Contents

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Historical introduction

Cytology (Gr. kytos, "cell", -logia, "science"), the study of cells, and **histology** (Gr. histos, "tissue", -logia, "science"), the study of tissues, are among the biological sciences. Therefore, we know cytology and histology of both plants and animals. From the perspective of medicine study of the microscopic structure of the human body has a particular importance. In histology laboratories samples from experimental animals are also processed. Organisms are present in a state of physiological (normal), which are healthy, but also pathological; thus, histology and cytology are further divided into normal and pathological. In 19th century, an already well-known German pathologist, **Rudolf Virchow** (1821 – 1902) found that the majority of pathological changes begin at the cellular level. By that, he actually established the basis for cytopathology and histopathology, and promoted the anatomical pathology to a major scientific discipline. The motto of his teachings was "***Omnis cellula e cellula***", that cell can only come from the cell. Virchow with his views certainly highlighted the significance of the cell and its importance in pathological processes.

The history of cytology and histology is relatively short, and is directly associated with the construction of the light microscope. Microscope, however, gave the basis for other medical or biological sciences, such as, bacteriology, parasitology, hematology, embryology, and others. The short history of histology is demonstrated by the fact that in 1882 still at Charles University in Prague it was taught together with anatomy. The first Czech Associate Professor of Histology and Embryology, **Jan Janošik** (1856 – 1927) habilitated in 1884. An independent histology institute at Charles University was not founded until 1886. The first Institute of Histology and Embryology in Slovakia was founded in 1922 by **Professor Zdeněk Frankenberger** (1892 - 1966) at the Faculty of Medicine of the Comenius University in Bratislava.

Currently the **light microscope** is the basic equipment of histological laboratories. With the help of the microscope we are able to see objects that are too small for the naked eye, i.e. they are smaller than the resolution capability of our eyes (smaller than 0.2 millimeters). Probably the ancient Greeks used a simple lens for magnifying different objects. First eyeglasses appeared in Europe in the 14th century, but the first microscope

was made few hundred years later, the interface between the 16th and the 17th century. History of the light microscope is brief, but it is full of contradictory claims. For example, two countries, The Netherlands and Italy are in disagreement on the place of origin of the microscope. The first microscope to be developed was the optical microscope, although the original inventor is not easy to identify. Evidence points to the first compound microscope appearing in the Netherlands in the late 1500s, probably an invention of eyeglass makers there. Probably Hans Jannsen and his son Zacharias in Middleburg, the Netherlands, somewhere between 1590 and 1618 made the first microscope (a single-lens one, with a total magnification only 10-times). Between the inventors of the microscope are also known Italian physicist, mathematician, astronomer and philosopher Galileo Galilei (1609), Italian physician and biologist Francesco Stelluci (1630) and the Italian physicist Francisco Fontana (1646). The first microscopes were imperfect due to significant spherical and chromatic errors. It is interesting that at that time each researcher and scientist had to cut his/her lens alone.

In biology, however, first time microscope was not used by a scholar or a biologist, but by a person without any knowledge in biology and medicine. It was a Dutch tradesman **Antonie Philips van Leeuwenhoek** (1632 - 1723), whose hobby was cutting lenses. Self-taught, he began his scientific career in his 40s, when he began making hundreds of tiny single-lens microscopes with a magnification of about 200 times. Pioneering the use of now-common microscopic techniques, he was the first human to see microbes and microscopic structures in animals, plants, and minerals. Over 50 years, he wrote only letters, more than 300 of them, and published half of them himself. More than a hundred were published in translation in the Royal Society's Philosophical Transactions. Leeuwenhoek's microscopes were highly protected, and the only person who could secretly use it was his assistant from the shop, Ham. And allegedly he was the first to describe the sperm.

Robert Hooke (1635 – 1703), an English physicist was first to introduced the word "cell" (*cellula*) into biological sciences. In his book "*Micrographia*" which was published in 1665 he used this term to refer to small cavities and pores in the cork, which shape was according to his opinion similar to "monk praying cells". However, Hooke had constructed

a microscope with its own light source. At that time it was just an oil lamp, whose light beams focused on the studied object by a container filled with water.

Imperfection of the microscopes of the 18th century caused the smaller animal cells to become later the focus of interest than those of the plants. The discoveries of the nucleus of animal cells (inside bird´s eggs) or the protoplasm of cells deserve a Czech scientist, the first professor of physiology at the Charles University in Prague, **Ján Evangelista Purkyně** (1787 - 1869). He was the most formidable person in Middle-Europe histology and physiology on the world who brought many scientific discoveries into the biological, physiological and biomedical sciences. Among the systems to which he paid attention it was mainly the nervous system and especially the cerebellum. The works of J. E. Purkyně, showed that the nervous system contains not only nerve fibers, but also cellular elements (in 1837 he described these cells as "small granules", because the term "cell" was not commonly used in human histology – it was two years before the introduction of the cell theory which describes the properties of cells as the basic units of all structure in all organisms). He was the first scientist to see and describe neurons containing black melanin granules in the part of middle brain called *substantia nigra* and the largest nerve cells in the cortex of cerebellum. Later, the Nobel Prize laureate Santiago Ramon y Cajal (1911), one of the most distinguished researchers of the nervous system, recommended in his monumental monography to designate these large neurons according an author who described them for the first time as "Purkynje cells". And also the part of the conducting system of the heart is named after Professor Purkyně ("Purkynje fibers").

The methods used in histologic laboratories are extremely diverse. Histological techniques and methods have undergone significant changes and improvements from tissue sampling gained by needles, surgery or squishing on a slide, until obtaining a fine and thin, often only few micrometer using a microtome. Microtome is a fine cutting instrument to obtain fine and thin, precisely cut sections of few micrometers that are previously prepared for sectioning and was first described by Purkinje's assistant Oschatz.

With a gradual development of the textile and leather industries, and improvements, different new staining methods were introduced

into cytology and histology to visualize different structures of cells and tissues. However, cytology and histology are experiencing the greatest developments and improvements in recent decades, with the use of the **electron microscope** (German physicist Ernst Ruska and the electrical engineer Max Knoll constructed the prototype electron microscope in 1931), **histochemical and immunohistochemical methods** (American pathologist Albert Coons was the first person to conceptualize and develop immunofluorescent techniques for labeling antibodies in the early 1940s), molecular biology techniques, as well as many other methods.

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1. Methods of study

1.1. Light microscopy

Stained sections are usually examined with the light microscope by transillumination. The microscope is composed of both mechanical and optical parts, and the light source.

The mechanical part is composed of *focus adjustment knob, stage position adjustment, and stage* as it is illustrated in Fig. 1.

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 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.

Resolution or *resolving power* 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 μm. This permits good images magnified 1000- 1500 times. Magnification is independent of 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 lenses.

1.1.1. Instructions for the use of a light microscope

1. Switch on the light and place the object-specimen on the stage.
2. Focus the specimen with low-power objective using the coarse adjustment.
3. Make sure that iris diaphragm of the substage condenser is widely open.
4. Apply the eyes onto the oculars and observe if the field is well illuminated.
5. Next, slowly and carefully focus up with a coarse adjustment until the object is brought and then use the fine adjustment to secure a sharp focus.

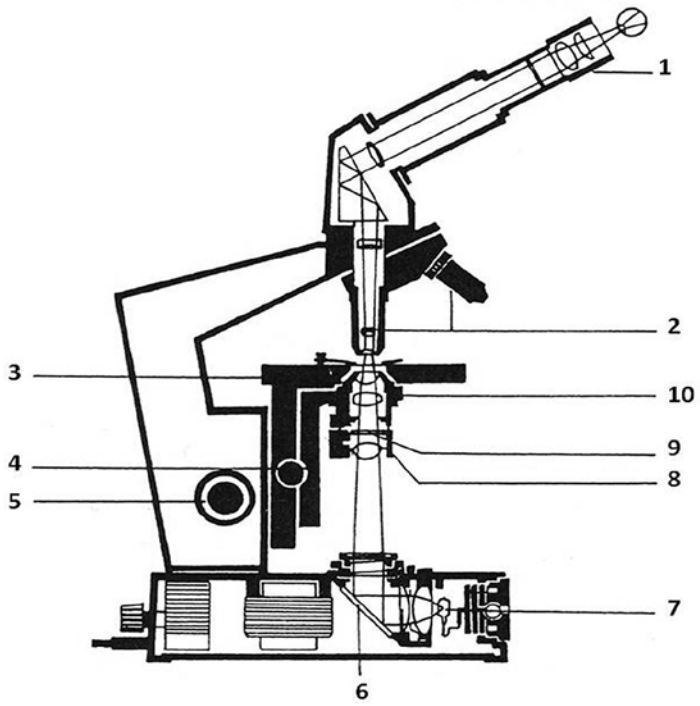

Fig. 1.
1-ocular lens, 2-objective lens, 3-stage, 4-stage position adjustment, 5-focus adjustment knob, 6-mirror, 7-lamp, 8-lens, 9-filter, 10-condenser

1.2. Types of microscopes

1.2.1. Phase contrast microscopy

The phase contrast microscope enables the examination of unstained cells and tissues and is especially useful for living cells. The principle of this microscopy takes advantage of the fact that light changes its speed and direction when passing through cellular and extracellular structures with different index of refraction. Light passing through areas of relatively high refractive index (denser areas) is deflected and becomes out of phase with the rest of the beam of light that has passed through the specimen. Dark portions of the image correspond to dense portions of the specimen, and light portions of the image correspond to less dense – light portions of the specimen. Two modifications of the phase contrast microscope

are the interference microscope, which also allows for quantitation of tissue mass, and the differential interference microscope (using Nomarski optics), which is especially useful for assessing surface properties of cells and other biologic objects.

1.2.2. Confocal microscopy

This type of microscopy uses laser and computer to produce three-dimensional image of living cells and tissue specimens. 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 very thin plane of section in a computer allows a three-dimensional image to be reconstructed.

1.2.3. Polarizing microscopy

In the polarizing microscope a polarizing filter, called the *polarizer,* is located between the light source and the specimen and a second polarizer, called the *analyzer* is located between the objective lens and the viewer. Both the polarizer and the analyzer can be rotated. The ability to rotate the direction of polarized light is called birefringence and is present in crystalline substances or substances containing oriented molecules – collagen, microtubules, microfilaments, striated muscle fibers, and crystalloid – Leydig cells.

1.2.4. Fluorescence microscopy

The fluorescence microscope utilizes the fact that certain molecules emit light after irradiation with ultraviolet light. This emission is in the visible portion of the spectrum. The fluorescent substances appear as brilliant 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 observer eyes.

Fluorescent compounds that have affinity for cell macromolecules may be used as fluorescent dyes. There are some naturally fluorescent substances – vitamin A, vitamin B_2, and porphyrins. Acridine orange is used as a fluorescent dye that can combine with DNA and RNA. In the fluorescence microscope the DNA-acridine orange complex emits a yellowish-green light, and RNA-acridine orange complex emits a

reddish- orange light. It is thus possible to identify and localize nucleic acids in the cells.

1.2.5. Electron microscopy

The electron microscopy is a system that theoretically permits very high resolution – 0.1 nm. This is 400 times greater than those achieved with light microscopy and allows magnification of up to 400,000 times.

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

A transmission electron microscope transmits a beam of electrons through an ultrathin section of tissue that has been cut via an ultramicrotome. Electrons are produced by high temperature heating of a metallic tungsten filament (cathode) in a vacuum. The emitted electrons are then accelerated by a potential difference between the cathode and anode - which is a metallic plate with a hole in the center. Some of the electrons pass through the central opening in the anode forming a constant beam of electrons. The beam can be deflected by electromagnetic fields in a manner similar to light deflection in glass lenses. The condenser focuses the beam at the object, the objective forms the image, and image is enlarged by projector and finally seen on a fluorescent screen or is projected to photographic plates (Fig. 2). Electrons are not visible by naked eye that is why a fluorescent screen or photographic plate must be used for recording the image as a black and white electron micrograph. Dark areas of an electron micrograph are usually called electron dense, whereas light areas are called electron lucent.

Scanning electron microscopy permits three-dimensional views of the surfaces of cells and tissues. In the scanning electron microscope the electrons do not pass through the object and it is not necessary to cut ultrathin sections. The beam of electrons is moved sequentially from point to point across the surface to be examined. The primary electron beam interacts with a thin metal coating on the surface of the specimen and produces reflected secondary electrons that are scanned in synchrony with the primary electron beam of microscope. The resulting picture is recorded photographically.

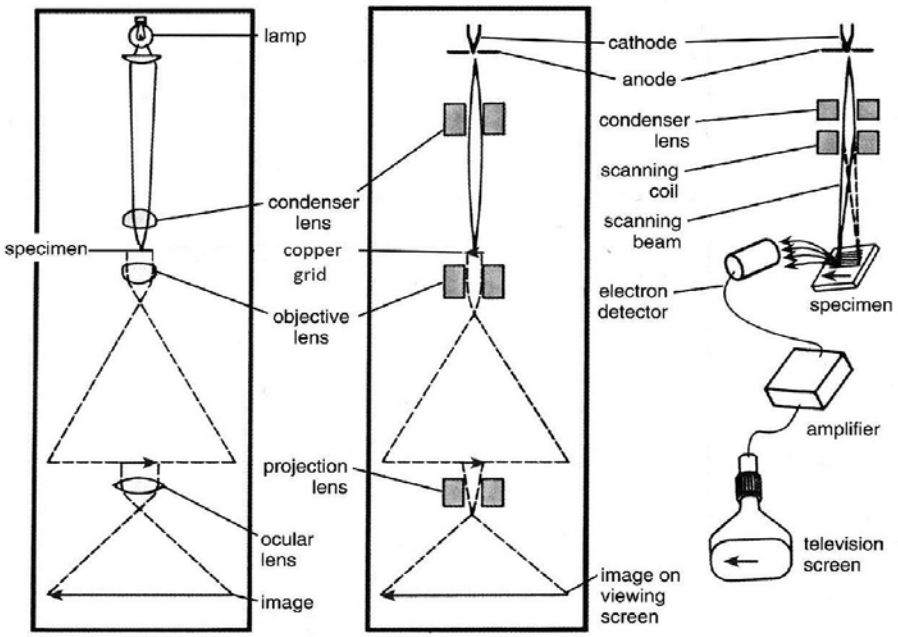

Fig. 2. Scheme comparing the optical path in different types of microscopes
A-light microscope, B-transmission electron microscope, C-scanning electron microscope

1.3. Preparation of tissues for microscopic examination

The direct examination of living cells and tissues is possible only to a limited extent. That is why, cells, tissues, and organs cannot be studied to advantage unless they are suitably prepared for microscopic examination. Histologic techniques are preparations of tissues for examination with the aid of light microscope. The ideal microscope tissue preparation should preserve the tissue on the slide so that it has the same structure and molecular composition as it had in the living body. There are 6 steps during preparation of permanent histologic slide:

1.3.1 Sampling

For histologic examination we can obtain tissue sample from cadaver during pathologic

dissection – *necropsy.*

Tissue sample from living organism is called – *biopsy.*
Methods of biopsy:

A. Needle biopsy – aspiration of small volume of the tissue with thick injection needle (liver, kidney, bone marrow).
B. Direct excision biopsy – section of small sample from the organ. Excision is a small fragment of the tissue. Its size is about 0.5 cm^3.
C. Curettage biopsy – with using curettage we can obtain superficial lining of hollow organs (endometrial lining of uterus).
D. Endoscopic biopsy – endoscope is an instrument used to obtain a view from the interior of the hollow organs – stomach, intestines.

Example:

Gastroscope – optical instrument used to inspect the interior of the stomach and it allows taking a small piece of tissue for microscopic examination and therapeutic procedures.

1.3.2. Fixation

Fixatives prevent tissue from postmortem degeneration and morphologic and molecular changes. The chemical substances used to preserve tissues are called fixatives. Fixatives also harden soft tissues.
Fixation is a fast coagulation of proteins in order to avoid tissue digestion from hydrolytic enzymes (autolysis).
Fixatives:
Physical fixatives – warm, freezing.
Freeze-drying method – a tissue is frozen and then dehydrated at low temperature in a high vacuum.
Chemical fixatives – the fixatives are used singly or in combination.
Simple chemical fixatives: 10% solution of formalin
alcohol
glutaraldehyd
osmium tetroxide - OsO_4
Mixtures: Bouin's fluid (picric acid, formalin)
Zenker's fluid (formalin, mercuric chloride $HgCl_2$, potassium dichromate $K_2Cr_2O_7$).

Characteristics of the good fixatives:

- penetrate easily to the tissue

- keep the structure of the tissue
- allow appropriate staining

1.3.3. Embedding

To obtain histologic sections that are sufficiently thin (about 5 μm), it is necessary to infiltrate the tissue after fixation with embedding substances:

- gelatin
- paraffin (for soft tissues)
- celloidin (for hard tissues)
- other plastic resins

Tissues and the embedding substances are sectioned together.

Paraffin embedding

1. Dehydration of tissue – the water is extracted from the excisions with graded series of ethanol from 70% up to 100% or acetone.
2. Ethanol replacement with paraffin solvents (benzene or xylene). As the tissue becomes impregnated with solvent, it usually become transparent-clearing.
3. The tissue is infiltrated with melted paraffin usually at 58°C. The whole tissue becomes filled with paraffin.
4. Infiltrated excisions are embedded in paraffin in small boxes. The paraffin hardens at room temperature.

1.3.4. Sectioning

Paraffin blocks

Paraffin blocks or tissue blocks are attached to the wooden or plastic pads. The small blocks of paraffin containing the tissue are sectioned with special instruments - microtomes. The slices – sections (5-10 μm) are spread on a glass slide and stained. Then are mounted in resin (canadian balm, entellan) and covered by thin glass - cover slip.

Celoidin blocks

Celoidin is used for hard tissues (bone, teeth, cartilage). Embedding in celoidin is used at room temperature. It is rather slow process and sections cannot be cut as thin as paraffin blocks.

Frozen excisions without embedding

On some occasions, only frozen tissue blocks are sectioned on freezing microtome to avoid the extraction of lipids in the process of dehydration. Sections

of fresh or fixed tissue may also be cut with a freezing microtome – cryostat after embedding in gelatin or without use of any embedding substance.

The frozen section method has advantage of great speed - often used for surgical biopsies during operation.

1.3.5. Staining

Most tissues in the human body are colorless, so observing them unstained in the light microscope is useless. Histological staining methods have been developed in accordance with their ability to stain the tissue components differently or to enhance the contrast between them. The sections may be stained by any of a great variety of combination of dyes. The dyes stain various tissue components more or less selectively. The dyes behave like acidic or basic compounds and have a tendency to form electrostatic linkages with ionizable radicals of the tissues.

Basic dyes (cationic) – hematoxylin, methylene blue, toluidine blue.

Acidic dyes (anionic) – eosin, orange G.

Some components of tissues are stained more readily with acidic dyes – *acidophilia* or with basic dyes – *basophilia*.

Acidophilic structures of tissues – erythrocytes, collagen fibers, cytoplasm.

Basophilic structures of tissues – nucleus, granular endoplasmic reticulum.

The most commonly used staining method is HEMATOXYLIN-EOSIN method (H&E).

Hematoxylin is a basic dye and stains basophilic material in sections in blue or purple.

Example: Nuclear structures are basophilic and they are stained by hematoxylin in blue or purple.

Eosin is an acidic dye and stains acidophilic material in sections pink or red.

Example: Collagen fibers are acidophilic/eosinophilic. They are stained by eosin in pink or red. Terms acidophilic and eosinophilic are synonymous words.

1.3.5.1. H&E staining method

a. DEPARAFINATION – dissolution of paraffin with xylene
b. REHYDRATION – with ethanol 100%, 96%, 80%, H_2O
c. STAINING
   - hematoxylin
   - H_2O

- acidic ethanol
- eosin
- H_2O

d. DEHYDRATION - ethanol 96%, 100% (ethanol removes water from sections)
e. CLEARING - with xylene.

1.3.6. Mouting in resin

Resin is transparent and keeps the color and structure of the section – canadian balm, entellan. The section with a drop of resin is covered by a cover slip and after evaporation of solvent (xylen) resin get hard and permanent histologic slide gives rise.

1.4. Histochemical methods

Demonstrate presence of chemical substances in the tissues. They include chemical reactions that permit identification of lipids, carbohydrates (glycogen, mucopolysacharide), proteins and enzymes (phosphatase).

1.4.1. Demonstration of polysaccharides and glycogen by PAS reaction

This method is based on oxidative reaction of **P**eriodic **A**cid with polysaccharides. The reaction gives rise to aldehyde groups that react with **S**chiffs reagent and produce *red reaction product* on the structures where polysaccharides are present.

This method demonstrates polysaccharides in the tissue sections = *PAS positive* (glycogen, glycoproteins, glycolipids).

1.4.2. Demonstration of lipids

Lipids are best revealed with dyes that are more soluble in lipids than in medium in which the dye is dissolved. In this process, frozen sections are immersed in alcoholic solutions saturated with the appropriate dye. The dye then migrates from the alcohol to the cellular lipid droplets. The dyes most commonly used for this purpose are Sudan IV and Sudan black. They confer red or black colors on the lipids.

1.4.3. Demonstration of enzymes

The enzymatic histochemical procedures are based on the production of intensely stained or electron-dense precipitates at the site of enzymatic activity.

Often acid phosphatase is demonstrated. This method is fast and specific. During this reaction acid phosphatase in the cells or tissues split the added naphtyl phosphate and resulted naphtol reacts with the diazonium salt giving rise to black or red precipitates.

1.5. Immunohistochemical methods

Immunohistochemistry is the localization of antigens in tissue sections by the use of labeled antibody as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

Albert H. Coons and his colleagues (Coons et al. 1941, 1955; Coons and Kaplan 1950) were the first to label antibodies with a fluorescent dye, and use it to identify antigens in tissue sections. With the expansion and development of immunohistochemistry technique, enzyme labels have been introduced such as peroxidase (Nakane and Pierce 1966; Avrameas and Uriel 1966) and alkaline phosphatase (Mason and Sammons 1978). Colloidal gold (Faulk and Taylor 1971) label has also been discovered and used to identify immunohistochemical reactions at both light and electron microscopy level. Other labels include radioactive elements, and the immunoreaction can be visualized by autoradiography.

Since immunohistochemistry involves specific antigen-antibody reaction, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes and tissue structures. Therefore, immunohistochemistry has become a crucial technique and widely used in many medical research laboratories as well as clinical diagnostics.

1.5.1. Target antigen detection methods

1.5.1.1. Direct method

The direct method is a one-step staining method and involves a labeled antibody reacting directly with the antigen in tissue sections. While this

technique utilizes only one antibody and therefore is simple and rapid, the sensitivity is lower due to little signal amplification, such as with indirect methods, and is less commonly used than indirect methods (Fig.3).

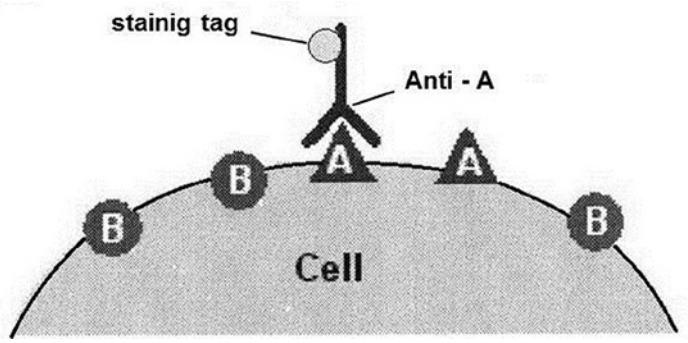

Fig. 3. The direct method of immunohistochemical reaction uses one labelled antibody, which binds directly to the antigen being stained for

1.5.1.2. Indirect method

The indirect method involves an unlabeled primary antibody (first layer) that binds to the target antigen in the tissue and a labeled secondary antibody (second layer) that reacts with the primary antibody. As mentioned above, the secondary antibody must be raised against the IgG of the animal species in which the primary antibody has been raised (Fig. 4).

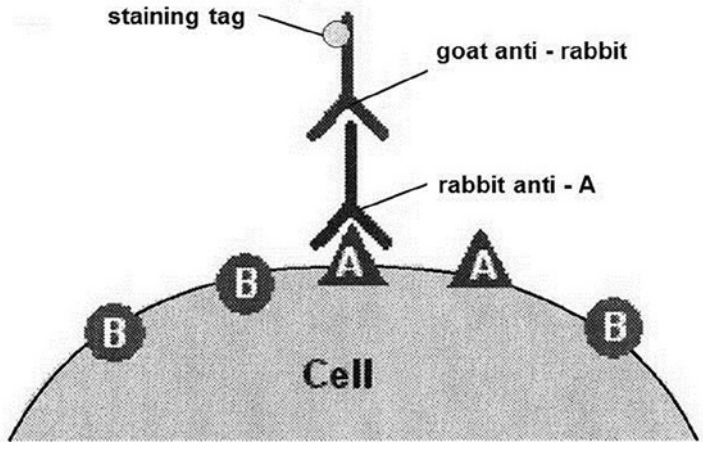

Fig. 4. The indirect immunohistochemical method uses one antibody against the antigen being probed for, and a second, labeled antibody against the first

This method is more sensitive than direct detection strategies because of signal amplification due to the binding of several secondary antibodies to each primary antibody if the secondary antibody is conjugated to the fluorescent or enzyme reporter. Further amplification can be achieved if the secondary antibody is conjugated to several biotin molecules, which can recruit complexes of avidin-, streptavidin or neutravidin protein bound-enzyme. The difference between these three biotin-binding proteins is their individual binding affinity to endogenous tissue targets leading to nonspecific binding and high background; the ranking of these proteins based on their nonspecific binding affinities, from highest to lowest, is: 1) avidin, 2) streptavidin and 3) neutravidin protein.

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2. Functional cytology

2.1. Introduction

The cell is the basic structural unit of life. There are two cell types; prokaryotic and eukaryotic. Multicellular organisms are composed of eukaryotic cells, which are characterized by the presence of a defined nucleus as opposed to prokaryotic cells. Besides the nucleus, eukaryotic cells contain other compartments called organelles. All cellular components are enclosed by a system of internal membranes. All organelles carry out a specific function in cell metabolism and growth. They have unique membrane structure, specific enzymes and ionic composition, which differ from the cells´ internal medium – the cytosol. Organelles allow multiple independent biochemical and metabolic processes to occur simultaneously inside a single cell.

Variations in cell structure account for the morphological variability of tissues in the body. Some organelles are present in all types of eukaryotic cells, such as the nucleus, mitochondria and endoplasmic reticulum. Other organelles can be specific to certain cell types, such as secretory vesicles in endocrine cells.

2.2. Cell structure

Understanding the structure of the cell is the key to understanding its function. Breakthrough in the understanding of mechanisms of disease comes parallel to the development of various methods which allow us to visualize and discover various structures within cells and tissues. For example, the identification of "hairy cell leukemia" as a distinct leukemia subgroup came in parallel with the description of thin (hair like) projections from the malignant cells.

Due to their small size, most cellular organelles and their substructures are not visible in conventional light microscopy. In routine light microscopy we examine the cell shape, size, staining properties. We can also appreciate the nucleus and large inclusion bodies. Visualization of other organelles requires more specialized and high resolution methods already explained in Chapter 1.

2.2.1. Surface membrane

Also called the plasma membrane is composed of a double layer of phospholipids containing various imbedded proteins such as transporters and surface receptors. Some cell types can contain additional types of lipids

which aid in performing a specific function; such as sphingomyelin in neurons. The type and amount of membrane proteins vary with the function of the specific cell and the type of membrane. This same principle applies to membranes inside the cell, enclosing different organelle compartments.

The cell membrane functions as a selective barrier, allowing the passage of oxygen and nutrients into, and waste and other products out the cell. The process of material exchange is dependent on the surface area to volume ratio. To increase the surface area in cells which require more material exchange cells can have many thin projections from their surface called microvilli. The semi-permeable character of the membrane allows certain ions and molecules to diffuse freely while restricting others. This helps maintain the proper ionic composition, pH and osmotic pressure of the cytosol. Transport of substances and ions is facilitated by selective transport proteins in the membrane, several of these proteins use energy from the hydrolysis of adenosine triphosphate (ATP).

In multicellular organisms, specialized areas of the plasma membrane form specific contacts and junctions between cells. These are explained in more detail in chapter 3. The membrane also contains various embedded receptor proteins for specific signaling molecules, such as hormones or neurotransmitters, inducing a specific cellular response, e.g. the receptor protein for adrenalin.

The plasma membrane in some specialized epithelial cells is polarized, that means that the structure of the apical domain is different from the baso-lateral one. Each domain contains a different sortiment of proteins. Detailed description of the structure and function of biomembranes is explained below.

2.2.2. Cytosol

The cytosol is the semifluid medium in which cell organelles are suspended. It contains many proteins and enzymes and is the site for several metabolic and other processes occurring in the cell. Its high protein content allows for the formation of complex protein aggregates. It is believed that the content of the cytosol is highly organized, with regional specificity and regulated intracellular movement.

2.2.3. Nucleus

The largest organelle of the eukaryotic cell and the house of most of the cell's DNA. It is defined by a double membrane, the outer of which is

continuous with the rough endoplasmic reticulum. The nuclear membrane contains pores which regulate material transport between the nucleus and the cytosol. The nucleus maintains it shape by a network of rigid fibers called the nuclear lamina, supporting the inner nuclear membrane. The inner nuclear space is called the nucleoplasm.

The nucleus contains the cell's DNA in the form of irregularly dispersed chromatin. Chromatin is packaged into chromosomes only during cell division. It also contains the nucleolus; a suborganelle where most of the cells ribosomal RNA is synthesized and ribosomes are partially assembled. A nucleus can have more than one nucleolus depending on type and stage of the cell cycle.

DNA and RNA synthesis in the nucleus depends on the phase of the cells cycle. Growing and differentiating cells are synthetically active, while resting cells have minimal nuclear activity.

2.2.4. Ribosomes

Ribosomes are a complex of ribosomal RNA and proteins, which carry out proteosynthesis by translating mRNA into an amino acid chain. Cells which produce lots of proteins have a large amount of ribosomes, they also have a prominent nucleolus, for example; pancreatic acinar cells.

Ribosomes can be free in the cytoplasm, or bound to the surface of the rough endoplasmic reticulum or the nuclear envelope. Free ribosomes synthesize simple proteins which do not need further modification. Bound ribosomes synthesize proteins which need to be modified in the endoplasmic reticulum and Golgi apparatus to serve their destined function. Ribosomes in a pancreatic acinar cell are mostly ER-bound**.**

A ribosome is composed of 3 different types of ribosomal RNA (rRNA) and about 55 proteins, arranged into a large and a small subunit. In this ribonucleoprotein complex, the biosynthesis of proteins is catalyzed by the RNA molecule itself and not by a protein enzyme. Elucidation the conformational structure of ribosomes facilitated the understanding of the molecular mechanisms of effect of antibiotics and the development of antibiotic resistance in various microorganisms.

2.2.5. Mitochondria

Mitochondria are the energy factories of the cell. They are large complex organelles which produce ATP through aerobic respiration mainly

from fatty acids and glucose. Mitochondria are enclosed by a system of double membranes with two very different compositions. The inner membrane is convoluted, with folds called cristae protruding into the central space -the matrix- of the organelle, thus significantly increasing its surface area.

The composition and structure of the mitochondrial double membrane system is crucial for the process of oxidative phosphorylation which forms ATP. The phospholipid bilayer of both membranes has a unique composition of phospholipids and embedded proteins which are precisely coordinated to preserve mitochondrial morphology and function. The outer membrane which is about 50% protein is permeable to relatively large molecules, while the inner membrane, almost 76% protein, is much less permeable. Proteins of the respiratory chain are embedded in the inner membrane

In addition to its various enzymes, the mitochondrial matrix contains ribosomes and circular DNA with which it can synthesize some of its own proteins and phospholipids. It is an autonomous organelle, which grows and reproduces on its own within the cell. All these features show resemblance to a prokaryotic cell, which supports the endosymbiont theory. The theory states that the early eukaryotic cell engulfed a prokaryotic cell and they formed an endosymbiont relationship, eventually merging into one organism; an eukaryotic cell with a mitochondrion.

A cell can have hundreds or even thousands of mitochondria correlating with its metabolic activity. In the living cell, mitochondria form a branched tubular network in the cytoplasm.

Defects in the complex mitochondrial structure and function can give rise to a heterogeneous group of diseases. These defects are caused by a mutation either in the nuclear or in the mitochondrial DNA, and by alteration in the synthesis of the lipid or protein component. Given that these diseases cause dysfunction of the respiratory chain of aerobic metabolism, tissues and organ highly dependent on aerobic metabolism are preferentially involved.

2.2.6. Endoplasmic reticulum (ER)

The ER is a network of closed membranes which carry out various metabolic processes. They are the main site for glycoprotein and lipid synthesis. It can be divided into:

1. Smooth endoplasmic reticulum: it lacks ribosomes, which gives it a smooth appearance. Its main function is to synthesize fatty acids and phospholipids. It participates in carbohydrate metabolism and the detoxification of drugs and other substances. This organelle is abundant in hepatocytes, where hydrophobic substances are detoxified by conjugation into more soluble compounds, which makes it easier to flush them out of the body. Introducing alcohol and other substances to the organism induces the proliferation of smooth ER and its detoxifying enzymes, leading to increased tolerance. Smooth ER also stores calcium ions, which play a role in the mechanism of muscle contraction and other calcium ion dependent processes.
2. Rough endoplasmic reticulum: is speckled with a large amount of ribosomes which provides its rough appearance. It synthesizes proteins; such as plasma membrane proteins and extracellular matrix proteins. The rough ER modifies and folds the primary polypeptide chains and attaches carbohydrates in the process of glycoprotein synthesis. It is especially abundant in organelles which secrete proteins, such as pancreatic acinar cells which secrete digestive enzymes.

2.2.7. Golgi apparatus

Proteins synthesized in the rough endoplasmic reticulum are transported into the Golgi apparatus where they are modified, sorted and then sent to other destinations. Proteins are transport from the rough ER to the Golgi apparatus by small vesicles called transport vesicles. Again, this organelle will be extensive in cells specialized in secretion.

The Golgi apparatus, situated near the nucleus, consists of a series flattened membrane sacs and surrounding vesicles. It has three parts, the cis, medial and trans; each containing different membrane bound enzymes. The protein vesicle fuses into the cis part, passes through the apparatus where the proteins are modified by enzymes depending on their structure and destination. The protein product is then released in a vesicle from the trans part to its destination. All secretory proteins and plasma membrane glycoproteins follow this pathway.

The process of directing newly made polypeptides to their proper destination is called protein sorting. It is a complex multi-signal guided

process involving the ER, Golgi apparatus and transport vesicles. Proper protein sorting is thought to be guided by a specific amino acid sequence in the proteins polypeptide chain.

The Golgi apparatus also plays an important role in the synthesis of carbohydrates, proteoglycans and other polymers. It also creates lysosomes.

2.2.8. Lysosomes

Lysosomes are small organelles bound by a single membrane and contain acidic environment with various hydrolytic enzymes which breakdown large molecules and digest "disposed of" cell constituents and foreign material**.** Lysosomal enzymes degrade polymers into smaller units, such as nucleic acids, peptides, saccharides and lipids. Digestion products such as monosaccharides and amino acids are released back to the cytosol.

Lysosomes can be primary; small, spherical and do not contain visible particles, or secondary; large with irregular shape, with visible particles of substances which are being digested. The process of degrading an aged organelle in a lysosome is called autophagy. With this process, the cell continuously recycles some of its components. Lysosomes are also used by immune cells to break down ingested material and cell debris.

The lysosomal structures and enzymes protect themselves from auto-digestion by specific protein structure and folding which protects target molecular groups. Leak of lysosomal enzymes to the cytoplasm does not cause auto-digestion because these enzymes are inactive in the slightly alkaline physiological cell pH.

Nonetheless, if all cellular lysosomes rupture and release their enzymes to the cytoplasm at once, they will breakdown cellular organelles and cause cell death. This process is called autolysis and is induced by several mechanisms, including tissue injury and death of the organism.

In practice, tissues excised for histological examination must be processed to prevent autolysis, this is facilitated by fixative solutions such as formalin. Formalin causes cross-linkage of proteins, mainly between lysine residues. This denaturation does not harm the structure greatly; it deactivates enzymes and preserves antigenicity, keeping cells in a life-like state for a long time.

The loss of lysosomal enzyme function causes lysosomal storage disease. Defective enzyme function leads to the accumulation of substances

and damage to cells and whole organs or systems, eventually with lethal consequences. For example; Tay-Sachs disease is a defect in the lysosomal enzymatic breakdown of certain glycolipids called gangliosides, which are abundant in nervous tissue. Accumulation of lipids in neurons causes nervous system damage with fatal outcome at a very young age.

2.2.9. Peroxisomes

Peroxisomes are specialized metabolic organelles bound by a single membrane, found in all animal cells except erythrocytes. They contain oxidases; enzymes which oxidize organic substances by removing a hydrogen atom and transferring it to oxygen, producing hydrogen peroxide, hence the name. They also contain catalases; enzymes which degrade hydrogen peroxide into oxygen and water.

These enzymatic reactions can be used for different functions. For example; the oxidation and breakdown of fatty acids through beta oxidation to be later used in respiration in mitochondria, and the detoxification of alcohol and other substances in the liver.

A genetic mutation leading to defective peroxisomal oxidation of very long chain fatty acids causes a disease called X linked adrenoleukodystrophy (ADL). Accumulation of very long chain fatty acids causes nervous system damage with varying severity of symptoms and a possible fatal outcome.

2.2.10. Cytoskeleton

The cytoskeleton is an extensive network of fibrous proteins in the cytosol, which gives the cell its shape and rigidity. They also organize structures and activities within the cell, and play an important role in cell motility; this includes changing of location of the whole cell and the movement of organelles within the cell.

Cytoskeletal fibrous proteins are made of polymers of small protein subunits held together by noncovalent bonds. Protein subunits assemble and breakdown through various mechanisms, some of which require energy from ATP. The cytoskeleton is composed of three types of fibers;

1. Microtubules, the thickest of fibers, made from tubulin. They are present in all eukaryotic cells. Provide shape and support and tracks along which organelles move. They aid in cell division through centrioles and are vital in cell motility structures such as

flagella and cilia which are present in specialized cells; such as sperm and respiratory epithelial cells respectively. Cilia can also act as a signal receiver; in vertebrates all cells have one cilium for receiving signals called the primary cilium, which is crucial in brain function and embryonic development.

2. Microfilaments, the thinnest of fibers made from actin. They are also present in all eukaryotic cells. They support the cell shape by forming a three dimensional network against the plasma membrane. Such network forms the core of cytoplasmic projections like the microvilli in intestinal epithelium. Microfilaments are most well known for their role in muscle contraction.
3. Intermediary filaments, with a diameter smaller than microtubules and larger than microfilaments. It consists of one or more rod shaped proteins of various types. They are more rigid and permanent than other types of fibers. Their principle function is structural support and they are present only in cells which display multicellular organization. A network of intermediary filaments also lines the interior of the nuclear envelope called the nuclear lamina. Nerve cell axons are strengthened by intermediate filaments.

The properties and functions of each type is summarized in Table 1.

Table 1. Types of cytoskeletal fibers and their function

Type	Microtubules	Microfilaments	Intermediate filaments
Structure	Hollow tubes made from columns of tubulin polymers	Two intertwined strands of actin polymers	Rod shaped proteins coiled into thicker wires
Protein subunit	Tubulin	Actin	Various types depending on the cell; e.g. Keratin in squamous cells
Function	Maintenance of cell shape (compression resistance) Cell motility (cilia and flagella) chromosome movement in cell division (centrioles) organelle movement within the cell	Maintenance of cell shape (tension bearing) change in cell shape muscle contraction cell motility (pseudopodia) cell division (cytokinesis)	Maintenance of cell shape (tension bearing) support of the nucleus and other organelles formation of nuclear lamina

2.2.11. Inclusion bodies

The cytoplasm also contains granules which are not bound by a membrane, called inclusion bodies. In a healthy cell, they are often deposits of substances related to the cells metabolism and function. For example; liver and muscle cells contain glycogen granules. Adipocytes are specialized

fat storing cells which contain a large cytoplasmic lipid droplet bound by a phospholipid monolayer. The lipid droplet pushes the nucleus and other organelles to the cells periphery, resulting in the cell appearing as a large empty bubble on standard Hematoxylin and Eosin stained slide.

2.3. Cell division

Reproduction of organisms has a cellular basis. Cells ability to produce more of their kind (cell division) is the basis of the continuity of life. Cell division is an integral part of the cell cycle, which is controlled by complex molecular mechanisms. Cell division resulting in two genetically identical daughter cells is called mitosis and is the most common type of cell division. Meiosis is a special type of cell division which produces gametes.

2.3.1. Mitosis

Mitotic cell division is the process of producing two genetically identical daughter cells, i.e. containing 2n complement of genetic material. During cell division, the DNA is duplicated and packaged into chromosome units. Mitosis is carried out by a specialized mitotic apparatus called the mitotic spindle. The apparatus captures the chromosomes and pulls them apart to opposite sides of the cell. This apparatus is temporary, it exists only during cell division, and is carried out by microtubule motor proteins and assembly dynamics. Although mitosis is a continuous process, it is subdivided into 4 phases as observed by light microscopy:

1. Prophase: duplicated condensed chromosomes are visible in the nucleus, later they are released to the cytoplasm as the nuclear membrane breaks and the nucleolus disappears.
2. Metaphase: the chromosomes are aligned along the equatorial plate of the cell and each chromatid is attached to the microtubular apparatus.
3. Anaphase: the chromatids are pulled to opposite poles of the cell by shortening microtubules, and the cell elongates to further separate its poles.
4. Telophase: the nucleus membrane forms around each new set of chromosomes, the nucleolus reappears and the cytosol divides by forming a cleavage furrow, pinching the cell into two. Division of the cytosol is called cytokinesis.

On light microscope, the visible morphological substrate of mitosis is the chromosomes. Based on its phase, mitosis presents with various morphological images. These are also affected by the cell type. The number of mitotic figures present in a sample is an indicator of the proliferative activity of the sampled tissue. In practice, mitotic count is a important indicator for cancer screening and assessment. It is classically performed manually by the histopathologist, there are also various automated techniques.

2.4. Cell differentiation

The process by which a less specialized cell becomes more specialized is called cell differentiation. It is a complex DNA-led process which creates diversity of cell types and functions during the development of the organism from a fertilized egg.

Cell differentiation is based on the pattern of gene expression. A cell can differentiate to a certain tissue based on which genes are potentially activated; i.e. cell potency. The zygote is a totipotent cell, which means that all its genes are potentially active, i.e. it can differentiate into any type of cell and form an organism. As it divides, daughter cells gradually lose their potency and commit to a certain lineage of differentiation. This means that only genes of that lineage are potentially active, finally giving rise to terminally differentiated organ specific cells.

Specialized cells in the organism usually have distinctive morphology depending on the specific spectrum of proteins and structures it contains to fulfil a given metabolic and biological function. Cell differentiation is led by a complex of extracellular regulatory signals and intracellular transcription control mechanisms.

Understanding cell differentiation was a key step in stem cell research. Embryonic stem cells are pluripotent cells from the blastocyst phase of development. The adult human tissue still contains a certain amount of stem cells, called adult stem cells. They are less potent than embryonic stem cells, but still have a great therapeutic potential. For instance, bone marrow transplantation for the treatment of leukemia is based on the replacement of malignant cells by adult blood stem cell collected from the bone marrow or peripheral blood of the donor.

2.5. Apoptosis

Apoptosis is programmed cell death. It is a vital mechanism for controlling multicellular development. It controls the population by balancing cell growth and multiplication, eliminates unnecessary cells, for example; during embryogenesis, fingers and toes are formed by apoptosis of cells in the intervening spaces. Apoptosis can be triggered for various reasons, such as cell infection or other type of damage, or physiologically when the cell reaches the end of its life span.

The cell can initiate apoptosis by receiving an extracellular signal, which binds to a specific receptor and initiates a second messenger cascade. Apoptosis can also be initiated from the inside of the cell, either from the nucleus or from the endoplasmic reticulum.

Apoptosis can be initiated through several pathways, depending on cell type and initiating signal. In the human cell, one of the pathways involves the mitochondria. Once apoptosis is initiated, enzymes such as proteases and nucleases are activated, which digest cellular structures and fragments DNA. The nuclear membrane disappears (a process called karyorrhexis), organelles are fragmented, the cell shrinks and the cytoplasm divides into small lobules and vesicles. These vesicles, called apoptotic bodies, are consequently engulfed by surrounding and specialized cells, leaving no trace or waste.

In light microscopy, the morphological picture of apoptosis begins with nuclear condensation, appearing dark blue on a HE slide, and DNA fragmentation into large chunks. The whole cell shrinks leaving an empty space around it, and then it is fragmented into irregular dark blue apoptotic bodies and consumed by other cells.

Signaling pathways for apoptosis are complex. They are regulated by several proteins and can be activated by several mechanisms, including the lack of an essential factor from the extracellular environment or the detection of an error in the cell cycle. The latter is a very important mechanism in the prevention of tumor formation. Proteins involved in cell surveillance by activating apoptosis are called tumor suppressor proteins. Today they are a leading target of cancer research.

(See also Chapter 22).

2.6. Organelles and disease

Rudolph Virchow, the father of pathology was the first to hypothesize disease at the cellular level. Today, we know that various diseases can be caused by defects in the structure and function of cellular components. Understanding organelle disorders is becoming increasingly important for pediatricians. Because many of these diseases are genetic mutations which impair a specific function of an organelle but is not immediately fatal, their symptoms mostly appear during childhood. Many of these diseases are commonly termed as metabolic diseases. Some of these diseases are shown in Table 2.

Table 2. Examples of organelle related diseases.

Structure	Related disease
Endoplasmic reticulum	Gene mutations causing protein misfolding cause various diseases (e.g. in cystic fibrosis the misfolded chloride channel protein is trapped and degraded in the ER)
Golgi apparatus	A group of congenital disorders of glycosylation (CDG) caused by gene mutation in glycosylation enzymes.
Nuclear envelope	Various mutations in genes coding for membrane proteins like lamin and emerin (e.g. Progeria)
Lysosomes	Lysosomal storage disease are a group of genetic disorders caused by absent lysosomal enzymes (e.g. Tay-Sachs, Gaucher and Pompe disease)
Mitochondria	Genetic mutations in nuclear or mitochondrial DNA causing a heterogeneous group of diseases (e.g. Leigh syndrome)

2.7. Biological membranes

As mentioned earlier, biological membranes are semipermeable membranes composed of a phospholipid bilayer with various embedded proteins. The basic accepted model for the membrane structure is the fluid mosaic model.

Phospholipids are amphipathic molecules; this means that they contain a hydrophilic and a hydrophobic part. The phospholipid bilayer is arranged so that the hydrophobic ends face each other forming the core, and the hydrophilic ends face the outer and inner surface of the cell. These leaflets are held together by hydrophobic forces, which are much weaker than covalent bonds. In the cell, phospholipids will spontaneously form a phospholipid bilayer structure, and will spontaneously seal to form closed spherical structures, i.e. with no ends, which will ensure the

stability of the phospholipid bilayer, and will result in the separation of two aqueous compartments.

The membrane structure ensures that the membrane leaflets are not rigid. Most of the lipids and some of the proteins can shift laterally in the plane of the membrane. These molecules are in constant motion, the smaller lipids shift faster and the larger proteins slower. This movement represents the "fluidity" of the membrane. Membranes need this fluidity for their proper function. Changes in membrane fluidity cause changes in its permeability, and the activity of its proteins.

Changes in membrane fluidity can be caused by temperature alteration. The types of lipids composing the phospholipid molecules play a decisive role in the maintenance of membrane fluidity of various organisms at different temperatures. Saturated hydrocarbon chains enhance membrane viscosity while unsaturated chains enhance fluidity. In the human body, at the relatively high temperature of 37º, a pure phospholipid bilayer is too fluid and unable to sustain proper consistency. This problem is overcome by the incorporation of cholesterol in the phospholipid bilayer.

Cholesterol is abundant in the plasma membrane of animal cells. It works as a fluidity buffer to maintain the proper consistency of the membrane. Its net effect varies with the temperature; in physiological conditions it causes the membrane to be less fluid. Its rigid steroid ring restricts the mobility of the outer portion of the hydrocarbon chain, while its hydrocarbon tail allows for more fluidity in the bilayer center. In lower temperatures, it prevents the binding and crystallization of hydrocarbon chains. Cholesterol in the phospholipid bilayer decreases the membrane permeability to small water soluble molecules.

The "mosaic" in the fluid mosaic model refers to membrane proteins, which are embedded in the phospholipid bilayer like a tile mosaic. Membrane proteins determine most of the membrane's functions. Different cell types have different membrane proteins, and different organelles have their specific sets of membrane proteins.

Based on the type of cell and membrane, the protein to lipid ratio varies significantly. For example; the inner mitochondrial membrane is 76 percent protein, while the myelin membrane around nerve axons is only 18 percent proteins. The high lipid content in myelin contributes to electrical insulation.

There are two types of membrane proteins:

1. Integral proteins: these are embedded in the phospholipid bilayer, most of which span the membrane – transmembrane proteins, and some of them extend only half way through the phospholipid bilayer.
2. Peripheral proteins: these are not embedded in the membrane, but are loosely attached to its surface, usually to an integral protein.

Some integral proteins are attached inside the cell to the cytoskeleton, and some other proteins are attached outside the cell to the extracellular matrix. This provides the cell with stronger framework. Aside from this, membrane proteins carry out several other functions, which are important for the membrane to work properly. Sometimes one protein has multiple functions. These functions include: cellular transport, enzymatic activity, signal transduction, cell recognition and intercellular joining.

Membranes also contain some carbohydrates. Some are attached to lipids – glycolipids, but most are attached to membrane proteins – glycoproteins. Both glycolipids and glycoproteins are mostly attached to the outer surface of the cells membrane, facing the environment. Membrane carbohydrates vary in different cells, allowing them to function as markers for distinguishing one cell from another, i.e. the process of cell recognition. This process is important for various functions such as immunity and cell sorting in embryogenesis. An example of glycoproteins is blood types A, B and O, which reflect the variation in carbohydrate chains of red blood cells membrane glycoproteins.

The fluid mosaic structure provides membranes with properties which are necessary for its function. Biological membranes are selectively permeable; substances do not cross the membrane indiscriminately. Non polar molecules such as hydrocarbons, carbon dioxide or oxygen can dissolve in the hydrophobic lipid layer and cross easily. Ions and hydrophilic molecules including glucose and water cross with greater difficulty and with a much slower rate. At this point, membrane embedded proteins play an important role; hydrophilic substances can cross by passing through transport proteins.

Some transport proteins work as a channel; they contain a hydrophilic tunnel through which certain polar molecules can pass; for example, aquaporins in the kidney. Other transport proteins work as a carrier;

when a specific substance is attached to them they change their shape to shuttle the substance across the membrane, for example; the glucose transport protein GLUT1.

Channel proteins which transport ions are called ion channels, many of which are gated channels, which means that they open and close in response to a stimulus. A stimulus can be an electrical impulse or the binding of a specific substance to the channel protein. Gated channels are important for the function of neurons in the nervous system.

2.8. Cellular transport

Traffic through biological membranes has several mechanisms:

1. Passive diffusion: passage of substances through the membrane in the direction of their concentration gradient.
2. Facilitated diffusion: passage of substances in the direction of their concentration gradient through specialized channel or carrier proteins, which are called uniporters. They allow substances such as amino acids, sugars and other small molecules to diffuse at a much higher rate than in passive diffusion. Because through the uniporter, transported molecules bypasses the hydrophobic core of the phospholipid bilayer. A typical example is the GLUT1 uniporter. It is a membrane transporter of glucose, which is present in most mammal cells.
3. Active transport: as opposed to the above, this mechanism requires energy to move substances across the membrane against their concentration gradient. Proteins used for this purpose are all carrier proteins rather than channel proteins. The most classical example of active transport is the sodium-potassium pump.

   The sodium-potassium pump is fueled by ATP. It pumps sodium ions out of the cell and potassium ions inside, against their concentration gradient. The function of this pump is to maintain an uneven distribution of ions on either side of the membrane, creating a difference in voltage, which is called the membrane potential. This electrical energy affects the traffic of charged substances across the membrane. In general, the concentration of K^+ ions in the cytosol is 35 times higher than in the blood, while the concentration of Na^+ in the cytosol is 12 times lower than in the blood.

Consequently, ions move across the membrane not only by the concentration gradient, but also by their electric potential. These two forces can act in the same or opposite directions.

Cells use ion pumps to create and maintain the membrane potential, and use channel proteins which selectively allow ions to move down their concentration gradient to create a difference in voltage. These principles are the basis for the understanding of many biological processes, for example; impulse transmission in the nerve cell.

4. Cotransport: cells also use the energy stored in the electrochemical gradient of ions such as Na^+ and H^+ to power the transport of other ions and small molecules against their concentration gradient. Several types of cotransporters regulate the cytosolic pH, such as the Na^+HCO3^-/CL^- cotransporter.

2.8.1. Vesicular traffic

Large substances such as proteins and polysaccharides need to be packaged into vesicles to be transported from organelles within the cell; such as from the ER to the Golgi apparatus, or be sent in or out of the cell through the plasma membrane. This process, like active transport, also requires energy.

Vesicle budding and fusion between membranes is a very complex, mostly protein guided process. Its mechanism is still not fully understood, but it plays a vital role in the sorting of external and internal proteins, and the import and export of materials to the cell. Cells also use vesicles for regenerating the plasma membrane.

2.8.2. Exocytosis

Exocytosis is when a cell spills material by fusion of a vesicle with the plasma membrane. This mechanism is used by many cell types with specific secretion. Secretion by exocytosis can be continuous or regulated. In continuous secretion, proteins are released from the Golgi apparatus to the plasma membrane where they are continuously secreted. In regulated secretion, cells store secretory proteins in vesicles inside the cell, and secrete them only when triggered by a specific stimulus. An example is the secretion of insulin from the β-islet pancreatic cells in response to the elevation in blood sugar levels.

In many instances, secretory vesicles are packed with inactive proteins (proproteins) which need further processing. These undergo further proteolytic processes within the vesicle to generate mature active proteins. An example is the conversion of proinsulin into insulin.

2.8.3. Endocytosis

Endocytosis is when a cell internalizes material from the surroundings by forming a vesicle around it from the plasma membrane. There are three subtypes of this process:

- Phagocytosis: when a phagocytic cell ingests large material such as bacteria for degradation. It is a highly specialized process, which can be performed only by few specialized cell types, such as macrophages.
- Pinocytosis: is nonspecific take up of droplets of extracellular fluids.
- Receptor mediated endocytosis: is triggered by the binding of a specific substance on a membrane receptor; for example, the mechanism by which cells take in cholesterol through the LDL receptor. Patients with familial hypercholesterolemia have a defective or completely lack this receptor, causing its accumulation in blood.

2.8.4. Transcytosis

Transcytosis is transcellular transport which combines endocytosis and exocytosis. Endocytosed material passes through the cell and is exocytosed from the plasma membrane on the other side of the cell. A typical example is the transfer of molecules through endothelial cells in capillaries.

2.9. Cell communication

Cells can in various ways examine its surrounding, communicate with other cells and react based on the information received. Survival of multicellular organisms depends on the intercellular communication network which regulates the physiology and coordinates different functions. Cells can create and react to a variety of signaling mechanisms, these can be:

1. Chemical signals: Molecules such as gases, amino acids, even macromolecules such as polypeptide hormones.
2. Electrical: difference in voltage across the membrane, such as voltage gated calcium ion channels in neurons.
3. Mechanical: physical pressure on the receptor, such as the cochlea in the ear.

In multicellular organisms cells can communicate over various distances; either by direct contact with nearby cells, or by messenger molecules with distant cells in a short or long range.

Different signaling mechanisms can be employed to communicate a specific message within one or several cells. For example, an electric impulse signal travels along the nerve cell axon, which can be regarded as long distance, and then triggers the release of chemical synaptic signaling to another cell, which can be regarded as local signaling.

Direct contact signaling between adjacent cells is achieved through:

1. Intercellular junctions; such as gap junctions in the lateral surface between adjacent epithelial cells, these junctions allow substances present in the cytosol to move freely between the cells.
2. Contact of surface membrane structures; this is called cell-cell recognition and is one of the basic mechanisms in immune response.

Signaling by extracellular secreted molecules is a complex, precisely regulated process. Extracellular signaling can be classified by range into:

1. Endocrine signaling; cells secrete special chemicals called hormones, which are carried by the blood stream and act on distant target organs and structures.
2. Paracrine signaling; cells release messenger molecules which target cells in close distance (local signaling). An example is the conduction of electrical impulse from a nerve cell to a muscle cell by neurotransmitters to induce muscle contraction.
3. Autocrine signaling; cells respond to substances which they themselves release, such as growth factors.

For communication to take place, the signaling cell must be able to synthesize and release the signaling molecule. On the other end, the recipient cell must be able to detect and receive the signal, translate it into

a series of intracellular processes, which will in turn initiate a specific response. Signal receiving can be summarized into three stages; reception, transduction and response.

2.9.1. Reception

A messenger molecule can only be detected by a cell which contains a receptor protein to which the messenger binds specifically, i.e. acts as a ligand. These ligands are called first messengers, as they perform the first step in communication. Most receptor proteins are transmembrane proteins, some however are located inside the cell.

Membrane protein receptors can be divided by their mechanism of action into three groups; the largest of which is G protein coupled receptors, for example β1 and β2 adrenergic receptors for adrenaline and noradrenaline. The second group is ion channel receptors or ligand gated channels, such as the calcium ion channel in neurons. The third group is comprised of enzyme linked receptors. Binding of a ligand activates enzymatic activity within the cell. Receptors can either have catalytic activity of their own, such as the insulin receptor which has tyrosine kinase activity, or they can interact by activating other cytosolic enzymes such as protein kinases. Examples of the latter are receptors for cytokines and interferons. The product of this enzymatic activity will be the first step in signal transduction.

Intracellular receptor proteins are found in the cytoplasm or the nucleus. To pass through the plasma membrane, the messenger molecules have to be small and hydrophobic. A typical example is steroid hormone receptors, such as the estrogen receptor.

Despite of the importance of protein receptors, the structure of many of them is still unknown. In general, of the estimated number of 300,000 different proteins, we have identified the 3D structure of less than 5 percent. Thus their functional interpretations and possibilities for target therapy are yet unknown.

Messenger molecules bind to receptors reversibly, and in a concentration dependent pattern. Therefore, the higher concentration of the molecule, the more receptors it occupies. This is one of the mechanisms for regulating the intensity of the desired effect.

Receptor proteins transmit information to the cell by changing its conformation, or aggregating with other surface proteins, or catalyzing a specific reaction. These changes will initiate transduction.

2.9.2. Transduction

Signal transduction is a cascade of specific intracellular interactions activated by the binding of a ligand to the receptor. It is usually a multistep pathway, which is a mechanism that facilitates signal amplification. Molecules involved in transduction are called second messengers.

Transduction can be initiated by the change of cellular concentration of second messengers. Two of the most common second messenger molecules are cyclic adenosine monophosphate (cAMP) and inositol triphosphate (IP3). These changes will trigger a cascade of intercellular interactions.

Most of transduction interactions are carried out by proteins, which can be activated by a simple reaction such as phosphorylation. These reactions often cause conformational changes in its structure. Proteins which act by phosphorylating other target proteins are called protein kinases and they play a major role in signaling pathways. Naturally, another group of enzymes which are vital to these processes are protein phosphatases, they deactivate proteins by the opposite mechanism; dephosphorylation.

2.9.3. Response

Signaling pathways lead to a certain cellular activity, either cytoplasmic or nuclear, for example; the regulation of protein expression by transcription, or cellular metabolism by altering enzyme activity, change in cell morphology, or the induction of cell division.

Signaling pathways form a branching network of interactions with multiple regulation mechanisms, which allow for high specificity and fine coordination of the response. The synthesis and release of many signaling molecules is subject to feedback control. Recipient cells also modulate their response by regulating the number and activity of its receptors. For example the cell can decrease its sensitivity to a messenger by decreasing the number of its surface receptors by endocytosis and then degradation by lysosomes. Similarly, increasing the sensitivity will depend on the synthesis of new receptor molecules.

Target cells also modify or degrade the ligand, therefore modifying or terminating their response. The lifetime of a hormone in the blood can vary from few seconds for catecholamines to several days for thyroxine.

Cellular response can be specific to a certain cell type based on its composition of receptors and transduction molecules; a first messenger molecule can cause two different responses in two different cell types. Similarly, a single transduction component in the same cell can participate in various different signaling cascades with different responses. It is still not yet clear how this specificity is determined. Structural biology remains a key component in the elucidation of these complex processes.

2.10. Methods and history of cell research

If we define a hypothetical cell as the unit of life, we also mean that it would be able to exist independently from other cells. Such cells may be obtained from embryonal (or tumor) tissues and can kept in culture for a long time. As a rule, however, the histologist observes groups of differentiated cells organized into tissues. Single cells obtained from tissues are 10-20 μm (0.02 mm) in size. Such single cells are for example seen in the smears taken from body fluids (blood) or from urogenital tract. The dissociated single cells are usually of different type reflecting their original occurrence in close contact with each other. The discovery that animal as well as human tissue are aggregates of individual cells was associated with the claim of the so called *cell doctrine* telling that the cells are bricks from which the bodies of *multicellular* organisms are composed. This discovery was closely related to the development of *light microscopy*. Even at a high power of view, the eukarytic cell appears as a relatively simple structure, consisting of a basophilic nucleus and an eosinophilic cytoplasm, in which the cell organelles can be seen by special staining techniques only. It should be mentioned here, that living animal cells as they appear within the body or in cell culture, are translucent and colorless. When observing living unstained cells at usual magnifications raging from 80x to 800x, we can observe the borders of individual cells surrounded by the cell membrane and a slightly contrasting nucleus. Using the so called phase contrast technique, we can observe some cellular organelles, such as secretory vacuoles and/or the lysosomes and occasionally the

cellular filaments and, of course, the chromosome movements during the cell division. The light microscope would not resolve details smaller than 0.2 μm (200 nm) even when using fixed and stained cells. The cells for histological examination are usually fixed in 4% formalin solution, for cytological examination mainly in alcohol. Fixation in formalin cross links the protein molecules changing the native (secondary and tertiary) conformations of individual protein molecules into linear filaments joined by $–CH_2-$ bridges. Fixation on one hand stops any metabolic and enzymatic activities (kills cells), but on other hand it preserves their structure in a stabilized state for long time. Alcohol (when concentrated to at least to 96%) removes water and dissolves the lipid bilayers. At *embedding*, which is necessary to prepare thin sections for histological examination, water must be always removed from the formalin fixed tissue by a series of alcohols (from 70% to absolute alcohol); then the tissue can be soaked by paraffin or other suitable embedding medium. After embedding, the tissue is cut into sections 4-7 mm thick, which are stained by suitable dies, most frequently with hematoxylin and eosin (H.E.), which is the basic stain for histology. Hematoxylin has an excellent affinity to DNA and RNA and reveals their presence within the nucleus. Other stains may be used for selective staining of organelles, fat droplets, glycogen and other cytoplasmic deposits.

