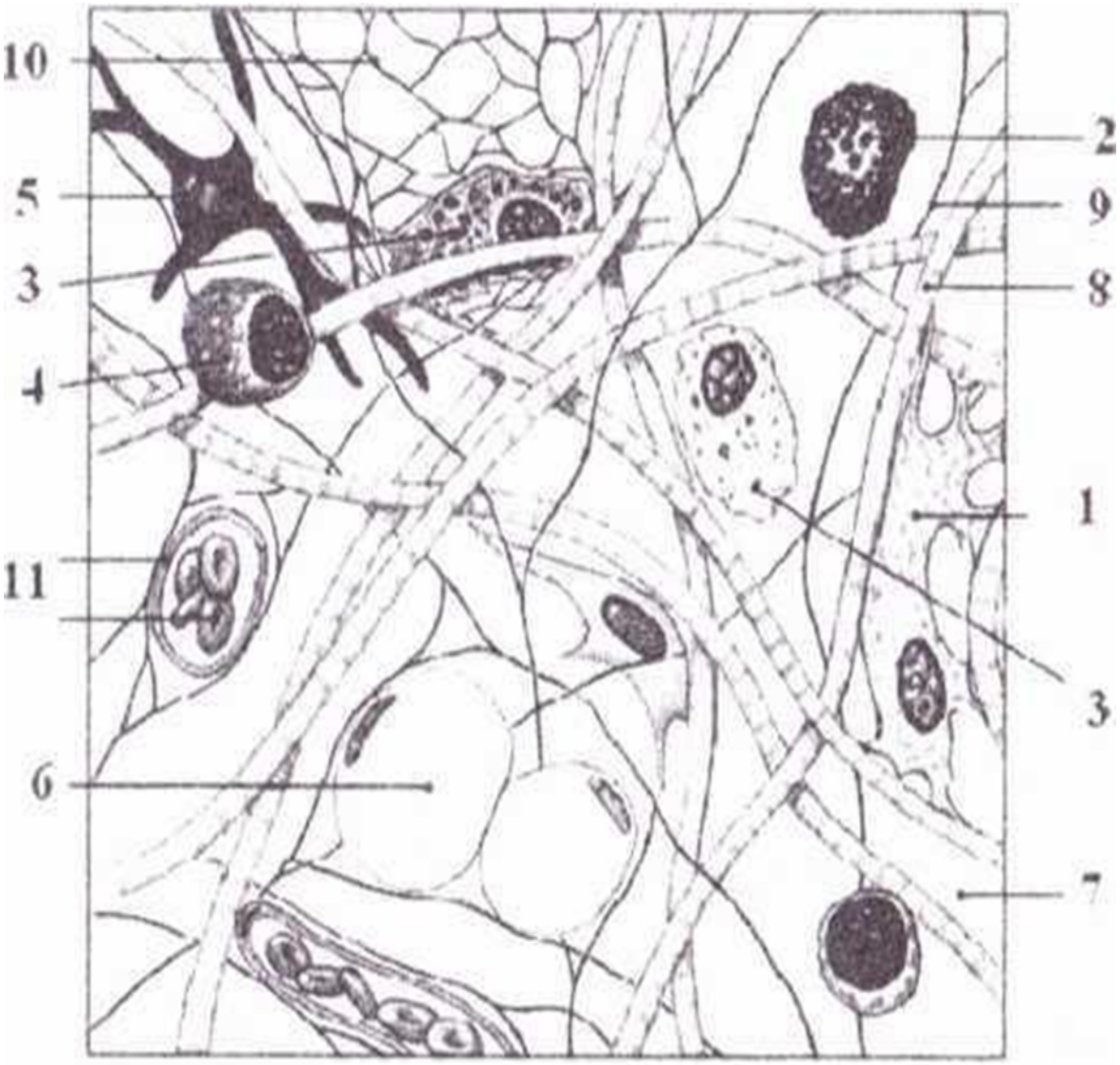
Practical lessons № 11

Questions for self-control

1.Connective tissues: origin, disposition, structure and functions.

Connective tissues: classification. Loose connective tissue: structure of cells, functions.

1. Fibroblast differon.
2. Macrophages (histiocytes).
3. Mast cells.
4. Plasma cells.
5. Fat cells (adipocytes), pigment cells (melanocytes), adventitional cells.
6. Dense connective tissue (regular, irregular): structure and functions.
7. Connective tissue with special properties (mucous, adipous, reticular, pigmental): their structure and functions.
8. Collagen fibers.
9. Elastic fibers.



The teacher's signature:

SKELETAL (SUPPORTING) TISSUES. CARTILAGES

Classification of the skeletal tissues

Cartilage
- hyaline,
- elastic,
- fibrocartilage.

Bone
- primary, immature, or woven bone,
- secondary, mature, or lamellar bone (compact bone),
- spongy bone.

Cartilage

The cartilage is a flexible connective tissue that consists of specialized cells called chondroblasts that produce a large amount of extracellular matrix composed of collagen fibers, abundant ground substance rich in proteoglycan, and elastic fibers. Unlike other connective tissues, cartilage does not contain blood vessels. The chondrocytes are supplied by diffusion, helped by the pumping action generated by compression of the articular cartilage or flexion of the elastic cartilage.

Cartilage functions

1. Movement (cartilage joins bones firmly together in such a way that a certain amount of movement is still possible between them).

2. Support (maintain shape of the organs: the C-shaped cartilaginous rings in the trachea and bronchi assist in keeping those tubes open).

3. Growth (hyaline cartilage is responsible for the longitudinal growth of the long bones).

Histogenesis of cartilage tissue (chondrification, chondrogenesis)

In embryogenesis, the skeletal system is derived from the mesoderm germ layer.

Chondrification (also known as chondrogenesis) is the process by which cartilage is formed from condensed mesenchymal cells, which differentiates into chondroblasts and begins secreting the molecules that form the extracellular matrix.

Steps in chondrification

- early stage of the chondrification centres formation from the mesenchymal cells;
- late stage of the chondrification centres formation: mesenchymal cells lose processes, become rounded and form densely packed cellular masses;
- differentiation of mesenchymal cells into cartilage-forming cells, chondroblasts, which begin to secrete the components of the extracellular matrix of cartilage;
- formation of isogenous groups of chondrocytes.

Growth of the cartilage

Two types of growth can occur in cartilage: appositional and interstitial.

1. **Appositional growth** results in the increase of the diameter or thickness of the cartilage. The new cells derive from the perichondrium and occur on the surface of the cartilage model.

2. **Interstitial growth** results in an increase of cartilage mass and occurs from within. Chondrocytes undergo mitosis within their lacuna, but remain imprisoned in the matrix, which results in clusters of cells called isogenous groups.

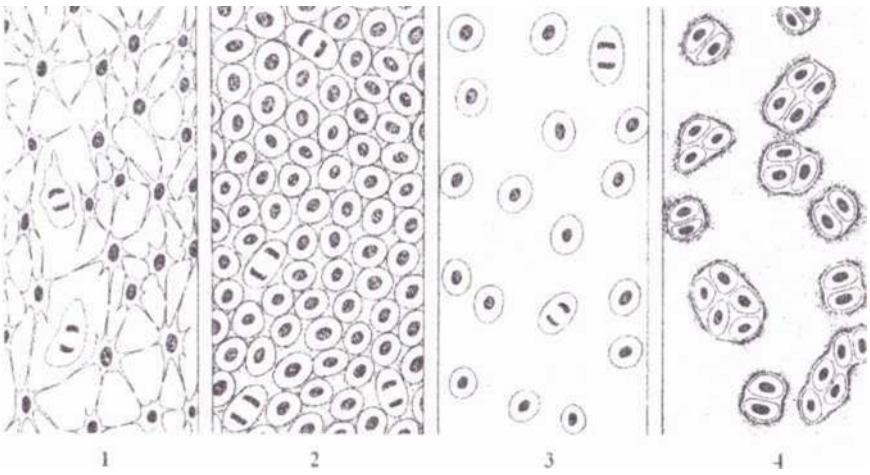


Figure 112. Stages of chondrogenesis. 1 - early stage of the chondrification centre formation, 2 - late stage of the chondrification centre formation, 3 - differentiation of mesenchyme cells into chondroblasts. 4 - formation of isogenous groups of chondrocytes (from Junqueira L.C., Carneiro J., 2005).

Repair of cartilage

Once damaged, cartilage has limited repair capabilities. Because chondrocytes are bound in lacunae, they cannot migrate to damaged areas. Also, because hyaline cartilage does not have a blood supply, the deposition of new matrix is slow. Damaged hyaline cartilage is usually replaced by fibrocartilage scar tissue.

Common plan of cartilage tissue structure

Cartilage consists of cells (chondroblasts**,** chondrocytes)
and **extracellular matrix** is composed of: fibers and ground substance

Cells of cartilage tissue

Chondroblasts are young cartilage precursor cells, capable to a proliferation and synthesis of intercellular substance of a cartilage. Cytoplasm of chondroblasts contains well-developed rough and smooth cytoplasmic reticulum, Golgi apparatus.

Chondrocytes occur singly or in **groups called isogenous groups** within spaces called lacunae. Chondrocytes are responsible for the secretion and maintenance of the matrix.

Differon of cartilage cells

Chondroblasts and chondrocytes are derived from chondroprogenitor cells (mesenchymal stem cell that can undergo mitotic division and differentiate into a chondroblast). **Chondroprogenitor cell => chondroblast => chondrocyte**

Extracellular matrix

The majority of the wet weight of cartilage, ranging any where 65 -80%, consists of water. The matrix is mainly composed of **proteoglycans,** which are large molecules with a protein backbone and *glycosaminoglycans (GAG)* side chains. Glycosaminoglycans are polysaccharides. This molecule fills all the spaces between the collagen fibers and holds water, thus plumping out the extracellular matrix and giving cartilage its resistance to compression and its resilience (ability to spring back into shape after load). The most common types of GAGs in cartilage are chondroitin sulfate and keratin sulfate. The matrix immediately surrounding the chondrocytes is referred to as the territorial matrix, or capsule, and stains darker than the interstitial matrix during slide preparation.

Cartilage covering

At the periphery of mature cartilage is a zone of dense connective tissue, ft is rich in collagen and contains numerous fibroblasts with cartilage- forming potential. This zone is called **perichondrium.**

Functions of the perichondrium:

1. trophic;
2. growth of the cartilage;
3. maintenance of the cartilage.

Types of cartilage

There are three different types of cartilage, each with special characteristics adapted to their function.

Hyaline cartilage

Hyaline cartilage (fig. 113) is the most abundant type of cartilage. The name hyaline is derived from the Greek word hyalos, meaning glass. This refers to the translucent matrix or ground substance. Hyaline cartilage is found in the wall of respiratory passages (nose, larynx, trachea, and bronchi), lining bones in joints (articular cartilage or, commonly, gristle) and is also present inside bones, serving as a center of ossification, or bone growth. In addition, hyaline cartilage forms most of the embryonic skeleton.

Structural features of the hyaline cartilage:

- presence of isogenous groups of chondrocytes;
- ground substance contains a dense network of collagen fibers',
- possesses a perichondrium.

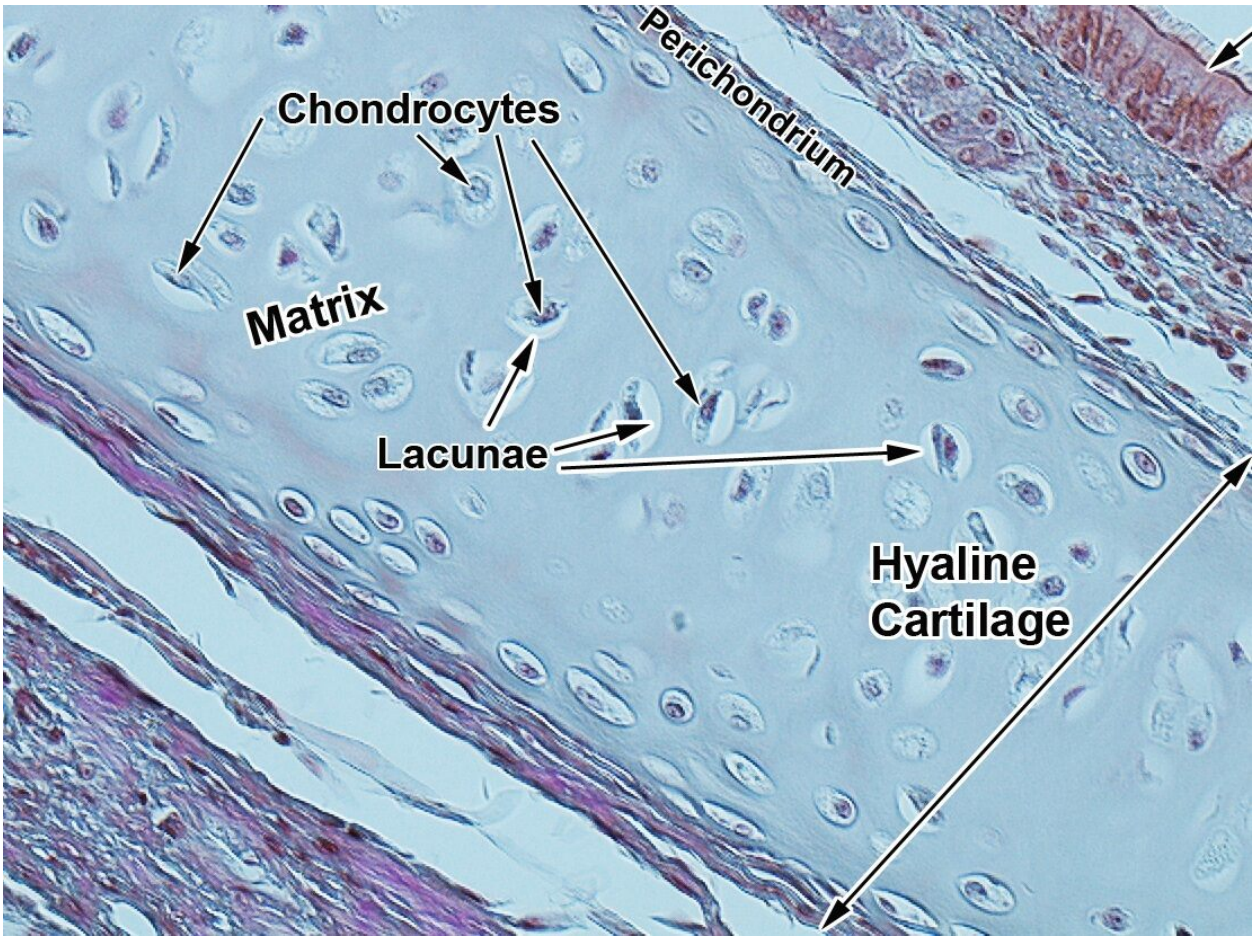


Figure 113. Hyaline cartilage.

Elastic cartilage

Elastic cartilage (fig. 114), also called yellow cartilage, is found in the pinna of the ear and several tubes, such as the walls of the auditory (Eustachian) tubes, larynx, and especially in the epiglottis (keeps food from entering the airways).

Elastic cartilage is similar to hyaline cartilage but contains elastic fibers scattered throughout the matrix. Elastic fibers can be demonstrated by standard elastin stain (orcein).

Structural features of the elastic cartilage:

- presence of singly chondrocytes or isogenous groups of chondrocytes;
- ground substance contains a dense network of branching and anastomosing elastic fibers;
- possesses a perichondrium.

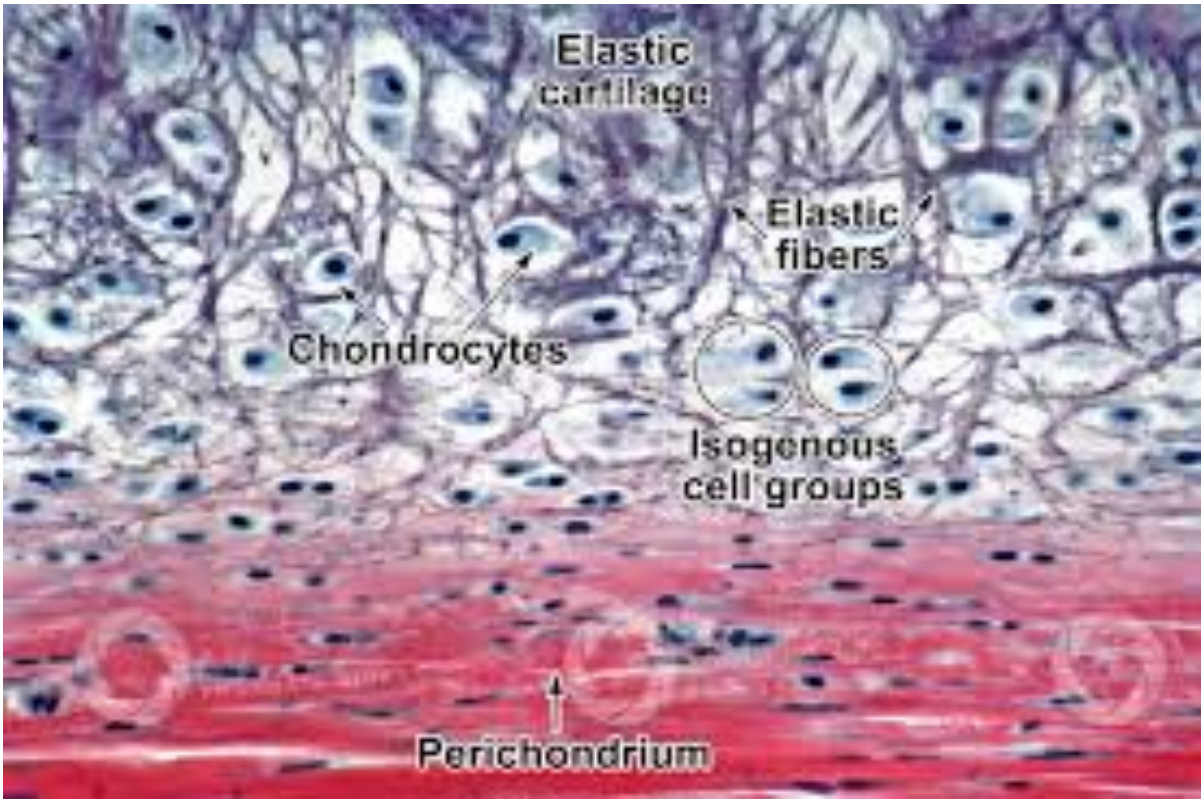


Figure 114. Elastic cartilage: isogenous groups of chondrocytes, elastic fibers, perichondrium

Fibrocartilage

Fibrocartilage is a specialized type of cartilage found in areas requiring tough support or great tensile strength, such as between the intervertebral discs, between the hip and pelvis bones, and at sites connecting tendons or ligaments to bones.

Fibrocartilage has characteristics intermediate between those of dense connective tissue and hyaline cartilage.

Structural features of the fibrocartilage (fig. 115):

- presence of singly chondrocytes or isogenous groups of chondrocytes arranged in long rows;
- ground substance contains a great number of collagen fibers oriented in the direction of the functional stresses;
- perichondrium is absent.

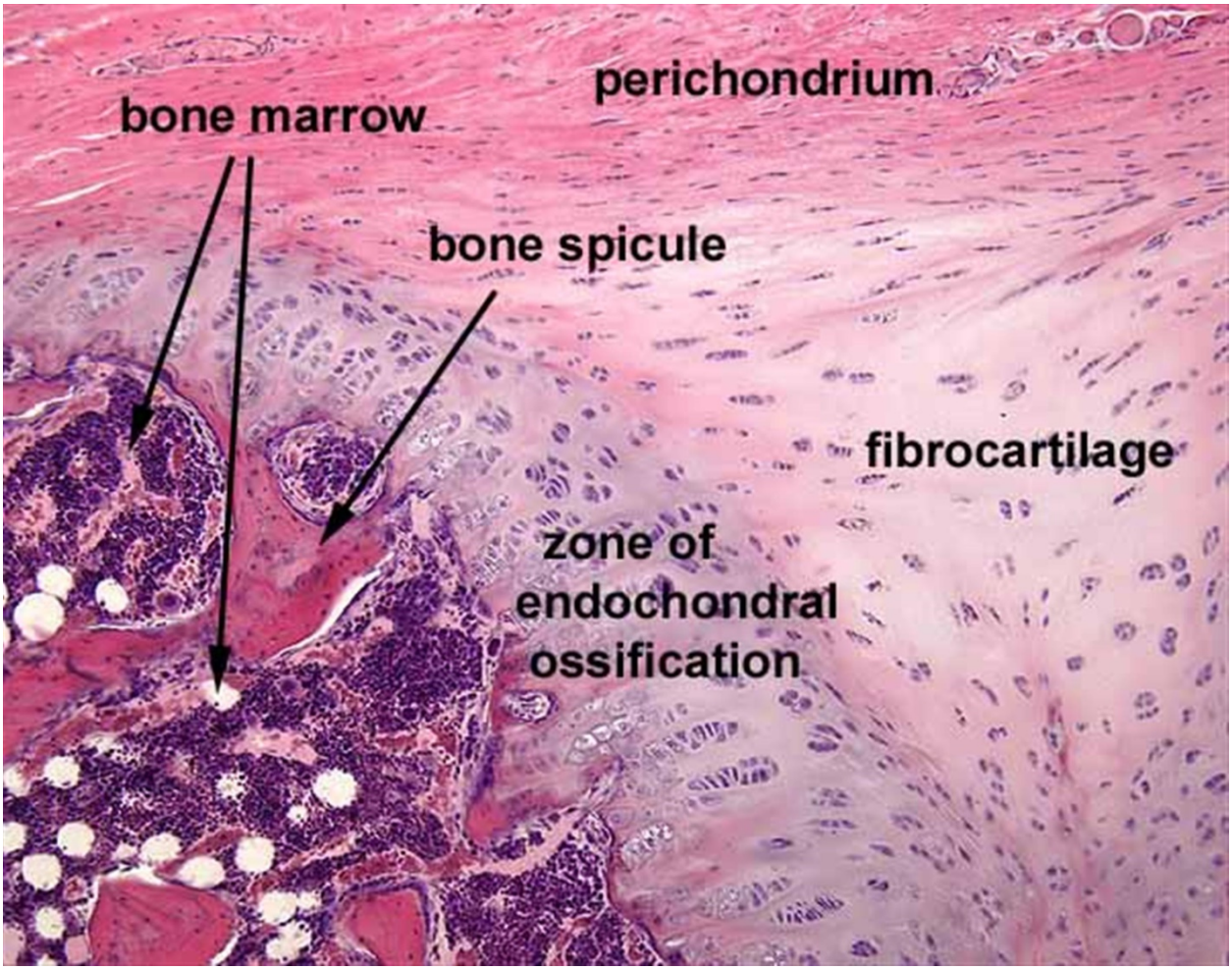


Figure 115. Fibrocartilage.

Nutrition of cartilage Cartilage is avascular.

Hyaline and elastic cartilages get nutrition from the blood vessels of the perichondrium; fibrocartilage - from blood vessels of surrounding connective tissue, articular cartilage from synovial fluid.

Practical lessons № 12

Questions for self-control

1. The general features of chondroid tissue.
2. Classification and functions of cartilages.
3. Morphofunctional characteristic of cartilages.
4. The structure and functions of perichondrium.
5. Hyaline cartilage: structure and functions.
6. Elastic cartilage: structure and functions.
7. Fibrocartilage structure and functions.
8. Diffferent types of cartilages growth.

Hyaline cartilage

Elastic cartilage

Fibrocartilage.

The teacher's signature:

BONE TISSUES

Bone tissue is a specialized type of connective tissue and is the main
element of the skeleton. It is composed of cells and an extracellular matrix in which fibers are embedded.

Bone tissue is unlike other connective tissues in that the extracellular matrix becomes calcified.

Functions of the bone tissue

1. formation of the adult skeleton;
2. participation in the movements of body;
3. protection of the vital organs of the cranial and thoracic cavity;
4. reservoir of ionic calcium essential for many cellular processes of the body; bone has several metabolic functions especially in calcium homeostasis.
5. protection of the hematopoietic bone marrow.

Common plan of structure and functions of bone tissue

Like other connective tissues, bone consists of cells and extracellular matrix (fibers, and ground substance) but differs because the extracellular matrix is calcified.

Bone is composed of:

1.Cells:

•osteoprogenitor cells;
•osteoblasts;
•osteocytes;
•osteoclasts;

2. Matrix:

•organic component (osteoid) - 50%;
•in organic component - 25%;
•water - 25%.

Cells of bone tissue

Osteoprogenitor cells are mesenchymal stem cell that can undergo mitotic division and differentiate into osteoblasts. Osteoprogenitor cells are located in the inner cellular layer of the periosteum (periostal cells), the endosteum and lining canals of the osteons. These cells are most active during bone growth.

Osteoblasts are derived from osteoprogenitor cells and are responsible for the synthesis of the organic components of bone matrix, which is called osteoid. Osteoblasts are associated with growing surface of bone. Osteoblasts have a size of 30-40 p.m., basophilic cytoplasm, a well-developed rough endoplasmic reticulum and Golgi complex. The function of these cells is to produce extracellular substance and collagen fibers.

When the cells are active they have a cuboidal appearance (); and when their activity declines, they flatten. The cells have cytoplasmic processes that bring them in contact with neighbouring cells. Their organelles are typical of protein secretory cells: they contain extensive rough ER, well developed Golgi complex and numerous secretory vesicles.

Osteocytes are the flat, almond-shaped cells of mature bone (). They reside in the lacunae of bone, only one osteocyte is found in each lacuna. Thin cylindrical spaces that house cytoplasmic processes are called canaliculi. Processes of adjacent cells make contact via gap junctions, which allow ions and small molecules to travel from cell to cell. Canaliculi also contain extracellular fluid carrying nutrients to nourish the osteocytes.

Differons of bone cells

Osteoblasts and osteocytes are derived from osteoprogenitor cells (mesenchymal stem cell that can undergo mitotic division and differentiate into an osteoblast).

Osteoprogenitor cell => osteoblast => osteocyte

Osteoclasts are derived from the fusion of blood-derived monocytes and thus belong to the mononuclear phagocyte system.

Pluripotential cell => myeloid multipotential cell => monocyte-colony- forming cell => promonocyte => monocyte => osteoclast

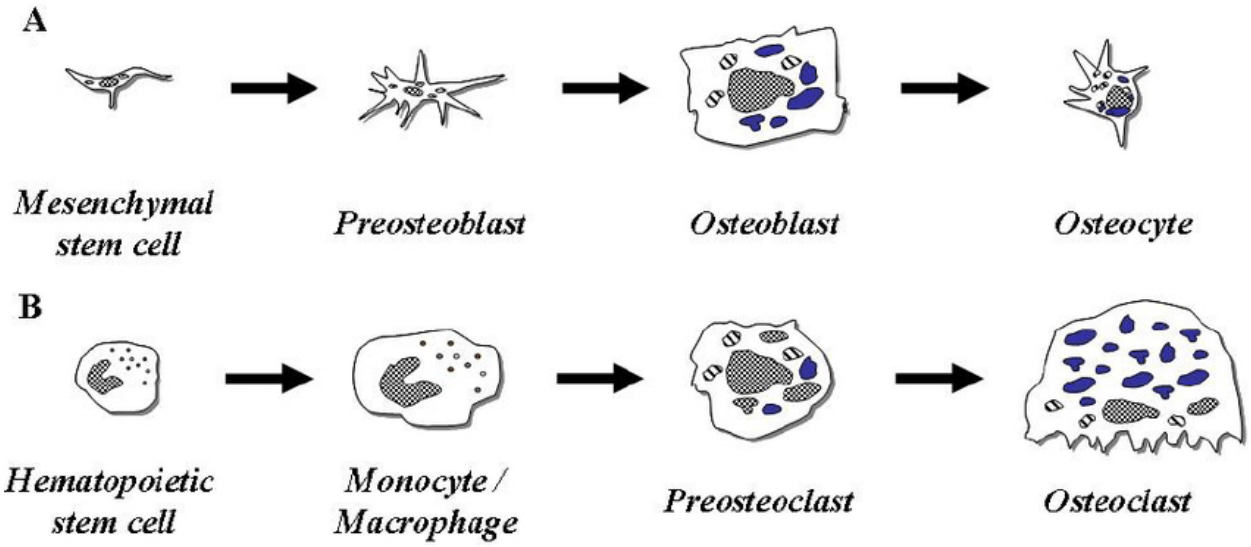


Figure 116. Diagram of development of osteoblasts, osteocytes and osteoclasts.

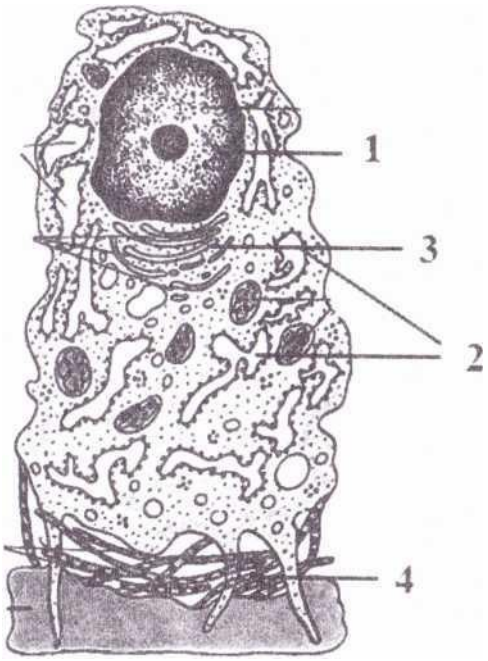


Figure 117. Schematic diagram of the osteoblast. 1 - nucleus, 2 - rough endoplasmic reticulum, 3 - Golgi complex, 4 - synthesized fibres

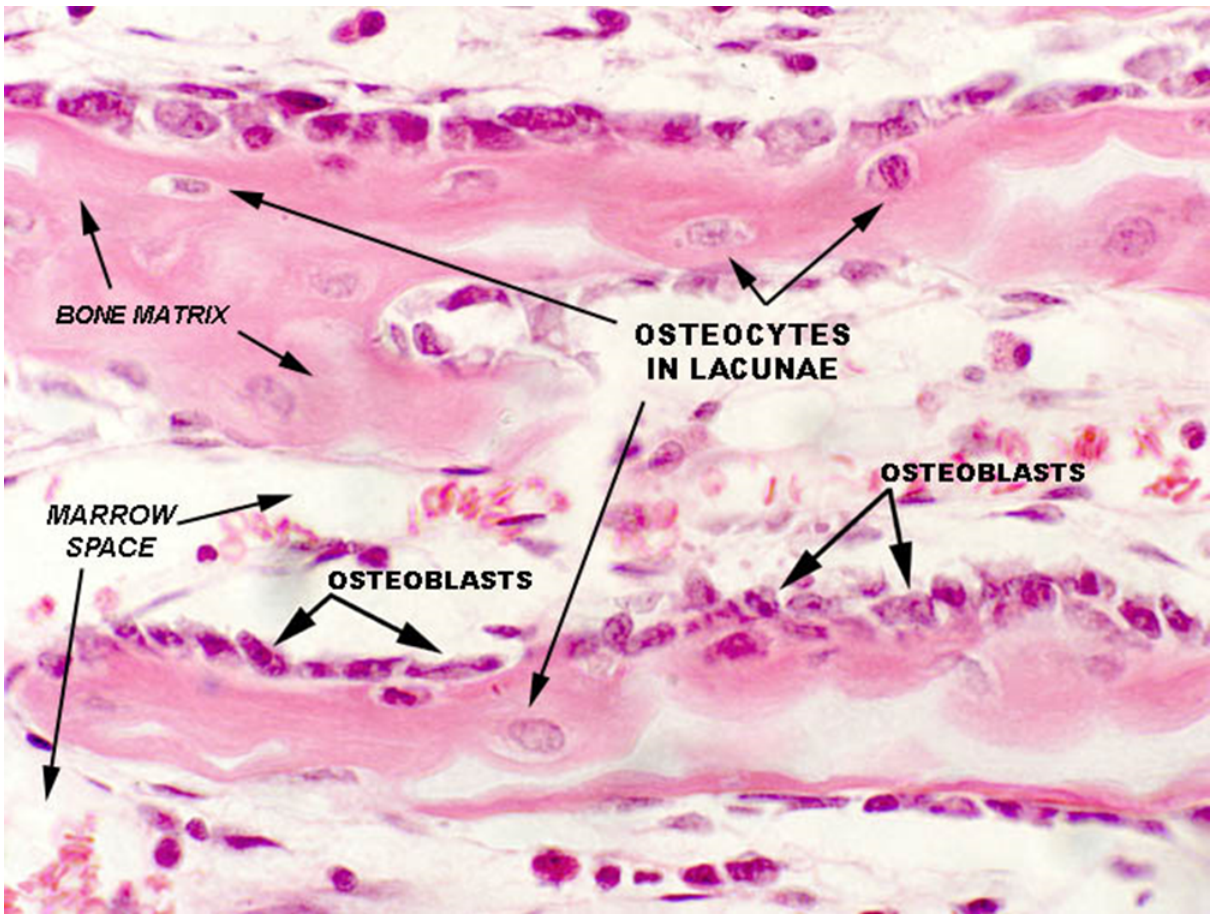


Figure 118. Development of woven bone.