Optical microscopy of fixed and paraffin embedded tissues dominated over hundred years (in the second half of 19th and the first half of 20th centuries), until the technique of frozen sections was introduced. This technology is especially useful for *histochemical reactions* due to preservation of protein domains, i.e. the secondary and tertiary conformations of protein remain unchanged. If the tissue sample has been quickly frozen below the critical temperature of -40°C to -70°C the cells show a quite satisfactorily preserved structure within cryostat sections, which have been properly fixed and stained. The technique of *cryostat* sectioning (omitting embedding), was first used for detection of enzyme activities. The corresponding reactions when using proper substrates and indicator substances can proceed within the sections itself. Due to formation of colored complexes (deposits) as result of such histochemical reaction, various enzyme activities could be properly localized, in contrast to the same or alternative reactions provided in tissue and/or organ extracts (biochemistry).

Furthermore, the proteins investigated according to their chemical function can be visualized as antigens using properly prepared mainly monoclonal antibodies. In cryostat sections both the continuous as well as the discontinuous antigenic determinants (epitopes) may be preserved and detected by means of fluorescent (labeled) antibody staining. The sections following acetone fixation is stained with the fluorescein labeled antibody and examined in a special *fluorescent microscope*. The fluorescencing die, linked to the antibody, emits green and/or orange fluorescence. After biding to its subcellular target, the antigen can be seen as a fluorescing object (granule) over the dark background provided that a suitable filter combination is used (cut off filters). More recently, highly specific histochemical staining techniques have been developed due to availability of commercial mono specific and/or monoclonal antibodies, which bind to a single well defined continuous antigenic determinant. The properties of the antigenic determinant are essential along with the excellent binding ability of antibody. As mentioned, only those antigens can be visualized in the fixed and embedded material, which were not destroyed during these procedures, i.e. which still remain preserved in an antibody reactive state. The new branch of optical microscopy called *immunohistochemistry*, evaluates the protein components possessing continuous antigens directly within cells. The reaction can be done hand in hand with the classical histological staining, since many commercial antibodies are already available for routine diagnostic work.

From the second half of last century, a new impetus for cytological work was given by introducing the transmission electron microscopy (EM). For transmission EM, small pieces of material are fixed in glutaraldehyde solution and embedded into epoxi resins. Ultrathin sections (50-100 nm thick) are cut, contrasted with osmium tetroxide and put on a copper grid, which is then inserted into the EM column, in which a beam of electrons moves between electromagnetic lenses in a similar way as the light spreads through the optical lens. The beam of electrons accelerated by a high voltage (100.000 V) either passes through and/or is in part reflected from the contrasted specimen. Depending on the contrast, which is related to the property of the glutaraldehyde fixed cellular structures to bind the osmium, black and white image appears on the fluorescent screen. The cell components appear either translucent or shows various intensity of

the gray and/or black contours. The resolution of the EM image reaches details up to 1-0.1 nm depending on the quality of the devise and/or that of the specimen. The lipid bilayer can be clearly distinguished not speaking about the relatively large ribosomes. Occasionally large protein complexes and or polymeric proteins can be recognized but nether the individual protein molecules. The viral particles are clearly visible within infected cells, and of course, the relatively large bacterial cells as well as their subcellular structures can be identified. Pathological protein deposits such as inclusion bodies and/or amyloid are visible in their ultrafine composition. Nevertheless, in order to detect individual proteins, an approach resembling immunofluorescent staining should be used. Proteins as antigens are can be visualized at EM level by means of antibodies labeled with colloidal gold. Also histochemical reactions can be performed for certain enzymes and corresponding reaction deposits can be localized by more precision than in the light microscope. For these special techniques, the specimens are not embedded into epoxi resin, but deeply frozen and cut on an ultracryocut device. The study of the three dimensional structure of microscopic objects is provided by special techniques such as scanning EM and freeze fracture. In both cases, the surface of the object (not the section) is shadowed by platinum or other heavy metal. The former approach needs a special EM equipment, called scanning EM (SAM), while latter needs suitable specimens prepared in a special manner (freeze etching). During the last decades there was a growing effort to interpret structure and function together in order to understand the unique design of cells and their components.

To sum up, light microscopy of fixed and stained eukaryotic cells detects the basophilic nucleus and the eosinophilic cytoplasm, in which organelles such as mitochondria, the Golgi apparatus, the cytocenter (centriole and related vicinity) and various vesicles (such as lysosomes) can be seen. During mitosis, light microscopy of living cells shows the movement of chromosomes and/or chromatides towards cellular poles, around which the nucleus is later on rearranged. Much more details can be seen by electron microscopy (in chromatin organization, within the nucleus, in the ER distinguishing the smooth and rough ER). The combination of microscopy with histochemistry and antibody labeling provided new insights into the function of ribosomes and polyribosomes, of lipid

bilayer, of secretion vesicles and lysosomes as well as of mitochondria. In addition, electron microscopy has opened a quite new insight into cell structure providing the opportunity to understand the functional importance of cell structures as objects of life.

In this review the basic knowledge of cell function has been presented in close association with the morphologic structures visible by microscopic examination. This means, the cell structure has been described here with the aim to understand its functional aspects. The latter are in more detail described in the textbook of biology, biochemistry and/or molecular biology, but as a rule, without explanation of relationships to cell structures. Thus, the purpose of our description, in purpose braking the traditional rules of classical histological textbooks, was to teach the student to understand and the link between classical morphology (histology) and the function of individual cell structures as referred to by biologists, molecular biologists, chemists, biochemists and other representatives of related scientific disciplines (genetics, molecular genetics, immunology etc).

Clinical correlations

Cytology in clinical terms refers to diagnostic cytology, or cytopathology. It is the examination of cell properties in sampled material. It is a sub-specialized field of pathology, which differs from conventional tissue biopsy (histopathology) by the general loss of intercellular relations. The diagnostician must rely on the morphology of individual cells, or small groups of cells, without background tissue to make a diagnosis.

Cytology today is a routine diagnostic method, which has its specific indications. It is used for screening such as in cervical smears or follow-up on different disease such as sputum from patients diagnosed with lung cancer. Its ultimate goal is to reach a definitive diagnosis. Cytology however is not a substitute to conventional histopathology, it should be regarded as a complementary part of the diagnostic procedure in combination with clinical, radiological and other laboratory data.

Major breakthrough in diagnostic histopathology and cytology was achieved by the development of monoclonal antibodies and immunohistochemistry. Specific antibodies which bind to a specific surface protein or other cellular structures are applied on the examined sample.

With this method, we can typify cells which cannot be differentiated using conventional staining methods, we can also detect the presence of a certain protein or cellular structure in the examined sample. Today, these methods play a pivotal role in the routine diagnosis and profiling of tumors, facilitating specific and targeted treatment methods; for example, the administration of tamoxifen in breast cancer patients requires immunohistochemical demonstration of the expression (positivity) of estrogen receptors in tumor cells.

The cytologist, examining a routine cytology slide by a light microscope, will evaluate the slides quality, background, cells shape and size, staining properties, nucleus shape and size, the nucleus to cytoplasm ratio, inflammatory infiltrate, infection and other factors based on the type of sample and clinical data. Immunohistochemistry can be used to identify and classify structures as in Table 3.

Table 3. Examples of the use of immunohistochemistry in targeting various cellular structures in routine practice

Type of immunostaining	Target structure	Clinical use
Various types of keratin (e.g. Cytokeratin 7)	Keratin protein in intermediate filaments	Differential diagnosis of epithelial and other tumors
Vimentin	Vimentin protein in intermediate filaments	Demonstration of mesenchymal differentiation in tumors
Desmin	Desmin protein in intermediate filaments	Demonstration of muscular differentiation in tumors
Clusters of differentiation (e.g. CD3, CD20)	Surface membrane proteins	Diagnosis and typing of lymphomas
Various types of hormone receptors (e.g. Estrogen, progesterone)	Hormone receptor proteins	Tumor typing and tumor response to treatment
Mismatch repair proteins (MMR)	Several proteins involved in DNA repair mechanisms (e.g. MLH-1, PMS-2)	Detection of MMR mutation in tumors and evaluation of tumor behavior and prognosis
Various infectious organisms (e.g. HPV, EBV)	Various antigens of infectious organisms	Demonstration of infectious agent

Cytopathology is divided into two branches based on the type of sample; exofoliative and aspiration cytology.

Exofoliative cytology examines cells that exfoliate from superficial or deep serosal or mucosal surfaces; samples can be obtained by:

1. Scraping from tissue surface. The most common and practical example is the Papanicolaou test, also known as the Pap smear. It is

a screening method for cervical cancer. Cells are collected from the uterine cervix and examined for abnormalities. Papanicolaou was one of the first to draw attention to the possibility of diagnosis using smear samples. Mortality rates due to cervical cancer has significantly decreased since the implementation of smear screening programs.

2. Body fluids: Any fluid sample can be spread on a slide and stained for cytological examination, it can also be centrifuged, and the pellet examined by various techniques. Fluid cytology includes:
   - Blood; the most classical of cytological methods is blood smears, first developed and implemented by Paul Ehrlich. Today, differential blood count is a part of the basic workup for any patient.
   - Serous effusions such as peritoneal, pleural and pericardial. Cytology has become a necessary routine examination of accumulated serous fluids, for example peritoneal cytology is essential for the staging of ovarian malignancies.
   - Cerebrospinal fluid; this method is mainly used in case of suspicion of infection of the central nervous system. It is also used for assessing primary malignant neoplasms and neurodegenerative disorders.
   - Urine; Standard urinalysis includes cytological evaluation of hematuria. Urine cytology is also used in screening for bladder cancer and follow-up on patients with urothelial cancer.

Aspiration cytology; fine needle aspiration cytology (FNAC) is a procedure where a fine needle is used to collect a sample of cells from a suspicious tissue mass. It can be used for almost any type of tissue, superficial and deep situated lesions.

In general, cytology sampling is a cost-effective minimally invasive procedure. Samples taken for cytology can be processed using several methods; the basic of which is classical histological slides. The material is spread to obtain a cellular thin slide of intact cells, and stained using specific histochemical methods as in conventional histology.

Another method is flow cytometry and immunophenotyping. Flow cytometry is a technique which provides quantitative and qualitative analysis of cells by measuring their optical properties. Immunophenotyping is the use of fluorescent dye conjugated antibodies to detect specific cell antigens. These methods are widely used in various medical fields like hematology, immunology, oncology and others.

Other methods include cytogenetics, molecular pathology and electron microscopy. Each method has specific indications and sampling requirements.

Cytological screening for cervical cancer

Traditionally, the Pap smear is prepared by spreading material scraped from the exocervix and endocervix by a brush onto a glass slide. Today the liquid based cytology (LBC) technique is becoming more popular. In the latter, the collected specimen is placed in a preservative fluid which causes erythrolysis, and then deposited on a slide, creating an evenly thin cellular monolayer and reducing possible artefacts. Samples are best collected at mid cycle, when the possibility for artefacts is minimal, such as samples obscured by menstrual bleeding.

A normal Pap smear will contain epithelial cells from the exocervix (squamous cells) and the endocervix (glandular cells), in addition to histiocytes, neutrophils, erythrocytes and some cervical mucus. Physiologically, the cytological findings correlate with the hormonal status of the woman (the phase of the cycle). This however can be obscured by the administration of various types of hormonal therapy. Therefore, providing clinical information is important for correct interpretation.

In addition to changes in the epithelial cells, the Pap smear will reveal various vaginal infections and vaginosis.

In a physiological Pap smear, there are three types of exocervical squamous cells present, based on their origin within the squamous cell layers;

- Superficial cells; these cells come from the outermost layer of the squamous epithelium. They are large, polygonal, with abundant transparent cytoplasm and small pyknotic nuclei. The cytoplasm can be eosinophilic (stain pink), or basophilic (stain blue).
- Intermediate cells; these come from the *stratum spongiosum*. They are also large and polygonal, their nucleus is vesicular, roughly the size of a neutrophil. Their cytoplasm is transparent, often basophilic and occasionally contains keratohyaline granules.
- Parabasal cells; they come from the basal layer of the squamous epithelium. They are smaller in comparison, which means that they have a higher nuclear/cytoplasmic ratio. They are round to

oval. The cytoplasm is basophilic, homogeneous and dense, with thickened edges. Sheets of these cells are commonly observed in atrophic smears, e.g. in postmenopausal women.

Endocervical glandular cells in a physiological Pap smear are usually tall, columnar, with slightly larger nuclei than squamous cells, with vesicular chromatin. They can be seen forming sheets of regular cells with regular nuclei, displaying a typical honeycomb pattern, in stripes (a picket fence arrangement) or as single cells. They can be divided into;

- Secretory cells; these contain intracellular mucin, which appears as granular or vacuolated cytoplasm.
- Ciliated cells; these cells display apical eosinophilic ciliae.
- Naked nuclei of endocervical glandular cells.

A normal Pap smear can also contain endometrial cells, these are small, cuboidal with hyperchromatic round, oval or bean shaped nuclei.

The cytologist will interpret the morphology and submit a descriptive diagnosis in addition to evaluating the adequacy of specimen. The report is issued in a standard format, called the Bethesda system. It is an international system of terminology and classification of cytological lesions, which provides clear directions and recommendations, therefore facilitates the communication between laboratories and clinicians.

2.11. Summary

Knowledge of the cell's structure is important for understanding its function. This is the basic step in understanding the whole complex of biological systems and their interactions.

Advancement of medicine is heading towards the molecular mechanism of disease. This in many instances sends us back to the cell, such as cellular proteins; enzymes or membrane transporters and the mechanism of their synthesis. This in turn leads us back to DNA, transcription and translation and the rest of genetic processes determined and occurring within the cell.

Diseases which were known for decades and centuries based on clinical nosological units are now being reclassified based on their molecular basis. Understanding the cell is the basic prerequisite for understanding

the complexity of the human organism, identifying mechanisms of pathogenesis and methods of treatment.

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3. Epithelial tissue

3.1. Introduction

The human body is composed of billions of cells. Cells form tissues, which are collections of cells with similar structural and functional characteristics. In general, the cell is the basic functional and structural unit of the body. The human body consists of four basic types of tissue:

- epithelial tissue
- connective tissue
- muscular tissue and
- nervous tissue

These tissues, which are formed by cells and extracellular matrix, do not exist as isolated units, but rather in association with each other and in variable proportions forming different organs and systems of the body. Tissues during development are derived from the embryonic germ layers. The three primary germ layers in the developing embryo are:

- ectoderm (the outer germ layer)
- mesoderm (the middle germ layer)
- endoderm (the inner germ layer)

3.2. General characteristic and function of epithelial tissue

3.2.1. Characteristic of epithelial tissue

1. Epithelium covers the exterior body surface and lines the internal cavities and tubes that communicate with the exterior (epithelium lines the digestive system, the respiratory system, the cardiovascular system and the urogenital system). The epithelium cover outer surface of the body – epidermis. Epithelium also forms the secretory portion of glands and their ducts.
2. Epithelial tissue is composed of closely aggregated cells with very little extracellular substance. Epithelial cells have tight lateral adhesions with specialized cell junctions between them called the junctional complexes.

3. Epithelial cells exhibit functional and morphological polarity.
4. Epithelial cells rest on a basement membrane, which is a noncellular layer, composed of glycoproteins and fibrils.
5. Epithelium is an avascular tissue; nerve fibers can pass freely in between epithelial cells.
6. Epithelium can develop from all three embryonic germ layers:
   - ectoderm – epidermis of skin, corneal epithelium, neuroepithelium;
   - endoderm – epithelium lining of the digestive tube and respiratory system, liver, and pancreas;
   - mesoderm – epithelium lining of the cardiovascular system (endothelium), serous membrane (mesothelium) as well as the urinary and reproductive system.
7. Most of the epithelial cells have the ability to regenerate (i.e. continuous wear and tear).
8. Specialized epithelial cells serve as receptors for special senses (smell, taste, hearing and vision).
9. Some epithelial cells are modified to contract and form myoepithelial cells.

3.2.2. Functions of epithelial tissue

The epithelial tissues have many functions depending on the site, shape and number of layers:

- protection (epidermis of skin, lining epithelium),
- secretion (production of hormons and enzymes),
- lubrication (mucus secretin cells),
- excretion (epithelium of the urinary system and sweat glands),
- absorption (epithelium of the digestive tube),
- special sensation (taste, smell and hearing - receive and transduce external stimuli),
- contraction (myoepithelial cells - branched cells that contain myosin and actin, specialized for contraction).

3.3. Structure and organization of epithelial tissue

3.3.1. Epithelial cell polarity

Epithelial cells exhibit functional as well as morphological polarity.

- Apical domain – always directed towards the exterior surface or the lumen of the tube or cavity;
- Lateral domain – communicates with the adjacent cells and is characterized by specialized attachment of the cell membranes;
- Basal domain - rests on the basal lamina anchoring the cells to the underlying connective tissue.

The properties of each domain are determined by specific membrane proteins. The structure of the membrane determines the functional polarity of all three cell domains.

3.3.1.1. Apical domain and its modifications

In many epithelial cells, the apical domain exhibits special structural surface modifications that have specific functions. The apical domain may contain specific enzymes, ion channels and carrier proteins. The core of surface modifications consists of actin filaments linked by other proteins, such as fimbrin, myosin, villin or spectrin. The following free surface modifications are recognized:

1. *Microvilli* - finger-like cytoplasmic projections on the apical surface of most epithelial cells, which increase the cell surface area. The microvilli are most developed in the absorptive cells (e.g. small intestine, kidney tubules). The core of each microvillus contains *actin* filaments that are cross-linked by *fimbrin. Myosin I* binds the actin filaments to the plasma membrane of the microvillus. Actin filaments are anchored to *villin* located in the tip of the microvillus. At the base of the microvillus the entering actin filaments are stabilized by *spectrin*, and with horizontal actin filaments, know as the *terminal web*.

   Epithelial cells in the body contain either numerous microvilli (as in syncytiotrophoblast), sparse microvilli (as in mesothelium) or very dense microvilli (as in the brush border of the kidney tubules and intestinal epithelium).
2. *Stereocilia* – extremely long processes that facilitate the absorption. Stereocilia are supported by *actin* filament bundles that are cross-linked by *fimbrin*. *Erzin* anchors the actin filaments to the plasma membrane. Stereocilia are present in the male reproductive

system (epididymis, proximal part of the ductus deferens), where they facilitate the absorption, and in the membranous labyrinth (hair cells) of the inner ear, where they serve as sensory receptors.

3. *Cilia* – motile cytoplasmic projections capable of moving fluid and various particles along the epithelial surfaces. Cilia possess an internal structure that enables their movement. Each cilium is composed of parallel *microtubules* that are bound together with other proteins (*tubulin, dynein*). In most ciliated epithelia, such as the trachea, bronchi or oviducts, cells may have several hundred cilia, all arranged in orderly rows.

   In the trachea, the cilia sweep mucus and trapped particulate material towards the oropharynx, where it is swallowed with saliva, and thus eliminated from the body.

   The cilia of epithelial cells in the uterine tube help move the fertilized ovum and fluid towards the uterus.

3.3.1.2. Lateral domain and its specializations

The lateral domain of epithelial cells is in close contact with the opposite lateral domain of the neighboring cells and participates in the structural integrity of epithelium. The integrity of epithelium is maintained by adhesion of cells to each other and by extracellular matrix. These adhesions are mediated by cell membrane proteins. It is also the site of barrier against the passage (diffusion) of substances across the epithelium.

The specific structural components are identified with the EM and are referred to as *junctional complexes*, visible as *terminal bars* with a light microscope. These complexes are responsible for binding the epithelial cells together and are classified into three types:

1. *Occluding junctions* called *zonula occludens* (tight junctions) link the cells and form an impermeable barrier. Zonula occludens involve a fusion of the adjoining cell membranes. This junction is perfomed by the intramembranous proteins, which mediate the adhesion of the adjacent cells.

   Occluding junctions have two main functions: prevention of diffusion of molecules between adjacent cells and prevention of lateral migration of specialized cell membrane proteins. Occluding junctions are

particularly evident between epithelial cells that have an absorptive role.

2. *Anchoring junctions* provide the mechanical stability of the epithelial cells by linking the cytoskeleton of one cell to the cytoskeleton of an adjacent cell. Anchoring junctions interact with actin and intermediate filaments and can be found not only on the lateral cell surface, but also on the basal domain (hemidesmosomes) of the epithelial cells. Two types of anchoring cell-to-cell junctions can be identified on the lateral cell surface:
   Zonula adherens – In the TEM, zonula adherens is characterized by 15 to 20 nm space between the opposite cell membranes. The cytoskeletal filaments of the adjacent cells are joined through intracellular link proteins, which attach the filaments to transmembrane link proteins. Zonula adherens is composed of the transmembrane adhesion molecule E- cadherin, whose molecules from the adjacent cells are linked by Ca^{2+} ions. Therefore, the morphologic and functional integrity of the zonula adherens is calcium dependent.
   Macula adherens or *desmosome* – interacts with the intermediate cytokeratin filaments. In nonepithelial cells are not cytokeratin filaments but other proteins, such as desmin or vimentin. Macula adherens is an anchoring cell-to-cell junction that provides a particularly strong attachment. On the cytoplasmic side of the plasma membrane of each of the adjoining cells there is a disk-shaped structure consisting of very dense material called the desmosomal attachment plaque and here anchors intermediate filaments. The intercellular space of the macula adherens is up to 30 nm wide and is occupied by a dense medial line of glycoproteins. Macula adherens was originally described in the epidermal cells.
3. *Communicating or gap junctions (nexus)* are specialized regions along the lateral side of plasma membranes that allow for selective diffusion of molecules between the adjacent cells and facilitate the direct cell-to-cell comunication without extracellular space. A gap junction consists of accumulation of transmembrane channels or pores. Ultrastructurally, they are seen as close (gap of 2 nm) appositions of the adjacent cell membranes. The pores in one cell membrane are precisely aligned with corresponding pores on the membrane of

an adjacent cell. Gap junctions permit the exchange between cells through the pores. They are present in the epithelial tissue, smooth and cardiac muscle and nervous tissue.

Lateral interdigitations.
The lateral surfaces of epithelial cells show a tortuous boundary due to infoldings along the border of each cell with its neighbour. These infoldings or interdigitations at the lateral surfaces of two adjoining cells increase the lateral surface area of these cells. Interdigitations are prominent in epithelia that are engaged in fluid and electrolyte transport, such as the intestinal epithelium.

3.3.1.3. Basal domain and its specializations

Cell membrane on the basal domain is affixed to the basal lamina by:

Hemidesmosomes - adhering junctions, which morphologically resemble half of a desmosome, but their biochemical composition is different. In desmosomes, the attachment plaques contain mainly cadherins, while in hemidesmosomes they consist of integrins.

Plasma membrane infolding – present on the basal surface plasma membrane and form deep invaginations. These folds are more evident in cells involved fluid or ion transport and are associated with high concentrations of mitochondria. The presence of basal folds and mitochondria in the basal cytoplasm gives rise to a striated cytoplasm. Basal folds are seen in kidney tubular cells as well as in the cells of the secretory glands.

3.3.2. Basement membrane – structure and function

The epithelial cells are separated from the underlying connective tissue by a specialized layer of extracellular matrix - the *basement membrane*, which is visible with light microscope as PAS-positive structure. The major structural components of the basement membrane are: collagen type IV, glycoproteins laminin and entactin and proteoglycan heparan sulfate. The basement membrane 20 – 100 nm thick layer is shown by electron microscopy to be composed of :

- *Basal lamina* – amorphous, adjacent to the epithelium and manufactured by the epithelial cells. Electron microscope displays its two parts*: lamina lucida*, which consists mainly of extracellular

glycoproteins laminin and entactin and *lamina densa*, which comprises of a meshwork of type IV collagen and proteoglycans, such as heparan sulfate.

- *Reticular lamina* – fibrous, situated below the basal lamina, manufactured by the cells of the connective tissue and responsible for affixing the basal lamina to the underlying connective tissue. It is composed of type I and type III collagen.

Although the basement membrane is described as exclusively associated between epithelium and connective tissue, similar PAS-positive structure can be demonstrated where other cell types come into contact with the connective tissue, such as round adipose cells, muscle cells and Schwann cells. The basement membrane has the following main functions: it acts as a molecular filter (permeability barrier) as well as a flexible firm support for the overlying epithelium. Epithelial cells are anchored to the basement membrane, while basement membrane is anchored by fibroreticular lamina to the adjacent extracellular matrix.

The thickness of the basement membrane depends on the functions of the epithelium:

- Small capillaries have a thin or fenestrated basement membrane.
- Basement membrane in the trachea is thick and serves as defense against bacterial invasion.
- The basement membrane of epidermis is thick and rugged to endure the constant stress sustained by the epidermis.
- In contrast, the glomerular basement membrane of the double epithelium in the Bowman´s capsule is thick, but lacks reticular lamina.

3.3.3. Classification of epithelium

Epithelia are divided into two main groups according to their structure and functions:

1. *Covering epithelia*
2. *Glandular epithelia*

3.3.3.1. Covering epithelia

The cells are organized in layers that cover the external surface or line the cavities of the body. They can be classified according to the

number of cell layers and the morphological features of the cells in the surface layer.

Simple epithelium - contains only one layer of cells, all of which are in contact with the basal lamina.

Pseudostratified epithelium - all cells are attached to the basal lamina, but only some cells (the tallest) reach the luminal surface. The cells of these epithelium are of different heights, their nuclei are located of different levels (appears as stratified epithelium). This epithelium is found in respiratory tracts, epididymis, in large excretory ducts of glands.

Stratified epithelium - contains more than one layer of cells, while only the basal layer is in contact with the basal lamina.

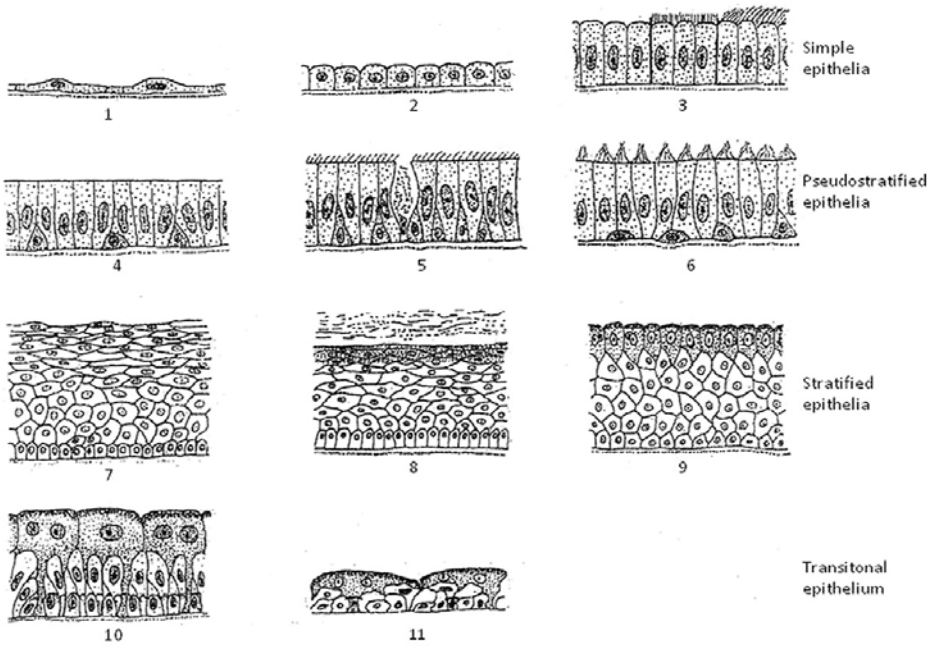

Fig. 1. Diagrams of covering epithelia

1-simple squamous epithelium, 2-simple cuboidal, 3-simple columnar-without specialization-with microvilli-with cilia, 4-pseudostratified columnar, 5-pseudostratified ciliated with goblet cells, 6-pseudostratified columnar with stereocilia, 7-stratified squamous nonkeratinizing, 8-stratified squamous keratinizing, 9-stratified columnar, 10-transitional (relaxed), 11-transitional (stretched)

Common types of covering epithelia in the human body

Type	Cell form	Main function	Examples of present
Simple	Squamous	Transport by pinocytosis, facilitates the movement of the viscera (mesothelium), secretion of active molecules	Lining of vessels and heart (endothelium), lining of cavities; pleura peritoneum, pericardium
	Cuboidal	Covering, secretion, lining	Covering the ovary, lining of kidney tubules, thyroid gl., pigment epith.
	Columnar	Protection, absorption, secretion, lubrication	Lining of the intestine, stomach, gallbladder, uterus, uterine tube
	Pseudostratified columnar particles trapped in mucus, lubrication	Protection, secretion, cillia-mediated transport of auditory tube, epididymis, vas deferens, male urethra	Lining of the nasal cavity, trachea, bronchi, larynx,
Stratified	Surface layer is squamous keratinized	Protection, prevention of water loss	Epidermis of the skin
	Surface layer is squamous nonkeratinized	Protection, secretion	Mouth, oesophagus,anal canal, vagina, larynx
	Cuboidal	Protection, secretion	Sweat glands, developing ovarian follicles
	Transitional: dome-like to flattened, depending on the functional stage	Protection, distensibility	Renal calyces, ureters, bladder, part of the urethra
	Columnar	Protection, secretion, absorption,	Conjuctiva, large excretory duct, portion of the male urethra

Simple epithelia can be squamous, cuboidal or columnar, according to the shape of cells:

1. *Simple squamous* epithelium is composed of a single layer of flat cells. Simple squamous epithelium lines the pulmonary alveoli, composed of the loop of Henle, and the parietal layer of the Bowmasn´s capsule in the kidney.

- *Endothelium* lines the blood and lymph vessels and cavities of the heart.
- *Mesothelium* lines the body cavities, such as peritoneal, pleural and pericardial cavities.

2. *Simple cuboidal* epithelium is composed of a single layer of cuboidal cells. Cuboidal epithelium covers the surface of the ovary and makes up the ducts of many glands as well as some of the kidney tubules. It also covers the choroid plexus, which helps produce the cerobrospinal fluid. Follicular epithelial cells in the thyroid gland can also assume a cuboidal shape, depending on their function. Often, cuboidal epithelial cells have many apical microvilli, which facilitate ion pumping and fluid transport.
3. *Simple columnar* epithelium is composed of a single layer of tall cells and present in areas where absorption occurs. This epithelium lines the digestive tube – stomach, small and large intestines, where it contains the absorptive cells and goblet cells. It is also found in the gallbladder and some glandular ducts and it lines the papillary collecting ducts in the urinary system. Also lines uterus and uterine tube. The columnar epithelial cells have many apical microvilli.

Pseudostratified columnar epithelium is composed of one layer of cells, all of which rest on the basement membrane, but only some of them extend to the apical surface; the nuclei appear to be lined in various levels. The best-known example of this epithelium is the cilliated pseudostratified columnar epithelium with goblet cells in the respiratory passages. Pseudostratified columnar epithelium is abundant in the male reproductive system – epididymis, vas deferens, prostate gland, seminal vesicles and the distal part of the male urethra (membranous and pendulous part).

Stratified epithelium is composed of several layers, from which only the basal layer of cells is in contact with the basement membrane. Stratified epithelium is classified according to the shape of cells in its superficial layer.

1. *Stratified squamous epithelium* - the cells closer to the underlying connective tissue are usually cuboidal or columnar. As they move to the surface, they become irregular in shape, the more superficial becoming very squamous. Stratified squamous epithelium exists in

locations exposed to chronic abrasion. The superficial layers are continuously sloughed and then replaced by cell division in the basal layer. Stratified squamous epithelium on the tongue and in the mouth and esophagus resists the abrasion caused by mastication, swallowing and the passage of food from the oral cavity to the stomach. Stratified squamous epithelium in the anal canal resists the abrasion of passing semi-solid feces. In the female reproductive system, it is present in the vagina and covers the uterine cervix.

2. *Stratified squamous keratinized epithelium* - covers dry surfaces, such as the skin, where it is called epidermis. Cells in the superficial layer contain the protein *keratin*. The apical layers change continuously to become flattened non-nucleated keratinized plates, whose cytoplasm is filled with *keratin*.
3. *Transitional epithelium* - stratified epithelium containing many layers of polyhedral cells, which is characterized by a surface layer of dome-like cells that are neither squamous nor columnar. The form of these cells changes according to the degree of distention of the urinary passages. Transitional epithelium undergoes a reversibile morphologic change during bladder destension and evacuation. It is found in the minor and major calyces, renal pelvis, ureters, urinary bladder and proximal urethra.

Neuroepithelial cells are cells of epithelial origin with specialized sensory functions (cells of taste buds, olfactory epithelial cells and statoacustic receptor cells).

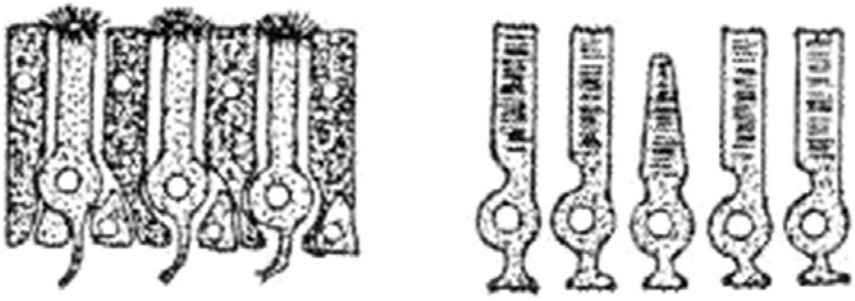

Fig. 2. Diagram of neuroepithelial cells
a. Olfactory sensory epithelium, b. Rods and cones of the retina

Myoepithelial cells are specialized for contraction. These epithelial cells are branched and contain myosin and many actin filaments. They are mostly present in the salivary, sweat and mammary glands, where they surround the secretory units.

3.3.3.2. Glandular epithelium

Glandular epithelia are formed by cells specialized to produce secretion. Glandular cells may secrete proteins (e.g. pancreas), lipids (e.g. adrenal and sebaceous glands) or comlexes of carbohydrates and protein (e.g. salivary glands). The mammary glands secrete all three of these substances. Glands arise during fetal life from the covering epithelia by means of proliferation and invasion of epithelial cells into the subjacent connective tissue. The secretory units along their ducts form the *parenchyma* of the gland and the elements of the connective tissue that invade and support the parenchyma are called the *stroma* of the gland.

Classification of glands

The epithelia that form the glands of the body can be classified according to various criteria.

- *Unicellular glands* - consist of an isolated glandular cell in the epithelium. An example of these are *goblet cells, which* represent the simplest form of exocrine gland dispersed in the epithelia lining of the digestive and respiratory tract. The secretions released by these mucous glands protect the linings of these tracts. Goblet cells derive their name from their shape, being that of a goblet. The expanded apical portion of goblet cells faces the lumen and contains secretory droplets; the nucleus is situated on its base.
- *Multicellular glands* - are composed of clusters of secretory cells and connective tissue elements. These secretory cells do not act alone, but as secretory organs.

  Multicellular glands are divided into two major groups according to where their products are released:

  - *Exocrine glands* - persist their connection with the surface epithelium, from which they originate. They secrete products onto the surface directly or through ducts or tubes that are connected to the surface.

- *Endocrine glands* - their connection with the surface is lost during development. Therefore, these glands are ductless and secrete products into the bloodstream. The products of endocrine glands are called *hormones*. However, the secretory substance of *Paracrine glands,* a special type of endocrine glands, does not reach the bloodstream, but affects other cells within the same epithelium. The secretory material reaches the target cells by diffusion through either the extracelular spaces or the immediately subjacent connective tissue.

Cells of exocrine glands exhibit three different mechanisms for releasing their secretory products:

- *Merocrine secretion* - secretory vesicles fuse with the plasma membrane and extrude their contens by exocytosis. This is the most common mechanism of secretion (e.g. pancreas, parotid gland).
- *Apocrine secretion* - the secretory product is released in the apical portion of the cell, surrounded by a thin layer of cytoplasm (e.g. lactating mammary gland – releases large lipid droplets of milk, sweat glands).
- *Holocrine secretion* - the secretory product accumulates within the maturing cells, which die simultaneously (e.g. sebaceous glands of skin).

Multicellular glands are classified according to the shape of their secretory units:

- *Tubular* – if the secretory portion is shaped like a tube (e.g. intestinal glands, stomach, sweat glands of skin). Tubular secretory portions may be straight, branched or coiled.
- *Alveolar (acinar*) – if the secretory portion has the shape of a sac (e-g- pancreas, parotid gland, paraurethral glands). Alveolar portion may be single or branched.
- *Tubuloalveolar* – if the tube ends in a sac-like dilatation (e.g. submandibular salivary gland). Tubuloalveolar portions are branched.

Various combinations of shapes of the secretory portions are found in the body.

According to the morphology of the ducts, multicellular glands are classified as:

- *Simple* – the ducts are not branched, or
- *Compound* – the ducts are branched.

Exocrine glands are further subclassified according the chemical composition of the secrete:

- *Mucous glands* - secrete mucin, a large glycosylated protein. Mucous cells are PAS-positive, their cytoplasm appears to be empty in HE – staining. Nucleus is usually flattened against the base of the cell.
- *Serous glands* - serous cells produce poorly glycosylated protein or pure protein. The nucleus is typically round or oval, cytoplasm is often intensively stained.
- *Mixed glands (mucoserous)* - contain both types of cells, mucous and serous.

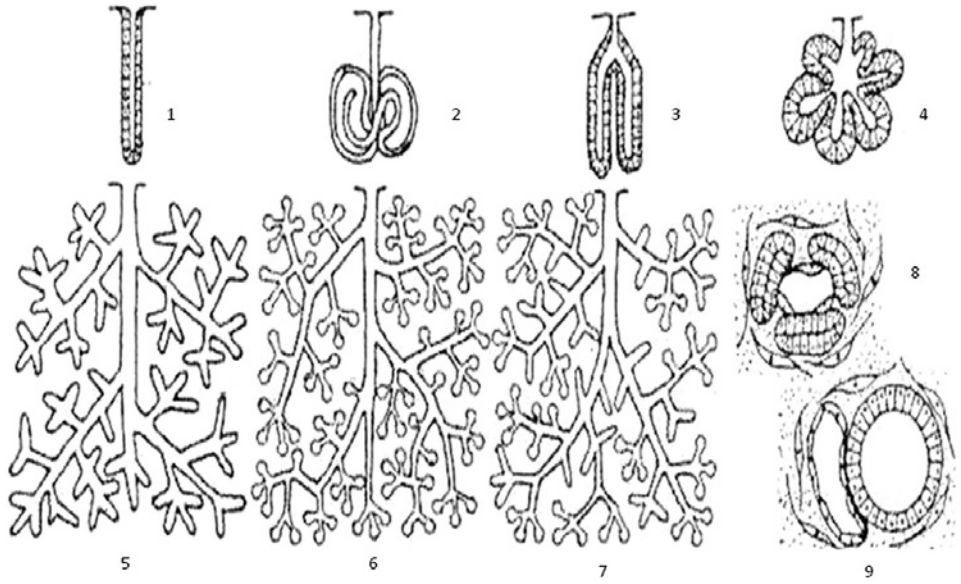

Fig. 3. Principal types of exocrine glands

1-simple tubular, 2-simple coiled tubular, 3-simple branched tubular, 4-simple branched acinar, 5-compound tubular, 6-compound acinar, 7-compound tubuloacinar, 8-endocrine gland-form cords or 9-follicles

3.4. Renewal of epithelial cells

Epithelial cells are renewed continuously by mitotic activity of the basal cells. Epithelium generally exhibits a high turnover rate related to its location and function. The replacement rate is characteristic of a specific epithelium. The cells lining the small intestine are replaced every 4 to 6 days by mitotic activity of cells in the base of crypts. The new cells then migrate to the tips of the villi, where they undergo apoptosis and slough off into the lumen.

Cells of the epidermis of skin are replaced at most sites over a period of approximately 28 days. Cells in the basal layer of the epidermis undergo mitosis to provide for cell renewal. Other epithelial cells are renewed in less time. In some epithelia, particularly in more complex glands, individual cells may live for a long time (liver, pancreas), while other epithelia are renewed periodically.

Control of glandular activity

Glands are usually sensitive to neural and endocrine control, though one form of control frequently dominates. For example, the salivary glands are principally under neural control. Exocrine secretion in the pancreas depends mainly on stimulation by hormones secretin and cholecystokinin. Neural and endocrine control is achieved by chemical substances called chemical messengers.

Clinical correlations

Under certain abnormal conditions, one type of epithelial tissue may undergo transformation into another type. This process, which is called *metaplasia*, is reversibile**.**

For example, pseudostratified cilliated columnar epithelium lining the bronchi of heavy smokers can be transformed into stratified squamous epithelium – *squamous metaplasia*.

In individuals with chronic vitamin A deficiency, epithelial tissues found in the bronchi and urinary bladder are gradually replaced by stratified squamous epithelium. These changes may be reversed when pathological insult is removed.

A tumor arising from the epithelial cells may be either benign or malignant. Malignant tumors originating in the covering epithelia are called

carcinomas, while those originating in the glandular epithelial cells are called *adenocarcinomas*.

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4. Connective tissue

Connective tissue can be found all over the body. Embryologically, connective tissue develops from embryonic mesoderm, which forms the multipotentional mesenchyme from which bone, cartilage, blood, hemopoietic cells and lymphoid cells develop. Most of the connective tissues in the body have a good vascular supply. The cells within connective tissue are not tightly packed together. There is more intercellular substance in connective tissue than in epithelial tissue.

Functions:

Connective tissues *connect and hold* tissues and organs to one another. Connective tissue *supports* the different organs and tissues together, it *transports nutrients and metabolites (*diffusion), has *immunological function* (mast cells, macrophages). *Inflammation is a specific function* of connective tissue (lymphocytes, neutrophils). *Mechanical support* (bone, cartilage) is very important function of connective tissue. Additional functions are *energy storage* (as fat), *hemopoiesis* (blood cells formation). After injury, connective tissues instrumental in *tissue repair*, specifically in *scar formation*. Scars are formed by fibroblast activity during tissue repair. The substance of the scar is collagen deposited by fibroblasts to replace damaged tissue.

4.1. Components of connective tissue

Connective tissue consists of cells and an extracellular matrix. Matrix consists of fibers, ground substance and tissue fluid.

4.1.1. Connective tissue cells

The resident cell population - Fixed cell are relatively stable.

These resident cells include fibroblasts, myofibroblasts, adipose cells, fat cells (lipocytes), reticular cells, pigment cells.

Wandering or transient cell population is lymphocytes, plasma cells, mast cells, neutrophils, eosinophils, basophils, monocytes.

Fibroblasts

The fibroblast is the principal cell of connective tissue. Fibroblasts are responsible for the synthesis of collagen, elastic and reticular fibers, and others elements of the extracellular matrix of connective tissue. The active fibroblast has ovoid pale staining nucleus. The cytoplasm is rich in rough endoplasmic reticulum. Fibrocyte is mature fibroblast, a smaller cell than the fibroblast, spindle-shaped with multiple processes, has darker elongated nucleus.

Myofibroblasts

Myofibroblasts are spindle-shaped cells that secrete collagen, but also have contractile proteins (actin, desmin) not seen in fibroblasts. Myofibroblasts are modified fibroblasts that demonstrate characteristics similar to those of both fibroblasts and smooth muscle cells. Fibroblasts and myofibroblasts are not easily distinguished by routine light microscopy. Electron microscopy – myofibroblasts have bundles of actin filaments and dense bodies similar to those of smooth muscle cells. Myofibroblasts differ from smooth muscle cells in that an external lamina (basal lamina) is absent.

In normal tissues, myofibroblasts are an inactive population of cells, for example in the alveolar septa of the lung, around the crypts of glands in the gut, in connective tissue of the capsule and trabeculae of the spleen. Myofibroblasts become active and proliferate and their role appears to be to repair defects resulting from tissue death (=fibrous scar). They are also found in several diseases characterized by fibrosis of tissues (fibrosis of the lung, cirrhosis of the liver)

Adipose cells, fat cells, adipocytes

The adipose cell is a connective tissue cell specialized to store neutral fat. They are large spherical cells with eccentric flat nucleus. Adipose cells are located in loose connective tissue as individual cells and groups of cells. When they accumulate in large numbers, they are called adipose tissue.

Unilocular adipose cells contain one large fat vacuole surrounded by a thin rim of cytoplasm. In routine histological techniques, alcohol removes lipid vacuole; each cell appears as a thin ring of cytoplasm with flat nucleus surrounding the vacuole.

Multilocular adipose cells contain numerous lipid droplets within cytoplasm (brown fat).

Reticular cells

Reticular cells are stellate-shaped cells with many large processes. The cells are connected with each other by processes and they form cellular reticulum. Some of these cells are phagocytic cells.

Pigment cells (melanocytes, melanophores, chromatophores)

They are small cells with many branching processes. They have shape similar to fibroblasts, but synthesize melanin pigment. Pigmentary cells come from neural crest. *Pigmented connective tissue cells,* know as *chromatophores,* and are common in non-human vertebrate skin. Chromatophores occur in isolated areas in humans. These areas include the dermis of the skin, the choroid and iris of the eye, and choroid plexus. Virtually all pigment cells contain brown or black melanin and are referred to as *melanocytes*. Melanophores are a special type of chromatophores.

Macrophages, Histiocytes

Macrophages are phagocytotic cells. *Connective tissue macrophages*, also known as *histiocytes*, are derived from blood cells monocytes. Monocytes migrate from the bloodstream into connective tissue, where they differentiate into macrophages. The main function of the macrophage is phagocytosis of bacteria and cell debris. Macrophages *has kidney – shaped dark nucleus*. They also contain endocytotic vesicles, phagolysosomes and other evidence of phagocytosis (residual bodies).

Mononuclear phagocyte system (Macrophage system)

It is a system of cells comprising all free and fixed phagocytes together with their ancestral cells including monocytes and their precursors in the bone marrow called *reticuloendothelial system (RES).*

Reticuloendothelial system is the class of cells that occurs in widely separated parts of the human body and that take up particular substances. These cells are part of the body´s defense mechanisms. Reticuloendothelial cells are derived from precursor cell in the bone marrow. These precursors develop into monocytes, phagocytic cells that are released into bloodstream. Some monocytes remain in the general blood circulation, but most of them enter body tissues, where they develop into much larger phagocytic cells called macrophages. In certain regions, macrophages have special names, e.g. Kupffer cells in the liver, microglial cells in the central nervous system, Langerhans cells of the skin, and osteoclasts in bone tissue. The reticuloendothelial cells also participate in body defense through immune

reactions. Another important function of the reticuloendothelial cells is the destruction of worn-out or abnormal cells and tissues. The reticular cells of the spleen in particular play a major role in the destruction of worn-out red blood cells and recycling of hemoglobin.

Table 1. Cells of the mononuclear phagocyte system

Cell Type	Location	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation phagocytosis of foreign substances and bacteria
Kupffer cell	Liver	Same as macrophages
Microglia cell	Nerve tissue of the central nervous system	Same as macrophages
Langerhans cell	Skin	Antigen processing and presentation
Dendritic cell	Lymph nodes	Antigen processing and presentation
Osteoclast	Bone (fusion of several macrophages)	Digestion of bone

Mast cells

Mast cells are large, ovoid cells with a spherical nucleus and cytoplasm filled with large, basophilic granules. After glutaraldehyde fixation, mast cell granules can be displayed with basic dyes, as toluidine blue. It stains the granules intensely and metachromatically (purple). Several vasoactive and immunoreactive substances are contained in mast cell granules.

Histamine and *slow-reacting substance of anaphylaxis (SRS*-A) increase the permeability of small blood vessels, causing edema in the surrounding tissue, increase contraction of smooth muscle in the pulmonary airways, causing bronchospasm. Mast cells release *eosinophil chemotactic factor (ECF*) and *neutrophil chemotactic factor (NCF),* which attracts eosinophils and neutrophils to the site of inflammation. *Heparin* is an anticoagulant; it can block numerous coagulation factors. Heparin is useful for treatment of thrombosis. Mast cells during activation release several secondary mediators *(leukotriens, prostaglandin D)*. These mediators are not stored in granules but are synthesized by the cell and released into the extracellular matrix.

Mast cells are numerous *in connective tissue of skin and mucous membranes* but not present in the brain and spinal cord. They are also present *in the capsules of organs and the connective tissue that surrounds the blood vessels*. Mast cells are also numerous *in the thymus*. Release of the chemical mediators stored in mast cells promotes the allergic reaction known as *immediate hypersensitivity reactions* because they occur within a few minutes after penetration by antigen of an individual previously sensitized to the same or a very similar antigen. A dramatic immediate hypersensitivity reaction is *anaphylactic shock*, a potentially fatal condition.

Plasma cells

Plasma cells are antibody-producing cells derived from B lymphocytes. They are found in loose connective tissue of gastrointestinal and respiratory tracts. They are also normal component of salivary glands, lymph nodes and hemopoietic tissue. Plasma cell has short life span of 10 to 30 days. It is an ovoid cell; the cytoplasm displays strong *basophilia* because of an extensive rough endoplasmic reticulum. The nucleus is spherical and typically offset or *eccentrically positioned*. It exhibits large clumps of peripheral heterochromatin alternating with clear areas of euchromatin. This arrangement resembles a *cartwheel* or analog *clock face*.

Basophils, neutrophils, eosinophils

Basophils leave the circulation in certain immune reactions and function in the connective tissue. In an acute inflammatory reaction, *neutrophils* migrate into the connective tissue.

Eosinophils may be observed in connective tissue during allergic reactions and parasitic infections.

Undifferentiated mesenchymal cells

These cells give rise to differentiated cells that function in repair and formation of new tissue and development of new blood vessels (neovascularization). *Pericytes (adventitial cells, perivascular cells*) are one type of undifferentiated mesenchymal cells, they are found around capillaries and venules. They are surrounded by basal lamina that is continuous with the basal lamina of the capillary endothelium. During the development of new vessels, pericytes may differentiate into the smooth muscle of the vessel wall.

Table 2. Cells of connective tissue

Cell Type	Function
Fibroblast, reticular cells	Production of fibers and ground substance
Plasma cell	Production of antibodies
Myofibroblast	Is responsible for wound closure after tissue injury
Eosinophilic leukocyte	Participation in allergic and vasoactive reactions, modulation of mast cell activities and the inflammatory process
Neutrophilic leukocyte	Phagocytosis of foreign substances, bacteria
Macrophage	Secretion of cytokines and other molecules, phagocytosis of foreign substances and bacteria, antigen processing and presentation to other cells
Mast cell and basophilic leukocyte	Liberation of pharmacologically active molecules (e.g. histamine)
Adipose (fat) cell	Storage of neutral fats, energy reservoir, heat production
Pigment cell (melanophore)	Synthesize pigment melanin

4.1.2. Connective tissue fibers

Connective tissue fibers are of tree types: collagen fibers, reticular fibers and elastic fibers. Connective tissue fibers are long, slender protein polymers that are present in variable proportions in the different type of connective tissue.

Collagen fibers

Collagen fibers are most abundant type of connective tissue fibers. They are flexible and have a remarkably high tensile strength. Collagen fibers are acidophilic; they are stained pink with eosin and blue with aniline blue.

When examined with the transmission electron microscope, collagen fibers appear as bundles of fine, thread-like subunits collagen fibrils. Within an individual fiber, the collagen fibrils are uniform in diameter. In different locations and different stages of development, the fibrils differ in size. In immature tissue, the fibrils may be as small as 15 or 20 nm in diameter. In dense connective tissue, they may measure up to 300 nm in diameter. When collagen fibrils stained with osmium are examined with the electron microscope, they exhibit a sequence of closely spaced transverse bands. This banding pattern reflects the fibril´s subunit structure, the size and

shape of the collagen molecule tropocollagen and arrangement of the molecules that form the fibril. Each collagen molecule is a triple helix composed of three polypeptide (alpha) chains. Amino acid in the chain is a glycine, a hydroxyproline or hydroxylysine.

There are 19 different types of collagens based on the combinations of (alpha) chains they contain. The various collagens are classified by Roman numerals I to XIX according to the chronology of their discovery. *Type 1 collagen* is found in loose and dense connective tissue (account for 90 % of body collagen). *Type 2 collagen* is present in hyaline and elastic cartilage, where it occurs as very fine fibrils. The molecules of collagen types 1, 2, 3, 5 and 11 aggregate to form banded fibrils. In contrast, *type 4 collagen* forms a nonfibrillar network that provides structural cohesion to the basal lamina. Collagen is produced by fibroblasts. The precursor of the collagen molecule within the fibroblast is *procollagen.* Production of the structural fibrils occurs outside the cell.

Table 3. Types of collagen

Type	Morphology	Distribution
I	large banded collagen fibers	skin dermis, tendon, bone, ligaments, fascia, fibrous cartilage, cornea, loose fibrous tissue
II	small banded collagen fibers	hyaline and elastic cartilage, vertebral discs, vitreous of eye
III	small banded collagen fibers	blood vessels, parenchymal organs, bone marrow, lymphoid tissues, smooth muscle, nerves, lung, fetal skin
IV	sheet-like layers	basement membranes, external laminae, lens capsule
V	thin fibrils	basement membrane of placenta, smooth and skeletal muscle
VI	thin fibrils	ubiquitous
VII	short striated fibrils	anchoring fibrils in basement membrane of skin and amnion
VIII	uncertain	endothelium
IX	uncertain	cartilage
X	uncertain	mineralizing cartilage
XI	uncertain	cartilage

Clinical correlations

The important role of collagens in the body can be illustrated by *collagenopathies* (collagen diseases), which are caused by a deficit

or abnormality in the production of specific collagens *(osteogenesis imperfecta).* Several diseases result from an overaccumulation of collagen. In *progressive systemic sclerosis*, almost all organs may present an excessive accumulation of collagen (*fibrosis*). This occurs mainly in the skin, digestive tract, muscles and kidneys. *Keloid* is a local swelling caused by abnormal amounts of collagen that form in scars of the skin.

Reticular fibers

Reticular fibers are named for their arrangement in network. Reticular fibers and collagen fibers consist of collagen fibrils. Unlike collagen fibers, reticular fibers are composed of *type 3 collagen*. In routinely stained HE preparations, reticular fibers cannot be identified positively. Reticular fibers are readily displayed by means of the periodic acid-Schiff (PAS) reaction. They are also revealed with special *silver-staining procedures* (*Gomori),* the fibers appear *black* thus, they are said to be *argyrophilic*. In loose connective tissue, networks of reticular fibers are found at the boundary of connective tissue and epithelium, as surrounding adipocytes, small blood vessels, nerves, and muscle cells. Reticular fibers also function as a supporting stroma in hemopoietic and lymphatic tissues (but not in the thymus). In these tissues, the collagen and the reticular fibers synthesize the reticular cells. Important exceptions to this general rule include the endoneurium of peripheral nerves, where Schwann cells secrete reticular fibers. The reticular and other collagen fibers synthesize smooth muscle cells of the tunica media of blood vessels.

Elastic fibers

Elastic fibers are fine, straight, stretchable fibers. They are thinner than collagen fibers and are arranged to form three-dimensional network. Elastic fibers allow tissues to respond to stretch and distension. Elastic fibers stain with eosin not well, therefore, they cannot always be distinguished from collagen fibers in routine hematoxylin eosin preparations. They can also selectively stain with special dyes, as *orcein or resorcin-fuchsin (red-brown).* Fibroblasts and smooth muscle cells produce elastic fibers. Elastic fibers are composed of two structural

components, a central core of amorphous elastin and surrounding fibrillin microfibrils. *Elastin* is a protein that is rich in proline and glycine. Elastin forms fibers of variable thickness or lamellar layers (as in elastic arteries). Elastin also contains desmosine and isodesmosine, two amino acids that are responsible for the covalent bonding of the elastin molecules to one another. With the transmission electron microscope, elastin appears as an amorphous structure of low electron density. *Fibrillin* is a glycoprotein that forms fine microfibrils. In mature elastic fibers, the *fibrillin microfibrils are located at its periphery.* In the tunica media of blood vessel walls, the elastic material of arteries is synthesized by smooth muscle cells, not by fibroblasts. In elastic arteries, the elastic material is in the form *fenestrated lamellae*. In contrast to elastic fibers, microfibrils are not found in the lamellae, only the amorphous elastin.

Clinical correlations

Marfan´s syndrome – one of the consequences of the disease is abnormal elastic tissue. The skin biopsy from a person with Marfan´s syndrome will show an absence of elastin-associated fibrillin microfibrils.

4.1.3. Ground substance of connective tissue

The ground substance is colorless, transparent, homogeneous, amorphous material composed of proteoglycans and glycosaminoglycans. Ground substance occupies the space between the cells and fibers in connective tissue. It is a viscous, clear substance and has high water content. In the light microscope, ground substance appears amorphous. In HE preparations, ground substance is lost because of its extraction during fixation and dehydration of the tissue. The result is an empty background, only cells and fibers are evident. Ground substance permits diffusion of oxygen and nutrients between the vessels and cellular components of the tissue. Ground substance consists of *proteoglycans and glycosaminoglycans*. They are responsible for the physical properties of ground substance.

Clinical correlations

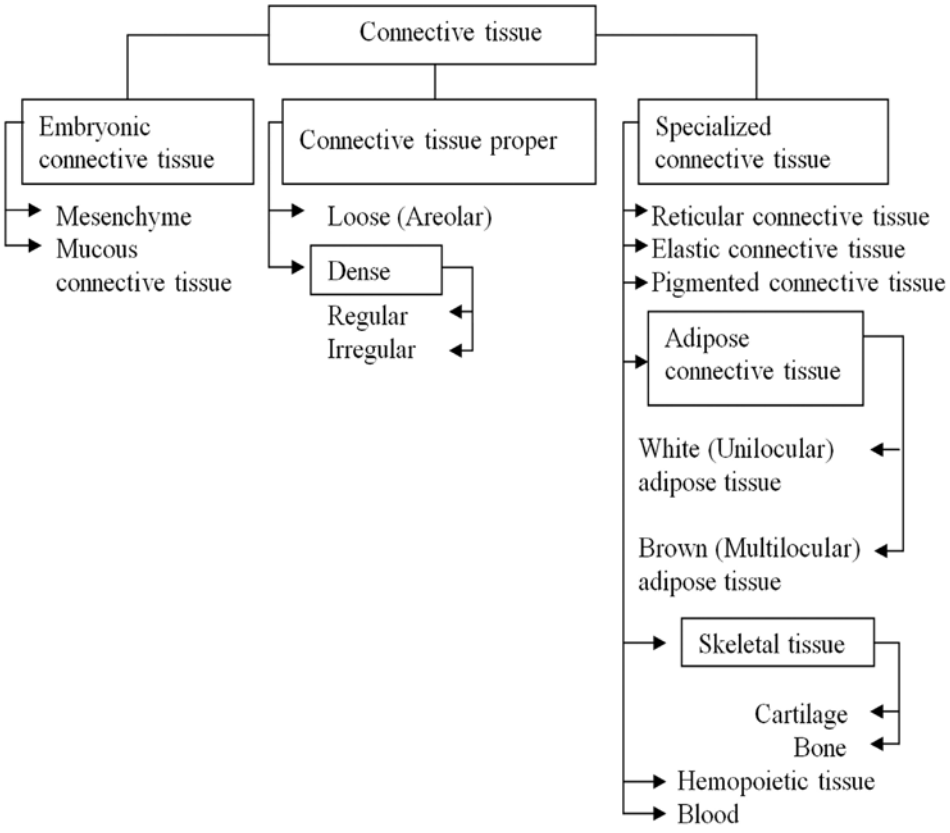
In several pathologic conditions, the quantity of tissue fluid may increase considerably, causing *edema.* Histologically, this condition

is characterized by enlarged spaces between the components of the connective tissue caused by the increase in liquid.

4.2. Classification of connective tissue

Classification of connective tissue is based on the composition and organization of its cellular and extracellular components and on its functions. Different types of connective tissue are responsible for a variety of functions. The term connective tissue includes a variety of tissues with differing functional properties but with certain common characteristics.

Table 4. Classification of connective tissue



4.2.1. Embryonic connective tissue

Embryonic connective tissue is present in embryo and within the umbilical cord.

Mesenchyme

Mesenchyme is found *in the embryo*. It arises from the mesoderm, the middle embryonic germ layer. Mesenchyme contains small, spindle-shaped cells. Processes extend from these cells and contact, forming a three-dimensional cellular network. The extracellular space is occupied by a viscous ground substance and very fine collagen (reticular fibers). Maturation and proliferation of the mesenchyme give rise not only to the various connective tissues of the adult but also muscle, the vascular and urogenital systems, and serous membranes of the body cavities.

Mucous connective tissue

It is present in the *umbilical cord* and in *the pulp of young teeth*. It consists of gelatin-like extracellular matrix *(Wharton's jelly),* thin collagen fibers and fibroblasts.

4.2.2. Connective tissue proper

It is divided into two general subtypes:

Loose (areolar) connective tissue

Loose connective tissue is *most common type connective tissue of human body*. It is a cellular connective tissue with thin and relatively sparse collagen fibers. The ground substance occupies more volume than the fibers (Fig. 1). It plays an important role in the diffusion of oxygen and nutrients from the small vessels and in the diffusion of carbon dioxide and metabolic wastes back to the vessels. Most cell types in loose connective tissue are wandering cells that migrate from local blood vessels in response to specific stimuli. This tissue is, therefore, the site of inflammatory and immune reactions. In areas of the body where foreign substances are present, large populations of defending cells are maintained. The loose connective tissue of mucous membranes of the respiratory and alimentary systems contains large numbers of these cells. Loose connective tissue *is located beneath epithelia, surrounds the organs and blood vessels, forms the papillary layer of the dermis, and it fills spaces between nerve fibers and muscle.*

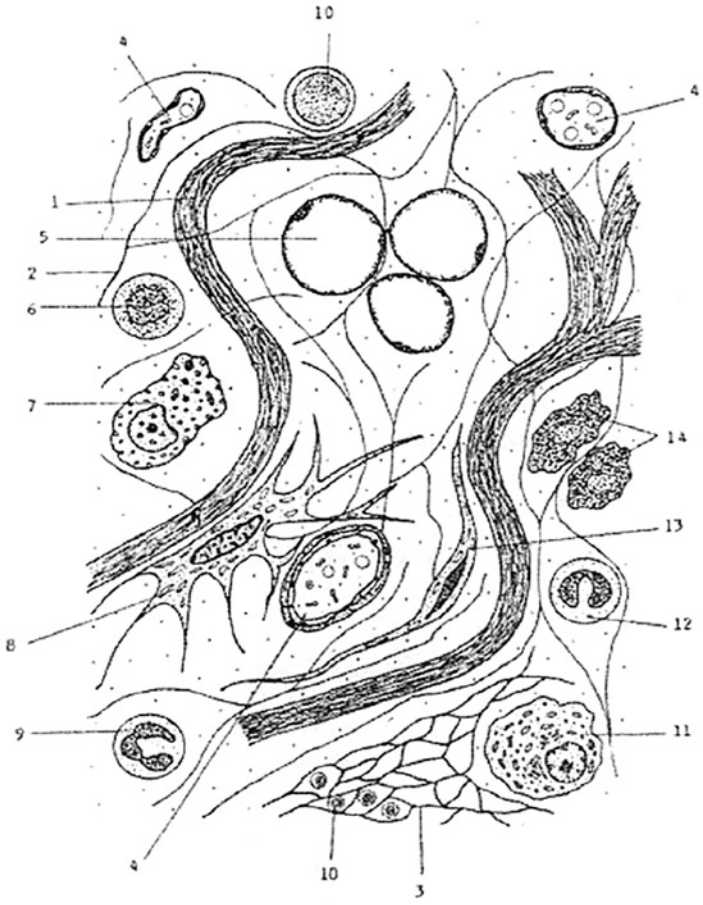


Fig. 1. Loose connective tissue
1-bundles of collagen fibers, 2-elastic fibers, 3-reticular fibers, 4-blood capillary, 5-adipose (fat) cell, 6-basophilic leukocyte, 7-macrophage, 8-fibroblast, 9-neutrophilic leukocyte, 10-lymphocyte, 11-plasma cell, 12-eosinophilic leukocyte, 13-fibrocyte, 14-mast cell

Dense connective tissue

Dense irregular connective tissue

Abundant fibers and few cells characterize dense irregular connective tissue. It contains mostly collagen fibers, fibroblasts and relatively little ground substance. *The fibers are arranged in bundles oriented in various directions* (thus the term irregular). Dense irregular connective tissue is presents in *the submucosal layer of the intestinal tract*, in the skin forms

deep *reticular layer of the dermis*. It is presents in *the capsules of organs* (spleen, testis), forms perichondrium, periosteum, septa, trabeculae and fasciae.

Dense regular connective tissue

The fibers of dense regular connective tissue are the prominent feature and there is little ground substance. In dense regular connective tissue, *the fibers are arranged in parallel array*, the *cells are packed between fiber bundles*. Dense regular connective tissue is the main component of *tendons, ligaments and aponeuroses*. It is found in *cornea of the eye*.

Tendons are structures that attach muscle to bone. They consist of parallel bundles of collagen fibers. Between these bundles are rows of fibroblasts called *tendinocytes*. In longitudinal sections, tendinocytes appears as rows of flattened basophilic nuclei.

The tendon is surrounded by a connective tissue capsule, the *epitendineum.* The tendon is subdivided into fascicles by *endotendineum*. It contains the small blood vessels and nerves of the tendon.

Ligaments join bone to bone. Ligaments consist of fibers and fibroblasts arranged in parallel. Some of the ligaments associated with the spinal column (ligamenta flava) contain many elastic fibers and fewer collagen fibers. These ligaments are called elastic ligaments.

Aponeuroses are broad, flattened tendons. The fibers of aponeuroses are arranged in multiple layers.

4.2.3. Specialized connective tissue

Reticular connective tissue

Reticular connective tissue is a special connective tissue that consists of *reticular cells* and the network of *reticular fibers*, made of type 3 collagen. Reticular connective tissue forms the structural framework in which the cells of the organ are suspended such as *lymph nodes, spleen and bone marrow*.

Elastic connective tissue

Elastic connective tissue is formed of *many elastic fibers* and fibroblasts. This type of connective tissue is present in *elastic ligaments*

(vocal cord, suspensory ligament of the penis) and *elastic membranes in wall of aorta*.

Pigmentary connective tissue

It is ordinary loose or dense connective tissue with large number of pigment cells (vascular tunic of the eye, dermis under mammary gland areolae). The stroma of the iris consists of a loose, pigmented and highly vascular connective tissue. Color of the iris depends on the quantity and arrangement of the pigment.

Adipose tissue

Adipose tissue is a specialized connective tissue containing *large numbers of adipocytes*. There are two types of adipose tissue, which derive their names from the color of the tissue and the number of lipid droplets found in the adipocytes: *white (unilocular) and brown (multilocular)*.

White (unilocular) adipose tissue

Unilocular adipocytes are main component of white adipose tissue. They are derived from undifferentiated mesenchymal cells associated with the adventitia of small venules. *Unilocular adipocytes* are spherical or oval large cells, 100 um or more in diameter. The mature adipocyte is characterized by *a single, large lipid vacuole* surrounded by a *thin rim of cytoplasm*. The lipid vacuole compresses the *nucleus to an eccentric position*, producing a *signet-ring appearance* in hematoxylin and eosin preparations. Adipose tissue is richly supplied with blood vessels and capillaries. Adipocytes are surrounded by reticular fibers (type 3 collagen), which are secreted by the adipocytes. White (unilocular) adipose tissue is the predominant type in adult humans. *Functions* of white adipose tissue include *energy storage, insulation and cushioning of organs*. This tissue has function in *the storage of lipids*. In the palms of the hands, on the plantar surface (sole) of the feet, beneath the visceral pericardium, in the orbits around the eyeballs and in gluteal region it has a structural, cushioning function. The nonlactating female breast is composed primarily of this tissue. *Unilocular adipose tissue* forms a layer called the *panniculus adiposus or hypodermis under the skin*. This subcutaneous layer has insulating function. Concentrations of

adipose tissue are found under the skin of the abdomen, buttock, axilla and thigh. Adipose tissue is located in the *greater omentum, mesentery, and retroperitoneal space and around the kidneys*. It is also found *in bone marrow* and between other tissues, where it fills spaces. *The distribution of white adipose tissue is different in males and females* and is part of the secondary sexual characteristics. White adipose tissue produces the hormone *leptin*. Biologic effects of leptin are inhibition of food intake, loss of body weight and stimulation of the metabolic rate. It acts on the central nervous system by binding to specific receptors, mainly in the hypothalamus.

Brown (multilocular) adipose tissue

The adipocytes of brown, multilocular adipose tissue are smaller than cells of white adipose tissue. The cytoplasm of the multilocular adipocyte contains *numerous fat droplets*. The *nucleus is in an eccentric* position, but *is not flattened*, as is the nucleus of a unilocular adipocyte. The mitochondria contain large amounts of cytochrome oxidase, which imparts the brown color to the cells. In humans, multilocular adipose tissue is present in large amounts *in the newborn (*2-5 % of the body weight in a newborn). The amount of brown adipose tissue decreases as the body grows. It then disappears from most sites except for regions around the kidney, adrenal glands, aorta and regions in the neck and mediastinum. Metabolism of lipid in brown adipose tissue *generates heath. Hibernating animals* have large amounts of brown adipose tissue.

Clinical correlations

The most common adipose tissue benign tumor is *the lipoma. Malignant adipocyte-derived tumors are liposarcomas.* Tumors of brown adipose tissue are called *hibernomas.*

4.2.4. Skeletal tissue

Cartilage and bone, like all other connective tissue, consist of *cells, fibers, and amorphous ground substance*. *The ground substance of bone is mineralized*, making the bone rigid and strong, but brittle. *The ground substance of cartilage* is not mineralized but is more like

very firm, making cartilage stiff and incompressible but more flexible than bone.

Functions:

Cartilage is a key tissue in most growing bones. Bones are the principal supports for the body. They protect vital organs in the skull and thoracic cavity. Bones contain a large store of calcium and bone marrow for hematopoiesis.

4.2.4.1. Cartilage

Cartilage is specialized connective tissue. It is an *avascular tissue* that consists of *chondrocytes* and highly specialized extracellular matrix. The cartilage matrix is composed of abundant ground substance and elastin and collagen fibers. The principal constituents of ground substance are proteoglycans, which consist of protein combined with complex carbohydrates such as *chondroitin sulfate* and *keratan sulfate*. The acidic sulfate groups account the basophilia of the matrix. The proteoglycans are themselves attached to hyaluronic acid to form giant molecules, which form a hard gel. They tie up almost all of the water molecules in cartilage and are attached as gigantic molecular complexes to collagen fibrils. Embedded in the matrix are either *collagen* (Type II) fibers or *elastic fibers*.

Three kinds of cartilage are distinguished on the basis of characteristics of the matrix:

Hyaline cartilage, characterized by matrix containing type 2 collagen fibers, proteoglycans and hyaluronic acid. *Elastic cartilage*, characterized by elastic fibers in the matrix. *Fibrocartilage*, characterized by abundant type 1 collagen in the matrix.

Hyaline cartilage

Hyaline cartilage is the most abundant type of cartilage. The matrix of hyaline cartilage has an amorphous, *glassy (or hyaline) appearance in living state*. Hyaline cartilage resists compression well, remodels itself trough life, trends to calcify with age, cartilage matrix is highly hydrated. Sixty to eighty percent of the net weight of hyaline cartilage is water.

Hyaline cartilage consists of the *chondrocytes* and the *ground substance*.

Chondrocytes

In hyaline cartilage, chondrocytes produce the extracellular matrix. In living cartilage *chondrocytes* lie in matrix spaces called *lacunae*. Chondrocytes are distributed either singly or in clusters called *isogenous groups*. The typical chondrocytes are spherical cells with large nucleus. Chondrocytes shrink during fixation.

Hyaline cartilage matrix

Hyaline cartilage matrix is produced by chondrocytes. It consists of collagen (predominantly type 2 collagen, type 9, 10, 11 and type 6 collagen), proteoglycans, (hyaluronic acid, chondroitin sulfate, keratan sulfate), noncollagenous proteins (anchorin C2, tenascin, chondronectin – which help to anchor chondrocytes to the matrix) and glycoproteins that give it its mechanical and biologic properties.

Ground substance components of hyaline cartilage matrix are not distributed uniformly. Ground substance is stained with basic dyes and with hematoxylin. The highest concentration of sulfated proteoglycans occurs immediately around the lacunae. This ring of *intensely staining matrix* is called *capsule or territorial matrix*. *The interterritorial matrix* has a lower concentration of sulfated proteoglycans and *stains less intensely* (Fig. 2).

In the adult, the hyaline cartilage is found on the *articular surfaces of joints* (articular cartilage), within the rib cage (*costal cartilage)* and it is also present in the *trachea, bronchi*, *larynx and nose*. In fetal development, hyaline cartilage is *precursor of bones* that develop by the process of endochondral ossification. The epiphyseal growth plate (epiphyseal disc) is the remaining cartilage as a growth site of the bone in length.

Perichondrium

The perichondrium surrounds hyaline cartilage. It consists of a *dense connective tissue*. It also serves as the source of new cartilage cells. When actively growing, the perichondrium appears divided into an *inner cellular layer*, which gives rise to new cartilage cells and an outer *fibrous layer.* There are *some exceptions that hyaline cartilage is surrounded by a perichondrium*: The *articular surfaces in joints* (areas where cartilage forms a free surface), *areas where cartilage makes direct contact with bone*, as in the nasal and costal cartilage.

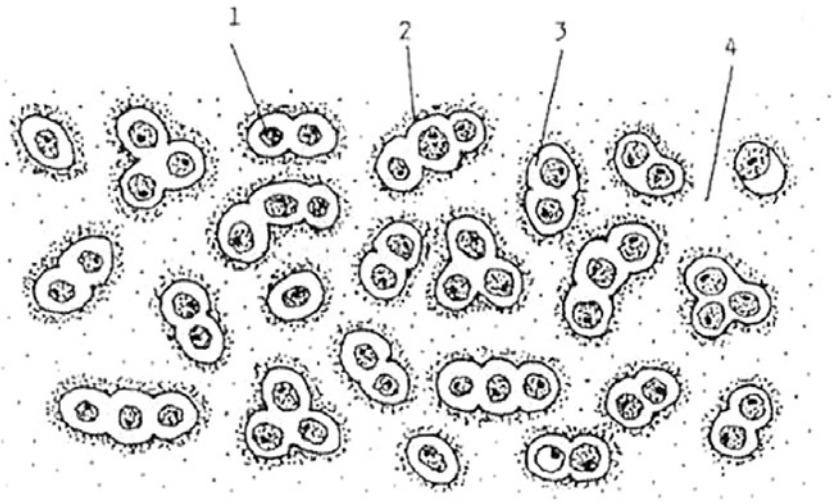


Fig. 2. Hyaline cartilage
1-chondrocyte, 2-isogenous groups, 3-territorial matrix, 4-interterritorial matrix

Growth of hyaline cartilage (Chondrogenesis)
Cartilage grows by two different processes:

Appositional growth. It is the process that forms new cartilage at the surface an existing cartilage. New cartilage cells, chondroblasts, are derived from the osteoprogenitor cells in inner portion of the surrounding perichondrium.

Interstitial growth. It is the process that forms new cartilage within an existing cartilage. New cartilage cells arise from the mitotic division of chondrocytes. Clusters of cells derived from a single chondrocyte within matrix designated as isogenous groups. The overall growth of cartilage thus results from both the interstitial secretion of new matrix by chondrocytes and appositional secretion of matrix by newly differentiated chondroblasts.

Repair of hyaline cartilage (Regeneration)

Cartilage has limited ability for repair. In adult man, cartilage repair following injury is a slow process. Complete reconstitution, although rare in the adult, is often more fully realized in children. Some repair can occurs, but only if the defect involves the perichondrium. The limited ability of cartilage to repair itself can cause significant problems in cardiothoracic surgery, when costal cartilage must be cut to enter the chest cavity.

Clinical correlations

Hyaline cartilage in the adult calcifies with time as a part of the aging process. *Calcification* normally occurs in the *epiphyseal cartilages* as on process of longitudinal bone growth. For example, in older individuals *portions of the cartilage rings in the trachea* are replaced by bone tissue. Calcification of the *costal cartilages* is a good example. Not all cartilages calcify, nasal and aural cartilages almost never do.

Asbestiform degeneration, frequent in aged cartilage, is due to the formation of localized aggregates of thick, abnormal collagen fibrils in this tissue.

Elastic cartilage

In addition to the normal components of hyaline cartilage matrix, elastic cartilage matrix also contains *elastic fibers* and sheets of elastic material. These fibers and lamellae are demonstrated in paraffin sections with special stains such as *resorcin-fuchsin and orcein*. The elastic material gives the cartilage elastic properties (Fig. 3).

Elastic cartilage is found in the *external ear (*auricle), the walls of *external acoustic meatus*, *the auditory (Eustachian) tube*, *the epiglottis* of the larynx and parts of the larynx.

The elastic cartilage is surrounded by a perichondrium. The matrix of elastic cartilage does not calcify during the aging process.

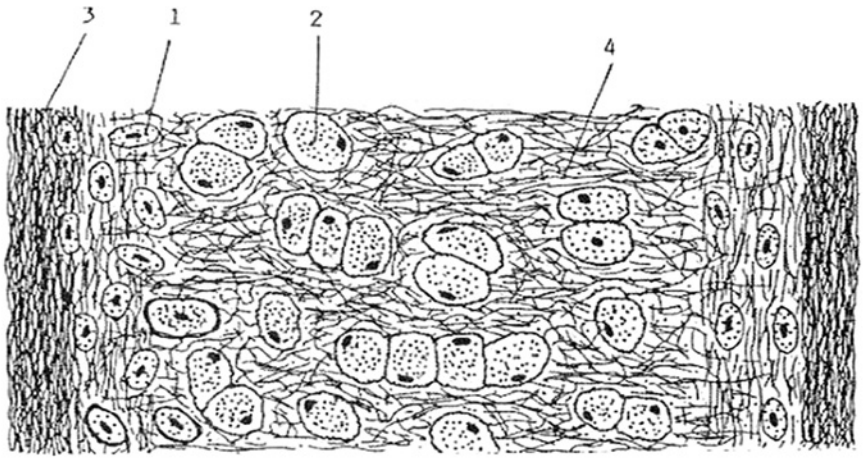


Fig. 3. Elastic cartilage

1-chondroblast, 2-chondrocyte 3-perichondrium, 4-elastic fibers

Fibrocartilage

Fibrocartilage is a combination of dense regular connective tissue and hyaline cartilage. The chondrocytes are dispersed among the collagen fibers, singly, in rows and in isogenous groups. Fibrocartilage is not surrounded perichondrium as in hyaline and elastic cartilage (Fig. 4). Fibrocartilage is present *in intervertebral discs, the symphysis pubis, articular discs of the sternoclavicular and temporomandibular joints, menisci of the knee joint, and certain places where tendons attach to bones.*

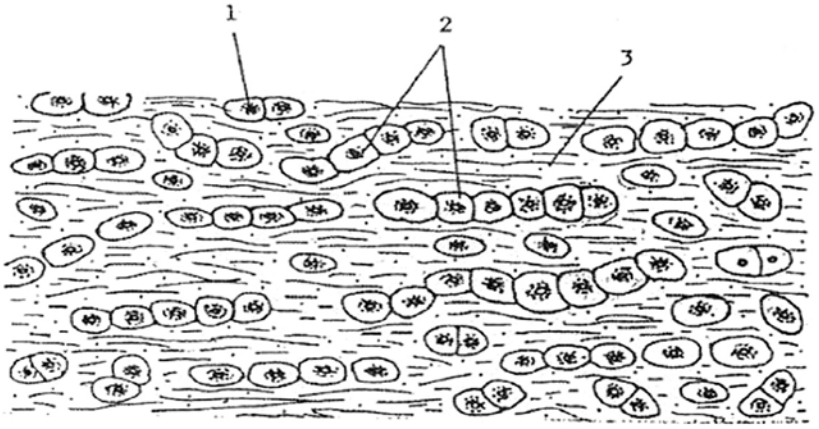


Fig. 4. Fibrocartilage

1-chondrocyte, 2-isogenous groups in rows, 3-collagen fibers

Intervertebral disks

They are interposed between the articular surfaces of successive vertebrae. Each disk contains a gelatinous center, called *nucleus pulposus*, which is composed of cells, derived from the notochord, within a hyaluronic acid-rich matrix. The cells disappear by the 20th year of life. The nucleus pulposus is surrounded by *the annulus fibrosus*. The annulus fibrosus exhibits a narrow peripheral zone of collagenous fibers and an inner zone of fibrocartilage.

Clinical correlations

A ruptured disk refers to a tear or break of the annulus fibrosus through which the gel-like nucleus pulposus extrudes. This condition occurs more

often on the posterior portions of the disks, in the lumbar portion of the back, where *the disk may dislocate, or slip ("slipped disk").*

Table 5. Type of cartilage

	Characteristics	Perichondrium	Location
Hyaline cartilage	Type II collagen, basophilic matrix, chondrocytes usually arranged in isogenous groups	Perichondrium present in most places. Exceptions: articular cartilages and epiphyses	Articular ends of long bones, nose, larynx, trachea, bronchi, ventral ends of ribs, fetal skeleton
Elastic cartilage	Chondrocytes single or in pairs Type II collagen, elastic fibers	Perichondrium present	Pinna of ear, walls of auditory canal, auditory tube, epiglottis, cuneiform cartilage of larynx
Fibrocartilage	Type I and II collagen, acidophilic matrix, chondrocytes arranged in parallel rows between bundles of collagen	Perichondrium absent	Intervertebral disks, articular disks, pubic symphysis, insertion of some tendons

4.2.4.2. Bone

Bone is the main constituent of the adult skeletal system. The adult skeleton consists of more than 200 bones. Bone is one of the hardest substances of the body. The hardness of bone makes it difficult to section. Special techniques for obtaining thin sections include grinding bone slices. Demineralized bone can be sectioned and stained by standard histological methods. Like cartilage, it is a skeletal connective tissue specialized for support and protection. The term "bone" is used for an organ composed largely of bone tissue but also containing blood vessels, nerves and bone marrow.

Functions:

Bone supports and protects the more fragile tissues and organs, harbors hematopoietic tissue (bone marrow). The skeleton contains 99% of the body calcium. Bone serves as the calcium reservoir, storing excess calcium and releasing it when it is needed.

Classification of bones according their shape:

Long bones (the femur). The diaphysis is the shaft of a long bone and the epiphysis is its bulbous end.

Short bones (carpal bones of the hand).

Flat bones (the skull, the sternum). They consist of two layers of compact bone and intervening layer of spongy bone.

Irregular bones (vertebra, sphenoid and ethmoid bones). They contain air spaces or sinuses.
Sesamoid bones (the patella). They develop within tendons.

Bone types based on microscopic observations:
Primary bone (immature bone, nonlamellar bone or woven bone).

It is the first bone tissue during the formation of new bone during fetal development and during bone repair of fractures.
Secondary bone (mature bone).

It is composed of bone lamellae, the matrix is more calcified and it is stronger than primary bone.

A layer of dense connective tissue, *the periosteum,* surrounds bone. *The periosteum* has two layers: an *outer fibrous* layer with typical fibroblasts and an *inner cellular layer*, which contains osteoprogenitor calls. The character of periosteum is different where ligaments and tendons attach to the bone. Collagen fibers from these structures extend directly into the bone tissue. These fibers are called *Sharpey´s fibers*. The marrow surface of compact bone and spicules of spongy bone are lined by thin layer of cells called *the endosteum* (endosteal cells). A thin layer, the endosteum, lines the surface of the bone facing the marrow cavity. Both layers possess osteogenic potency. Following injury, cells in these layers may differentiate into osteoblasts (bone forming cells).

Bone cells

Bone tissue is the structural component of bones. Bone is a connective tissue characterized by a mineralized extracellular matrix. It is composed of cells, fibers and ground substance. Cells of bone are:
Osteoprogenitor cells.

They are stem cells found in the endosteum and in the inner cellular layer of the periosteum. These spindle – shaped cells have ovoid to elongate nuclei. There are two types of osteoprogenitor cells: One gives rise to osteoblasts, the other to osteoclasts.
Osteoblasts.

They are cuboidal, bone-forming cells with large, round nucleus and basophilic cytoplasm. Osteoblasts may form a low columnar „epitheloid

layer“ at sites of bone deposition. They synthesize and secrete all the organic components of bone matrix. When an osteoblast is completely surrounded by matrix, it is called an osteocyte. Osteoblast precursors derive from embryonic mesenchyme.

Osteocytes.

Osteocytes are differentiated bone cells found in cavities in the bone matrix called lacunae. They long, thin cytoplasmic processes are found in canaliculi. Osteocytes are isolated from one another by the impermeable bone matrix and contact one another at the tips of their processes, often through gap junctions. Osteocytes contain less endoplasmic reticulum and are smaller than osteoblasts.

Osteoclasts.

Osteoclasts are bone - resorbing cells that lie on bony surfaces in depressions termed Howship´s lacunae. They are very large cells (up to100 μm) and multinucleated (2-50 nuclei per cell), with acidophilic cytoplasm containing lysosomes, mitochondria and a well develops Golgi complex. The osteoclast surface facing the depression exhibits a ruffled border of plasma-membrane foldings. The cells release lytic enzymes. These break down bone matrix and release minerals, a process called bone resorption. Osteoclasts are stimulated by parathyroid hormone (produced by the parathyroid gland) and inhibited by calcitonin (produced by cells of the thyroid gland). Osteoclasts respond to PTH by enlarging their ruffled borders and increasing their activity, resulting in increased blood calcium levels. Calcitonin, which decreases blood calcium, reduces surface ruffling and osteoclasts activity. Osteoclasts precursors derive from blood monocytes. They arise by the fusion of monocytes.

Bone matrix

Bone matrix contains organic components, or osteoid and inorganic components, or bone minerals.

Organic components.

Osteoid constitutes about 50 % of bone volume and 25 % of bone weight. It is composed of fibers and unmineralized ground substance. Type 1 collagen fibers constitute 90-95 % of the osteoid.

Inorganic components.

Bone mineral makes up about 75 % of bone weight. It is composed of calcium and phosphate, with some bicarbonate, citrate, magnesium and potassium. Calcium and phosphate form crystals of hydroxyapatite. Calcification begins a few days after the deposition of organic bone substance by the osteoblasts. About 75 % of the hydroxyapatite is deposited in the first few days of the process, but complete calcification may take several months. Hydroxyapatite crystals and collagen fibers are embedded in the acid ground substance, which is composed of proteins, carbohydrates and small amounts of proteoglycans and lipids.

Histological organization of bone

Lamellar bone is composed by *lamellae.* Lamellar bone forms trabecular and compact bone. Lamellae are microscopic structures. They are thin sheets of the bone tissue. *Osteocytes* are found in small hollows within matrix, they are called *the lacunae*. They processes lie in *canaliculi* extending radially from each lacuna.

In mature compact bone, lamellae form structural units a *Haversian systems or an osteons*. In the osteon, *concentric lamellae* (8-15) surrounding a central *Haversian canal*. This canal contains connective tissue, nerves and blood vessels. The system of canaliculi that opens to the Haversian canals serves for the passage of the substances between the osteocytes and blood vessels. *Haversian canals run parallel to the surface* and along the long axis of the bone. The collagen fibers in the concentric lamellae in an osteon are laid down parallel to one another in any given lamella but in different directions in adjacent lamellae. This arrangement imparts great strength to the osteon. Between the osteons are irregular areas of *interstitial lamellae*. They are parts of older Haversian systems. Lamellar bone is also found at sites other than osteons. *Circumferential lamellae* follow the entire inner and outer circumferences of the shaft of a long bone. Perforating canals (*Volkmann´s canals)* are channels in compact bone through which blood vessels and nerves travel from the periosteal and endosteal surfaces to reach osteonal canal and *connect osteonal canals to one another*. Volkmann´s canals are not surrounded by concentric lamellae.

Adult bone occurs in two basic types, spongy and compact. Early developmental forms of both types consist of *woven* bone. During reorganization and growth, woven bone will in time by replaced by lamellar bone. Lamellar bone forms both spongy and compact bone, which are the two macroscopically recognizable bone forms.

Compact bone (dense bone, cortical bone)

It forms the thick *diaphyseal cylinder of long bones*, a thin covering over the epiphyses and the tables of the flat bones of the skull. Compact bone is always composed of secondary bone.

The diaphyseal compact bone consists of *outer circumferential lamellae*, intermediate zone of *osteons*, *interstitial bone lamellae* and *inner circumferential lamellae*. The external surface is covered by dense irregular connective tissue, the *periosteum*, which is attached to the outer circ*umferential lamellae* by *Sharpey´s fibers*. Blood vessels of the periosteum enter the bone via large nutrient canals *Volkmann´s canals*. They convey blood vessels to the *Haversian canals* of *osteons* and interconnect adjacent Haversian canals (Fig. 5).

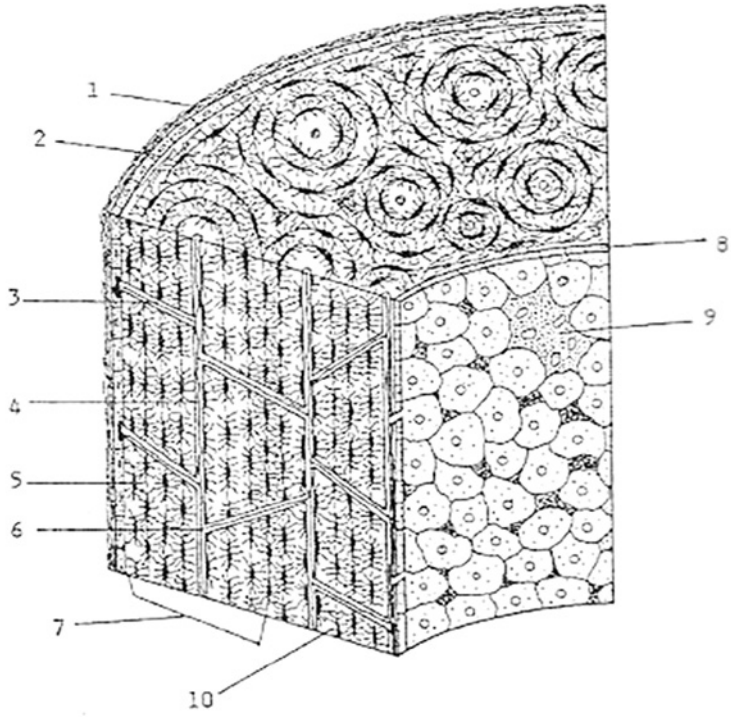


Fig. 5. Diaphyseal compact bone

1-outer fibrous layer of the periosteum, 2-inner cellular layer of the periosteum, 3-Volkmann´s canal, 4-Haversian canal, 5-osteocyte, 6-blood capillary, 7-osteon, 8-endosteum, 9-osteoclast, 10-bone lamellae

Spongy bone (cancellous bone, trabecular bone)

Mature spongy bone is structurally similar to mature compact bone. It forms a fine three dimensional lattice with many open spaces. The branching and anastomosing lamellae or spicules of bone are between the spaces. These spaces contain *bone marrow*. Spongy bone is found at the core of the epiphyses of mature long bones, at the core of short bones and between the thick plates of the flat bones of the skull, where it is called the *diploe.*

Clinical correlations

Especially during growth, bone is sensitive to nutritional factors. Calcium deficiency in children causes *rickets (rachitis),* a disease in which bone matrix does not calcify normally. The bones not only grow more slowly but also become deformed. The primary cause of rickets is a vitamin D deficiency. Vitamin D is required for proper calcium absorption from the gut. In the absence of vitamin D, dietary calcium is not properly absorbed, resulting in hypocalcemia, leading to skeletal and dental deformities and neuromuscular symptoms.

Calcium deficiency in adults (most frequently due to vitamin D deficiency) gives rise to *osteomalacia,* characterized by deficient calcification of recently formed bone and partial decalcification of already calcified matrix. *Osteoporosis* is caused by decreased bone formation or increased bone resorption. It is characterized by decreased bone mass, the ratio of mineral to matrix is normal. Osteoporosis is most often seen in chronically immobilized patients and postmenopausal women. In osteoporosis, there is increased bone fragility and susceptibility to fracture.

Bone formation, ossification

Bone tissue initially formed in the skeleton of a developing fetus is called immature bone. On the basis of its collagen fiber arrangement, such bone is designated nonlamellar or woven bone. This immature bone is later replaced by secondary, lamellar bone.

Development of bone is classified as endochondral and intramembranous:

Intramembranous ossification

Intramembranous ossification occurs within *membrane-like mesenchymal condensation*. In intramembranous ossification, bone is formed by

differentiation of mesenchymal cells into osteoblasts. Osteoblasts begin to synthesize and secrete osteoid, which later becomes mineralized. This initial site of bone formation is termed *the primary ossification center*. Newly formed bone matrix appears in histological sections as irregularly shaped *spicules* and *trabeculae*. Bone trabeculae are cover by a layer of osteoblasts. The older, calcified portion of trabeculae contains osteocytes surrounded by bone matrix. Bone tissue formed by this process is called membrane bone or intramembranous bone. Only a few human bones form in this way. *Flat bones of the skull, the mandible and the maxilla are membrane bone.*

Endochondral ossification

In the embryo most bones are formed by the transformation of *hyaline cartilage model* with the general shape of the bone, a process called endochondral ossification. Basic steps in the formation of an endochondral bone. The cells within the perichondrium (now the periosteum) differentiated into osteoblasts. Osteoblasts form a thin periosteal bone collar of membrane bone around the cartilage model. The first bone tissue in an endochondral bone forms by intramembranous ossification. Structural and functional changes begin in the cartilage model. *The chondrocytes* near the collar *proliferation*, forming long columns (isogenous groups). *The chondrocytes hypertrophy* and the *matrix becomes calcified*. The calcified cartilage matrix is removed by chondroclasts and primary bone marrow cavity is formed. The periosteal bud is a small cluster of blood vessels and perivascular tissue from the periosteum that penetrates the primary marrow cavity. Osteoprogenitor cells and bone marrow stem cells divide and differentiate into osteoblasts, which form osteoid and *primary bone.* The primary bone is later resorbed and replaced by secondary lamellar bone. In long bones, the process occurs first near the middle of the diaphysis, forming *the primary ossification center*. *The secondary ossification centers* form late, by the same process, in the epiphyses. In humans, the first bone to ossify is the clavicle.

Histological appearance of developing endochondral bone is characterized by zones:

1. *Zone of resting (reserve) cartilage* - it is composed of typical hyaline cartilage.

2. *Zone of proliferation* - it contains *columns (isogenous groups)* of flattened chondrocytes.
3. *Zone of hypertrophy* - the chondrocytes are enlarged and rounded.
4. *Zone of calcified cartilage* - the enlarged cells begin to degenerate, the matrix becomes calcified and in HE-stained sections is more basophilic.
5. *Zone of resorption* (line of erosion) - it borders directly on the primary marrow cavity.
6. *Zone of ossification* (osteoid zone, zone of developing trabeculae) - this zone is characterized by osteoid, osteocytes within the bone matrix and a monolayer of osteoblasts on the surface of the newly formed primary bone.
7. *Zone of calcifying trabeculae* (ossiform zone) - this zone contains immature primary bone.

Epiphyseal growth plate is a thin transverse disc of hyaline cartilage between the diaphysis and the epiphyses, which separates the epiphyseal and diaphyseal cavities. By continuing cartilage production, the epiphyseal plate provides the basis for *growth in the length of the bone*. Bone formation and bone resorption go hand in hand during the growth of bone.

Growth of bones

Bones increase in size from birth into early adulthood. During this growth, the bone tissue is continuously remodeled.

Growth occurs in two directions:

Growth in length of long bones is due primarily to the proliferation of chondrocytes in the resting cartilage and in the zone of proliferation of the epiphyseal plates, under the influence of growth hormone. At puberty, growth hormone levels decline and endochondral bone gradually overtakes and replaces the remaining cartilage, a process termed closure of the epiphyseal plates.

Growth in girth occurs by proliferation and differentiation of osteoprogenitor cells in the inner layer of the periosteum and deposition of new ossified tissue on the outer surface of the bone.

Fractures and bone repair

Bone fracture tears vessels in the periosteum, endosteum and Haversian and Volkmann´s canals, causing local hemorrhage and *clot formation between the broken ends of the bone*. Fibroblast and capillaries then proliferate and grow into the site of injury. New loose connective tissue is formed and cartilage forms in parts of it. The dense connective tissue and newly formed cartilage grow, covering the bone at the fracture site, producing *a callus*. The cartilage in the callus calcifies and is replaced by bone as in endochondral ossification. In healthy individuals, this process usually takes from six to twelve weeks. The time required for complete healing depends on the site and extent of the injury and is longer in older people.

Bone remodeling

Living bone is continually being remodeled. Bone remodeling is a life long process where old bone is removed from the skeleton (bone resorption) and new bone is added (bone formation). Remodeling responds also to functional demands of the mechanical loading. The reorganization of bone may not result in macroscopically visible changes of bone structure. During this process new osteons are formed. Parts of older Haversian systems represent the interstitial lamellae in compact bone. The process of bone remodeling is extremely active during childhood. In the first year of life, almost 100 % of the skeleton is replaced. In adults, remodeling proceeds at about 10 % per year.

Modeling is when *bone resorption and bone formation occurs on separate surfaces*. Bone modeling occurs during birth to adulthood and is responsible for gain in skeletal mass and changes in skeletal form.

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5. Muscle tissue

Muscle tissue is composed of elongated cells or fibers containing special contractile proteins responsible for contraction. Muscle tissue is classified into three types according to structure, function and location. *Striated muscles* exhibit cross striations in the light microscope and include both skeletal and cardiac muscle.

1. *Striated skeletal muscle* occurs mainly in association with bones and is responsible for movement of the skeleton and for maintenance of body position. It forms also part of the tongue, pharynx and diaphragm. These muscles play a role in speech, swallowing of the food and breathing. Its contraction is quick, strong and under voluntary control.
2. *Striated cardiac muscle* occurs exclusively in the heart (myocardium). Its contraction is quick, strong, rhythmic and involuntary.
3. *Smooth muscle* does not exhibit cross-striations in the light microscope and is found in the wall of hollow organs (e.g., stomach, intestines, blood vessels). Its contraction is slow and involuntary.

5.1. Embryonic origin

Most muscle tissue develops from *mesoderm* that gives rise to *mesenchymal cells.* Mesenchymal cells differentiate into muscle cells through a process of accumulation of myofilaments in the cytoplasm.

- *Skeletal muscles* develop from *paraaxial mesoderm***,** organized into myotomes in somites. Muscles of the head develop from *mesenchyme of branchial arches.*
- *Cardiac muscles* develop from *cardiogenic mesoderm.*
- *Smooth muscles* develop from *splanchnic mesoderm*, with exception of iris where smooth muscle arises from neuroectoderm.

5.2. Regeneration of muscle tissue

Cardiac muscles have no regeneration capacity in adults. Damaged cardiac muscle is replaced by connective tissue scars after infarct.

Skeletal muscles have limited regeneration. After injury of muscle tissue small *satelite cells* **(**situated between sarcolemma and basal lamina) become activated, proliferate and differentiate to new myoblasts. If basal lamina is complete myoblasts fuse to form myotubes and mature to muscle fibers. If basal lamina is not complete, fibroblasts proliferate and form connective tissue scar.

Smooth muscles are able to regenerate. After injury smooth muscle cells undergo mitosis. Regeneration of smooth muscle in vascular wall occurs from undifferentiated cells in tunica adventitia, or from pericytes.

Under *physiological condition* smooth muscle cells proliferate in the uterus during menstrual cycle and pregnancy. This process is under hormonal control.

Hyperplasia – increased number of cells (proliferation).

Hypertrophy – increased size of the cells (increased volume).

5.3. Special terminology in muscle tissue

Special terms applied to muscle include prefixes *sarco-, myo-*.

Sarcolemma (cell membrane), *sarcoplasm* (cytoplasm), *sarcoplasmic reticulum* (smooth endoplasmic reticulum), *sarcosomes* (mitochondria), *sarcomere* (repeating functional unit of contraction). *Myofilaments* (composed of contractile proteins: actin and myosin), *myofibrils* (higher arrangement of myofilaments, with characteristic striation pattern). *Myoblasts* (cells differentiated from mesenchymal cells) after aggregation and end-to-end fusion produce multinucleated *myotubes*, that gives rise to *myofibers* (skeletal muscle fibers). *Myoglobin* is an oxygen binding protein.

5.4. Skeletal muscle

Basic morphologic unit of skeletal muscle is multinucleated (syncytium) muscle fiber with cross striation visible under light microscope. Nuclei are situated directly beneath the sarcolemma (Fig. 1).

5.4.1. Organization of skeletal muscles

Skeletal muscles consist of striated muscle fibers held together by connective tissue. The ends of muscle fibers insert to tendons, that are attached to bones and makes body movement.

The whole muscle is surrounded by *dense conective tissue* sheath externally – *epimysium*. Connective tissue septa arising from epimysium devide the muscle to *bundles (fascicles)*. Fascicles containing several muscle fibers are functional units that work together and are surrounded by *perimysium*. Individual muscle fibers are surrounded by basal lamina and fine network of reticular fibers – *endomysium*. Connective tissue carries nerves and blood vessels. Endomysium contains small-diameter blood vessels and capillaries; fine terminal branches of nerve fibers are present as well.

Sequence in organization of skeletal muscles is as follows: skeletal muscle – muscle fascicle – muscle fiber – myofibrils – myofilaments.

5.4.2. Histology and ultrastructure of skeletal muscle fiber

Skeletal muscle fibers are large, elongated, cylindrical and unbranched. In cross section have polygonal shape with diameter of 10 to 100 μm. Their lenght varies from a few of milimeters (stapedius muscle) to 100 cm (sartorius muscle).

Based on the morphology, biochemistry and histochemistry, skeletal muscle fibers can be classified as type I (red) and type II (white, has 3 subtypes). In humans, skeletal muscles are composed as a mixture of different types of these fibers.

Type I fibers (slow oxidative) contain many mitochondria, high levels of mitochondrial oxidative enzymes and large amount of myoglobin (limb muscles in mammals, long back muscles in humans adapted to the long and slow contraction, maintaining erect body position). Type II fibers are related to rapid and discontinuous contraction. They contain less of myoglobin (muscles of digits, extraocular muscles).

5.4.2.1. Light microscopic structure

Skeletal muscle fibers (Fig. 1) in the longitudinal section have many oval nuclei situated directly below the sarcolemma. Eosinophilic sarcoplasm with lighter (isotropic) and darker (anisotropic) bands is typical characteristic of skeletal muscle fibers. In the cross sections, fibers are visible like polygonal cells with no or more rounded nuclei below the sarcolemma and eosinophilic cytoplasm with fine granular appearance (presence of myofibrils).

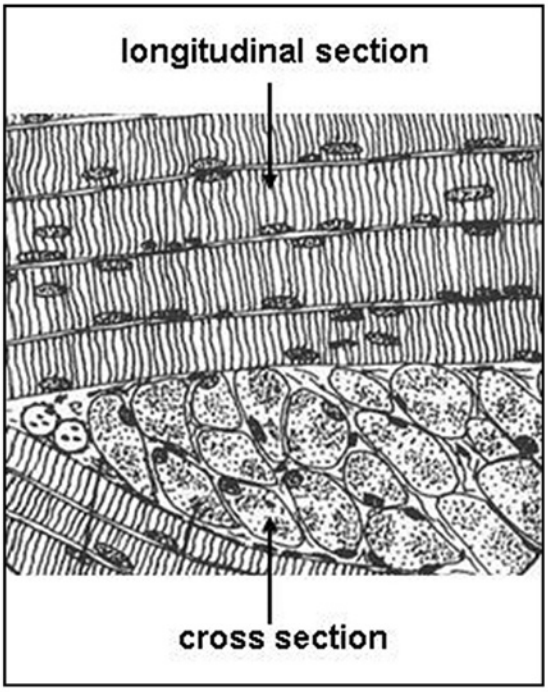

Fig. 1. Multinucleated skeletal muscle fibers in the light microscope in longitudinal and cross sections. Oval nuclei are situated below the sarcolemma.

5.4.2.2. Electron microscopic structure

Externally, of *sarcolemma* is found a delicate layer of basal lamina. Internally, below sarcolemma are oval nuclei. Sarcolemma forms finger-like invaginations at the level of A – I bands called *transverse tubules (T tubules)*. *Sarcoplasm* in mature muscle fibers is reduced and contains *myofibrils* arranged in long axis of the muscle fiber. Each myofibril is surrounded by specially organized network of *sarcoplasmic reticulum* (SR) tubules. At the junction between A and I bands tubules of SR fuse to form ring-like channel called *terminal cisterna.* Sarcoplasmic reticulum serves as a reservoir for Ca^{+2} ions. Two terminal cisternae together with one T tubule form specialized complex, known as the *triad.* Alongside the myofibrils a lot of *mitochondria* are present (Fig. 2). Sarcoplasm contains pigment *myoglobin* (oxygen-binding protein), *glycogene* and *lipofuscin granules*. There is no Golgi complex, nor granular endoplasmic reticulum in the sarcoplasm of matured muscle fibers.

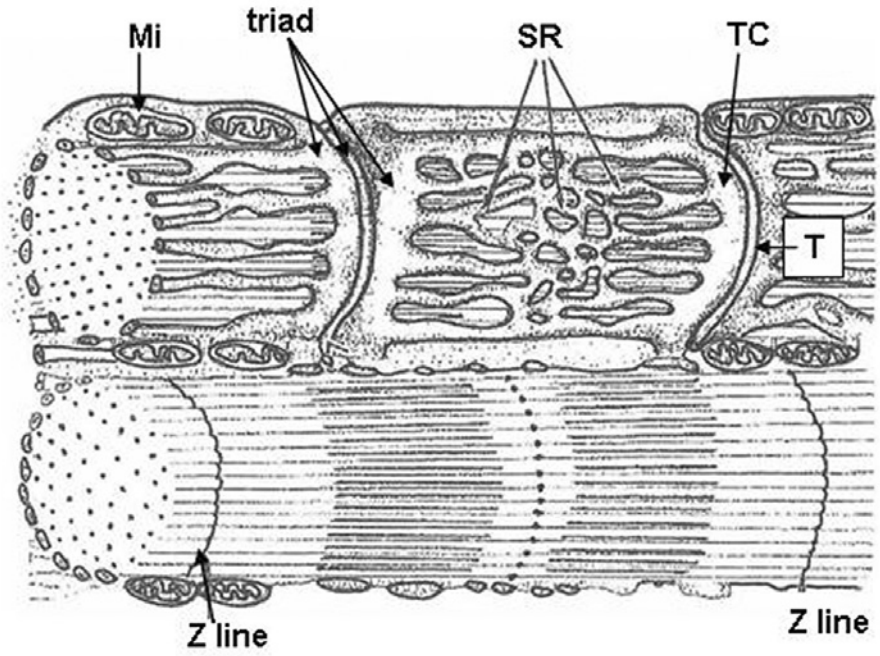

Fig. 2. Skeletal muscle fiber is composed of several myofibrils, each surrounded by sarcoplasmic reticulum (SR) and mitochondria (Mi). Tubules of SR anastomose in the middle of A band. Two terminal cisternae (TC) of sarcoplasmic reticulum and one T- tubule (T) form triad.

5.4.2.3. Myofibrils

The structural and functional unit of muscle fiber is the myofibril. Sarcoplasm of muscle fiber contains about 80 % of myofibrils, each 1 – 2 μm thick, oriented longitudinally with long axis of the muscle fiber.

Myofibrils are composed of myofilaments with regular banding pattern giving typical histological feature – *cross-striations.* Cross-striations are visible on myofibrils in H&E staining and also in living muscle in polarizing microscope, in which they show alternating dark and light bands. These bands are called *A bands (anisotropic,* dark, birefringent) and *I bands (isotropic,* pale, monofringent). Each I-band is bisected by a dark transverse line (electron-dense), called *Z line*. The smallest repetetive functional unit between two Z lines is a *sarcomere* (Fig. 3). The sarcomere in relaxed mammalian muscle measures 2 to 3 μm and during the maximal contraction is reduced to 1μm or may be stretched to 4 μm.

Thin, *actin filaments,* which form I bands, are attached to Z line by special protein *α-actinin.* A band is situated in the centre of the sarcomere. In the middle of the A band is a paler segment called *H band* and dark line - *M line*. H band contains only thick – *myosin filaments,* its length changes during contraction. Periphery of the A band contains actin filaments penetrating between myosin filaments. In skeletal muscle six actin filaments surround one myosin filament.

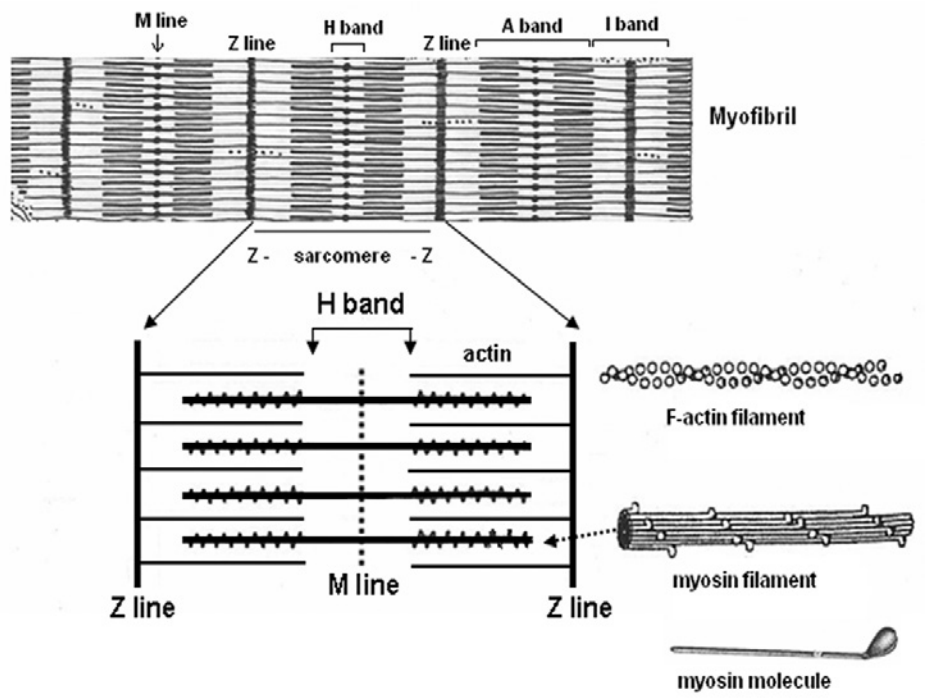

Fig. 3. Structure and position of myofilaments in the sarcomere in striated muscles. Molecular composition of thin actin filaments and thick myosin filaments is shown at right.

5.4.2.4. Myofilaments

A. *Thin actin filaments* have several components.

1. *Filamentous actin (F-actin)* is a polymer composed of *globular actin (G-actin)* monomers. Each thin filament consists of a double helix of F actin strands.

2. *Tropomyosin* is a long, thin, double helical polypeptide that run in the groove between F-actin molecules. In resting muscle mask the myosin-binding sites on the actin molecule.
3. *Troponin* is a complex of three globular proteins. *Troponin C (TnC)* binds calcium ions necessary for starting of contraction. *Troponin T (TnT)* attaches troponin complex to a specific site of tropomyosin. *Troponin I (TnI)* inhibits actin-myosin interaction (Fig. 4).

B. *Thick myosin filaments* are composed of two polypeptide chains. A myosine molecule is a long, golf stick-shaped. It has straight part (rod) – *light meromyosin* and globular part (head) – *heavy meromyosin* (Fig. 5). A thick myosin filament consists of hundreds of myosine molecules with head parts projecting from the thick filament in a spiral pattern. The head portion of heavy meromyosin has an *ATP-binding site* and *actin-binding site*. It also demonstrates *ATP-ase activity*. Rod-shaped reagions overlap, does not have globular projections and form H band.

C. *Accessory proteins* have regulatory and binding function during contraction and relaxation of muscle (α-actinin, nebulin, titin, desmin, dystrophin, etc.).

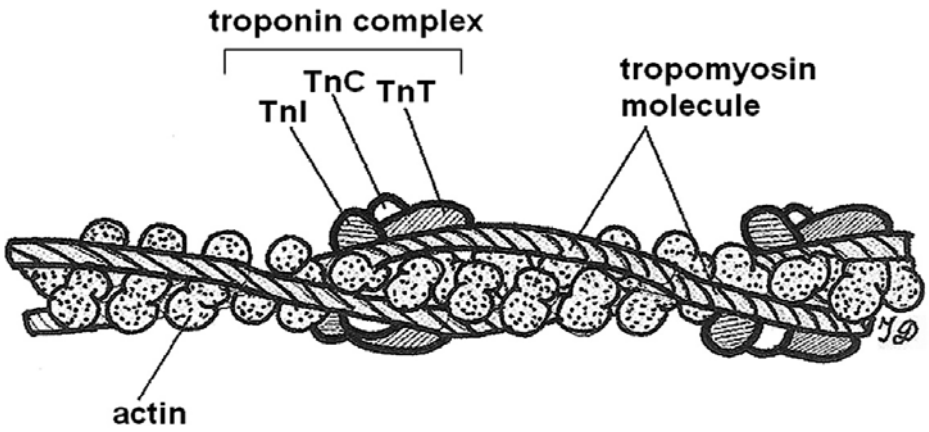

Fig. 4. Schematic drawing of the thin actin filament composed of actin, tropomyosin and troponin complex.

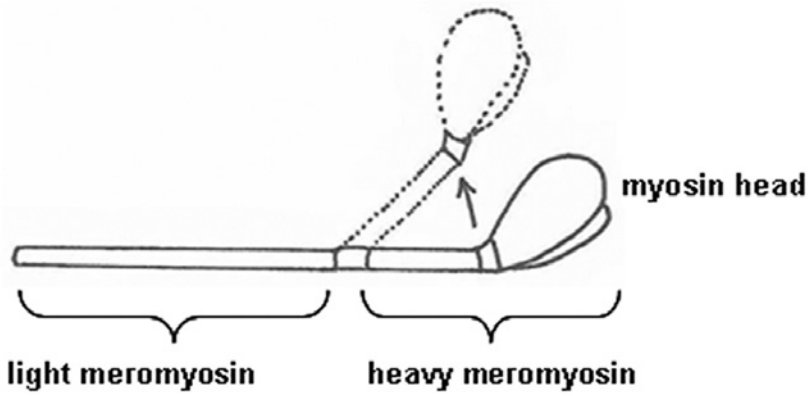

Fig. 5. Myosin molecule is composed of rod-like part (light meromyosin) and myosin head connected with flexible neck part (heavy meromyosin).

5.4.3. Contraction of skeletal muscle

During the contraction, each sarcomere shortens, but myofilaments retain their original length. Contraction is a result of thin actin filaments movement (sliding and overlapping) between thick myosin filaments (Fig. 6).

Mechanism of contraction

1. Muscle fibers are innervated by motor end-plate (myoneuronal junction). After releasing of neurotransmitter (acetylcholine) the sarcolemma is depolarized.
2. Transverse (T) tubules spread depolarization to terminal cisternae of sarcoplasmic reticulum (SR).
3. Depolarization of SR results the release of Ca^{+2} ions.
4. Ca^{+2} ions are bound to TnC subunit.
5. Spatial configuration of the three troponin subunits changes and drives tropomyosin molecule deeper into the groove of the actin helix.
6. In that manner actin is free to interact with the head of myosin molecule.
7. ATP provided by mitochondria binds to myosin heads, as a result of the hydrolysis of ATP, the energy is released and produces movement of myosin head toward G actin molecule (actin-myosin bridging).

8. Movement of myosin heads pulls the actin filaments along the myosin filaments. As a result of repeating actions, actin filaments are pulled into the A-band, thus shortening the sarcomere (Fig. 6).

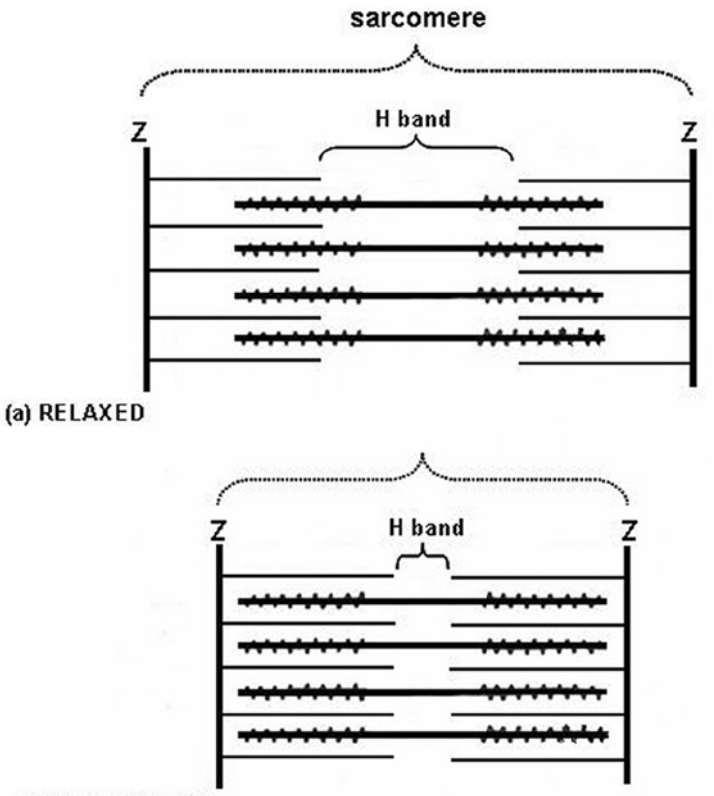

Fig. 6. Sarcomere in the relaxed stage (a) showed wide H band and isotropic bands. During contraction (b) the myosin heads pull the thin actin filaments into the A band. The sarcomere becomes shorter and also isotropic bands and H band shorten. Anisotropic band does not change its length.

5.4.4. Skeletal muscle innervation

Motor innervation

Skeletal muscles are innervated by motor neurons (efferent α-motor neurons) that originate in the spinal cord or brainstem. Single neuron can innervate one muscle fiber (delicate movement of eye muscles) or hundreds of muscle fibers. In the case of multiple innervation a single nerve fiber makes several nerve endings (presynaptic membrane of neuromuscular

junction). Loss of innervation causes muscle fiber (and muscle) *atrophy* and total loss of function in the denervated muscle.

Sensory innervation

Muscle spindles, encapsulated sensory receptors in skeletal muscles provide information about the degree of tension in a muscle and its position. Muscle spindle consists of modified muscle fibers and neuron terminals (afferent sensory nerve fibers and γ efferent nerve fibers).

Clinical correlations

Muscular atrophy. After denervation, skeletal muscle fibers decrease in diameter (in neurodegenerative diseases or after injury of nerve fibers).

Duchenne muscular dystrophy is a genetic disorder caused by a deficiency of *dystrophin* – membrane associated cytoskeletal protein responsible for maintaining of fiber mechanical integrity during contraction. Symptoms of muscular weakness start at the age 3 -5 years mainly in boys.

Myasthenia gravis is an autoimmune disorder characterized by progressive muscular weakness caused by reduction of functionally active *acetylcholine receptors* in the sarcolemma of myoneuronal junction.

5.5. Cardiac muscles

Basic morphologic unit of cardiac muscle is cardiac muscle cell (cardiomyocyte) with cross striation in the cytoplasm and centrally located oval nucleus (Fig. 7).

Cardiac muscle cells can be divided according their function to three types:

1. cardiac contractile cells
2. cardiac conducting cells (Purkinje fibers, modified cardiac muscle cells which generate and conduct electrical impulses through the heart)
3. cardiac endocrine cells (in the atria of the heart) contain granules with atrial natriuretic hormone (ANH) in their sarcoplasm. ANH is a polypeptide hormone involved in the homeostatic control of body water, sodium and potassium.

5.5.1. Light microscopic structure of cardiac cells

Cardiac muscle cells are cylindrical with extended processes connected end to end by intercalated discs (Fig. 7, 8). Cardiac cells often

bifurcate (branch) at their ends and bind to the neighbouring chain of cells, thus creating *branched fiber-like appearance*. Cardiac muscle cells are approximately 15 μm in diameter and from 85 to 100 μm in length. Sarcoplasm exhibits cross striation, centrally located one or two paler nuclei with perinuclear accumulation of pigment lipofuscin.

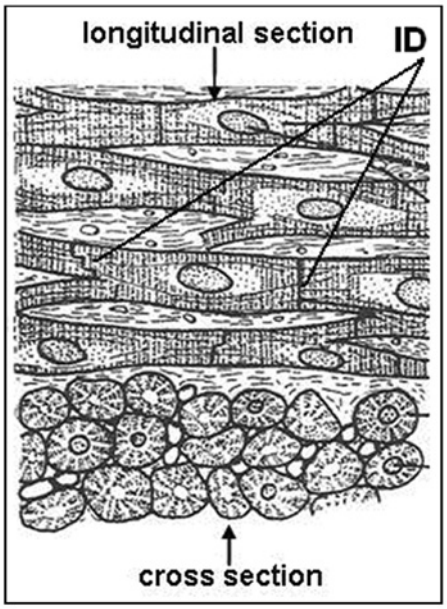

Fig. 7. Cardiac muscle cells in the light microscope in longitudinal and cross sections. Oval nuclei are situated in the centre of the cell. Cells are connected by intercalated discs (ID).

5.5.2. Electron microscopic structure

Myofilaments arranged to myofibrils are oriented longitudinally. Numerous and large mitochondria are densely packed between the myofibrils. Energy providing mitochondria form 40% of the whole volume of cell sarcoplasm. Glycogen granules that store energy are also localized between myofibrills. Sarcoplasmic reticulum of cardiac muscle is not as well organized as that of skeletal muscle. Small expansion of sarcoplasmic reticulum (like terminal cisterna) is connected to the T tubule to form a *diad* at the level of Z line. Passage of Ca^{2+} ions from the lumen of the T tubule to the sarcoplasm of a cardiac cell is essential to initiate contraction cycle, identical to those in skeletal muscle.

5.5.3. Structure and function of intercalated discs

Intercalated discs represent junctions between cardiac muscle cells. In the light microscope intercalated discs are visible as dark-stained transverse lines between cardiac cells.

In the electron microscope intercalated discs represent junctional complexes that appear as straight lines or have a step-like pattern. In the step-like pattern two portions can be described: transverse and lateral (Fig. 8).

Transverse portion contains:

1. *Fascia adherens (adhering junction* serves for attachment of actin filaments in the terminal sarcomere anchored into the sarcolemma.
2. *Maculae adherentes (desmosomes)* bind the cardiac cells together to prevent their pulling apart under constant contractile activity.

Lateral portion consists of *gap junctions* (nexus, communicating junctions) providing ionic and molecular transport between adjacent cells. This exchange permits cardiac muscle chains to act as a syncytium, allowing the signal to pass in a wave from cell to cell with following contraction.

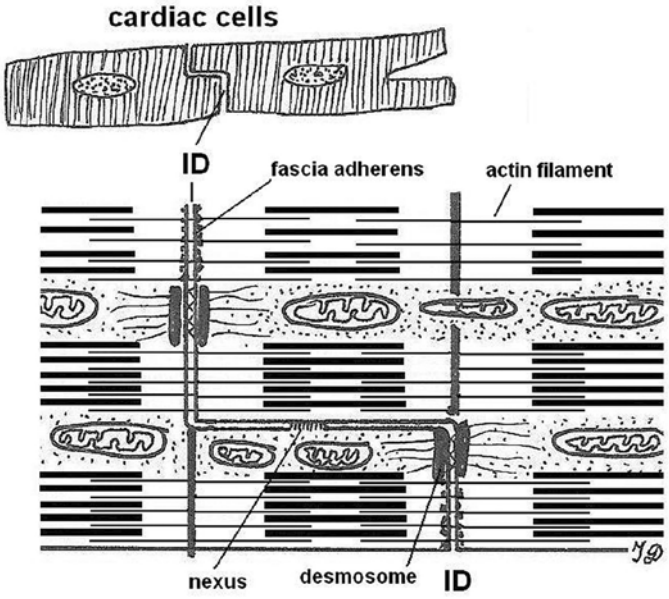

Fig.8. Detail of step-like intercalated disc (ID). Transverse portion serves for firm attachment of cardiac cells by desmosomes and fascia adherens anchors actin filaments. Lateral portion contains nexus providing ion transport between adjacent cells.

5.6. Smooth muscle

Smooth muscle consists of fusiform cells with no cross striations in the light microscope. Smooth muscle cells are present in the wall of hollow organs and blood vessels. Contraction of smooth muscle is involuntary and slow.

5.6.1. Structure in the light microscope

Basic morphologic unit of smooth muscle is fusiform (spindle-shaped) smooth muscle cell, with eosinophilic cytoplasm without cross striations. Centrally located *nucleus is oval or rod-like* (Fig. 9). After contraction of the cell nucleus has a corkscrew appearance (Fig. 10). The cells range in length from 20 μm in the wall of arterioles to about 200 μm in the wall of the intestine or 500 μm in the wall of uterus during pregnancy. Diameter in the central part of the cell is about 8μm in tapered ends 2 μm and less. Nucleus is elongated and centrally located. When the nucleus is included in a cross section of a smooth muscle cell, it appears as a round.

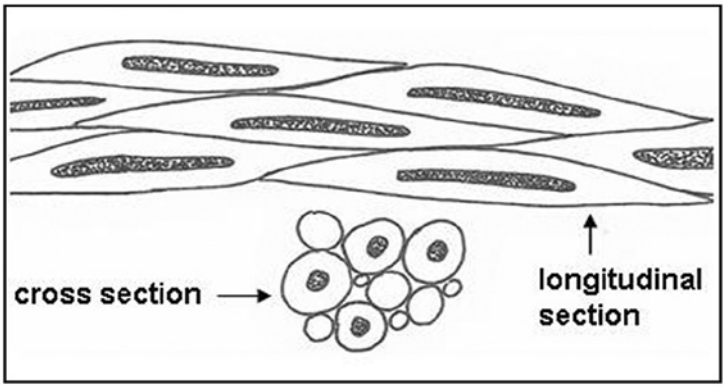

Fig.9. Smooth muscle cells in the light microscope in longitudinal and cross sections. Rod-like nuclei are situated in the centre of the spindle-shaped muscle cell.

5.6.2. Structure in the electron microscope

Smooth muscle cell is surrounded by sarcolemma and externally by basal lamina. Smooth muscle cells make contact with neighbouring cells by gap junctions. Sarcoplasm contains actin and myosin myofilaments

and intermediate filaments desmin and vimentin. At the poles of elongated nucleus are mitochondria, polyribosomes, cisternae of rough endoplasmic reticulum and Golgi apparatus. Sarcoplasmic reticulum is rudimentary. A characteristic feature for smooth muscle cells is the presence of large number of the cell membrane invaginations – *caveolae*. Caveolae are included in transport of Ca^{2+} ions from external environment. Caveolae, vesicles and sparse profiles of smooth endoplasmic reticulum beneath sarcolemma are considered as a T tubules in smooth muscle.