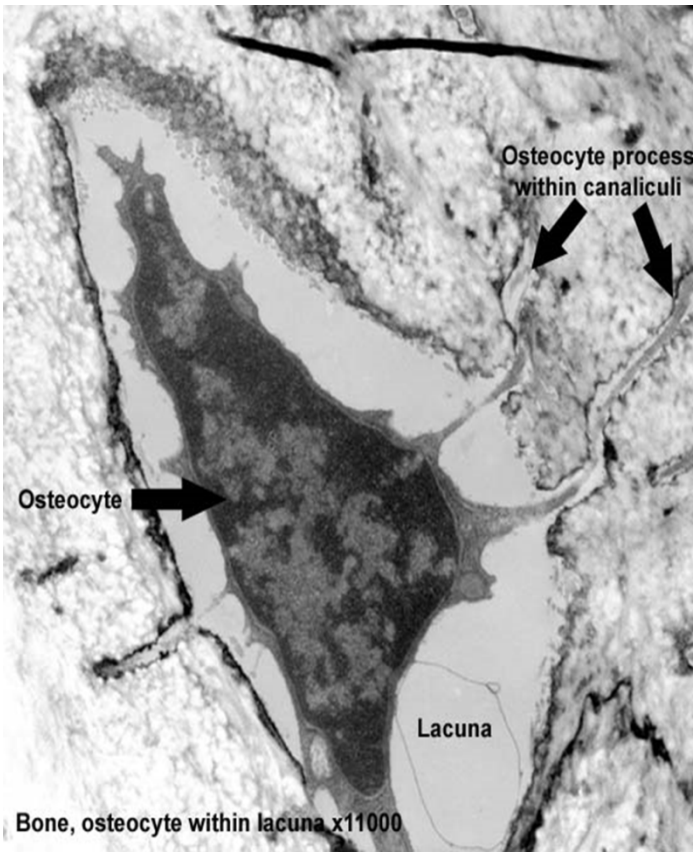


Figure 119. Osteocyte in lacune.

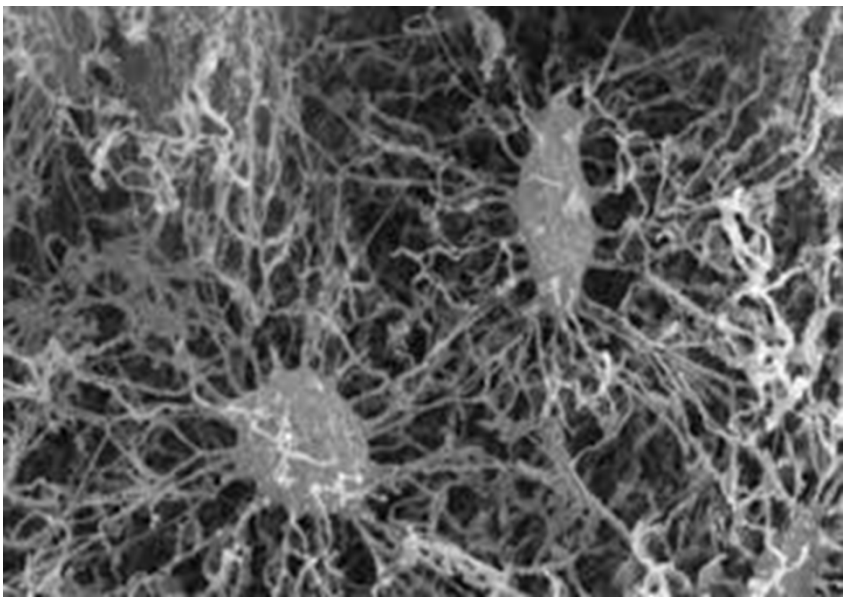


Figure 120. Osteocyte in lacune.

Osteocytes have flattened nuclei and eosinophilic cytoplasm with small amount of rough endoplasmic reticulum and Golgi complex. Function of osteocytes is maintenance of bone matrix.

Osteoclasts (fig. 121-123) are very large, motile, multinucleated, bone- resorbing cells. Osteoclasts are derived from blood monocytes of the blood. They contain 5-50 nuclei and acidophilic, foamy cytoplasm with the high maintenance of lysosomes, mitochondria, well development Golgi complex and some rough endoplasmic reticulum. Osteoclasts lie in a small cavity called lacunae, formed from the digestion of the underlying bone.

Function of the osteoclasts is maintenance of the calcium homeostasis (lysosomal enzymes are released by exocytosis and degrade the organic components of bone).

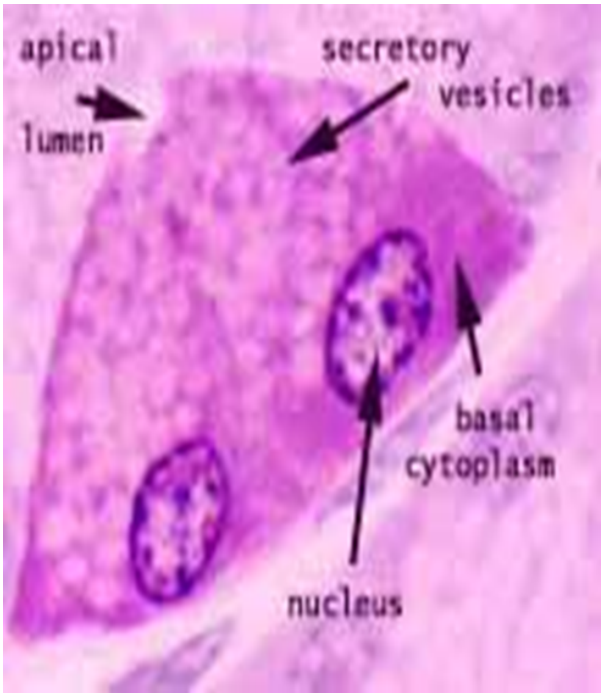


Figure 121. Osteoclasts in lacune.

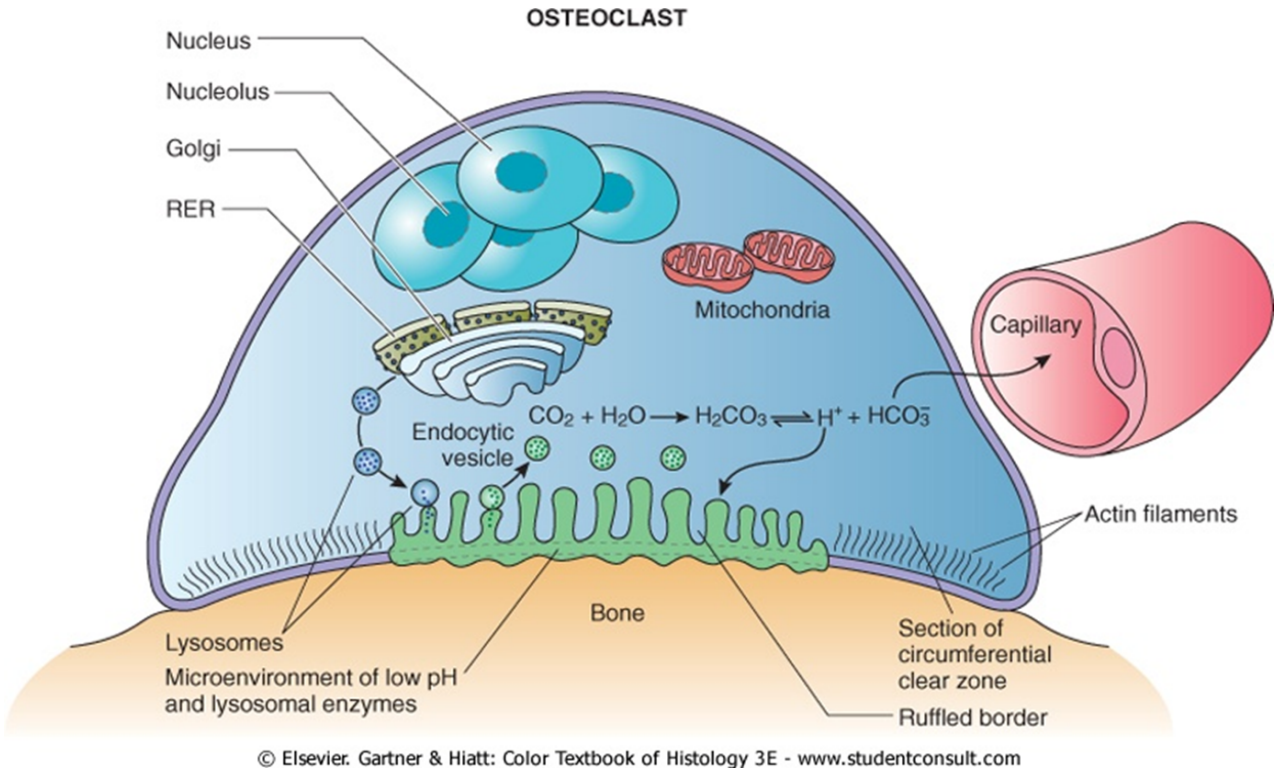
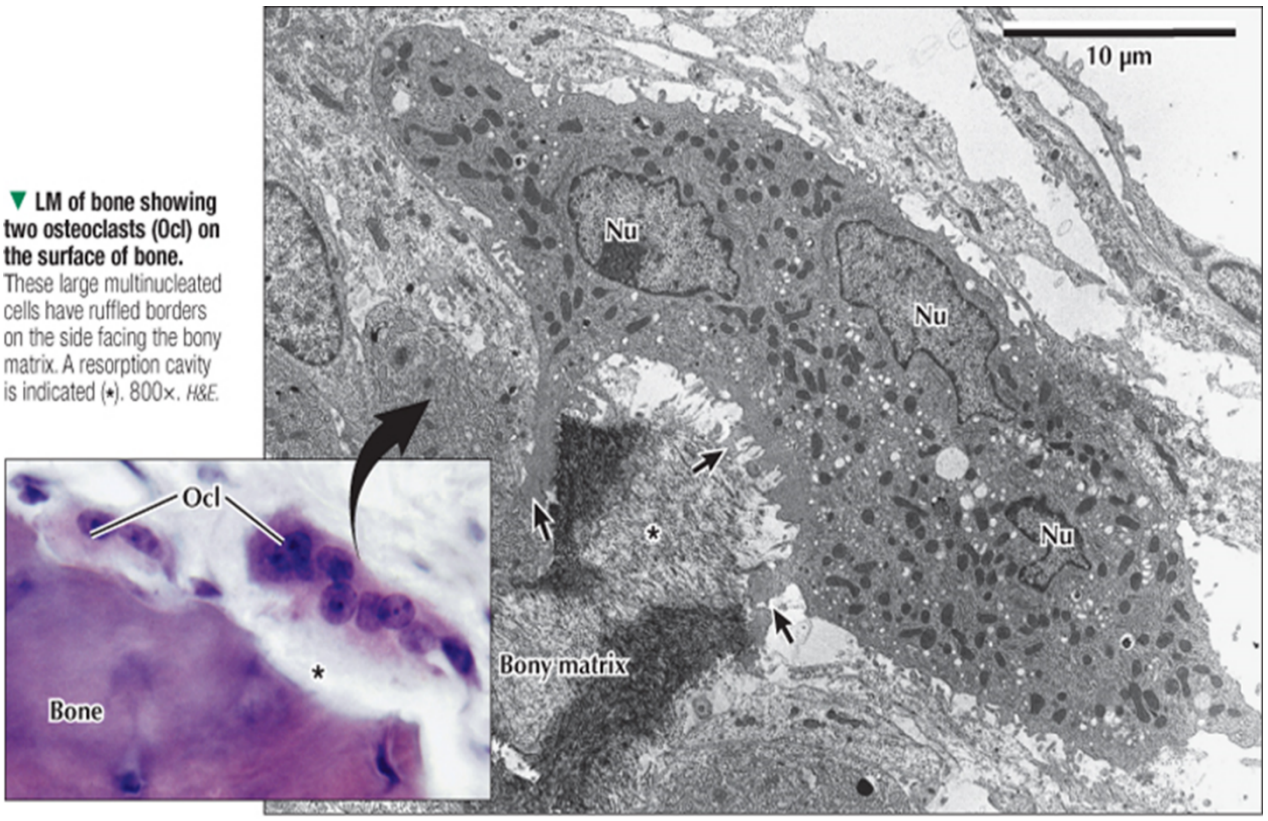


Figure 122. Structure of osteoclasts.

Two hormones affect osteoclastic activity:

- Parathyroid hormone produced by the parathyroid gland that increases osteoclastic activity and results in elevated blood calcium levels.
- Calcitonin produced by the thyroid gland that decreases osteoclastic activity and results in reduced blood calcium levels.



▼ **LM of bone showing two osteoclasts (Ocl) on the surface of bone.** These large multinucleated cells have ruffled borders on the side facing the bony matrix. A resorption cavity is indicated (*). 800×. *H&E.*

▲ **EM of an osteoclast in the process of resorbing bone.** This large cell contains many mitochondria, lysosomes, and vesicles of various sizes, and several nuclei (**Nu**). Its cell membrane has an irregular outline, with a prominent ruffled border (**arrows**) in contact with bone that is being resorbed (*) in Howship lacuna. Parts of the bony matrix under the border are undergoing demineralization and dissolution. 3300×. *(Courtesy of Dr. W. L. Hunter)*

Figure 123. Osteoclasts.

Bone matrix

The bone matrix has two main components:

- **Inorganic component (70%)** is composed mainly of calcium and phosphorus in the form of hydroxyapatite (P04)b(0H)2) crystals.

- **Organic component (30%):** collagen makes up over 90% of the organic component, which is called osteoid; collagen forms collagen fibers.

Bone coverings

- **Periosteum** is the external covering of bone. Periosteum consists of two layers:

1. outer fibrous layer that contains many blood vessels. Branches of the blood vessels penetrate the inner layer of periosteum to enter Volkmann's canals and eventually communicate w'ith the vessels in the Haversian canals;

2. inner cellular layer that contains osteoprogenitor cells that have osteogenic potential.

- **Endosteum** is thin layer of osteoprogenitor cells, osteoblasts and a small amount of connective tissue that lines all internal surfaces of cavities within bone including the Haversian canals and marrow spaces.

Functions of periosteum and endosteum

1. Nutrition of bone tissue
2. Repair or growth of bone
3. Mechanical, supporting (periosteum provides mechanical connection of the bone with tendons and muscles).

Types of bone tissue

Primary, immature, or woven bone

Primary, immature, or woven bone is the first type of bone formed during fetal development, bone repair, and tissue turnover.

Characteristics of primary bone are abundant osteocytes, a low mineral content, and an **irregular organisation of collagen fibers** (fig.124). Woven bone is mechanically weak.

It is temporary and is replaced by secondary bone tissue.

Localization.

- embryo skeleton:
- suture of the flat bones of the skull (in adults),
- tooth sockets (in adults)

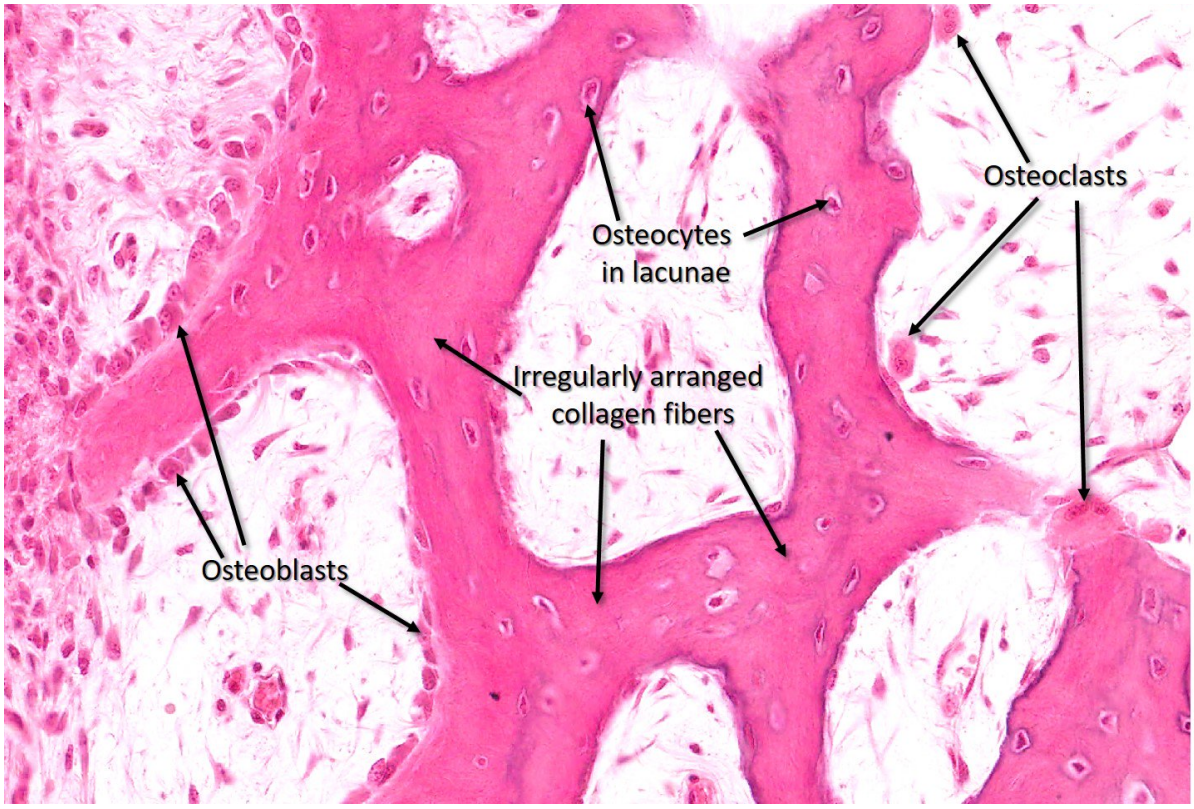


Figure 124. Structure of woven bone.

2. **Secondary, mature, or lamellar bone**

Secondary bone characteristically contains collagen fibers arranged in lamellae that are parallel to each other or concentrically organized around a vascular channel (fig. 125).

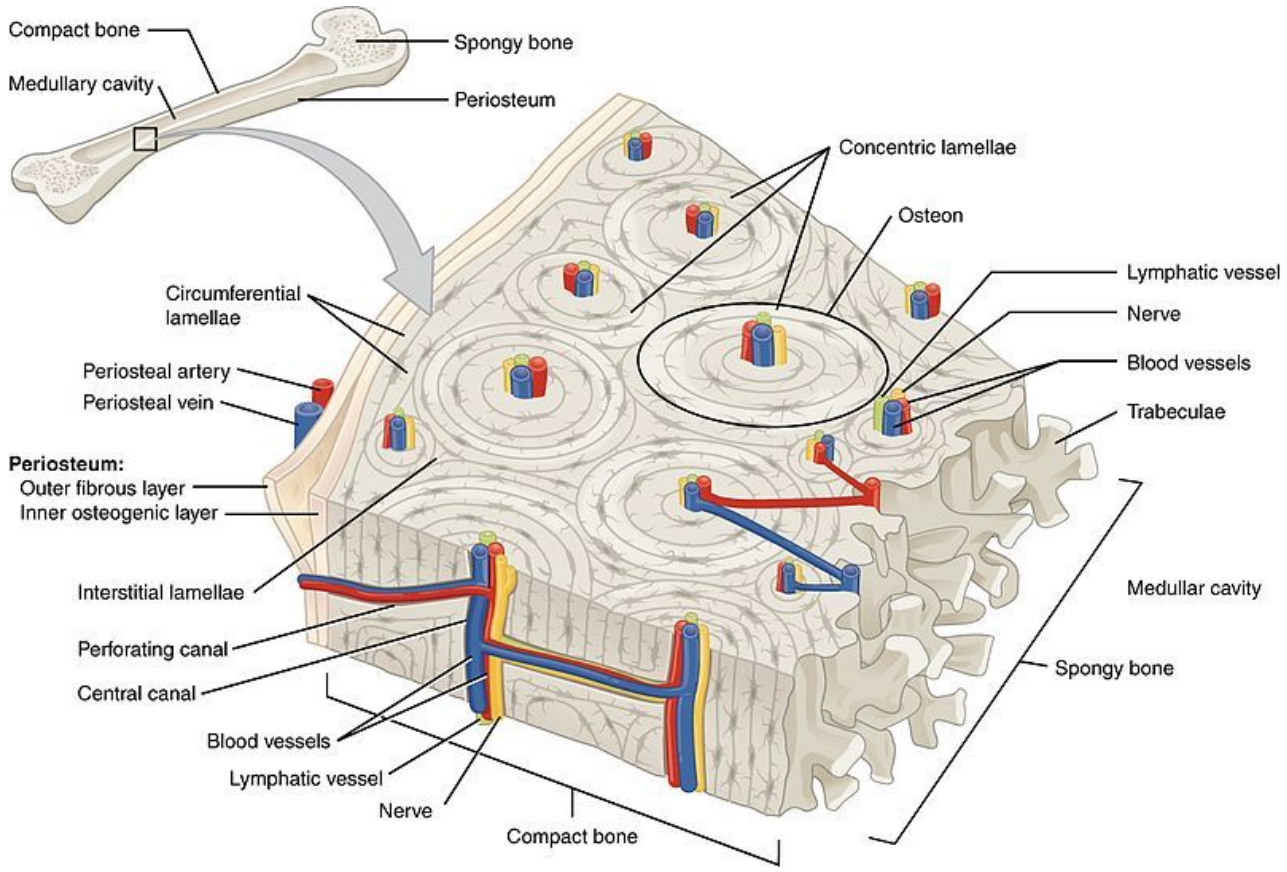


Figure 125. Schematic diagram of structure of secondary bone.

Mineralized matrix of lamellar bone consists of the lamellae, each of which contains collagen fibers that **are parallel** to each other. Fibers of the next **lamellae lay under an angle to each other**. The lamellae contain lacunae housing osteocytes, which are nourished by diffusion of nutrients that travel through canaliculi from the marrow cavity.**Lacunae contain osteocytes**, are found between and sometimes within the lamellae.Canaliculi house cellular processes belonging to osteocytes and permit communication between lacunae and with the Haversian canals. Bone consists of dense areas without cavities - compact hone - and areas with numerous interconnecting cavities - cancellous (spongy) bone.

In long bones, the bulbous ends - **epiphyses - are composed of spongy** bone covered by a thin layer of compact bone. The cylindrical part - **diaphysis - composed of compact bone.**

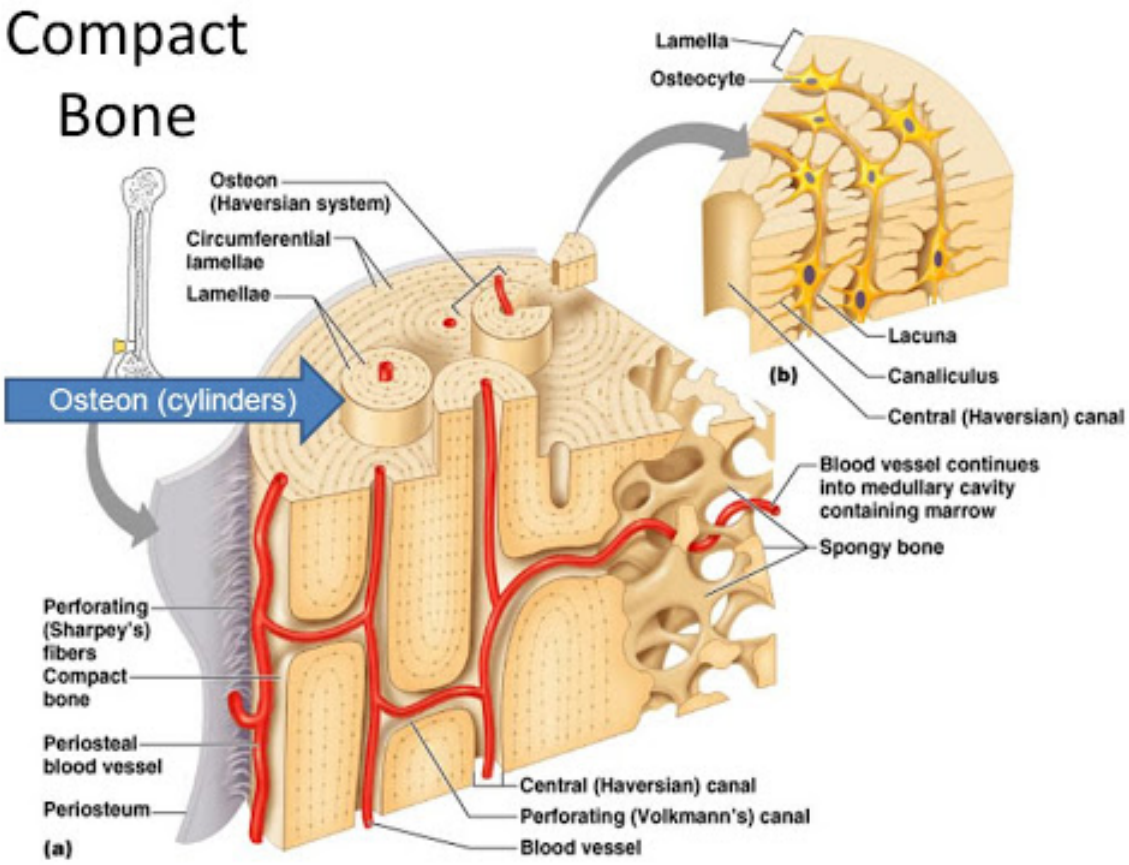


Figure 126. Schematic diagram of structure of secondary compact bone.

Short bones have a core of spongy bone surrounded by compact bone.

Compact bone consists of cylindrical units called osteons or Haversian system (fig.126) comprising of:

1. **Haversian canals** are tubes located in the center of osteons, run parallel to the long axis of the bone, and are united to other canals. Canals contain blood vessels and nerves.

2. Concentric bony lamellae surround the Haversian canal. The lamellae consist of fine collagen bundles in calcified matrix.

3. Osteocytes are located in the lacunae and their processes extend into canaliculi.

4. Volkmann's (perforating) canals are vascular canals containing blood vessels, and connect Haversian canals with periosteum and marrow cavity.

In compact bone (e.g., the diaphysis of long bones) the lamellae exhibit a typical organization consisting of (fig. 125-126):

1. **outer circumferential lamellae** that are deep to the periosteum and form the outermost region of the diaphysis,

2. **haversian systems** (lamellae arranged around an osteonie (Haversian) canals),

3. **interstitial lamellae** that lie between osteons,

4. **inner circumferential lamellae** that completely encircle the marrow cavity.

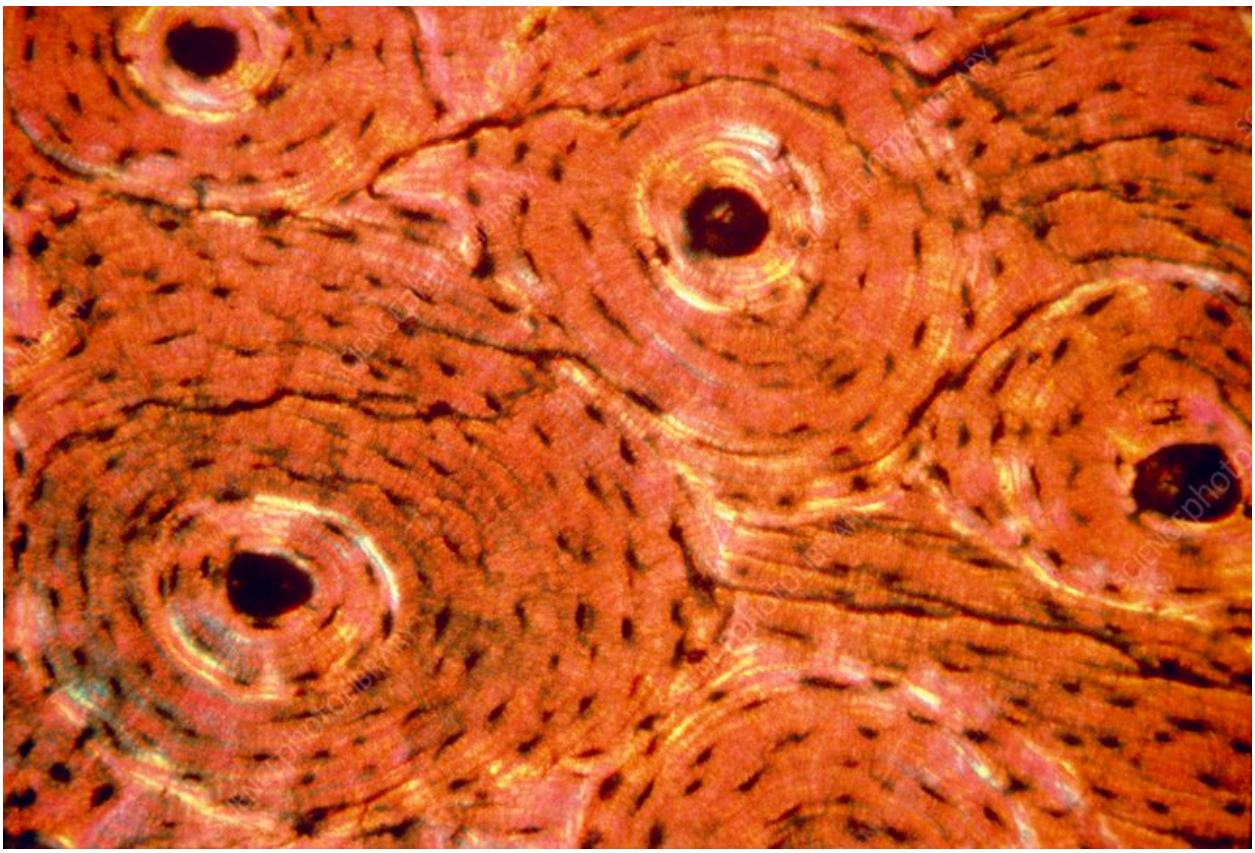


Figure 127. Structure of secondary compact bone.

Spongy bone consists of branching bone trabeculae project out from the internal surface of compact bone into the marrow cavity.

Spongy bone **typically does not contain osteons**. Trabeculae are only a few cell layers thick and **contain irregularly arranged lamellae.**

*Figure 128. Structure of s**pongy** bone.*

Histogenesis of bone (ossification)

Bone can be formed in two ways:

1. by direct mineralization of matrix secreted by osteoblasts (**intramembranous ossification) - development from mesenchyme ,**

2. by deposition of bone matrix on a preexisting cartilage matrix (**endochondral ossification) - development from hyaline cartilage.**

Intramembranous ossification

Intramembranous ossification is responsible for the formation of most flat bones.

Steps in intramembranous ossification (fig. 129) are:

Development of ossification center .

1. mesenchymal stem cells proliferate and aggregate in richly vascularised connective tissue (the primary ossification center), where they differentiate into osteoblasts;

2. osteoblasts secrete bone osteoid, some become surrounded and trapped by the newly formed matrix and are now called osteocytes.

3. calcification:

- osteoid is calcifies to form spicules of spongy bone;
- inorganic salts carried in by the blood vessels;
- salts are deposited in an orderly fashion as fine hydroxyapatite crystals intimately associated with the collagen fibers;collagen fibers in the developing spicules are randomly oriented (primary bone); remaining connective tissue among the spicules is penetrated by growing blood vessels and the undifferentiated mesenchymal cells give rise to bone marrow cells.

-

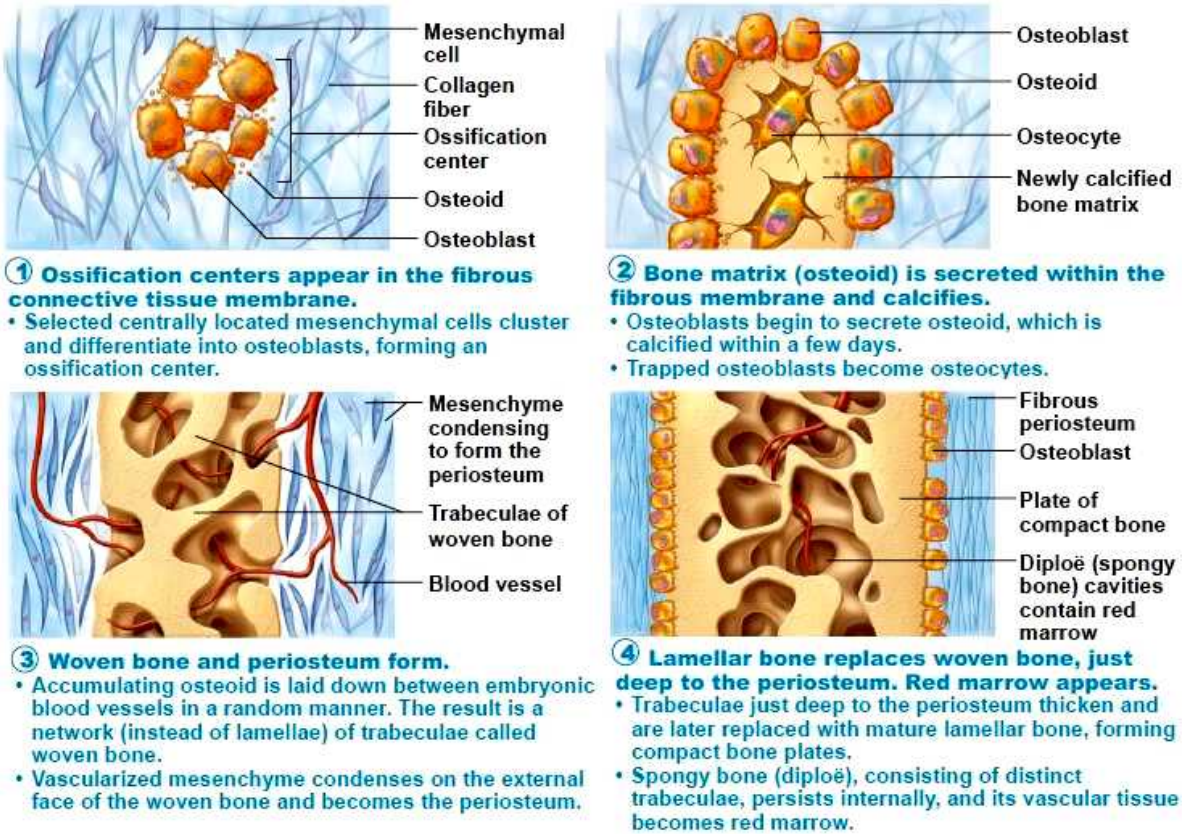


Figure 129. Intramembranous ossification.

4. **Formation of trabeculae:.**

- spicules unite to form trabeculae;
- ossification centers grow radially and finally fuse replacing the original connective tissue.