Two types of *dense bodies* appear in smooth muscle cell. One is *membrane associated* and the other *cytoplasmic*. Both contain *α-actinin* and provide an attachment site for the thin actin filaments and intermediate filaments (Fig. 10, 11).

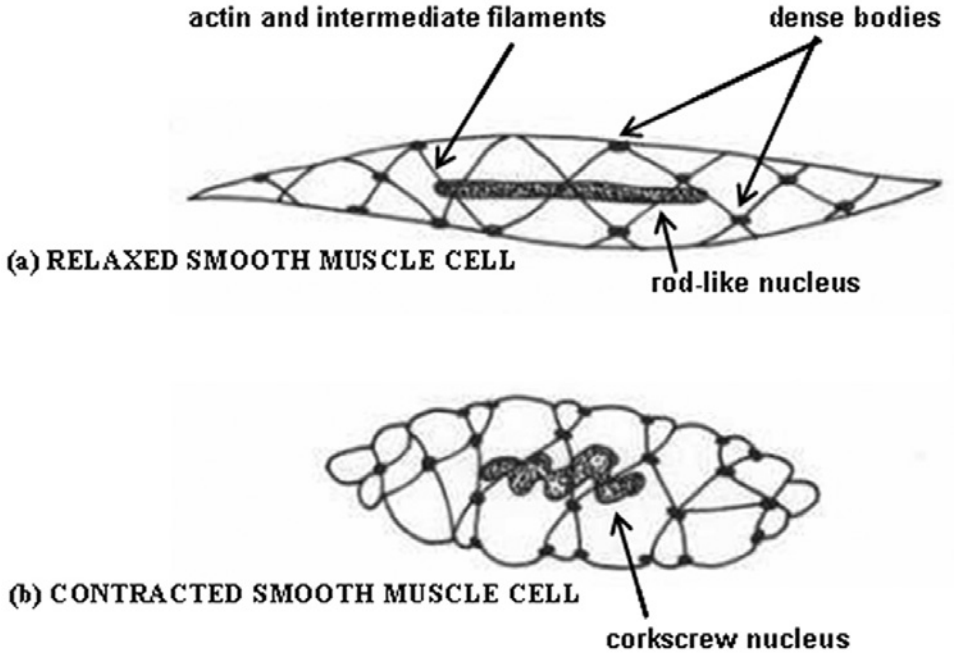

Fig. 10. Smooth muscle cell has actin and myosin filaments arranged „criss-cross". Actin filaments and intermediate filaments (desmin) are attached to dense bodies situated in the sarcoplasm and below sarcolemma. After contraction muscle cell is shorter and nucleus has corkscrew appearance.

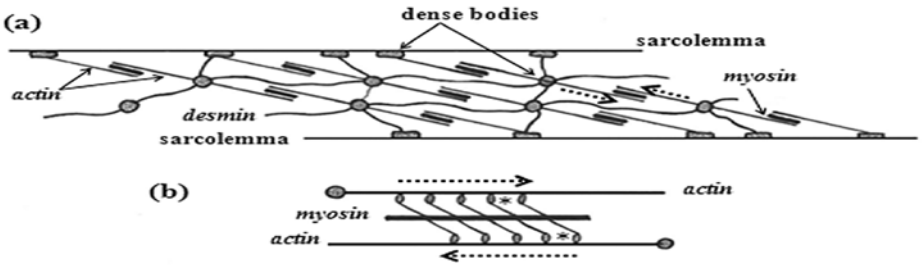

Fig. 11. (a) Detailed organization of myofilaments and intermediate filaments in smooth muscle cells. Dense bodies associated with sarcolemma (rectangle —), cytoplasmic dense bodies (circle ●). (b) Direction of actin filaments movement (arrow) after bending and attachment of myosin heads (*) to actin.

5.6.3. Function of smooth muscle cells

Secretion

Smooth muscle cells have well developed rER and Golgi apparatus involved in synthesis of collagen type IV (basal lamina) and type III (reticular fibers), elastic fibers, proteoglycans and glycoproteins.

Contraction

Smooth muscle cells are specialized for slow and prolonged contraction. Contraction is regulated by (1) postganglionic neurons of the autonomic nervous system (sympathetic and parasympathetic) or by (2) hormones (e.g. oxytocin stimulates uterine contraction during parturition).

Nerve fibers pass through connective tissue that surrounds groups of muscle cells. The neurotransmitters released by the nerve endings reach the muscle cells by diffusion. Not all smooth muscle cells are exposed directly to neurotransmitters. Coordination of smooth muscle contraction is provided by gap junctions (nexus) between smooth muscles cells.

5.6.4. Mechanism of contraction

1. Extracellular calcium ions are transported to smooth muscle cells through invaginations of the cell membrane - *caveolae*.
2. Ca^{2+} ions are bind to protein *calmodulin* that has function like TnC in striated muscles (smooth muscle cells have no troponin complex!).
3. *Ca^{2+} - calmodulin complex* activates *myosin light chain kinase* (MLCK).

4. MLCK activates phosphorylation of *regulatory light chain* of the myosin molecule.
5. Myosin heads are attached to actin filaments. In the presence of ATP, the myosin heads bend and pull the actin filaments (Fig. 11). This mechanism is dependent on myosin light chain phosphorylation that leads to cross-bridge formation between the myosin heads and the actin filaments. Hence, smooth muscle cells are contracted.

Because contractile filaments are oriented obliquely to the long axis of the cell (Fig.11), their contraction shortens the cell and produces corkscrew-shape of the nucleus (Fig. 10).

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6. Nerve tissue

All living cells have the ability to react to stimuli. Nerve tissue is specialized to react to stimuli and to conduct impulses to various organs in the body which bring about a response to the stimulus. Human nerve tissue is made up of more than 100 million specialized nerve cells called *neurons* and many more supporting *neuroglial cells.* Neurons are easily stimulated and transmit impulses very rapidly.

The nervous system is divided anatomically into the *central nervous system* (CNS) and the *peripheral nervous system* (PNS). Central nervous system consists of the brain, the spinal cord, and neural parts of the eye. Peripheral nervous system consists of peripheral nerves, nerve ganglia, nerve endings, and receptors.

6.1. Neurons

The vast majority of neurons is generated before birth. Persisting stem cells give rise to a small number of new neurons throughout the lifetime of mammals, including humans. The permanent addition of neurons may be important for the maintenance and plasticity of some parts of the CNS, but it is insufficient to replace neurons that die because of disease or acute damage to the CNS. Neurons should last a lifetime. Mature neurons are not mitotically active, i.e. they do not divide.

Nerve cell or neuron is a basic structural and functional unit responsible for the reception, transmission, and processing of stimuli.

6.1.1. Shape of neurons

The shape of neurons is different. There are star shaped, pyramidal, pear shaped, spherical, angular, and many other types of nerve cells.

According to the number of processes can be distinguished: (Fig. 1)

1. Bipolar neurons – have two processes. These neurons are typical of the visual, auditory, and vestibular system.
2. Pseudounipolar neurons have only one short process leaving the spherical cell body. This is branched into two processes – peripheral

and central one. Pseudounipolar cells are localized in sensory ganglia of cranial and peripheral nerves.

3. Multipolar neurons display many processes attached to a polygonal-shaped cell body. The processes include a single axon and more than one dendrite. Typical examples are motor neurons in the ventral horns of spinal cord or pyramidal cells in cerebral cortex.

   Based on the length of the axon the neurons with a long axon are called Golgi type I, when the axon extends beyond the limits of the dendritic tree, and with a short axon Golgi type II, when the axon terminates in the immediate area of the cell body and does not extend beyond the limits of dendritic tree.

Neurons and their processes are variable in size. Some are very large with cell body measuring up to 150 μm (motor neurons in the ventral horns of spinal cord), other are among the smallest cells in the human body 4 - 5 μm in diameter (granule cells of cerebellar cortex).

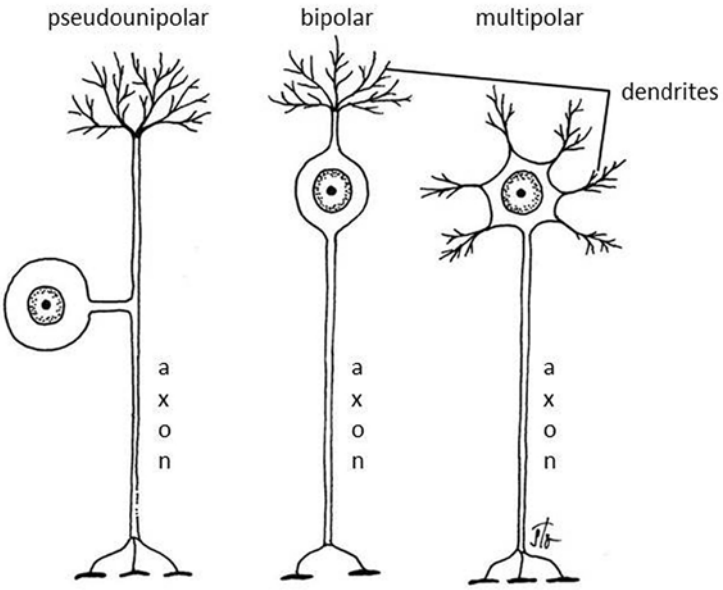

Fig.1. Types of neurons according to the number of processes

6.1.2. Classification of neurons according their function

1. *Motor neurons* (efferent) – control the function of peripheral organs like skeletal muscle tissue, exocrine and endocrine glands.

2. *Sensory neurons* (afferent) – are involved in reception of sensory stimuli from the environment and from within the body.
3. *Interneurons* – establish relationships among other neurons, forming complex functional networks or circuits.
4. *Neurosecretory neurons* – synthesize and release hormons (hypothalamus).

6.2. Morphological characteristic of nerve cell

Although all nerve cells conform to the general principles, there is a wide range of structural diversity. Routine staining methods, such as hematoxylin and eosin staining method, are of little value in the light microscopic study of normal nervous tissue. Special histologic techniques are used (Nissl stains) in which cationic dyes, such as cresyl violet, toluidin blue, and thionin are bound to nucleic acids. They therefore demonstrate nuclei and nucleoli and granular endoplasmic reticulum - like the basophilic Nissl substance in the cytoplasm of nerve cells.
Most neurons consist of three parts: dendrites, cell body, and axon.

6.2.1. Dendrites

Dendrites are part of the receptive surface of the neuron. As a rule, neurons have one to several primary dendrites, which emerge from the cell body. Primary dendrites may divide into secondary, tertiary as multiple treelike branches. Dendrites are thicker at the beginning, can be smooth, or they can be studded with small, mushroom-shaped appendages, which are called dendritic spines.

6.2.2. Cell body

The cell body, or perikaryon, or soma is enclosed by a cell (plasma) membrane. It is primarily a trophic centre with receptive capabilities. Nerve cells are responsible for the reception, transmission, and processing of stimuli. Neural activity and its control requires the expression of many genes. This is reflected in the large, spherical, euchromatic (pale – staining) nuclei of most neurons, with prominent nucleoli. Basophilic granules called *Nissl bodies* are found in the cytoplasm of the cell body. In the electron microscopy Nissl bodies represent granular endoplasmic

reticulum and are responsible for proteosynthesis. The *Golgi complex* is located near the nucleus and consists of parallel smooth cisternae and vesicles arranged circularly or in a half moon shape. *Mitochondria* are abundant in the perikaryon but especially in the axon terminals. Within the cell body and its processes special kinds of microtubules - *neurotubules* and extremely fine *neurofilaments* extend from the dendrites into the axon. Neurofilaments belong to the group of intermediate filaments with a diameter of 10 nm. The bundles of neurofilaments, when impregnated with silver, form neurofibrils visible with the light microscope. Nerve cells occasionally contain the inclusions of pigments - melanin, and lipofuscin.

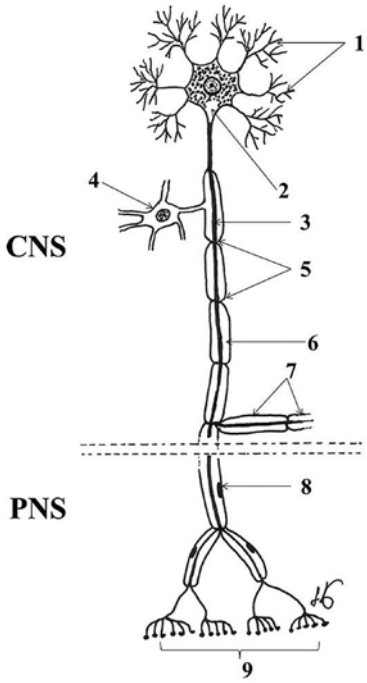

Fig. 2. Diagram of a myelinated neuron
1-dendrites, 2-axon hillock, 3-axon, 4-oligodendrocyte, 5-nodes of Ranvier, 6-internodal segment, 7-collateral with myelin sheath, 8-nucleus of Schwann cell, 9-telodendron

6.2.3. Axon

Each neuron has only one *axon* that emerges from the perikaryon (Fig. 2). The axon is surrounded by axolemma, and contains axoplasm

without any basophilic structures. Has a constant diameter, and may have 2-3 branches called collaterals as it travels through the nervous tissue to its destination. The axon is the "transmitting" process of the neuron. The point of origin of the axon from the perikaryon is called the *axon hillock*. Portion of the axon between the axon hillock and the point at which myelination begins is called the *initial segment*. This is the site where excitatory and inhibitory stimuli impinging on the neuron are summed, resulting in the decision to spread nerve impulse or not. Ion channels in the axolemma, typical for this localization, are important in generating the change in electrical potential that constitutes action potential.

The axon can be surrounded by the *myelin sheath,* which forms a whitish, non-cellular, fatty layer around the axon (phospholipids, glycolipids, cholesterol). Axon together with glial sheath is called *nerve fiber*.

The distal portion of the axon is naked – without myelin sheath and usually branched. This part constitutes the terminal arborization – *telodendron.* Each branch makes contact on the next neuron in dilatation called *bouton or axon terminal*, forming structure designated as *synapse.* Synapse transmits nerve impulses to the next neuron in the circuit.

6.2.4. Designation of groups of neurons and axons

In the CNS, functionally and structurally related neurons form aggregates of nerve cells called *nuclei.*

Between the neuronal cell bodies are spaces called the *neuropil.* The term neuropil designates an area with packed dendrites, axonal branches with abundant synapses, and glial cells.

Clusters of neurons arranged in a layer form a *stratum* or *lamina* – cerebral cortex.

Bundles of axons in the CNS are called *tracts, fasciculi*, or *lemnisci* – corticospinal tract, medial lemniscus.

In the PNS, a cluster of neurons forms *ganglia* – sensory or motor. Axons derived from a ganglion are included in peripheral nerves.

6.3. Synapses

The synapse is a specialized area of contact, where the transmission of nerve impulses takes place. Synapses are morphologically contacts

between a bouton formed by the terminal arborization enlargements of the axon of presynaptic neuron, and the nerve cell surface of another – postsynaptic neuron. The boutons – axon terminals, contain mitochondria and numerous small vesicles with a diameter of 20 – 60 nm. These occur exclusively in nerve tissue and are termed *synaptic vesicles*. Synaptic vesicles contain substance so called neurotransmitter. A transmitter is described as a substance that is released synaptically by one neuron and which affects another cell in a specific manner.

According to the site of contacts there are *axodendritic synapses* where axon and dendrite are in contact, *axosomatic synapses* where axon and the cell body are in contact, and *axoaxonic synapses* where two axons are in contact (Fig. 3).

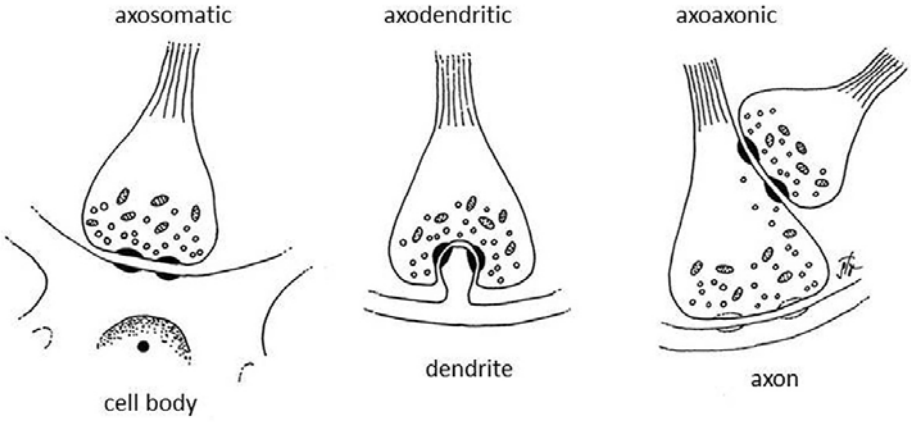

Fig. 3. Types of synapses

The synapses are composed of three parts:
1. presynaptic part (axon),
2. synaptic cleft (wide 20-30 nm),
3. postsynaptic part (dendrite, perikaryon, axon).
Synaptic vesicles in the presynaptic part contain neurotransmitters and these typically accumulate (Fig. 4) in the presynaptic part close to the site of contact between the bouton and the postsynaptic neuron. The most frequent neurotransmitters are: acetylcholine; monoamines - dopamin, adrenalin, noradrenalin, serotonin, histamine; aminoacids – glutamate, GABA, lysine, glycin; peptides – substance P, endogenous opioids.

The morphology of synaptic vesicles is different. In the central nervous system can be find spherical, flattened or elliptical vesicles. In the electron microscopy one distinguishes clear, dense or dense-core vesicles. Synapses that release acetylcholine, always contain clear vesicles, and synapses that release noradrenaline or dopamine (catecholamines) always contain dense-core vesicles.

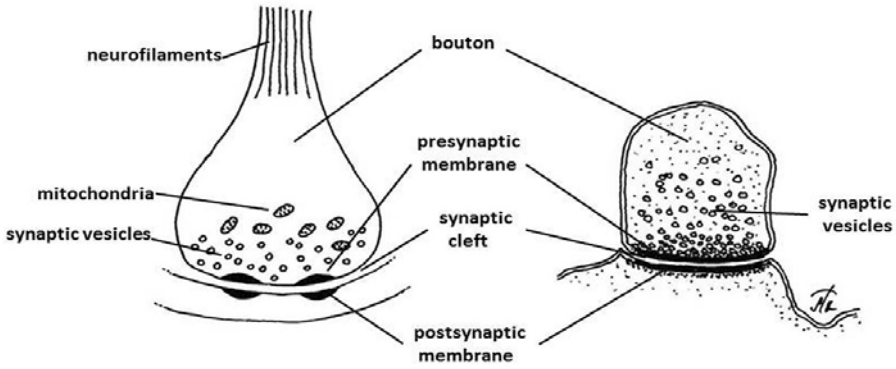

Fig. 4. Synapse

The release of the neurotransmitter from the synaptic vesicles into the synaptic cleft, mediates the transfer of information from the pre- to the postsynaptic neuron. Both the release of the synaptic vesicles and the mediation of the response to the transmitter require membrane-associated specializations – the presynaptic and postsynaptic densities that occur on the inner surface of the presynaptic and postsynaptic membranes. The molecular machinery that is needed to mediate the events occurring at *excitatory synapses* differs from those at *inhibitory synapses*. Different morphological appearances of the synapses accompany the functional differences. The pre- and postsynaptic densities are typically symmetric at inhibitory synapses. The postsynaptic density is thicker than the presynaptic density at asymmetric synapses, which are typically excitatory.

6.3.1. Neurotransmitters

Neurotransmitters either excite or inhibit the postsynaptic neuron. The most prominent excitatory transmitter in the central nervous system is L-glutamate. The most prominent inhibitory transmitter in the central nervous system is GABA (gamma-amino butyric acid). Other main

neurotransmitters are e.g. dopamine, serotonin, acetylcholine, noradrenaline and glycine. Each neuron uses only one of the main transmitters, and this transmitter is used at all synaptic boutons that originate from the neuron. One or more of the minor transmitters (there are several dozens of them - such as endogenous opioids, endorphins, somatostatin, NO, substance P, cholecystokinin) may be used together with a main transmitter. These low molecular substances have been shown to occur in the cytoplasm of neurons and may function as neuromodulators. The excess of transmitters is removed by appropriate enzymes.

6.3.2. Functional characteristic of synapse

The membranes of nerve cells are considered to be polarized i.e. on opposite side of the cell membrane is created an unequal electrical charge. When the nerve impulse is initiated in the initial part of the axon, ion channels are open and the unequal electrical charge-potential returns to zero and the membrane is depolarized (Fig. 5).

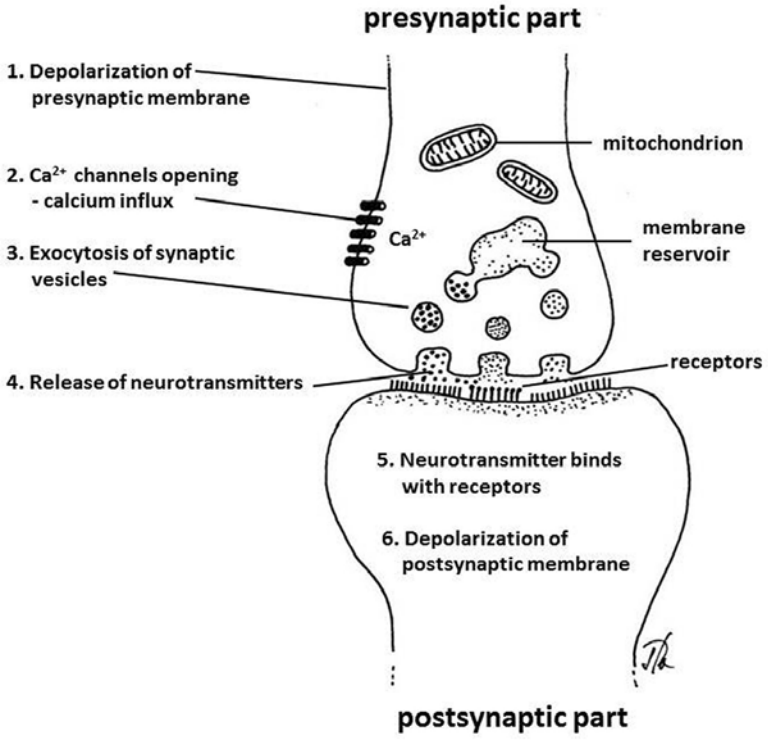

Fig. 5. Synaptic transmission

Depolarization opens calcium channels in the presynaptic part, promoting a calcium influx to the cytoplasm. Calcium influx then triggers the exocytosis of neurotransmitters from synaptic vesicles into the synaptic cleft. The released neurotransmitters react with the receptors at the postsynaptic part, promoting depolarization of postsynaptic membrane. By this way the transmission of nerve impulses occurs. Transmission is realized only in one direction from one neuron to another. Once used, neurotransmitters are removed by enzymatic breakdown, for example acetylcholine is broken by acetylcholine esterase. This is important because prevents an undesirable sustained stimulation of postsynaptic neuron.

6.4. Neuroglial cells

6.4.1. Neuroglial cells in CNS

Within the CNS there are four types of glial cells (Fig. 6):

1. Oligodendrocytes
2. Astrocytes
3. Microglial cells
4. Ependymal cells

Glial cells are 10 times more abundant in the mammalian nervous system than neurons. Only the nuclei of glial cells are seen in routine histologic preparations. Heavy-metal staining (Cajal method, Golgi method, Hortega method) or immunocytochemical methods are necessary to demonstrate the shape of the entire glial cell.

6.4.1.1. Oligodendrocytes

Oligodendrocytes produce and maintain the myelin sheath in central nervous system. The cells are small with darker and smaller nuclei than those of astrocytes. The cytoplasm contains an extensive Golgi apparatus, many mitochondria and a large number of microtubules. From the cell body arise a few and less branched processes. Each of these processes wrap around axons, producing only one *internodal segment* of myelin sheath that is formed by concentric layers of fused oligodendrocyte plasma membranes. The periodic gaps between the intermodal segments are the *nodes of Ranvier.* The cell body of oligodendrocyte with the nucleus is

situated at some distance from the axon it myelinated. Myelin sheath provides the electrical insulation of axons in the central nervous system.

6.4.1.2. Astrocytes

Astrocytes are the largest star-shaped neuroglial cells. They contain bundles of intermediate filaments made of glial fibrillary acidic protein. Astrocytes bind neurons to capillaries and to pia mater. Two categories of astrocytes are identified: *protoplasmic astrocytes* with numerous, short, branching cytoplasmic processes and *fibrous astrocytes* with fewer and relatively long, thin, and straight processes. Nuclei of astrocytes are large, ovoid, and lightly stained. Protoplasmic astrocytes reside predominantly in gray matter and fibrous astrocytes in white matter.

Astrocytes play a role in the movement of metabolites and waste material to and from neurons. They also regulate ionic concentration in the intercellular compartment – neuropil, thus maintaining the microenvironment and modulating activities of neurons. In addition, surrounding astrocytic end-feet contribute to the formation of blood-brain barrier, cover the nodes of Ranvier, surround the synapses and form the limiting membrane of perivascular glia and limiting membrane of superficial glia.

6.4.1.3. Ependymal cells

The ependyma consists of two types of cells: 1. *ependymal cells* and 2. *tanycytes.* Ependymal cells form the lining of the ventricular cavities of the brain and central canal of the spinal cord. They are low columnar or cuboidal cells, in some locations ciliated and functionally facilitate the production, absorption and movement of cerebrospinal fluid. The apical surfaces of these cells are tightly bound by junctional complexes, but unlike this typical feature of epithelium, ependymal cells lack a basement membrane.

In some areas a long process leaves the basal part of these cells and penetrates the surrounding nerve tissue – tanycytes.

Ependymal cells also cover the choroid plexus in the brain ventricles where form simple cuboidal (choroidal) epithelium resting on the basement membrane. Choroid plexus is involved in production of cerebrospinal fluid.

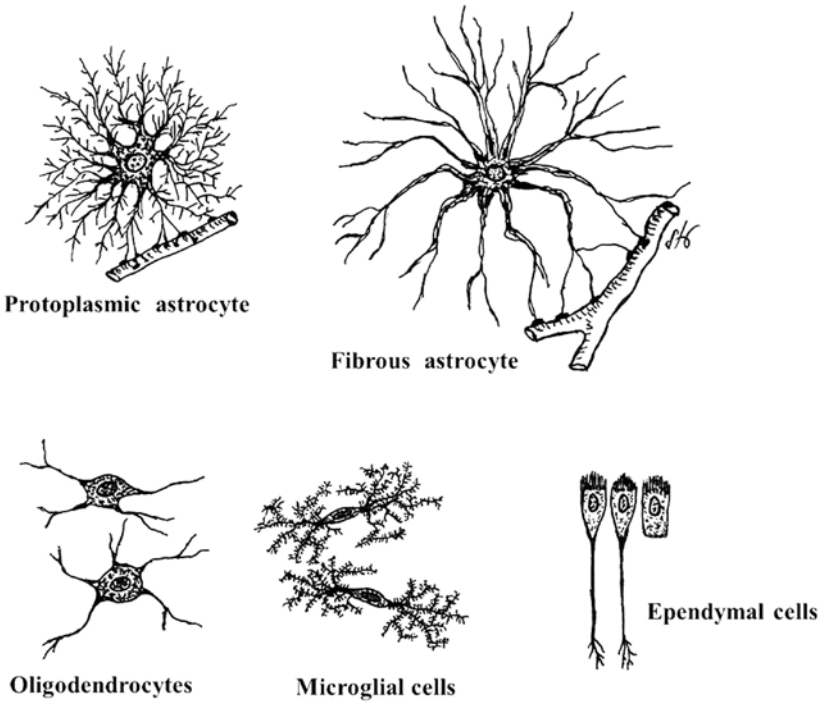

Fig. 6. Neuroglial cells in CNS

6.4.1.4. Microglial cells

Microglial cells are small elongated cells with short many times branched irregular processes. In contrast to spherical nuclei of other glial cells, microglial cells possess dense elongated nuclei. Microglial cells have phagocytic activity and are considered part of the mononuclear phagocytic system and during development originate from bone marrow precursor cells. When activated, microglia acts as phagocytic cells and antigen presenting cells. In addition, secrete a number of immunoregulatory cytokines and remove unwanted cellular debris caused by central nervous system lesions, and apoptotic structures during development of nervous system.

6.4.2. Neuroglial cells in PNS

Within the peripheral nervous system another two types of neuroglial cells are present:

1. *Schwann cells*
2. *Satellite cells*

6.4.2.1. Schwann cells

Schwann cells have the same function as oligodendrocytes in CNS i.e., produce a lipid rich layer called the myelin sheath around the axons in PNS. Each Schwann cell is responsible for creation of only one internodal segment. Its presence ensures the rapid conduction of nerve impulses. Schwann cells also clean up cellular debris and guide the regrowth of PNS axons after injury.

6.4.2.2. Satellite cells

Satellite cells are small, star or spindle shaped flattened cells with short processes and dark nuclei. They are attached to the basement membrane. The satellite cells surround pseudounipolar cells of spinal ganglia and provide structural and metabolic support for these cells. Both, Schwann and satellite cells have the same embryonic origin from neural crest.

6.5. Myelin sheath

Myelin sheath (Fig. 7) gives rise during the process called myelination. In the central nervous system oligodendrocytes are responsible for myelin sheath formation. The processes of oligodendrocytes wrap around axons. Each process forms only one internodal segment of myelin sheath i.e. one oligodendrocyte forms many internodal segments around the same or different axons because have many processes. Between internodal segments of myelin sheath are situated small gaps, *nodes of Ranvier*, where the axon is naked. The myelin sheath prevents the nerve action potential from being propagated continuously along the axon and the axon potential travels by jumping from one to another node. This mode of conduction, known as saltatory conduction, greatly enhances the conduction velocity of axons.

In the peripheral nervous system, at the beginning the axon is enclosed within Schwann cell cytoplasm that lies along the axon. Later the *mesaxon* is created and a sheet like extension of this mesaxon then wraps around the axon in a concentric motion. The first layers of myelin contain sparse amounts of Schwann cell cytoplasm - the so called *Schmitt - Lanterman clefts*. With the electron microscope, closely aligned inner surfaces of Schwann cell membranes give rise to the *major dense line* and outer

surfaces of closely apposed membranes give rise to the *intraperiodic lines* in the myelin sheath. The outer mesaxon represents invaginated plasma membrane of Schwann cell on the surface of myelin sheath where also the nucleus and a small amount of cytoplasm of Schwann cell are situated.

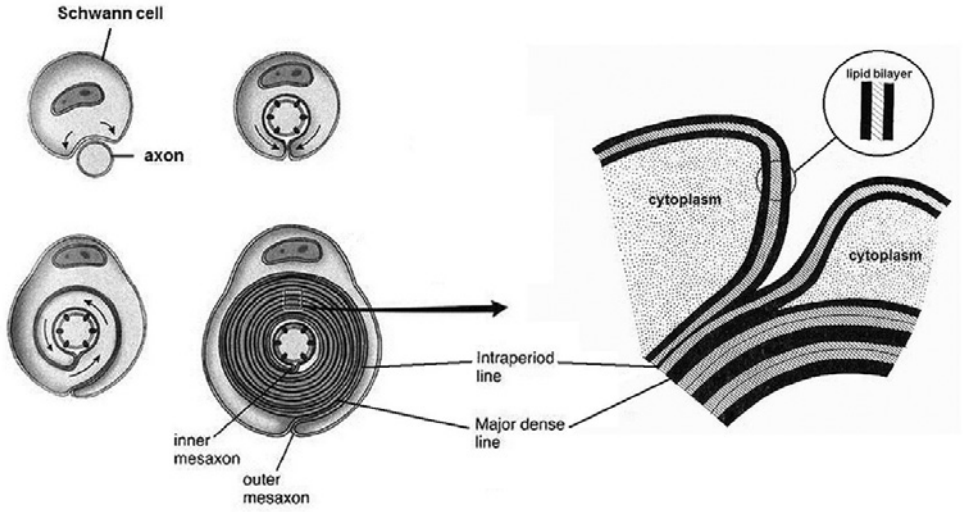

Fig. 7. Stages in the formation of myelin sheath by Schwann cell. The axon initially lies in a groove on the surface of Schwann cell. A sheet like extension of mesaxon membrane then wraps around the axon forming multiple membrane layers.
The inset shows formation of major dense and intraperiodic line.

Clinical correlations

Parkinson´s disease **-** slowly progressive neurologic disorder caused by the loss of dopamine secreting cells in the substantia nigra and basal ganglia of the brain. Main symptoms are: tremor in the limbs, rigidity in muscles, abnormal walking and slowness of thought.

Demyelinating diseases are characterized by damage to the myelin sheath.

Multiple sclerosis – preferential damage of myelin in CNS and destruction of oligodendrocytes. The myelin basic protein appears to be the major autoimmune target in this disease. Symptoms depend on the area in CNS in which myelin is damaged. Deficits of vision, loss of cutaneous sensation, lack of muscle coordination and movement.

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7. Circulatory system

Anatomy introduction

The circulatory system is frequently divided into the *cardiovascular system*, which consists of the heart and blood vessels, and the *lymphatic system,* which consists of lymph vessels, lymph nodes and lymphatic organs.

The heart is a hollow, fibromascular organ of conical or pyramidal form. It has the apex – *apex cordis,* the base – *basis cordis* and surfaces – *sternocostal, diaphragmatic and pulmonary surface.*

The heart is covered by *pericardium.* It is localized in the thoracic cavity behind the body of sternum and occupies the middle mediastinum (space between two lungs). Approximately 2/3 of the heart lies to the left of the midline. The heart divided into two parts, right and left. Right heart consists of *right atrium* and *right ventricle* connected by right atrioventricular opening with *tricuspid valve (right atrioventricular valve).* Left heart consists of *left atrium* which opens to the *left ventricle* through the left atrioventricular opening with *bicuspid valve (mitral* or *left atrioventricular valve).* The right and left heart are separated by *interatrial* and *interventricular septum.* Right atrium accepts *superior* and *inferior vena cava,* returning the venous blood from the body. *The pulmonary trunk* with *pulmonary valve (semilunar)* outflows from the right ventricle, and pumps the venous blood to the lungs. Left atrium receives *4 pulmonary veins,* they carry the arterial blood from the lung. Left ventricle possesses *ascending aorta* with *aortic valve (semilunar)* and as a powerful pump forces the arterial blood out (to systemic arteries of the body).

Blood vessels

All vessels that carry blood away from the heart are called *arteries.* Vessels which carry blood toward the heart are *veins.* Arteries contain blood that is rich in oxygen (oxygenated or arterial blood). Veins carry blood that is deficient in oxygen (deoxygenated or venous blood). Between arteries and veins lies the *capillary network.*

Lymphatic system is described in chapter 15.

The circulatory system comprises the cardiovascular and lymphatic system. The fluids which are transported though the circulatory system: blood and lymph. The heart, blood vessels form cardiovascular system. Central organ is the heart that pumps oxygenated blood to the body and deoxygenated blood to the lungs. Lymph nodes and lymphatic vessels form lymphatic system.

7.1. Heart

The heart is separated by an interatrial septum and an interventricular septum into the right and left side. The interatrial septum is thinner than the interventricular septum. Each side consists of two chambers: an atrium and a ventricle. Valves guard the exits of the chambers, preventing backflow of the blood.

The right atrium has blood from the superior and inferior venae cavae. The blood passes through the right atrioventricular valve into the right ventricle.

The right ventricle pumps deoxygenated blood by the pulmonary trunk to the lungs for gaseous exchange.

The left atrium receives the oxygenated blood from the lungs via the pulmonary veins.

From the left atrium, the blood passes through the left atrioventricular valve to enter the left ventricle.

The left ventricle pumps blood into the aorta, for distribution to the tissues of the body.

7.1.1. General structure of the heart wall

The heart wall is composed of three layers: the endocardium, the myocardium, the epicardium

7.1.1.1. Endocardium

It lines chambers and covers valves, chordae tendineae and papillary muscles. The endocardium is variable in the thickness, thickest in the atrium, thinnest in the ventricles.

It is composed of four layers:

1. The inner layer is formed by simple squamous epithelium called endothelium. Endothelial cells rest on a basal lamina.
2. The subendothelial loose connective tissue with collagen fibers, elastic fibers, fibroblasts and ground substance.
3. The middle layer is the thickest endocardial layer which contains connective tissue with smooth muscle cells and elastic fibers. These fibers are arranged parallel with the surface.
4. The deeper layer is called the subendocardial layer and consists of loose connective tissue. This layer contains blood vessels, nerves and Purkinje fibers which form the part of impulse-conducting system of the heart.

The subendocardial layer is continuous with endomysium of the cardiac muscle cells.

7.1.1.2. Myocardium

It is thickest layer of the heart, consisting of striated muscle cells called cardiomyocytes.

There are three populations of cardiac muscle cells:

1. *The contractile cells* which are approximately 15 μm in diameter and 80 to 100 μm in length. These cells have only one or two centrally located nuclei. Adjacent cardiac muscle cells have intercalated disks which are formed by junctional complexes such as fasciae adherentes, maculae adherents and gap junctions. Cardiac muscle cells are subdivided in to atrial and ventricular muscle cells. Cardiac muscle cells of the right atrium and left atrium are smaller than those of the ventricles.
2. *The impulse-generating cells* which are specialized noncontractile cardiac muscle cells. These cells are specialized to control the rhythmic contraction.
3. *The atrial muscle cells* contain neuroendocrine granules with hormone called atrial natriuretic factor. It causes natriuresis, diuresis, renal vasodilatation. Atrial natriuretic hormone also decreases blood pressure by inhibiting renin secretion in the kidney and aldosterone secretion in the adrenal glands.

The myocardium of the ventricles is thicker than that of the atria. Cardiac muscle cells attach the myocardium to the fibrous skeleton. Between cardiac muscle cells are numerous free nerve endings.

7.1.1.3. Pericardium

The pericardium is double-walled sac that surrounds the heart and the roots of great vessels. It is divided into fibrous and serous pericardium. The serous pericardium has parietal and visceral layers. Visceral layer of the pericardium which consists of mesothelium and the subepicardial layer of loose connective tissue also called epicardium. It is outer layer of the wall of the heart. The subepicardial layer contains the blood vessels, nerves and ganglia. In this layer also fat is stored.

Visceral and parietal pericardial layers enclose space the pericardial cavity, which contains serous fluid (normally no more than 50 ml) for lubricating the layers of the pericardium and permit friction - free movement of the heart within pericardial cavity during its contraction.

7.1.2. Cardiac fibrous skeleton

The cardiac fibrous skeleton forms the base for the valves as well as the side of origin and insertion of the cardiomyocytes. It is composed of dense connective tissue with irregular orientation of collagen fibers and nodules of fibrous cartilage.

The cardiac skeleton includes fibrous valve rings surrounding the valve orifices, called *the annuli fibrosi,* two *fibrous trigones* connecting the rings and the *septum membranaceum* (the membranous part of the interventricular and interatrial septa).

The valve rings support the base of each valve leaflet. The valve rings on the left side of the heart are thicker than on the right side.
The functions of the cardiac fibrous skeleton are follows:

a) Provides attachments for cardiac muscle cells.
b) Act as an electrical insulator for free flow of electrical impulses.

7.1.3. Valves of the heart

The heart has:

1. *The tricuspid valve*, between right atrium and right ventricle consists of three leaflets.
2. *The pulmonary (semilunar) valve*, between pulmonary artery and right ventricle.
3. *The mitral valve*, between left atrium and left ventricle consists of only two leaflets and is referred as the bicuspid valve.
4. *The aortic (semilunar) valve*, between aorta and left ventricle.

The atrioventricular valves prevent regurgitation of blood back into atria. The semilunar valves located in pulmonary trunk and aorta, prevent backflow into the heart.
The leaftets of the both atrioventricular valves have a similar histological structure.

The central core of valve is formed by dense connective tissue containing numerous elastic fibers covered by endothelium on both sides. At

the base of the leaflet are continuous with the fibrous rings surrounding the orifices. The heart valves are attached by chordae tendineae to papillary muscles in the ventricular wall. The leaflets of the aortic semilunar valve are thinner than those of the atrioventricular valves. Valve cusps are normally avascular. Several diseases may induce angiogenesis in the valve. The valves become rigid and inflexible due to insufficiency or stenosis of valvular orifices.

7.1.4. Impulse-conducting system of the heart

The rhythmic contractions of the atria and ventricles are result from impulses generated within the heart itself.

The impulse-conducting system of the heart consists of:

a) the sinoatrial node (S-A node)
b) the atrioventricular node (A-V node)
c) the atrioventricular bundle
d) the right and left crus

These parts are composed of modified cardiac muscle cells, which are specialized to conduct of impulses.

The impulse for contraction is initiated at the *sinoatrial node,* which is termed a pacemaker of the heart. It generates cardiac impulses about 70 per minute.

The sinoatrial node is located at the junction of the superior vena cava and the right atrium. It is a 6 – to 7 mm^3 mass of modified cardiac muscle sells.

The impulse is then spreads to *the atrioventricular node,* located in the septal wall near the tricuspid valve. This node initiates the marginally later contraction of the ventricles.

The atrioventricular node contains specialized cardiac muscle cells which transmit impulses via *the atrioventricular bundle*.

The atrioventricular bundle divides into right and left branches and travels in the subendocardial layer as *Purkinje fibers,* which transmit impulses to the cardiac muscle cells at the apex of the heart.

The structure of the impulse-generating cells

Modified cardiac muscle cells of the impulse-generating and conducting system are smaller than normal working cardiac muscle cells, do not have intercalated disks, but connect with each other by desmosomes, contain few

myofibrils. Purkinje fibers are large fibers with high glycogen content. They have more sarcoplasm with myofibrils predominantly located to the periphery.

Clinical correlations

Rheumatic heart valve disease - is condition develops as result of rheumatic fever. Valve defects may be related to improper closing (incompetency) or improper opening (stenosis).

Pericarditis is infection in the pericardial cavity that the space is obliterated.

Ischemic heart disease is related to atherosclerosis of the coronary vessels. Atherosclerotic plaques reduce the lumina of coronary vessels. Partial obstruction of the coronary arteries reduces the supply of oxygen to the myocardium.

7.2. Components and the functions of the circulatory system

The circulatory system composed of:

a) *The cardiovascular system,* which distributes blood. It is formed by the heart and blood vessels. It is closed in humans.
b) *The lymphatic system*, which distributes lymph. The lymph, lymph nodes and lymphatic vessels form the lymphatic system. This system is not closed.

7.2.1. Cardiovascular system

This system is the first system to function in the embryo. Blood begin to circulate by the end of the third week. Blood vessels first appear on the yolk sac. These primitive vessels form a primitive cardiovascular system.

The cardiovascular system includes the pulmonary circulation and the systemic circulation.

The pulmonary circulation transfers blood from the heart to the lungs and them back to the heart. It consists of:

a) The pulmonary arteries - carry deoxygenated blood to the lungs.
b) The pulmonary capillaries in the lungs, which are specialized for gas exchange.
c) The pulmonary veins - return oxygenated blood to the heart.

The systemic circulation carries blood with nutrients, gases, hormones from the heart to all tissues of the body. It helps stabilize homeostasis.

It consists of:

a) The elastic arteries.
b) The muscular arteries.
c) The arterioles carry blood to anastomosing network of capillaries.
d) The capillaries, which enable the actual exchange of water and metabolities between blood and tissues.
e) The venules-small vessels that receive drainage from the capillary network and carry blood to veins.
f) The large veins empty into the superior and inferior venae cavae and carry blood back to the heart.

7.2.2. Lymphatic vascular system

The function of the lymphatic vascular system is to return the fluid of the tissue to the blood stream.

7.2.3 Blood vessels

Blood vessels are: arteries, capillaries, veins.

The wall of all blood vessels, except the capillaries has the same histological structure, but the proportion of the components varies with function of each type of vessels.

Histological plan of blood vessels

The layers of all blood vessels (except capillaries) are: tunica intima, tunica media and tunica adventitia. In veins these layer are less clearly defined.

a) *The tunica intima* consists of highly specialized endothelial cells which are present in all blood vessels. Endothelial cells sit on a basal lamina.
b) *The tunica media* consists of layers of smooth muscle cells. In arteries is relatively thick and predominant. Between the smooth muscle cells are interspersed elastic, reticular fibers and proteoglycans. All components are produced by the smooth muscle cells. The contraction

of the muscle cells in this layer of small arteries and arterioles reduces diameter. The relaxation of smooth muscle cells increases diameter of the vessels.

Muscular arteries have the internal elastic lamina. Capillaries, postcapillary venules do not have this lamina.

c) *The tunica adventitia.* It is composed of fibroblasts, collagen fibers (collagen type I) and elastic fibers. In arteries it is demarcated from the media by a variably distinct external elastic lamina. This layer is the most prominent in the wall of the veins. The smooth muscle cells may present, particularly in veins. Within tunica adventitia of large arteries and veins are vasa vasorum which send branches into the tunica media to supply. The tunica adventitia also carries autonomic nerves for contraction of the smooth muscle cells in the wall.

Blood supply of the blood wall

Large vessels have vessels of vessels (vasa vasorum). Vasa vasorum are more frequent in veins than in arteries and provide metabolites to the adventitia and to part of the media. The tunica intima and the most internal region of the media are devoid of vasa vasorum. These layers receive nutrition and oxygen by diffusion from the blood that circulates into the lumen.

7.2.3.1. Arteries

Arteries are classified into elastic arteries, muscular arteries and arterioles based on their size and morphological characteristic of the tunica media.

Elastic arteries (large arteries)

The elastic arteries help to stabilize the blood flow. The ventricles of the heart pump blood into the elastic arteries during systole of the cardiac cycle.

Aorta, pulmonary trunk and branches of aorta such as the brachiocephalic, common carotid artery, the subclavian artery, vertebral, pulmonary and the common iliac arteries are *elastic or large arteries.*

The main components of elastic arteries are fenestrated elastic membranes in the tunica media.

Organization of tissues in the wall of elastic arteries:

a) The tunica intima

It is relatively a thick layer. It is composed of *endothelium* and *subendothelial layer*. Endothelium is a special type of simple squamous epithelium which rest on a basal lamina. The cells are joined by tight junctions and gap junctions. The cells are elongated with their long axis to the direction of blood flow. Endothelial cells too provide smooth surface and secrete II, IV and V collagens, laminin and endothelium-derived relaxing factor was later identified as nitric oxide. Endothelial cells play an important role in the structural and functional integrity of the vascular wall. Endothelial cells also have antithrombogenic function. The functions of the endothelial cells may change in response to various stimuli (bacteria, viruses, hypoxemia). Endothelial cells also secrete various factors called interleukins that affect the activity of while blood cells dooring inflammation. Activated endothelial cells are also responsible for the pathogenesis of vascular diseases. Various factors released from endothelial cells stimulate migration smooth muscle cells from the tunica media to the tunica intima where smooth muscle cells produce extracellular matrix and increase the thickness of the tunica intima. Atherosclerotic lesions also are characterized by focal thickening of the tunica intima.

The integrity of the endothelial layer is an important antithrombogenic mechanism. The intravascular coagulum (thrombus) produces obstruction of the local blood flow. The embolus, solid masses from thrombus, may detach and be carried by the blood to obstruct distant blood vessels.

Subendothelial layer is composed of loose connective tissue with collagen, elastic fibers and few smooth muscle cells. These cells secrete extracellular ground substance as well as fibres. The internal elastic lamina is also present, but is indistinct. It has fenestrations for diffusion of substances to nourish.

b) The tunica media

The tunica media is the thickest layer and consists of concentric fenestrated elastic laminae and smooth muscle cells which are less abundant. The fenestrations on elastic laminae facilitate the diffusion of substances. In the alder adult the number and the thickness of the

elastic laminae are increased. The smooth muscle cells are spindle shaped with elongated nuclei. These cells synthesize the extracellular matrix, collagen and elastic fibers. They may proliferate and migrate to the tunica intima and play role in normal repair as well in pathologic processes in the vascular wall. The external elastic lamina also is indistinct.

c) *The tunica adventitia*

It is relatively thin layer that contains fibroblasts, macrophages, type I collagen fibers and longitudinal arranged elastic fibres. Vasa vasorum also are abundant. They supply the tunica adventitia and the part of the tunica media with oxygen and nutrients. Lymphatics and nerves are also present in this coat.

Muscular arteries or medium sized arteries

The elastic arteries branch to form medium-sized muscular arteries. The wall of muscular arteries consists of:

a) *The tunica intima*

It is thinner than that in the elastic arteries. The endothelial cells are oriented parallel to the longitudinal axis of the artery. Subendothelial loose connective tissue contains few smooth muscle cells. Internal elastic lamina is distinct prominent.

b) *The tunica media*

Generally, in the tunica media elastic material decreases and the predominant smooth muscle cells. It is thick layer that contains several layers of smooth muscle cells. Small muscular arteries have three to four layers of smooth muscle cells. Large muscular arteries may have many layers of smooth muscle cells about 40. Interspersed within smooth muscle cells are elastic fibers, collagen fibres type III which form bundles, and chondroitin sulfate. The extracellular components are produced by smooth muscle cells. Tunica media is separated from tunica adventitia by external elastic lamina.

c) *The tunica adventitia*

It is also thick layer which consists of collagen fibres, elastic fibres and ground substance which is produced by fibroblasts. Collagen and elastic fibres are oriented longitudinally. This layer contains nerves and blood vessels called vasa vasorum that supply blood to vascular wall.

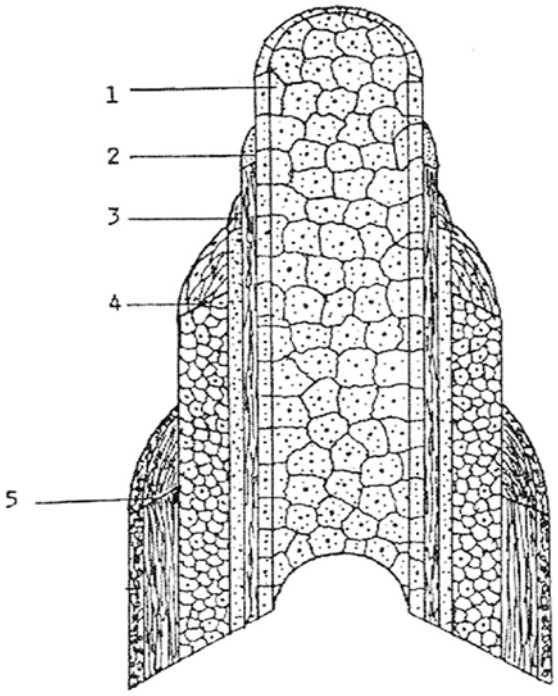

Fig. 1. Structure of the artery
1-the endothelium, 2-the subendothelial connective tissue, 3-the internal elastic lamina, 4-the tunica media with smooth muscle cells, 5-the tunica adventitia

Certain arteries have some variation in structure of the wall according to function and their location.
Examples:
The umbilical arteries that have tunica media composed of two thick smooth muscle cells layers, with indistinct internal elastic lamina.

Arterioles

The arterioles are the smallest arteries with a diameter of less than 0.1 mm.
Terminal arterioles give rise to the capillaries. The arterioles deliver blood to the capillaries that connect the arterioles with the venules or the smallest veins.
The arterioles control blood flow to the capillary by contraction of the muscle cells.

The arterioles branch into small vessels surrounded by a discontinuous layer of smooth muscle cells, the metarterioles, which branch into capillaries.

Organization of the tissues in the wall of arterioles:

a) *The tunica intima*
   It is formed by endothelium. No subendothelial loose connective tissue is found. Internal elastic lamina is absent.
b) *The tunica media*
   It is composed of layers of circular muscle cells. Larges arterioles consist of 2-5 layers of smooth muscle cells. In smaller arterioles these layer is composed of a single smooth muscle cells layer. Arterioles do not have internal elastic lamina. Contraction of smooth muscle in the tunica media reduces the diameter of arterioles (vasoconstriction). The relaxation of smooth muscle cells increases the diameter of the vessels (vasodilatation).
c) *The tunica adventitia*
   It is a thin layer of loose connective tissue that contains few elastic fibres and fibroblasts. In the smallest arterioles, the adventitia is insignificant.

Sensory structures in the arteries
Specialized sensory structures are found in the wall of the large arteries. There are: *carotid sinuses, carotid bodies* and *aortic bodies.* Nerve endings in these structures monitor blood pressure.
The carotid sinus is a baroreceptor located in the wall of the internal carotid artery just distal to the bifurcation of the common carotid artery. It detects changes in blood pressure and relay the information to the brain.
The carotid bodies are a small oval structure, which act as chemoreceptor monitoring changes of levels in oxygen, carbon dioxide and concentration of hydrogen ion. They are located at the bifurcation of the common carotid artery. The carotid bodies are composed of the type I and the type II cells. The type I cells contain adrenaline, dopamine and serotonin. The type II cells are supporting cells. The afferent nerve fibers carry impulses to the central nervous system.
The aortic bodies are located on the arch of the aorta between the right subclavian and the right common carotid artery and between the left cammon carotid artery and the left subclavian artery.

7.2.3.2. Capillaries

There are small thin-walled blood vessels with diameter of 9-10 μm. They form blood vascular network. The richness is related to the metabolic activity of the tissue. For examples: the liver, the kidney, cardiac muscle have very rich capillary network. Less capillary network is in dense connective tissue. The capillaries are suited for the exchange of metabolites and gases between cells and the bloodstream.

Classification of the capillaries

Structure of the capillaries varies in different tissues and organs. The human body has three types of capillaries: *continuous capillaries, fenestrated* and *sinusoidal (discontinuous) capillaries.*

1. *The continuous capillaries (somatic)* are the most common type. These types of capillaries are lined by solid endothelial layer cells. Endothelial cells rest on continuous basal lamina. They found in connective tissue, nervous tissue, exocrine glands and in muscle tissue.
   In some continuous capillaries pericytes may be associated with endothelial cells. The pericytes surrounds the capillary with cytoplasmic processes and form an incomplete covering. These cells have mesenchymal origin.
2. *The fenestrated capillaries (visceral)* have fenestrations in the endothelial cells. Endothelial cells rest on continuous basal lamina. Fenestrae are about 60-80 nm in diameter, which provide channels across the wall. Many fenestrae are closed by a very thin diaphragm. The diaphragm is thinner than plasma membrane and does not have the trilaminar structure. These types of capillaries also have pinocytotic vesicles. Fenestrated capillaries are found in the endocrine glands, the small intestine and the glomeruli of kidneys.
3. *The discontinuous capillaries (sinusoidal capillaries)* are discontinuous with wide and irregular lumen. Sinusoidal capillaries are found in the liver, the spleen, the bone marrow. Between individual endothelial cells are gaps and basal lamina may be incomplete or absent. Discontinuous capillaries may have specialized cells. For example: In the liver sinusoidal macrophages (Kupffer cells).

Arteriovenous anastomoses or shunts

In certain regions of the body participate in regulation of blood flow by direct communication between arterioles and venules**.**

In tissues (in the skin of nose, lips, in erectile tissue of penis) are direct routes between the arteries and the veins that divert blood from the capillaries. Arteriovenous anastomoses serve in thermoregulation at the body surface. Changes in diameter of arteriovenous anastomoses regulate blood pressure.

7.2.3.3. Veins

The veins are classified into three groups on the basis of their diameter and wall thickness: large veins, medium- sized veins and venules.

General characteristic of the veins

The diameters of the veins are larger than those of corresponding arteries. The veins are low pressure vessels, venous blood flow is passive. They may contain valves in the lumen that prevent retrograde blood flow. They are folds of the tunica intima, which protrude into the venous lumen like sails.

The valves consist of connective tissue with network of collagen fibres covered on the both surfaces by a layer of the endothelium. This fiber network is anchored in the wall. Valves are abundant in the veins of the limbs.

The veins also have three concentric layers: the tunica intima, the tunica media, the tunica adventitia.

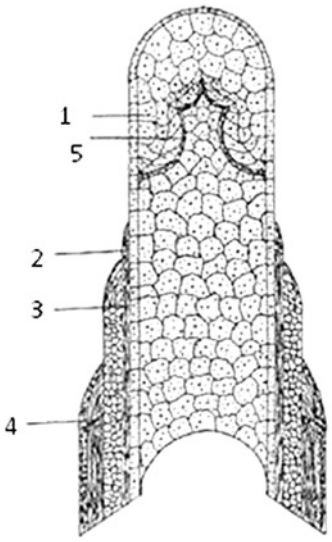

Fig. 2. Structure of the vein

1-the endothelium, 2-the internal elastic lamina, 3-the tunica media, 4-the tunica adventitia, 5-the valve

Large veins

Large veins are: the venae cavae and pulmonary, portal, renal, internal, jugular, iliac and azygos veins.

In large veins, the tunica adventitia is relating thick and tunica media is relative thin.

The wall of large vein consists of:

a) *The tunica intima*
   It consists of endothelium, basal lamina and small amount of subendothelial connective tissue and smooth muscle cells. Boundary between tunica intima and tunica media is not clear.
b) *The tunica media*
   This layer is relatively thin and contains smooth muscle cells, collagen fibers, fibroblasts.
c) *The tunica adventitia*
   It is the thickest layer in large veins, containing collagen, elastic fibers, fibroblasts and smooth muscle cells which run longitudinally. This layer is well-developed. Vasa vasorum are present.

Medium veins

Their diameter of up to 10 mm and valves are typical in these veins.

The wall of medium vein consists of:

a) *The tunica intima* consists of endothelium, basal lamina a thin subendothelial layer with smooth muscle cells.
b) *The tunica media* is thinner than the some layer in medium sized arteries.
c) *The tunica adventitia*- the thickest of tunics, is composed of longitudinally arranged collagen bundles and elastic fibers with a few scattered smooth muscle cells.

Venules

Classification of the venules

The venules are classified into*: postcapillary venules and muscular venules.*

a) *The postcapillary venules* which are 15-20 μm in diameter. Their wall has endothelial cells with receptors for vasactive substances.

These types have pericytes. Pastcapillary venules empty into muscular venule. The postcapillary venules in the lymph nodes, located in the deep cortex are lined by cubical endothelial cells and are called *high endothelial venules*. These venules participate in the transmural migration of lymphocytes into lymphatic tissue.

b) *The muscular venules* are located distal to the postcapillary venules. They have continuous endothelial layer. Their tunica media contains several layers of smooth muscle cells. Pericytes are progressively replaced by smooth muscle cells. Muscle cells forming a continuous layer as diameter increases. Mascular venules drain into the smallest veins.

Atypical veins

The veins in certain locations have atypical wall. For example: veins in the retina, veins in maternal part of the placenta, veins in trabeculae of the spleen, sinuses of the dura mater. These veins do not possess smooth muscle cells and as a result, do not have a tunica media.

Inervation of blood vessels

Most blood vessels contain network of unmyelinated sympatic nerve fibres-vasomotor nerves with neurotransmitter norepinephrine. In the veins, nerve endings are located in the tunica adventitia and the tunica media.

Clinical correlations

Atherosclerosis is the most common abnormality of blood vessels. The lesion develop in the intima a consist of thick layer of fibrous connective tissue containing scattered smooth muscle cells, macrophages, cholesterol crystals. The deposition of plaque within the wall of large arteries, results in reduced blood flow. Blood stasis and clotting (thrombosis) may lead to occlusion of the vessels. Hypertension is often associated with atherosclerotic vascular disease.

Varicose veins – are enlarged, tortuous veins in the legs of older persons and develop as incompetence of the valves.

Esophageal varicose veins occur in the lower end of the esophagus.

Hemorrhoids are varicose veins at the terminus of the anal canal.

Aneurysm – occurs most often in large vessels such as the aorta.

7.2.4. Lymphatic vessels

The lymphatic vessels convey fluid from the tissues to the blood. This fluid is called lymph and it circulates toward the heart. The lymphatic vessels serve as adjuncts to the blood vessels.

The lymphatic vessels are present throughout the body except in the central nervous system, lens, corpus vitreum, cornea. There are few present in the orbit, internal ear, cartilage and bone.

The lymphatic vascular system is an open system because in that is no pump and no circulation of lymph. Lymph moves primarily by compressions of the lymphatic vessels and by contractions of surrounding skeletal muscles on their wall.

Classification of the lymphatic vessels

Lymphatic capillaries

The lymphatic capillaries are small thin-walled blind end tubes, which are lined by endothelial cells and discontinuous basal lamina and the attachment of anchoring filaments, which reach to the endothelial cells on one side and to the connective tissue on the other side. Discontinuous basal lamina can be correlated with their high permeability. Their endothelial cells have no fenestrations in their cytoplasm. The lymphatic capillaries are without valves. The lymphatic capillaries are numerous in the loose connective tissues of the mucous membranes. The lymphatic capillaries absorb the proteins and electrolytes that leave blood capillaries. They usually form drainage systems. The lymphatic capillaries turn into larger lymphatic vessels.
As lymphatic vessels become larger, the wall is thicker.

Medium sized lymphatic vessels

They possess valves that prevent backflow of the lymph.
The wall consists of:

a) *Tunica intima* is lined with simple squamous epithelium- endothelium
b) *Tunica media*, which contains smooth muscle cells, which run circular and longitudinal
c) *Tunica adventitia* contains connective tissue

Large lymphatic vessels

The largest lymphatic vessels are *the thoracic duct* and *the right lymphatic trunk*. Large lymphatic vessels empty into the large veins near the heart.

The lymphatic vessels join to form the thoracic duct and the right lymphatic duct. The right duct collect lymph from the upper right of the body and the thoracic duct collect lymph from the left side and lower right part of the body. The lymphatic ducts are similar in structure to large veins, but they have thinner walls and lack a clear boundary between the 3 tunics. They passes valves, have larger luminae.

The wall of large lymphatic vessels is formed by:

a) *The tunica intima* with endothelial cells.

b) *The tunica media* containing smooth muscle cells.

c) *The tunica adventitia* is relatively underdeveloped and consists of longitudinally oriented smooth muscle cells and collagen fibers. In this layer are also small vasa vasorum similar to the vasa vasorum of the arteries.

The lymph is returned to the blood, it passes through lymph nodes, where it is exposed to the cells of the immune system. Afferent lymphatic vessels deliver lymph into lymph nodes, where it is filtered and it leaves by efferent lymphatic vessels. The lymphatic vessels also act as an integral component of the immune system.

Clinical correlations

Malignant tumor cells are spread throughout the body by lymphatic vessels and may produce secondary tumor. Examination of the lymph nodes and removal of lymph nodes in the pathway with associated lymphatic vessels play role in preventing secondary growth of the tumor.

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8. Bone marrow

Bone marrow (BM) is gelatinous, highly cellular and vascular tissue located in cavities of spongy bones. The surface of bone trabeculae is covered by single layer of flattened endothelium – like cells (endosteum, composed of osteoblasts, osteoprogenitor cells and occasional osteoclasts). In the adult, BM is found in the skull, sternum, scapulae, clavicles, ribs, vertebrae, pelvic bones and long bones. It is one of the largest organs in body. The weight of BM is 1600-3700 g, it constitutes about 5% of total body weight.

Function:

BM is responsible for the formation of blood cells (hematopoiesis) and their delivery into circulatory system. BM is also source of uncommitted lymphocytes and components of mononuclear phagocyte system. The production of blood cells is enormous with an estimated daily output of about 2.5 billion of erythrocytes, a comparable number of platelets, some 50-100 billion granulocytes (1.0 billion/kg body weight per day).

Hematopoiesis begins early in embryonic development. During the second week of gestation, angiogenic cell clusters (blood islands) appear in yolk sac. In the six week, hematopoiesis begins in the liver (hepatic phase) and during the second trimester in the spleen (splenic phase). Both hepatic and splenic phases continue until the end of gestation. Hematopoiesis begins in the BM (myeloid phase) by the end of second trimester.

8.1. BM structural components

- Blood vessels and nerves
- Reticulum cells and fibres
- Fat cells
- Hematopoietic cells
- Lymphoid cells

8.1.1. Blood vessels and nerves

Blood supply of BM is derived from nutrient arteries which penetrate bony shaft and branch in hematopoietic tissue to from a number of

capillaries. Capillary system opens into extensive meshwork of large thin – walled sinusoids. The sinusoids empty into central vein, which is drained by emissary veins via the nutrient canal. Physiologically, portions of the sinusoidal channels are partially or completely collapsed within the rigid bony cage. BM possess sinusoidal capillaries with discontinuous basal lamina, it may provide the exit route for mature blood cells into the circulation. Nerve fibres accompany marrow blood vessels but rarely found. BM has no lymphatic vessels.

8.1.2. Reticulum cells and fibres

The normal BM contains only delicate network of fine reticulin fibres best visualized by the Gomori staining. Reticulin fibres are secreted by branched fibroblasts, which are derived from embryonic mesenchyme. Fibroblasts and reticulin fibres constitute the microenvironment for developing of blood cells. There are also collagen fibres and the large molecular weight proteins laminin and fibronectin which bind the hemopoietic cells to the fibroblasts and marrow stroma. Fibroblast can be converted to fat cells and may be phagocytic. Proteoglycans dominate in ground substance.

8.1.3. Fat cells

In normal BM, fat cells occupy about 1/3-1/2 of BM volume in ilium. These cells have supporting and filling function in spongy bone cavities. In case of sudden need, fat cells can be replaced by hematopoietic tissue very quickly. The proportion of fatty tissue increases with advancing age. According to total amount of fat cells, we can macroscopically distinguish two types of BM:

a) Yellow marrow – composed mainly of mature, fat cells. Hematopoietic cells are decreased and accompanied by an increase in fat cells. Yellow BM is found in old people (around 70 and more). It may be reactivated if the need arises for icreased hematopoiesis.
b) Red marrow – made up mainly of hematopoietic cells. The red color is determined by cells of the erythrocytic series as well as by the amount of blood in sinusoids. Red BM is normally present in young and middle age people.

8.1.4. Hematopoietic cells

Hematopoiesis is the term, which is applied to the process of production of the formed elements of the blood. Process of hematopoiesis is a continuum of proliferation and progressive differentiation from stem cell to mature form. Hematopoiesis takes place in the extravascular parts of the BM. The normal values of cells in the peripheral blood are maintained by the hematopoietic tissues.

All types of blood elements arise from pluripotential hemopoietic stem cells (PHSCs). These cells account for about 0.1% of the nucleated cells of BM. PHSCs give rise to two populations of multipotential hemopoietic stem cells (MHSCs): colony-forming unit-spleen (CFU-S) and colony-forming unit-lymphocyte (CFU-Ly). Both of them produce various progenitor cells. CFU-S cells are responsible for manufacturing of the myeloid cell lineages (erythrocytes, granulocytes, megakaryocytes and monocytes). CFU-Ly give rise lymphoid cell lines, T- and B-lymphocytes.

The hematopoietic tissue is distributed in the BM spaces in the extravascular compartment and shows characteristic topography.

Erythroid precursors are associated with the marrow sinusoids in the central regions of the intertrabecular cavities. The nucleated precursors (normoblasts) are found in small and large clusters (erythrons). A macrophage with long cytoplasmic processes is usually located in the vicinity of a medium to large cluster of 5 or more erythroid cells. Cytoplasmic processes extend between the red cell precursors. This macrophage usually contains hemosiderin and some cellular and nuclear debris. It appears to play supporting role in normal red cell production. As the erythroid elements mature, they migrate outwards along the cytoplasmic projections of the macrophage. Less differentiated elements lag behind. The nucleus is extruded prior to to entry into the blood circulation. Nuclear fragments are phagocytosed by perivascular macrophages. The myeloid: erythroid ratio is 2:1 to 5:1.

Megakaryocytes are present in relatively small number, but they are prominent because of their size. They are the largest cells normally present in the BM. Their size ranges from 12-150 μm. Three stages are recognized in megakaryocytes at maturation: the megakaryoblast (12-20 μm), the promegakaryocyte (20-80 μm) and the mature megakaryocyte (80-150 μm). Mature forms possess large single highly lobulated nucleus. On progression

through these maturational stages, cytoplasmic basofilia decreases. The cytoplasm of mature megakaryocyte is eosinophilic and variably granular. These elements are located next to sinusoids. Megakaryocytes give rise platelets (thrombocytes) by fragmentation of their cytoplasm. Each megakaryocyte can produce several thousand platelets. The remnants of cytoplasm and nucleus of megakaryocyte degenerate and are phagocytosed by macrophages.

Early (immature) *granulopoietic precursors* are situated in proximity to bone trabeculae and arteries, they form the granulocytic generation zones. More mature granulocytes are located in central intertrabecular regions. Myeloblast, promyelocyte, myelocyte, metamyelocyte, band and segmented forms are all identifiable in BM sections. Generally granulopoiesis is very effective, so that practically all the cells produced reach blood circulation.

8.1.5. Lymphoid cells

Lymphoid cells belong to the normal cells population in BM. They are dispersed among the hematopoietic and fat cells or aggregated to form lymphoid nodules. The incidence of nodules increases with age. Under normal circumstances, lymphoid nodules are situated in central regions of marrow cavities. Plasma cells are also a normal component of the cell population in the BM. Their characteristic location is along small blood vessels.

8.2. BM cellularity

BM cellularity refers to both hematopoietic and fat cells and it indicates the relative amount of these two cellular components.

In normocellular BM, ratio between hematopoietic and fat cells is approximately 50:50±10% with respect to age.

Hypocellular BM indicates a reduction in hematopoiesis and corresponding increase in normal fat cells. Thus, the major part of each marrow cavity is represented by fat cells.

Hypercellular BM is characterized by increase in hematopoietic elements which replace fat cells. Hypercellular marrows may show either a normal distribution of cells with predominance of mature forms or a predominance of immature cells.

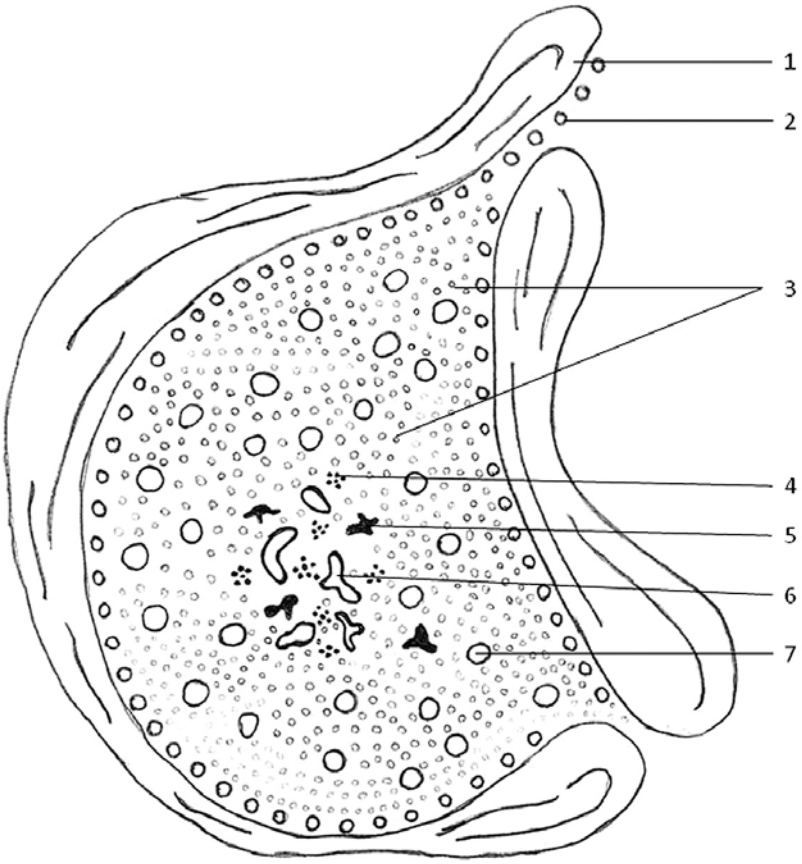

Fig. 1. Topography of bone marrow cells

1-bone trabeculae, 2-early (immature) granulopoiesis, 3-more mature and mature granulopoiesis, 4-clusters of normoblasts (erythrons), 5-megakaryocytes, 6-blood vessels (sinusoids), 7-fat cells

Clinical correlations

In past decades, there is a great increase in the number of bone marrow biopsies (BMB). Indication for taking BMB have increased especially in hematology and oncology. The posterior and anterior iliac crests are the preferred sites from which BMB are obtained. For this, special needles are used.

The description of the BM (e.g. to make a diagnosis) should include:

- overall cellularity
- red cells

- hemosiderin content
- granulocytes
- E:G ratio (nucleated red cells: granulocytes)
- megakaryocytes
- eosinophils
- plasma cells
- lymphocytes
- stromal reaction, vascular lesions, inflammatory reaction, necrosis, granulomas, fibrosis, bone lesions
- foreign cells and parasites.

In diagnostic procedure, conventional stainings, histochemical and immunohistochemical methods, as well as molecular-biology approaches are used. Following the description of the BM, a diagnosis is made. Other informations, such as clinical findings, blood count and blood smears should also be taken into account.

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9. Blood

Blood is a bodily fluid, which connects all cells, tissues, and organs of human body. It delivers substances such as nutrients and oxygen to the cells, and transports metabolic waste products away from those same cells by means of unidirectional flow within the lumen of blood vessels, within the *enclosed circulatory system*. Note that only two places where the blood with all its constituents leaves blood vessels and pours out into the extravascular space are the red pulp of the spleen and placenta (see dedicated chapters). Blood is a *dark red, viscous, slightly alkaline fluid* that accounts for 7-8% of human's total body weight, with females generally having less blood volume than males (an adult man has a blood volume of approximately 5 liters). From histological point of view, blood is a specialized type of connective tissue. As every other tissue that is made of cells and of the extracellular matrix, blood is also composed of two parts: *formed blood elements – haemocytes* ("blood cells" – leukocytes, erythrocytes and thrombocytes), and of the blood *plasma* – the liquid intercellular component (equivalent of the extracellular matrix of connective tissue). We preferentially use term "formed blood elements", because, in fact, thrombocytes (platelets) and erythrocytes are not cells by definition. These components may be separated by centrifugation if blood is collected in the presence of anticoagulants. The blood plasma contains minerals (inorganic salts) and organic compounds (including amino acids, lipids, vitamins, proteins, hormones, glucose, etc.). Water of the blood plasma is freely exchangeable with that of the body cells and other body fluids, and maintains the normal state of hydration of all tissues. Blood has many significant functions, which ensure and maintain homeostasis of the body, among them transport of nutrients (e.g., glucose, amino acids, fatty acids), and oxygen (bound to hemoglobin) directly or indirectly to the cells, transport of metabolic waste products (e.g., urea, lactic acid) and carbon dioxide (also bound to hemoglobin) away from cells and tissues, transport of humoral agents and cells of the immune system, delivery of hormones and other signaling and regulatory substances (e.g., cytokines) to and from the cells and tissues. *Thanks to coagulation factors in the blood*

plasma, blood is responsible for the coagulation. Blood maintains pH, osmotic pressure, blood volume and maintains a constant body temperature (see Table 1). Hence this fact, the examination of *peripheral blood smears* under the light microscope, and biochemical analysis of the blood plasma are of crucial diagnostic importance, and are one of basic diagnostic methods.

Table 1. Main functions of the blood

transport of: respiratory gases, nutrients, metabolites, biologically active agents, metabolic waste products
protection: immunological function (leukocytes, antibodies, antigens, some proteins - immunoglobulins), prevention of massive blood loss by coagulation (platelets, coagulation factors)
maintance of a steady state of organism's internal environment (homeostasis): body temperature, acid-base balance (as a buffer), osmotic pressure balance, blood volume balance
signaling and messaging: hormones, cytokines
hydration functions

Blood plasma is a pale yellow liquid component of the blood. It makes up about 55% of the body's total blood volume. More than 90% of plasma by weight is water (91-92%), around 7-8% are attributable to proteins (albumin, fibrinogen, globulins), and up to 3% is composed of other organic substances (e. g., sugars, lipids, vitamins, hormones, enzymes) and inorganic salts (minerals) (e. g., Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}). *Blood serum* is a blood plasma without coagulation factors (fibrinogen and the others), but includes all other proteins and organic substances not used in the process of blood coagulation, as well as electrolytes, antigens and hormones.

Hematocrit (packed cell volume) is the ratio of the volume of red blood cells (erythrocytes) to that of the whole blood. Its is expressed either as a percentage (conventional) or as a decimal fraction (SI units). The units (L/L) is implied. Physiological values of hematocrit in percentage are 45% ± 5% for men and 39% ± 4 % for women. It can be measured directly by centrifugation of the whole blood (noncoagulated) or indirectly as the product of the mean corpuscular volume (MCV) times red blood cell count in automated instruments. During centrifugation, erythrocytes are separated from the plasma. Between the erythrocyte sediment and the plasma, a thin white opaque layer, referred to as the *buffy coat* is formed

by platelets and leukocytes, and constitute about ≤1% of the total blood volume.

Erythrocytopenia generally refers to an abnormally low hematocrit (decreased number of red blood cells, typically accompained with decrease in the amount of hemoglobin in the blood = *anemia*), as opposed to *polycythemia*, which generally refers to an abnormally high hematocrit (increased number of red blood cells).

Hypervolemia, or fluid overload, is the medical condition where there is too much fluid in the blood. The opposite condition is *hypovolemia,* which is too little fluid volume in the blood. Common causes of hypovolemia are loss of blood (external or internal bleeding), loss of blood plasma (e.g., severe burns) or loss of body sodium and consequent intravascular water; e.g. diarrhea or vomiting.

9.1. Development of blood elements

Providing a short and brief overview of the development of blood elements (*formation haemocytorum*) during the ontogenesis of a human is not an easy task. It is partially due to the fact that during prenatal development, the location of *hemopoiesis* (hematopoiesis) changes several times, from extraembryonic tissues into the body of an embryo. Hemopoiesis first takes place in the extraembryonic splanchnic mesoderm within the wall of the yolk sac from the 17th day after fertilization, when the blood islands (*insulae sanguineae sacci vitellini*) start to appear. Primitive hemopoietic stem cells of the yolk sac are the precursors for nucleated erythrocytes, megakaryocytes and primitive macrophages. The wall of the yolk sac serves as a hemopoietic organ until the end of the second month after fertilization. From then, hemopoiesis starts to relocate into the body of fetus itself - with liver and the red pulp of the spleen (*textus haematopoieticus in hepate et pulpa rubra splenis*) to mention those most important. And then, to a minor extent, even thymus and a connective tissue around the aorta (abb. AGM – aortic, gonad and mesonephros region) serve as next hemopoietic organs. During the 4th week of the prenatal development, the liver primordium is colonized with hemopoietic stem cells. Only after the 10th week of prenatal development, these stem cells colonize

the bone marrow. However, the bone marrow takes over the function of hemopoietic organ from the liver only at the very end of pregnancy. Red bone marrow subsequently becomes the sole organ of hemopoiesis in postnatal period in humans. Under the pathological conditions such as anemia, myeloproliferative syndromes, chronic myeloid leukemia, return of hemopoiesis into liver and spleen (extramedullary hemopoiesis) can be observed often.

Hemopoietic stem cells (*cellulae haemopoieticae precursoriae*) are undifferentiated derivate of the middle germ layer – the mesoderm. They are capable of dividing and re-differentiate into all mature blood cells, even into mast cells (monophyletic theory = all of the blood elements arise from one common stem cell). Lymphoid lineage multipotent stem cells (for future adult lymphocytes) and myeloid lineage multipotent stem cells (for future erythrocytes, granulocytes, monocytes, macrophages, and megacaryocytes) originate from the same pluripotent stem cell (often termed shortly as hemocytoblast).

Erytropoetin, a hormon-like substance produced by fibroblasts of the connective tissue of the cortical part of kidney which is released during hypoxia, *thrombopoetin* produced by hepatocytes of the liver, and various cytokins and growth factors (so-called *colony-stimulating factors*) produced by bone marrow itself are important in the regulation and balancing of hemopoiesis in general. Macrophages tightly control the production and clearance of red blood cells. In erythroblastic islands, macrophage residents of the bone marrow provide erythroblasts with interactions that are essential for erythroid development, and provide a proper environment for promoting erythropoiesis.

9.2. Erythrocytes

Red blood cells (erythrocytes) are the most numerous amongst all formed blood elements. Normal quantity of erythrocytes per litre of blood is 4.3 – 5.3 x 10^{12} in males and 3.8 – 4.8 x 10^{12} in females. The red blood cells are produced in bone marrow from stem cells continuously at a rate of about 2-3 million cells per second. *Hypererythrocytosis* is a state when numbers of erythrocytes are higher than mentioned above (during pregnancy, while in high altitudes, abuse of erythropoetin in

sports). Reduced number of erythrocytes is called *erythrocytopenia*, which is usually accompanied with reduced level of hemoglobin – anemia. Note, that in fact, erythrocytes are not cells by definition, they lack cell nucleus and most of the cell organelles (e.g., mitochondria, endoplasmic reticulum, Golgi apparatus).

When studying blood smear of a healthy person under the light microscope, red blood cells are seen as homogeneous circular, biconcave disc-shaped, anuclear pink to red cells of nearly uniform size, ranging from 6 – 8 μm in diameter (7.4 – 7.5 μm in average). These normal-sized erythrocytes are called *normocytes*. However, even under the physiological condition, individual cells as small as 5.5 μm (microcytes), and as large as 9.5 μm (macrocytes), can be seen. This is called *physiological anizocytosis*. Thanks to their shape (biconcave disc), and loss of cell nucleus and other cell organelles, the center of erythrocytes is thinner and brighter than its periphery (as there is less cytoplasm). Shape of the biconcave disc is mantained by special membrane skeleton made of protein fibres of *spectrin and actin*. Spectrin is believed to play an important role in the erythrocyte membrane‘s ability to deform elastically. Analysis of electron micrographs of spectrin reveals that its α and β subunits are twisted around a common axis, forming a double helix with two fold rotational symmetry. Elastic deformation of the cell is mediated by transient extension of the helix by mechanical forces. The membrane of a red blood cell consists of spectrin tetramers connected to actin junctional complexes, forming a two-dimensional six fold triangular network anchored to the lipid bilayer. Erythrocytes are good example as how the morphology of a cell is often interconnected with its function – in this case – transport of gases. 97% of transported oxygen in the blood is bound to hemoglobin molecule. Hemoglobin is gas transporting protein molecule that makes up 95% of a red cell. Each red cell has about 270,000,000 iron-rich hemoglobin molecules. The hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain closely associated with a non-protein heme group. A heme group consists of an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin. During the perinatal period, hemoglobin consists of 80% of fetal hemoglobin (HbF - α2 γ2 chain) and only of 20% of adult hemoglobin (HbA1c - chain α2 β2).

After the birth, exchange of fetal hemoglobin for adult hemoglobin occurs within 6-12 months.

The rest 3% of oxygen (which is not bound to hemoglobin molecule) is dissolved in the blood plasma. This physically dissolved oxygen is detected by receptors in the cells of the kidney, ascertaining the possible hypoxia, what afterwards could lead to release of erythropoetin. Typical shape of erythrocyte is a best possible compromise between the surface area and the volume of the cell. Any abnormally shaped red blood cell (oval, pear-shaped, teardrop-shaped, helmet-shaped, and irregularly shaped) is a *poikilocyte*, and variation in shape is called *poikilocytosis*. On the outer surface of the erythrocyte is glykocalyx, composed of oligosaccharide chains. This structure is the basis for various *blood groups*.

Young red blood cells are particularly flexible and deformable due to their plasma membrane – they pass easily through the capillaries, which diameter may be smaller than their own size. The life span of erythrocytes is about 120 days. Old erythrocytes have a more rigid and less flexible plasma membrane and therefore are rather retained in the spleen, which has a special arrangement of blood circulation (see dedicated chapter) thus being able to filter overaged red blood cells. The spleen is therefore called as „cemetery of red blood cells."

Erythrocytes do not have typical cell organelles, they do not containcell nucleus, endoplasmic reticulum, and mitochondria; it is basically just a unit wrapped by plasma membrane filled with 33% solution of hemoglobin. This hemoglobin can reversibly bind oxygen (to form oxyhemoglobin) and carbon dioxide (to give carbaminohemoglobin). Under the pathological conditions, the hemoglobin may be irreversibly bound to the carbon monoxide (if present in inspired air), forming carboxyhemoglobin. The affinity of hemoglobin to CO is up to 200-times higher than that of oxygen. Therefore even relatively low concentration of CO in the atmosphere can cause human death. In addition, hemoglobin has a very important function in maintaining a steady blood pH by acting as a buffer.

A brief overview of erythropoiesis is provided in Table 2. Note, that in different literature, there are considerable inconsistencies in naming developmental stages of future erythrocytes

Table 2. A brief overview of development of erythrocytes

proerythroblast	derived from myeloid stem cell, large basophilic nucleus, basophilic cytoplasm, with diameter up to 20 μm
basophilic erythroblast	intense basophilic cytoplasm – many ribosomes, similar to its precursor, but smaller
polychromatic erythroblast	cytoplasm still basophilic (presence of ribosomes), but also partially acidophilic (hemoglobin concentration is increasing)
oxyphilic normoblast	hemoglobin predominant within the cytoplasm, nucleus is small and condensed (prepared for expulsion)
reticulocyte	without nucleus, remnants of ribosomes (after special staining they appear as a web-like structure within the cytoplasm, giving its name to the cell) are present, can be seen in peripheral blood smear (up to 1% of all red blood cells)
normocyte	mature, normal-sized erythrocyte

9.3. Leukocytes

White blood cells (leukocytes) are the second most abundant formed elements of the blood, and the only ones with nucleus, however, they make up a very small part of the blood‘s volume - normally only about 1% in healthy people. Leukocytes are not limited to blood. They occur elsewhere in the body as well, most notably in the connective tissue proper, spleen, liver, and other lymphoid organs, where they are moving by amoebic movement. *Diapedesis* (transmigration) is the process in which leukocytes penetrate through the wall of blood vessels. However, once in connective tissue, white blood cells cannot return back into the bloodstream except for lymphocytes, which have the ability to re-circulate, and constantly move between the lymphoid tissue and the blood, using specialized blood vessels called post-capillary venules. These are lined with cuboidal endothelial cells instead of squamous endothelial cells as in other blood vessels. Leukocytes are produced mostly in bone marrow from the same stem cells that produce red blood cells. Their count is $3.5 – 10.5 \times 10^9$ / liter of blood, and varies during the day. It is the lowest in the morning, and the highest in the afternoon. The increased number of leukocytes is referred to as *leukocytosis*, and the reduced number as *leukopenia*. Leukocytes are cells that in different ways participate in our immune responses.

Leukocytes can be divided into two major groups. One group includes cells that contain specific granules in their cytoplasm that are well visible

under the light microscope, and also less visible non-specific granules, therefore they are called *granulocytes*. The second group includes cells that do not have specific granules, but possess only non-specific azurophilic granules instead, therefore they are called *agranulocytes*. Because granulocytes usually possess segmented nuclei, they are also named as polymorphonuclear leukocytes, while agranulocytes have spherical nucleus (with or without a notch), therefore, they are referred to as mononuclear leukocytes. According to the dyeability of their cytoplasmic granules, granulocytes are further divided into neutrophilic granulocytes (neutrophils), eosinophilic granulocytes (eosinophils) and basophilic granulocytes (basophils). Agranulocytes are further divided into lymphocytes and monocytes. The relative percentage of each white blood cell type is referred to as the differential white blood cell count, known as the *leukogram.*

9.3.1. Granulocytes

9.3.1.1. Neutrophils

Neutrophils are the most abundant (40% to 70%) type of white blood cells in humans and form an essential part of the innate immune system. They live shortly in the blood (only hours), and are highly motile. Their extremely short lifespan and their total number in the blood reveal their high renewal rate; nevertheless, 10^{11} produced neutrophils daily is indeed quite an unimaginable number. Their size varies from 12 to 15 μm with pale pink cytoplasm that contains many fine, lightly stained red-violet granules. These specific granules contain enzymes such as alkaline phosphatase, collagenase, or lactoferrin.

Neutrophils, as a members of PMN (polymorphonuclear) leukocytes, have a multilobed (segmented) nucleus (number of lobes varies from 1 to 5). These lobes (segments) are connected with each other by thin chromatin bridges. The nucleolus disappears as the neutrophil matures, a phenomenon that is seen only in few other types of nucleated cells. In the cytoplasm, the Golgi apparatus is small, mitochondria and ribosomes are sparse, and the rough endoplasmic reticulum is absent. Neutrophils show hypersegmentation (many segments of the nucleus) as they mature, so the oldest cell possess 5-lobed nucleus, and, in contrary, youngest neutrophils are called band neutrophils (band cells or stab cells) with

nucleus having only one lobe. The so-called *Hink's number* tells us the average number of segments of neutrophils (their degree of maturity). Under normal conditions, Hink's number is about 2.7. When the value of Hink's number is significantly below the average of 2.7, we are talking about Arnet's shift to the left. This means that the blood harbours much more young neutrophils, which generally indicates some current acute infection (mainly of bacterial etiology). If the value of Hink's number is significantly above the average, we are talking about Arnet's shift to the right, which suggests the depression of hematopoiesis. In females, it is possible to find small appendage from one of the lobes of the nucleus, which is the inactived X chromosome, and is known as the "neutrophil drumstick", or the *Barr body* (named after discoverer Murray Barr).

Neutrophils are one of the first-responders among inflammatory cells to migrate towards the site of inflammation; thus, pathogen is likely to first encounter a neutrophil. They migrate through the blood vessels (diapedesis), then through the interstitial tissue, following chemical signals in a process called *chemotaxis*. They are the predominant cells in the pus, accounting for its whitish/yellowish appearance. Neutrophils are recruited to the site of injury within minutes following trauma, and are the hallmark of acute inflammation. When circulating in the blood and unactivated, neutrophils are spherical. Once activated, they change shape and become more amorphous or amoeba-like and can extend pseudopods as they hunt for antigens. Neutrophils act as professional phagocytes; therefore, Ilya Ilyich Mechnikov, who discovered the process of phagocytosis (1883), described them as *microphages*. They have several methods for directly attacking micro-organisms: phagocytosis and release of soluble anti-microbials (including proteins contained within specific granules) being the most important. In addition, they express and release cytokines, which in turn amplify inflammatory reactions thanks to several other cell types.

9.3.1.2. Eosinophils

Eosinophils are slightly greater than the neutrophils (12 – 17 μm in diameter) and account for 2% to 6% of total white blood cells. Together with neutrophils, they create another component of the immune system responsible for combating multicellular parasites. Along with basophils and mast cells, they are important mediators of allergic responses and asthma

pathogenesis, and are associated with disease‘s severity. Eosinophils are also able to phagocyte, but they absorb only the immune (antigen-antibody) complexes. They persist in the circulation for 8–12 hours, and can survive in the tissue for an additional 8–12 days in the absence of stimulation.

The cell nucleus is typically bilobed (two segments, similar to eye-glasses). In the cytoplasm, there are many relatively large specific granules containing many chemical mediators, such as major basic protein, which is regarded as one of the most important ones, causing the eosinophilic color of these granules. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues. Electron microscopy studies have shown that the granules of eosinophils are composed of the crystalline core with basic protein with a high content of arginine. After processing, the dye is concentrated in small granules within the cytoplasm giving a brick red color of the cytoplasm.

An increase in eosinophils is called *eosinophilia*. Several causes are known, with the most common being some form of allergic reaction or parasitic infection (typically seen in people with parasitic infestation of the intestines). Eosinopenia is a state where the number of eosinophils is lower than normal.

9.3.1.3. Basophils

Basophils are the least common of the granulocytes, representing only about 0.01% to 1% of the circulating white blood cells, and are the smallest (8 – 11 μm in diameter). Their nucleus is tend to be kidney-shaped or sigmoid but it is often obscured with basophilic specific granules rich in heparin (an anticoagulant) and histamine (a vasodilator), which promote blood flow to tissues, causing swelling or edema. Origin and molecular regulation of basophils differentiation remain not fully understood. In contrast to other myeloid lineages, a specific basophil growth factor has not been discovered yet. Furthermore, it is still unclear whether basophils possess a lineage-restricted progenitor or whether they share a common ancestor with mast cells, eosinophils, or even megakaryocytes.

Basophils’s role is essential in the propagation of *allergic reactions* and in causing allergic symptoms and, as well as eosinophils, they play a role in parasitic infections.

9.3.2. Agranulocytes

9.3.2.1. Monocytes

Monocytes belong to agranulocytes and develop from myeloid stem cells in the bone marrow. Then they enter the blood, where they circulate for a few days before migrating to different tissues. In the tissue, they further mature into *macrophages* responsible for phagocytosis of foreign antigens or cellular debris, or *antigen-presenting dendritic cells*.

Monocytes are the largest blood cells (averaging 15–20 μm in diameter), and they count for 1 – 9% of total leukocytes. The nucleus is relatively large, kidney or bean-shaped (also C-shaped) wih a well visible notch, and slightly eccentric position, with less heterochromatin as lymphocytes. Cytoplasm of monocytes is grayish and contains only non-specific granules (which are, in fact, lysosomes).

Monocytes and macrophages are the same cells, but in a different stage of development. Monocytes are localized within the blood stream, macrophages represent a motile cell population of connective tissue proper. Both play important roles in the immune defence, inflammation and tissue remodelling, and they do so by phagocytosis, antigen processing and presentation, and by production of cytokines.

The primary role of monocytes was considered to scan the environment and replenish the pool of tissue macrophages and dendritic cells. Recent advances in immunology research have discovered that monocytes are heterogenic and can be divided into numerous subsets based on specific surface markers (note that their recognition is possible only via methods of immunohistochemistry). Most of monocytes are equipped with a set of Toll-like receptors and scavenger receptors, recognizing pathogen-associated molecular patterns and removing microorganisms, lipids, and dying cells via phagocytosis. Inflammatory monocytes selectively traffic to the sites of inflammation, produce inflammatory cytokines, myeloperoxidase and superoxide, and contribute to local and systemic inflammation.

Elevated levels of monocytes are seen in tissue breakdown or chronic infections, carcinomas, leukemia or lymphomas. The causes of monocytopenia include acute infections, stress, treatment with glucocorticoids, aplastic anemia or hairy cell leukemia.

9.3.2.2. Lymphocytes

Lymphocytes also belong to agranulocytes, since they possess only non-specific granules. They are relatively small, spherical cells, the size of which is comparable to a red blood cell (small-sized lymphocytes have only 6 to 8 μm, less numerous medium and large-sized lymphocytes have from 10 to 18 μm). However, they are of enormous significance and they are *key cells of the immune system*. Larger cells are stimulated cells or memory cells which are about to differentiate into effector cells. Lymphocytes count for 20% - 40% of all leukocytes in the blood. The three major types of lymphocyte are T-lymphocytes, B-lymphocytes and natural killer (NK) cells. It is impossible to distinguish between various types of lymphocytes in a peripheral blood smear without recognition of their typical surface markers (receptors) using immunocytochemistry.

The nucleus is relatively large and occupies almost the entire cell volume. It contains a large amount of heterochromatin, causing its dark blue color. The slightly basophilic cytoplasm forms only a thin rim around the nucleaus, and is staind pale blue in peripheral blood smear.

B-lymphocytes are responsible for specific humoral immunity (antibody production). In birds, they mature in a specialized primary lymphoid organ organ called the “bursa of Fabricius”. The name “B-lymphocyte” comes from the name of this organ. In humans like in all mammals, B-lymphocytes are produced and mature in the *bone marrow*. B-lymphocytes after stimulation (activation) further divide and differentiate into either plasma cells or memory cells. This process takes place within secondary lymphoid follicles via different developmental stages (for more detailed information see chapter Lymphatic tissue and organs). Memory cells (memory B-lymphocytes) can persist in the blood for months or years, but after contacting known antigens, they are quickly activated, producing antibodies and defend the organism against the pathogen (principle of acquiring immunity after overcoming some infections or after active immunization). Plasma cells are found within connective tissue as wandering (free) cells of connective tissue, where they produce antibodies.

T-lymphocytes are responsible for specific cellular immunity. They are called T-lymphocytes because they mature in the thymus. T-lymphocytes can be further divided into several sub-groups. *Helper T-lymphocytes*

(expressing CD4 antigen) produce chemicals known as cytokines. The individual subpopulations of helper T-lymphocytes can stimulate B-lymphocytes to proliferate and differentiate into plasma cells, and can stimulate higher activity in macrophages and NK-cells to regulate non-specific immunity. *Cytotoxic T-lymphocytes* (expressing CD8 antigen) produce special enzymes (perforins and granzymes), which damage the virus-infected cells, foreign cells (eg. after transplantation) and tumor cells. *Natural regulatory T-lymphocytes* suppress the immune response with immediate cooperation with the antigen-presenting cells. *Memory T-lymphocytes* provide the body immune memory; carry information about a particular antigen.

A separate group of lymphocytes are *NK cells* (natural killer cells), which count for about 5 to 10% of lymphocytes circulating in peripheral blood. It is believed that NK cells do not mature in the thymus, although they have a very similar function to cytotoxic T-lymphocytes. They are primarily responsible for the destruction of virus-infected cells and tumor cells.

9.4. Myelopoiesis

Myelopoiesis is the regulated formation of myeloid cells including granulocytes (neutrophils, eosinophils, and basophils) and monocytes (also macrophages, and dendritic cells). During the embryonic development of mammals, myelopoiesis occurs in a stepwise fashion that begins in the yolk sac and ends up in the bone marrow. During this process, early monocyte progenitors colonize various organs such as brain, liver, skin, and lungs and differentiate into resident macrophages of those organs that will self-maintain throughout the life. Dendritic cells are constantly replenished from a bone marrow precursors, but can also arise from monocytes under inflammatory conditions. A granulocyte differentiates into a distinct cell type by a process called *granulopoiesis*. In this process it first transforms from a common myeloblast (myeloid progenitor) to a common promyelocyte. This promyelocyte gives rise to a unique myelocyte that for the first time can be classified as an eosinophil, basophil, or neutrophil progenitor based on the histological staining affinity. Overview of myelopoiesis is presented in Table 3.

Table 3. A brief overview of development of granulocytes

Myeloblast	it is derived from myeloid stem cell, it is similar to proerythroblast. It represents only a short, transitional phase of development, so it is rarely found in the blood smear
Promyelocyte	second largets cell in the bone marrow (after megacaryocytes)
Neutrophilic, eosinophilic and basophilic myelocyte	there is an increasing number of specific granules. Mitotic division
Neutrophilic, eosinophilic and basophilic metamyelocyte	already post-mitotic cells, they contain specific granules
Adult neutrophilic, eosinophilic and basophilic granulocyte	

Table 4. A brief overview of development of monocytes

Monoblast	it is derived from the myeloid stem cell
Promonocyte	various developmental stages are hardly distinguishable in the specimen from the bone marrow
Monocyte	circulates in peripheral blood
Macrophage	in organs and tissues, it is localized within the connective tissue

9.5. Lymphopoiesis

All lymphocytes originate, during the process called *lymphopoiesis*, from a common lymphoid progenitor before differentiating into their distinct lymphocyte types. The differentiation of lymphocytes follows various pathways in a hierarchical pattern. Precursors of B-lymphocytes mature in the bursa-equivalent organs, which in humans is the bone marrow itself and/or gut-associated lymphoid tissue (GALT), namely the Peyer‘s patches of the intestine, while precursors of T-lymphocytes migrate to and mature in a distinct organ, called thymus. Following the maturation, lymphocytes enter the circulation and peripheral lymphoid organs (e.g. the spleen and lymph nodes) where they survey for invading pathogens and/or tumor cells. They live from weeks thru several years to a whole lifetime, which is very long time period compared to other leukocytes.

Table 5. A brief review of development of lymphocytes

Lymphoblast	is derived from lymphoid stem cell
B- prolymphocyte	matures in bone marrow and GALT system
T- prolymphocyte	matures in thymus

9.6. Thrombocytes (platelets)

In addition to erythrocytes and leukocytes, one can still see clusters of anuclear cytoplasmic fragments (plates) that are stained basophilic in a stained peripheral blood smear. These are *thrombocytes (platelets)*. They are biconvex anuclear discoid subcellular fragments, with a lense-like shape, 2–3 μm in greatest diameter and their normal quantity is 150 – 400 x 10^9 per litre of blood, with men as a group having a slightly higher mean values than women. The average life span of circulating platelets is 8 to 9 days. Old platelets are destroyed by phagocytosis in the spleen and in the liver.

Membrane of platelets invaginates to form an open canalicular system. On their surface, platelets possess glycoprotein-rich zones required for platelet adhesion, activation, and aggregation in the process of hemostasis (see below). The central region of the platelet is darker and is referred to as *granulomere* (rich in platelet granules), while the outer area (periphery) is lighter and is referred to as *hyalomere* with microtubules, microfilaments and a spectrin-based membrane skeleton, allowing the platelets to maintain their discoid shape. Cytoplasm of platelets contains also remnants of megakaryocytic (see below) smooth endoplasmic reticulum organized into a dense tubular system, and mitochondria.

Platelets originate from large cells (30-150 μm) with multilobed nuclei called *megakaryocytes* that reside primarily in the bone marrow. During maturation, megakaryocytes in a cytoskeletal-driven process, extend long branching processes - designated proplatelets - into sinusoidal blood vessels where they undergo fission to release platelets. As the nascent platelet matures, its content of granules and organelles is delivered as a stream of individual particles moving from the megakaryocyte cell body to the proplatelet tip. Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone produced in the kidneys and the liver. An average of 10^{11} platelets are produced daily in a healthy adult.

The main function of platelets is to minimize the blood vessel injury and taking part in hemostasis; the process of stopping bleeding at the site of interrupted endothelium trough the formation of the platelet plug *(primary hemostasis)*, which is also associated with the activation of the coagulation cascade resulting in deposition and linking of fibrin *(secondary hemostasis)*. However, platelets also play a secondary role in angiogensis and innate immunity.

Low platelet concentration is called *thrombocytopenia* and occurs due to either decreased production or increased destruction. Elevated platelet concentration is then called thrombocytosis and is either congenital, reactive (to cytokines), or due to unregulated production.

Table 6. A brief overwiev of development of thrombocytes

Megakaryoblast	it is derived from myeloid stem cell
Promegakaryocyte	
Metamegakaryocyte	
Megakaryocyte	up to 100-150 μm, contains a huge multilobed nucleus (polyploidy - may have up to 64 chromosome sets)
Thrombocyte	anuclear fragments of cytoplasm of the megakaryocytes

9.7. Blood smear (blood film examination)

A blood smear (peripheral blood smear or blood film) is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow various blood elements to be examined microscopically. Examination of the blood smear (film) is an important part of investigating hematological problems (disorders of the blood) and, occasionally, of detection of parasites within the blood such as malaria and filaria. The reliability of the information obtained depends heavily on well-made and well-stained smears that are systematically examined.

Clinical correlations

Chronic lymphocytic leukemia (lymphoid, lymphatic, CLL) is a malignant, monoclonal disorder characterized by progressive accumulation of immunologically incompetent small B lymphocytes with low-density surface immunoglobulin. The term “chronic” comes from the fact that it

typically progresses more slowly than other types of leukemia. In Western countries, CLL is the most common type of leukemia, comprising about one-quarter of all the leukemias. CLL is observed most frequently in adults with male predominance. Approximately one-third of patients are asymptomatic at time of presentation and the diagnosis is discovered only when a routine blood test is performed or by trephine biopsy.

Bone marrow biopsy reveals three distinctive microscopic patters of infiltration: interstitial, nodular, and diffuse. There may be extensive replacement of normal bone marrow by lymphocytes, reading 30-95% of the marrow cell total. Patients with nodular or interstitial pattern of infiltration have a better prognosis. The blood count in CLL shows an absolute lymphocytosis (between 20-200 x 10^9/l) and peripheral blood has a characteristic lymphoid morphology. Patients with CLL show a wide range of symptoms and sings (enlargement of lymph nodes, splenomegaly, hepatomegaly, thrombocytopenia with bruising and skin purpura, infections, loss of apetite, fatique, night sweats, etc.).

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10. Skin

Anatomy introduction

Skin covers the entire external surface of the body, including the external auditory meatus, the lateral surface of tympanic membrane and the vestibule of the nose. It is the largest organ in the body, occupying almost 2 m^2 of surface area. The skin consists of the *epidermis* and the *dermis (corium).* The *subcutis* forms the connection with the structures beneath the skin (fascias, periosteum). It is useful to distinguish between two major classes of skin: these are, *thin hairy skin,* which covers the greater part of the body, and *thick, hairless (glabrous) skin,* covers the palms of the hands, soles of the feet, and palmar surface of the digits. The surface of the skin and its deeper structures show various lines and ridges. Their arrangement is genetically determined and is characteristic for each particular individual. This fact forms the basis of *finger printing.* Appendages of skin are *hairs, nails* and *glands.*

Skin (cover, integumentum, cutaneous layer) is an extensive organ, which covers the entire outer surface of the body, with the exception of the cornea and conjunctiva. Skin varies in structure and thickness in different sites according to specific functions (e.g., 0.5 mm on the eyelids, more than 5 mm on the midle of the upper back, typical thickness is 1 to 2 mm). This complex covering is essential to life. In addition, there are its appendages such as sweat glands, sebaceous glands, hair and nails.

Function:

Skin has six major functions:

1. Regulation of body temperature
2. Protection against invasion of microorganisms, external various injuries and desiccation
3. Sensation – skin is the largest sensory organ, it contains a variety of receptors (e.g. temperature, touch, pressure and pain)
4. Metabolic functions – absorption of UV (ultraviolet) radiation for vitamin D synthesis, subcutaneous adipose tissue is important store of energy
5. Excretion from sweat glands
6. Sexual signaling (attractant)

10.1. Skin consists of three main layers firmly attached to one another

a) the epidermis – an outer stratified squamous keratinized epithelium which is able to self – regenerate, it is derived from the ectoderm, part of skin in direct contact with external environment
b) the dermis – a dense irregular connective tissue with supporting and nourishing functions, derived from the mesoderm, it contains blood vessels, nerves and sensory receptors
c) the hypodermis (subcutis) – a subcutaneous loose connective tissue containing varying amounts of fat, but also contains the larger vessels which supply and drain the dermal blood vasculature

10.1.1. Epidermis

The stratified squamous keratinized epithelium of epidermis is composed of four populations of cells:

- Keratinocytes
- Melanocytes
- Langerhans cells
- Merkel cells

Keratinocytes are the predominant cell type in epidermal layer. They are called as keratinocytes because their main product is protein keratin forming intermediate filaments. Three remaining cell types are interspersed among keratinocytes in specific locations.

The surface keratinized layer of epidermis is shed continuously and is therefore replenished by the proliferation, maturation and migration of cells from the epidermal basal (germinal) layer. The process of keratinocyte maturation varies from site to site. Slow turnover range from 30-50 days, being more faster in areas exposed to heavy frictional forces (e.g. soles), it takes from 25 to 30 days. The turnover period is markedly shortened in some diseases of skin, mainly in psoriasis. The phases of this dynamic turnover of keratinocyte maturation (keratinization) is represented by five histomorphological layers:

10.1.1.1. Stratum basale (basal layer)

It is the most basal layer of cells in epidermis, rests on a thick basement membrane next to the dermis. Basal layer consists of single layer

of cuboidal or low columnar cells. The basal aspect of each cell is bound to the basement membrane by hemidesmosomes. Apical domain of these cells is bound to adjacent cells by desmosome junctions. Stratum basale cells are highly proliferative, they undergo repeated mitoses (germinal layer of the epidermis). This layer is partially responsible for cell renewal in the epithelium. Mitosis occurs mostly during the night. Histological specimens are produced during the day, and therefore mitotic figures are rarely seen in histological section of skin. When new cells arise by mitosis, the previous cell are pushed surface ward to join cells of stratum spinosum.

10.1.1.2. Stratum spinosum (prickle cell layer)

It is the thickest part of epidermis, consists of relatively large and polyhedral prickle cells with central round nuclei and prominent nucleoli. The thickness of stratum spinosum varies from five to ten cell layers. The prickle cells are separated by narrow spaces and are in contact with each other by system of intercellular bridges. These intercellular connections are formed by small cytoplasmic projections from the cell surfaces terminating in large number of desmosomes. Active protein synthesis is salient feature of these cells. Prickle cells produce a fibrillar protein cytokeratin, which aggregates to from intracellular fibrils called as tonofibrils. Bundles of tonofibrils radiate from central part of cytoplasm toward highly interdigitated cytoplasmic projections. Tonofibril groups cause the cytoplasm to become eosinophilic. Cytoplasm of stratum spinosum cells also possesses secretory membrane-coating granules (lamellar granules) housing lipid substancies. The cells of the upper prickle cell layer are flatter than the polyhedral cells located deeply.

10.1.1.3. Stratum granulosum (granular layer)

The stratum granulosum is composed of three to five layers of flattened, nucleated and irregulary shaped keratinocytes. The cells of this layer contain numerous granules of keratohyalin which are stained intensely with various basic dyes including hematoxylin. Granules are without a limiting membrane and are associated with the tonofibrils. The process of keratinization begins in granular cells by combination of keratohyalin and tonofibrils to form mature keratin. The outermost

aspect of granular layer is characterized by cell death due to rupture of lysosomal membranes.

In addition, keratinocytes in this layer also posses small round, membrane-coating granules (keratinosomes or Odland bodies). The lamellar content of these granules is discharged by exocytosis into the extracellular spaces. It is lipid-rich substance and is related to the epidermal barrier function as waterproof zone and probably has a role in cellular cohesion.

10.1.1.4. Stratum lucidum

The stratum lucidum is present only in thick type of skin as intermediate layer above the stratum granulosum and beneath the stratum corneum. It consist of flat, translucent layers of dead cells. These cells lack nuclei and organelles, but they contain coarse keratin filaments and protein eleidin. The inner aspect of the plasma membrane is thickened by deposition of non-keratin protein involucrin.

10.1.1.5. Stratum corneum (cornified layer)

The stratum corneum is the most superficial layer of epidermis. Its thickness is ranging from few cell layers (e.g. on the eyelid) to several hundred cell layes (e.g. on the sole of the foot). The cells of cornified layer are dead, flattened and desiccated with a thickened plasma membrane. They are filled with mature keratin. Desmosomes are only present between cells in the deeper aspect of this layer. Towards the surface, the desmosomes are disrupted, the outermost layers are loosely attached to each other. The desquamation is ready to start. The surface cells in the stratum corneum are constantly shed through normal abrasion. On the other hand, they are replaced by new keratinocyte that are formed by cell division and migrate from the germinative layer below.

Epidermal layers (epidermal stratification) are a result of keratinization process. Highly proliferative and non-keratinized cells are found next the basement membrane and non-proliferative, dead, completely keratinized cells comprise the outermost layer. Between these extremes, the layers in the intermediate stages of keratin accumulation and maturation are present.

According to the thickness of the epidermis, two types of skin are recognized:

1. Thin skin covers most of the body skin. Epidermis is 0.075 to 0.150 mm thick. Stratum corneum is thin. Stratum lucidum does not appear in this type of skin. It has sebaceous glands, sweat glands and hair follicles.
2. Thick skin possesses all five above mentioned epidermal layers. It covers the palms and soles. The epidermal thickness is 0.4 to 0.6 mm. It lacks sebaceous glands and hair follicles, but it has numerous sweat glands.

10.2. Nonkeratinocytes in the epidermis

The stratified squamous epithelium of epidermis contains three other cell types in addition to keratinocytes.

10.2.1. Langerhans cells

They originate from precursors in the bone marrow and belong to the mononuclear phagocyte system, located in all epidermal layers but predominantly are scattred in the stratum spinosum. They represent 2% to 4% of the epidermal cells (800/mm^2). These cells possess an oval or irregular pale nucleus and pale cytoplasm with cytoplasmic processes. Characteristic morphological feature of Langerhans cells are rod-like Birbeck granules. Sometimes, the end of the rod forms spherical vesicle, it gets appearance of a tennis racquet. Functionally, these cells are antigen-presenting cells. They are mobile and migrate to paracortical zone of regional lymph node interacting with T-cells.

Unlike melanocytes, which multiply after repeated exposure to ultraviolet light, Langerhans cells decrease in number after such insult. This is possibly a contributing factor in ethiopathogenesis of cancers.

10.2.2. Melanocytes

Melanocytes are neuroectodermally-derived cells producing pigment melanin which is responsible for brown coloration of skin. They are also involved in protection of skin against ultraviolet radiation. They are mainly located among the cells of the stratum basale of epidermis and

are in contact with basement membrane. Their number varies in different regions of skin representing around 3% of the epidermal cells (ranging from 800 to 2300/mm^2). Melanocytes are round to columnar shaped with numerous long cytoplasmic processes extending into stratum spinosum. One melanocyte and associated keratinocytes form an epidermal-melanin unit. Rough endoplasmic reticulum of melanocyte produces enzyme tyrosinase which is packed by Golgi apparatus to form oval granules called as melanosomes. The amino acid tyrosine enters melanosomes, where it is converted by tyrosinase into melanin pigment. Then melanosomes are transported by cytoplasmic projections to keratinocytes and they form in supranuclear region protective barrier between nucleus and sun ultraviolet rays (apical cap).

Racial and ethnic differences in skin color are caused by several factors, including the number of melanocytes present and the types and sizes of melanin granules.

10.2.3. Merkel cells

Merkel cells are scattered only in the basal layer of epidermis. Morphologically, they resemble melanocytes having irregular, lobulated nuclei and cytoplasmic processes among keratinocytes. Merkel cells form synaptic junctions with unmyelinated sensory peripheral nerve ending and are most abundant in areas of greatest tactile sensitivity, such as the fingertips and lips (mechanoreceptors). These cells contain round membrane-bound neuroendocrine-type granules.

10.3. Dermis

The dermis lies beneath the epidermis, is derived from mesoderm and is composed of two layers: - loose connective tissue papillary layer

- dense connective tissue reticular layer

The epidermal – dermal boundary is the basement membrane. Dermis contains a series of folds called dermal papillae which increase the contact area between the epidermis and dermis. By this way, the adhesion between these layers is strengthen.

10.3.1. Papillary layer

The papillary layer is associated with the dermal papillae. It is composed of loose connective tissue (type III collagen-reticulin, elastic fibres, collagen fibres) forming loose network. There are numerous fibroblasts, macrophages, mast cells, plasma cells and other common connective tissue components. Papillary layer also houses many capillaries, which extend to epidermal – dermal boundery. These capillaries are responsible to regulate body temperature and to supply the avascular epidermis by nourishment.

10.3.2. Reticular layer

The reticular layer is deeper and much denser than superficial papillary layer. Collagen fibres are arranged in coarser bundles intermingled with network of thick elastic fibres. Proteogly can extracellular matrix fills the interstices of this layer. Cellular elements resemble those found in papillary layer. With aging, the elastic fibres of the dermal region of the skin lose normal elasticity and cause the skin wrinkles.

10.4. Hypodermis

The hypodermis is a thick layer of subcutaneous connective tissue containing adipose tissue.

10.5. Skin appendages

The skin appendages are sweat glands, sebaceous glands, and hair follicles. All are derived from the epidermis, invade the deeper dermis and hypodermis during embryogenesis, where are located permanently.

10.5.1. Sweat glands

The sweat glands are distributed throughout the body. They are specialized for the production of sweat, which cools the human body by evaporation, and other complex secretions.

10.5.2. Apocrine sweat glands

In humans, apocrine sweat glands are restricted to the axilla, the area around the genitalia and the nipples of the mammary glands.

Cuboidal and collumnar secretory cells deep in these glands produce viscous secretion, which is expelled by stimulation of myoepithelial cells, merocrine mechanism of secretion is used. This product is odorless, but distinctive odor develops due to action of cutaneous bacteria. The ducts of apocrine sweat glands empty into canals of the hair follicles. The ceruminous glands of the external auditory canal and the glands of Moll in the eyelid are modified apocrine sweat glands.

10.5.3. Eccrine sweat glands

The eccrine sweat glands are distributed throughout the surface of the human body. They are coiled shaped simple tubular glands with coiled ducts. The secretory portion is surrounded by myoepithelial cells.

Glands contain dark cell producing a mucus-rich substance and clear cells producing a watery secretion. Merocrine mode of secretion is found also in eccrine type of sweat glands. When heat radiation from the body is inadequate, sweating begins. The body cools as the water in sweat evaporates.

10.5.4. Sebaceous glands

All sebaceous glands are associated with hair follicles and present on most body surfaces. They secrete an oily substance (sebum). The gland is connected to the shaft of a hair follicle by single short duct. Sebaceous glands are lobular with clusters of acini. Each acinus is composed of outer layer of basal cells and central group of cells filled with lipid droplets. These lipid-laden cells become part of secretion product (holocrine secretion). Sebaceous glands are partially under control of sex hormones and increase their activity after puberty.

10.5.5. Hair

The hairs are produced by deep epithelial ingrowths that form hair follicles. It is terminated as bulbous cores indented by a connective tissue papilla. This is richly supplied with nerve endings and small blood vessels. Bulbous portion contains numerous actively proliferating cells (germinal matrix), which produce the hair shaft and the internal root sheath. Scattered melanocytes are also found in germinal matrix.

Internal root sheath is made from soft keratin (continuation of cornified layer). It is composed of three layers.

External root sheath is a tubular invagination of the epidermis, which consists of basal, spinosum and granular layers. Outside of external root sheath is a thick basement membrane (known as the glassy membrane).

Hair shaft is composed of three layers of highly organized keratin:

- an inner medulla (central core, soft keratin)
- an outer cortex (surrounds the medulla, keratinized cells)
- cuticle (superficial layer, hard keratin).

10.5.6. Nail

Nails protect the dorsal surfaces of the distal parts of the fingers and toes. They are composed of keratinized epithelial cells forming plates of hard keratin. Nail plates rest on the epidermis (nail bed). The proximal end of the nail is called nail matrix or nail root. The nails grow up by proliferation and differentiation of nail matrix cells. The nail matrix is located beneath the proximal nail fold. The stratum corneum of the proximal nail fold forms the eponychium (cuticle). White crescent nail part at the proximal end is called lunula. Finger nails grow continuously at the rate of about 0.5 mm/week. Toe nails grow more slowly.

10.6. Wound healing (skin)

Introduction

Wound healing is the replacement of damaged and dead tissue by living one. The dead cells and all other tissue debris caused by injury must be removed. The phagocytic cells of the inflammatory reaction are responsible for cleaning process in this area. Following the inflammatory response, three mechanism complete wound healing: contraction, repair, and regeneration. In healing of skin wound, part of the defect is closed by wound contraction, part by granulation tissue, and part by regeneration of surface epithelial cells.

10.6.1. Wound contraction

Contraction is a result of the action of myofibroblasts, it is the mechanical reduction in the size of the wound defect. Wound contraction

is most prominent in the skin. The magnitude of this process depends on the size, shape and site of the wound. Myofibroblast appear in the wound area two or three days after injury. These cells share features intermediate between those of a fibroblast and a smooth muscle cells.

Myofibroblasts migrate into the wound and their active contraction decreases the size of the defect.

10.6.2. Repair

Repair is the replacement of dead tissue by granulation tissue which gradually mature to permanent scar. If only surface lining epithelium is affected, it is called as erosion. Erosions heal by regeneration, it is proliferation of surrounding epithelial cells which covers this superficial defect. If the injury extends to deeper part of the skin, to connective tissue of dermis, the fibroblasts are activated and proliferate. Active fibroblasts synthesize and secrete all extracellular matrix components (fibres and ground substance). Fibroblast proliferation is partially influenced by the presence of macrophages.

Conspicuous vascular proliferation starts 48 to 72 hours after injury. Endothelial cells in proximity to injury undergo mitotic division and form solid sprouts extending from preexisting vessels. Inside of new cell cytoplasm, the vacuoles are formed. The fusion of several vacuoles produces a lumen. The vascular sprouting anastomose with each other to form a new capillary network. Frequently, new capillaries protrude from the surface of the wound as minute red granules. Therefore it is named as granulation tissue.

Proliferation of fibroblasts and formation of new capillaries is most salient feature of wound healing in early phase. Inadequate vascularity causes poor fibroblast proliferation. Early wound healing is characterized by inflammatory cells, including macrophages, by some debris, and accumulation of active fibroblast and numerous capillaries.

The production of collagen by fibroblast starts within 24 hours of the injury. In early phase, type III collagen predominates. Later, by day 7 to 8, type I collagen is prominent, it also becomes the major collagen of mature scar tissue. Collagen rapidly turns over at the healing site. More types of cells, such as fibroblasts, epithelial cells,

neutrophils, and macrophages produce enzyme collagenase, which participates in degradation of collagen during wound healing. In early phases of healing, type III collagen is produced but it is removed by collagenase activity. This activity of collagenase remains elevated for more months. After initial stages of healing, capillaries are resorbed, cells are decreased in number, tensile strength is established, and collagen fibres and bundles are reoriented according to new lines of stress. This reorientation is achieved by removal of initially produced type III collagen and by deposition of new one, type I collagen. The collagen production and accumulation show a steady rate, despite of collagenase activity. The maximum of collagen accumulation is reached by 2-3 months after injury, but the tensile strength of the wound continues to increase more months. For example, the tensile strength of the dermis after one week healing is only about 10% that of normal skin. During the next 4 weeks, the tensile strength increases to about 80% of normal skin. The weakness in early phases of healing process is associated with the synthesis of type III collagen. When type III collagen is removed and replaced by type I collagen, tensile strength is improved.

By accumulation of type I collagen, many of the newly formed vessels are obliterated and absorbed. This devascularization (vascular involution) occurs in a few weeks. By this way, a richly vascularized maturing scar is transformed into more mature avascular and pale scar. Maturation (organization) of scar is characterized by devascularization decrease in collagenase activity and collagen degradation, and decrease in number of active fibroblast.

Mature scar consist of dense, almost avascular and parvicellular extracellular matrix with predominance of type I collagen.

10.6.3. Regeneration

By definition, the regeneration is the replacement of lost or destroyed tissue and cells by newly formed similar tissue and cells. It is accomplished by proliferation of the adjacent living cells. The best example to demonstrate regeneration is healing of skin.

As the consequence of wound, the epidermal cells at its edges lose contact with other epithelial cells and with their basement membrane.

This lost of contact trigger the migration of epithelial cells at the wound margins. Simultaneously, the cells of stratum basale adjacent to the migrating cells undergo mitoses. Final result of this coordinated migration and mitotic activity of epidermal cells is the gradual covering of epidermal defect. The epidermal cells not only divide, but they produce all basement membrane components and the continuity of this lamina is restored.

Clinical correlations

Some individuals are predisposed to excessive accumulation of collagen fibres and ground substance during wound healing. It caused by prolonged activity of fibroblast. Histologically, there are abundant and irregular collagen bundles, capillaries and above mentioned fibroblasts. Scar tissue does not undergo proper maturation. The result of this process is hypertrophic elevated immature scar known as a keloid.

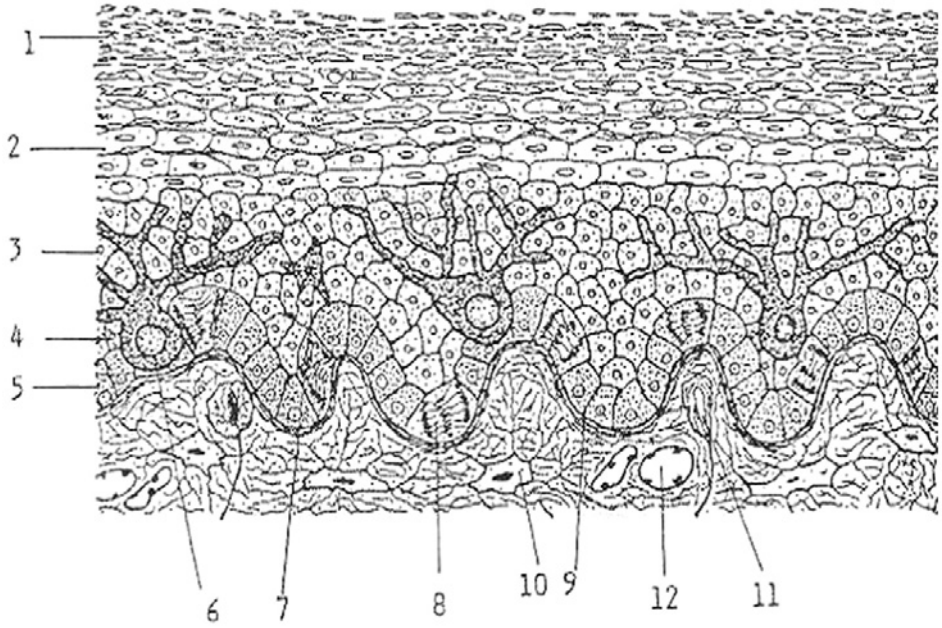

Fig. 1. Schematic diagram of thick skin

1-stratum corneum, 2-stratum lucidum, 3-stratum granulosum, 4-stratum spinosum, 5-stratum basale, 6-melanocyte, 7-Langerhans cell, 8-mitosis, 9-basement membrane, 10-fibroblast, 11-nerve ending, 12-capillaries

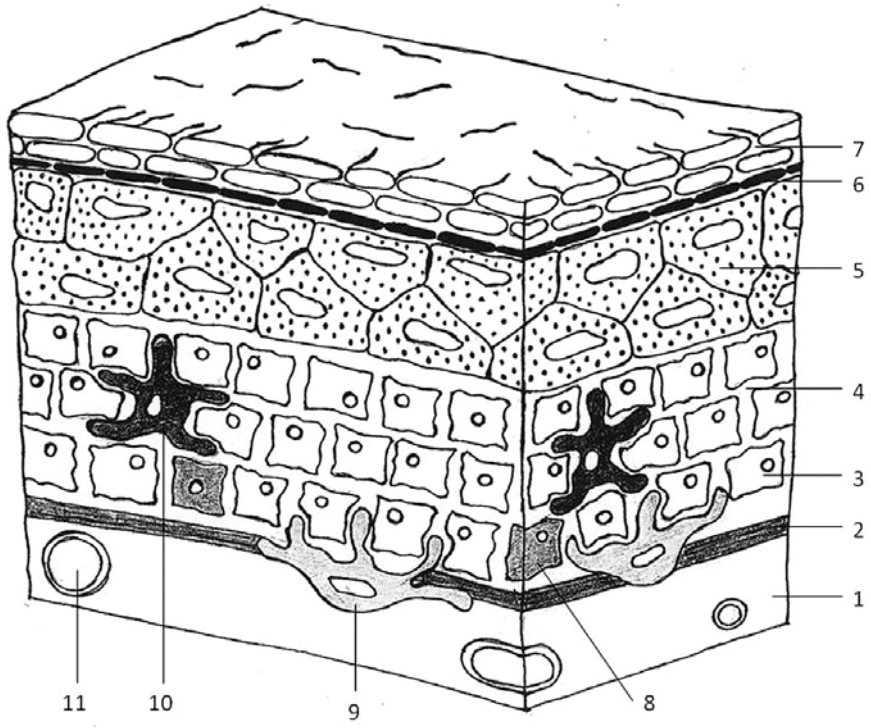

Fig. 2. Thick skin architecture

1-dermis, 2-basement membrane, 3-stratum basale, 4-stratum spinosum, 5-stratum granulosum, 6-stratum lucidum, 7-stratum corneum, 8-Merkel cell, 9-melanocyte, 10-Langerhans cell, 11-blood vessel

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11. Breast

Anatomy introduction

Female breasts (mammary glands) are secondary sexual features of females. Developmentally they are derived from sweat glands. Breast shape and size depend upon genetic, racial and dietary factors. It is a complex structure composed of adipose tissue surrounding epithelial secretory tissue. The breasts are situated on the anterior surface of the thorax and extend from the level of the 3rd through 7th rib. Just below the center of the breast lies the *nipple (mammary papilla)* where the milk ducts open. The papilla is surrounded by the *areola,* which is more darkly pigmented than the neighboring skin.