5. **Development of periosteum**.

- portion of bone that does not undergo ossification becomes the periosteum and endosteum. Later, there is a partial replacement of the woven bone with a lamellar compact bone. Osteoclasts migrate along the blood vessels and destroy the woven bone and the osteoblasts in its place build a plate consisting of osteons.

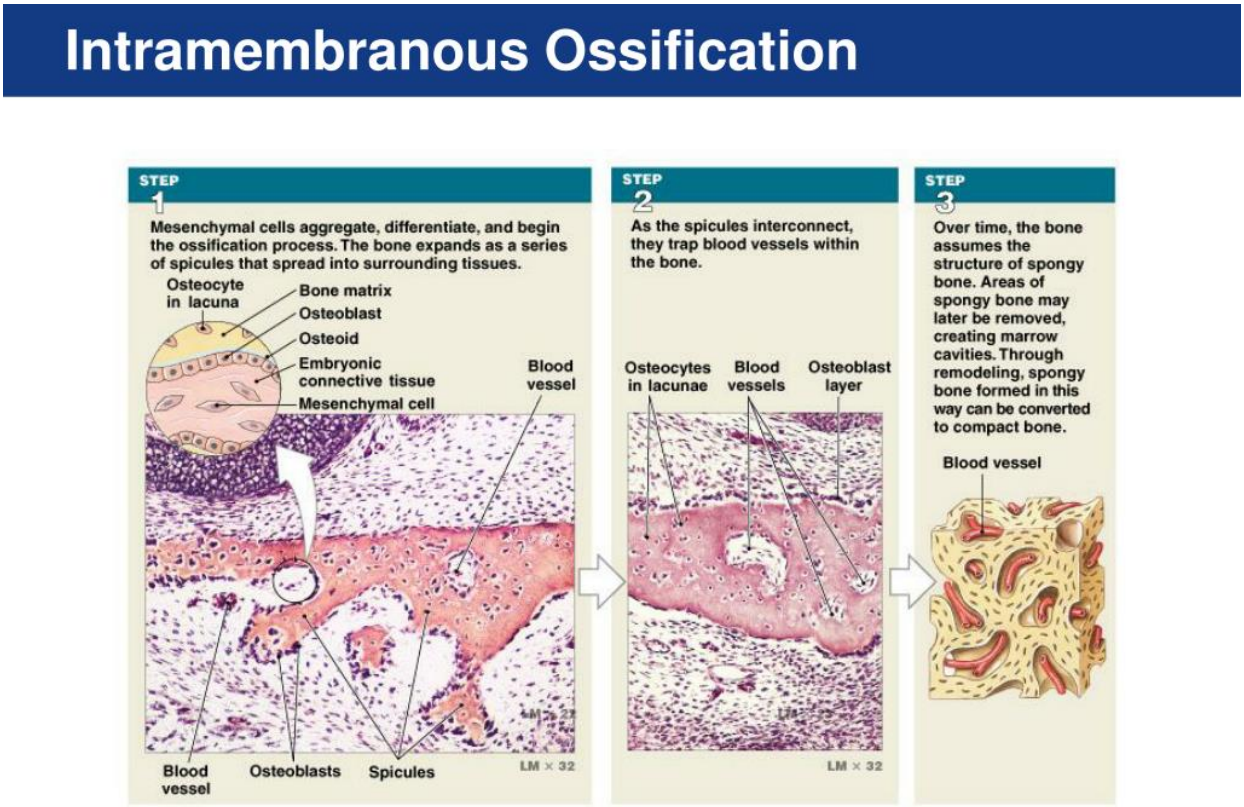


Figure 130. Intramembranous ossification.

Endochondral ossification

Endochondral ossification is responsible for the formation of short and long bones.

This process begins with a hyaline cartilage model whose shape resembles a small version of the bone to be formed.

Steps in endochondral ossification (fig. 131) are:

1. Development of cartilage model.

-process of endochondral ossification begins with a hyaline cartilage model whose shape resembles a small version of the bone to be formed.

-Growth of cartilage model

2. **Development of the** primary (diaphyseal) ossification center:

-formation of a thin woven **bony collar (periosteal collar) around the diaphysis** by intramembranous ossification;

- perichondrium of the template becomes the periosteum;

-invasion of the diaphysis by blood vessels that carry osteoprogenitor cells from periosteum that mature into osteoblasts;

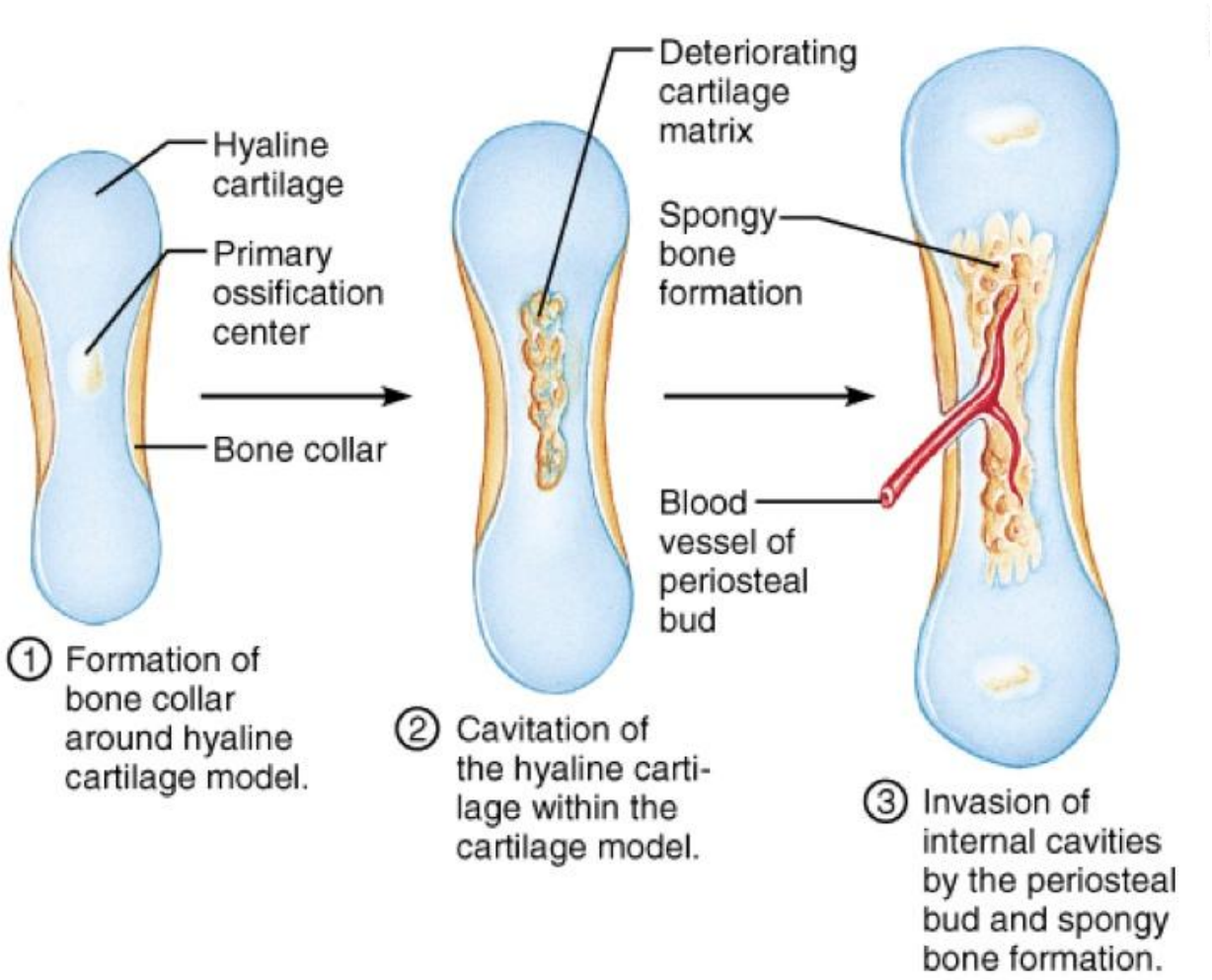


Figure 131

- osteoblasts secrete osteoid, and cartilage matrix begins to calcify;
- chondrocytes hypertrophy and die (because there is no diffusion of nutrients across the bone matrix;
- osteoclasts form a primary marrow cavity, and incoming blood vessels carry in bone marrow cells;
- compact bone is formed.

3. **Development of the secondary (epiphyseal) ossification center.**

- blood vessels infiltrate the epiphysis;
- chondrocytes of the epiphysis hypertrophy and die upon ossification;
- osteoblasts start building trabecular bone.

4. Formation of articular cartilage and epiphyseal plate . Cartilage remains in two places:

- articular cartilage: hyaline cartilage covering joint surfaces that remains throughout life;
- epiphyseal plate: the cartilage of the epiphyseal plate continues to grow and is continuously replaced by newly formed bone matrix resulting in elongation of bone.

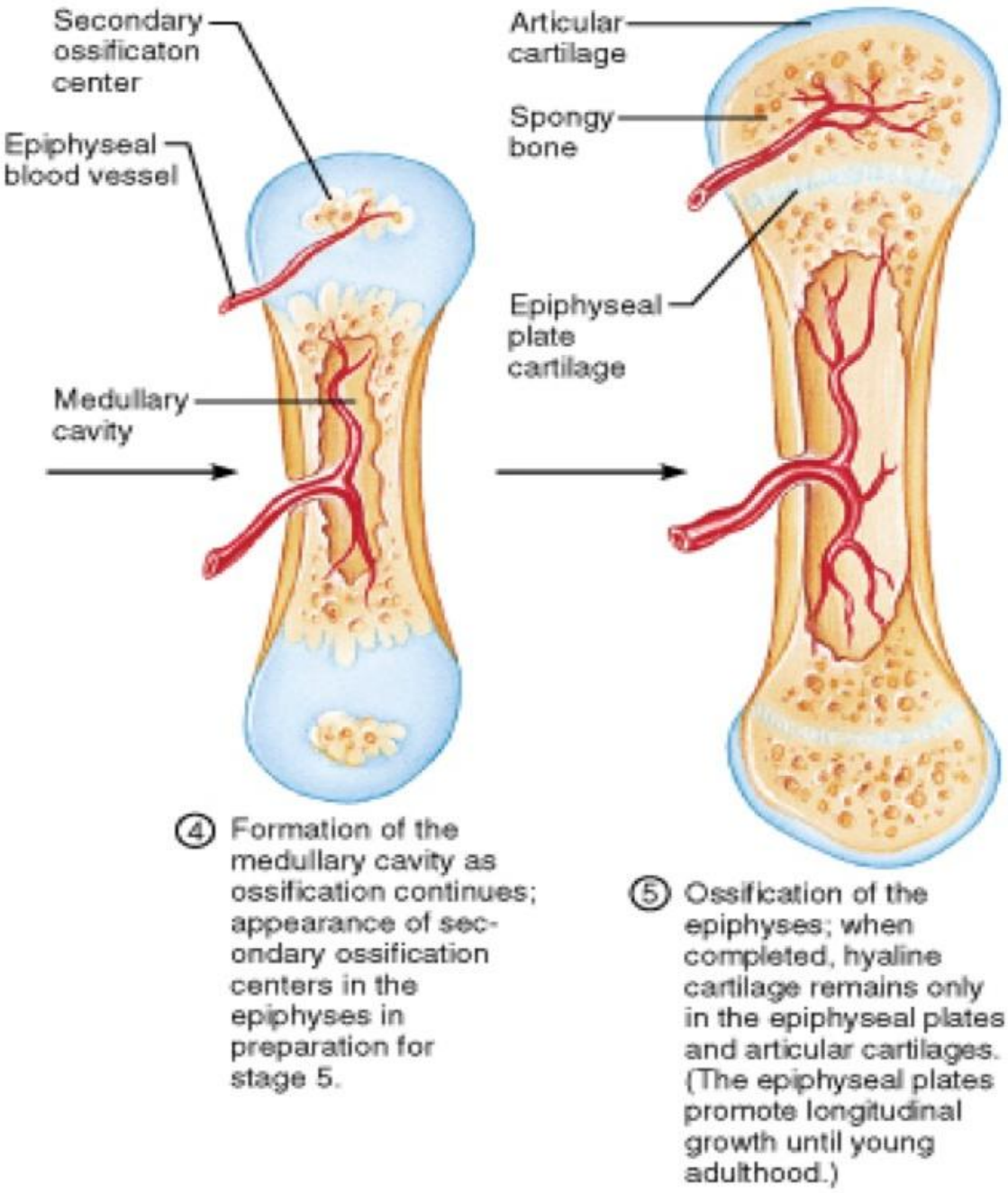


Figure 132. Endochondral ossification.

The epiphyseal plate is divided into zones (fig. 133):

1. resting zone: hyaline cartilage without morphological changes;

2. zone of proliferation: chondrocytes dividing rapidly that from columns of stacked cells parallel to the long axis of the bone;

3. zone of maturation / hypertrophy: large chondrocytes whose cytoplasm has accumulated glycogen and narrow areas of matrix between lacunae;

4. zone of ossification: osteoprogenitor cells invade the area and differentiate into osteoblasts, which secrete bone matrix onto the calcified cartilage matrix. Chondrocytes here die when they can no longer receive nutrients via diffusion. This is because the calcified matrix is much less hydrated than hyaline cartilage.

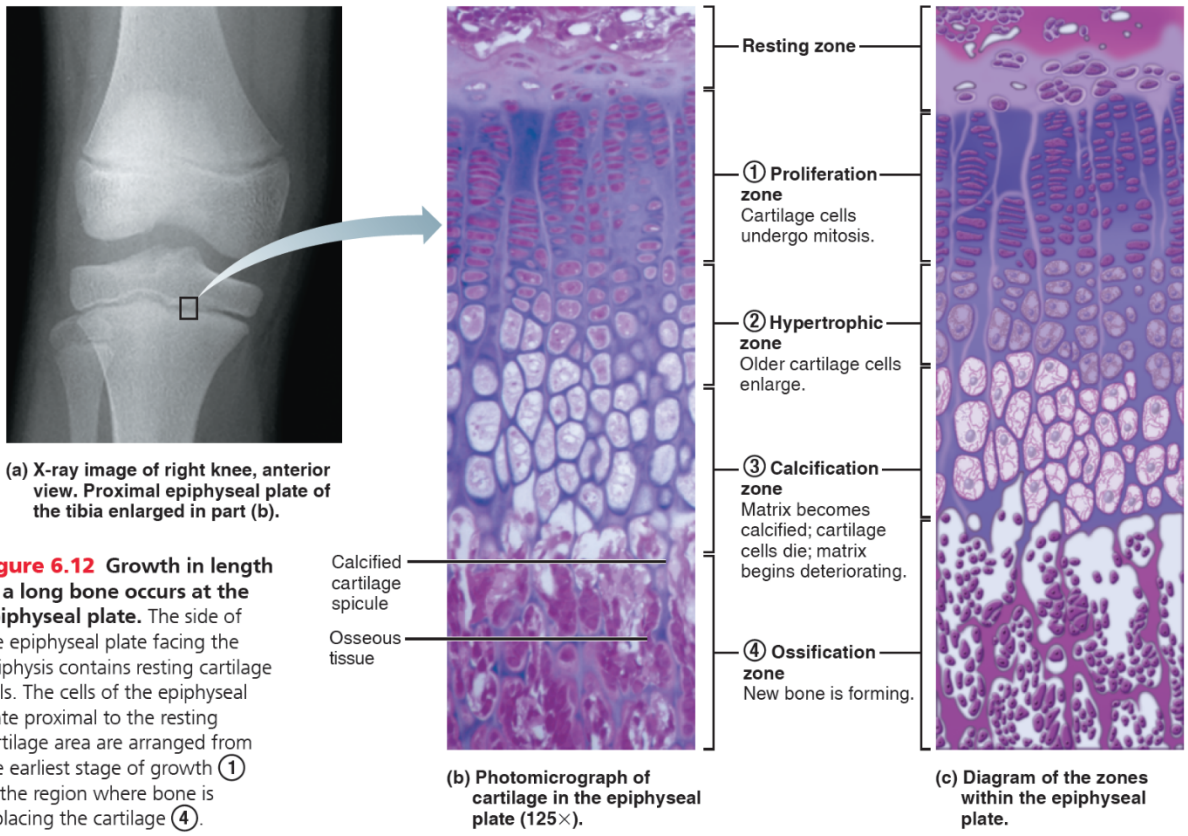


Figure 6.12 Growth in length of a long bone occurs at the epiphyseal plate. The side of the epiphyseal plate facing the epiphysis contains resting cartilage cells. The cells of the epiphyseal plate proximal to the resting cartilage area are arranged from the earliest stage of growth ① to the region where bone is replacing the cartilage ④.

Figure 133. Zones of the epiphyseal plate.

Bone growth

Bone length is dependent upon the activity that occurs in the epiphyseal plate. Bone growth stops when the cartilage of the epiphyseal plate ceases proliferation and bone development continues to unite the diaphysis and epiphysis.

An increase in bone width occurs by a process called appositional growth. Bone is produced by the periosteum (intramembranous ossification) on the external surface of the bone collar, and at the same time bone is removed from the internal surface causing the marrow cavity to increase in size.

During infancy and childhood the most important stimulus of epiphyseal plate activity is growth hormone, which is released from the anterior pituitary gland. Excessive amounts of growth hormone result in excessive height (pituitary gigantism) and deficits of growth hormone result in diminished height (dwarfism).

Normal bone growth is dependent on proper dietary intake of protein, minerals and vitamins. A deficiency of vitamin D prevents calcium absorption from the GI tract resulting in rickets (children) or osteomalacia (adults). Osteoid is produced but calcium salts are not deposited, so bones soften and weaken.

Bone remodeling. In a growing person bone deposition exceeds bone resorption.

In adulthood after the closure of the epiphyseal plates, bone deposition is balanced with bone resorption.

- Osteons are replaced by osteoprogenitor cells and osteoblasts from the periosteum.
- Trabeculae are replaced by osteoprogenitor cells and osteoblasts from the endosteum.
- Bone resorption is accomplished by osteoclasts.
- If bone resorption exceeds bone deposition then osteoporosis will occur.

Fracture repair The bone matrix is destroyed and the bone cells adjoining the fracture die.

The damaged blood vessels form a blood clot.

The blood clot, damaged bone matrix, and dead cells are removed by macrophages.

Granulation tissue forms in the site of the blood clot and condenses into connective tissue and later into a fibrocartilaginous callus.

At the same time, osteoprogenitor cells of the periosteumare activated and become osteoblasts that begin to deposit new bone. The new bone, which is a meshwork of trabeculae of primary bone, forms a bone callus around the fracture site.

A similar activation of cells of the endosteum results in deposition of bone around the fibrocartilaginous callus that is slowly eroded away and replaced by bone (endochondral ossification).

The spongy bone uniting the bones is transformed into compact bone by osteoblastic deposition of bone matrix, which gradually obliterates the spaces among the trabeculae.

Resorption of excess bone by osteoclasts reestablishes the marrow cavity and the normal surface contours of the bon

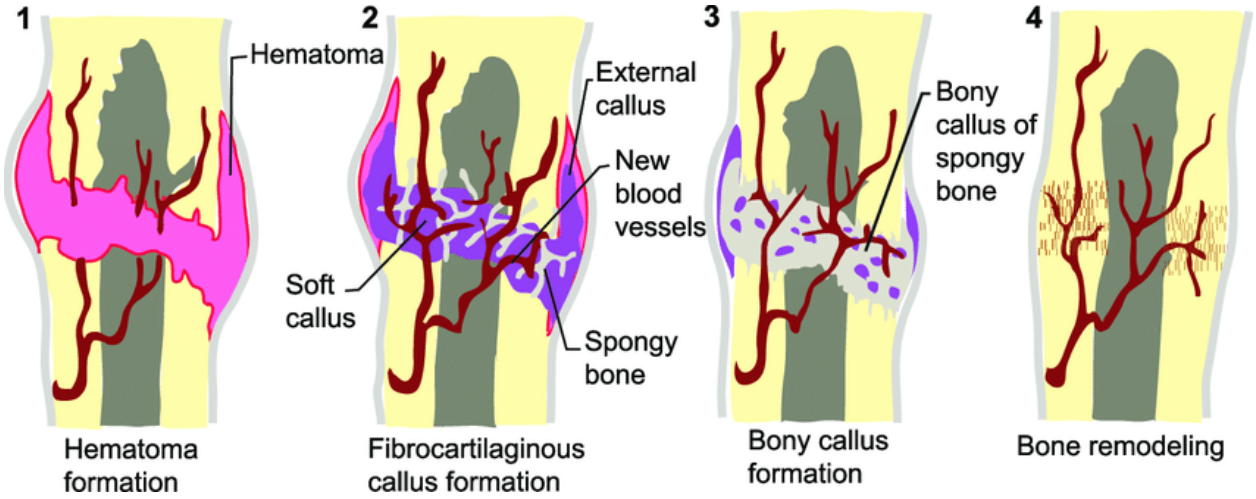
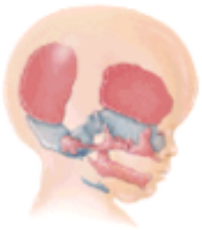


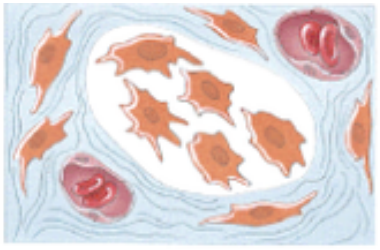
Figure 134. Fracture repair.

Practical lessons № 13

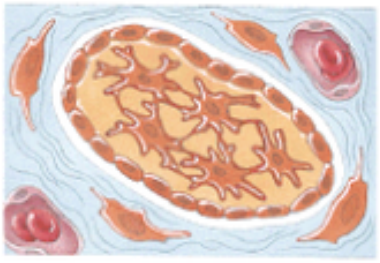
Questions for self-control.

1. Morphofunctional characteristic of osteoblasts.
2. Morphofunctional characteristic of osteocytes.
3. Morphofunctional characteristic of osteoclasts.
4. Osteon as a morphofunctional unite of compact bone. Structure of the dyaphysis.
5. Steps in intramembranous ossification.
6. Endochondral ossification.
7. The epiphyseal plate.
8. Growth and regeneration of bones.

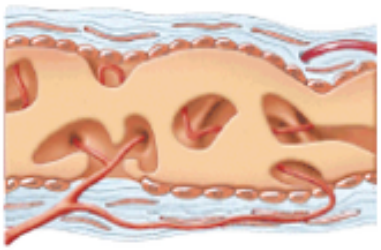




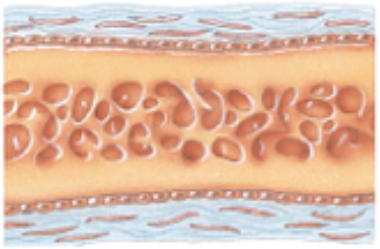
1



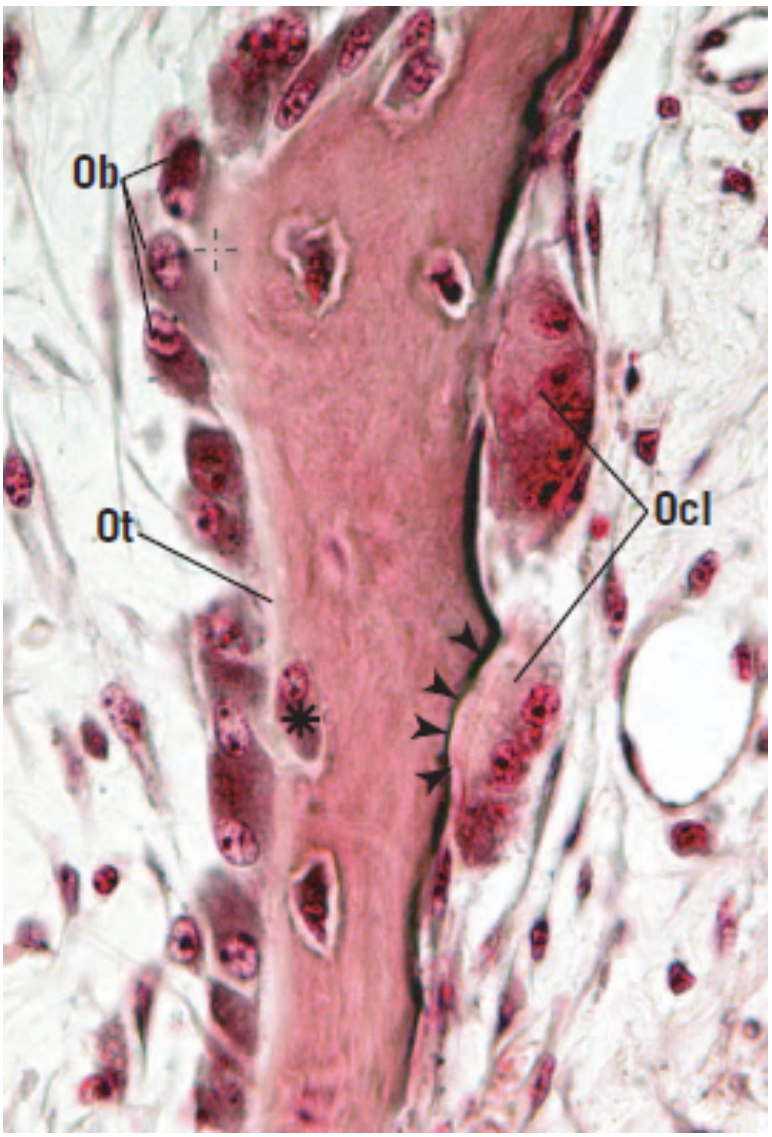
2

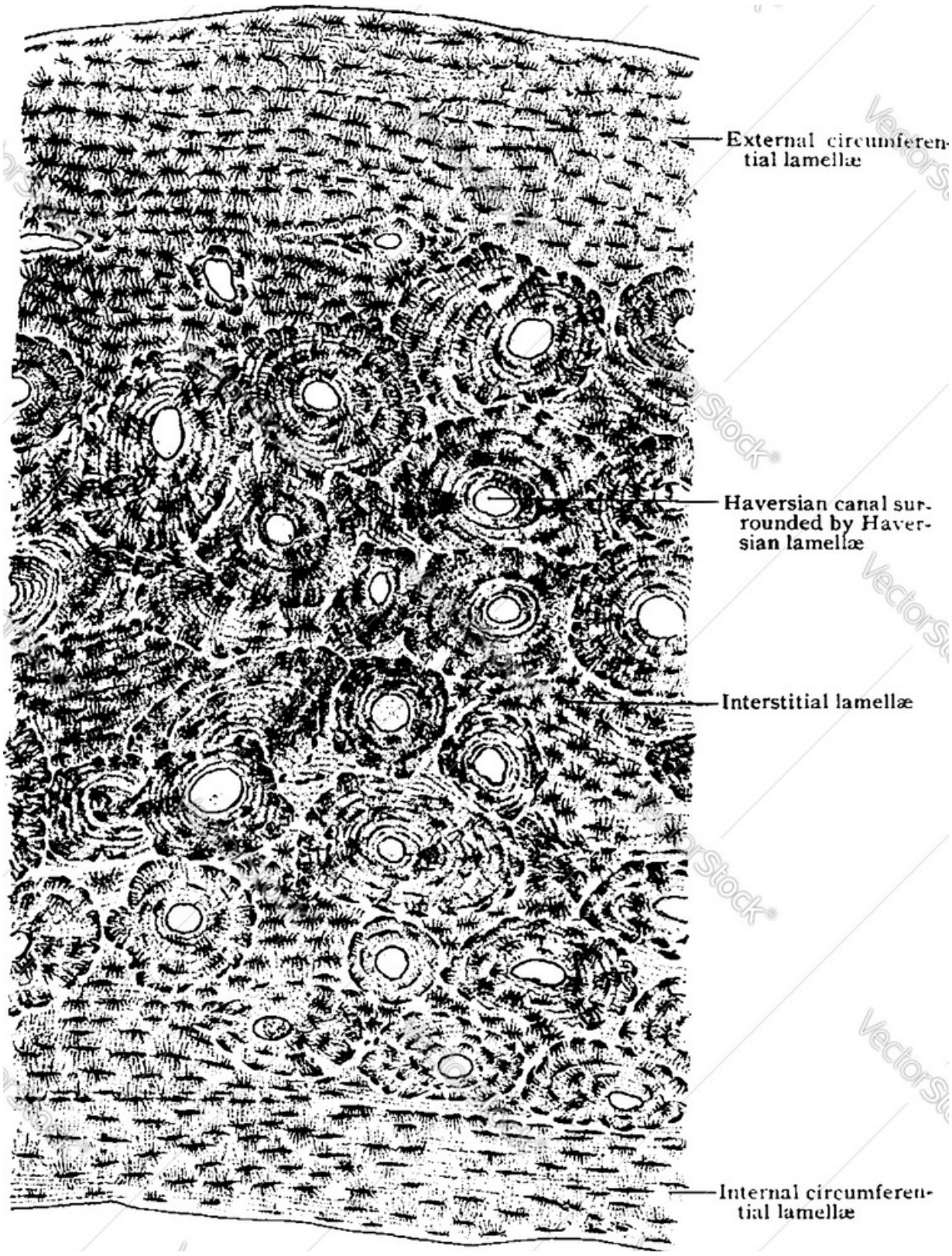


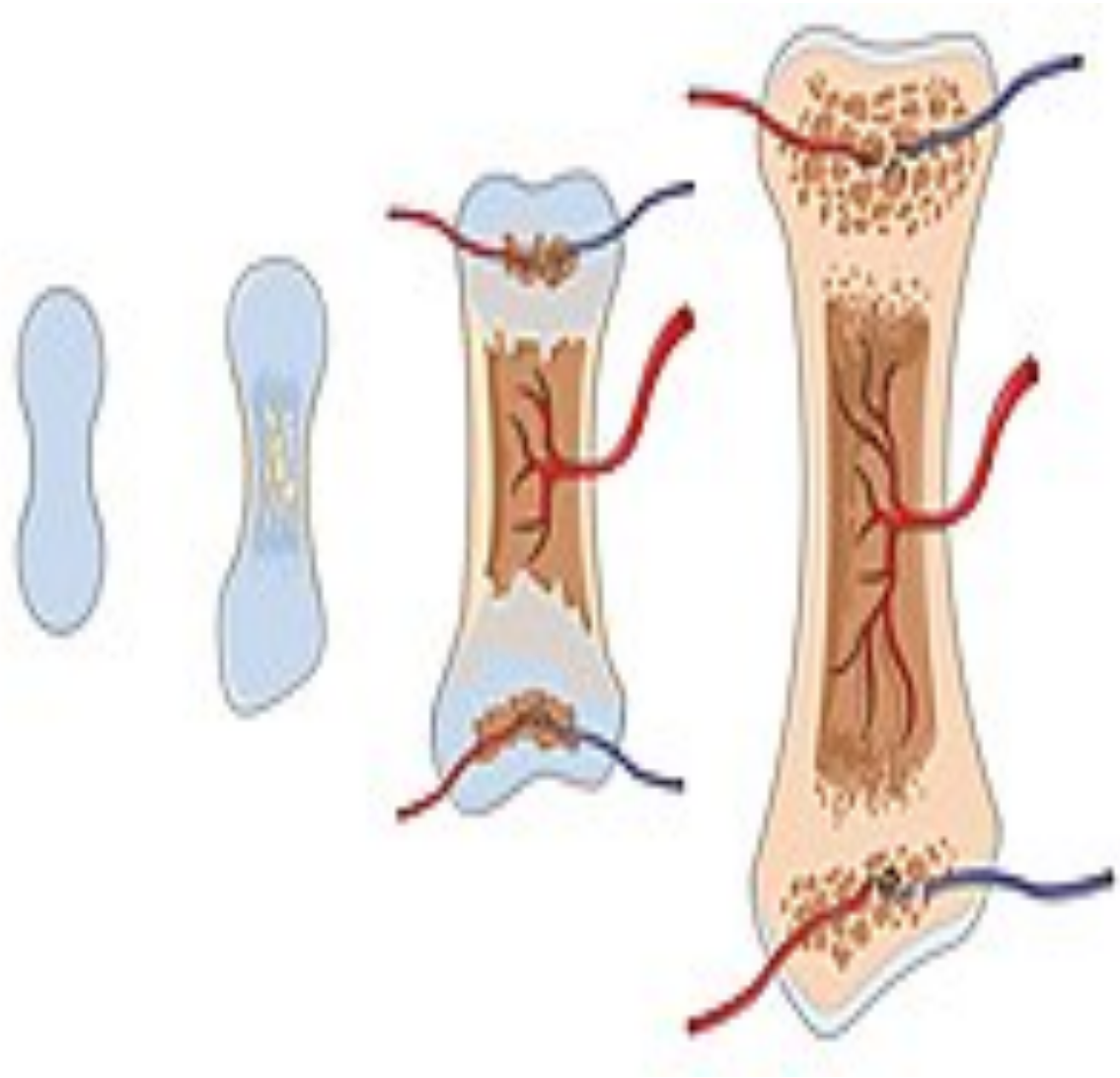
3

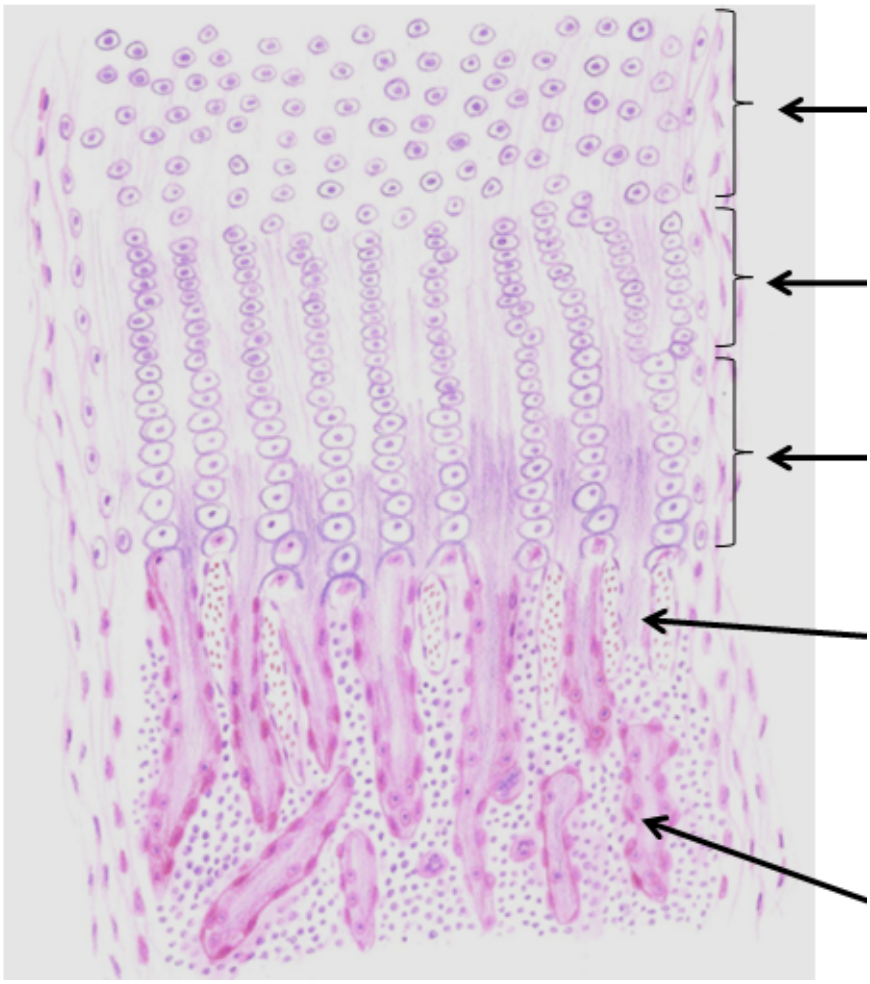


4


Ob
Ot
Ocl


External circumferential lamellæ
Haversian canal surrounded by Haversian lamellæ
Interstitial lamellæ
Internal circumferential lamellæ





The teacher's signature:

MUSCLE TISSUES

Muscle tissues represent group of tissues of a various origin and a constitution, united on the basis of the common sign - contractile ability. Cytoplasm of muscle cells and muscle fibers contain actin and myosin, two contractile proteins that generate force and produce movement.

The function of muscle tissue is to contract and thus produce motile forces that have two general consequences:

- movement and support of body parts,
- transport of materials within the body.

The cytoplasm of muscle cells is called **sarcoplasm**, the smooth endoplasmic reticulum is called **sarcoplasmic reticulum**, the cell membrane, or plasmalemma is called **sarcolemma**.

Classifications of the muscle tissues

1. Morphofunctional classification (based on the structure and function of the muscle tissue)

1. Striated muscle tissues
   - skeletal (somatic, voluntary) muscle;
   - cardiac muscle;
2. Smooth (visceral) muscle

2. Histogenetic classification (based on the origin of muscle tissue)

1. muscle tissue of somatic type arises from myotomes of somites; forms a skeletal muscle, is cross-striated;
2. muscle tissue of coelomic type arises from the cardiogenic area of splanchnopleuric mesoderm; forms a cardiac muscle (myocardium), is cross- striated;
3. muscle tissue of mesenchymal type arises from a mesenchyme, forms a musculature of an internal organs and vessels, is smooth;
4. muscle tissue of ectodermic type arises from ectoderm, forms myoepithelial cells located in the alveoli of exocrine glands and muscles of the iris.

Skeletal muscle tissue

Skeletal muscle is a form of striated muscle tissue existing under control of the somatic nervous system.

Function of skeletal muscle tissue is the movement of the skeleton and organs. Skeletal muscle tissue forms skeletal musculature, tongue, pharynx upper 2/3rd of esophagus, anal canal, and lower part of vagina.

Skeletal muscle tissue is made up of individual components known as muscle fibers or **myosymplasts**.

Muscle fibers are morphofunctional unit of the skeletal muscle. Muscle liber is very long (up to 30 cm) cylindrical **multinucleated** fiber with a diameter of 10-100 pm. **Nuclei** are oval in shape, **reside along the cell periphery**. Each muscle fiber is surrounded by a basal lamina.Skeletal muscle fibers are multinucleate structures that arise by fusion of mononucleate myoblasts.

Components of the muscle fiber.

1. Myosymplasts,
2. Mononucleate satellite cells associate with the muscle fiber and reside within the muscle basal lamina (fig.135).

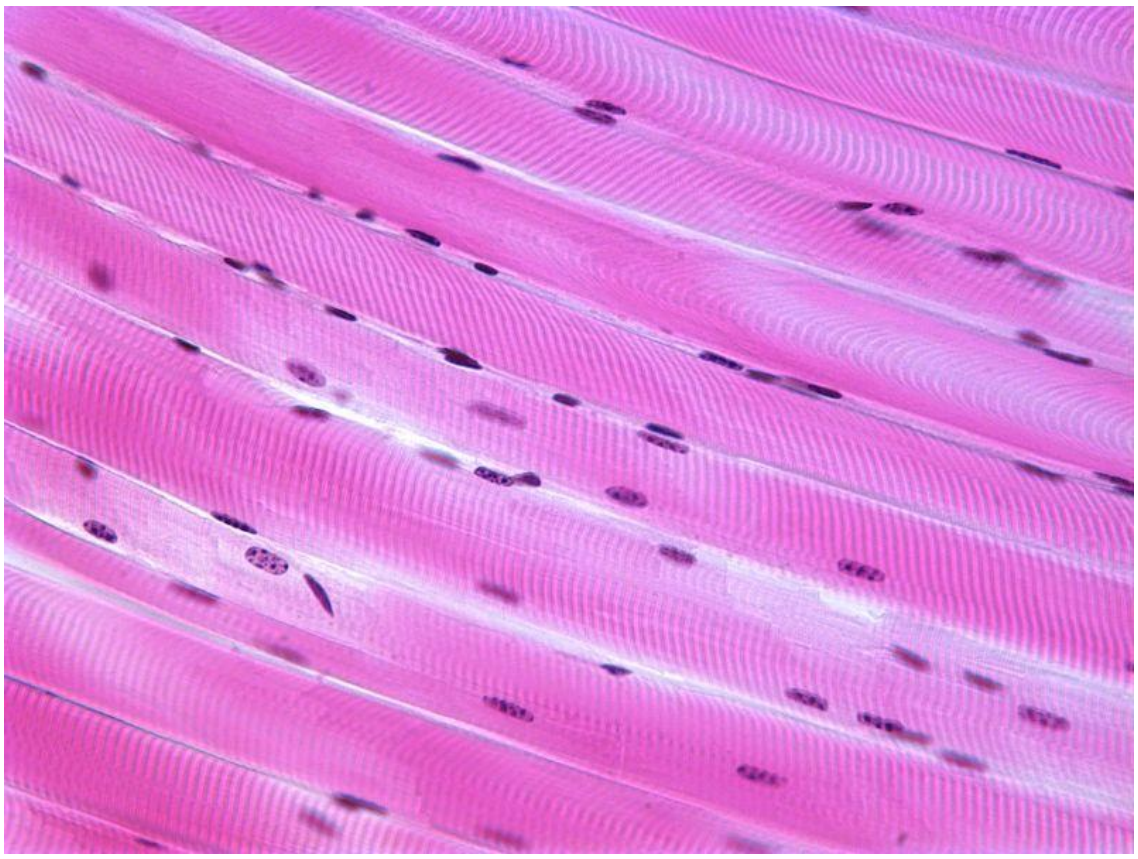


Figure 135. Skeletal muscle.

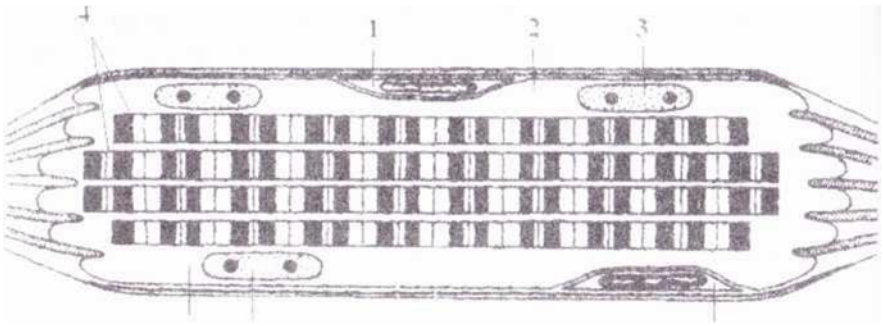


Figure 136. Schematic diagram of skeletal muscle fiber. 1 - satellite cells, 2 - sarcoplasm, 3 - nuclei of myosymplast, 4 - myofibrils

The **sarcoplasm** of a myosymplast contains all general organelles (except for centrioles) and some special organelles, and also inclusions. These structures form functional systems.

1. contractile system,
2. conducting system,
3. supporting system,
4. system of energy production.

Contractile system

Muscle fibers contain many myofibrils, running parallel to one another and to long axis of the muscle fibers.

Myofilaments are polymers of two types:

- **thick filaments**, which are composed principally of **myosin**, and
- **thin filaments**, which are composed of **actin,** thin filaments also contain other proteins including tropomyosin and troponin, which regulate contraction. The registered packing of many

myofilaments produces the myofibril, which shows the striations that are visible by light and electron microscopy (fig. 137).

Arrangement of thin and thick filaments in a myofibril. Each skeletal muscle fiber exhibits along its length alternate dark bands (anisotropic or A-bands) and light bands (isotropic or l-bands) (fig. 138).

The sarcomere is the basic contractile unit of a myofibril. Each myofibril contains many sarcomeres in tandem.

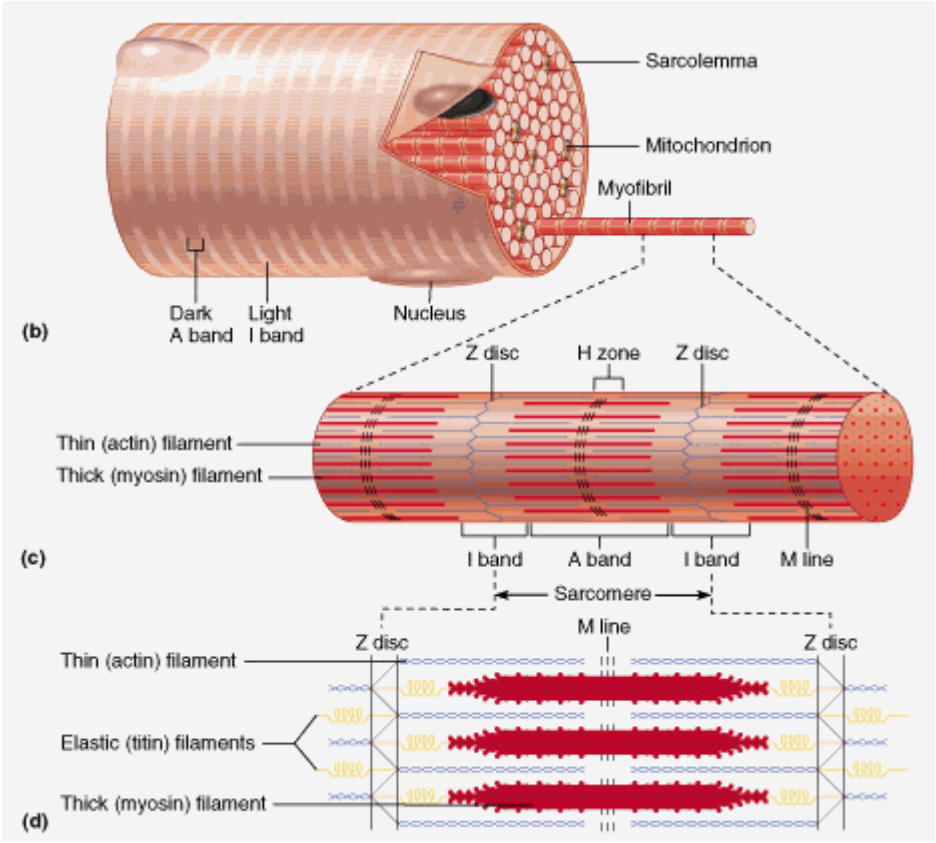


Figure 137. Skeletal muscle fibers.

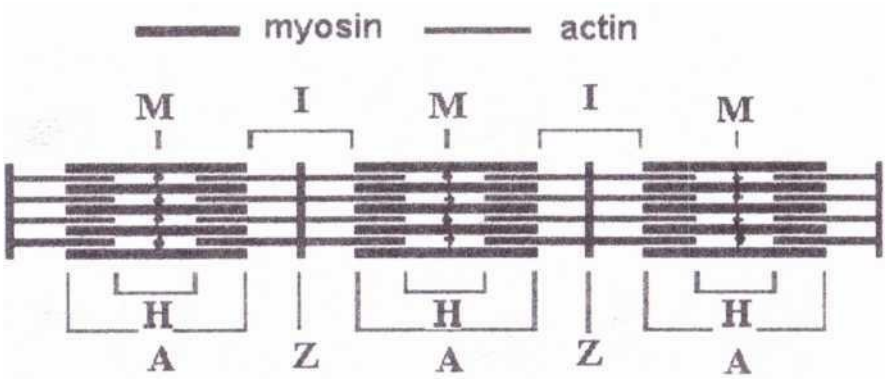


Figure 138. Diagram of contractile system of skeletal muscle fiber. M-M-line, Z-Z-line, A-anisotropic or A-band. I - isotropic or 1-band

The **thick filaments occupy the A-band**, the central part of the sarcomere.
The **thin filaments run between** and parallel to the thick filaments.
One of their ends attached to the Z- line.

A- band has two parts:

-central part (H band) containing only myosin (thick) filaments: they are held together in the M- line situated in the center of H- band,

-lateral parts containing both thick and thin filaments.

I-band consists of only actin (thin) filaments; the free ends of actin filaments extend into outer part of A- band.

In cross section **6 thin filaments surround only 1 thick filament**.

Muscle contraction results from the coordinated contraction of the sarcomeres. The contraction of sacromeres is mediated by the relative movement (sliding) of the thick and thin filaments.

Regulation of contraction

Contraction in skeletal muscle is regulated by the activities of two membrane systems (conducting system) (fig. 139).

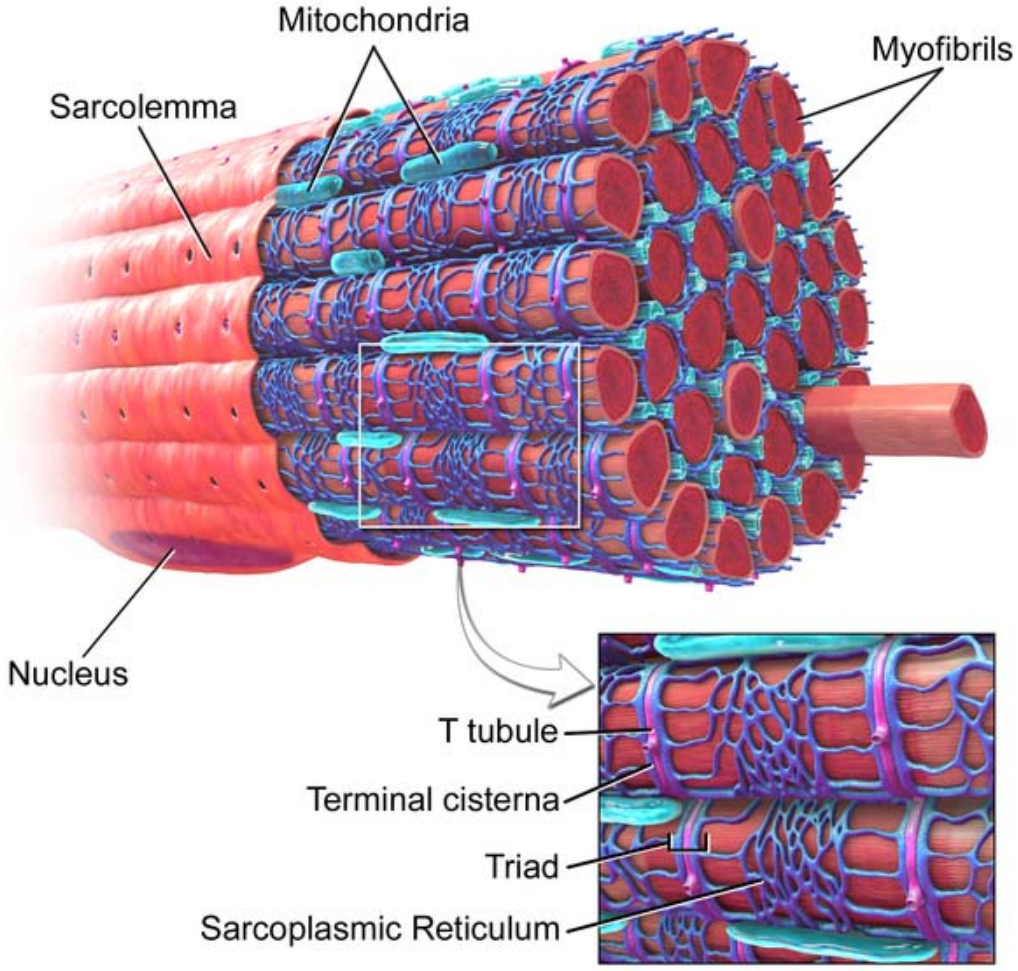


Figure 139. Diagram of conducting system of skeletal muscle fiber.

- **T-tuhules** (or transverse tubules) are transverse, deep tubular invaginations of the plasma membrane which surround myofibrils at the junction of the A and I band. They are responsible for synchronous contraction of all sarcomeres.

- **The sarcoplasmic reticulum (SR)** is a large membrane network derived from ER that surrounds each myofibril between the T-tubules.

-**The terminal cisternae** are flat regions of the SR adjacent to the T- tubules. The SR serves as a calcium store.

T tubules together with two cytoplasmic cisterns form triads.

Nerve-induced depolarization propagates into the cytoplasm by the T- tubule system inducing release of calcium from the terminal cisternae of the sarcoplasmic reticulum; this induces contraction through interactions with troponin, a regulatory' protein associated with the thin filaments. Subsequent uptake of calcium by the SR, in turn, results in relaxation.

Supporting system of a muscle fiber includes the special elements of cytoskeleton, providing locating of myofibrils inside a fiber, and also a sarcolemma connected to them.

System of energy production includes

-mitochondria, which produce ATP, necessary for exercise of muscle work, and also

-trophic inclusions (glycogen) serve as a depot of energy that is mobilized during muscle contraction.

Mitochondria in a myosymplast settle down as chains under a sarcolemma and between myofibrils.

Histogenesis of skeletal muscle (myogenesis)

Skeletal muscle arises from the paraxial mesoderm that is present either side of the neural tube in two wide strips of loose unconnected cells or mesenchyme.

Skeletal myogenesis proceeds through three stages :

- determination of precursor muscle cells, called myoblasts;
- proliferation and in some cases migration of myoblasts; and
- differentiation of myoblasts into mature muscle.

Somites are collections of embryonic mesodermal cells, some of which become determined as myoblasts. After formation of the neural tube, each somite forms a dermamyotome, which gives rise to skin and muscle, and a sclerotome, which develops into skeletal structures. Myoblasts form at each edge of a dermamyotome. Lateral myoblasts proliferate and migrate to form premuscle nwsses in the limbs, where they differentiate into long, multinucleate skeletal muscle cells responsible lor muscle contraction, called myotubes. Axial myoblasts form the myotome. The dermatome gives rise to skin elements (dermis), and the myotome to axial muscle.

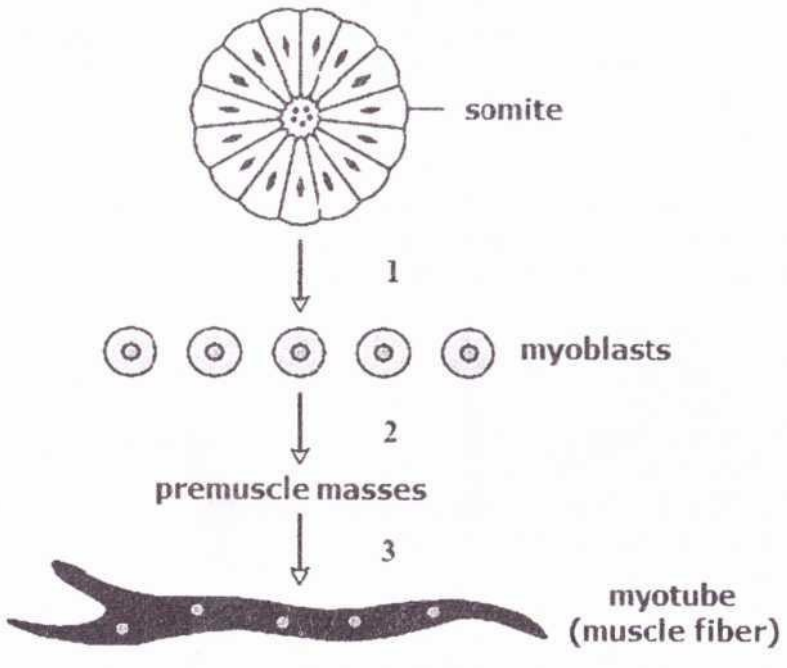


Figure 140. Schematic diagram of stages in development of skeletal muscle. 1 - myoblast determination, 2 - myoblast proliferation and migration, 3 - differentiation into muscle.

Organization of skeletal muscle

The skeletal muscle consists of fascicles of the muscle fibers connected together by system of connective tissue components (fig. 141).

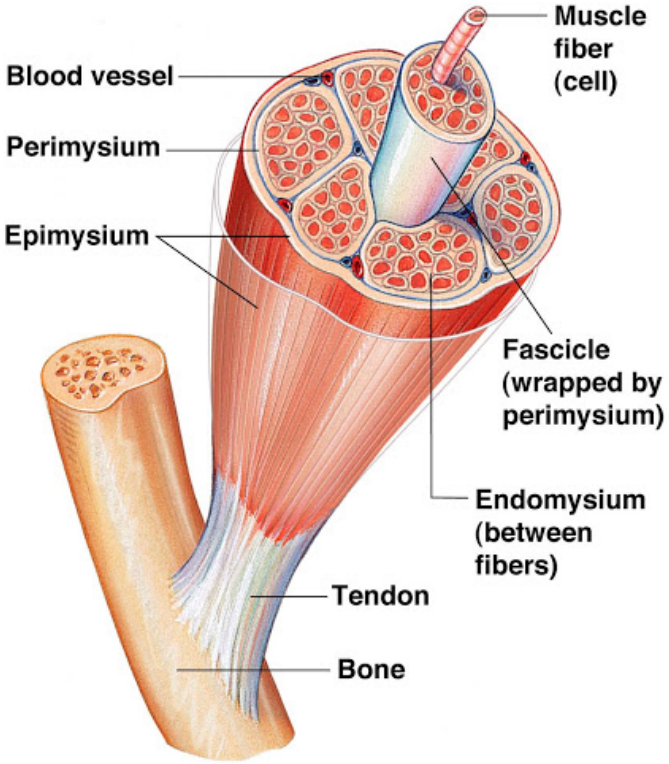


Figure 141. Organization of skeletal muscle.

System of connective tissue components:

Endomysium is delicate layer of loose connective tissue surrounded each muscle fiber.

Perimysium is loose connective tissue surrounded the groups of muscle tissue (fascicles).

Epimysium is connective tissue surrounded the muscle as a whole.

Innervation of skeletal muscle

Skeletal muscle has **efferent (motor)** and **afferent (sensitive)** innervation. A single nerve fiber (axon) can innervate one muscle fiber, or it may branch and be responsible for innervation 160 or more muscle fibers. A single nerve fiber and all the muscles it innervates are called a motor unit.

Cardiac muscle tissue

Cardiac muscle is a type of involuntary striated muscle tissue found in the walls of the heart, specifically the myocardium.

Function of the cardiac muscle tissue

Coordinated spontaneous rhythmic contractions of cardiac muscle cells in the heart propel blood out of the atria and ventricles to the blood vessels of the left/body/systemic and right/lungs/pulmonary circulatory systems. This complex of actions makes up the systole of the heart.

Morphofunctional unit is cardiac muscle cell.

Cardiac muscle cells are long, cylindrical; they are 15 pm in diameter and 85-100 pm in length. Cardiac muscle cells have one or two pale-staining, centrally located nuclei. Sarcoplasm contains organelles and inclusions that form functional systems:

1.contractile system,

2.conducting system,

3.supporting system,

4.system of energy production.

Contractile system

The ends of the cardiac muscle cells are split into branches which form three-dimensional cytoplasmic network. Between the muscle cells delicate loose connective tissue have capillary network. Contractile systems of skeletal fibers and cardiac muscle cells have many structural similarities.

Conducting system

Conducting system of a heart contains:

1. **sarcoplasmic reticulum** is not well development, does not form terminal cisterns,
2. **transverse (T)** tubules are wide, together with elements of sarcoplasmic reticulum form diads composed of one T-tubule and one cytoplasmic cistern, which are found in range Z- lines.

Supporting system

The ends of adjacent cardiac cells are connected through an intercellular junctional complex called the intercalated disks. It provides anchorage for the myofibril and permits rapid spread of contractile stimuli. It is visible in the light microscope as a dense line and is comprised of several structural elements.

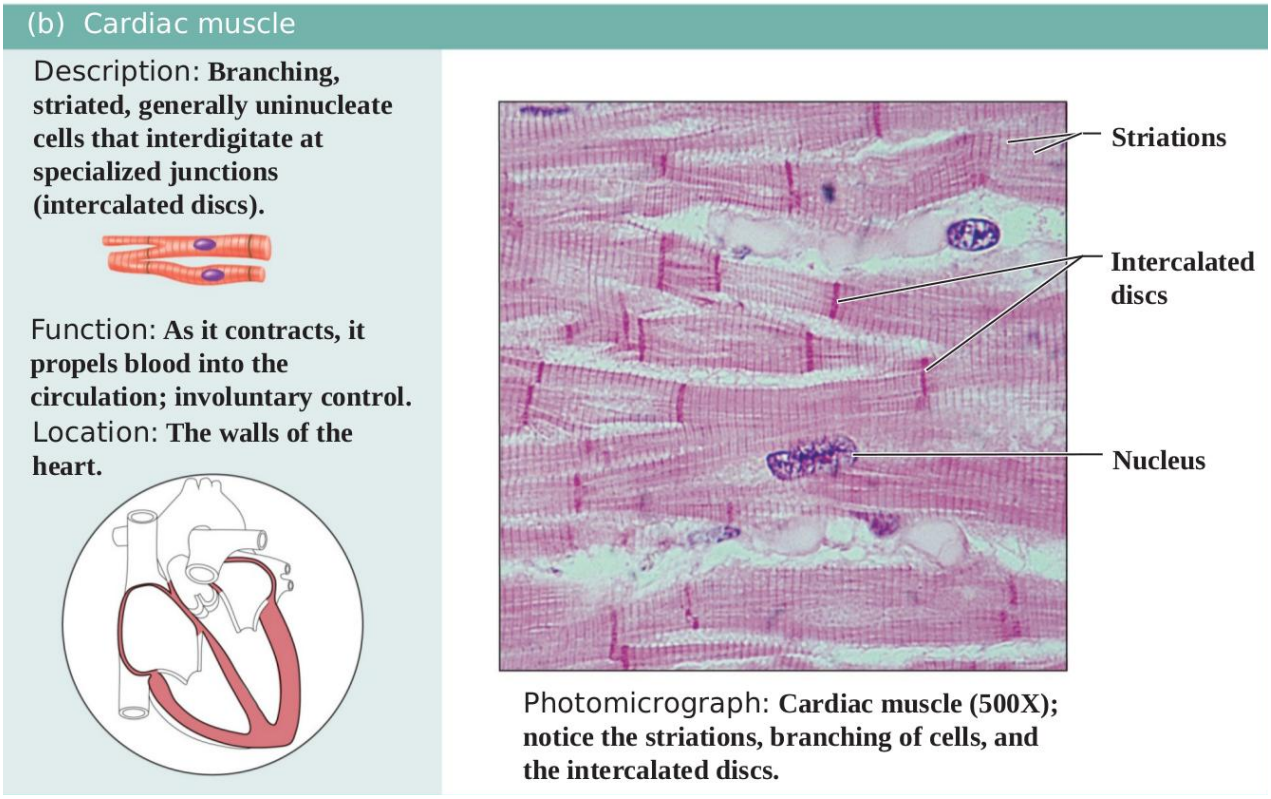


Figure 142. Cardiac muscle tissue.

The intercalated disc consists of three types of contacts (fig. 143):

- transverse portion, which runs across the fibers at right angles:
  - fascia adherens,
  - desmosomes,
- lateral portion, which runs parallel to the myofilaments:

gap junctions (nexuses).

Fascia adherens and desmosomes carry out mechanical function; nexus carries out electrical connection of cardiac muscle cells. Nexuses provide fast conduction of impulses from cell to cell.

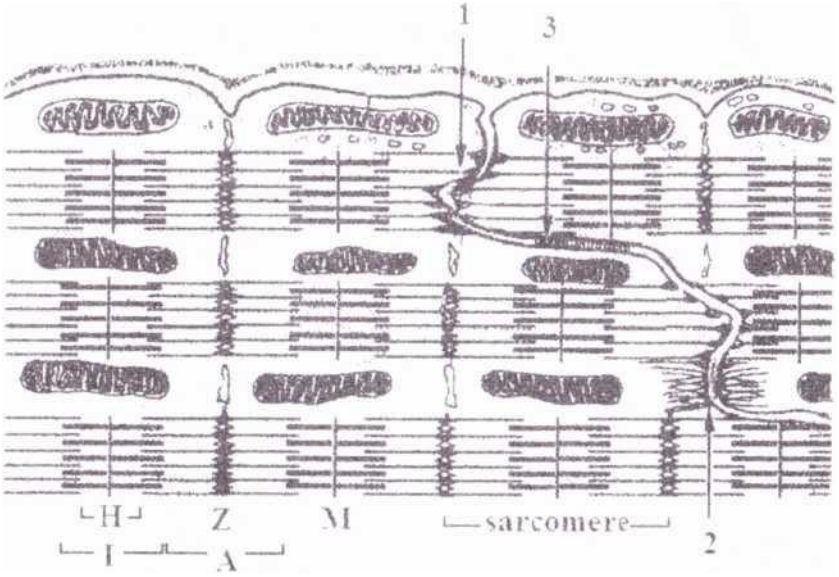


Figure 143. Schematic diagram of intercalated disk of cardiac muscle cell.
1 - fascia adherens, 2 - desmosome, 3 - gap junction.

System of energy production

The system of energy production is submitted by mitochondria and inclusions (glycogen, triglycerides). Very numerous and large mitochondrion lay series between myofibrils, at poles of nucleus and under sarcolemma.

Types of the cardiac muscle cells

The cardiac muscle cells are subdivided on three types:
- contractile;
- conducting;
-secretory (endocrine).

1.Contractile cardiac cells form the basic part of a myocardium and are characterized highly developed contractile system (fig. 144);

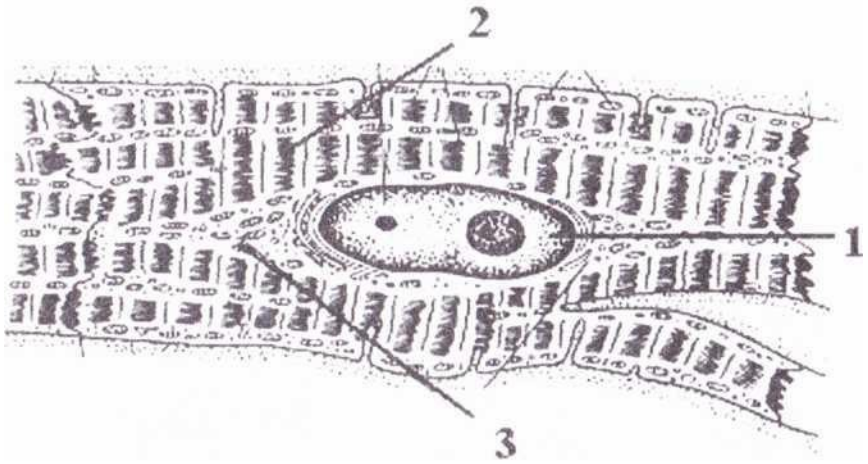


Figure 144. Contractile cardiac cell. 1 - nucleus, 2 - myofibrils, 3 - mitochondria

2.Conducting cardiac cells have ability to generation and conduction of electrical impulses through an impulse-generating and conducting system of a heart. They are characterized by weak development contractile system, a light sarcoplasm and large nucleus.

3.Secretory (endocrine) cardiac cells are found in right atrium and are characterized by weak development of contractile system. In their sarcoplasm near to poles of a nucleus there are granules containing hormone (atrial natriuretic factor, auriculin, or atriopeptin).

Histogenesis of cardiac muscle

The source of development of a cardiac muscle is the cardiogenic plate

- thickening of splanchnopleuric mesoderm located at the cranial end of the embryo. Cardiac precursor cells which lie in a horse-shoe shape configuration in the plate coalesce to form two endocardial tubes. These tubes are then forced into the thoracic region due to cephalic and lateral foldings where they fuse together forming a single. Cells of endocardial tube can differentiate into endocardium which lines the heart chamber and valves and the myocardium which forms the musculature of the ventricles and the atria.

Smooth (visceral) muscle tissue

Smooth muscle is an involuntary **non-striated muscle**.

Function of smooth muscle tissue

Smooth muscle may contract spontaneously or as in the gut special pacemakers cells interstitial cells of Cajal produce rhythmic contractions. Contraction and relaxation can be induced by a number of physiochemical agents (e.g., hormones, drugs, neurotransmitters - particularly from the autonomic nervous system).