The breasts (mammary glands) are highly modified apocrine sweat glands. They develop embryologically along two lines, the milk lines, extending from the axillae to the groins. In humans, only one gland develops on each side of the thorax, however accessory breast tissue may be found rarely anywhere along the milk lines. The breasts of male and female follow a similar course of development until puberty. Then the female breasts develop under the influence of pituitary, ovarian and other hormones.

The breasts are compound tuboalveolar glands composed of 15-20 distinct lobes. Each lobe consists of a duct system with its own separate opening in the nipple, lobes are separated from each other by adipose tissue and collagenous connective septa.

Until the menopause, the breasts undergo cyclical changes in activity, which are controlled by the hormones of the ovarian cycle. After menopause, the breasts, like the other female reproductive tissues, undergo progressive atrophy and involution (mamma senilis).

Function:

Mammary glands produce and secrete milk providing the high-quality nourishment for newborn. Milk contains proteins, lipids, carbohydrates, immunoglobulins, minerals and electrolytes. Milk production is most essential function of mammary glands.

11.1. Nipple and areola

The nipple is round protuberance covered by stratified squamous epithelium. It consists of collagenous connective tissue, elastic fibres and large portion of smooth muscle cells. The contraction of these muscle cells causes erection of the nipple. The nipple is supplied by rich network of blood vessels and abundant sensitive nerve endings. Therefore it is the area of sexual arousal. On the surface of the nipple, there are 15-20 small openings of lactiferous ducts.

The nipple is surrounded by circular pigmented areola. This area enlarges and darkens during pregnancy. It contains large sebaceous glands, sweat glands and areolar glands of Montgomery.

11.2. Resting (nonsecreting, non-pregnant woman of reproductive age) mammary gland

The mammary lobes are arranged radially at different depths around the nipple forming clusters which look like bunches of grapes. Each lobe is drained by independent lactiferous duct which opens on the nipple surface. Just beneath opening, duct is dilated into lactiferous sinus, it serves for milk storage. The main lobe duct branches repeatidly to form numerous terminal ducts, each of them gives rise to cluster of blind-ending terminal ductules. Cluster of terminal ductules creates mammary lobule. One lobule and its terminal duct is called as terminal duct-lobular unit. The lobules are separated from each other by dense collagenous interlobular tissue. The intralobular connective tissue surrounds terminal duct and terminal ductules within each lobule. This connective tissue is less collagenous and rich in blood and lymph vessels.

Lactiferous duct and lactiferous sinus are covered by stratified cuboidal epithelium, whereas lactiferous duct near the opening on nipple surface is lined by stratified squamous keratinized epithelium. Smaller ducts are lined by simple columnar cells.

11.3. Mammary gland during pregnancy

Mammary gland structure changes in pregnancy. The production of estrogens and progesterone by corpus luteum and later by placenta

causes proliferation of terminal ductule epithelium to form greatly increased number of secretory acini (alveoli). Proliferation of terminally located mammary structures is also dependent on prolactin, human chorionic somatomammotropin (prolactin-like hormone produced by placenta), thyroid hormone and corticosteroids. The lining epithelium of acini can vary from low columnar to cuboidal, it is surrounded by a meshwork of myoepithelial cells resided on basement membrane. As pregnancy processes, the acini enlarge and begin to produce colostrums (protein – rich fluid). Within a few days after birth, secretion of colostrums is replaced by secretion of milk. Milk secretion is controlled by hormone prolactin, which is secreted by acidophils of the anterior pituitary gland.

11.4. Lactating mammary gland

After parturition, the levels of estrogene and progesterone decrease and prolactin starts to stimulate milk production in conjuction with other hormones. During lactation, acinar lining cells differentiate into secretory cells that secrete the components of milk. These secretory cells possess aboundant rough endoplasmic reticulum, well-developed Golgi apparatus and mitochondria, lipid droplets, and many vesicles containing proteins and lactose. Myoepithelial cells in acini and ducts contain numerous contractile microfilaments which contract to help express secreted milk from these structures. Contractions of myoepithelial cells are directed by oxytocin produced by posterior lobe of the pituitary. By this way, oxytocin iniciates the milk ejection reflex.

Clinical correlations

Carcinoma of the breast is common in women, almost all are adenocarcinomas. They are derived from the glandular epithelium of the terminal duct-lobular unit. Carcinomas occur most commonly in the upper outer quadrant of the breast.

The breast contain an extensive drainage system made up of many small blood and lymphatic vessels. Carcinoma cells may invade these vessels and spread away from their site of origin (metastases). Lymphatic spread to the axillary lymph nodes is most common. Spread by blood

stream gives rise to metastases in the lungs, bones, brain, ovaries, liver, and adrenal glands.

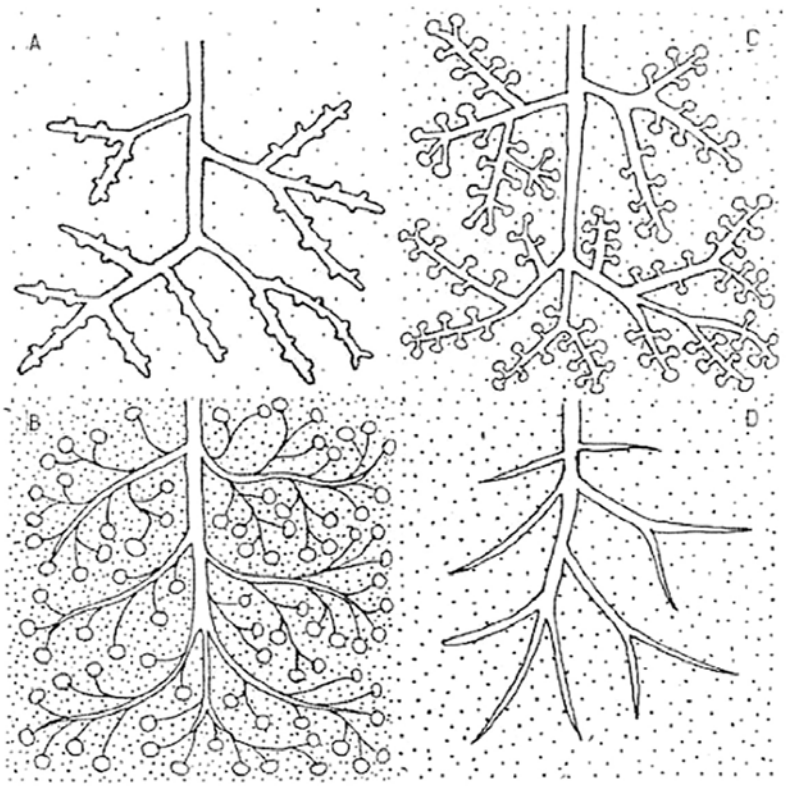

Fig. 1. Glandula mammae
A-mamma nonlactans, B-mamma graviditatis, C-mamma lactans, D-mamma senilis

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12. Endocrine system

Anatomy introduction

The endocrine system produces hormones into the blood, which transports them to their target tissues, where they are controlled by feedback mechanisms. The endocrine system is slow and less localized, through its effects is specific and often prolongated. It consists of the *endocrine glands*, *diffuse neuroendocrine system* and other *hormone-producing cells* which form components of other systems (e.g. pancreatic islets, certain renal cells, ovarian cells).

Within the endocrine glands we distinguish:

Hypophysis (pituitary gland) – reddish-gray, ovoid body. It belongs to hypothalamus and lies within the hypophyseal fossa of the sphenoid bone. The hypophysis consists of two parts *adenonohypophysis* and *neurohypophysis,* which differ in origin, function and structure.

- *Adenohypophysis* – synthesizes hormones (*somatotropin-STH, lactogenic hormone-LTH, adrenocorticopropin-ACTH, thyrotropin-TSH,* etc.).
- *Neurohypophysis* – here the hypothalamic hormones are stored (*vasopressin-ADH, oxytocin*).

Epiphysis (pineal gland) – small, ovoid structure, which belongs to epithalamus (diencephalon) and is localized between the superior colliculi of the midbrain. It produces the hormone *melatonin* and regulates the circadian rythms.

Thyroid gland – is located anteriorly in the neck, between the fifth cervical and the first thoracic vertebrae. It has *right* and *left lobes* connected by a narrow *isthmus.* Thyroid gland produces *the thyroxine.*

Parathyroid glands – are small glands, four in number. They are localized posteriorly to the thyroid gland. Parathyroid glands synthetize *parathormone.*

Suprarenal (adrenal) glands – two small bodies, are situated on the top of kidney (superior renal pole). Suprarenal gland consists of *cortex* and *medulla.* The cortex produces the *corticoids* and the medulla synthesizes *adrenaline* and *noradrenaline.*

All glands have rich arterial blood supply.

The endocrine system is made up of glands that produce and secrete hormones, chemical substances produced in the body that regulate the activity of cells or organs. The hormones are released into the bloodstream

and may affect one or several organs throughout the body. Blood circulation allows hormones to act on target cells or target organs that may be at a distance from the site of their secretion. General properties of endocrine glands are their ductless nature, their vascularity, and usually the presence of intracellular vacuoles or granules storing their hormones. Hormones are carried to their final destination via connective tissue spaces and vascular system. Cells of the endocrine system release over 100 hormones that are chemically divided into three classes: steroids, proteins and amino acid analogues and derivates.

There are exist three types of secretion of hormones:

1. paracrine secretion – cell producing hormones may act a short distance on target cell,
2. juxtacrine secretion – interaction between molecule of hormone secreted by one cell and receptor of adjacent cell,
3. autocrine secretion – cell secretes molecules that act on themselves or on the same type of cell.

The endocrine system acts with nervous system to coordinate the body's activities. However, the endocrine system produces a slower and more prolonged response than nervous system, both systems act simultaneously on the same target cells and some nerve cells secrete hormones. Endocrine system is represented by following organs:

- Pituitary gland (hypophysis)
- Pineal gland (epiphysis)
- Thyroid gland
- Parathyroid gland
- Islets of Langerhans
- Adrenal glands (suprarenal glands)

Many organs specialized for other function, such as the heart, kidney, gastrointestinal tract and reproductive organs contain endocrine cells which have secondary endocrine functions.

12.1. Pituitary gland (Hypophysis cerebri)

Pituitary gland is located in the sella turcica of the sphenoid bone that is covered by diaphragma sellae (dural fold). Pituitary is connected to the brain by the infundibulum.

Hypophysis is partitioned into two parts: *adenohypophysis* (anterior pituitary), secreting most of the hormones and *neurohypophysis* (posterior pituitary), which stores and releases hormones from the hypothalamus.

These parts have different embryological origin. The anterior lobe derives from the ectoderm of the oropharynx forming the hypophyseal pouch (Rathke‘s pouch). The posterior lobe has neuroectodermal origin. It is derived from the floor of the third ventricle of brain. Both parts are joined and encapsulated into a single gland.

All hormones of adenohyphophysis regulate other endocrinne glands and are responsible for regulating, reproduction, growth and metabolism. The pituitary gland is under the control of the hormons from the hypothalamus. These hormons stimulate or inhibit the cells of the hypophysis.

12.1.1. Blood supply of pituitary gland

The blood supply of hypophysis is derived from *superior and inferior hypophyseal arteries.*

Both arteries arise from the internal carotid arteries.The superior and the inferior hypophyseal arteries are connected by the trabecular artery.

The superior hypophyseal artery form *a primary capillary plexus* –capillary network with fenestrated endothelial cells in the median eminence and infundibular stem. Primary capillary plexus receives the hormons of the neurosecretory cells in the nuclei of the hypothalamus. Primary capillary plexus drain into *hypophyseal portal veins,* which run along the pars tuberalis and give rise to *the secondary capillary plexus* in the adenohypophysis (anterior hypophysis). The primary and secondary capillary plexuses linked by the portal veins form hypothalamohypophyseal portal system.

The hypothalamohypophyseal portal system play role in regulation of hypophyseal functions and enables:

a) The integration of the hypothalamus with the adenohypophysis.
b) The transport of hypothalamic hormones from the primary capillary plexus to the hormon-secreting cells of the adenohypophysis.
c) The transport of hormones from adenohypophysis into secondary capillary plexus to the blood circulation.

The inferior hypophyseal arteries supply the posterior lobe and form third capillary plexus.

The anterior lobe of the pituitary gland is supply by postganglionic fibers of the autonomic nervous system.

12.1.2. Microscopic structure of the hypophysis

The hypophysis comprising of two glands: the adenohypophysis and the neurohypophysis with different functions.

12.1.2.1. Adenohypophysis

It has three following parts*: pars distalis, pars tuberalis, pars intermedia*

The pars distalis is the largest part consisting of specialized hormon-secreting cells that release several hormones. This part accounts 75 % of the mass of adenohypophysis.

The hormon-secreting cells are organized in clumps and cords separated by sinusoids (fenestrated capillaries) with large diameter, which form the secondary capillary plexus. The release of adenohypophyseal hormones into the fenestrated capillaries occurs by exocytosis. Vesicles containing hormone empty into the perivascular spaces near capillaries and secretory material enters the blood circulation by diffusion. All components are supported by connnective tissue stroma.

Parenchymal cells of the pars distalis have an affinity for histologic dyes and are divided into two groups: *chromophobes and chromophils.*

Chromophobes are smaller, poorly staining cells. They may be recognized in clusters throughout the adenohyphophysis. Chromophobes do not produce hormones.

Chromophils are subdivided into *acidophils (*staining with acid dyes) and *basophils* (staining with basic dyes)

The most abundant cells in the pars distalis are acidophils.

Acidophils (granules stain pink to red with eosin) are subdivided into:

a) *somatotrophs* (somatotropic cells) - constitute 50% of cell population and have oval shape with round, centrally located nuclei.
Somatotrophs produce somatotopin or growth hormone (STH or GH). The action of somatotropin is indirect. This hormone act on the liver and kidney and production of somatomedins act on epiphyseal plates

in long bones. A stimulatory effect is caused by somatotropin-releasing hormone and an inhibitory effect is produced by somatostatin.

b) *mammotrophs* (mammotropic cells) - constitute 15 to 20 % of the parenchymal cell and are large, polygonal cells with oval nuclei. The hypertrophy and hyperplasia of these cells occur during pregnancy and lactation. Mammotrophs produce prolactin (PRL). This hormone initiates milk formation, stimulates secretion of casein, lactalbumin, lipids into the milk. A stimulatory effect of the prolactin is exerted by prolactin-releasing hormone (PRH) and thyrotropin-releasing hormone (TRH). The main inhibitor is dopamine.

Basophils are subdivided into three groups : *one group* of basophils *(thyreotrophs)*, produces the thyroid-stimulating hormone (TSH), *another cells (gonadotrophs)* produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and *third group* (*corticotrophs)* produces corticotropin hormone (ACTH) and lipotropic hormone (LPH)

a) *Thyreotrophs (thyrotropic cells)* constitute 5 % of the cell population in the adenohypophysis. These cells are large, polygonal cells with eccentric round nuclei. Thyroid-stimulating hormone (TSH) stimulates the follicular cells of the thyroid gland to production of thyroglobulin and hormones of thyroid gland. The production of TSH is inhibited by increased concentrations of the thyroid hormones.

b) *Gonadotrophs (gonadotropic cells)* constitute 10 % of the parenchymal cells in the adenohypophysis. These cells are small, oval with round, eccentric nuclei. These cells produce two hormones: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH in the female stimulates follicular development in the ovary. In the male, FSH stimulates spermatogenesis in the testis. The release of both hormones is regulated by gonadotropin-releasing hormone (GNRH) from the hypothalamus. LH in the female regulates the maturation of ovarian folicle, ovulation and the formation of the corpus luteum. In the male LH act on the Leydig cells of the testis.

c) *Corticotrophs (corticotropic cells)* also constitute 15-20 % of total cells population in the adenohypophysis**.** These cells are polygonal, medium-sized cells with round, eccentric nuclei that produce adrenocorticotropin hormone (ACTH). This hormone controls the

function of the zona fasciculata and the zona reticularis in adrenal cortex. ACTH stimulates the production of cortisol and androgens. ACTH release is regulated by corticotropin-releasing hormone (CRH) produced by the hypothalamus.

Hormones produced in the hypothalamus
The hormon-secreting cells of the pars distalis are controlled by *hypothalamic releasing and inhibiting hormones***,** manufactured in the hypothalamus. These hormones are carried along axons and in the median eminence are stored and enter the primary capillary plexus which are drained by the hypophyseal portal veins.

The hormones liberated in the median eminence are:
a) *thyrotropin-releasing hormone* (*TRH)*, stimulates the release of thyrotropin and prolactin
b) *corticotropin-releasing hormone (CRH),* stimulates the release of B lipotropin and corticotropin
c) *somatotropin-releasing hormone (SRH) (growth hormone-releasing hormone GHRH)* stimulates the release of somatotropin
d) *gonadotropin-releasing hormone (GnRH),* stimulates the release of luteinizing hormone
e) *prolactin-releasing hormone (PRH),* stimulates the release of prolactin
f) *prolactin-inhibiting hormone (PIH),* inhibits the release of prolactin
g) *somatostatin* inhibits release of growth hormone and thyrotropin
The hormon-secreting cells also are controlled by the direct effect of hormones and by nerve impulses.

The pars tuberalis is a region surrouding the infundibulum. This part is highly vascularized region and contains gonadotropic cells, which are arranged in clusters or cords. These cells secrete follicle-stimulating hormone and luteinizing hormone.

The pars intermedia is rudimentary region containing follicles filled with colloid (remnant of the Rathke‘ pouch). This part constitutes about 3 % of the adenohypophysis and contains small groups of chromophobes and basophils. Basophils secrete α- melanocyte-stimulating hormone (α-MSH). Hormon increases melanocyty activity.

Negative feedback conrol

Another control of the adenohypophysis is negative feedback. Inhibitory control of the rate of secretion of thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH) is affected by secretions of the target organs.

12.1.2.2. Neurohypophysis

It consists of:

a) The pars nervosa (neural lobe).
b) The infundibulum, which is formed by the median eminence and the infundibular process.

The infundibulum connects it with hypothalamus.

Neurohypophysis does not contain hormon-secreting cells. It is a storage site for hormones of the neurons of the supraoptic and paraventricular nuclei of the hypothalamus.

Histological structure of the neurohypophysis

The neurohypophysis contains:

a) The glial cells known as pituicytes-branched cells, occupy 25 % of volume. Their nuclei are round or oval. Pituicytes have processes that surround the axons of the neurosecretory cells. The processes terminate in the perivascular space and serve a supporting role to that of astrocytes in CNS.
b) The unmyelinated axons with nerve endings.
   These unmyelinated axons arising from the neuroendocrine cells located in the supraoptic and paraventricular nuclei form the hypothalamohypophyseal tract.
c) Fenestrated capillaries derived from the inferior hypophyseal artery.

12.1.3. Hypothalamohypophyseal tract

The cell bodies of neurosecretory neurones located in the hypothalamus in the supraoptic and paraventricular nuclei secrete the neurosecretory material consisting from two hormones: antidiuretic hormon (ADH) or vasopresin and oxytocin. Each hormone is a small peptide of nine amino acid residues. These hormones form neurosecretory granules that aggregate to form Herring bodies. Herring bodies are visible in the light microscope

by specific technique such as chrome-hematoxylin. Neurosecretory granules are present in the terminal endings of the axons of the pars nervosa. Fenestrated capillaries are present to the nerve endings.

Each hormone is joined to a binding protein called neurophysin. ADH has neurophysin I and oxytocin has neurophysin II. The hormone-neurophysin komplex is transported to the neurohypophysis where it is stored. When the hormone is released into the blood circulation, a specific binding protein - the neurophysin complex is broken, and hormon is carried to target organs. Hormones must pass through two basal laminae and hormones are released by exocytosis from axon. Hormones stored in Herring bodies are released as needed by the organism.

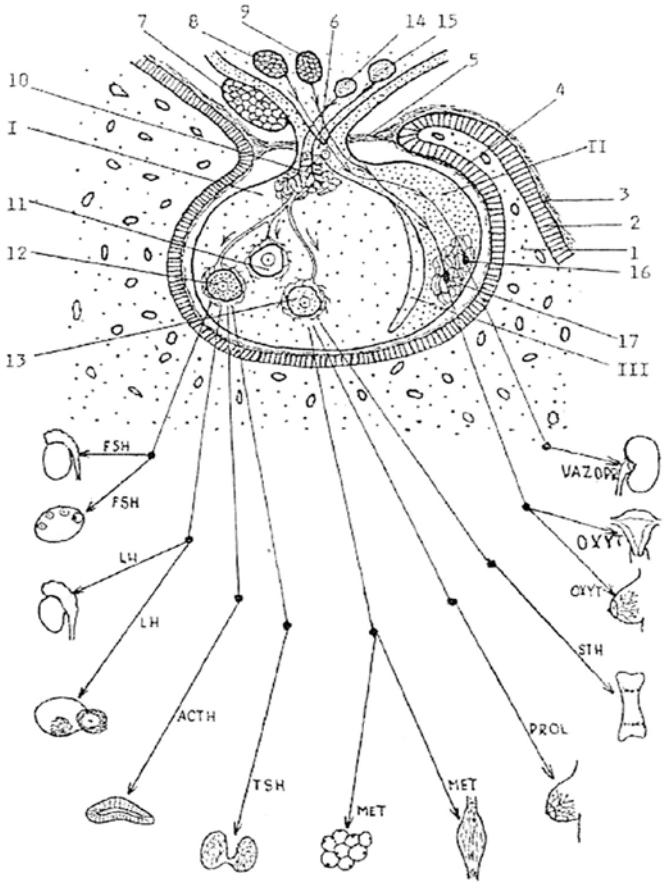

Fig. 1. Structure and effect of various hormones of the hypophysis
I-the pars distalis, II-the pars nervosa, III-the pars intermedia

1-the sphenoid bone, 2-the compact bone, 3-the periostium 4-the sella turcica, 5-the diafragma sellae, 6-the infundibulum, 7-the chiasma optic nerve, 8-the supraoptic nucleus, 9-the paraventricular nucleus, 10-the median eminence, 11-chromophobes, 12-basophils, 13-acidophils, 14-the ventrolateral nucleus, 15-the dorsomedial nucleus, 16-the neurosecretory granule, 17-the neurosecretory granule with vasopresin and oxytocin

Actions of the hormones of the neurophypophysis

The effect of *vasopresin* is to increase the permeability to water in the distal convoluted tubule and collecting ducts of the kidney. This hormone also includes the contraction of the smooth muscle of small arteries and arterioles and elevates blood pressure.

O*xytocin* stimulates contraction of uterine smooth muscle during menstruation and parturition. It also stimulates the myoepithelial cells of the alveoli and lactiferous ducts in the mammary glands during nursing to eject milk.

Clinical correlations

Adenomas are tumors of the hypophysis-usually benign although may compress neighboring structures (for example the optic nerves). Adenomas can produce growth hormone, prolactin, adrenocorticotropin, thyroid- stimulating hormone.

Hypersecretion of somatotropin in tumors that originate in the somatotrops produces *gigantism* in children because the epiphyseal plates are active. If excessive somatotropin secretion occurs in adult, when the epiphyseal plates are inactive, *acromegaly* develops. A decrease in the secretion of growth hormone during childhood results in hypopituitary *dwarfism.*

The hypothyroidism characterized by reduced metabolism, temperature, mental rethardy occurs during a deficity in secretion of thyroid stimulating hormone-TSH (congenital hypoplasia of the hypophysis).

In the absence of production of antidiuretic hormone is produced *diabetes insipidus* (produce up 20 liters of urine per day-polyuria).

12.2. Pineal gland

Pineal gland (epiphysis cerebri, pineal body) is reddish-gray flattened, cone-shaped organ about the size of a grain of rice (5–8 mm in length

and 3–5 mm at width) develops from neuroectoderm on the roof of the diencephalon, situated in the posterior of the third ventricle. The pineal gland regulates the daily rhytms of bodily activities.
The pineal body consists of a lobular parenchyma of several types of cells. The gland's surface is covered by a pia mater sends connective tissue septa into the pineal body, subdividing it into lobules. The septa containing blood vessels and unmyelinated nerve fibers penetrate the pineal tissue. Cell population is represented mainly by pinealocytes (about 95% of the cells). The pinealocytes have a slightly basophilic cytoplasm with large, light and round nuclei with prominent nucleoli. They produce and secrete hormone melatonin stimulated by darkness and inhibited by light. The astrocytes of the pineal gland are a specific type of cells characterized by dark stained elongated nuclei and long cytoplasmic processes.
Characteristic property of the pineal gland is a presence of brain sand or corpus arenaceum (acervulus cerebri) small calcium-containing concretions in the pineal parenchyma, which increase in size and number with age, good midline-marker in radiology and computer-assisted tomography.

12.3. Thyroid gland

Thyroid gland (thyroid) is one of the largest endocrine glands in the body. It is butterfly-shaped organ and is composed of two cone-like lobes connected via the isthmus. The organ is located on the anterior side of the neck, lying against and around the larynx and trachea. It originates from the foregut endoderm. The gland is cover by a loose connective tissue capsule that sends septa into the parenchyma divided it into lobules. Septa carries rich network of blood vessels, and lymphatic vessels and nerves as well.

The thyroid gland is the only endocrine gland which store a large amount of secretory product. Thyroid gland synthetises thyroid hormones, principally triiodothyronine (T3) and thyroxine or tetraiodothyronine (T4). These hormones regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. T3 and T4 are synthesized utilizing both iodine as well as tyrosine. The thyroid gland also produces a hormone calcitonin, which plays a role in calcium homeostasis.

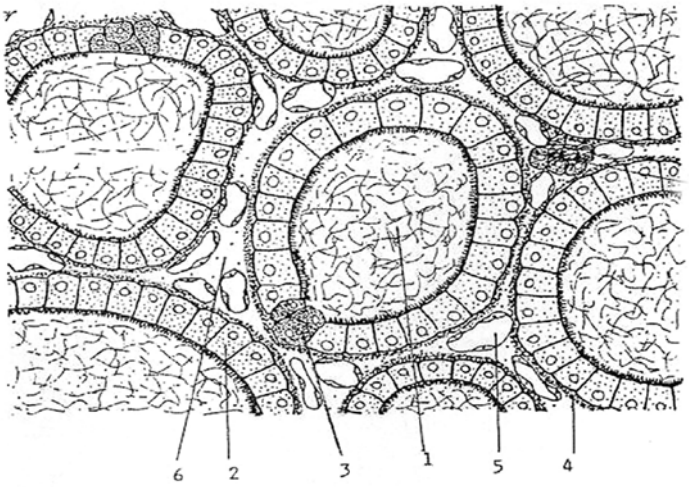

Fig. 2. Scheme of thyroid gland
1-follicles filled with colloid, 2-follicular cells, 3-parafollicular cells, 4-plasma membrane, 5-capillary, 6-stroma

The thyroid is composed of spherical follicles with the core being occupied by gelatinous substance – colloid. Colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin. The follicles are line by a single epithelial layer of follicular (principal) cells, which secrete T3 and T4. When the gland is not secreting T3/T4 (inactive), the epithelial cells are squamous. When active, the epithelial cells become cuboidal to low columnar cells. Parafollicular cells (C – cells) are located among follicular cells and in spaces between the spherical follicles. These cells are larger and less stained like follicular cells and secretes hormone calcitonin.
Thyroid gland is under control of thyroid stimulating hormone (TSH) from the pars distalis of the adenohypophysis, which stimulate release of thyroid hormones. Thyroid hormones inhibit the synthesis of the TSH maintaining the adequate quantity of T3 and T4 in organism.

12.3.1. Synthesis of thyroid hormones

The triiodothyronine (T3) and thyroxine (T4) is synthesized in the thyroid gland follicular cells as follows:

1. Thyroglobulin is synthesized in the ER of thyroid epithelial cells and secreted into the lumen of the follicle – colloid, which serves as essential pool.
2. Iodine, or more accurately iodide (I^-), is taken up from blood by thyroid epithelial cells, which have on their outer plasma membrane a transport protein sodium-iodide symporter (NaI) or "iodine trap". Once inside the cell, iodide is transported into the lumen of the follicle along with thyroglobulin.
3. Iodide is activated (oxidized) by enzyme thyroid peroxidase, an integral membrane protein present in the apical plasma membrane of thyroid epithelial cells.
4. The thyroid peroxidase iodinates the tyrosyl residues of the thyroglobulin within the colloid ("organification of iodide") resulting in formation of monoiodotyrosine and diiodotyrosine. Their coupling produces the hormones T3 and T4.

Thyroid-stimulating hormone (TSH) released from the pituitary gland binds the TSH receptor on the basolateral membrane of the cell and stimulates the endocytosis of the colloid. Endocytosed vesicles are then digested by lysosomal enzymes.
The lysosomal enzymes cleave the T4 from the iodinated thyroglobulin. These vesicles are then exocytosed, releasing the thyroid hormones.

Clinical correlations

Hyperthyroidism – overactive tissue within the thyroid gland, resulting in overproduction and an excess of circulating free thyroid hormones: thyroxine (T4), triiodothyronine (T3), or both. The most common cause of hyperthyroidism is a disease called „Graves' disease", which is considered to be an autoimmune disease and results from excess stimulation of the thyroid gland and usually presents with symptoms such as an enlarged thyroid (goitre), protruding eyes (exopthalmos), palpitations, excess sweating, diarrhea, weight loss, muscle weakness and unusual sensitivity to heat.
Hypothyroidism – insufficient production of thyroid hormone by the thyroid gland. Hypothyroid disorders occur when the thyroid gland is inactive or underactive as a result of improper formation from birth, or the removal in whole or the removal in part of the thyroid gland. Symptoms include

abnormal weight gain, tiredness, baldness, temperature intolerance (both heat and cold), and palpitation.

12.4. Parathyroid glands

Parathyroid glands are four small oval bodies about the size of a grain of rice, located at the posterior surface of the thyroid gland and derived from pharyngeal pouches. The major function of the parathyroid glands is to maintain the body's calcium level within a very narrow range, so that the nervous and muscular systems can function properly.

Each parathyroid gland is surrounded by a thin connective tissue capsule send septa into a gland. The parenchyma is composed of two cell types. The most numerous type, the chief cells are small polygonal cells with light nucleus and weekly stained acidophilic cytoplasm. The chief cells synthetise parathyroid hormone (PTH, parathormone), pivotal for normal calcium concentrations in the fluids and tissues of the body. Less frequent oxyphil cells are larger and their cytoplasm is strongly acidophilic, the nucleus is small and uniformly intense basophilic. With increasing age, secretory cells are replaced with adipocytes.

Clinical correlations

Hyperparathyroidism – overactivity of one or more of the parathyroid lobes, which make too much parathyroid hormone causing a potentially serious calcium imbalance and leads to hypercalcemia, kidney stones, osteoporosis, and various other symptoms.

Hypoparathyroidism – is decreased function of the parathyroid glands, leading to decreased levels of parathyroid hormone. The consequence, hypocalcaemia, is a serious medical condition.

12.5. Islets of Langerhans

Islets of Langerhans or pancreatic islets are a small part (about 1 to 2%) of the mass of the pancreas, and are distributed throughout the pancreas. Pancreatic islets originated from the endoderm, i.e., they have the same embryonal origin as the acinar exocrine tissue. Normal human pancreas contains about one million of these islets. They appear as

irregularly shaped or rounded clusters of endocrine tissue located within the exocrine pancreatic tissue. Each islet consists of lightly stained cords of polygonal or rounded cells, which form an irregular network and is extensively vascularized. A very fine capsule of delicate reticular fibers surrounds each islet, to separate it from the exocrine pancreatic tissue.

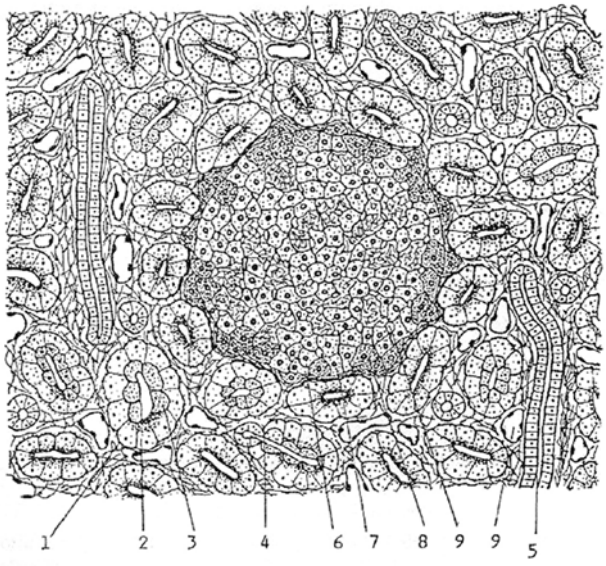

Fig. 3. Structure of islet of Langerhans in pancreas
1-pancreatic acinus, 2-acinar cell, 3-centroacinar cells, 4-intercalated duct, 5-intralobular duct, 6-plasma membrane, 7-A–cell, 8-B–cell, 9-capillary

Hormones produced in the islets of Langerhans are secreted directly into the blood flow by (at least) five different types of cells:

1. Alpha cells (α–cells, A–cells) producing hormone glucagon (15–20% of total islet cells) are usually located at the periphery of islets.
2. Beta (β–cells, B–cells) cells producing insulin (65–80%) are located centrally in islets.
3. Delta cells (δ–cells, D–cells) producing somatostatin (3–10%) are scattered and less numerous.
4. PP cells (F–cells) producing pancreatic polypeptide (3–5%) occurs rarely.
5. Epsilon cells (ε– cells) producing ghrelin (<1%)

Insulin and glucagon work synergistically to keep blood glucose concentrations normal. An elevated blood glucose concentration results

in the secretion of insulin-producing beta cells and glucose is transported into cells. The uptake of glucose by liver, kidney and brain cells is by diffusion and does not require insulin. The glucagon-producing alpha cells remain quiet, and hold on to their hormone. A fall in blood glucose leads to a pronounced decrease in insulin secretion. The alpha cells become active and deliver glucagon to the blood.

Clinical correlations

Diabetes mellitus or simply known as diabetes is a group of metabolic diseases characterized by high blood glucose levels, that result from defects in insulin secretion, or action, or both. There are three main types of diabetes:

a) type 1 (insulin-dependent or juvenile diabetes) – autoimmune disease that results in partial or total destruction of insulin-producing beta cells, leading to insulin deficiency,
b) type 2 (insulin-independent or adult diabetes) – is the most common type characterized by insulin resistance which may be combined with relatively insulin deficiency, occurs at later stage in life and is frequently associated with obesity,
c) gestational diabetes – is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy, resembles type 2 diabetes in several aspects and may improve or disappear after delivery.

12.6. Adrenal glands

The adrenal (suprarenal) glands are orange-colored paired endocrine glands which are located on the top of both kidneys, embedded in connective tissue capsule and surrounded by an adipose tissue. The adrenal glands are triangular or half-moon shaped and measure about 4–6 cm in length and 1–2 cm in height. Each adrenal gland is composed of two distinct structures, outer adrenal cortex and inner medulla. Both of tissues are very richly vascularized. The adrenal cortex is devoted to the synthesis of corticosteroid hormones, medulla secretes catecholamines.

Despite their organization into a single gland, the medulla and cortex are morphologically and functionally different endocrine organs, and

have different embryological origins. The medulla derives from ectoderm (neural crest), while the cortex develops from mesoderm.

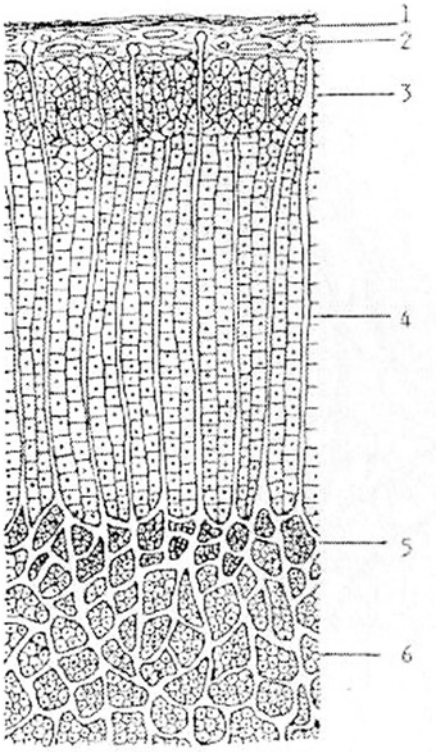

Fig. 4. Schematic view of the adrenal gland
1-adrenal capsula 2-subcapsular artery 3-zona glomerulosa, 4-zona fasciculata, 5-zona reticularis, 6-adrenal medulla

The adrenal cortex is partitioned into three concentric layers of steroid-synthesizing cells: zona glomerulosa (about 15% of the cortex), zona fasciculata (about 65-80% of the cortex) and zona reticularis (about 10% of the cortex). Although the boundaries between these zones are somewhat indistinct, each has a characteristic arrangement of cells. The outermost layer is the zona glomerulosa. Cells within this zone appear columnar or pyramidal in shape arranged in irregular arched or rounded cords. The zona fasiculata is the middle and is the largest of the three zones in the cortex. Cells in the fasiculata are polyhedral and usually have a foamy, spongy cytoplasm due to abundant lipid droplets, therefore

are called spongyocytes. They are arranged in distinctively straight cords that radiate toward the medulla. Cortical capillaries are usually prominent within the fasiculata. The innermost zone of the cortex is the zona reticularis. Cells within this zone are arranged in cords that project in many different directions and anastomose with one another. Their cytoplasm contains numerous lipofuscin pigment granules.

Medullary parenchymal cells in the adrenal medulla are known as chromaffin cells. They are modified sympathetic postganglionic neurons, which lost their processes during embryonal development. Less numerous are parasympathetic ganglion cells. The cells are arranged in clusters, usually around medullary veins. They are seen to have a granular cytoplasm due to hormone-containing secretory granules contain mostly the catecholamines epinephrine and norepinephrine.

Table 1. Hormones of the adrenal glands

region	zones	hormone	effect	regulating hormone
adrenal cortex	zona glomerulosa	mineralocorticoids (aldosterone)	acts on the kidney promoting the reabsorption of sodium ions (Na+) and water into the blood helps maintain normal blood pressure	renin angiotensin system
	zona fasciculata	glucocorticoids (cortisol, corticosterone)	raising the level of blood glucose by stimulating gluconeogenesis anti-inflammatory effect on the body	adrenocorticotropin (ACTH)
	zona reticularis	androgens (dehydroepiandrosterone - DHEA), glucocorticoids (cortisol)	stimulates or controls the development and maintenance of male characteristics like accessory male sex organs and development of male secondary sex characteristics	adrenocorticotropin (ACTH)
adrenal medulla		catecholamines (adrenaline or epinephrin, noradrenaline or norepinephrin)	prepare the body for physical activity (fight-or-flight response) -increase in the rate and strength of the heartbeat resulting in increased blood pressureblood shunted from the skin and viscera to the skeletal muscles, coronary arteries, liver, and brain; rise in blood sugar; increased metabolic rate; bronchi dilate; pupils dilate; hair stands on end („gooseflesh“ in humans); clotting time of the blood is reduced; increased ACTH secretion from the anterior lobe of the pituitary	sympathetic nervous system

12.6.1. Blood supply

The adrenal glands have a rich blood supply. Each gland is supplied by the superior, middle and inferior suprarenal arteries, which arise

from the inferior phrenic artery, abdominal aorta and renal artery, respectively. The branches of these arteries forming two groups: the cortical arterioles and the medullary arterioles. The cortical arterioles supply the capsule and forming sinusoidal cappilaries, that irrigate the cortex, and the medullary arterioles, which pass through the cortex and form a cappilary network in the medulla. The venous blood originating from sinusoidal capillaries in the cortex and together with medullary capillaries form the central medullary vein. The medullary veins emerge from the hilum of each gland before forming the right and left suprarenal veins, which join the inferior vena cava on the right side and the left renal vein on the left side.

Clinical correlations

Overexpression of products of the adrenal cortex may lead to several disorders. Hyperaldosteronism – is a medical condition where too much aldosterone is produced by the adrenal glands, which can lead to lowered levels of potassium in the blood. Primary hyperaldosteronism are conditions in which the adrenal gland releases too much of the hormone aldosterone. Most cases of primary hyperaldosteronism are caused by a noncancerous tumor of the adrenal gland. In secondary hyperaldosteronism, the excess aldosterone is caused by something outside the adrenal gland that mimics the primary condition.

Cushing's syndrome – is the result of the overproduction of corticosteroids by the adrenal glands. Disease may arise from excessive cortisol levels in the blood which may be the result of taking glucocorticoid drugs, or by tumors of the pituitary glands, adrenal glands that produce cortisol or ACTH or from tumors or cancer arising elsewhere in the body.

Primary adrenal insufficiency, also called Addison's disease, occurs when the adrenal glands are damaged and cannot produce enough of the hormone cortisol and often the hormone aldosterone. This can be caused by a disorder of the adrenal glands, autoimmune disorder. The disorder causes the body's immune system to gradually destroy the adrenal cortex. Addison's disease affects one to four of every 100,000 people, in all age groups and both sexes. Secondary adrenal insufficiency occurs when the pituitary gland fails to produce enough ACTH, a hormone that stimulates the adrenal glands to produce cortisol.

12.7. Endocrine cells in gastrointestinal tract

The gastrointestinal tract is the largest endocrine organ in the body and the endocrine cells within it are referred to collectively as the enteric endocrine system. The enteric hormones are represents mostly by:

- Gastrin – secreted by G-cells from the pylorus and duodenum of stomach plays an important role in control of gastric acid secretion.
- Somatostatin – secreted by D-cells from the pylorus of stomach are essential for inhibition of gastric acid secretion.
- Cholecystokinin – small intestinal hormone secreted by I–cells stimulates secretion of pancreatic enzymes and bile.
- Secretin – another hormone secreted from small intestinal epithelial cells (S–cells); stimulates secretion of bicarbonate rich fluids from the pancreas and liver.
- Serotonin – secreted by enterochromaffin (EC) cells located in epithelium of stomach, small and large intestinum are responsible for increased gut motility.
- Histamin – secreted by enterochromaffin-like cells (ECL) found in the fundus of the stomach stimulate the production of gastric acid.

12.8. Testes

The respective functions of the testes are to produce sperm (spermatogenesis) and to produce androgens. Leydig cells produce and secrete testosterone and other androgens important for sexual development, puberty and secondary sexual characteristics.

12.9. Ovaries

Ovaries secrete hormones estrogen and progesterone. Estrogen is responsible for the appearance of secondary sex characteristics of females at puberty and for the maturation and maintenance of the reproductive organs in their mature functional state. Progesterone functions with estrogen by promoting menstrual cycle cyclic changes in the endometrium.

12.10. Kidneys

The human kidney is also an endocrine gland secreting hormones erytropoietin, calcitriol and enzyme renin. Erythropoietin is released in response to hypoxia (low levels of oxygen at tissue level) in the renal circulation. Calcitriol the activated form of vitamin D promotes intestinal absorption of calcium and the renal reabsorption of phosphate. Renin as a part of renin-angiotensin-aldosterone system is involved in the regulation of aldosterone levels.

12.11. Placenta

Hormones produced by the placenta include human chorionic gonadotropin (hCG), human placental lactogen (hPL) as well as steroids (estrogens; progesterone). Hormone hCG produced by fetal trophoblast has occurred through the first three months of pregnancy and binds to the corpus luteum to prevents luteal regression. Placental lactogen has been used as an indicator of fetal wellbeing and growth and steroids stimulate uterine growth and endometrial lining of the uterus during pregnancy.

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13. Respiratory system

Anatomy introduction

The respiratory system can be divided into upper and lower divisions on the basis of function and embryological development. The upper consists of the *nose* with *nasal cavities, paranasal sinuses, nasal* and *oral part of pharynx*. The lower respiratory tract includes the *larynx, trachea, bronchi* and the *lungs* (right and left).

Nose – the external nose has the *root*, *dorsum*, *apex* and two *alae nasi*. Alae nasi open inferiorly by two ellipsoidal apertures – the *external nares (nostrils).* The framework of external nose is formed by bones of skull and by cartilages.

Nasal cavities – are separated from each other by *nasal septum.* Each nasal cavity opens by posterior nasal aperture, *choana* to nasopharynx. The lateral walls of nasal cavities are enlarged by 3 elevations, *nasal conchae*. Conchae are thin bones covered by a mucous membrane. There is a nasal passage, *meatus,* beneath each concha.

Paranasal sinuses – include bilaterally paired sinuses inside the frontal bone – *frontal sinus,* the sphenoid bone – *sphenoidal sinus,* the maxilla – *maxillary sinus* (the biggest one) and ethmoid bone – *ethmoidal sinus (ethmoidal air cells).* All sinuses communicate with the nasal cavity and open into its lateral walls under the conchae.

Pharynx – has 3 parts: *nasal part (nasopharynx)* (extension:base of skull - C2) – communicates with the nasal cavities through choanae, *oral part* (C2 - C4) - opens to the oral cavity and *laryngeal part* (C4 - C6) which communicates with laryngeal cavity.

Larynx – it is an air passage and an organ of phonation. It extends from the tongue to the trachea. Cartilages form the skeletal framework of the larynx. The primary source of phonation are *vocal folds* situated inside the *laryngeal cavity.*

Trachea – consists of horseshoe-shaped *tracheal cartilages* connected by *annular ligaments.* Trachea has *cervical* and *thoracic part.* At the end (level Th4-5) trachea bifurcates (tracheal bifurcation) into 2 *principal bronchi* (right and left).

Lungs – are situated on either side of the heart and other mediastinal structures. Each lung has an *apex, base* and *surfaces.* Right lung has 3 lobes and the left one only 2 lobes. At the hilum of the lung *(hilum pulmonis)* the structures of *the root of the lung* enter and leave the lungs (principal bronchi, pulmonary artery, pulmonary veins, bronchial vessels and nerves, lymph vessels). Principal bronchi divide inside the lungs into *lobar bronchi* and lobar bronchi into *segmental bronchi.* Ramification of bronchi forms the *bronchial tree.* Each lung is covered by *pleura,* a serous membrane sac. Part of pleura adheres closely to the pulmonary surface, it is *pulmonary (visceral) pleura.*

The continuation of visceral pleura lines the corresponding part of thoracic wall, it is *parietal pleura.*

The respiratory system consists of three portions:
An air passages.
A respiratory portion for gas exchange between blood and air.
A mechanism of ventilation, controlled by the inspiratory and expiratory movements of the thoracic cage.

Air passing through the respiratory passages must be conditioned before reaching the terminal respiratory units. Conditioning of the air occurs in the conducting portion of the system and includes warming, moistening and removal of particulate material. Mucous and serous secretions play role in the conditioning process.
The principal functions of respiratory system are:
- air conduction, air filtration and gas exchange,
- the larynx is used to produce speech,
- the olfactory mucosa in the nasal cavities carries stimuli for the sense of smell,
- regulation of immune responses to inhaled antigens,
- hormone production and secretion.

The air passages of the respiratory system consist of a *conducting portion and a respiratory portion.*

13.1. Air conducting portion

The conducting passages include those located outside as well as within the lungs. The passages *external to the lungs* consist of: *nasal cavities and associated sinuses, nasopharynx and oropharynx, larynx, trachea, paired main (primary) bronchi*. Within the lungs, the main bronchi are branching to give rise *bronchioles*. The internal bronchi and the bronchioles constitute the *bronchial tree*.

13.1.1. Nasal cavities

The functions of the nasal cavity and paranasal sinuses is *warming* and *moistening* air and *filtering dust particles* present in the inspired air. The nasal cavities are paired chambers separated by a bony and cartilaginous

septum. Each cavity communicates anteriorly with the external environment through the *nares (nostrils*) and posteriorly with the nasopharynx through the *choanae.* The chambers are divided into three regions: *vestibule, respiratory segment, and olfactory segment.*

Vestibule of the nasal cavity

The vestibule is lined with stratified squamous epithelium, a continuation of the skin of the face, and contains a variable number of stiff hairs, vibrissae. Sebaceous glands are also present in the vestibule. Posteriorly the stratified squamous epithelium become thinner and undergoes to the pseudostratified epithelium that characterizes the respiratory segment.

Respiratory segment of the nasal cavity

The respiratory portion is lined by a *pseudostratified ciliated epithelium with goblet cells* supported by the lamina propria, which consists of connective tissue with *seromucous glands*. The lamina propria has a rich *superficial venous plexus*. Incoming air is warmed by blood in the venous plexus and moistened by secretions of the seromucous glands and goblet cells. The highly vascular nature of the nasal mucosa accounts for common bleeding *(epistaxis*) after trauma or acute inflammation *(rhinitis).* The lamina propria is continuous with the periosteum or perichondrium of bone or cartilage forming the wall of the nasal cavities. Projecting into each nasal cavity from the lateral wall are three curved plates of bone covered by mucosa: the superior, middle and inferior turbinate bones or *conchae.* They determine airflow disturbance to facilitate warming and moistening of air. The medial wall of the respiratory segment, *the nasal septum*, is smooth.

Paranasal sinuses (maxillary, frontal, ethmoid and sphenoid) are air-containing cavities within the bones of the skull. The sinuses are lined by thin pseudostratified columnar epithelium. Sinuses communicate with the nasal cavity by openings. The ethmoid sinuses open beneath the superior concha and maxillary sinus opens under the middle concha.

Olfactory segment of the nasal cavity

The olfactory area is present on the roof of the nasal cavity. It is lined with a specialized olfactory mucosa. In humans, its the total surface area is only a few square centimeters.

The mucosa of the olfactory area consists of pseudostratified ciliated columnar epithelium, but it contains very different cell types.

The olfactory epithelium consists of three cell types: *olfactory cells (bipolar neurons), basal cells* (stem cells that differentiate into olfactory cells) and *supporting or sustentacular cells*. The underlying lamina propria contains the venous plexus, the olfactory glands of Bowman and nerve bundles (called *fila olfactoria).*

The olfactory cell has two regions:

An apical region (the dendrite) characterized by an apical knob bearing nonmotile olfactory cilia. *Olfactory cilia* contain odorant receptors that bind to *odorant-binding proteins* (produced by the gland of Bowman) carrying an inhaled odorant particle.

On the opposite site, olfactory cells form small *fascicles of unmyelinated axons.* Axons penetrate the cribriform plate of the ethmoid bone and synapse with neurons in *the olfactory bulb*. The axons of the olfactory cells converge to one or more glomeruli and interact with dendrites of *mitral cells*. Axons from mitral cells form the *olfactory tract* (olfactory nerve or cranial nerve I), which carries olfactory information to the olfactory cortex.

Clinical correlations

Anosmia refers to deprivation of the sense of smell by disease or injury. *Sinusitis* is an inflammatory process of the sinuses. Chronic sinusitis and bronchitis are components of *immotile cilia syndrome*, which is characterized by defective ciliary action.

13.1.2. Pharynx

The pharynx connects the nasal and oral cavities to the larynx and esophagus. The posterior portion of the nasal cavities is *the nasopharynx,* which at the level of the soft palate becomes *the oropharynx.* The auditory tubes *(Eustachian tubes),* extending from the middle ear, open into the lateral walls of the oropharynx. *The nasopharynx* is lined by a pseudostratified columnar epithelium and changes into nonkeratinizing squamous epithelium at the oropharynx. *The mucosa-associated lymphoid tissue* is present beneath the nasopharyngeal epithelium, forming *Waldayer´s ring*. The concentrations of lymphatic nodules form *the nasopharyngeal tonsils (adenoids).*

13.1.3. Larynx

The larynx is the passageway for air between the oropharynx and trachea. It consists of *hyaline and elastic cartilages*, intrinsic and *extrinsic laryngeal muscles.* In addition to serving as a conduit for air, the larynx serves as *organ for speech (phonation*) and closes the trachea during swallowing to prevent food and saliva from entering the airway. The Adam´s apple is a nickname for part of the larynx formed by the thyroid cartilage.

The larynx can be subdivided into three regions:

The supraglottis, which includes *the epiglottis, false vocal cords (or folds) and laryngeal ventricles.*

The glottis, consisting of the *true vocal cords (or folds) and the anterior and posterior commissures.*

The subglottis, the region below the true vocal cords.

True vocal cords (folds)

The true vocal cords are two folds of mucosa that project into the lumen of the larynx. True vocal cords control the flow of air through the larynx and vibrate to produce sound. During forced inspiration, the true vocal cords are abduced and the space between the true vocal cords widens. During phonation, the true vocal cords are adduced and the space between the vocal cords changes into a linear slit. The vibration of free edged of the cords during passage of air between them produces sound.

A stratified squamous epithelium covers the true vocal cords.

The lamina propria of the true vocal cords consists of:

A superficial layer containing extracellular matrix and few elastic fibers. This layer is known as *Reinke´s space.*

An intermediate layer with many elastic fibers.

A deep layer with abundant elastic and collagen fibers. Intermediate and deep layers constitute *the vocal ligament.*

The lamina propria is usually rich in mast cell. There are no seromucous glands in the lamina propria of the true vocal cords. Reinke´s space and the epithelial covering are responsible for vocal cords vibration. *Reinke´s edema* results when viral infection, trauma (laryngeal endoscopy) cause fluid to accumulate in the superficial layer of the lamina propria. The thyroarytenoid muscle lies lateral and adherent to each of the vocal cords.

False vocal cords (folds)

The false vocal cords are located above the true vocal cords. Above the vocal cords is an elongated recessus in the larynx called the ventricle. Immediately above the ventricle is another pair of mucosa folds, the false vocal cords. They and ventricle are important in creating sound resonance. The ventricle and false vocal cords are lined by *pseudostratified columnar ciliated epithelium with goblet cells.* The connective tissue of lamina propria contains mixed *seromucous laryngeal glands.*

Epiglottis

The epiglottis is part of the larynx. It is composed of *elastic cartilage*. The epiglottis projects the rim of the larynx, extends into the pharynx and therefore has both *a lingual and laryngeal* surface. *The lingual or anterior surface of the epiglottis is covered by a nonkeratinized stratified squamous epithelium* and many seromucous glands in the lamina propria, especially near its connection with the base of the tongue. The upper part of the posterior surface of the epiglottis is covered by a nonkeratinized stratified squamous epithelium, which merges into a transition zone and appears as stratified columnar epithelium. *The lower part of the posterior surface is covered by pseudostratified columnar ciliated epithelium with goblet cells.* Near the base are scattered taste buds. The lamina propria includes some mucous and serous glands.

13.1.4. Trachea

The trachea is a short tube about 2.5 cm in diameter and about 10 cm long. It is the major segment of the conducting portions of the respiratory system. The trachea divides into the *two primary bronchi (extrapulmonary bronchi)* entering the hilum of each lung. On entering the lungs, the primary bronchi become *the intrapulmonary bronchi,* which branch to give rise to *the lobar bronchi (secondary bronchi)* that supply the two lobes of the left lung and tree lobes of the right lung. Bronchial division subdivides each lobe into bronchopulmonary segments. The bronchopulmonary segment is the gross anatomic unit of the lung that can be removed surgically. The lumen of the trachea is held open by a series of C-shaped hyaline cartilage (of 16 to 20) that are stacked on one another to form a supporting structure. Fibroelastic

tissue and smooth muscle (the trachealis muscle) bridge the gap between the free ends of the cartilages at the posterior border of the trachea, adjacent to the esophagus.
The wall of trachea consists of: mucosa, submucosa, layer of cartilage and muscle, and adventitia.

Mucosa

The trachea and main bronchi are lined by *pseudostratified columnar ciliated epithelium*.
Several types of cells can be identified in this epithelium:
Columnar ciliated cells - are about 30% of the total cell population.
Goblet cells - the apical portion of the cell contain mucus secretion that is released by exocytosis into the lumen forming part of a protective mucus blanket. They are about 30% of the total cell population.
Basal cells rest on the basal lamina but do not extend to the lumen. This cells function as stem cells population for the epithelium. Basal cells are about 30% of the total cells population.
Cells of Kulchitsky (neuroendocrine cells) also resting on the basal lamina and are predominantly found at the bifurcation of lobar bronchi. Kulchitzky cells secrete peptide hormones. Under pathological conditions, these neuroendocrine cells give rise to bronchial carcinoid tumors within the bronchial mucosa.
Lamina propria is a loose connective tissue containing many elastic fibers. It is separated from the dense connective tissue of the submucosa by a condensation of elastic fibers.

Submucosa

The submucosa is dense connective tissue. It contains blood vessels, fat cells and seromucous glands.

Layer of cartilage and muscle

A stack of *C-shaped hyaline cartilage* (of 16 to 20) forms the framework of the trachea. They are surrounded by perichondrium. *The trachealis muscle* (smooth muscle) connects the free ends of the hyaline cartilages.

Adventitia

Adventitia consists of loose connective tissue. It contains fat cells, nerves, blood vessels and the paratracheal lymph nodes. This layer is continuous with tissue of the mediastinum.

13.1.5. Bronchi

A distinguishing feature between trachea and bronchi is the replacement of hyaline cartilage rings by *irregular plates of cartilage* in bronchi. Differences in the histology develop as the bronchi become smaller. The amount of cartilage decreases, the amount of smooth muscle and elastic fibers in lamina propria increases, g*oblet cells* in the lining epithelium become *less numerous. Epithelium in bronchi* becomes reduced in height and is *simple columnar with less cilia.*

Extrapulmonary bronchi resemble the trachea in histological structure. *Intrapulmonary bronchi (secondary, lobar bronchi)* and subsequent passages are surrounded by lung tissue. They are histologically similar to the trachea and primary bronchi. Within the pulmonary parenchyma, segmental bronchi branch to form a system of airways known as the bronchial tree. The bronchi subdivide within the lung into secondary or lobar bronchi. The lobar bronchi divide into smaller segmental bronchi. The terminal branches of lobar bronchi are called bronchioles.

13.1.6. Bronchioles

Bronchioles are intralobular airways with *diameter 1 mm or less. Bronchioles lack cartilage and glands*, but a few goblet cells may be found in the initial portions. The pseudostratified ciliated columnar epithelium decreases in height to become simple columnar-to-cuboidal ciliated at the terminal bronchioles. The lamina propria is composed of smooth muscle and elastic and collagenous fibers.

Terminal bronchioles

Terminal bronchioles give rise to respiratory bronchioles. Terminal bronchioles are lined by a *ciliated cuboidal epithelium with Clara cells. Clara cells* represent 80% of the epithelial cell population of the terminal bronchiole. Non ciliated Clara cells appear in the epithelial layer with a single layer of ciliated cuboidal cells. The apical region of Clara cells contains secretory granules, mitochondria and numerous vesicles. Clara cells secrete a component of the surfactant material covering the alveoli and presumably also regulating the transport of chloride ions.

Respiratory bronchioles

Respiratory bronchioles represent the transition from the conducting to the respiratory portion of the lung. The mucosa of the respiratory bronchioles

is similar to that of terminal bronchioles, except for the *presence of alveoli interrupting the continuity of the wall of the bronchiole*. *The low cuboidal epithelium* is replaced by squamous alveolar epithelial cells. *A terminal bronchiole* and *the associated region of pulmonary tissue* that it supplies constitute *a pulmonary lobule.* A pulmonary lobule includes the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. *The pulmonary acinus* is the portion of the lung *supplied by a respiratory bronchiole.* Therefore, pulmonary acini are subcomponents of a respiratory lobule.

13.2. Respiratory portion of the lung

The respiratory portion is that part of respiratory tract in which gas exchange occurs. It includes: respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli. Terminal bronchioles give rise to three generations of respiratory bronchioles (0.5 to 0.2 mm in diameter) (Fig. 1).

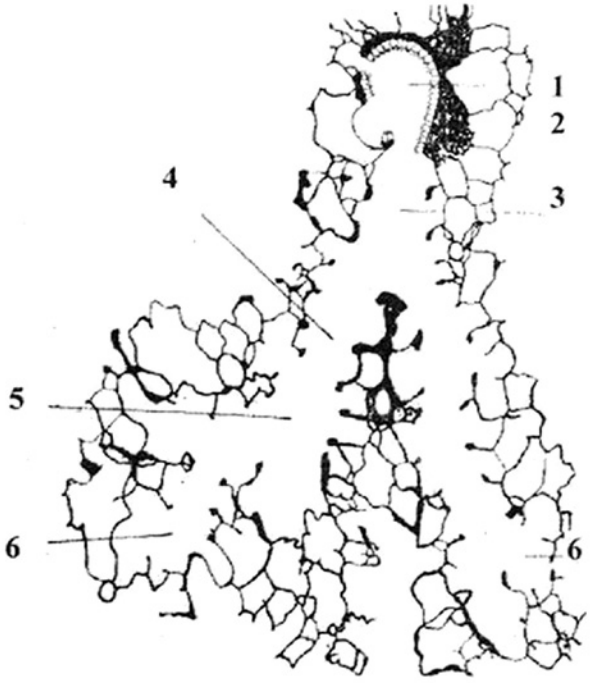

Fig. 1. Respiratory portion of the lung
1-terminal bronchiole, 2-wall of the terminal bronchiole, 3-respiratory bronchiole, 4-alveolar duct, 5-alveolar duct, 6-alveolar sac

Respiratory bronchioles

The respiratory bronchiole subdivides to give rise to an alveolar duct. The alveolar duct is continuous with the alveolar sac.

Alveolar ducts

The alveolar ducts are similar to the respiratory bronchioles from which they branch. The walls of the ducts are provided with so *many openings into the alveoli* that the wall appears discontinuous. *Bundles of smooth muscle* are seen around the openings into the alveoli.

Alveolar sacs, alveoli

Alveolar ducts branch to form two or more alveolar sacs. Alveolar sacs are formed by the alveoli.

Alveoli are *the terminal portions of the bronchial tree.* They are responsible for the spongy structure of the lungs. Within these structures, oxygen and carbon dioxide are exchanged between the air and the blood. The alveolus is the functional unit of the pulmonary acinus. About 300 million air sacs, or *alveoli*, in each lung are found. *Each alveolus opens into an alveolar sac*. *A few of them open directly into the respiratory bronchiole*. This particular feature distinguishes the respiratory bronchiole from the terminal bronchiole, whose wall is not associated with alveoli. Each alveolus has a thin wall. It is lined by simple squamous epithelial cells.

The alveolar epithelium consists of two cell types:

Type I alveolar cells (squamous alveolar cells), representing about 40% of the epithelial cell population but lining 90% of the alveolar surface. These cells are very thin, often only 25 nm in thickness. The main role of these cells is to provide a barrier of minimal thickness that is permeable to gases.