Location of smooth muscle tissue

Smooth muscle tissue found within:

- tunica media layer of large and small arteries (except large elastic arteries) and veins,
- urinary bladder,
- uterus and male and female reproductive tracts,
- gastrointestinal tract,
- respiratory tract,
- ciliary muscle and iris of the eye.
- glomeruli of the kidneys contain a smooth muscle-like cell called the mesangial cell.

Smooth muscle is fundamentally different from skeletal muscle and cardiac muscle in terms of structure, function, excitation-contraction coupling, and mechanism of contraction.

Morphofunctional **unit of the smooth (visceral) muscle tissue is smooth muscle cell (fig.145).**

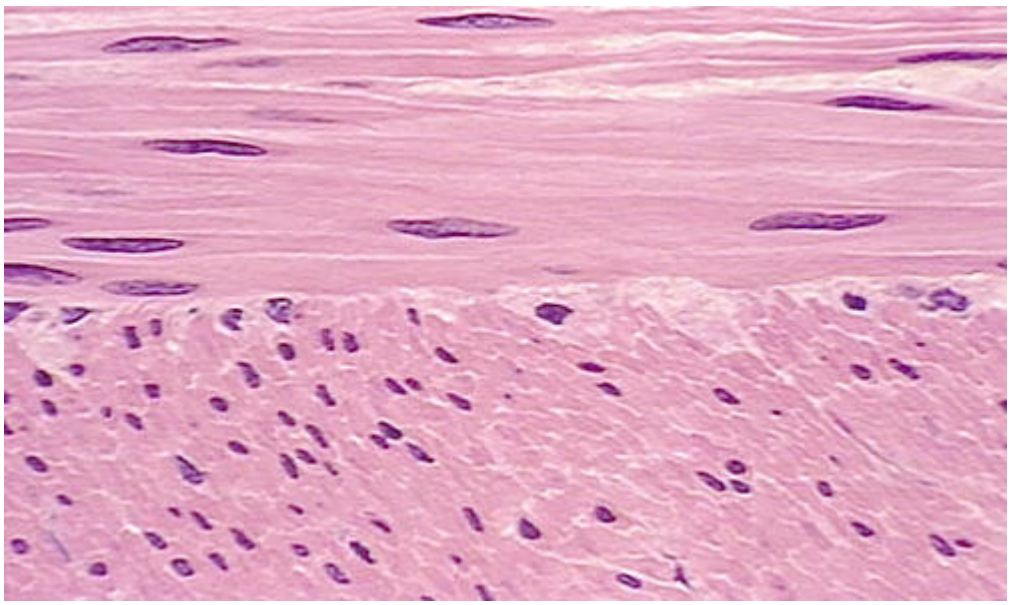


Figure 145. Smooth muscle.

Smooth muscle cell is long spindle-shaped cell Smooth muscle cell is from 20 pm in small blood vessels to 500 pm in the pregnant uterus in length; greatest diameter is about 6 pm.

Nucleus is **single, rod-shaped, and central in position**; in contracted condition of muscle the nucleus may become wrinkled.

Sarcoplasm contains concentrated at the pole of the nucleus **mitochondria**, polyribosomes, cisterns of rough endoplasmic reticulum, and Golgi complex.

Sarcoplasm consists of functional systems:

1.contractile system,

2.conducting system,

3.supporting system,

4.system of energy production.

Contractile system

Contractile system is submitted by thin actin and thick myosin

filaments which not form myofibrils. The bundles of myofilaments crisscross obliquely through the cell, forming a lattice like network . The myofilaments till most of the cytoplasm. They are **not organized into sarcomeres**. There is neither banding nor T tubules, however, smooth muscle cells do have a sarcoplasmic reticulum that can sequester and release calcium.

Each cell is surrounded by a connective tissue sheath.

Conducting system

Conducting system contains:

- **sarcoplasmic reticulum** which in these cells consists of system of vesicular and tubular structures, and also

-numerous flask-shaped **invaginations of plasmolemma** (caveolae).

Caveolae contain high concentrations of calcium, they contact to elements of sarcoplasmic reticulum. T-tubules are absent.

Supporting system

Supporting system of smooth muscle cell of is submitted by its

sarcolemma, a basal membrane, system of elements of cytoskeleton and connected with them dense bodies.

The sarcolemma of each smooth cell is surrounded with basal membrane with thin reticular, collagen and elastic fibers;

Dense bodies are the oval structures laying along the lengthy axis of smooth cell loosely in its sarcoplasm or connected with inner surface of a sarcolemma.

System of energy production

System of energy production is submitted by mitochondria and inclusions (glycogen, lipids) which scission provides reception of energy. Small mitochondrion lay at poles of a nucleus and under a sarcolemma.

Innervation of smooth muscle tissue

Smooth muscle is innervated by both sympathetic and parasympathetic nerves of autonomic system. Nerve terminals are found out only on separate cells. Depolarization is transferred to the next smooth muscle cells by means of gap junctions.

Histogenesis of smooth muscle tissue

The source of the development of smooth muscle is mesenchyme.

On a measure differentiation cells elongate, in them proteins of contractile system and cytoskeleton start to be synthesized.

In immature smooth muscle cells are strongly advanced rough endoplasmic reticulum and Golgi complex.

Regeneration of muscle tissues

Three types of adult muscle exhibit varying potentials for regeneration after injury.

Cardiac muscle has no regenerative capacity beyond early childhood. Defects or damage (e.g., infarcts) in heart muscle are generally replaced by the proliferation of connective tissue, forming myocardial scars.

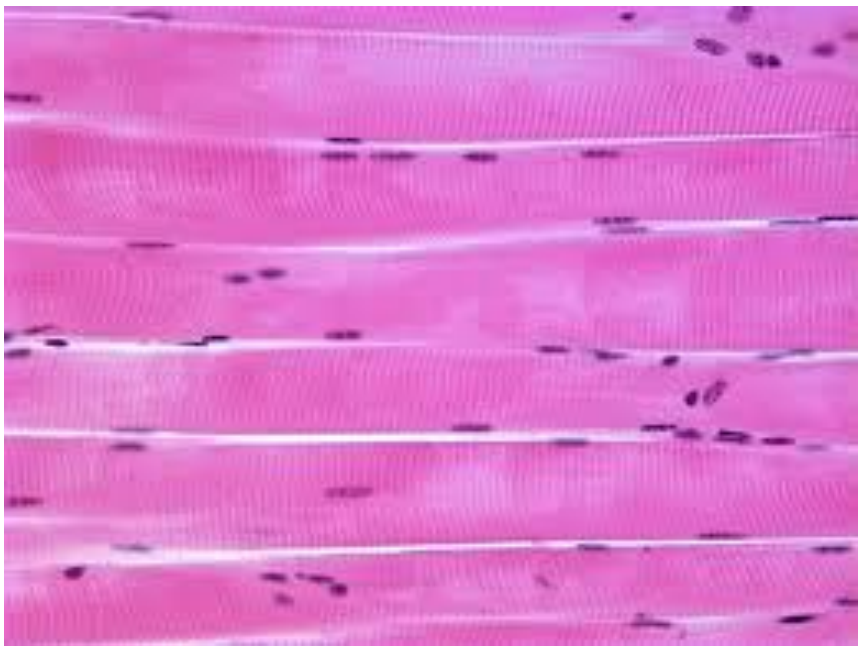
In skeletal muscle, although the nuclei are incapable of undergoing mitosis, the tissue can undergo limited regeneration. The source of regenerating cells is believed to be the satellite cells.

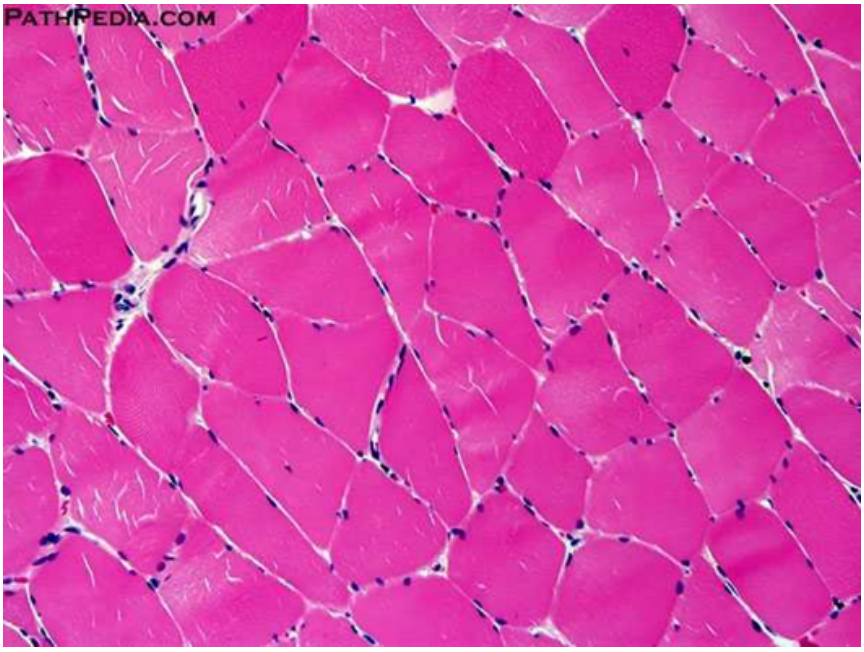
Smooth muscle is capable of an active regenerative response. Mononucleate smooth muscle cells and pericytes from blood vessels undergo mitosis and provide for the replacement of the damaged tissue.

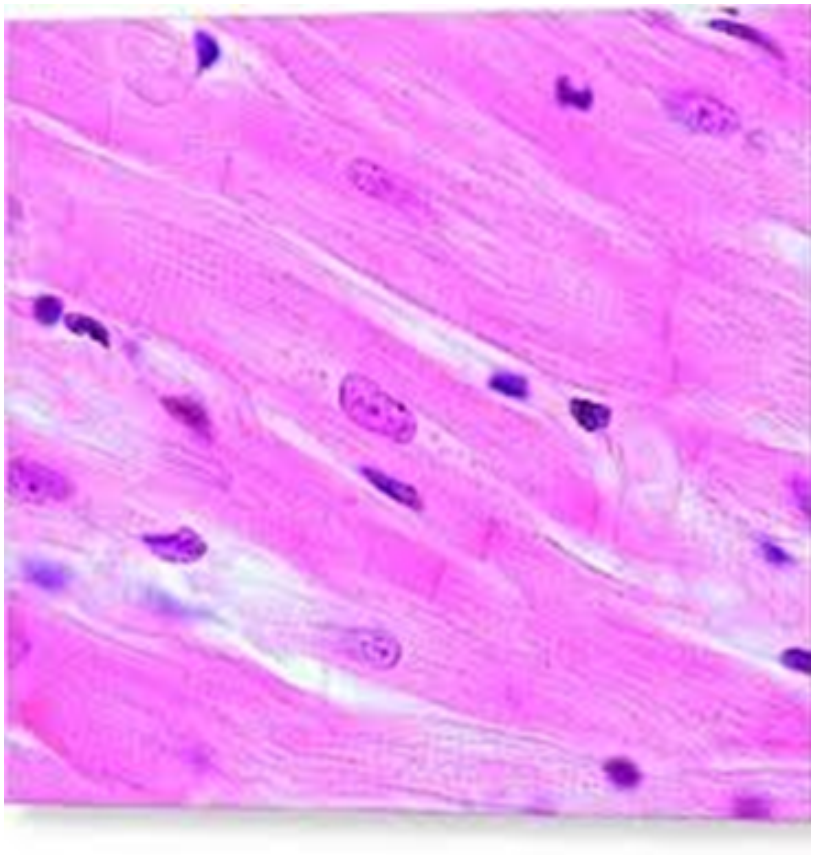
Practical lessons № 14

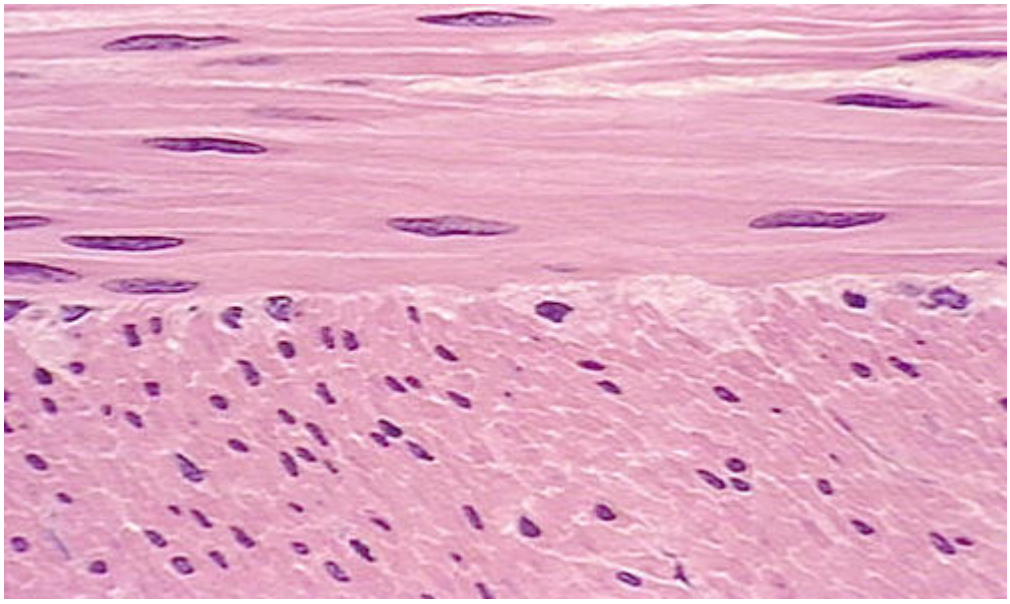
Questions for self-control

1. Morphofunctional and phylogenetic classification of muscle tissue
2. Smooth muscle: structure, localisation and functions.
3. Sceletal muscle: structure, localisation and functions.
4. Components of the muscle fiber.
5. The sarcomere.
6. Regulation of contraction.
7. Regeneration of muscular tissue.
8. Organization of skeletal muscle.




PATHPEDIA.COM





The teacher's signature:

NERVE TISSUE

The nervous tissue is the main component of the nervous system

- the brain, spinal cord, and nerves - which regulates and controls body functions. It is composed of the neurons, which transmit impulses, and the neuroglial cells, which assist propagation of the nerve impulse as well as provide nutrients to the neuron.

Functions of the nervous system are sensory input, integration, controls of muscles and glands, homeostasis, and mental activity.

Development of nerve tissue (neuruiation)

The neuruiation (fig. 12.1) is the formation of the embryonic neural plate and its transformation into the neural tube.

Stages of neuruiation:

1. formation of neural plate, cells of the neural plate can be distinguished as elongated cells in the dorsal region of the ectoderm (shaping); than the neural plate forms the neural folds (folding);
2. formation of neural groove: the dorsal edges of the plate thicken, forming the neural groove;
3. elevation and convergence of neural folds,
4. formation of neural tube:

Neural tube gives arise to the central nervous system. Cells lateral to the neural groove from the neural crests (fig. 146).

Although derived from ectoderm, the neural crest has sometimes been called the “fourth germ layer” because of its importance.

Neural crest cells give rise to a variety of important structures in the adult body. As neuruiation proceeds and the neural tube begins to form, neural crest cells are groups of cells positioned on the top (dorsal) edges of the forming neural folds. Once the neural tube has formed and invaginated, the neural crest cells form a distinct population of cells resting on top of (just dorsal to) the neural tube.

Cells of the neural crests undergo migration and give rise to:

- skin pigment cells (melanocytes);
- neurons of the dorsal root ganglia of spinal nerves;
- autonomic nervous system (both sympathetic and parasympathetic ganglia);
- Schwann cells responsible for myelination of peripheral nerves; adrenal medulla;
- some bones and cartilage in the lower jaw

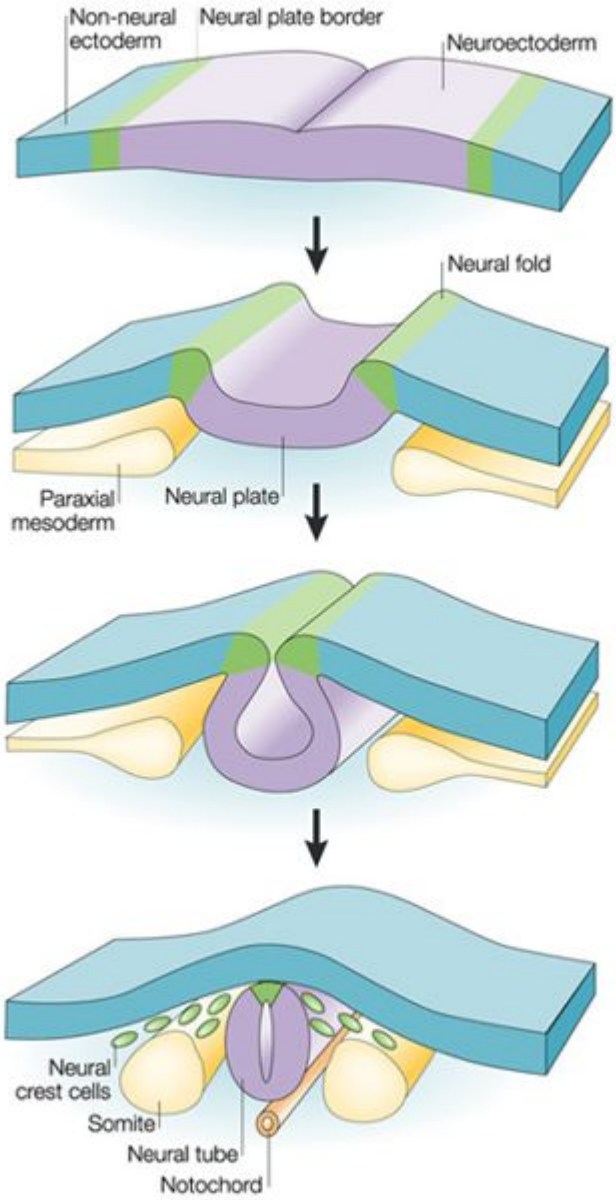


Figure 146. Schematic diagram of neurulation.

Neurons (nerve cells)

The neuron consists of:

1. cell body **(perikaryon**) containing the nucleus surrounded by cytoplasm;
2. processes:

- **dendrites**, which are multiple elongated processes specialized in receiving stimuli from the environment, sensory epithelial cells, or other neurons;
- **axon** which is a single process specialized in generating or conducting nerve impulses to other cells (nerve, muscle, and gland cells); all axons originate from a short pyramidal-shaped region, the axon hillock.

Cell bodies are situated in grey matter of CNS, in sensory and autonomic ganglia outside PNS.

Neurons are very variable in size - from 5 pm in cerebellum to 130 pm in the anterior horn of spinal cord. They have rounded, oval, star-shaped, or pyramidal shape. Nuclei of neurons are large, spherical, pale staining and centrically placed with a prominent nucleolus. Cytoplasm contains Nissl bodies, neurofibrills, Golgi complex, mitochondria, inclusions. The highly developed rough

endoplasmic reticulum organized into aggregates of parallel cisterns and ribosomes between them appear under the light microscope as **basophilic granules called Nissl bodies**. Nissl bodies are present in cell body and dendrites, are absent in axon.

Neurofibrils (stained by silver salts) are cytoplasmic fibrils in the cell body where they form a branching network, and extend into all processes. Each microfibril consists of collections of neurofilaments. They provide mechanical support and stability to the cell. Golgi complex is well development, perinuclear in position. Mitochondria are numerous in the cytoplasm and extend into all processes.Inclusions of pigment, such as iipofuscin, are residua! of undigested material by lysosome.

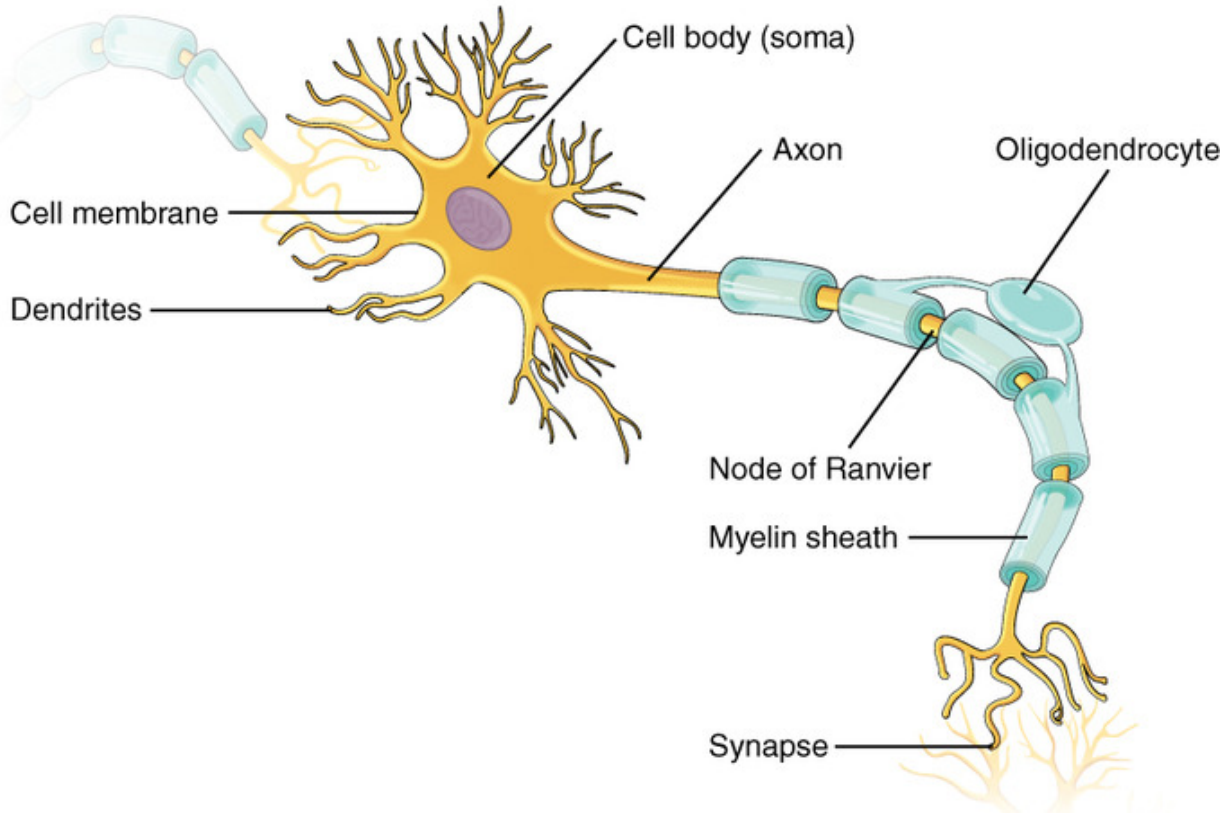


Figure 147. Structure of the neuron.

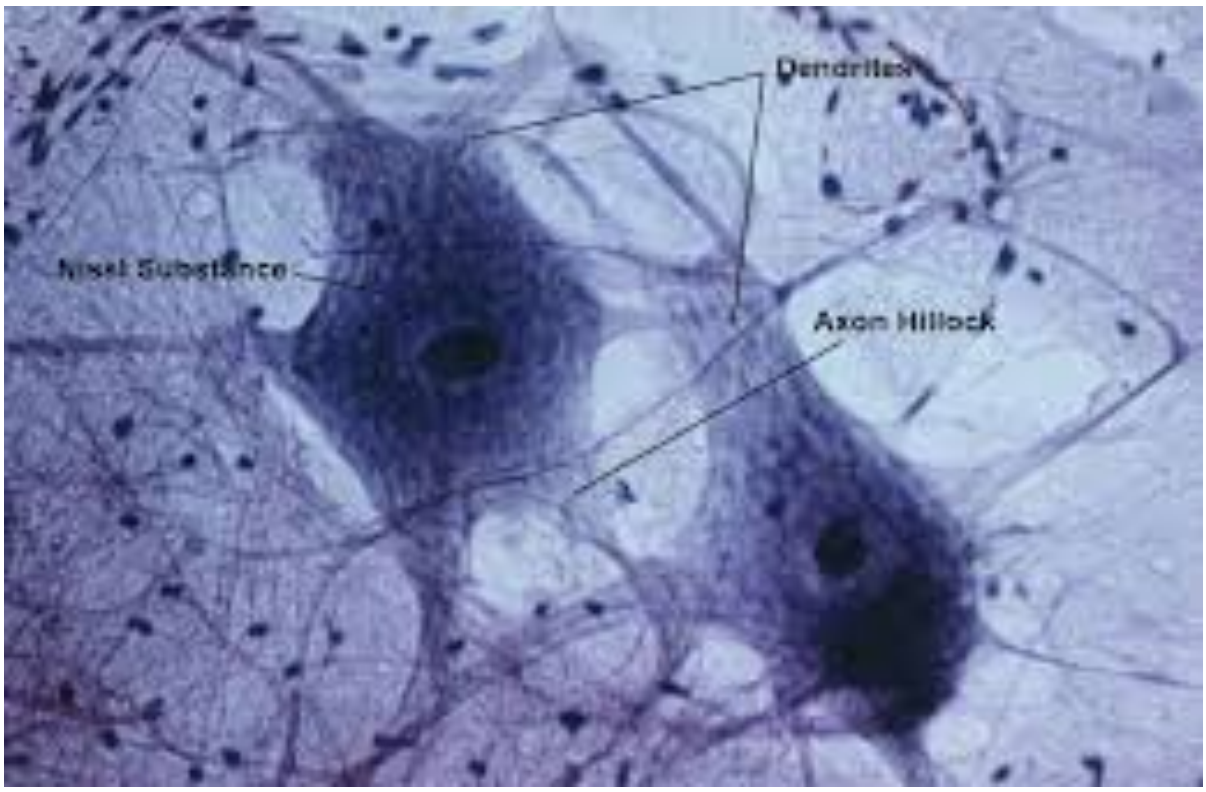


Figure 148. Nissl bodies in the neuron.

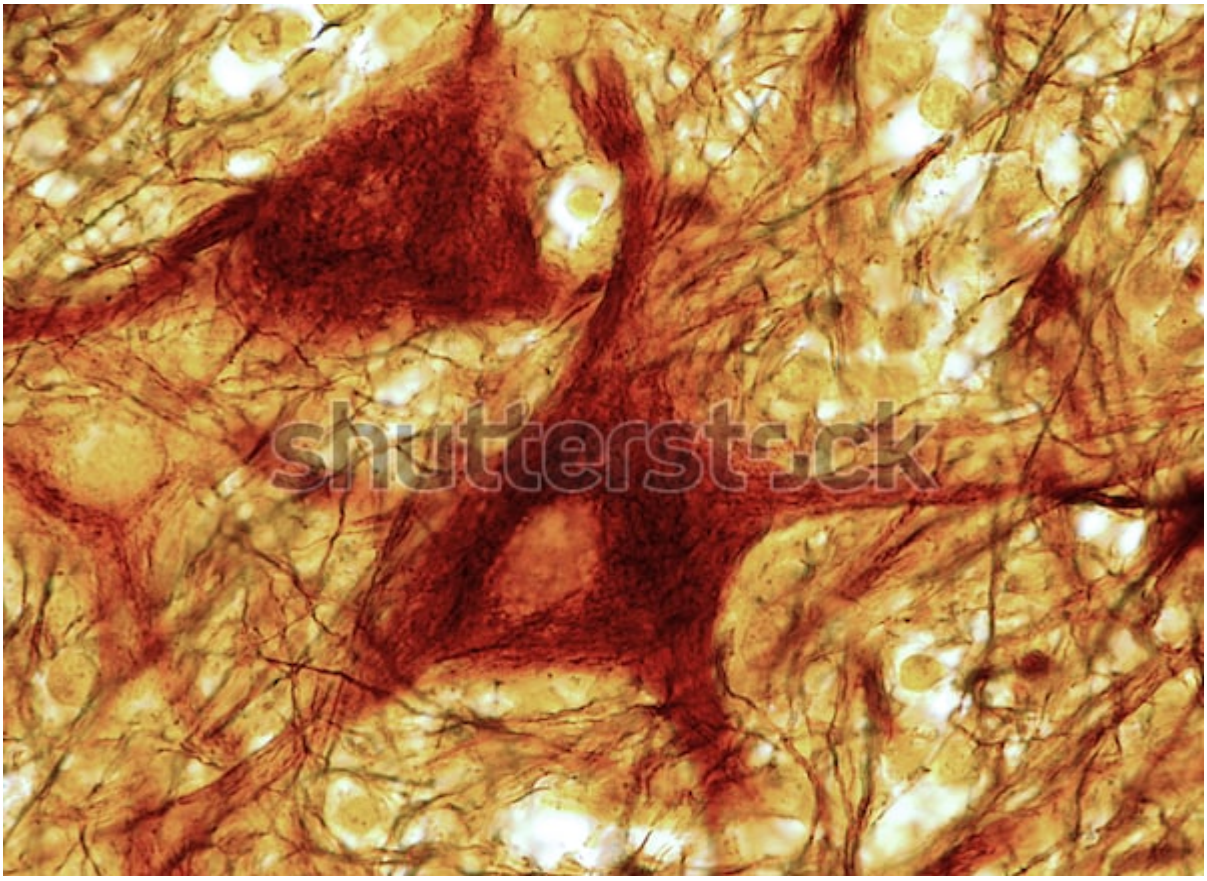


Figure 149. Neurofibrils in the neuron.

Classifications of the neurons

Morphological classification is based on number of processes (fig. 150):

1. **Unipolar** (neuroblasts)

2. **Pseudounipolar** neurons have a single process, which then forms a T- shape. Location, sensory ganglia except vestibular and cochlea.

3. **Bipolar** neurons have one axon and one dendrite.Location:cochlear and vestibular ganglia, olfactory neuroepithelium, retina.

4. **Multipolar** neurons have more than two processes, one process being the axon and others dendrites. Location: either motor or interneurons.

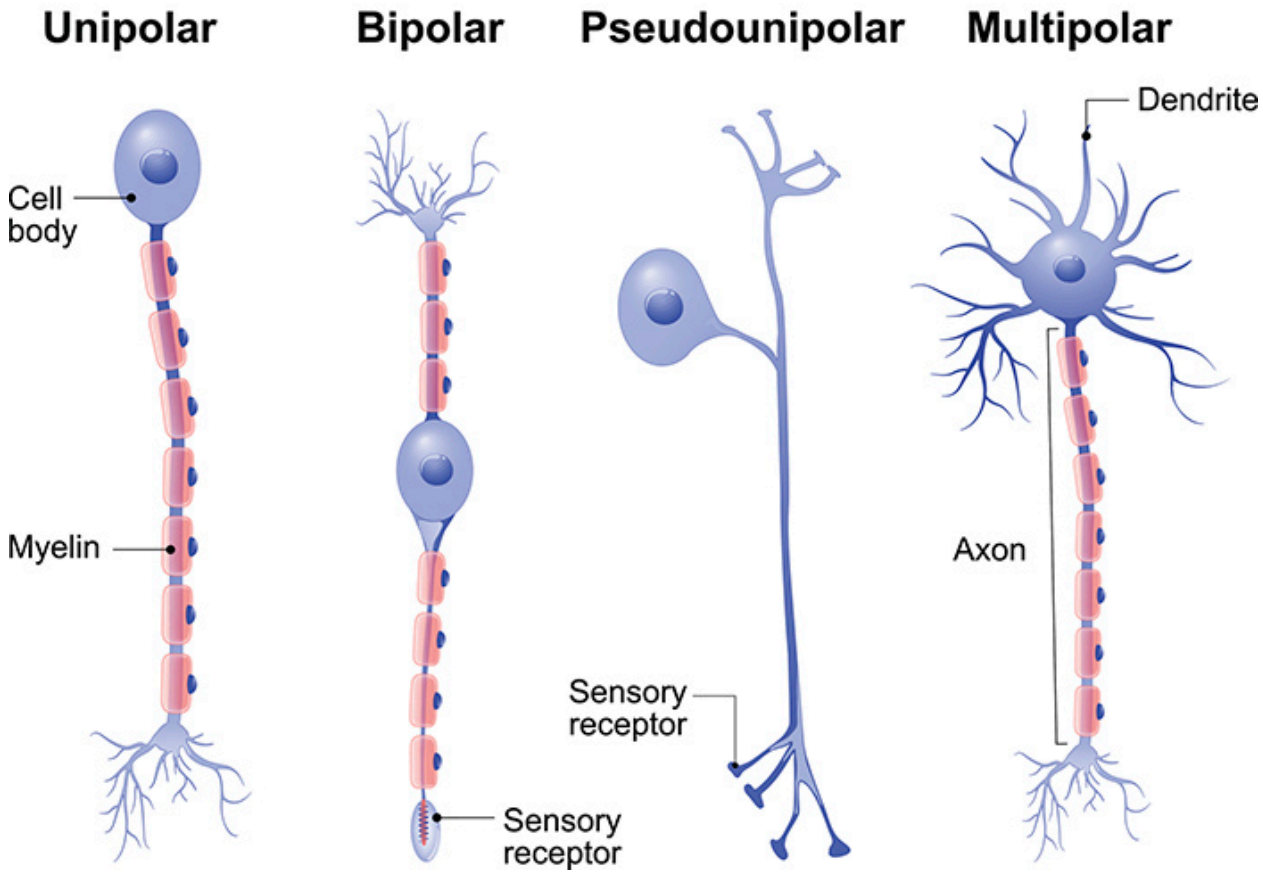


Figure 150. Main types of neurons.

Functional classification is based on functional relations.

1.**Sensory (afferent)** neurons are involved in the reception of sensory stimuli from the environment and from within the body. They are mostly pseudounipolar neurons; their cell bodies lie outside CNS in sensory ganglia.

2.**Interneurons** establish relationship among other neurons, forming complex functional networks.

3.**Motor (efferent)** neurons control effecter organs such as muscle fibers and exocrine and endocrine glands.

Glial cells

Nervous tissue has no intercellular matrix, and glial cells furnish a microenvironment suitable to neuronal activity.

Functions of the glial cells:
-supporting,
- delimitative,
-trophic,
- secretory,
-protective.

Classification of the glial cells
1. Macroglia:
- oligodendrocytes;
- astrocytes;
- ependymal cells.
2. Microglia: glial macrophages.

1. Macroglia

Oligodendrocytes produce the myelin sheath that provides the electrical insulation of neurons in the central nervous system. These cells have a few' small processes that wrap around axons (fig.151), formed a myelin sheath. The same function is performed by Schwann cells in the peripheral nervous system.

Types of the oligodendrocytes in the peripheral nervous system:

Schwann cells have the same function as oligodendrocytes but are located around axons in the peripheral nervous system; satellite cells of ganglia are flattened cells that form a capsule around each ganglion cell.

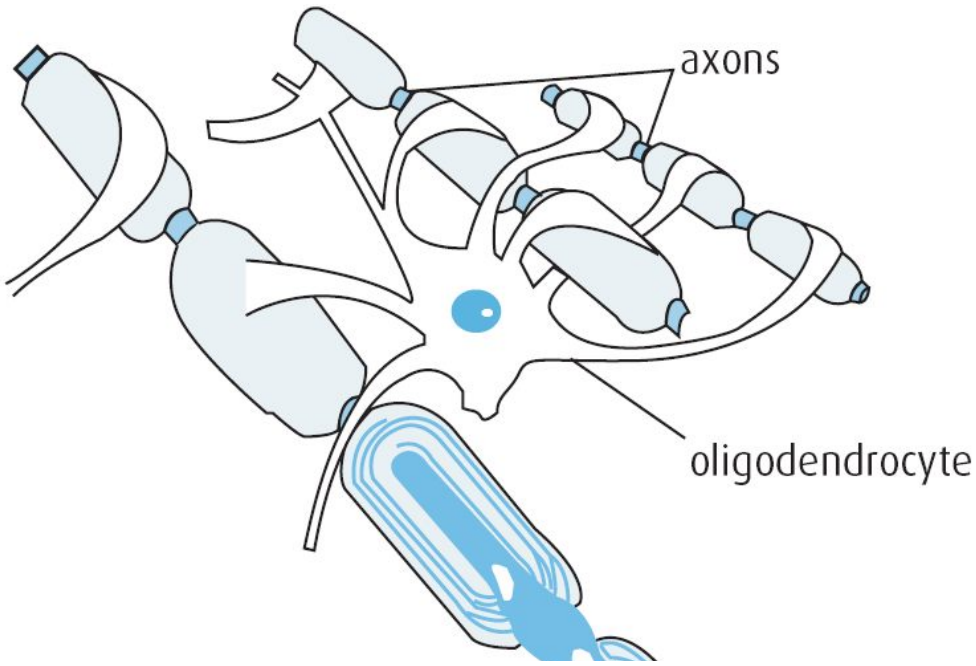


Figure 151. Oligodendrocytes.

Astrocytes are star-shaped cells with profuse branching processes, they contain large, pale, round nucleus.

Astrocytes with few long processes are called **fibrous astrocytes**; they are located in the white matter (fig. 152). Processes are attached to capillaries by perivascular feet.

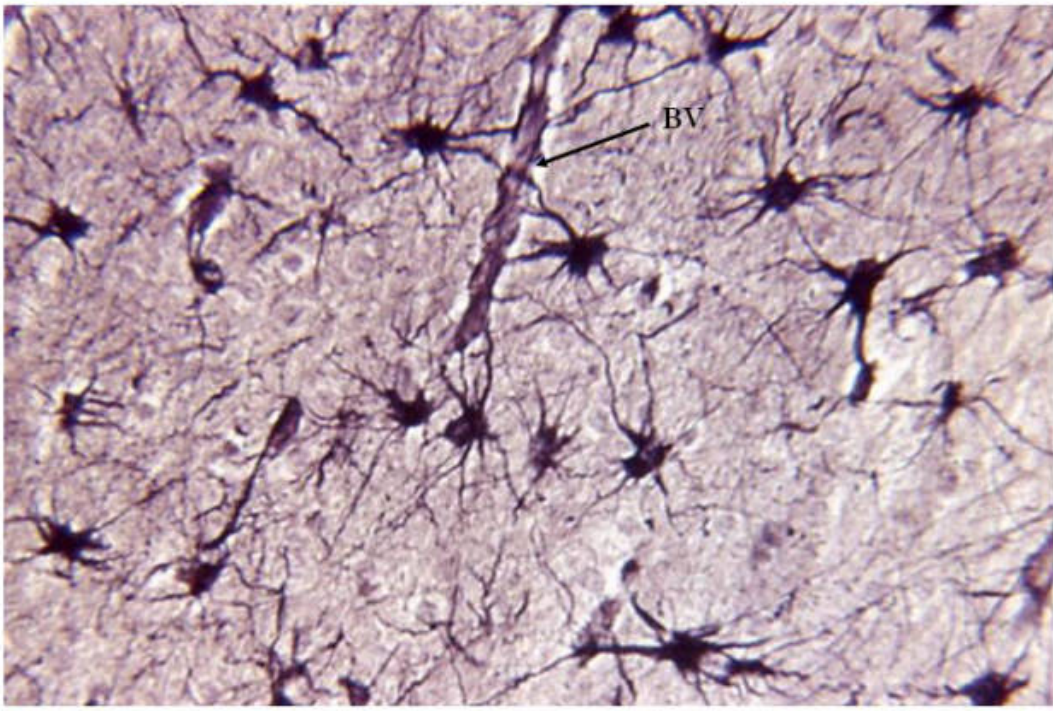


Figure 152. Fibrous astrocytes.

Protoplasmic astrocytes with many short-branched processes are found in the grey matter (fig. 153-154). Many of them processes are attached to pia mater; some extend to blood vessels and are termed perivascular feet.

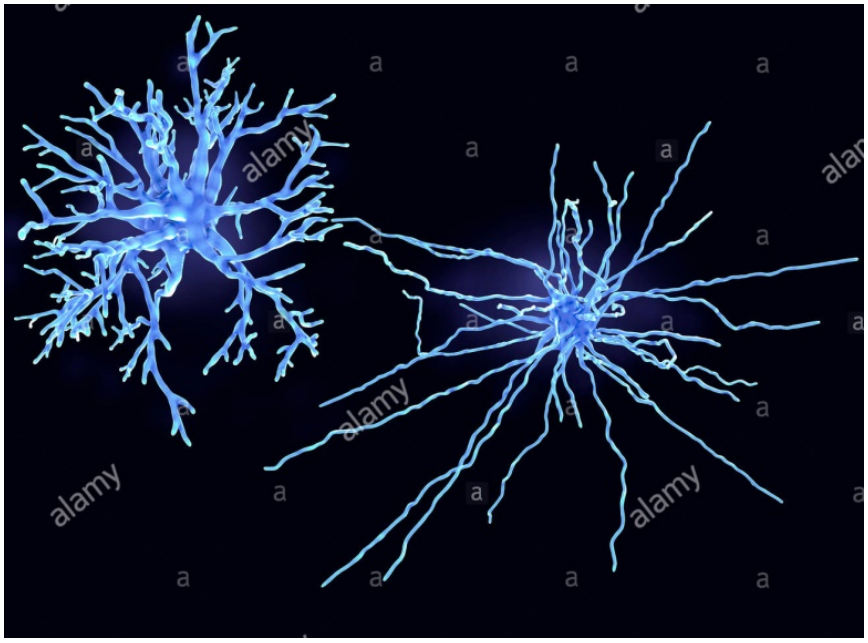


Figure 153. Protoplasmic astrocytes and fibrous astrocytes.

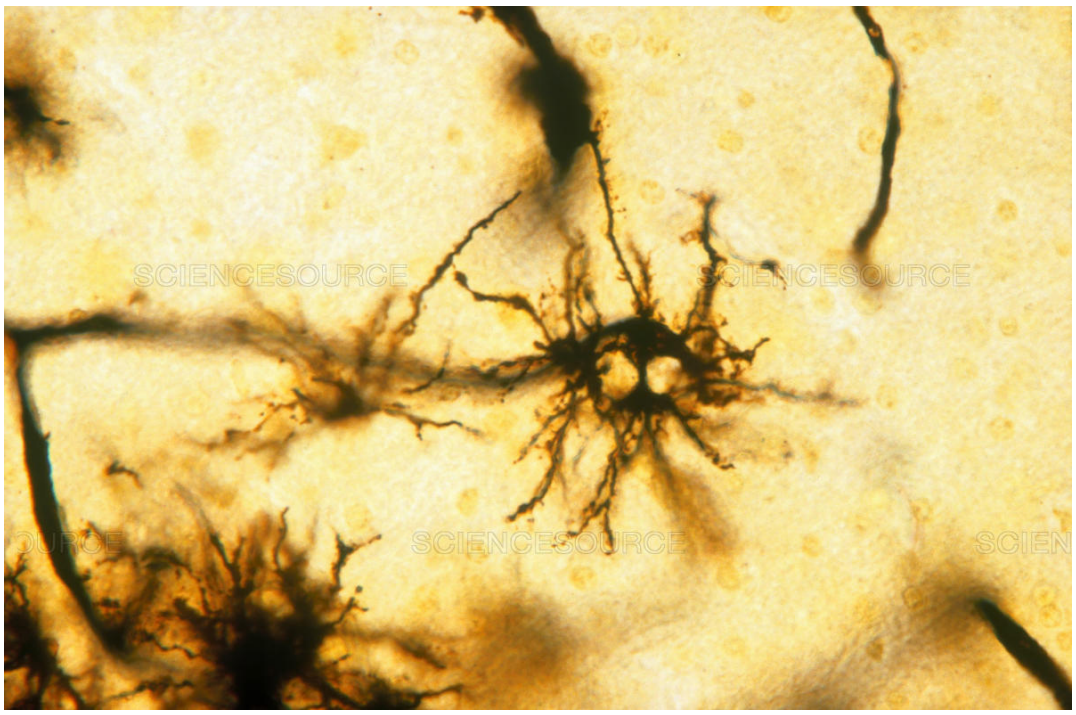


Figure 154. Protoplasmic astrocytes.

Ependymal cells are low columnar ciliated epithelial cells that line the cavities of central nervous system (fig. 155).

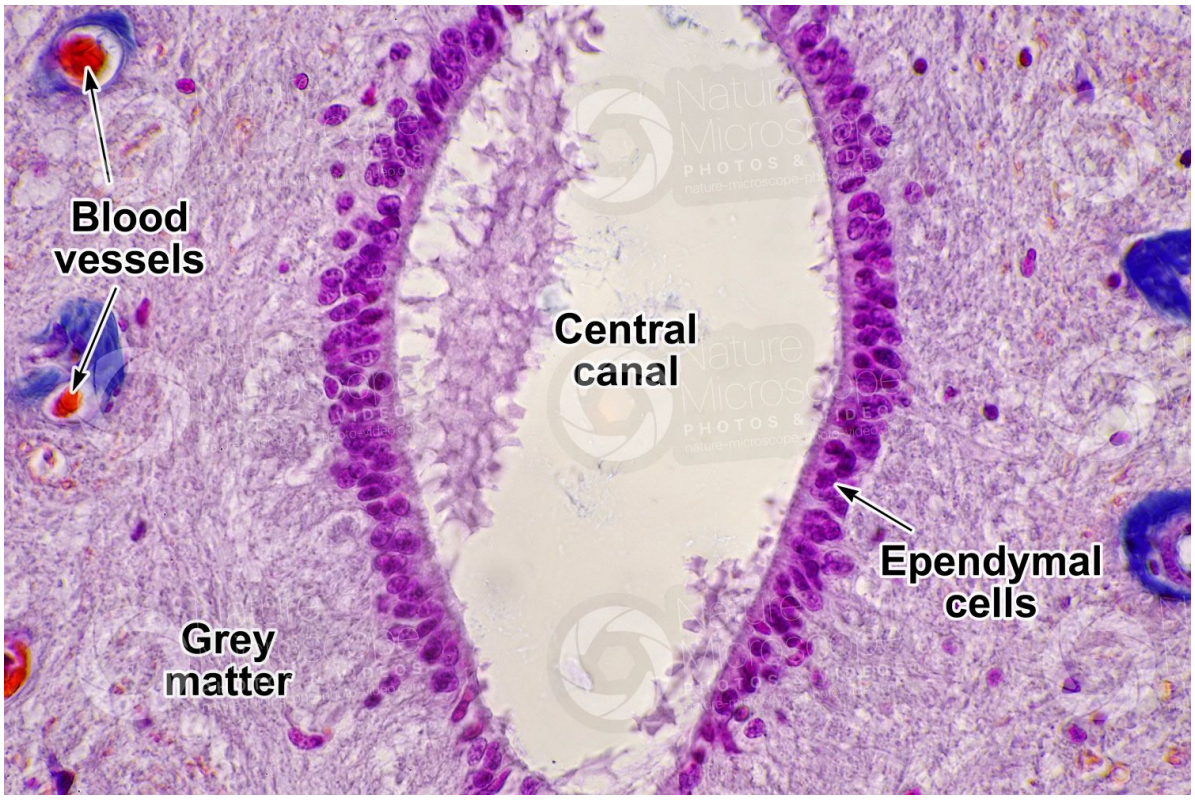


Figure 155. Ependymal cells.

Cilia of their apical surfaces circulate cerebrospinal fluid (CSF) around the central nervous system. Their apical surfaces are also covered with microvilli, which absorb CSF. Ependymal cells are CSF producing cells.

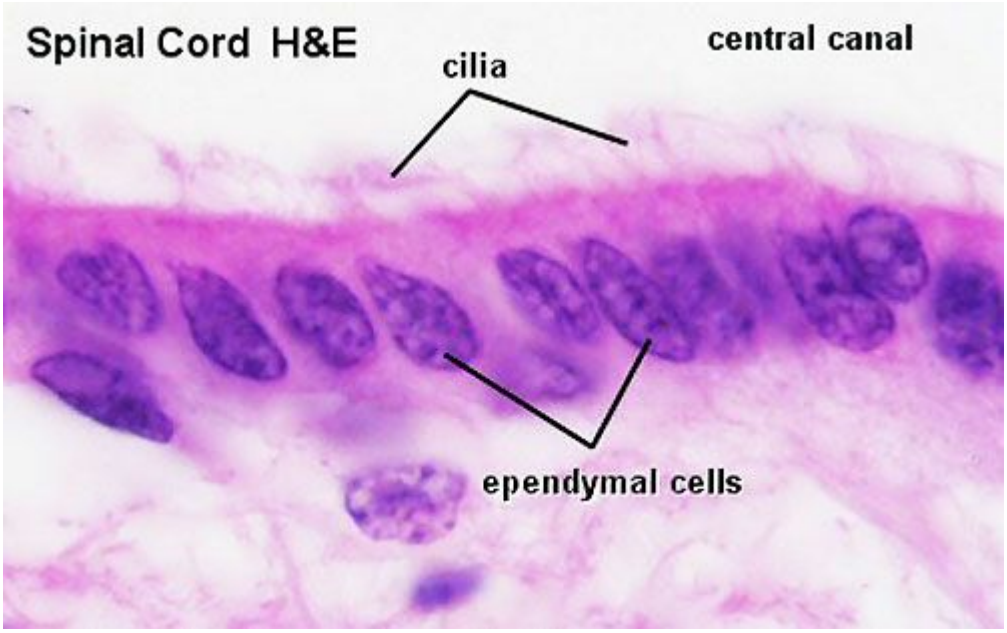


Figure 156. Ependymal cells

Within the brain's ventricles, a population of modified ependymal cells and capillaries together form a system called the choroid plexus, which produces the CSF.

2. Microglia

Microglia is small elongated cells with short irregular processes (fig.157). Microglia is phagocytic cells that represent the mononuclear phagocytic system in nervous tissue, are derived from precursor cells in the bone marrow. They are involved with inflammation and repair in the adult CNS.

Microglia acts as antigen-presenting cells; they secrete a number of immunoregulatory cytokines.

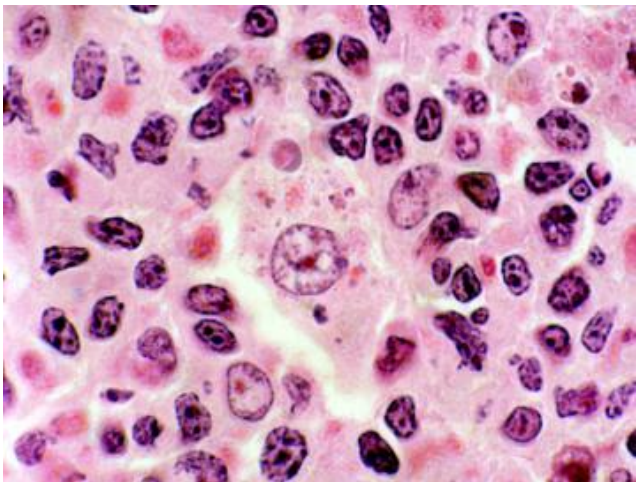
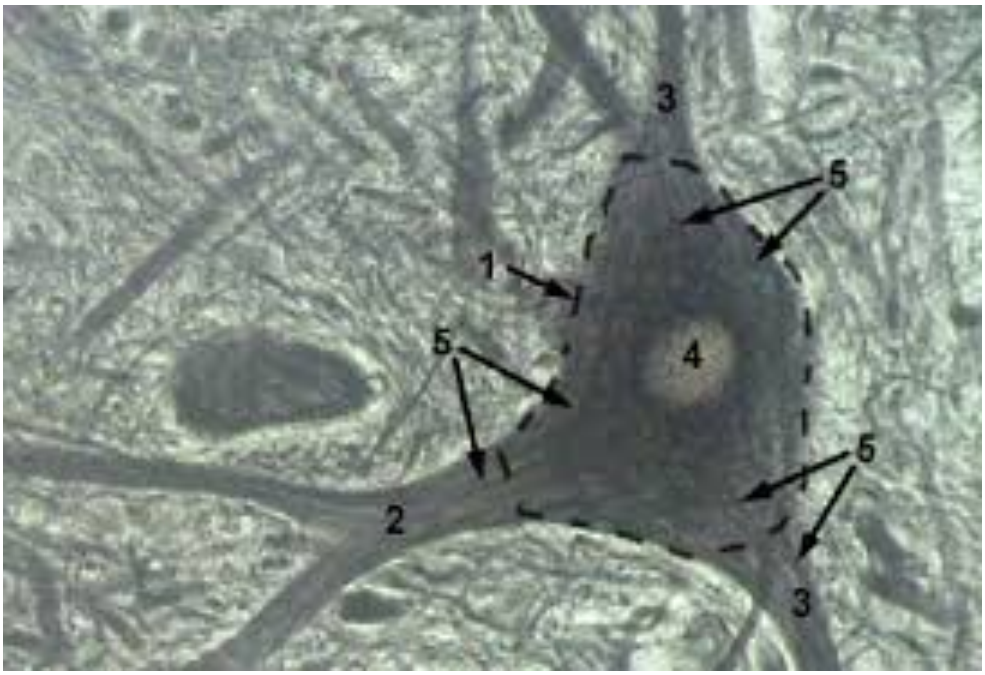


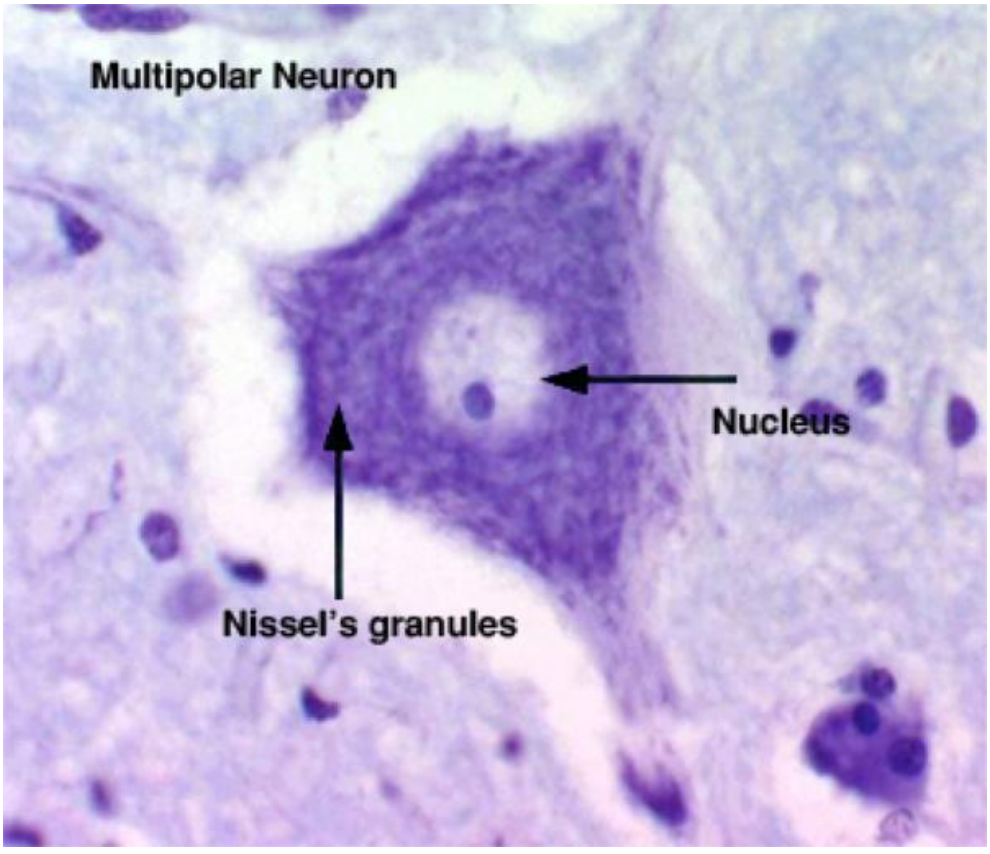
Figure 157. Microglia cells.

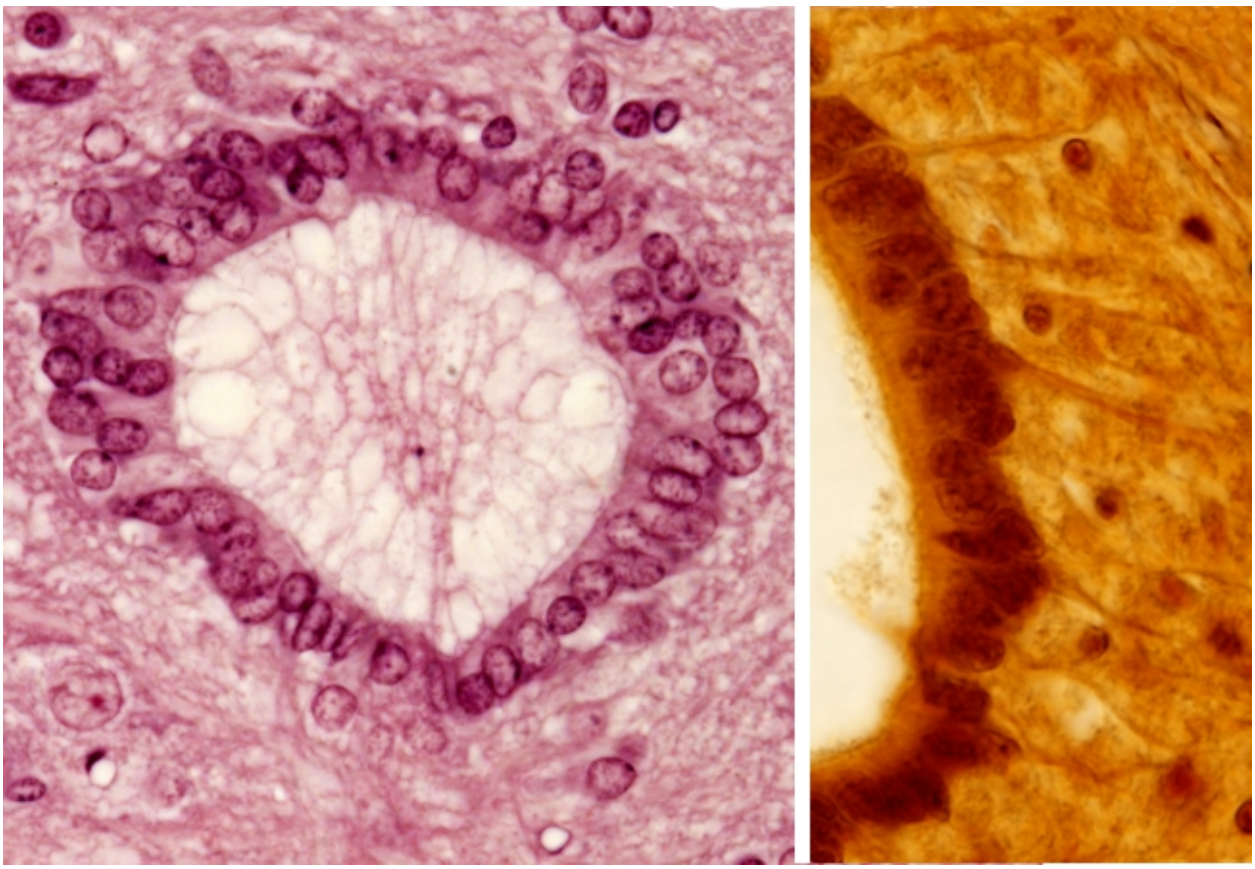
Practical lessons № 15

Questions for self-control

1. Morphofunctional classification of neurocytes.
2. Nissl bodies, neurofibrills.
3. Neuroglia: classification, structure and functions.
4. Astrocytes.
5. Ependymal cells.
6. Oligodendrocyte.







The teacher's signature:

NERVE FIBERS

Nerve fibers consist of **axons (axial cylinders**) enveloped by a special sheath derived from glial cells.

Classification of nerve fibers is based on the structure of their sheath.

Distinguish 2 types of the nerve fibers:

1. unmyelinated,

2. myelinated.

Unmyelinated nerve fibers

Location: at the adult mainly in composition of **autonomic nervous system**. They are characterized by low speed of carrying out of nervous impulses (0.5-2 m/s).

Unmyelinated nerve fibers are formed by dipping the axial cylinder (axon) in cytoplasm of the Schwann cell (fig. 158).

Thus the plasmolemma of Schwann cell invaginates, surrounding an axon, and forms duplication - mesaxon. Quite often in cytoplasm of one Schwann cell there can be till 10-20 axial cylinders. Such fiber reminds an electrical **cable** and consequently refers to as a fiber of **cable type.**

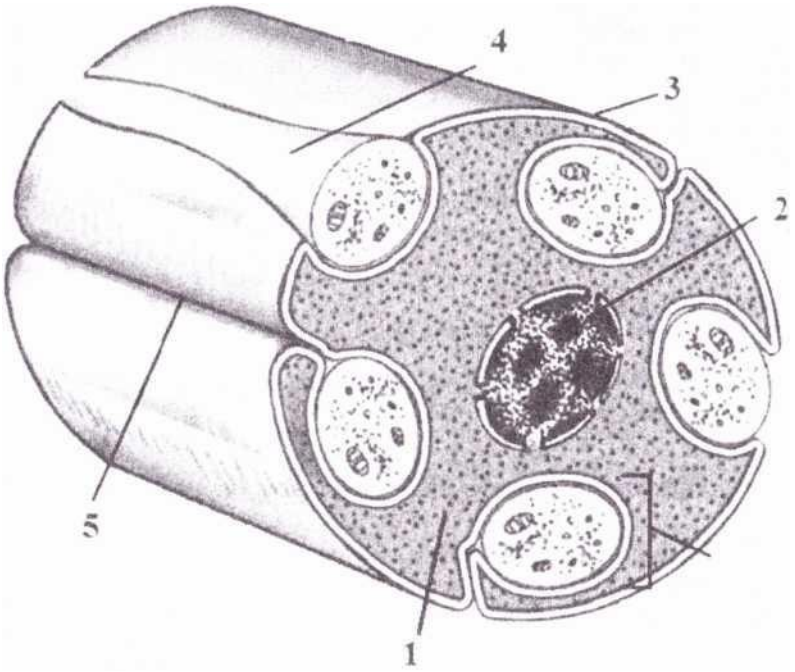


Figure 158. Schematic diagram of the unmyelinated nerve fiber. 1- cytoplasm of Schwann cell, 2 - nucleus of Schwann cell, 3 - cytolemma of Schwann cell, 4 - axial cylinders (axons), 5 - mesaxon.

Myelinated nerve fibers

Location: at CNS and PNS and are characterized by high speed of carrying out of nervous impulses (5-120 m/s).

In myelinated nerve fiber the axial cylinder is surrounded with a myelin sheath around of which the thin layer including cytoplasm and a nucleus of Schwann cell - neurolemma (fig.159).

The process of formation of myelin begins with the invagination of a single axon into a Schwann cell (fig.160); a mesaxon is then formed. As myelination proceeds, the mesaxon rotates around the axon thereby enveloping the axon in concentric layers of Schwann cell cytoplasm and plasma membrane. Thus myelin sheath is concentric layers of Schwann cell membrane. An axon comes in contact with many Schwann cells along its length.

Each Schwann cell forms myelin sheath over a short segment of the axon.

Between the Schwann cells there are short intervals at which the axon is not covering by a myelin. These points are nodes of Ranvier (fig.161).

The distance between two nodes is called an internode and consists of one Schwann cell.

The action potential travels by jumping from node to node. This mode ol conduction is called salutatory conduction.

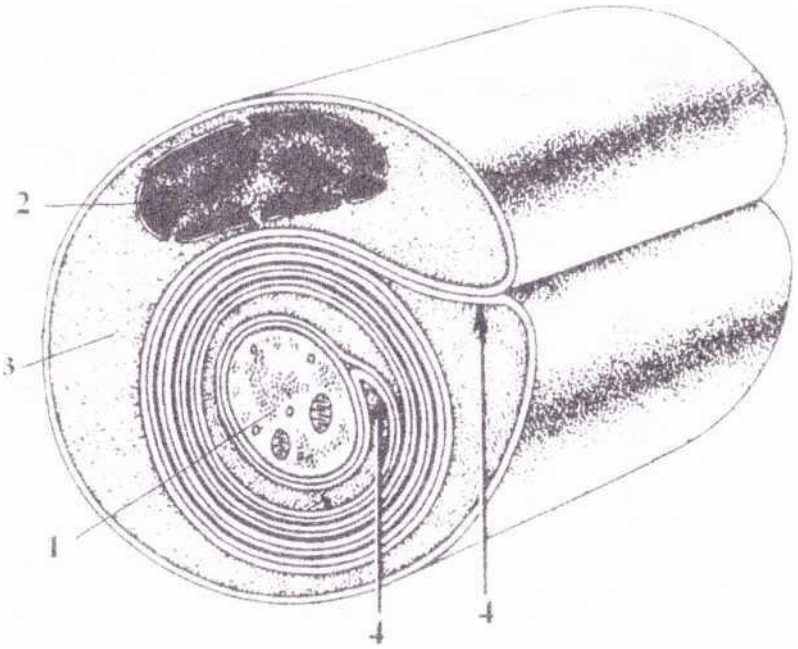


Figure 159. Schematic diagram of the myelinated nerve fiber. Cross section. 1 - axial cylinder (axon), 2 - nucleus of Schwann cell, 3 - cytoplasm of Schwann cell, 4 - mesaxon.