Type II alveolar cells (great alveolar cells, septal cells) 60% of the cells, covering only 10% of the alveolar surface area. These cells *secrete pulmonary surfactant.* They are located *at the angles formed by adjacent alveolar septa*. Type II alveolar cells are polygonal-shaped and extend beyond the level of the epithelium. The free surface of Type II alveolar cells is covered by *short microvilli*. The cytoplasm contains *dense membrane-bound lamellar bodies*, representing secretory granules containing pulmonary surfactant. *The pulmonary surfactant* is a thin layer of fluid that covers the alveolar surface. The surfactant, a lipid-protein complex that reduces

the surface tension of the alveolus and prevents alveolar collapsing. *The pulmonary surfactant lowers the alveolar surface tension* at the air-fluid interface and thus reduces the tendency of the alveolus to collapse at the end of expiration. Clara cells, located in terminal bronchioles, also secrete pulmonary surfactant (Fig. 2).

The structure of the alveolar walls is specialized for diffusion between the external and internal environments. *Each wall lies between 2 neighboring alveoli* and is therefore termed *an interalveolar septum or wall*. *An alveolar septum consists of* 2 squamous epithelial layers between which lie capillaries, elastic and collagen fibers, macrophages, fibroblasts, and mast cells. The endothelial lining of the capillaries is continuous and not fenestrated. The capillaries and connective tissue matrix constitute the interstitium. Additional cells of the alveolar septa are *the alveolar macrophages* also called *dust cells.* They are derived from bone marrow monocytes and are often seen *in the alveolar lumen*. The interalveolar septum may contain *one or more pores (10-15 μm in diameter).* They may equalize pressure in the alveoli or the collateral circulation of air when a bronchiole is obstructed.

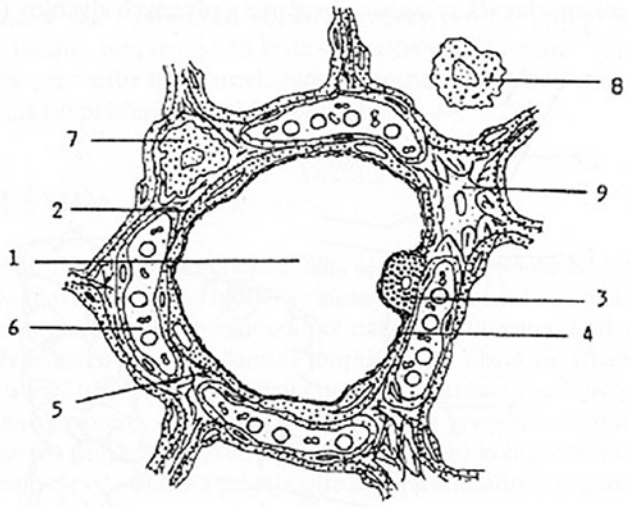

Fig. 2. Structure of alveolus

1-alveolus, 2-type I alveolar cell, 3-type II alveolar cell, 4-respiratory epithelium, 5-basal lamina, 6-blood capillary, 7-macrophage, 8-alveolar macrophage (dust cell), 9-fibroblast

Air-blood barrier (Blood-air barrier)

The lung is gas-exchanging organ for the provision of O_2 to the blood and removal of CO_2 from the blood. Gas exchange by passive diffusion occurs across the air-blood barrier. The total thickness of this layer varies from 0.1 to 1.5 μm (Fig. 3).

Air-blood barrier consists of:

- the cytoplasm of type I alveolar cells
- a dual basal lamina, synthesized by type I alveolar cells and endothelial cells
- the cytoplasm of the endothelial cells
- the plasma membrane of red blood cells

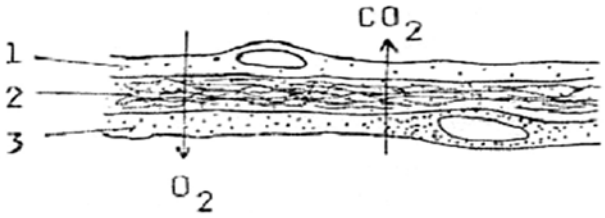

Fig. 3. Air – blood barrier

1- type I alveolar cell, 2-basal lamina, 3- endothelial cell

Clinical correlation

The respiratory distress syndrome of the newborn is a life-threatening disorder of the lungs caused by a *deficiency of surfactant.* It is associated with prematurity and is the leading cause of mortality among premature infants. The immature lung is deficient in both the amount and composition of surfactant. In the respiratory distress syndrome the alveoli are collapsed and the respiratory bronchioles and alveolar ducts are dilated and contain edema fluid.

13.3. Pleura

The pleura is the serous membrane covering the lung. It consists of two layers: *a visceral layer, and a parietal layer.* These layers are continuous in the region of the hilum. Both membranes are composed

of mesothelial cells resting on a fine connective tissue layer containing collagen and elastic fibers. *The visceral* layer is closely attached to the lung. *The parietal* layer is thicker and lines the inner surface of the thoracic cavity. *The parietal* layer is also lined by *the mesothelium*. A very thin liquid film in between the visceral and parietal layers permits the smooth gliding of one layer against the other.

Clinical correlation

Under normal conditions, the visceral pleura glides smoothly on the parietal pleura during respiration. During *an inflammatory process*, characteristic friction sounds can be detected during the physical examination. In certain pathologic states, the pleural cavity can become, a real cavity, containing liquid or air in its interior. If fluid accumulates in the pleural cavity *(hydrothorax)*, the lung collapses gradually. The presence of air in the pleural cavity *(pneumothorax),* caused by a penetrating wound, also collapses the lung. *Mesothelioma* is a tumor that originates in the mesothelial cells lining of the pleura, the peritoneum and the pericardium.

Lung tumors, which have historically had their highest incidence in males, are mainly of epithelial origin. The *squamous cell carcinoma* is principal lung tumor type. It is related to the effects of cigarette smoking on the bronchial and bronchiolar epithelial lining.

Chronic obstructive pulmonary disease is characterized by progressive and often irreversible airflow limitations. It includes emphysema and asthma.

Emphysema is permanent enlargement of the air spaces distal to the terminal bronchioles, associated with the destruction of their walls. Elastic fibers are important components of bronchioles and alveolar walls. A loss of elasticity and breakdown of elastic fibers give rise to emphysema. As a result of the loss of elastic fibers, the small airways tend to collapse during expiration, leading to chronic airflow obstruction and secondary infections.

Asthma, characterized by both reversible bronchoconstriction of the smooth muscle bundles encircling the bronchiolar lumen and mucus hypersecretion by goblet cells triggered by allergens or autonomic neural factors, leads to a reduction in the lumen of the airways. Wheezing, cough, and shortness of breath (dyspnoe) are classic symptoms.

Cystic fibrosis is a recessive genetic disease affecting children and young adults. A characteristic of the disease is the production of abnormally thick mucus by epithelial cells lining the respiratory and gastrointestinal tracts. Respiratory disease results from the obstruction of the pulmonary airways by thick mucus plugs, followed by bacterial infections.

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14. Digestive system (alimentary canal)

Anatomy introduction

The organs of the digestive system are specialized for the digestion and absorption of food. The digestive system consists of a tubular gastrointestinal tract and accessory digestive organs. The digestive organs are situated partly within the head and neck, and partly within the trunk. The digestive system starts by the *oral cavity.* It contains *salivary glands, oral* and *laryngeal part of pharynx, esophagus, stomach, small intestine, large intestine* and 2 large intestinal glands – *liver* and *pancreas.*

Oral cavity is divided into the *vestibule of the oral cavity* and *proper oral cavity.* Vestibule of oral cavity is bordered by *lips* and *cheeks;* against the proper oral cavity by *upper* and *lower dental arch* with *teeth.* The main content of proper oral cavity is the *tongue.*

Salivary glands – we distinguish large and small salivary glands. Large are: *parotid, sublingual* and *submandibular gland.* All are paired. Small glands are localized in the tongue, palate, mucosa of cheeks, lips, etc.

Pharynx – oral cavity opens into the *oral part of pharynx* where *the palatine tonsils* are located. Oral part continues to *laryngeal part of pharynx* which communicates with laryngeal cavity. Pharynx fluently continues to esophagus.

Esophagus – convey the food to the stomach. It measures about 25-30cm, and has three portions: *cervical, thoracic* and *abdominal portion.* The last one merges into the stomach. Esophagus lies first behind the trachea, then in the posterior mediastinum.

Stomach – the *cardiac portion* is the continuation of the end of esophagus. The main part is the *body,* its upper part is *fundus.* Body of stomach merges into *pyloric part.* Pyloric part opens into the duodenum (first part of small intestine) by *pyloric orifice (pylorus).* Stomach is situated in the epigastric, umbilical and left hypochondriac regions.

Small intestine – extends from the pylorus to the ileocecal orifice, where it joints the large inestine. Small intestine has 3 parts, *duodenum, jejunum* and *ileum.* Its average length is 5m.

Large intestine – extends from the end of ileum to the anus. It has following parts: *cecum* with *vermiform appendix, ascending colon, transverse colon, descending colon, sigmoid colon* and *rectum.* Rectum is subdivided into 2 parts, *rectal ampulla* and *anal canal.*

Liver – it acts as an exocrine gland in respect of production of *bile.* The bile is collected in *gall bladder.* The liver lies under the right dome of diaphragm and occupies a substantial portion of the abdominal cavity. It is essential organ for life.

Pancreas – extends transversely across the posterior abdominal wall from the duodenum to the spleen, behind the stomach. The pancreas is composed of two different types of tissues, *exocrine* type which produces pancreatic juice, and *endocrine*

type which secretes hormones *glucagon* and *insulin.* The pancreas with its function is essential for life.

The digestive system consists of the digestive tube (the oral cavity, pharynx, esophagus, stomach, small and large intestines, rectum and anus), and associated glands, i.e. salivary glands, liver and pancreas. The *upper portion* of the digestive tract that comprises of the *oral cavity, pharynx and esophagus*, is responsible for food intake, preparation of food for swallowing and partial digestion of complex carbohydrates as well as conveyance of food from the oral cavity to the stomach. The *lower portion* of the digestive tract consists of the *stomach, small intestine and large intestine.* This portion is responsible for digestion and absorption. In the large intestine, the constituents of undigested food are dehydrated and mixed with mucus, while the feces pass out of the body through the rectum and anal canal.

14.1. General structure of the digestive tube

The digestive tube has certain common structural characteristics. Histologically, it consists of four principal layers: *mucosa, submucosa, muscularis and adventitia or serosa*. These layers are similar throughout the length of the digestive tract, but display regional modifications and specializations.

Mucosa is the inner layer encircling the lumen and shows significant variations along the digestive tube. It is further subdivided into:

- *Lamina epithelialis* – the epithelium is variable; the upper portion has stratified squamous epithelium, the lower portion is lined with simple columnar epithelium.
- *Lamina propria* – a loose connective tissue rich in blood and lymph vessels, sometime also containing glands and lymphoid tissue. The abundant lymphoid nodules protect the organism from bacterial invasion. The lamina propria is a zone rich also in macrophages and plasma cells.
- *Lamina muscularis mucosae* – consists of a thin inner circular and outer longitudinal layer of smooth muscle cells. This layer separates the mucosa from the submucosa.

Submucosa is composed of a denser fibroelastic connective tissue with many blood and lymph vessels and a submucosal plexus of autonomic nerves called *Meissner's submucosal nerve plexus*. This plexus houses the postganglionic parasympathetic nerve cell bodies and controls the motility of the mucosa as well as the secretory activities of its glands. This layer also contains lymphoid tissue and glands (esophagus and duodenum).
Muscularis externa is usually inner circular and outer longitudinal sublayer, composed of smooth muscle (except the upper part of the esophagus). It is responsible for peristaltic activity, which moves the content of the lumen along the digestive tube. Between the circular and longitudinal sublayers lies the *myenteric (Auerbach´s) nerve plexus*. The myenteric plexus also houses some postganglionic parasympathetic nerve cell bodies. Between the two muscle layers there are blood and lymph vessels in the connective tissue.
Serosa is a thin layer of connective tissue, covered by simple squamous epithelium (*mesothelium*). This is the region of alimentary tube, that is intraperitoneal.
In places where the alimentary canal is retroperitoneal, serosa is replaced by thick *adventitia,* which consists of connective tissue containing vessels and nerves, but without mesothelium.

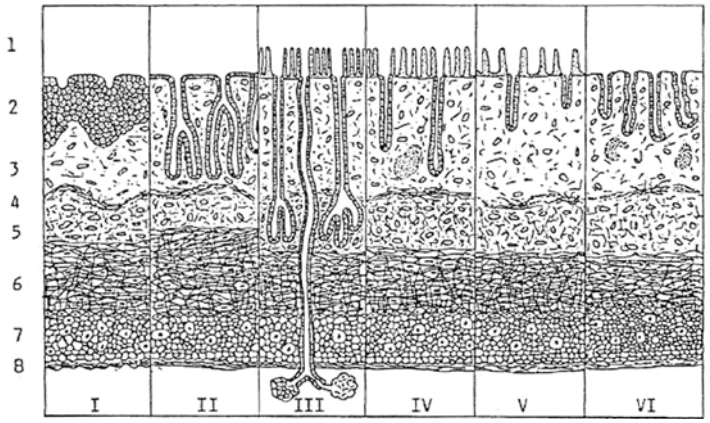

Fig. 1. Diagram of general organization of the alimentary canal
I-Esophagus, II-Stomach, III-Duodenum, IV-Jejunum, V-Ileum, VI-Colon
1-lumen-here extend villi, present in the small intestine, 2-epithelium, 3-lamina propria, 4-lamina muscularis mucosae, 5-submucosa, 6-muscularis externa-circular, 7-muscularis externa-longitudinal, 8-serosa

Glands of the digestive system

The digestive system is associated with numerous glands, which can be located in or empty into the mucosal epithelium.

- The simplest glands called *goblet cells* are unicellular and prominent in the intestine.
- The glands are usually located within the lamina propria. Large glands may extend into the submucosa.
- Large extrinsic glands such as the salivary glands, liver and pancreas have ducts that empty into the gastrointestinal tract.
- These glands are with mucous and serous secretion and produce digestive enzymes, ions, bile acids and antibacterial substances.

14.2. Functions of the digestive tube

The functions of the digestive tube are ingestion, digestion and absorption of food as well as elimination of the unusable parts of these materials. The first step in the complex process known as digestion occurs in the mouth, where food is moistened with saliva and broken down by teeth into smaller pieces; saliva also initiates the digestion of carbohydrates. Digestion continues in the stomach (food is transformed into a viscous mass – *chyme*) and small intestine, where food is absorbed. Water absorption occurs in the large intestine.

The main functions of the epithelial lining of the digestive tract are to:

- provide a selectively permeable barrier between the contents and the tissues,
- facilitate the transport and digestion of food,
- promote the absorption of this digestion,
- produced mucus for lubrication and protection,
- produce hormones that effect the activity of the digestive system.

The lamina propria and the submucosal layer have a protective function meaning that the abundant lymphoid nodules in the lamina propria and the submucosal layer protect the organism from bacterial invasion. The necessity for this immunological support is especially obvious where is simple vulnerable epithelium. The lamina propria is rich in macrophages and lymphoid cells, which produce antibodies (mainly immunoglobulin A). The IgA present in the digestive system

is resistant to proteolytic enzymes and can coexist with proteases in the lumen.

The muscularis mucosae promote the movement of the mucosa independent of other movements of the digestive tract, thus increasing its contact with food. The contractions of the muscularis are generated and coordinated by nerve plexuses, which are composed of multipolar visceral neurons that form small parasympathetic ganglia and a rich network of pre- and postganglionic fibers of the autonomous nervous system. The number of these ganglia along the digestive tube is variable; they are more numerous in regions of the greatest motility.

14.3. Histological structure

14.3.1. Oral cavity

The oral cavity (mouth) includes the tongue, teeth, gingiva, soft and hard palate, major and minor salivary glands and tonsils. The oral cavity is surrounded by *lips*, which form its anterior boundary and *palatoglossal folds* forming the posterior boundary.

The oral cavity is lined with stratified squamous partially keratinized (parakeratinized) epithelium, depending on the region. The epithelium protects the oral mucosa from damage during mastication. Parakeratinized epithelium is present mostly in the gingiva and hard palate. The remainder of the oral cavity is lined with nonkeratinized epithelium. The lamina propria in the gingiva and hard palate is a dense connective tissue, contains diffuse small salivary glands. Lamina propria rests directly on the bony tissue. The ducts of the three pairs of major salivary glands (parotid, submandibular and sublinqual) open into the oral cavity, delivering saliva to moisten the mouth. These glands also manufacture and release the enzyme *salivary amylase* (break down carbohydrates), antibacterial agents *lactoferrin, lysozymes* and *immunoglobulin (IgA*).

The posterior opening of the oral and nasal cavities is surrounded by aggregations of lymphatic nodules that form the tonsils. The lymphatic tissue is organized into a tonsillar ring (Waldeyer's ring) that includes the palatine tonsils, tubal tonsils, pharyngeal tonsils and lingual tonsils.

14.3.1.1. Lips

The core of the upper and lower lip is skeletal muscle that is responsible for lip mobility. The lips have three regions. The *external (skin) aspect* is covered with thin skin, associated with sweat glands, hair follicles and sebaceous glands. This region is continuous with the *vermilion zone*, a pink region, which is also covered by thin skin that is devoid of sweat glands and hair follicles, while occasional, nonfunctional sebaceous glands are present here. The mucosa is highly developed and forms papillae that contain capillary loops. The *internal (mucous) aspect* is always wet and lined with stratified squamous nonkeratinized epithelium. The lamina propria is a dense connective tissue and houses numerous minor, mostly mucous salivary glands.

14.3.1.2. Palate

The palate separates the oral and nasal cavity, is composed of the hard palate, the soft palate and the uvula. The *soft palate* is movable consisting of skeletal muscle (responsible for its movements) and covered by tunica mucosa. The oral surface of the soft palate is covered by a stratified squamous nonkeratinized epithelium. The lamina propria is a dense connective tissue, housing minor mucous salivary glands. The posterior extension of the soft palate is called the uvula. The *uvula* has a similar histological structure that soft palate. The *hard palate*, positioned anteriorly, is immovable and its core is bone. The oral aspect contain stratified squamous keratinized or (parakeratinized) epithelium. The lamina propria is a dense connective tissue. Its postero lateral aspect contains minor mucous salivary glands.

14.3.1.3. Tongue

The tongue is a mass of voluntary striated muscle covered by a mucous membrane. The striated muscle fibers of the tongue are arranged in three interweaving planes – longitudinal, vertical and transvers. The muscle fibers are grouped in bundles and separated by a connective tissue, with a variable amount of adipose tissue, and salivary glands in between. This arrangement gives the tongue a great mobility in order to manipulate with food as well as for swallowing and speaking. The tongue has a dorsal surface, a ventral surface and two lateral surfaces. The mucous membrane is smooth on the lower (ventral) surface of the tongue. The submucosa

can be distinguished on the ventral surface, while the dorsal surface has no submucosa.

The dorsal surface of the tongue is divided into: the anterior two thirds, the posterior one third and the root. The anterior and posterior regions are separated from each another by a shallow V-shaped groove, the *sulcus terminalis*. The anterior region contains numerous lingual papillae. The dorsal surface of the tongue behind the sulcus terminalis is without papillae, typically exhibiting mucosal ridges or *crypts*. The most posterior portion is known as the *root of the tongue*. The lamina propria of this part contains aggregations of lymphoid nodules that form the *lingual tonsils*.

The dorsal surface anteriorly is irregular, because the lamina propria forms a great number of small eminences called the *papillae*. They are all located anteriorly to the sulcus terminalis on the dorsal and lateral aspect of the tongue, there are recognized filiform, fungiform, foliate and circumvallate papillae.

The filiform papillae are the most numerous papillae located all over the dorsum of the tongue. They are tall, narrow, covered with stratified squamous keratinized epithelium, particularly at their tips, and help to scrape food off the surface. The filiform papillae do not contain any taste buds.

The fungiform papillae resemble a mushroom - they have a narrow stalk and a smooth dilated upper part. These papillae are irregularly interspersed among the filiform papillae. The fungiform papillae contain scattered taste buds (detecting sweet taste). The epithelium is stratified squamous nonkeratinized. The lamina propria forming the core of these papillae is highly vascularized.

The circumvallate papillae are the largest lingual papillae located in front of the *sulcus terminalis,* the human tongue has about 8 to 12 of these papillae. Each papilla is surrounded by a circular furrow lined with stratified squamous epithelium containing numerous taste buds. On the other hand, taste buds are not present on the dorsum of the papillae. The ducts of the lingual serous salivary glands (*Ebner´s glands*) open into the base of the circular furrow.

The foliate papillae, which are rudimentary in humans, are located along the posterolateral aspect of the tongue. In younger individuals, they are easily detected and contain many taste buds in the epithelium, but these taste buds degenerate by the second or third year of life.

Taste buds. Taste buds are intraepithelial taste receptors that detect tastants (sweet, sour, bitter, salty). The surface of the tongue and the posterior aspect of the oral cavity contain approximately 3,000 taste buds. Taste buds are also present on the glossopalatine arch, the soft palate, the posterior surface of the epiglottis and the posterior wall of the pharynx. In a histological section, taste buds appear as oval and pale staining structures within the stratified epithelium of the tongue and the oral mucosa. A small opening onto the epithelial surface at the apex of a taste bud is called the *taste pore*.

Each taste bud consist of three cell types:

- *Sensory (neuroepithelial) cells* are elongated and extend from the basal lamina to the taste pore. The apical surface of these cells is connected to the neighboring neuroepithelial or supporting cells by tight junctions. At their base, they form synapses with fibers of afferent sensory neurons. Their turnover time is about 10 days.
- *Supporting cells* are less numerous. They are also elongated and extend from the basal lamina to the taste pore. Their apical surface contains microvilli and possesses tight junctions. The turnover time of these cells is also about 10 days.
- *Basal cells* are small cells located near the basal lamina. They are the stem cells for the other two cell types.

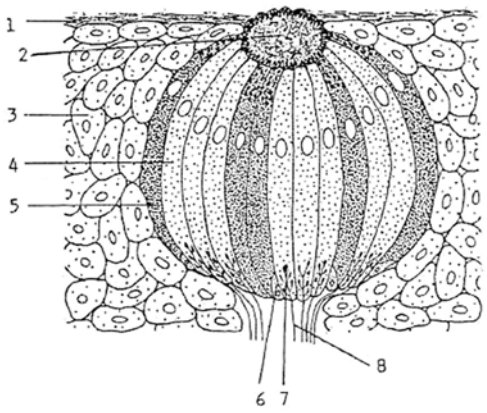

Fig. 2. Diagram of a taste bud

1-surface epithelial cells, 2-taste pore, 3-epithelial cells, 4-sensory sells, 5-supporting cells, 6-basal cells, 7-synapse with afferent fibers, 8-afferent nerve fibers

14.3.1.4. Teeth

Teeth are a major component of the oral cavity and as such, they are essential for the beginning of the digestive process. Humans develop two sets of teeth*: 20 deciduous (milk) teeth,* which are gradually replaced by *32 permanent (adult) teeth*.

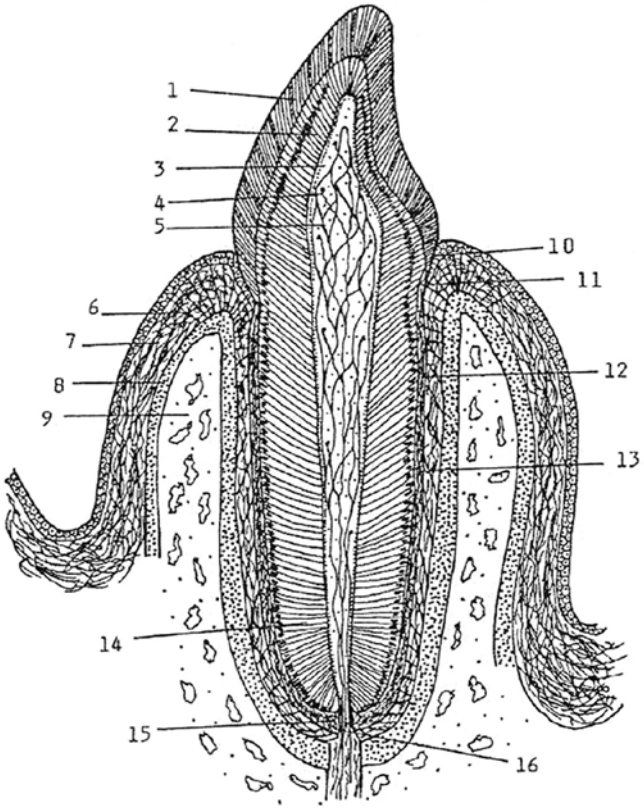

Fig. 3. Tooth – longitudinal section
1-enamel, 2-dentin-showing dentinal tubules, 3-odontoblasts, 4-pulp-nerve ending, 5-pulp-capilary, 6, 7-gingiva, 8-periosteum, 9-alveolar bone, 10-gingival sulcus, 11-cervix-juncture of enamel and cementum, 12-cementum, 13-periodontal ligament, 14-odontoblasts, 15-apex, 16-apical foramen

Each tooth, whether deciduous or permanent, has a *crown, cervix and root*. A *crown* is part that is visible when the tooth is in situ in the oral cavity, a *root* is the part embedded in the bony cavity (alveolus) of the maxilla and mandibulae, and a *cervix* is located between the crown and the

root. Also, each tooth is suspended in its bony *alveolus* by a *periodontal ligament*. The periodontal ligament is a dense collagenous connective tissue, composed of type I collagen. The *gingiva* and its epithelium are also involved in providing support for teeth.

Tooth is structurally composed of three layers of specialized calcified substances: *enamel, dentin and cementum*, which enclose of the *pulp* and the *root canal*. The root canal communicates with the periodontal ligament via a small opening at the tip of each root, where the blood, lymph vessels as well as nerves enter and leave the pulp. Dentin surrounds the pulp cavity and the root canal. On the crown, it is covered by enamel, while on the root it is covered by cementum. Enamel and cementum meet at the cervix of the tooth.

Enamel is the hardest substance in the body that consists of about 96% of inorganic salts - calcium hydroxyapatite and 4% of organic material and water. The organic constituents of enamel are the keratin-like glycoprotein *enamelin*. Enamel is translucent and its coloration is reflecting the color of the underlying dentin. Enamel is a unique tissue produced by cells known as *ameloblasts.* Unlike the bone, it is a mineralized material derived from epithelium. Also, enamel is a non-vital substance; because the ameloblasts die before teeth erupt into the oral cavity and the body cannot repair it.

Dentin is composed of 70% of calcium hydroxyapatite, 20% of organic materials and about 10% of bound water. Most of the organic substance is type I collagen associated with proteoglycans and glycoproteins. Dentin is produced by *odontoblasts* that**,** unlike ameloblasts, maintain associated with dentin during the whole life of the tooth. Odontoblasts are located at the periphery of the pulp and their cytoplasmic processes occupy the *dentinal tubules*, i.e. spaces within dentin. During dentinogenesis, the odontoblasts manufacture dentin every day. Dentin is sensitive to several stimuli, such as heat, cold, acidic pH and trauma, all of which are perceived as pain. Although the pulp is highly innervated, while dentin has a few unmyelinated nerve fibers.

Cementum is the third type of mineralized tissue of the tooth. Cementum is restricted to the root and is similar in composition to the bone, although Haversian systems and blood vessels are absent. Cementum is composed of 50% of calcium hydroxyapatite and 50% of organic material and

bound water. Again, most of the organic material is composed of type I collagen with associated proteoglycans and glycoproteins. Cementum is produced by cells known as *cementocytes* or *cementoblasts*, which are cells with the appearance of osteocytes. These cells are situated within the lacunae and are responsible for the formation of cementum, line cementum at its interface with the periodontal ligament and continue to elaborate cementum during the whole life of the tooth. Collagen fibers of the periodontal ligament – *Sharpey´s fibers* are embedded in cementum and suspend the tooth in its bony socket. Cementum can be resorbed by *odontoclasts.* During exfoliation, the replacement of deciduous teeth, odontoclasts resorb cementum of the rooth.
The pulp of the tooth consist of a loose gelatinous connective tissue that is rich in ground substance that contains proteoglycans and glycosaminoglycans and is richly vascularized and innervated. Blood vessels and myelinated nerve fibers enter the apical foramen and divide into numerous branches. Some nerve fibers lose their myelin sheaths and extend into the dentinal tubules. The pulp fibers are also sensitive to pain. The pulp communicates with the periodontal ligament through the apical foramen, a small opening at the tip of each root through which vessels and nerves enter and leave the pulp. The pulp is surrounded by dentin.

Structures associated with teeth. The structures associated with teeth are the periodontal ligaments, the alveolar bone and the gingiva.
Periodontal ligament is a dense collagenous connective tissue, suspend the tooth in its alveolus, located in the region between cementum of the root and the bony alveolus. This richly vascularized connective tissue contains fibroblasts, i.e. cells that manufacture collagen. Leukocytes, plasma cells, mast cells and macrophages are also frequently present in the periodontal ligament.
Alveolus is the bony socket in which the tooth is suspended by the fibers of the periodontal ligament and houses the roots of teeth.
Gingiva is a mucous membrane firmly bound to the periosteum of the maxillary and mandibular bones. It is composed of stratified squamous epithelium and the lamina propria contains numerous papillae. A very specialized part of this epithelium, the junctional epithelium, is bound to the tooth enamel by means of a cuticle that resembles a thick basal

lamina and forms the epithelial attachment. Between the enamel and the epithelium is the gingival sulcus.

Clinical correlations

Tooth caries is usually the result of accumulation of microorganisms in and on slight defects of the enamel surface. As bacteria metabolize nutrients on the tooth surface, they produce acids that begin to decalcify the enamel. The bacteria and toxins that they release enlarge the caries. Fluoride increases the hardness of enamel, making it more resistant to caries.

Dentin sensitivity is mediated by sensory nerve fibers that are closely associated with odontoblasts, their processes, and dentinal tubules. Disturbance of the tissue fluid within the dentinal tubules sends a signal to the brain, where it is interpreted as pain.

Cementum does not resorb as the bone does. Hemorrhage of the pulp is evident clinically as a dark discoloration of the tooth. Due to the fact that the pulp may recover, hemorrhage should not be the sole indicator of root canal treatment.

14.3.1.5. Pharynx

The pharynx represents the communication between the nasal region and the larynx. The cavity of the pharynx is incompletely divided by the soft palate and the uvula into two regions: an upper nasal part and a lower oral and laryngeal part. The pharyngeal wall consists of three layers: mucosa, muscularis and adventitia. The pharynx in the region continuous with the esophagus is lined with stratified squamous epithelium and pseudostratified columnar ciliated epithelium with goblet cells in the region close to the nasal cavity. The mucosa of the pharynx has many small mucous salivary glands and contains a diffuse lymphoid tissue and lymphoid nodules that form the pharyngeal tonsils. The muscle of the pharynx is skeletal and irregularly arranged.

14.3.2. Esophagus

The esophagus is a muscular tube that conveys the masticated food from the mouth cavity to the stomach. Transport is achieved by the peristaltic contraction and relaxation of the esophageal sphincters (upper

and lower). In general, the esophagus has the same layers as all other parts of the digestive tube – mucosa, submucosa, muscularis externa and adventitia.

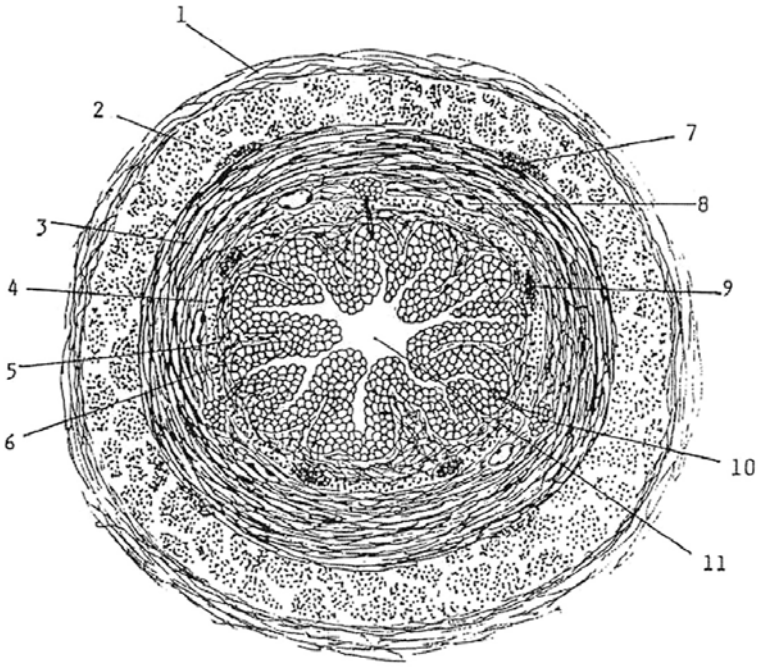

Fig. 4. Esophagus
1-adventitia, 2-muscularis externa-longitudinal layer, 3-muscularis externa-circular layer, 4-submucosa, 5-lamina propria, 6-epithelium, 7-myenteric plexus, 8-capilary, 9-submucosal plexus, 10-submucosal gland, 11-lumen

The mucosa of the esophagus is lined with a *stratified squamous nonkeratinized epithelium* resistant to abrasion. Within the epithelium, antigen-presenting cells known as *Langerhans cells* are interspersed, which phagocytize and degrade the antigens into small polypeptides. Langerhans cells migrate to the regional lymph nodes, where they present an antigen to the lymphocytes.
The lamina propria is unremarkable. It consists of a loose connective tissue. In the lamina propria near the stomach there are groups of esophageal cardiac glands, which secrete mucus.
The muscularis mucosae consist of only one layer of longitudinally organized smooth muscle cells is thicker near the stomach.

The submucosa of the esophagus consists of a fibroelastic connective tissue. Both the mucosa and the submucosa in the undistended esophagus form *longitudinal folds* that give the lumen an irregular outline. As the bolus of food moves down the esophagus, the folds disappear transiently. The submucosa houses mixed tubuloacinar glands, present only in its upper regions - *esophageal glands proper*, secretions of which facilitate the transport of food and protect the mucosa. The ducts of these glands open into the lumen of the esophagus. The submucosa also contains blood and lymph vessels as well as a nervous submucosal plexus. The submucosal plexus is in the vicinity of the inner circular layer of the muscularis externa.

The muscularis externa of the esophagus is composed of both skeletal and smooth muscle, and it is arranged in two layers: an inner circular and an outer longitudinal. The muscularis externa of the upper third of the esophagus contains mostly skeletal muscle, the middle third contains both skeletal and smooth muscle and the lowest third only smooth muscle. The nervous myenteric plexus is situated between the inner circular layer and the outer longitudinal layer and regulates the activity of the muscularis externa.

The adventitia or serosa of the esophagus is the outermost layer of the loose connective tissue. The esophagus is covered by adventitia until the diaphragm. The region of the esophagus situated intraperitoneally is covered by serosa, which is a thin connective tissue layer surrounded by a simple squamous epithelium*, the mesothelium***.**

Clinical correlations

A bolus entering into the esophagus is conveyed by peristaltic action of the muscularis externa into the stomach. The esophagus has two sphincters – *the upper and lower esophageal sphincter*. The upper esophageal sphincter participates in the initiation of swallowing, while the lower esophageal sphincter *prevents the reflux* of the gastric contents back into the esophagus from the stomach. Sometimes, the lower end of the esophagus is lined with poorly resistant columnar epithelium and thus, reflux causes chronic inflammation or ulceration and difficulty in swallowing (*dysphagia)*. This condition can lead to fibrosis of the lower esophagus.

At the lower end of the esophagus, submucosal venous plexuses drain into the systemic venous system and the portal venous system. An increase in pressure in the portal venous system caused by chronic liver disease results in the dilation of the submucosal venous sinuses and the formation of *esophageal varices*. Rupture of these varices or ulceration of the mucosa can produce a hemorrhage into the esophagus and the stomach. As the esophagus passes through diaphragm, it is reinforced by muscular fibers. In some people, this development is abnormal causing a gap in the diaphragm around the wall of the esophagus that permits herniation of the stomach into the thoracic cage known as *hiatal hernia*. This condition commonly affects young and middle-age women in particular. Also, excessive gastric distention, fatty meals, smoking and drinking tea and coffee all cause relaxation of the esophageal sphincter, thus facilitating the reflux of the gastric contents.

14.3.3. Stomach

The stomach is the most dilated region of the alimentary canal, whose main functions are to continue the digestion of carbohydrates initiated in the mouth and to initiate the digestion of proteins (pepsin), digests triglycerides. The stomach is responsible for the transformation of the ingested food, by muscular activity and the acid and enzymes secreted by the gastric glands, into a viscous mass – *chyme*, which is then released gradually into the duodenum. The mucosa and submucosa of the undistended stomach display longitudinal folds known as *rugae,* which disappear in the distended stomach. The stomach produces *hydrochloric acid*, *intrinsic factor* and *gastric lipase* that digests triglycerides and produces *paracrine hormones*.

The stomach has four regions:

- *cardia*, which is a narrow region at the gastroesophageal junction;
- *fundus*, a dome-shaped region frequently filled with gas;
- *body (corpus)*, being the largest portion responsible for the formation of chyme; and
- *pylorus*, a funnel-shaped portion where a thick pyloric sphincter controls the release of chyme into the duodenum.

Histologically, only three regions are differentiated in the stomach, because the fundus and body are identical in their microscopic structure.

In general, the stomach has the same major layers as those described in the digestive tube.

The mucosa, all regions of the stomach are lined by *simple columnar epithelium*, which secrete alkaline mucus. The mucus layer is a gel-like substance that adheres to the lining epithelium and protects it from autodigestion, because hydrochloric acid, pepsin and lipase all act as endogenous aggressors to the lining epithelium. The epithelium of the stomach invaginates into the lamina propria, forming *gastric pits* known as *foveolae gastricae*, which are shallowest in the fundus and deepest in the pyloric region. The surface columnar cells continue into the gastric pits, forming their epithelium. The bases of pits contain regenerative cells.

The lamina propria is a loose and highly vascularized connective tissue and has a rich population of fibroblasts, plasma cells, mast cells, lymphocytes and occasional smooth muscle cells. Lymphoid nodules may be located in the pyloric region. The lamina propria is occupied by closely aggregated tubular glands characteristic for each region of the stomach. These glands extend from the base of the gastric pits and reach the muscularis mucosae, each gland opening into a pit.

Muscularis mucosae is a layer of smooth muscle, separating the mucosae from the underlying submucosa.

Submucosa of the stomach is connective tissue containing a rich network of blood and lymph vessels. The cell population of the submucosa resembles that of any connective tissue, containing macrophages, plasma cells and mast cells, and it is infiltrated by lymphocytes. The submucosal nerve plexus is located in the vicinity of the muscularis externa.

Muscularis externa of the stomach is composed of three layers of smooth muscle. The innermost oblique layer is not well defined except in the cardiac region. The *middle layer* is clearly evident and is especially pronounced in the pyloric region, where it forms the *pyloric sphincter*. The outer longitudinal muscle layer is most evident in the cardiac region and the body of the stomach, but it is poorly developed in the pylorus. The myenteric plexus is located between the middle circular and the outer longitudinal layer.

Serosa is a thin outermost layer of loose connective tissue covered by simple squamous epithelium – mesothelium.

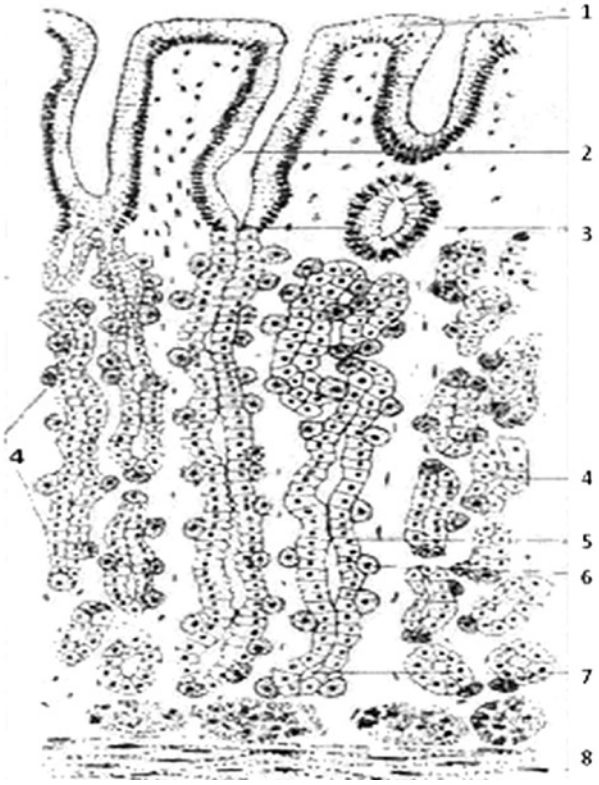

Fig. 5. The gastric glands in the fundus
1-surface lining cells, 2-pit, 3-isthmus of gland, 4-body of gland, 5- chief cells, 6-parietal cell, 7-fundus of gland, 8-lamina muscularis mucosae

Glands of the stomach

Histologically, three types of glands are recognized, depending on their location: the *cardiac glands*, found in the cardiac region, the *gastric* or *fundic glands*, having the widest distribution, and the *pyloric glands*, present in the pyloric region. Each gastric gland has an *isthmus* (a constricted part near the opening), a *neck* (the main part of the tubular gland) and a *fundus - base* (the dilated end of the gland). The gastric glands secrete HCl and digestive enzymes as well as primarily mucus and substances with endocrine and paracrine effect, though only regionally.

The gastric glands are composed of the following types of cells:

- *Chief (zymogenic) cells* – the most abundant secretory cells, located primarily deep in the body and the fundic portion of the tubular

gastric glands. These columnar or pyramidal cells display a basophilic cytoplasm (with an abundant rough endoplasmic reticulum), basally located nuclei and apically situated secretory granules containing the inactive enzyme *pepsinogen* that is released into the lumen. After being released into the acid environment of the stomach, pepsinogen is rapidly converted into the highly active proteolytic enzyme *pepsin*. In humans, the chief cells also produce gastric *lipase.*

- *Parietal (oxyntic) cells* – present mainly in the upper half of the gastric glands. They are the largest, rounded or pyramidal cells, with centrally located spherical nucleus and an eosinophilic cytoplasm. Parietal cells have abundant mitochondria (indicate their metabolic processes) and a smooth endoplasmic reticulum. They also have a deep invagination of the apical plasma membrane, forming intracellular canaliculi. In the resting cells, the tubulovesicular structures are in the apical region. At this stage, the cells have only a few microvilli. When the cells are stimulated, tubulovesicles fuse with the cell membrane to form the canaliculi and more microvilli. Parietal cells produce *hydrochloric* acid *(HCl)* and *gastric intrinsic factor*, a glycoprotein necessary for the absorption of vitamin B_{12} in the ileum.
- *Mucous neck cells* – present in clusters or as single cells between parietal cells in the neck of the gastric glands. They are irregular in shape (cuboidal or low columnar), with a basally located nucleus and secretory granules near the apical surface. These cells produce mucus.
- *Stem (regenerative) cells* – low columnar cells with oval nuclei, having a high rate of mitosis. Stem cells that move upward replace the pit and surface mucous cells and have a turnover time of 4 to 7 days. Stem cells that migrate more deeply into the glands differentiate into mucous neck cells, parietal, chief and enteroendocrine cells. These cells are replaced much more slowly than the surface mucous cells.
- *Enteroendocrine cells (DNES cells - diffuse neuroendocrine system cells, APUD cells -amine precursor uptake and decarboxylation)* – small cells that are dispersed among the other epithelial cells in the neck and base of the gastric glands. Endocrine cells manufacture endocrine, paracrine and neurocrine hormones, which are then released into the lamina propria. Peptide hormones produced by enteroendocrine cells have the following functions: regulation of water, electrolyte and

enzyme secretion, regulation of gastrointestinal motility and stimulation of the release of other peptide hormones. We recognize: *D cells - somatostatin producing cells*, which inhibit the release of hormones by DNES cells in its vicinity, *G cells - gastrin producing cells*, which stimulate HCl secretion, gastric motility and proliferation of regenerative cells in the stomach and are abundant in the pyloric region and *EC cells - enterochromaffin cells*, which produce *serotonin* (increases peristaltic movement) and *histamin* (stimulates HCl secretion).

Clinical correlations

Tumors, which arise from enteroendocrine cells are called *carcinoids* and are responsible for clinical symptoms caused by the overproduction of serotonin.

14.3.3.1. Cardia

The lamina propria contains simple or branched tubular *cardiac glands*, where we can find most of the mucous neck cells producing mucus and lysozyme (enzyme that attacks the bacterial wall) and a few parietal cells secreting HCl.

14.3.3.2. Fundus and body

The lamina propria of the fundus and body contains branched tubular *gastric glands*, which open into the bottom of the gastric pits. The gastric glands contain all types of cells located in the isthmus, neck and base. The isthmus of these glands contains mucous cells, undifferentiated stem cells and parietal (oxyntic) cells. The neck contains stem cells, mucous neck cells and parietal cells. The base primarily contains chief cells and parietal cells. Gastroenteroendocrine cells are dispersed in the neck and base of the gastric glands.

14.3.3.3. Pylorus

The pylorus has deep gastric pits extending approximately halfway down into the lamina propria, pyloric glands have shorter coiled secretory portions. The glands secrete mucus as well as enzyme lysozyme. The pyloric glands contain predominantly mucous cells, enteroendocrine cells that are intercalated among the mucous cells, *G cells,* which release gastrin and *D cells*, which secrete somatostatin.

Clinical correlations of the surface epithelial cells

The stomach normally contains an acid solution, but the mucosa is protected from the damaging effects of the acid by various mechanisms, including the presence of a thin layer of mucus on the surface of the epithelial cells. In some circumstances, the protective mechanisms break down and the acidic gastric contents damage the mucosa. Such damage leads to the formation of a shallow ulcer. Continued exposure leads to the formation of a deep or chronic ulcer. Stress, ingested substances such as aspirin, nonsteroidal anti-inflammatory drugs, ethanol and some microorganisms (e.g. Helicobacter pylori) can also disrupt the epithelial layer and lead to ulceration. An ulcer is a disruption of the mucosal integrity that leads to an excavation due to active inflammation.

Clinical correlations of parietal cells

Achlorhydria is characterized by the absence of parietal cells. Consequently, the gastric intrinsic factor is not secreted and this lack of intrinsic factor can lead to vitamin B_{12} deficiency with the subsequent development of *pernicious anemia* (a disorder of the erythrocyte – forming mechanism), usually caused by atrophic gastritis. Due to the fact that the liver has an extensive reserve of vitamin B_{12}, the disease is often not recognized for a long time after the actual deficiency of this vitamin.

14.3.4. Small intestine

The small intestine has three segments: *duodenum* (mainly retroperitoneal), *jejunum* and *ileum* (a movable intestinal segments suspended by a mesentery). Although these segments are similar histologically, their minor differences permit their identification.

The small intestine is the site of terminal food digestion, nutrient absorption and endocrine secretion, where the process of digestion is completed. The nutrients are absorbed by tall columnar epithelial cells called *enterocytes.* The inner surface of the small intestine is enlarged by the formation of plicae circulares, villi, microvilli and crypts of Lieberkühn.

Plicae circulares (Kerckring's valves) are transverse folds of mucosa and submucosa that form semicircular elevations into the lumen. These

permanent fixtures are present in the duodenum, jejunum, and the proximal half of the ileum. Plicae are most developed in the jejunum.
Villi are 0.5 – 1.5 mm long finger-like extensions of the lamina propria into the lumen. The core of each villus is a loose connective tissue containing capillary loops, blindly ending lymphatic vessels (lacteals vessels) and a few smooth muscle cells.
Microvilli are modifications of the apical plasmalemma of the columnar epithelial cells (enterocytes) covering the intestinal villi.
Crypts of Lieberkühn are simple tubular intestinal glands, which also increase the inner surface of the small intestine. These crypts are the invaginations of the epithelium into the lamina propria. They are composed of simple columnar epithelium (similar to surface absorptive cells), stem regenerative cells, goblet cells, Paneth's cells and enteroendocrine cells.

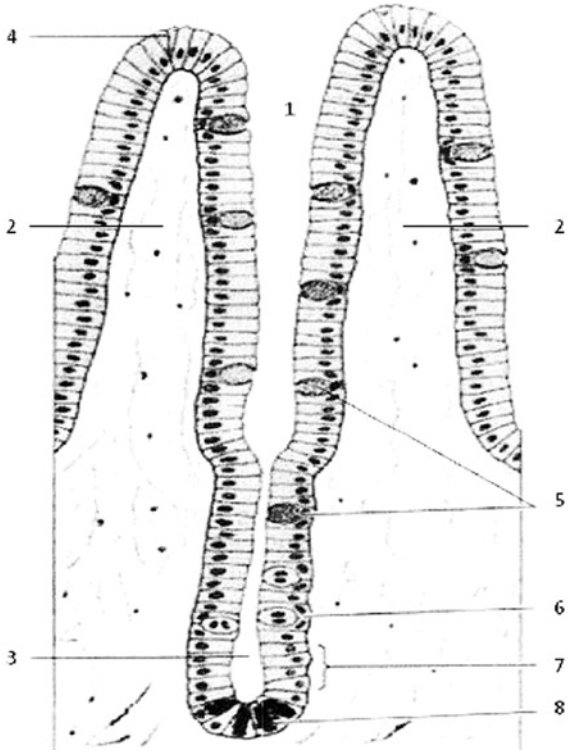

Fig. 6. Schematic diagram illustrating the villi and crypt of the small intestine
1-lumen, 2-villus, 3-crypt of Lieberkühn, 4-surface columnar epithelium, 5-goblet cells, 6-enteroendocrine cells, 7-stem cells, 8-Paneth´s cells

The wall of the small intestine consists of four layers: mucosa, submucosa, muscularis externa and serosa.

Mucosa – The intestinal mucosa is composed of three layers: simple columnar epithelium, lamina propria and lamina muscularis mucosae. The *simple columnar epithelium* covering the villi and is continuing into the intestinal glands (crypts). There are recognized – absorptive cells, goblet cells, stem cells, enteroendocrine cells and Paneth´s cells.

- *Absorptive cells* or *enterocytes* - the most numerous cells, tall columnar with an oval nucleus in the basal half of the cell. At the apex of each cell there are microvilli, approximately 1 μm long. Microvilli have an important physiological function of increasing the area of contact between the intestinal surface and the nutrients. The absorptive cells are covered with a thick glycocalyx layer. The glycocalyx layer not only protects the microvilli from autodigestion, but its enzymatic components participate also in the terminal digestion of dipeptides and disacharides into their monomers. The lateral cell membranes of the enterocytes form zonulae occludentes, zonulae adherentes, desmosomes and gap junctions with adjacent cells. An even more important function of the enterocytes is the absorption of water and nutrients produced by the digestive process. These cells re-esterify the fatty acids into triglycerides, form chylomicrons and transport the bulk of the absorbed nutrients into the lamina propria. Enterocytes have a short life span.
- *Goblet cells* – unicellular glands interspersed between the absorptive cells. The duodenum has the smallest number of goblet cells, while their number increases towards the ileum. These cells produce mucin, whose main function is to protect and lubricate the lumen of the intestine. Goblet cells have a short life span.
- *M cells (microfold cells)* – antigen presenting cells, specialized epithelial cells overlying the lymphoid nodules (more abundant above the Peyer's patches). M cells phagocytose and transport antigens from the lumen to the lamina propria. They represent an important link in the intestinal immunological system. The basement membrane under M cells is discontinuous, facilitating the transit between M cells and the lamina propria.

- *Regenerative cells* – stem cells that extensively proliferate to repopulate the epithelium of the crypts and the epithelium covering the villi.
- *Enteroendocrine cells, DNES cells* – various types of DNES cells represent approximately 1% of cells covering the villi and the lining of the crypts of the small intestine and produce paracrine and endocrine hormones. D cells produce somatostatin, enterochromaffin cells produced serotonin, G cells produced gastrin, while other cells produced secretin, neurotensin, cholecystokinin and motilin.
- *Paneth´s cells* – exocrine cells located in the basal portion of the intestinal crypts with secretory eosinophilic granules accumulated in their apical cytoplasm. These pyramid-shaped cells occupy the bottom of the crypts, manufacture the antibacterial agent lysozyme and may also play a role in controlling the intestinal flora. Unlike other cells of the intestinal epithelium, Paneth's cells have a comparatively long life span (20 days) and secrete lysozyme continuously.

The *lamina propria* forms the core of the villi and is composed of a loose and highly vascularized connective tissue. There are numerous intestinal crypts of Lieberkühn, also rich in lymphoid cells, which help protect the intestine. The lamina propria contains smooth muscle cells that are responsible for the rhythmic movements of the villi important for absorption. The lamina propria and the submucosa of the small intestine contain aggregates of lymphoid nodules known as *Peyer´s patches*, being most present in the ileum. Each patch consists of 10 – 200 nodules and is visible to the naked eye as an oval area. Instead of absorptive cells, its covering epithelium consists of *M cells*.

The *lamina muscularis mucosa* of the small intestine is composed of an inner circular and an outer longitudinal layer of smooth muscle cells. Muscle cells from the inner circular layer enter the villus and extend through its core to the tip, as far as the basement membrane. During digestion, these muscle cells rhythmically contract, thus shortening the villus.

Submucosa – The submucosa of the small intestine is composed of a connective tissue with a rich vascular and lymphatic supply and Meissner´s nerve plexus. The submucosa of the duodenum houses glands known as *Brunner´s glands (duodenal glands*). Brunner´s glands are branched, tubuloalveolar glands that secrete a mucous, alkaline fluid.

This fluid helps neutralize the acidic chyme that enters the duodenum from the pyloric region of the stomach. These glands also manufacture the polypeptide hormone *urogastrone* (known as human epidermal growth factor). Urogastrone inhibits the parietal cells and amplifies the rate of mitotic activity in the epithelial cells.

Muscularis externa **-** The muscularis externa of the small intestine is composed of an inner circular and an outer longitudinal layer of smooth muscle cells. The Auerbach´s myenteric nerve plexus is located between these two muscle layers. The muscularis externa is responsible for the peristaltic activity of the small intestine.

Serosa – Except for the second and third parts of the duodenum, the entire small intestine is invested by serosa.

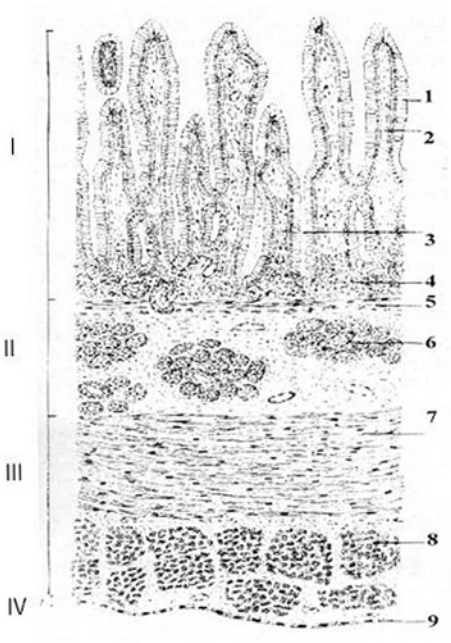

Fig. 7. Organization of the small intestine

I-mucosa, II-submucosa, III-muscularis externa, IV-serosa

1-surface columnar epithelium, 2-villus, 3-crypt of Lieberkühn, 4-lamina propria, 5-lamina muscularis mucosae, 6-glands of Brunner, 7-muscularis externa-circular, 8-muscularis externa-longitudinal, 9-mezotelium

Regional differences of the small intestine

The *duodenum* is the shortest segment of the small intestine. It receives bile from the liver and digestive juices from the pancreas. The duodenum differs from the jejunum and ileum in that its villi are broader, taller and more numerous. The duodenum has fewer goblet cells than the other segments and it has *Brunner´s glands* in the submucosa.

The *jejunum* has narrower, shorter and sparser villi than the duodenum. The number of goblet cells is bigger than in the duodenum.

The *ileum* has the sparsest, shortest and narrowest villi of the three regions of the small intestine. The lamina propria of the ileum houses lymphoid nodules known as *Peyer´s patches*.

Histophysiology of the small intestine

The main functions of the small intestine are digestion and absorption, while it also exhibits immunological and secretory activities and participates in the movement of the chyme along the small intestine.

The chyme that enters the duodenum is digested by enzymes produced by the glands of the oral cavity and the stomach. Digestion is intensified in the duodenum by enzymes from the exocrine pancreas. In the small intestine, the final breakdown of proteins and carbohydrates occurs. Lipids are emulsified by bile salts into small fat globules that are then split into monosacharides and fatty acids. The surface absorptive cells absorb approximately 6 to 7 l of fluid, 30 to 35 g of sodium, 0.5 kg of carbohydrates and proteins and 1 kg of fat every day.

Secretory activity of the small intestine – the glands secrete mucus. Brunner´s glands and crypts of Lieberkühn produce almost 2 l of slightly alkaline fluid per day. The DNES cells of the small intestine produce numerous hormones that affect the movement of the small intestine and regulate HCl secretion and release of pancreatic secretion.

The smooth muscle of the small intestine participates in its movement.

The mucosal surface of the gastrointestinal tract is exposed to many microorganisms. The first protective barrier against the penetration of these microorganisms is formed by the intercellular tight junctions made by the epithelial cells. In addition, the gastrointestinal tract contains plasma

cells, macrophages and a very large number of lymphocytes located in the mucosa and submucosa. The ileum contains permanent clusters of lymphoid nodules known as *Peyer´s patches*.

14.3.5. Large intestine

The large intestine is subdivided into the *cecum, colon, rectum and anus*; the *appendix* being a small, blind process of the cecum. The cecum and the colon have an identical histological structure. The wall of the large intestine is composed of the same four layers as the stomach and the small intestine: mucosa, submucosa, muscularis and serosa or adventitia.

Mucosa – The mucosa of the large intestine has neither folds, except in its distal portion (rectum), nor villi. The crypts of Lieberkühn are long and lined with highly abundant goblet cells, less absorptive cells and a small number of enteroendocrinne cells. These crypts also contain regenerative cells, whose rapid mitotic activity replaces the epithelium of the crypts and the mucosal surface cells every 6 to 7 days. No Paneth's cells are present in the crypts of the large intestine. The histological structure of the large intestine is well suited to carry out its main functions: absorption of water, formation of the fecal mass and production of mucus. Mucus lubricates the intestinal surface, thus facilitating the movement of feces and covers bacteria and particulate matter.

The lamina propria of the large intestine extends between the densely packed straight intestinal crypts. It is rich in lymphoid cells and lymphoid nodules that frequently extend into the submucosa. This richness in lymphoid tissue is related to the abundant bacterial population of the large intestine.

S*ubmucosa* – The submucosa is composed of a loosely arranged connective tissue. As elsewhere, it contains blood and lymphatic vessels, Meissner´s nerve plexus and lymphatic follicles.

Muscularis externa – Muscularis externa comprises of an inner circular and an outer longitudinal layer. The circular layer is typically as all other parts of the digestive tube. The outer longitudinal layer is not continuous along the surface, but rather gathered into three narrow bands known as *taeniae coli*. The constant tonus maintained by the taeniae coli puckers the large intestine into sacculations called *haustra coli***.** The Auerbach´s nerve plexus lies in the connective tissue separating the two muscle layers.

Adventitia and serosa – The colon has parts situated both retroperitoneally and intraperitoneally. The intraperitoneal part has serosa, a loose connective tissue with mesothelium, displaying numerous adipose tissue processes - the *appendices epiploicae*. The retroperitoneal part has adventitia.

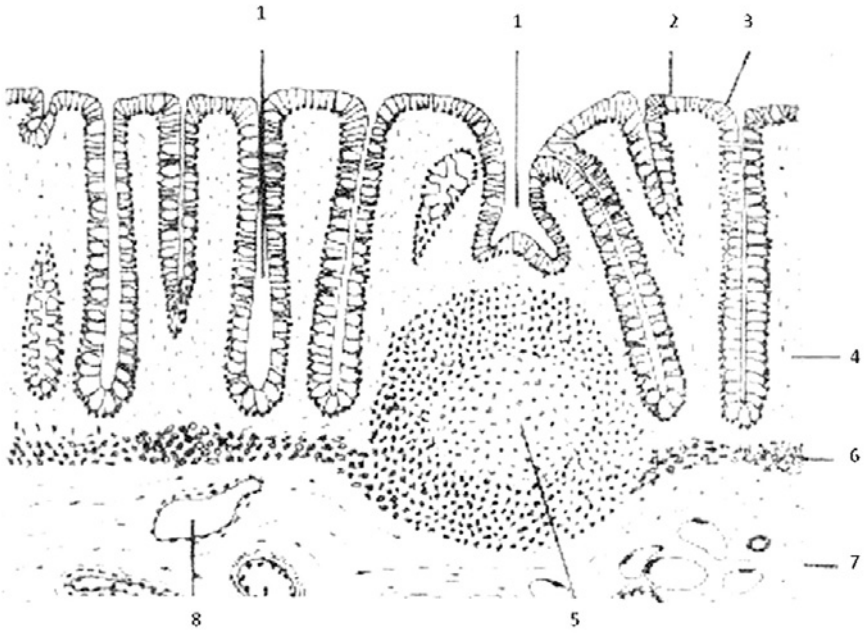

Fig. 8. Schematic diagram of the large intestine
1-crypts of Lieberkühn, 2-goblet cell, 3-surface columnar epithelium, 4-lamina propria, 5-solitary lymph nodule with germinal center, 6-lamina muscularis, 7-submucosa, 8-lymphatic vessel

14.3.5.1. Rectum and anal canal

The histological structure of the rectum resembles that of the colon, but the crypts of Lieberkühn are deeper and their number is lower. The crypts contain columnar absorptive cells and goblet cells.
The anal canal is a constricted continuation of the rectum.
The mucosa displays longitudinal folds - anal columns (rectal columns of Morgagni). About 2 cm above the anal opening, simple columnar epithelium is replaced by stratified squamous epithelium. The lamina propria is a fibroelastic connective tissue, which houses anal glands at the rectoanal junction and circumanal glands at the distal end of the anal

canal. The lamina propria contains hair follicles and sebaceous glands. The muscularis mocosae is composed of an inner circular and an outer longitudinal layer of smooth muscle cells.
The submucosa of the anal canal is composed of fibroelastic connective tissue and contains a plexus of large veins. There is an internal hemorrhoidal plexus and an external hemorrhoidal plexus.
The muscularis externa consists of an inner circular and an outer longitudinal layer of smooth muscle cells. The inner circular layer becomes thickened to form the *internal anal sphincter muscle*. The outer longitudinal layer continues, surrounding the internal anal sphincter. The skeletal muscle of the floor of the pelvis forms the *external anal sphincter muscle* that surrounds the internal anal sphincter. The external anal sphincter is under a voluntary control and exhibits a constant tonus.

Clinical correlations

An excessive dilatation of the vessels of the submucosal venous plexuses of the anal canal results in the formation of *hemorrhoids*, a condition common in people older than 50 years of age and during pregnancy. This condition may manifest as painful defecation, appearance of fresh blood with defecation and anal itching.
Malignant tumors in the large intestine are usually derived from its glandular epithelium *(adenocarcinomas).*

Histophysiology of the large intestine

The main functions of the large intestine are the absorption of water, electrolytes and gases, formation of fecal mass and production of mucus. The absorption of water is passive, following the active transport of sodium out of the basal surface of the epithelial cells. Feces are composed of water, dead bacteria, roughage, fat, inorganic substances, undigested protein, dead cells and bile pigment. The odour of feces varies with the individual and is the result of diet and bacterial flora, which produce variable amounts of indole, hydrogen sulfide and mercaptans. Bacterial byproducts include riboflavin, vitamin B_{12}, thiamin and vitamin K. Bacterial action in the colon produces gases released as *flatus.* The large intestine holds 7 to 10 l of gas each day, of which only 0.5 to 1 l is expelled as flatus, while the remainder gets absorbed by the lining epithelium.