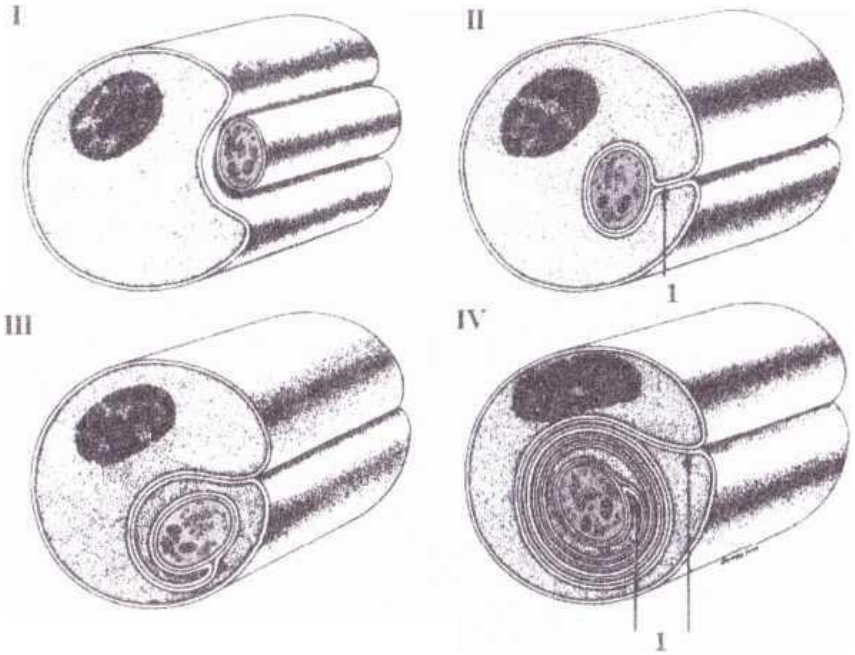


Figure 160. Consecutive phases of myelin formation in nerve fibers. I - invagination of a single axon into Schwann cell, II - formation of a mesaxon, III - rotation of the mesaxon around the axon, IV - enveloping the axon in concentric layers of Schwann cell cytoplasm and plasma membrane, 1 - mesaxon

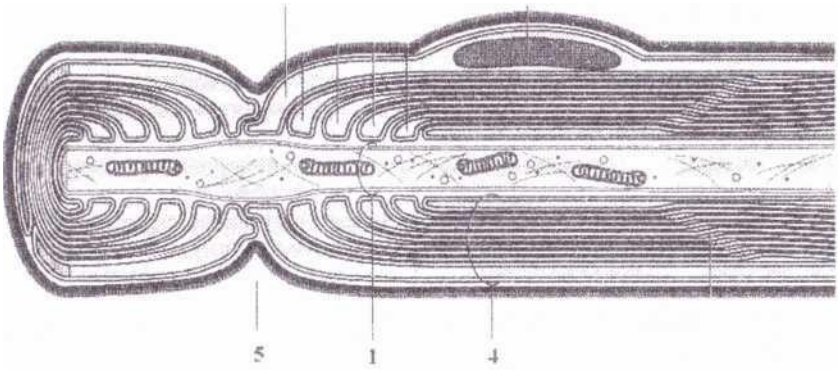
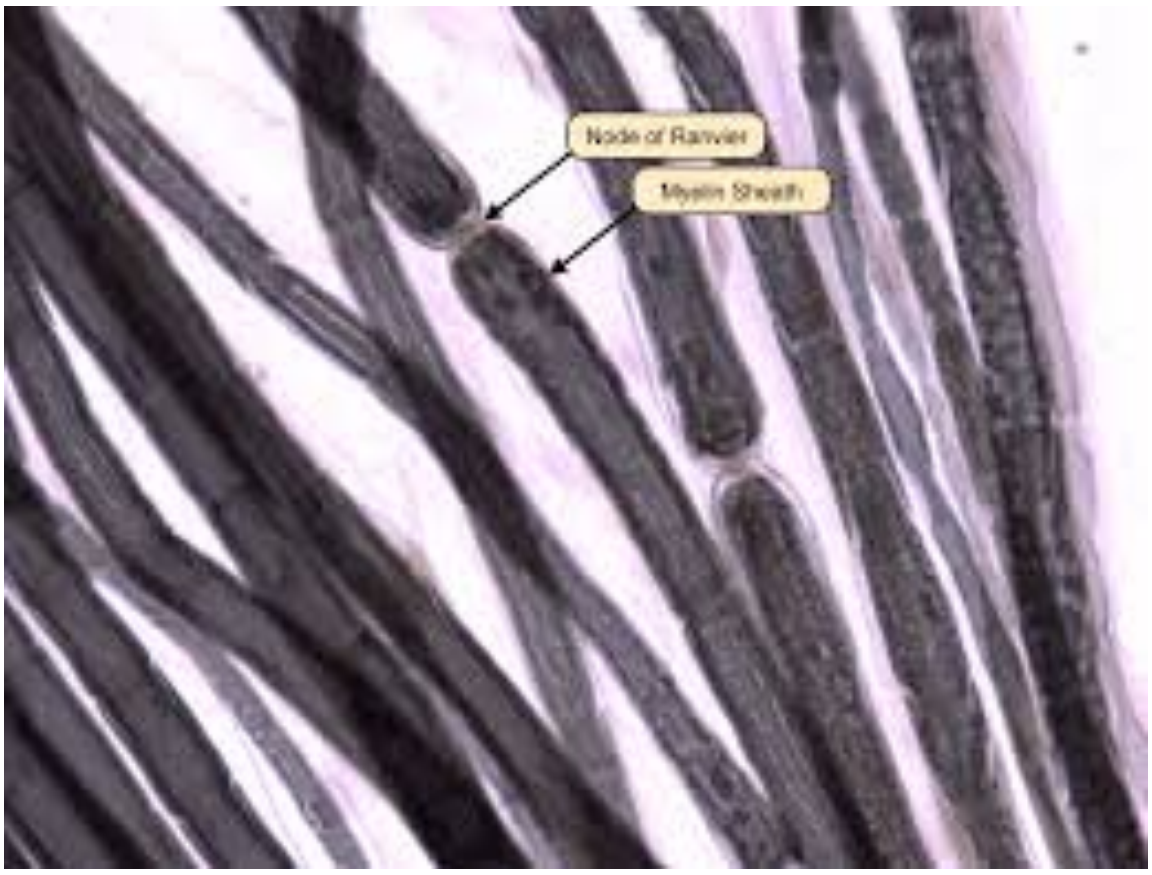


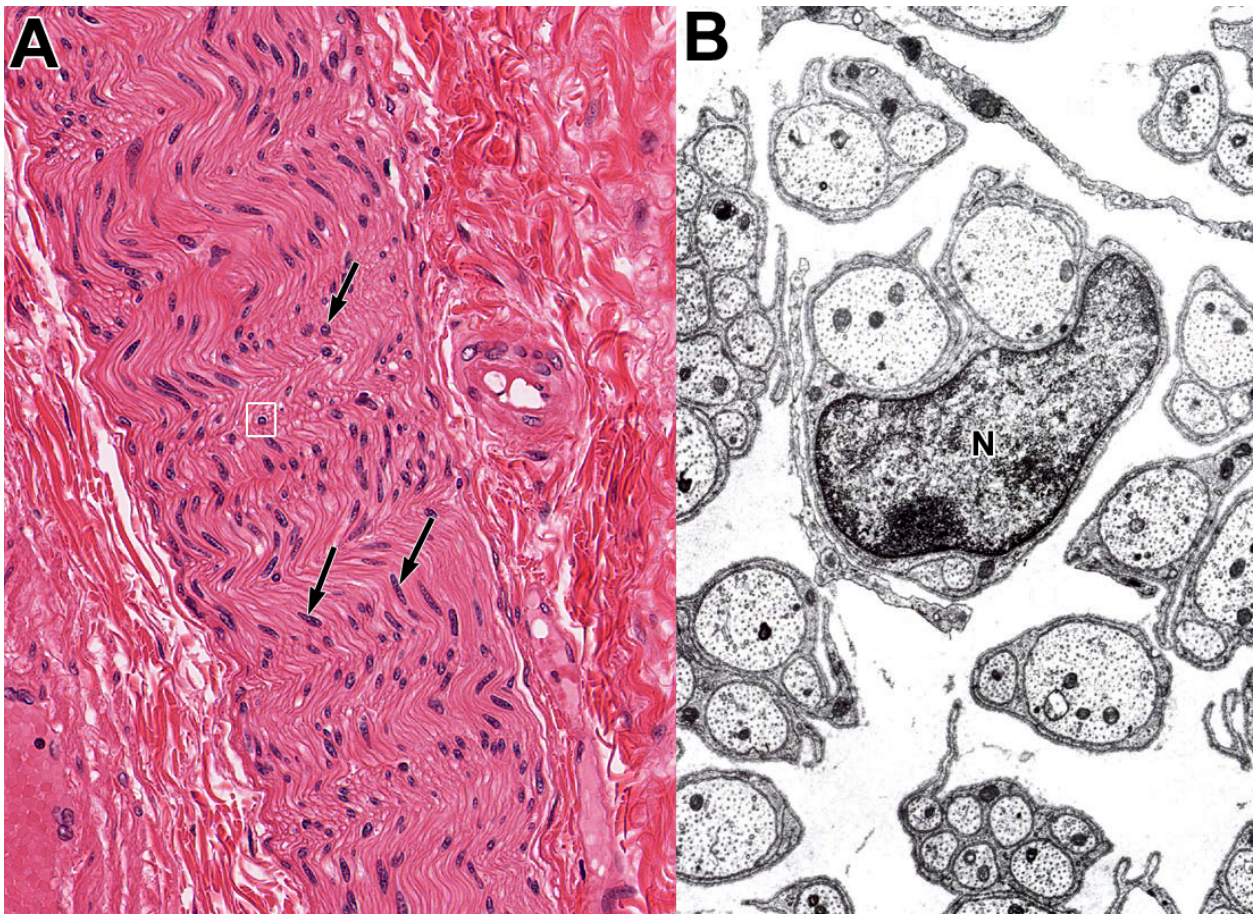
Figure 161. Myelinate nerve fiber. Longitudinal section. 1 - axial cylinder (axon), 2 - nucleus of Schwann cell, 3 - cytoplasm of Schwann cell, 4 - myelin sheath, 5-node of Ranvier.

Practical lessons № 16

Questions for self-control

1. Unmyelinated fibres: structure and functions.
2. Myelinated fibres: structure and functions.





The teacher's signature:

NERVE ENDINGS

Nerves end either on some other neurons, or in some peripheral organs (skin, joint capsules, muscles and glands).

Nerve endings (terminals) are subdivided on three types:

1**. transneuronal contacts (synapses)** provide a functional connection between neurons;

2. **receptor (sensory) endings** accept stimuli from an external and an internal environment are on dendrites.

3. **motor (efferent) endings** transfer signals from nervous system to organs (muscles, glands), are on axons.

Synapses

Synapse is the junction between neurons, where the nerve impulse passes from neurons to the next.

Synapses may be classified morphologically as:

- axodendritic, occurring between axon and dendrites;
- axosomatic, occurring between axon and the cell body;
- axoaxonic, occurring between axon and axon;
- dendrodendritic, occurring between dendrite and dendrite.

Synapses also may be classified as

- **chemical**, in which conduction of impulses is achieved by the release of chemical substances (neurotransmitters);

- **electrical**, which contain gap junctions that permit movement of ions between cells and consequently permit the direct spread of electrical current from one cell to another.

Chemical synapse (fig. 162) consists of:

- axon terminal **(presynaptic terminal)** that delivers the impulse;
- part of another cell where a new impulse is generated **(postsynaptic terminal**);
- thin intercellular space **(synaptic cleft).**

Most synapses transmit the impulse by releasing neurotransmitters.

These are chemical substances that induce the transfer of the nervous impulse to other neurons. Synapses which transmit nerve impulses through neurotransmitters are chemical synapses.

Presynaptic terminal contains **synaptic vesicles**.

Synaptic vesicles contain one of the neurotransmitter substances - acetylcholine, norepinephrine, dopamine, serotonin, glycine and gamma-amino-butyric acid (GABA).

Postsynaptic terminal contains postsynaptic web with **receptors.**

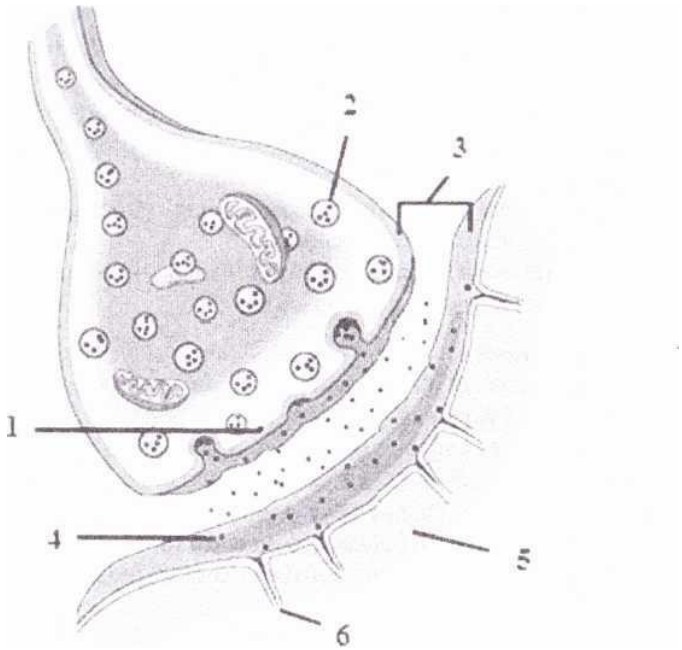


Figure 162. Schematic diagram of synapse. 1 - presynaptic membrane of axon. 2 - synaptic vesicles, 3 - synaptic cleft, 4 - neurotransmitter, 5 - postsynaptic terminal, 6 – receptors

Histophysiology of synapse

1. Depolarization of presynaptic membrane.
2. Opening of calcium channels.
3. Exocytosis of synaptic vesicles with neurotransmitter.
4. Release of neurotransmitter into synaptic cleft.
5. Reaction of neurotransmitter with receptors of postsynaptic region.
6. Depolarization of postsynaptic membrane.

Receptor (sensory) endings (terminations)

Receptor (sensory) nerve terminations accept signals from an external environment **(exteroceptors**) and an internals **(interoceptors).**

Functional classification of sensory nerve endings is based on nature of stimuli: mechanoreceptors, chemoreceptors, thermoreceptors, pain receptors (nociceptors).

Morphological classification of sensory nerve endings is based on features of their structural constitution:

1. **free,**
2. nonfree

-nonencapsulated,
-encapsulated

Free nerve endings

Free nerve endings are devoid of myelin and Schwann sheaths (fig.163)

They receive pain, tactile and temperature sensations.

Distribution, epithelium and connective tissue of skin.

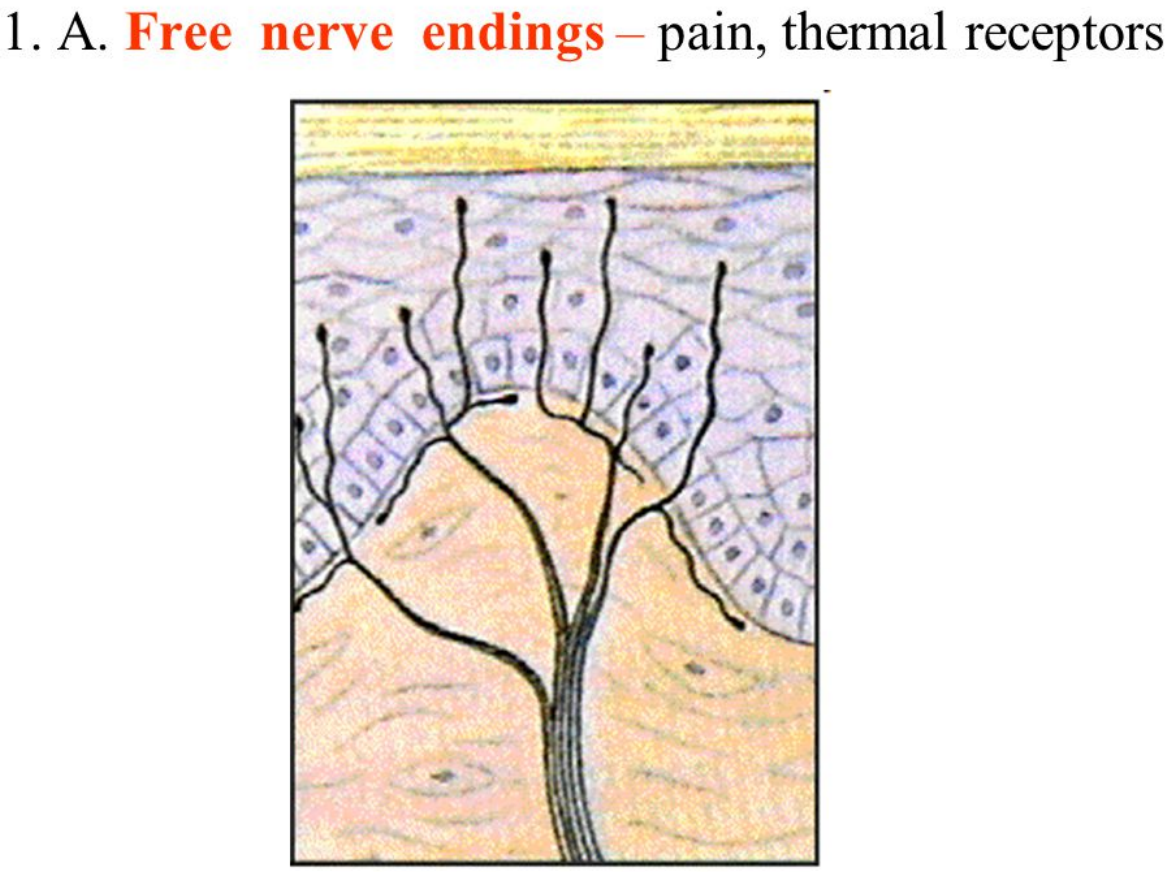


Figure 163. Free nerve endings.

Figure 164. Free nerve endings.

Nonfree nerve endings

Nonfree nonencapsulated nerve endings consist of dendrite branchings surrounded with Schwann cells.

Distribution, connective tissue of a skin (derma), lamina propria of mucosa.

The various types of nonfree encapsulated nerve endings are:

1. Pacinian corpuscle contains capsule consisting of concentrically arranged lamellae like the leaves of an onion (fig. 165). It receives pressure and vibration sensations.

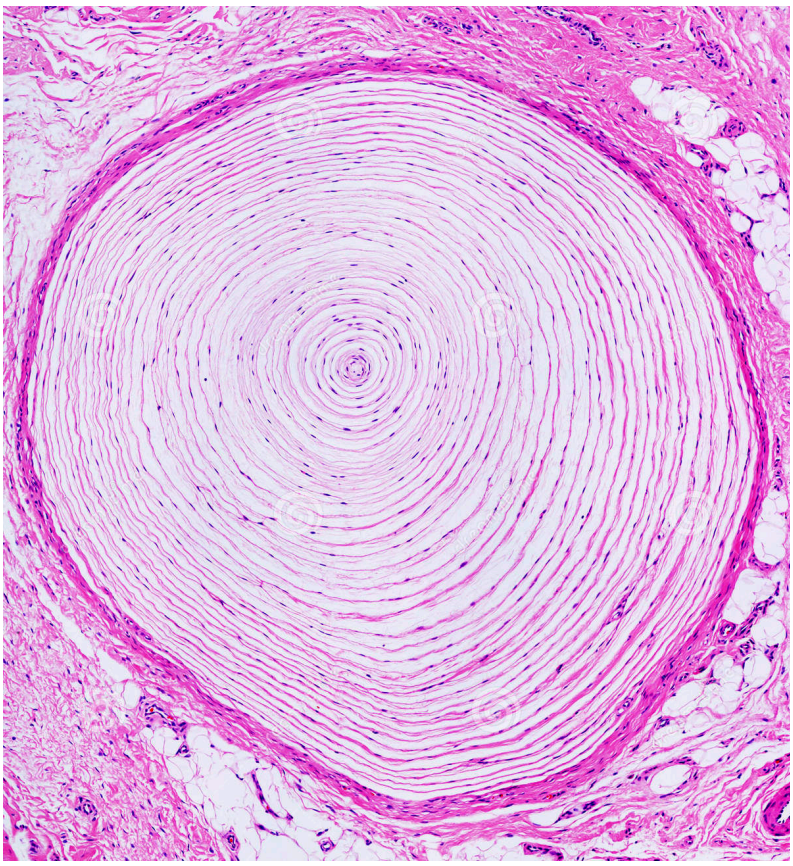


Figure 165. Pacinian corpuscle.

Distribution: connective tissue of internal organs, skin.

2**. Meissner's corpuscle** is touch receptor, has elliptic shape, contains the spiral terminals of afferent axons, surrounding by flattened Schwann cells which form several irregular lamellae and thin capsule (fig. 166).

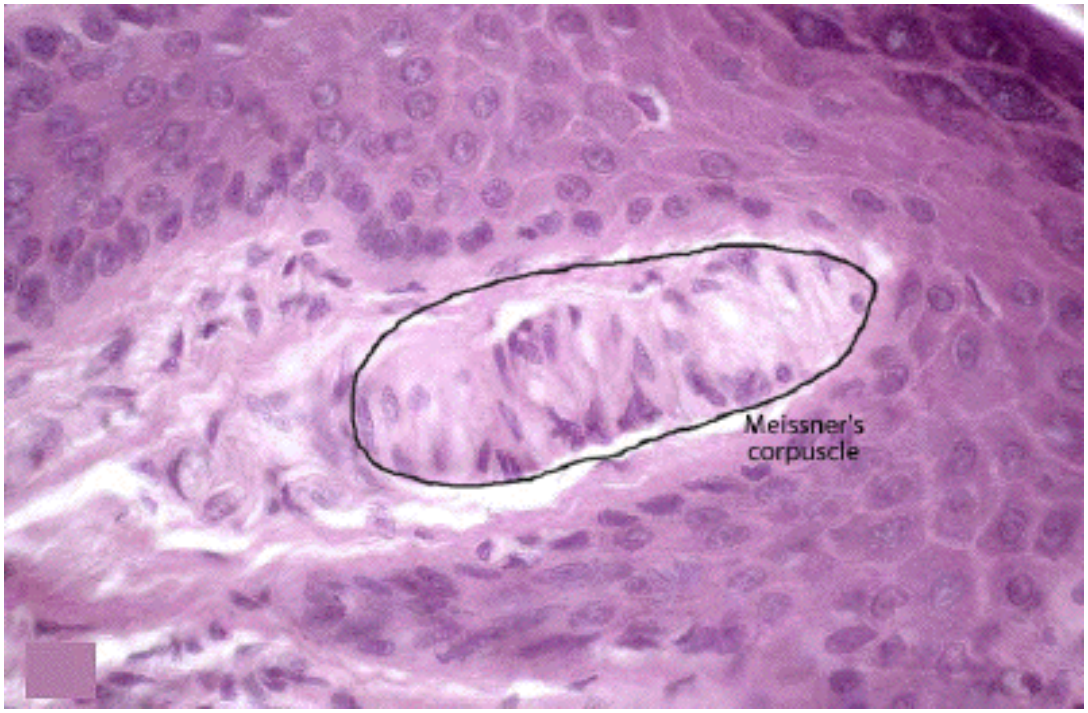


Figure 166. Meissner's corpuscle.

Distribution: papillary layer of dermis.

3. **Ruffini corpuscles** (fig. 167) are elongated, spindle-shaped capsular specializations are located deep in the skin, as well as in ligaments and tendons. The long axis of the corpuscle is usually oriented parallel to the stretch lines in skin; thus, Ruffini corpuscles are particularly sensitive to the cutaneous stretching produced by digit or limb movements. They account for about 20% of the receptors in the human hand, are sensitive to skin stretch, and contribute to the kinesthetic sense of and control of finger position and movement.

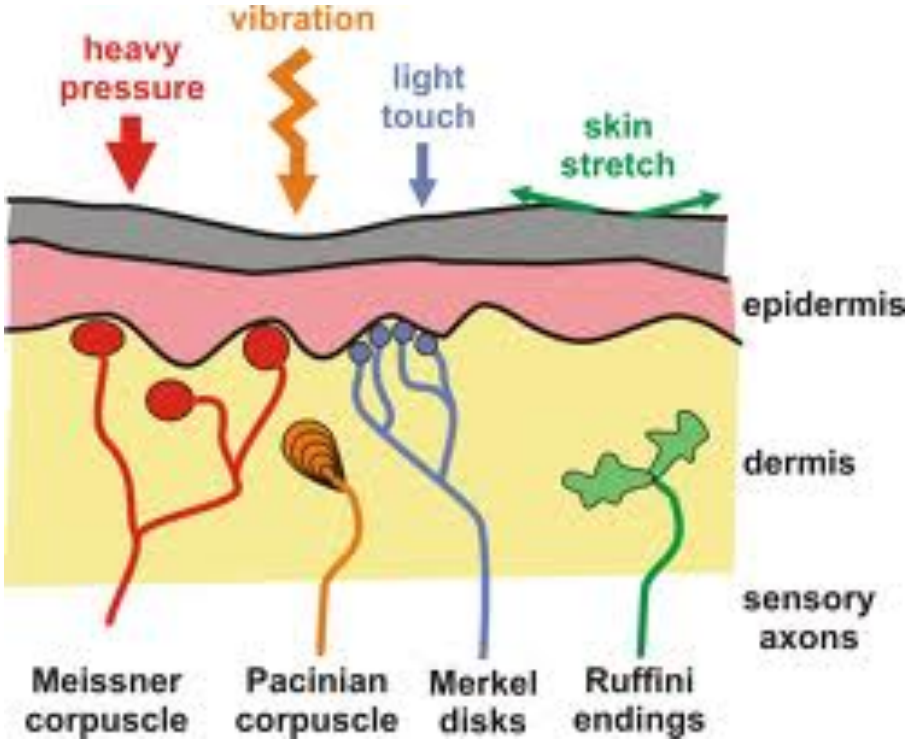


Figure 167. The various types of nonfree encapsulated nerve ending .

4. **End-bulb of Krause** (fig. 168) has round shape, contains glial cells and capsule. It receives cold sensation.

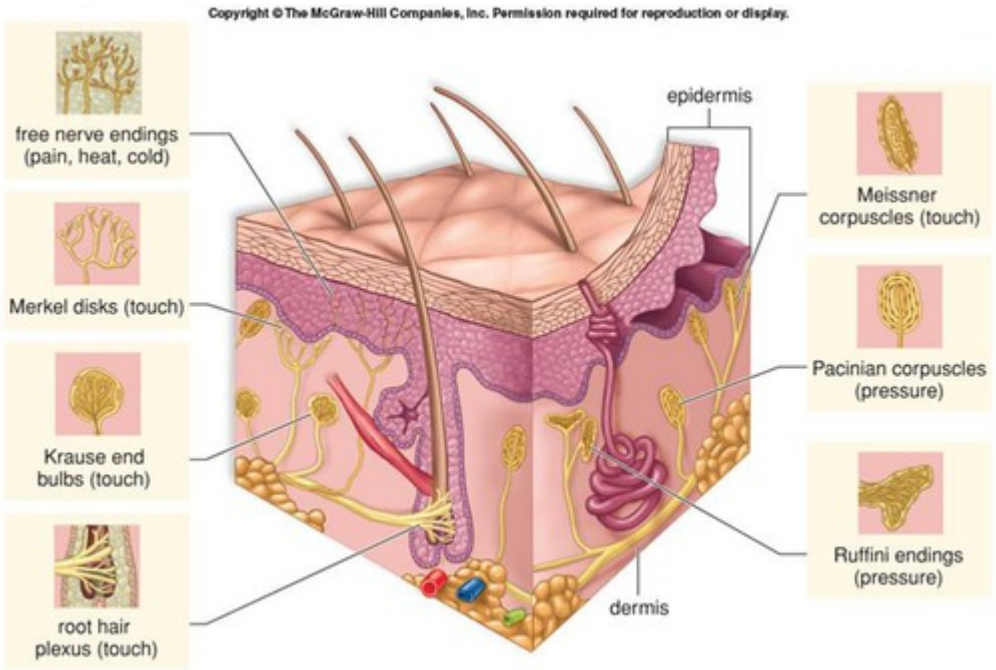


Figure 168. The various types of nerve ending

Distribution: papillary layer of dermis, lamina propria of mucosa of oral cavity.

5. **Neuromuscular spindles** (fig. 169) are sensory end organs in skeletal muscle. They consist of 6-14 modified skeletal muscle fibres called intraf usal fibres which are of two types - nuclear bag and nuclear chain fibres; they are supplied both by motor and sensory fibres. This complex of muscle and nerve is enclosed in a connective tissue capsule and is known as muscle spindle; they give information about the length of muscle.

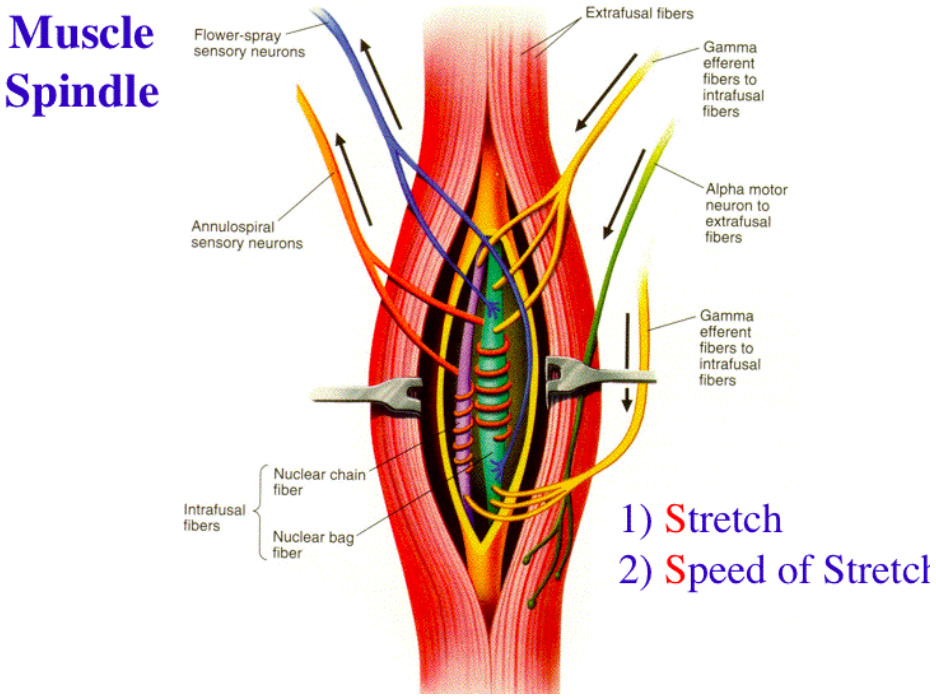


Figure 169. Structure of the neuromuscular spindles.

6. **Neurotendinous organ of Golgi** is spindle structure locating in region of connection of skeletal muscles fibers with collagen fibers of tendons. Exaltation of receptors arises at a distension of a tendon during a muscular contraction.

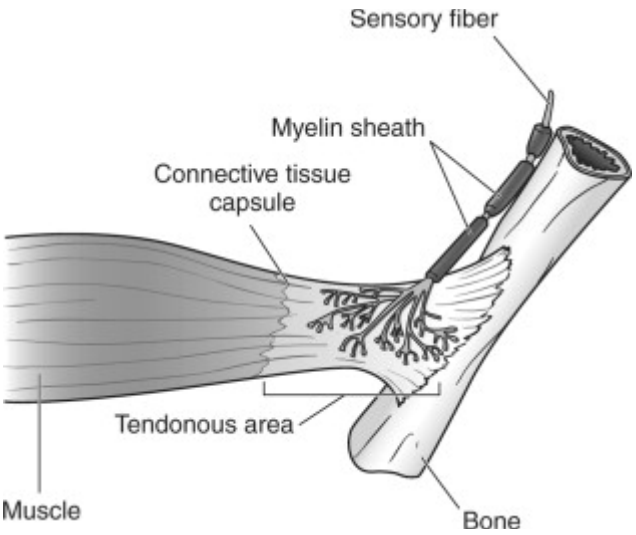


Figure 170. Structure of the neurotendinous spindles.

Motor (efferent) endings

The various types of motor (efferent) endings are:

1. **Motor end plate** (in skeletal muscle) (fig. 171) is the junctional area between the motor nerve terminal and the skeletal muscle. It consists of two parts, a nervous and a muscular, separated by a cleft; the relationship is a c lose apposition of axolemma and sarcolemma.

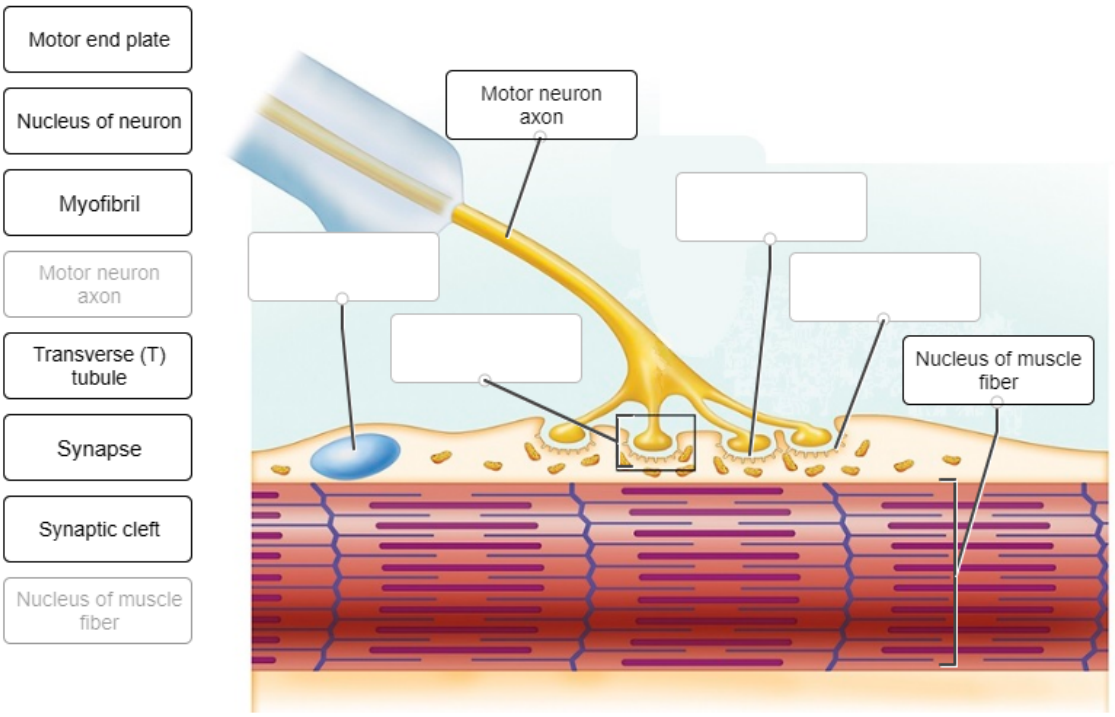


Figure 171. Structure of the motor end plate (in skeletal muscle).

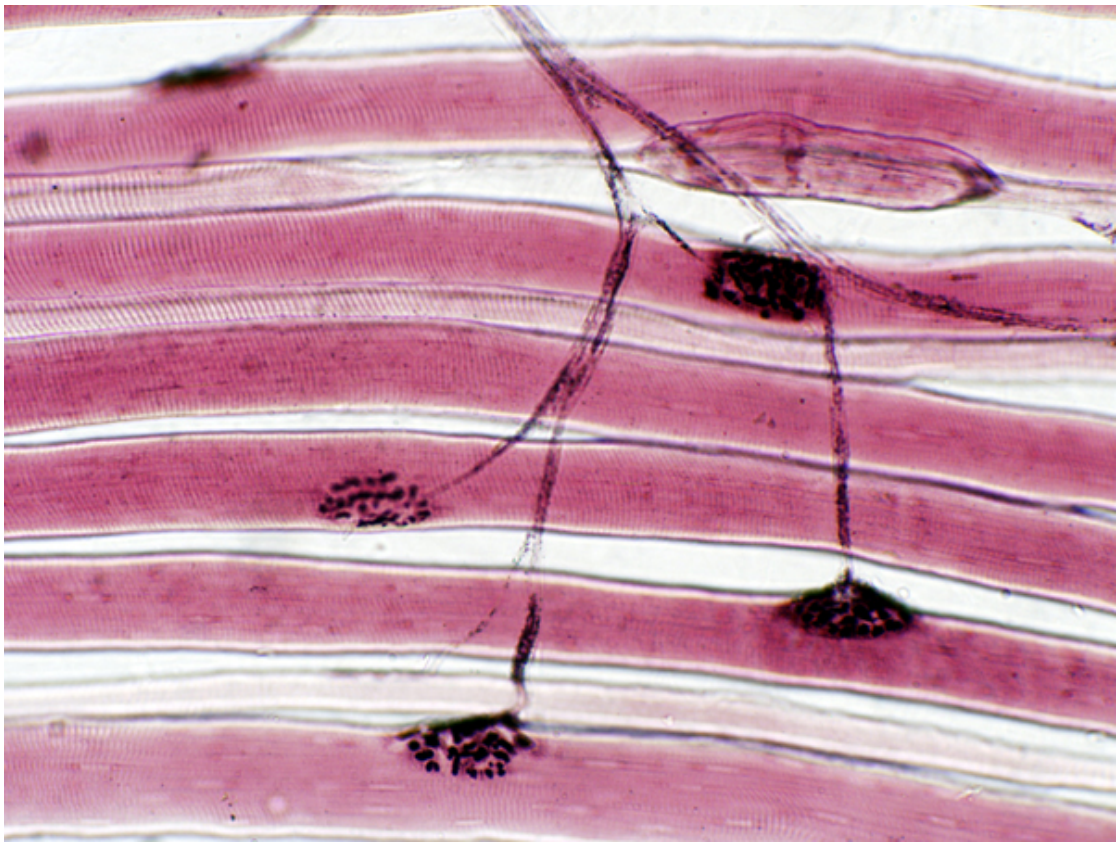


Figure 172. Structure of the motor end plate (in skeletal muscle).

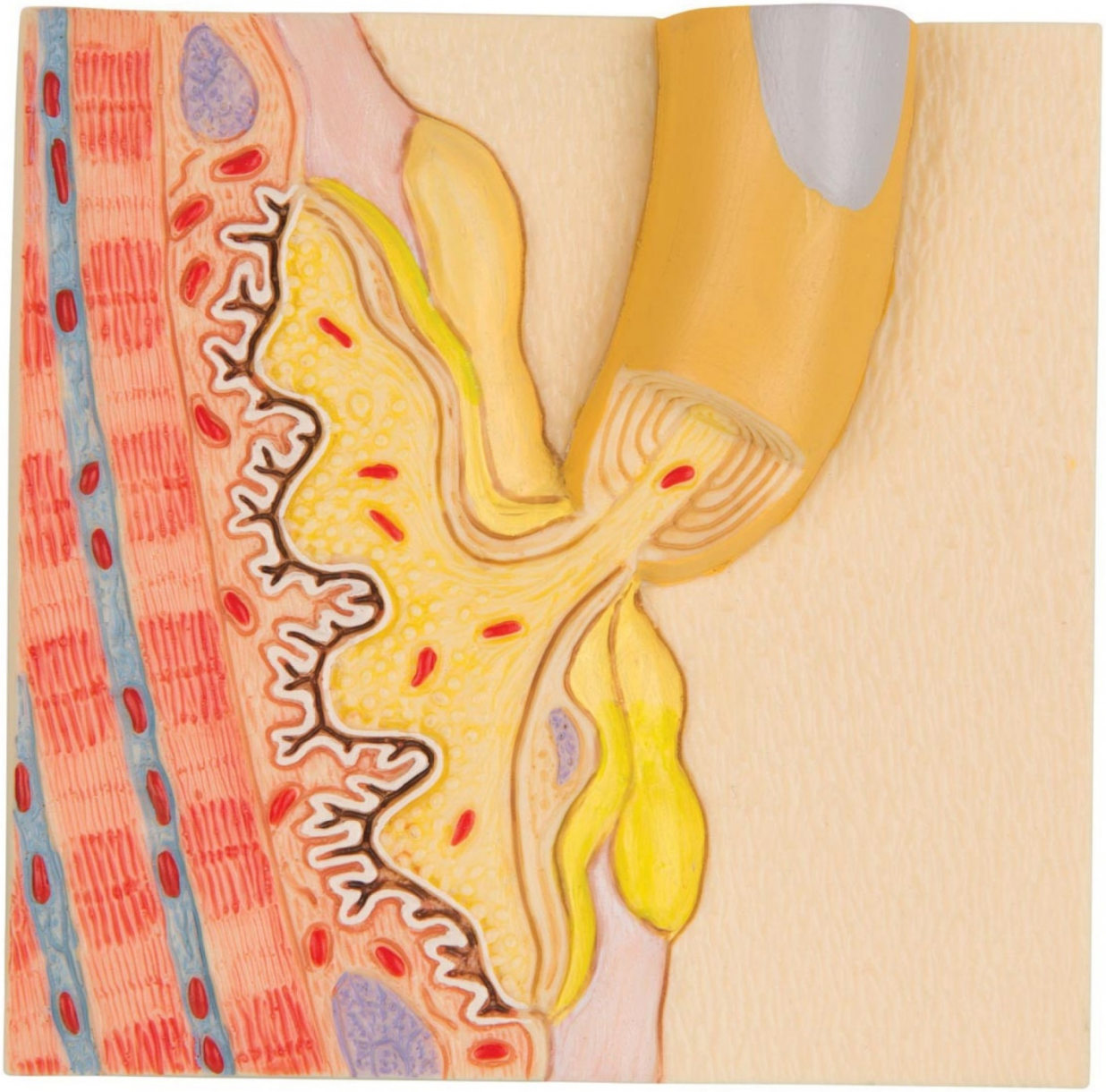
Each muscle fiber receives one motor end plate, but each axon by virtue of its branching supplies several muscle fibers. A motor neuron together with all the muscle fibers which it innervates is called a motor unit.

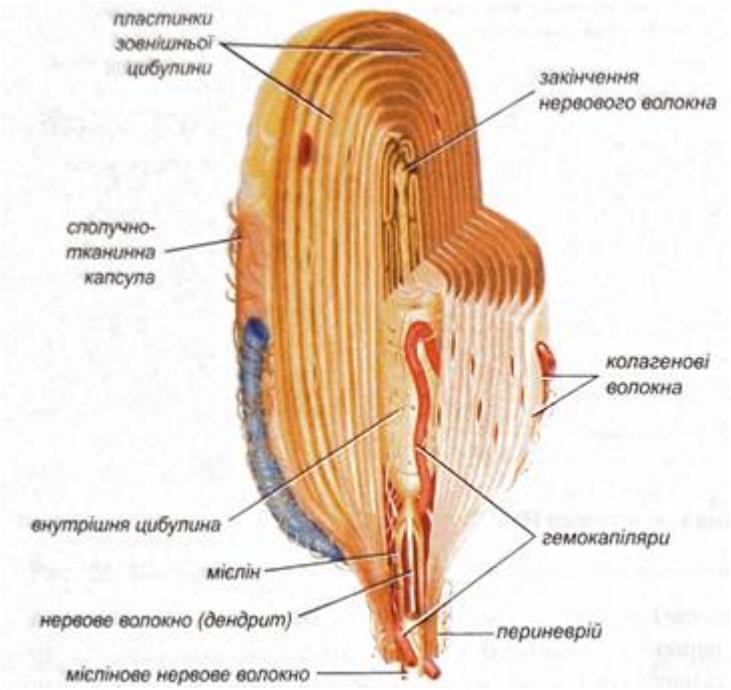
2. In cardiac and smooth muscles, the nonmyelinated fibers end by one or more terminal knobs on the plasma membrane, whereas in gland cells they end among the epithelial cells.

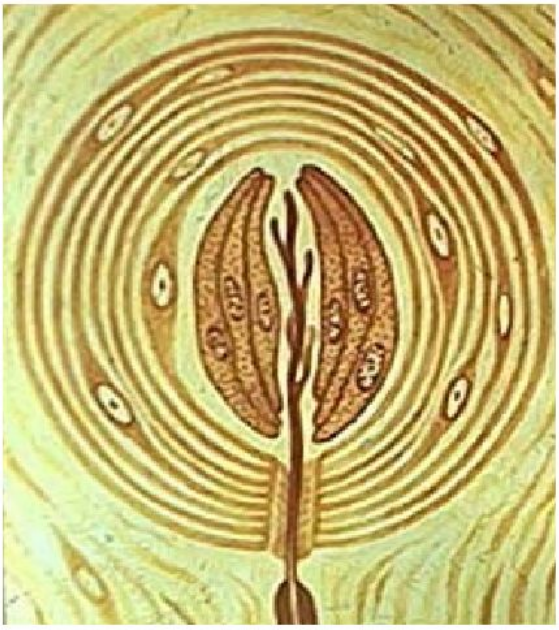
Practical lessons № 17

Questions for self-control

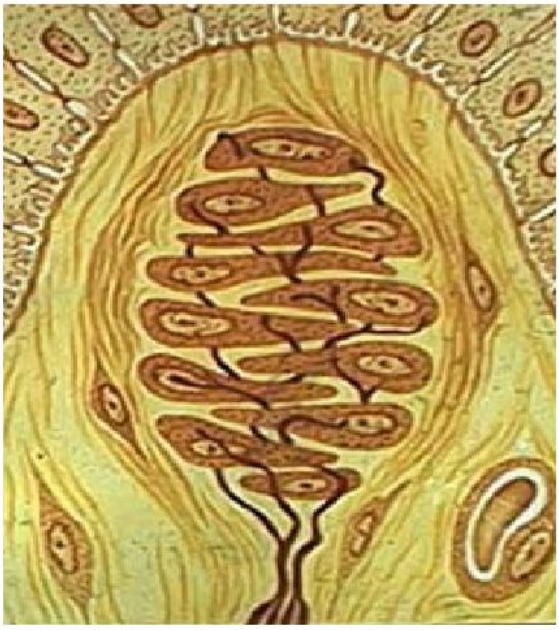
1. Classification of nervous endings.
2. Receptors: classification, structure and functions.
3. Synapses: classification, structure and functions.
4. Effectors: classification, structure and functions.
5. Neuromuscular spindle.
6. Motor end plate.



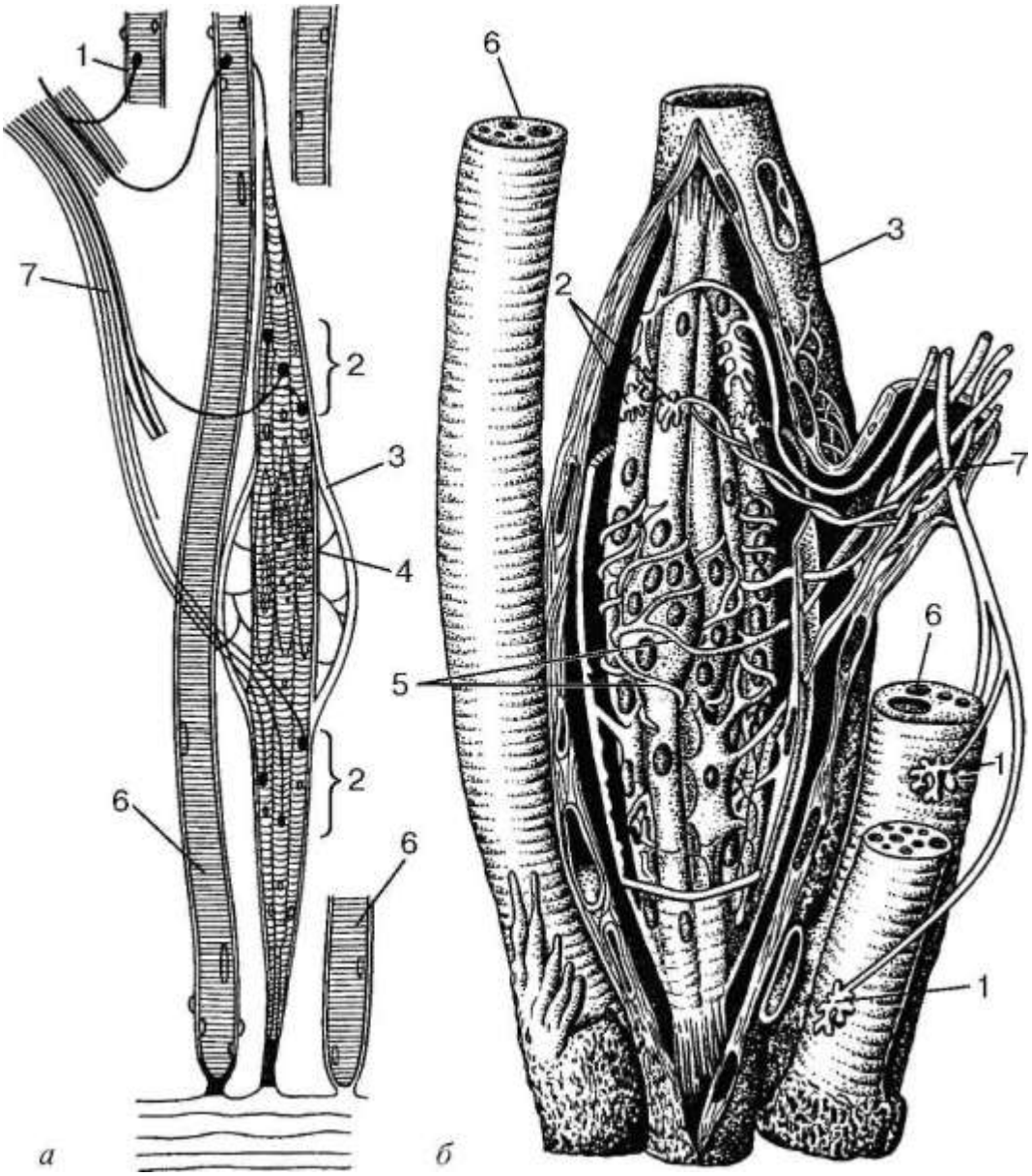




Пластинчатое тельце Фатера-Пачини (давление)



Осязательное тельце Мейснера



The teacher's signature:

STRUCTURE OF PERIPHERAL NERVE

The individual nerve fibers are held together by connective tissue organized into three components (fig. 173):

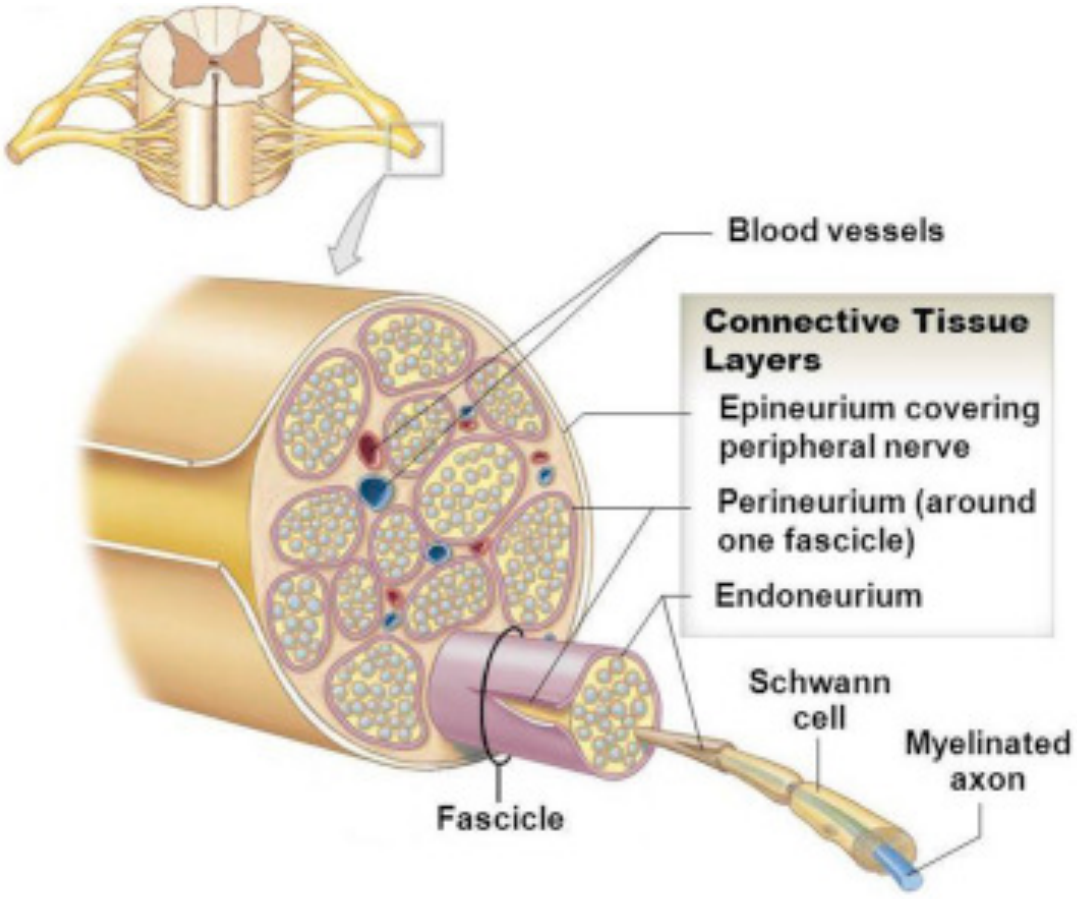


Figure 173. Schematic diagram of peripheral nerve.

1. **endoneurium** is a thin layer of loose connective tissue, surrounding each individual nerve fiber;
2**. perineurium** surrounds each bundle of nerve fibers;
3**. epineurium** includes the dense irregular connective tissue that surrounds a peripheral nerve.

The nerves establish communication between brain and spinal cord centers and the sense organs and effectors (muscles, glands, etc.).

Axonal Damage

Wallerian Degeneration. Wallerian degeneration refers to the changes that occur distally to the site of damage on an axon. Because protein synthesis occurs primarily in the neuronal cell body, the segment distal to the damaged site on the axon is affected profoundly. Initially, the axon swells up and becomes irregular. Later, the axon and the terminal are broken down into fragments that are phago-cytosed by adjacent macrophages and Schwann cells (Fig.2 A-D). Myelin is converted into fine drops of lipid material in the Schwann cells and is extruded from these cells; it is removed by macrophages in the PNS and microglial cells and invading macrophages in the CNS.

Alterations (similar to those mentioned earlier) may also be present in the proximal segment of the axon up to the first node of Ranvier.

Chromatolysis. Sectioning of an axon may produce changes in the cell body, and if the injury is close to the cell body, the neuron may degenerate. The cell body swells up due to edema and becomes round in appearance, and the Nissl substance gets distributed throughout the cytoplasm. This process is known as chromatolysis (Fig.172). The nucleus moves from its central position to the

periphery due to edema. The degenerative changes start within hours and are complete within a relatively short time (about a week).

Anterograde Transneuronal Degeneration. Anterograde transneu-ronal degeneration occurs in the CNS when damage to a neuron results in the degeneration of another postsynaptic neuron closely associated with the same function (Fig.174). For example, damage to an optic nerve results in the degeneration of the lateral geniculate neurons receiving inputs from this nerve. **Retrograde Transneuronal Degeneration**. Retrograde transneu' ronal degeneration occurs in neurons sending inputs to an injured neuron. In this situation, terminals of the neuron synapsing with a chromatolytic neuron withdraw and are replaced by processes of glial cells. The neuron, from which the inputs to the chromatolytic neuron arise, eventually degenerates (Fig.174).

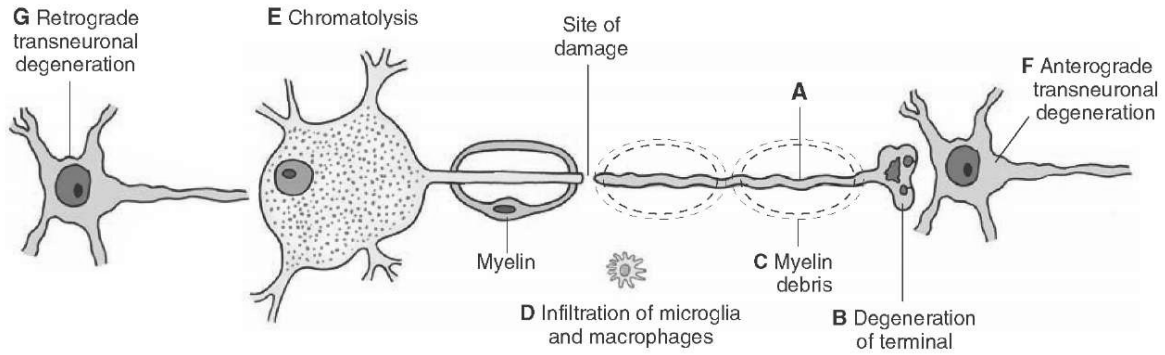


*Figure 174. Schematic diagram of **regeneration** peripheral nerve.*

Recovery of Neuronal Injury (Regeneration)

In the adult CNS**,** if the damage is not severe and some neuronal cell bodies are spared, sprouting of axons does occur, but this process ceases within a short time (about 2 weeks). Astrocytes proliferate at the site of injury in a random fashion and form a scar which acts as a barrier for axonal sprouts. Furthermore, astrocytes may not release growth factors that are needed for axonal growth, and oligodendrocytes may release substances that retard axonal growth. In this situation, regeneration of axonal tracts does not occur, and normal functions of the neurons are not restored. However, in peripheral nerves, an axon can regenerate satisfactorily if the endoneurial sheaths are intact. In this situation, the regenerating axons reach the correct destination, and the chances of recovery of function are reasonable. The growth rate of an axon has been estimated to be **2 to 4 mm per day.**

The ganglia are aggregations of cell bodies of neurons located outside the CNS. There are two types of ganglia - sensory and autonomic**. Sensory (dorsal root, spinal) ganglia**. Sensory ganglia lie along the vertebral column by the spine (fig. 175), contain pseudounipolar cell bodies of sensory neurons.

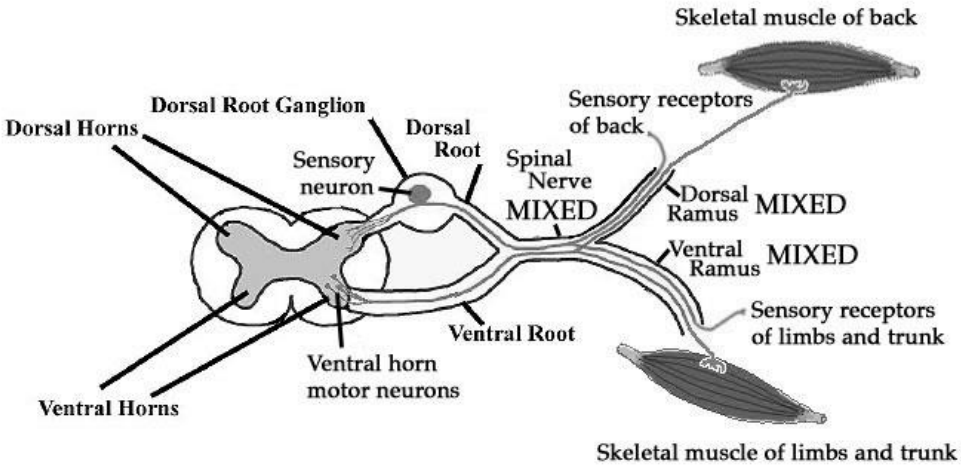


Figure 175. Location of sensory (dorsal root) ganglia.

Sensory ganglia (Dorsal Root Ganglion) of the spinal nerves are called dorsal root ganglia or spinal ganglia. Ganglia associated with cranial nerves are called cranial ganglia. Each spinal ganglion **has a thin connective tissue capsule** within which the nerve cel are peripherally placed (fig. 176). Ganglion cells will typically be several times larger than other cells in the ganglia. The perikaryon is very large and surrounds a large and light nucleus. Only the cells immediately surrounding the ganglion cells as one flattened layer are satellite cells. With a lot of luck you may see the process of a ganglion cell as it passes out of the capsule of satellite cells.

The dorsal root ganglion contains the cell bodies of sensory neurons that bring information from the periphery to the spinal cord. These neurons are pseudounipolar and contain an axon-like process that bifurcates with one branch extending toward the periphery and the other branch heading toward the grey matter of the spinal cord. **Fibers heading toward the periphery leave the ganglion** through the spinal nerve, where they run together with motor fibers. Fibers leading to the spinal cord travel through the dorsal root.

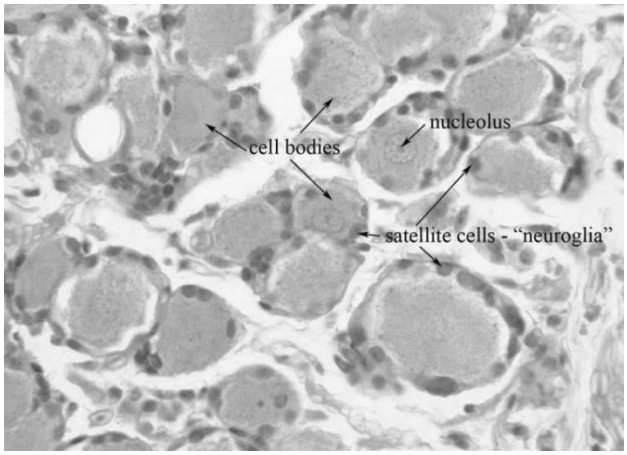
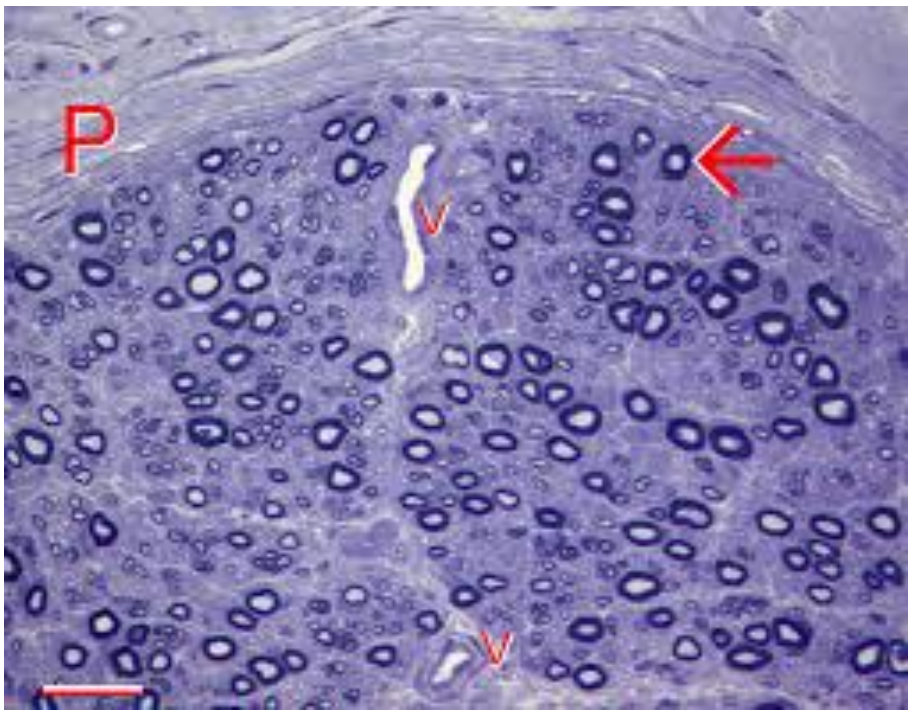


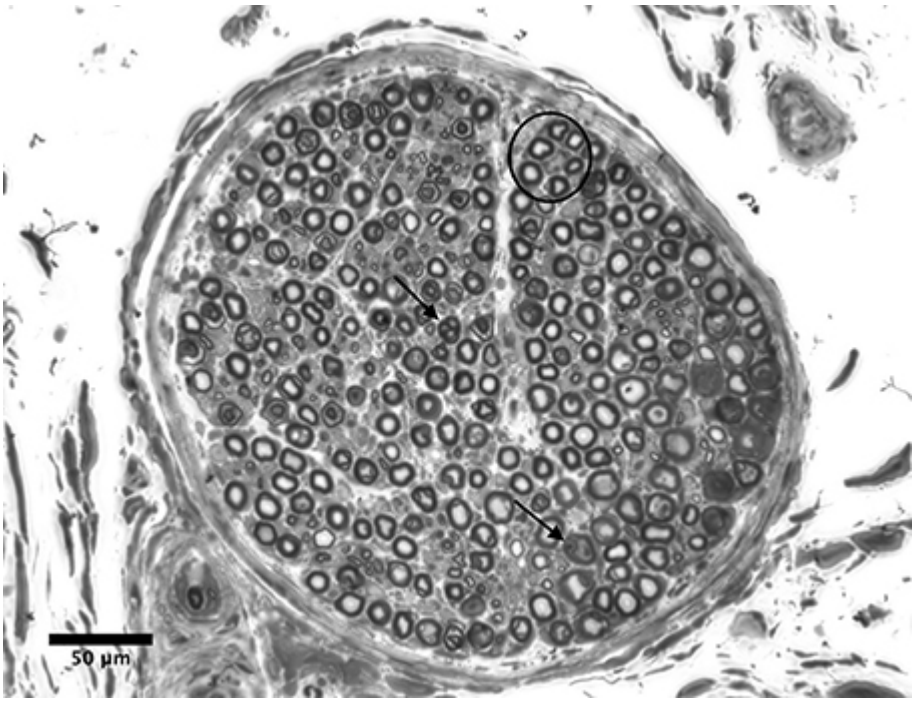
Figure 176. Photomicrograph of sensory ganglion.

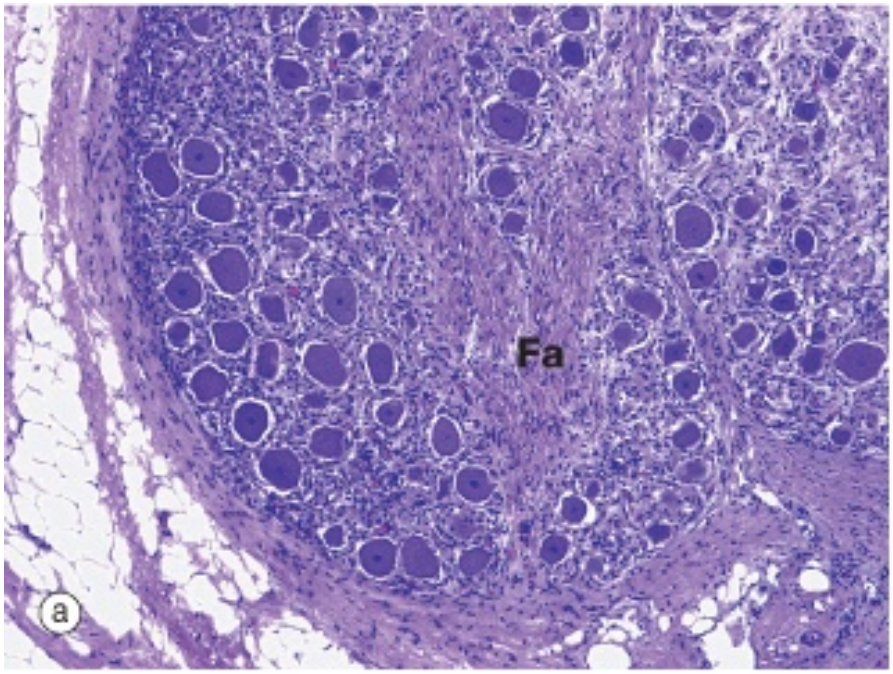
Practical lessons № 18

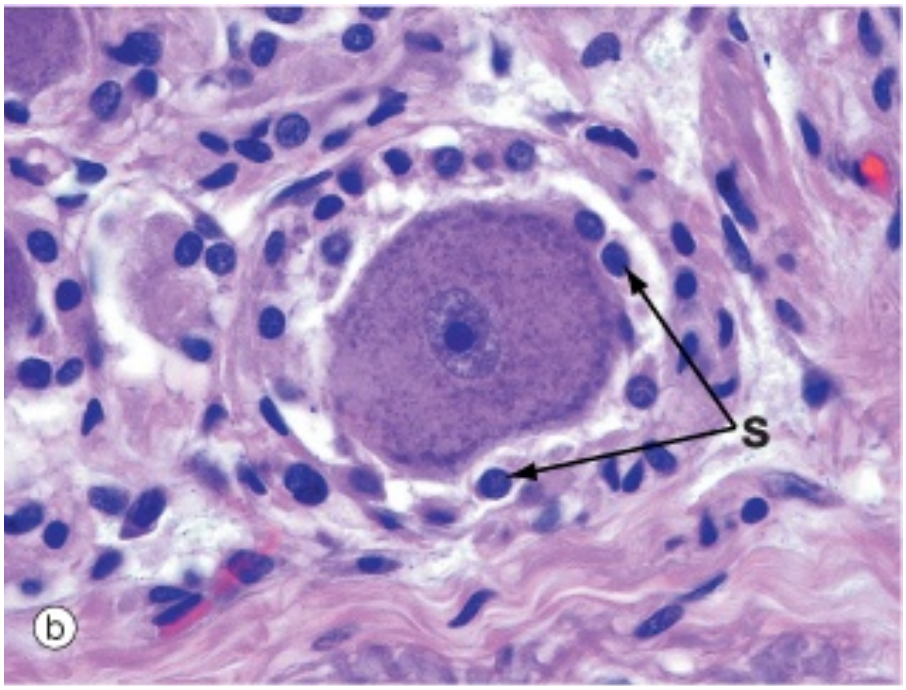
Questions for self-control

1. Structure of of peripheral nerve.
2. Axonal Damage.
3. Structure of spinal nodes (ganglion).


P
V
V


50 µm





The teacher's signature:

Erica Dobryanska

HISTOLOGICAL WORKBOOK. PART I.
Methodological Developments For Laboratory Classes

Monograph

Published: 30.12.2025.
Typeface: Times New Roman.
RS Global Sp. z O.O., Warsaw, Poland, 2025
Numer KRS: 0000672864
REGON: 367026200
NIP: 5213776394