The large intestine also secretes mucus and HCO_3-. Mucus facilitates the compaction of feces, while HCO_3- adheres to the mucus and acts as a buffer, protecting the mucosa.

14.3.5.2. Appendix

The appendix is a 5 to 6 cm long diverticulum of the cecum. Its general structure is similar to that of the large intestine, though it contains fewer and shorter crypts of Lieberkühn. The appendix has a relatively small, narrow and irregular lumen that is usually occupied by debris. The mucosa of the appendix is composed of simple columnar epithelium consisting of surface absorptive cells, goblet cells and M cells. The lamina propria is a loose connective tissue with shallow crypts of Lieberkühn. The crypts are lined with surface absorptive cells, goblet cells, regenerative cells, numerous DNES cells and infrequent Paneth´s cells. The lamina propria and submucosa contain numerous big lymphoid nodules and occasionally also fatty cells. The appendix has no teniae coli and is invested by a serosa.

Clinical correlations

The appendix frequently becomes the site of inflammation, *appendicitis*, which is more common in teenagers and younger people and also occurs more frequently in men than in women. Appendicitis is usually caused by the obstruction of the lumen. The clinical signs are nausea and vomiting, fever, tense abdomen and elevated leukocyte count. If the condition is not treated within 1 to 2 days, the appendix may rupture and lead to the peritonitis.

14.4. Cell renewal in the alimentary canal

The epithelial cells of the gastrointestinal tract are replaced with new cells produced by mitosis of the stem cells. This mitotic activity provides for a continuous cell renewal. In the esophagus, the stem cells are located in the basal layer of the esophageal epithelium. In the stomach, the surface mucous cells are renewed every 3 to 5 days by the mitotic activity of cells in the neck of the gastric glands. The surface mucous cells are ultimately shed into the stomach lumen and substituted with new cells. In the small intestine, the stem cells are located in the lower half of

the intestinal crypts. The renewal time for the absorptive and goblet cells is 5 to 6 days. Enteroendocrine cells and Paneth cells are also derived from the stem cells and they live approximately 4 weeks. All epithelial cells in the large intestine arise from stem cells located at the bottom of the intestinal crypts. The turnover time is about 6 days for the absorptive cells and goblet cells and 4 weeks for enteroendocrine cells. The newly produced cells from this proliferative zone in each region move and undergo maturation and replace cells population of each region, and then are ultimately shed into the lumen.

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15. Glands associated with digestive system

In the oral cavity are: salivary glands. In the abdominal cavity are: liver and pancreas.

15.1. Salivary glands

Salivary glands are exocrine glands producing saliva which has digestive, lubricating and immunologic function. The glands are divided according anatomical position and histological structure to minor and major salivary glands.

15.1.1. Minor salivary glands

Minor salivary glands are located in the lamina propria in the oral mucosa**,** have short ducts and secrete saliva continuously.

- labial – seromucous gl.
- buccal – seromucous gl.
- palatine – seromucous gl.
- retromolar – seromucous gl.
- lingual: a. seromucous apical gl.
  b. mucous Weber´s gl. (close to the lingual tonsil)
  c. serous Ebner´s gl. (close to the vallate papilla)

15.1.2. Major salivary glands

Major salivary glands are paired glands with long ducts that empty to oral cavity, secrete saliva after gustatory or olfactive stimulation.

- *parotid* – serous gl.
- *submandibular* **–** seromucous gl. - serous cells predominate
- *sublingual* – seromucous gl. - mucous cells predominate

15.1.3. Function of the salivary glands

Salivary glands produce saliva about 1.2 L per day. Saliva contains: water, mucus, enzymes, ions, antibodies.

Function of saliva:

- lubrication of the oral cavity

- moistening of dry food to aid swallowing
- initial digestion of carbohydrates by α-amylase
- buffering of the content in oral cavity by bicarbonate ions
- chemical stimulation of taste buds by dissolved food
- immune defence (IgA produced by plasma cells)
- antibacterial effect (lysozyme and lactoferrin act against bacteria)

15.1.4. Structure of the salivary glands

Salivary glands consist of secretory portion and duct system.

Secretory portion:

Secretory portion consist of two types of *secretory cells*: serous and mucous (Fig. 1).

1. Serous cells are protein secreting cells, organized into the *acini.* Acinus is an oval structure with narrow lumen in the centre. Serous cells are pyramidal in shape with a broad base resting on the basement membrane and a narrow apical surface facing to the lumen of the acinus.

In the light microscope the cytoplasm of basal compartment of serous acinar cells is basophilic because of accumulation of rough endoplasmic reticulum (rER) and free ribosomes. Apical part of the cell contains secretory granules containing enzymes that are stained with eosin. Oval nucleus is centrally located.

Plasma membrane infoldings are present at the basal side of cells in the transmission electron microscope (TEM). Neighbouring cells are connected by junctional complexes. Most organelles (rER, Golgi apparatus, mitochondria) are located in the basal and perinuclear cytoplasm.

2. Mucous cells are mucus (glycoprotein) producing cells usually of cuboidal or columnar shape. Their nuclei are oval and pressed at the basal part of the cell. Mucous cells are organized as *tubules*.

In the light microscope the apical cytoplasm usually appears pale because of accumulation of mucus containing granules. Because the mucus is lost in H&E–stained paraffin sections, the apical portion of the cell usually appears pale.

In TEM preparation, the rER, mitochondria and Golgi apparatus are present at the basal cytoplasm and mucus granules are accumulated in the apical part of the cell.

Around the serous acini and mucous tubules are myoepithelial cells. *Myoepithelial cells* are contractile cells with numerous processes resting on the basement membrane of secretory epithelial cells and also of the epithelial cells of proximal part of duct system (intercalated and striated). Myoepihelial cells after their contraction help to move secretory material toward excretory duct. In the light microscope it is possible to distinguish only their flattened nuclei near the basement membrane of epithelium.

Duct system:

The lumen of the secretory parts - acinus or tubule is continuous with the duct system ordered in this sequence - intercalated duct, striated duct, interlobular duct, and main duct.

Intercalated ducts are continuous with the lumens of serous acini. They are lined by low cuboidal epithelial cells with eosinophilic cytoplasm. Several of these ducts join to form intralobular - striated duct.

Striated ducts are lined by simple cuboidal epithelium that becomes columnar epithelium when it approaches interlobular duct. Cytoplasm of the duct epithelium is eosinophilic, oval nuclei are centrally located. There are infoldings of the basal plasma membrane („striations“) of the cell associated with elongated mitochondria. Striations are morphologic and functional specialization for active ion transport.

Function of intercalated ducts:

- secretion of bicarbonate ions into the acinar product
- absorption of Cl^- from the acinar product

Function of striated ducts:

- reabsorption of Na^+ from the primary secretion
- secretion of K^+ and HCO_3^- into the secretion

If more Na^+ is resorbed than K^+ is secreted, the saliva becomes *hypotonic.*

Serous glands have well-developed intercalated and striated ducts that help to modify the serous secretion (change concentration of ions to release hypotonic saliva).

Mixed glands with predominance of mucus secretion have no intercalated and less striated ducts, because their secretion is not modified.

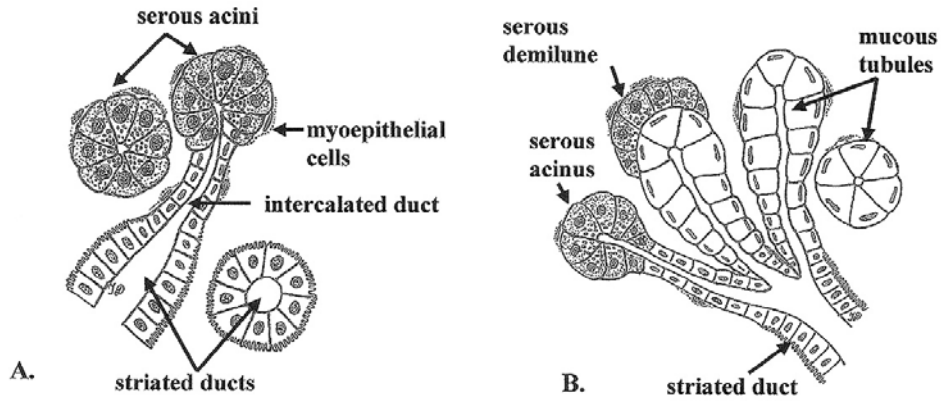

Fig. 1. Salivary glands. Schematic drawing of the salivary gland with secretory portions and duct system in transverse and longitudinal sections. Serous acini are composed of pyramidal cells with rounded nuclei. The apical pole of cells contains protein-rich secretory granules. Mucous tubules are composed of pale stained mucous cells with flattened nuclei at the basal pole. Short intercalated ducts are lined by simple cuboidal epithelium. The striated ducts are composed of simple columnar epithelium with basal striations.

The *major salivary glands* are paired glands. The glands are enclosed in a *capsule* of connective tissue and internally are divided into *lobules* by *connective tissue septa*. Blood vessels and nerves enter the glands at the hilum and gradually branch out into the lobules.
There are three major salivary glands: parotid gland, submandibular gland and sublingual gland.

A. Parotid gland is a serous, branched acinar gland in humans.

It is situated outside of the oral cavity, located subcutaneously, below and in front of the ear. The main excretory duct – *parotid duct* opens at the parotid papilla on the mucosal surface of the cheek opposite the second upper molar tooth.

Secretory lobules are composed of serous acini, numerous intercalated ducts and distinct branched striated ducts. There are connective tissue septa between lobules that contain interlobular ducts. Large amount of adipose tissue is a typical feature of the parotid gland.

B. Submandibular gland is a branched tubuloacinar gland. It is mixed, seromucous gland with predominating serous acini.

The gland produces about 70% of the whole saliva secretion. The gland is located in the submandibular triangle of the neck. The excretory *submandibular duct* opens at a small prominence (sublingual caruncle) on the floor of the oral cavity. Secretory lobules are composed of numerous serous acini, less amount of mucous tubules and numerous striated ducts. The mucous tubules are often capped with a *serous demilune*, a layer of serous cells resembling a half moon (Fig. 1). Excretory activity is stimulated both by sympathetic and parasympathetic inervation.

C. Sublingual gland is a branched tubuloacinar gland. It is mixed seromucous gland with predominating mucous tubules.

The gland is located inferior to the tongue at the floor of the oral cavity. It has a number of small and short ducts - *sublingual ducts* that empty to the submandibular duct or directly into the oral cavity.

The sublingual gland consists mostly of mucous tubules capped with serous demilunes. Intercalated ducts are rare and striated ducts are short.

Special sensory fibers of the chorda tympani (branch of facial nerve) provide taste sensation from the anterior two-thirds of the tongue.

Innervation of major salivary glands. Although the facial nerve (cranial nerve VII) passes through parotid gland, it does not supply its parasympathetic innervation. Parotid gland receives its *parasympathetic input* from the glossopharyngeal nerve (CN IX). Submandibular and sublingual glands receive their *parasympathetic input* from the facial nerve (CN VII). Direct *sympathetic* innervation of the salivary glands takes place via preganglionic nerves in the thoracic segments T1-T3. Parasympathetic stimulation produces water-rich, serous saliva. Sympathetic stimulation leads to the production of a low volume, enzyme-rich saliva.

15.1.5. Development of the salivary glands

Secretory part and duct system developes from oral cavity epithelium. Initially, the gland grows as solid cords of cells into the mesenchyma.

Parotid gland is of ectodermal origin. Submandibular and sublingual glands are of endodermal origin.

Clinical correlations

Tumors of salivary glands are adenomas of epithelial origin, carcinomas originated from ducts or connective tissue tumors.

Parotid gland: mumps (parotitis) is a painful swelling of parotid gland caused by the mumps virus. This viral infection can also damage facial nerve.

15.2. Liver

The liver is the largest organ in the human body, weighing 1.5 kg. It is located in the upper right and partially upper left quadrants of the abdominal cavity, protected by the ribcage.

15.2.1. Structure of the liver

*Connective tissue stroma***.** The liver is covered by *connective tissue capsule (Glisson´s capsule***)** that becomes thicker at the hilum, where the portal vein and hepatic artery enter the liver and hepatic ducts and lymph vessels exit. Branches of the blood vessels and ducts are surrounded by a small amount of connective tissue found in *the portal spaces* between the liver lobules. Network of delicate *reticular fibers* surrounds the hepatocytes and sinusoidal capillaries.

Parenchyma. Liver cells – *hepatocytes* are arranged in plates one or two cell thick and are separated by sinusoidal capillaries (Fig. 3).

Hepatocytes are large, polygonal cells measuring 20 – 30 μm. They constitute about 80% of the cell population in the liver. The cytoplasm of hepatocytes is generally eosinophilic. In the light microscope presence of the rough endoplasmic reticulum (rER) is visible like basophilic granulation in the cytoplasm. Accumulation of glycogene in the hepatocytes gives foamy appearance to the cytoplasm after hematoxylin eosin staining. Glycogene granules are PAS positive. The cells have one or two nuclei and prominent nucleoli.

- In TEM cytoplasm of hepatocytes is rich in cisternae of *rER* and multiple *Golgi complexes* (50/cell). Those organelles are involved in protein synthesis (albumin, globulins, fibrinogen, prothrombin, enzymes) and bile secretion.
- Numerous *mitochondria* (1000 per cell) are necessary for energy metabolism.

- Hepatocytes have many of *peroxisomes* (300/cell) involved in degradation of hydrogene peroxide; peroxisomes contain also enzymes for detoxification of alcohol, brake down of fatty acids, gluconeogenesis.
- *Smooth endoplasmic reticulum* (sER) may be extensive, but varies with metabolic activity (contains enzymes for degradation of toxins, drugs, alcohol; synthesis of cholesterole, lipids). The sER undergo hypotrophy after administration of alcohol, phenobarbital, anabolic steroids, chemotherapeutic agents used to treat cancer.
- *Lysosomes* of hepatocyte may contain pigment granules (lipofuscin), digested cytoplasmic organelles, iron (ferritin).

Hepatocytes are arranged in plates that anastomose with one another. The cells are polygonal in shape and their sides can be in contact either with sinusoids *(sinusoidal face)* or neighboring hepatocytes *(lateral faces).* A portion of the lateral faces of hepatocytes is modified to form *bile canaliculi* (bile capillaries). Their wall is formed by hepatocyte plasmalema projecting to the lumen with microvilli (Fig. 2).
Microvilli are present abundantly on the *perisinusoidal spaces (spaces of Disse).*

- Perisinusoidal space lies between the hepatocytes and sinusoids and serves for exchange of metabolites between blood and liver cells. *Sinusoids* are low pressure vascular channels that receive blood from terminal branches of the hepatic artery and portal vein at the periphery of liver lobules and deliver it into central veins. Sinusoids are fenestrated with incomplete basal lamina, lined with endothelial cells.
- The wall of sinusoids is populated by numerous *Kupffer cells,* a type of fixed macrophages. Their functions are to metabolize aged erythrocytes, digest haemoglobin (this function is gratelly increased after splenectomy).
- The other cell-types in perisinusoidal space are the stellate cells *(Ito cells).* They store fat and vitamin A. In pathologic conditions, they differentiate into myofibroblasts and synthesize collagen.
- *Hepatic lymph* originates in the perisinusoidal space. As blood flows through the sinusoids, a considerable amount of plasma is filtered into the space between endothelium and hepatocytes (the space of Disse), providing a major fraction of the body's lymph.

15.2.2. Blood supply

A. Functional blood supply

About 75% of the blood entering the liver is venous blood from the portal vein. *Portal vein* branches repeatedly to small venules called *interlobular veins – distributing vv.* (run arround the periphery of the lobules) that branch to the *sinusoidal capillaries* (run between plates of hepatocytes) – and to the *central veins. Sublobular vv.* (collect blood from the central veins) converge to form – *hepatic vv*. that leave the liver and empty to the *inferior v. cava.* Portal system conveys blood from the stomach, pancreas and spleen and blood containing nutrients absorbed in the intestines. Nutrients are accumulated and metabolized in the liver. Toxic material is neutralized and eliminated.

B. Nutritive blood supply

The remaining 25% of the blood supply in the liver is an arterial blood from the hepatic artery. *Hepatic artery* sends branches in the same course as the veins**.** *Interlobular aa. – distributing aa. – sinusoidal capillaries – central veins*. Arterial and portal venous blood is mixed in the sinusoidal capillaries. The main function of the arterial system is to supply liver cells with oxygen.

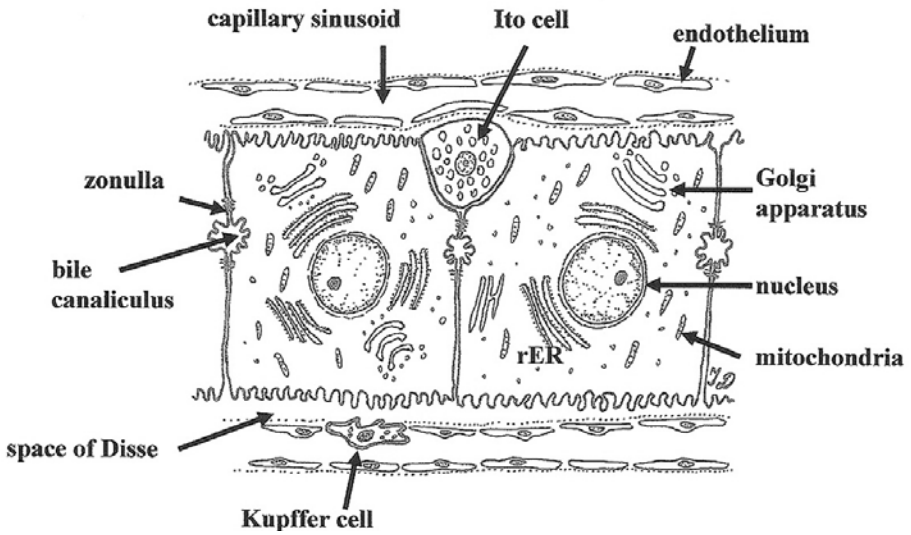

Fig. 2. Liver. Schematic structure of hepatocytes shows organelle-rich cytoplasm and centrally placed oval nuclei. The surface of hepatocytes is in contact with the sinusoids, through the space of Disse. Between neighbouring hepatocytes are tubular spaces – bile canaliculi, the first portion of the bile duct system.

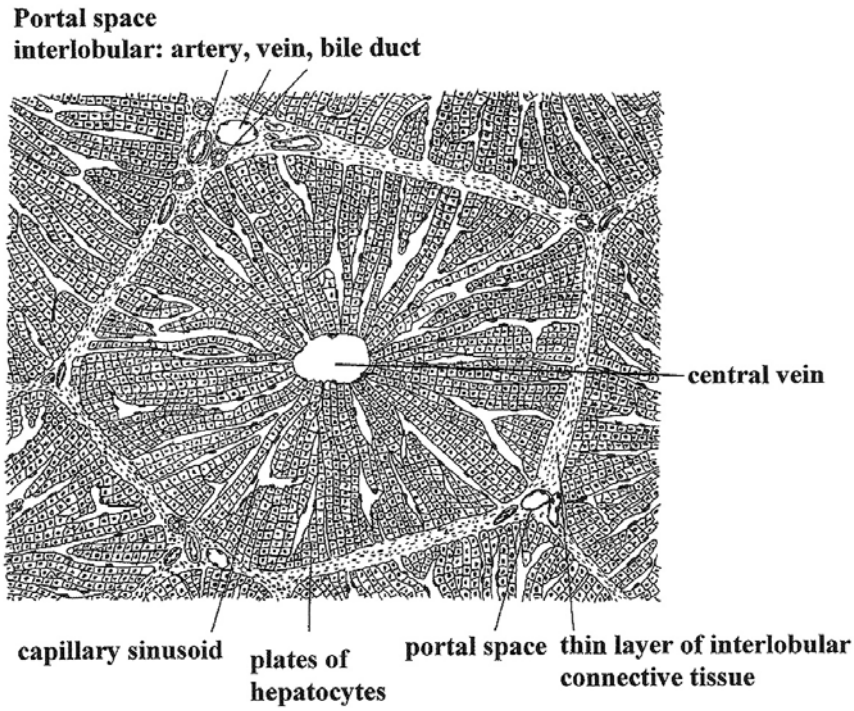

Fig. 3. Liver. Classic liver lobule has hexagonal shape. In the centre is the central vein, plates of hepatocytes are radially arranged. Portal space contains interlobular artery, vein and bile duct.

15.2.3. Morphologic and functional units of the liver

Liver lobule

Basic structural unit of the liver is the *classic liver lobule* (Fig. 4). Classic liver lobule has a polygonal (hexagonal) shape with centrally located thin-walled *central vein (CV)* and anastomosing plates of hepatocytes arranged radially from the central vein to the periphery of the lobule. Plates of hepatocytes are separated by sinusoidal capillaries that drain into the central vein. In healthy human liver, lobules are not separated by connective tissue. Small amount of interlobular connective tissue present at the angles of the neighbouring liver lobules is called *portal space (PS)* in which *portal triad* is situated. Human liver contains three to six portal spaces per one lobule, each contains three structures: *interlobular vein* (a branch of the portal vein), *interlobular artery* (a branch of hepatic artery) and *interlobular bile duct.*

Portal lobule

Portal lobule is the *exocrine functional unit* of the liver. The major exocrine function of the liver is a bile secretion. The portal lobule is a triangular block of the tissue (Fig. 4). Its outer margins are imaginary lined between the three central veins (CV – CV – CV) with centrally located portal triad (PS). The bile is drained from the three adjoining liver lobules into the interlobular bile duct.

Liver acinus

Liver acinus is *the smallest structural unit of the liver parenchyma showing correlation between blood perfusion, metabolic activity and liver pathology.* Liver acinus is supplied by circumlobular vein between two portal spaces (PS). It has diamond-shape limited by angles of area CV-PS-CV-PS (Fig. 4). According the proximity of the hepatocytes to the circumlobular vein the liver acinus is subdivided into three zones. Cells in zone I are the first to receive oxygen, nutrients and toxins. It means that a variety of pathologic processes lead to lesions that reflect acinar structure; for example, necrosis of hepatocytes at the periphery of the acinus.

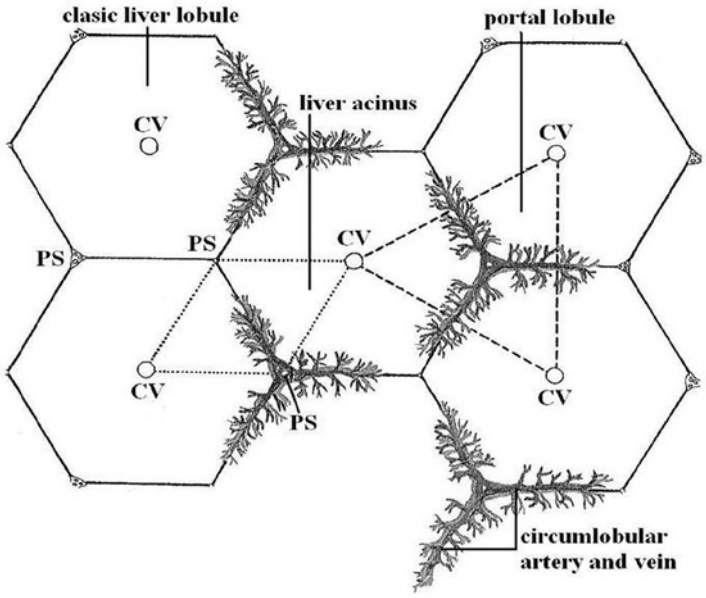

Fig. 4. Liver. Schematic drawing illustrating the territories of the classic liver lobules, portal lobules and liver acini.

Function of the liver

- plays an important role in the uptake, metabolism, storage and distribution of nutrients (carbohydrate, lipid, cholesterol metabolism) and vitamins (A, D, K)
- liver produces most of the body circulating plasma proteins (albumin, globulins, lipoproteins, glycoproteins, prothrombin, fibrinogen)
- degrades drugs and toxins
- liver has exocrine function, produces bile and releases it by the duct system to the gallbladder where it is concentrated. Bile is excreted to the duodenum by bile duct. Bile salts emulsify lipids and help their digestion and absorption in the gut.

15.2.4. Biliary tract and gallbladder

The bile produced by the liver cells flows through the:

- Intrahepatic ducts: *bile canaliculi* (between hepatocytes), *bile ductules of Herring* (at the periphery of the lobules), *interlobular bile ducts* (in the portal space) join to form *right* and *left hepatic ducts* that join to form the *common hepatic duct* at the hilum of the liver.
- Extrahepatic ducts carry the bile to the gallbladder by *cystic duct* and to the duodenum by *bile duct.*

The *gallbladder* is another important structure in the biliary system. This is a sac-like structure adhering to the liver which has a duct (cystic duct) that leads the bile directly into the bile duct. When bile is not flowing into the intestine, it is stored in gallbladder. The gallbladder *concentrates* and *stores the bile.*

Microscopic structure of the gallbladder

In empty gallbladder *tunica mucosa* forms mucosal folds lined with *simple columnar epitheli*um. This epithelium is specialized for water and ion absorption. The *lamina propria* is a loose connective tissue rich in fenestrated capillaries and venules. Mucous glands are present in the neck region of lamina propria mucosae.

Tunica muscularis is composed of spirally arranged smooth muscles connected by collagen and elastic fibers. Contraction of the muscles helps to transport bile to the cystic duct.

Tunica subserosa is present externally to the tunica muscularis. This layer contains large blood vessels and autonomic nerves in the connective tissue. *Tunica adventitia* is present at the surface where gallbladder is attached to the liver. The unattached surface is covered by a *tunica serosa* (visceral peritoneum) consisting of loose connective tissue and *mesothelium*.

15.2.5. Development of the liver

Liver and gallbladder are developed from *endodermal* evagination of the *foregut.* Hepatocytes and duct system are of endodermal origin. Connective tissue is of mesenchymal origin. Kupffer cells are derived from the monocytes. Ito cells are derived from the mesenchyme.

Clinical correlations

The most common diseases of the liver include: infections such as hepatitis A, B, C, E, alcohol damage, fatty liver, cirrhosis, cancer, drug damage (especially paracetamol). However, the liver parenchyma has a great capacity to regenerate.

Abnormal production of bile acids may lead to the formation of gall stones (cholelithiasis) that can block bile flow.

15.3. Pancreas

The pancreas is an elongated gland that lies in the epigastrium. Anatomically is divided to head, body and tail. Expanded *head* part lies in the C-shaped curve of the duodenum. It is joined to duodenum by connective tissue. The *body* lies behind the stomach and the *tail* is in contact with the spleen.

The pancreas is an exocrine and endocrine gland.

- The exocrine portion produces and releases digestive enzymes into the duodenum.
- The endocrine portion synthesizes and secretes hormones glucagon and insulin into the blood. These hormones regulate metabolism of glucose, lipids and proteins.

The exocrine pancreas is a *serous acinar gland*. Its structure resembles the parotid gland. The distinct features are: absence of striated ducts in the pancreas and cell masses of endocrine tissue (*Langerhans islets*).

15.3.1. Structure and function of exocrine portion

The secretory units are serous acini lined by simple columnar epithelium of pyramidal-shaped serous cells with narrow luminal surface and broad base surface. These cells have spherical nucleus, and are typical protein secreting cells. Basal part of the cell contains a lot of cisternae of rER and free ribosomes that give basophilic staining of the cytoplasm. Well developed Golgi apparatus is present at the apical cytoplasm and is involved in packing of secretory granules. The number of eosinophilic zymogene granules (inactive enzymes) is present in apical part of cytoplasm (Fig. 5). Serous cells produce different digestive enzymes and proenzymes:

- proteolytic peptidases: trypsinogen, chymotrypsinogen, procarboxypeptidase, proaminopeptidase
- nucleolytic enzymes: ribonuclease, deoxyribonuclease
- lipases: triacylglycerol lipase, phospholipase
- elastase
- amylolytic enzymes: α-amylase

The pancreatic enzymes are activated only after they reach the lumen of the small intestine (e.g. intestinal absorptive cells convert trypsinogen to trypsin; trypsin then catalyzes the conversion of the other inactive enzymes).

Duct system of the exocrine pancreas

Another characteristic detail is the presence of *centroacinar cells* in the centre of the serous acini. The centroacinar cells constitute the beginning of the *intercalated duct* that lies outside the acinus (Fig. 5). Intercalated ducts are lined by simple flattened or low cuboidal epithelium and drain into *intralobular ducts*. Intralobular ducts are lined by simple cuboidal epithelium. There are no striated ducts in the pancreas.

Branching network of intralobular ducts drains into the larger *interlobular ducts,* which are lined by simple columnar epithelium. Interlobular ducts run in connective tissue septa and drain into *main pancreatic duct*, which runs in long axis of the pancreas and empties to the duodenum at the major duodenal papilla. A second large duct is the *accessory pancreatic duct*, empties at the minor duodenal papilla.

- Pancreas secretes about 1 L of fluid per day. The acini secrete a small volume of enzyme-rich fluid. The *intercalated ducts add a large volume of fluid rich in sodium and bicarbonate.* Bicarbonate serves to

neutralize the acidity of the chyme (partially digested food) and by this way establish the optimal pH for the activity of pancreatic enzymes.

- *Pancreatic secretion is under hormonal control* of enteroendocrine cells of the duodenal mucosa (secretin, cholecystokinin) and *neural control* (vagus nerve)**.**

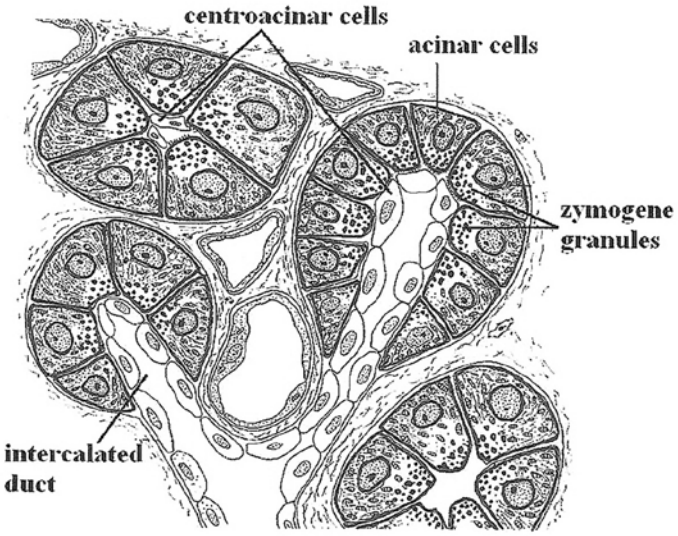

Fig. 5. Pancreas. Exocrine portion is formed by acini lined by columnar cells rich in rER at the basal pole of the cell. Apical pole of the acinar cell is rich in inactive enzymes – zymogene granules. Centroacinar cells are continuous with low cuboidal epithelium of intercalated ducts.

15.3.2. Structure and function of endocrine portion

Langerhans islets are clusters of endocrine cells embedded in exocrine tissue. The islets constitute about 1 to 2% of the whole volume of the pancreas. Islets are composed of a few or hundreds of cells. Polygonal cells are arranged in irregular cords surrounded by rich network of fenestrated capillaries. In H&E staining cells of Langerhans islets appear pale surrounded by more intensely stained exocrine tissue. Different types of cells can be distinguished by Malory-Azan staining and immunohistochemical methods. *A-cells* produce *glucagon, B-cells* produce *insulin, D-cells* produce somatostatin, *PP-cells* produce *pancreatic polypeptide; and ghrelin-secreting cells.* Hormones are secreted directly into the blood flow.

15.3.3. Development of the pancreas

The pancreas develops from the caudal part of the foregut, therefore acinar cells, duct system and endocrine cells are of endodermal origin. The fetal pancreas begins to secrete insulin at 10 weeks.

Clinical correlations

The most common diseases of the pancreas include: acute and chronic pancreatitis – inflammatory condition of the exocrine part of the pancreas that results from the injury to acinar cells.

Pancreatic cancer is a malignant neoplasm. About 95% of exocrine pancreatic cancers are adenocarcinomas with high mortality rate.

Diabetes mellitus type I is a chronic autoimmune disease, in which the immune system acts against insulin-secreting B cells.

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16. Lymphatic tissue and organs

Anatomy introduction

Lymphatic system is closely interrelated anatomically and physiologically to the circulatory system. It is widely dispersed in the body and fills variety of functions, including the drainage of tissue fluid, the removal of foreign matter and the immune responses of the lymphocytes and other cells. It consists of lymphatic vessels, lymph nodes, and the lymphatic organs - the spleen and the thymus. Lymph from most tissues is clear and colorless. The lymph from the small intestine is dense and milky, due to the presence of lipid molecules. In this case the lymph is called *chyle.* Lymphatic capillaries are present in most tissues and organs except avascular structures, which are: epidermis, hair, nails, cornea, lens, corpus vitreum and cartilages. The exception concerns also the central nervous system and bone marrow. Lymphatic capillaries joint into larger vessels which pass to lymph nodes.

The lymph nodes are arranged in regional groups. Each regional group has its region of drainage. Through the major collecting ducts (*thoracic duct* and *right lymphatic duct*) lymph finally returns to venous system.

The spleen is situated in the upper left hypochondric region of the abdominal cavity, between the diaphragm and the fundus of stomach. This organ has two major functions: the *removal of ageing erythrocytes* from the circulation and *production of lymphocytes and antibodies* as a secondary lymphatic organ. Both of these functions are shared with other organs, so the spleen is not essential to survival.

The thymus is primary lymphatic organ. It lies in the superior and anterior mediastinum, in front is the sternum. Behind the thymus lies the heart with pericardium. The tymus is largest in the early part of life, up to the age about 15. With age it becomes thinner, greyer and smaller. Later the adipose tissue infiltrates the organ. The main function of thymus is production of T-lymphocytes.

16.1. Overview of the cells of lymphatic system

The human organism has a compex system of cells (the immune system) that has the ability to distinguish “self” (the organism´s own molecules) from “non-self” (foreign molecules). In general, *immunity* is the reaction of these cells and tissues to foreign (non-self) substances or pathogens such as viruses, bacteria, parasites, tumor cells, proteins or polysaccharides. From the morphologic point of view, the *lymphatic*

(immune) system is a diffuse system with overall weight of about 1 kg. The lymphatic system includes all cells, tissues, lymphatic vessels and organs in the body that contain aggregates of basic cells of immune system called *lymphocytes*, as well as various *supporting cells* (other white blood cells, mast cells, macrophages and antigen-presenting cells). The overall number of all cells of the immune system is 10^{12}.

Cells of the immune system, especially lymphocytes, are distributed throughout the body either as a single cell, as isolated accumulation of cells, as distinct non-encapsulated lymphatic nodules in the connective tissue of digestive, respiratory, urinary or reproductive systems, or as encapsulated individual lymphatic organs (lymph nodes, spleen and thymus).

The two key cell components of the lymphatic system are lymphocytes and supporting (accessory) cells. *Lymphocytes* are cells that mediate specific immunity against foreign molecules, cells and microorganisms. Most of them are small (6 to 8 μm), and only small percent of them are of medium or large size (9 to 16 μm). These spherical cells have a densely stained round nucleus and a thin ring of blue-gray (basophilic) cytoplasm. The larger the cell, the more cytoplasm is visible. Lymphocytes can be classified into three major types based on their immunologic functions. Note, that these types are indistinguishable in conventional light microscopy examination. They are easily recognized only by monoclonal antibodies against surface markers called *clusters of differentiations* (CD; for more information see immunology textbooks):

- *B- lymphocytes* (B- cells) – originate from precursor cells in the bone marrow. Antigen-stimulated (activated) B- lymphocytes undergo cell division and differentiate into antibodies-secreting plasma cells and memory B- cells. *Memory B- cells* can live for a long time (months or years) in circulating blood in inactive state. They can differentiate into *plasma cells* (with eccentrically located "clock-face" nucleus, abundant cytoplasm with extensively developed rough endoplasmic reticulum), which produce antibodies (immunoglobins) to participate in the *humoral immune response*,
- *T- lymphocytes* (T- cells) – are responsible for cell-mediated immunity. They originate from precursor cells which migrate from the bone marrow to the thymus through the blood circulation. In thymus

they undergo cell division to generate a large number of developing lymphocytes differentiated to naïve (virgin) T- lymphocytes. These cells migrate to the secondary lymphatic organs where they may be activated by exposure to foreign antigens. Activated T- lymphocytes can differentiate into both long-living memory T- cells and effector T- cells. Effector T- cells include:

- Helper T- lymphocytes – assist other lymphocytes by secreting immune chemicals called cytokines (interleukins). Cytokines stimulate proliferation and differentiation of B- lymphocytes into plasma cells (are essential for initiating antibody-mediated immune response against extracellular pathogens), as well as differentiation of macrophages into activated macrophages,
- Cytotoxic T- lymphocytes – produce special enzymes (perforins and granzymes), which mediate apoptosis of virus-infected cells, foreign cells, transplanted cells, or cancer-transformed cells,
- Suppressor (regulatory) T- lymphocytes – may decrease or inhibit the functions of helper and cytotoxic T- lymphocytes, and thus modulate immune response,

- *Null cells* – lymphocytes which lack surface markers that are characteristic for B- and T- lymphocytes. They include pluripotent hemopoietic stem cells and natural killer (NK) cells. The function of NK-cells is similar to cytotoxic T- lymphocytes (attack virally infected and tumor cells).

Clinical correlations

The acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) and is characterized by significant immunosupression associated with malignancies, degeneration of the central nervous system, and opportunistic infections. HIV infects macrophages, dendritic cells, and predominantly T-helper lymphocytes (CD4).

In addition, various *supporting (accessory) cells* have an important function in immune responses, too. The basic types of supporting cells are:

- White blood cells – they can migrate throughout the vessels into connective tissue, most of them have fagocytic activity (neutrophils, eosinophils and monocytes which can develop into macrophages),

- Reticular cells - create reticular connective tissue of lymph nodes and spleen,
- Thymic epithelial cells – create suitable microenvironment for T- lymphocytes development within the thymus,
- Macrophages and other antigen-presenting cells (dendritic cells of spleen and lymph nodes, Langerhans cells of epidermis, Kupffer cells of liver) - derived from monocytes (mononuclear phagocytic system). Antigen presenting cells interact with T-helper lymphocytes to facilitate immune response. B- lymphocytes and thymic epithelial cells can also present antigen, but they do not belong to the mononuclear phagocytic system. Activated macrophages in connective tissue destroy phagocyted bacteria and foreign antigens. In some cases, macrophages can fuse to form multinuclear giant cells called Langerhans giant cells, which isolate pathogens from the body.
- Mast cells – large, ovoid, connective tissue cells (20 to 30 μm in diameter), which arise from the bone marrow and contain vasoactive and immunoreactive substances (e.g., histamine, heparine, leukotrienes, neutrophil chemotactic factor). The secretions of mast cell granules can result in immediate hypersensitivity reactions, allergy, and anaphylaxis.

16.2. Mononuclear phagocytic system

All the cells in the body that have the ability to phagocytose corpuscular material belong to mononuclear phagocytic system. The only exception is the neutrophil granulocytes because they are small in size and termed as microphages. All members of mononuclear phagocytic system have the same origin (are formed from monocytes) and the same function (phagocytic activity provides the body with a strong defense against foreign antigens). Cells of mononuclear phagocytic system are:

- Monocytes of blood,
- Macrophages of connective tissue (so-called histiocytes) and macrophages of reticular connective tissue of spleen, lymph node and bone marrow,

- Kupffer cells in liver (the largest population of fixed macrophages in human body),
- Alveolar macrophages of lungs (often called dust cells),
- Multinuclear osteoclasts of bone (responsible for bone resorption),
- Microglial cells (Hortegas´ glial cells) of central nervous system,
- Langerhans cells of the epidermis of skin,
- Mezangial cells of renal corpuscles of kidney (it is not clear, because some authors mentioned, that mezangial cells are derived from smooth muscle cells and not from monocytes).

In the past, this cell population has been termed as *reticulo-endothelial system*, because histologists think, that reticular cells of spleen and lymph nodes and endothelial cells lining blood sinusoids of liver have ability to phagocytose, too. But this hypothesis appears to be false; all phagocytic cells of these organs are de facto macrophages derived from blood monocytes.

16.3. Overview of the organs of lymphatic system

The organs of lymphatic system are classified into two categories:

- *Primary (central) lymphatic organs* – their role is to provide a suitable microenvironment for the proliferation, differentiation and selection of B- and T- lymphocytes. They are responsible for the development and maturation of lymphocytes into mature, immunocompetent cells (they can distinguish between self and non-self antigens). In humans the *bone marrow* (a site of B- lymphocytes education) and *thymus* (a site of T- lymphocytes education) constitute the primary lymphatic organs. In birds (but not mammals) the B- lymphocytes develop in specialized primary lymphatic organ, associated with cloaca, the *bursa of Fabricius*. According to some authors, the mucosa of intestines can be also a place where B- lymphocytes differentiate into immunocompetent cells,
- *Secondary (peripheral) lymphatic organs* – are the sites where immune responses occur. They include encapsulated organs (*lymph nodes* and *spleen*), partially encapsulated organs (the *tonsils*), and aggregates of lymphocytes and various supporting cells in the lungs, the mucosa of

digestive tract, and the mucosa of reproductive system. This diffuse lymphatic tissue is collectively called MALT – *mucosa-associated lymphatic system*.

B- and T- lymphocytes can leave the bloodstream through specialized vessels in the lymphatic organs called *high endothelial venules*. These specialized venules are lined by simple cuboidal or columnar endothelial cells instead of the typical simple squamous endothelial cell type.

16.4. Diffuse lymphatic tissue and lymphatic nodules

Lymphoid tissue is a special type of connective tissue characterized by a rich-supply of lymphocytes. It exists free within the loose connective tissue of mucous membranes of various organs or is surrounded by capsules, forming lymphatic organs (discussed in next chapters). Because lymphocytes have very little cytoplasm and densely stained nucleus, lymphatic tissue filled densely with such cells stains dark violet in hematoxylin and eosin stained sections (image resembling a stray poppy).

16.4.1. Mucosa-associated lymphatic tissue

Mucous membranes of the gastrointestinal, respiratory, and genitourinary tracts are open to the external environment, so they harbor the body's largest, most diverse populations of pathogens. The diffuse lymphoid tissue common in the loose connective tissue of lamina propria mucosae of various organs is known as *mucosa-associated lymphatic tissue* (MALT). Based on location MALT may be subdivided into:

- GALT – gut-associated lymphatic tissue (includes aggregates of lymphatic nodules in the terminal ileum called Peyer´s patches, *appendix vermiformis*, and tonsils). The gut-associated lymphatic tissue (GALT) consists of both isolated and aggregated lymphatic follicles and is one of the largest lymphatic organs, containing up to 70% of the body's lymphocytes. They were termed as *Peyer's patches* in the terminal ileum after their detailed description by the

Swiss pathologist Johann Conrad Peyer in 1677. The fetal human small intestine contains in average 60 Peyer's patches and their numbers steadily increase reaching a maximum of 240 at puberty. The regions immediately adjacent to the lymphatic follicles are covered by a special epithelial cells, called *M cells* (microfold cells). M cells captured antigens from the guts lumen and transfer them to macrophages located inside the Peyer's patches.

- BALT – bronchus-associated lymphatic tissue (located in the walls of bronchi, in lungs, especially in regions where bronchi and bronchioles bifurcate),
- NALT – nose-associated lymphatic tissue,
- VALT – vulvovaginal-associated lymphatic tissue.

16.4.2. Primary and secondary lymphatic nodules

In lymphatic tissue groups of lymphocytes are arranged as spherical masses called lymphatic nodules (follicles), containing primarily B-lymphocytes. Lymphatic nodules consisting chiefly of small lymphocytes (inactivated B- lymphocytes) are called *primary nodules*. Primary nodules which are formed mostly before birth in the absence of foreign antigens. Primary nodules become *secondary nodules* (Fig. 1) on exposure to foreign antigens and have distinctive features include:

- *Germinal center* - located in the central region of the nodule. It is the active site where B- lymphocytes encounter antigens and continue to proliferate and develop into centroblasts, centrocytes, and antibodies-secreting plasma cells. The germinal center is a morphologic indication of lymphatic tissue response to foreign antigen. Another cell populations located in germinal centers of lymphatic nodules are follicular dendritic cells (with multiple, thin, hair-like cytoplasmic processes that interdigitate between B-lymphocytes), macrophages and reticular cells.
- *Mantle zone (corona)* – an outer ring of small lymphocytes that encircles the germinal center.

Note that lymphatic nodules contain mostly B- lymphocytes and their activated form; T- lymphocytes are located in the tissue between nodules. Under normal conditions no lymphatic nodules are found in the thymus.

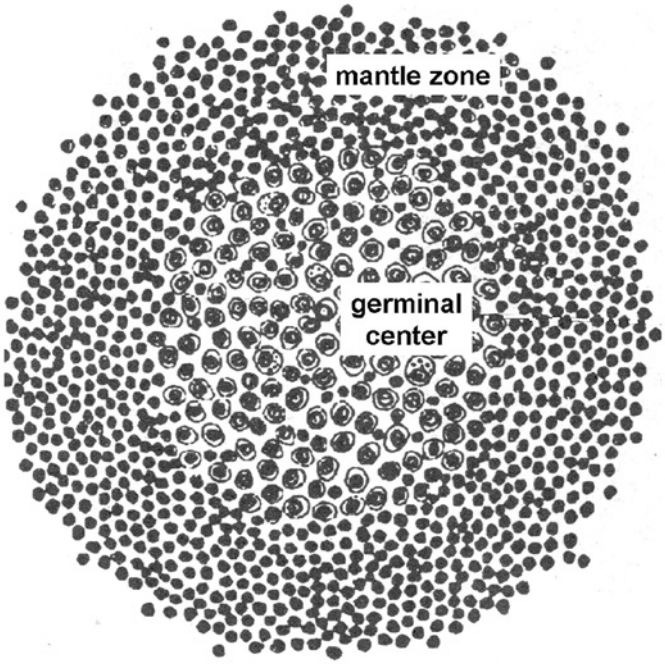

Fig. 1. Secondary lymphatic nodule

16.4.3. Tonsils

The tonsils (palatine, pharyngeal, and lingual) are aggregates of lymphatic nodules, which are partially encapsulated and partially covered by epithelium. Tonsils guard the entrance to the oral pharynx. They form a ring of lymphatic tissue called *anulus Waldeyeri* according to German anatomist Wilhelm Waldeyer-Hartz (1836-1921). Tonsils (in opposite to lymph nodes) do not possess afferent lymphatic vessels.

The palatine tonsils are paired and are located in the posterolateral walls of the oral cavity. They are covered by stratified squamous epithelium and have 10 to 20 epithelial invaginations that penetrate the tonsil deeply, forming *tonsillar crypts* (Fig. 2). The crypts frequently contain food debris, desquamated epithelial cells, dead leukocytes, and bacteria. The deep aspect of each palatine tonsil is isolated from the surrounding connective tissue by a dense connective tissue capsule. The parenchyma of the tonsil is composed of numerous lymphatic nodules, many of which display germinal centers, indicate B- lymphocyte activation. The palatine tonsil is evolutionary the youngest lymphatic organ (it is found only in

mammals). In humans it develops in the second pharyngeal pouch (*sinus tonsillaris*), which is infiltrated with lymphocytes during the 3rd month of prenatal development.

The pharyngeal tonsil is a single tonsil situated in the posterior root of the nasopharynx. It is usually covered by ciliated pseudostratified columnar epithelium and separated from underlying structures by a thin partial capsule. It is similar to palatine tonsil; their parenchyma is composed of lymphatic nodules, some of which have germinal centers. Instead of crypts, the pharyngeal tonsil has shallow, longitudinal *pleats* (infoldings).

The lingual tonsil is situated along the posterior surface of the tongue (*radix tonguae*) and is covered by stratified nonkeratinized squamous epithelium with crypts. Ducts of the mucous (Weber´s) glands of the posterior third of the tongue (minor salivary glands) often open into the base of these crypts. The parenchyma of this tonsil has the same basic features as that of palatine tonsils (lymphatic nodules, which frequently have germinal centers).

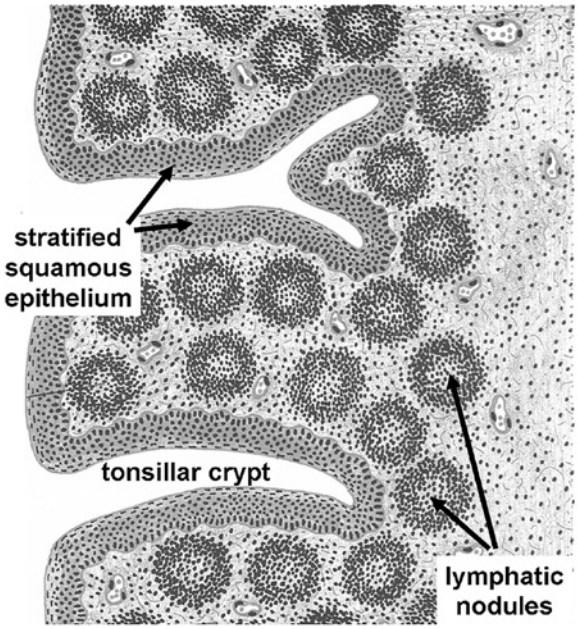

Fig. 2. Microscopic structure of palatine tonsil

Clinical correlations

Palatine tonsils are common sites for infections, such as acute or recurrent *tonsillitis* (amygdalitis), especially in children. It is results

from infection with bacteria such as Streptococcus or viruses such as Epstein-Barr virus. Surgical removal of inflammated palatine tonsils (tonsillectomy) may be a choice in some children or adults with recurrent tonsillitis. The inflammated pharyngeal tonsil is called the *adenoid* and can be removed surgically, too (adenoidectomy).

16.5. Spleen (lien, splen)

16.5.1. Introduction into histology of the spleen

In the past, spleen was described as "*organum mysterii plenum*" for its "mystic and mysterious" functions. The fact that spleen has no openings (only for vessels) might have complicated the investigation of its function. First hypotheses described it as a place of black bile degradation. According to Hippocrates (460-380 BC), the most famous physician of antiquity, the black bile was one of the four liquids regulating the human body. It was supposed to cause bad mood and sadness, melancholy. It is not precisely known when exactly the first information concerning the importance of the spleen in the defense against infections appeared. Spleen enlargement during febrile diseases and severe infections could have pointed at its protective role in human body. The question about the importance of the spleen for human life is still emerging ever since the first splenectomy made by Adrian Zaccareli in 1549.

The spleen is the largest encapsulated lymphatic organ in humans, weighing about 120-150g. It represents the most abundant accumulation of lymphatic tissue in the human body. With regard to its structure and function, the spleen is considered as two separate organs. On the cut surface of the fresh spleen, the unaided eye can distinguish *white pulp*, which appears as small, pale islands of lymphoid tissue, and *red pulp*, which appears bright red because of the large number of erythrocytes. The white pulp of the spleen is an important part of the immune system (immunologically monitors the blood), whereas the red pulp is connected to the bloodstream and is engaged in phagocytosis of senescent, damaged, or genetically altered (e.g., sickle cell disease) red blood cells.

The first recognizable sign of the developing spleen is a condensation of mesoderm-derived mesenchyme within the dorsal mesogastrium

(developing later into the greater omentum) at the end of the 4th week after fertilization. Thus, the spleen is not an endoderm derivate of the digestive tube, as are most of the organs in the abdominal cavity. Rotation of the stomach and growth of the dorsal mesogastrium during 6th and 7th weeks causes the translocation of the developing spleen from the midline to the left side of the abdominal cavity. The primordium of the spleen is continuously growing and separated from the dorsal mesogastrium. The spleen thus becomes an intraperitoneally located organ.

The spleen is an intraperitoneal organ, with a smooth serosal surface (simple squamous epithelium of peritoneum covers the capsule of the spleen). It is attached to the retroperitoneum by ligaments containing its vascular supply (*a. splenica* in the past the so-called *a. lienalis* and *v. splenica*,). The splenic artery, splenic vein, and efferent lymphatic vessels (the spleen has no afferent lymphatic vessels!) are found at the hilum. The spleen of adults normally lies in the left diaphragm arch, between the bottom of the stomach and the diaphragm. The longitudinal axis of the spleen is oriented along the 10th rib, and the width of spleen lies in the range of the 9th to 11th rib. The entire organ, including its lower pole, is covered by the costal arch and is not palpable in healthy individuals.

16.5.2. Functional overview of the spleen

Most of the functions of the spleen deal with its relationships with the blood flow. It filters, produces, modifies, destroys and protects the blood corpuscles.

- *Filtration of blood* – macrophages of the reticular tissue of cords of Billroth phygocytose cellular debris, parasites, bacteria and senescent or damaged red blood cells,
- *Metabolism of hemoglobin and iron* – red blood cells phagocyted by macrophages are destroyed within phagolysosomes and hemoglobin is catabolized into its heme and globin portion. Heme is converted to bilirubin and transported into liver via splenic vein; iron-atoms are retrieved from hemoglobin, conveyed to the bone marrow by a transport protein transferrin and are used in the formation of new red blood cells,
- *Immune defense* – foreign antigens (bacteria and viruses), circulating in blood stream, may stimulate a strong immune response in the spleen.

The white pulp of spleen is a place of activation and proliferation of B- and T- lymphocytes, and production of antibodies against antigen present in blood flow,

- *Prenatal hemopoiesis* – in the fetus spleen forms blood elements. Hemopoietic stem cells migrate into the developing spleen during the 1st trimester of pregnancy. The spleen works exclusively as a hemopoietic organ until the 14th week of development. The hemopoiesis gradually decreases and stops in the 8th month of prenatal development.
- *Blood reservoir in some animal species (not in humans)* – stimulation of sympathetic nerve fibers causes contraction of smooth muscle cells of the capsule. This results in constriction of the spleen, which discharges stored blood into the systemic circulation.

16.5.3. Microscopic structure of the spleen

The spleen (Fig. 3) surface is covered by a capsule (*capsula fibrosa lienis*) from dense irregular connective tissue with elastic fibers and smooth muscle cells (or myofibroblasts). The number of smooth muscle cells or myofibroblasts in capsule varies with the animal species. In many mammals the spleen holds large volumes of red blood cells in reserve. In these species, contraction in the capsule and trabeculae helps discharge stored red blood cells into the systemic circulation. In humans, splenic capsule contains only few smooth muscle cells and the spleen does not function as a blood reservoir. The capsule sends trabecular network (*trabeculae lienis*) from connective tissue into the substance of the spleen to form a supportive framework. The trabeculae carry blood vessels (trabecular arteries and veins) and nerves from and to the splenic red pulp.

The spleen is composed of reticular tissue containing fibroblastic reticular cells, many B- and T- lymphocytes, macrophages, antigen presenting cells and other blood elements. Splenic parenchyma, called *pulp*, has two components, the white pulp and the red pulp. These two regions are separated from each other by the marginal zone.

White pulp forms a typical lymphatic tissue around central arteries and contains mostly B- and T- lymphocytes, macrophages and other supporting cells of the immune system. White pulp consists of two major components:

- *Periarterial lymphatic sheath* (PALS) - surrounding central arteries, which is a thymic-dependent zone (housing T- lymphocytes), similar to inner cortex of a lymph node,
- *Lymphatic nodules (follicles)*, which are present at places where central arteries branch. In spleen they are so-called *Malpighian corpuscles* (according to Italic physician Marcello Malpighi, 1628-1694) and housing B- lymphocytes. Lymphatic nodules may display germinal centers, indicate of antigenic challenge. The nodules with pale germinal centers are so-called secondary nodules where B- lymphocytes actively differentiate into large cells (lymphoblasts and later plasma cells). The dark ring around the germinal center is the mantle zone where small inactive B- lymphocytes are hosted.

The borderline between the white and red pulp is formed by *marginal zone*. This zone is composed of T- and B- lymphocytes, plasma cells, macrophages and antigen-presenting dendritic cells. Additionally, numerous small vascular channels, *marginal sinuses*, are present in the marginal zone. The marginal zone is the site where the immune response is initiated (antigen presenting cells sample the foreign material traveling in blood and macrophages attack microorganism present in the blood) and where lymphocytes exit the bloodstream to repopulate the spleen.

The red pulp of the spleen consists of splenic cords (cords of Billroth, according to Theodor Billroth, German surgeon, 1829-1894) which are located between branched vascular channels, the splenic venous sinuses (sinusoids).

- *Splenic cords of Billroth* consist of three dimensional network of reticular tissue (reticular cells which manufacture type III of collagen, the reticular fibers) with numerous free cells, such as macrophages, lymphocytes, plasma cells, granulocytes, and mostly erythrocytes which are responsible for typical color of this part of the spleen in the fresh state. Splenic macrophages phagocytose damaged red blood cells. The iron from destroyed red blood cells is used in the formation of new red blood cells. Splenic macrophages begin the process of bilirubin (a toxic catabolic product of hemoglobin) breakdown and iron reclamation.

- *Splenic venous sinuses* are discontinuous vascular spaces lined by a special long rib-shaped endothelial cells (sinuses-lining cells) oriented in parallel along the long axis of the sinuses. Each splenic sinus is covered by a discontinuous basal lamina and circumferentially arranged ribs of reticular fibers around the endothelial cells (like a barrel hoops). Processes of macrophages extend between the endothelial cells into the lumen of the sinuses to monitor the passing blood for foreign antigens. The spaces between the endothelial cells of the splenic sinuses are 2-3 μm and only flexible blood cells (younger erythrocytes with flexible plasma membrane) are able to pass easily from the cords of Billroth into the lumen of the sinuses.

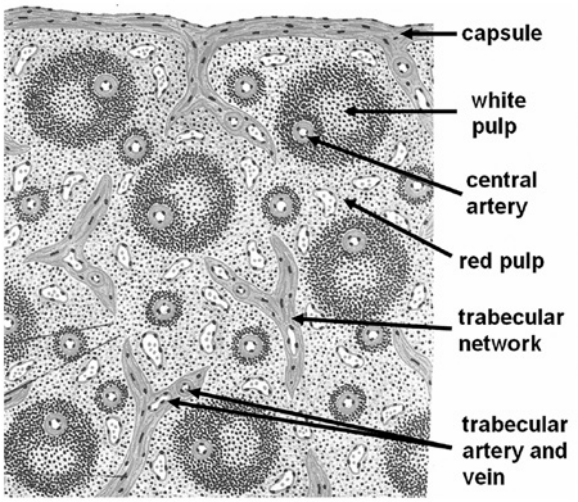

Fig. 3. Microscopic structure of spleen

16.5.4. Blood microcirculation in the spleen

The spleen gets blood from the *splenic artery*, branching to *trabecular arteries* (*arteriae trabeculares*) after entering the splenic hilum. Arteries after leaving the connective tissue of trabeculae change to *central arteries* (*arteriae centralis*), surrounded by a periarterial lymphatic sheath of white pulp. After leaving the white pulp, the central arteries branch at a right angle to numerous and relatively straight arterioles called *penicillar arterioles* (*arteriolae penicilattae*). Finally, the arterioles form capillary network (arterial capillaries) of red pulp and marginal zone. Before the final branching, the arterial capillaries are surrounded by aggregation of

macrophages; so-called Schweigger's and Seidl's sheaths ("encapsulated or sheated capillaries"). These sheaths are only discreetly formed in humans' spleen. Blood flow through the splenic red pulp can take two routes (Fig. 4). In *closed circulation* the arterial capillary's walls are directly related to the walls of sinuses, so that blood is always enclosed by the endothelial lining of vessels. In *open circulation* the penicillar arterioles and arterial capillaries end blindly in the red pulp, while the blood freely flows out into the spaces of cords of Billroth. After passing through the three-dimensional network of cords the blood subsequently gets into the sinuses by penetrating through the openings in their walls. In humans spleen both type of blood microcirculations are found, but the open system prevails, and only the smaller part runs in the closed system. Open circulation is engaged in a very sensitive blood purge from foreign antigens by numerous macrophages residing in cords of Billroth. For a closed circulation, rapid blood flow as well as the presence of small number of macrophages in sinuses is characteristic. Both types of microcirculation were observed parallel not only in humans, but also confirmed in several animals such as dogs or rats. From splenic sinuses the blood runs into veins of the red pulp, from there to trabecular veins and to splenic vein.

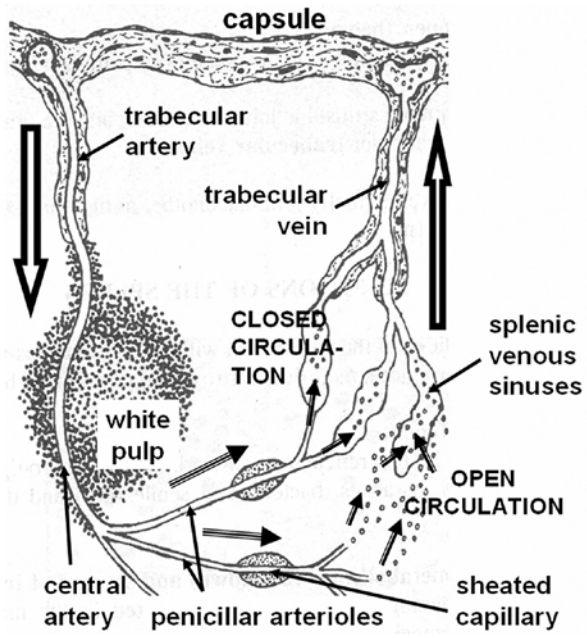

Fig. 4. Open and closed circulation in the spleen

Clinical correlations

Because the spleen is a fragile organ, major trauma (e.g. automobile accident) may be fatal due to massive intraperitoneal hemorrhage after the rupture of the splenic capsule. Although the spleen has many important functions, none of them is essential to life since surgical removal of the spleen, or *splenectomy* (e.g. for rupture of the spleen capsule, certain anemias, platelet disorders or malignant tumors of the spleen) does not cause death in the normal, healthy individual. After splenectomy, increased numbers of red blood cells with Howell-Jolly bodies (nuclear fragments that are normally removed from red blood cells as they pass through the splenic sinuses) are observed in the blood stream. Splenectomy in adults usually has no clinical consequence, but in children it may lead to increased occurrence and severity of infections.

A common cause of spleen enlargement, or *splenomegaly*, is portal hypertension resulting from cirrhosis of the liver. The spleen of affected patients is modestly enlarged, weighing sometimes up to 500g.

During fetal life the spleen's red pulp also works as a place of hemopoiesis. However, also in postnatal period, in some diseases of the blood and hemopoietic organs (e.g., chronic myeloid leukemia, myeloproliferative syndromes or anemia) the red pulp of the spleen may contain areas of *extramedullary hemopoiesis*.

Lobular spleen (*lien lobatus*) belongs to the normal variants in the shape of the spleen and it is not related to any pathological state of this organ. The clefts and notches can be sharp and occasionally have depths of 2–3 cm. These "cosmetic" abnormalities have no clinical significance, but must be distinguished from splenic ruptures due to trauma. Such variations in the shape of the spleen are residues of the splenic lobules during fetal development.

Accessory spleens (*lienes accesorii, splenunculi*) can be found in 10–20% of the population. They may be single or multiple, but there are seldom more than six. Accessory spleens are usually the size of a cherry (1–1.5 cm in diameter), and are commonly found in the hilum of the spleen (75% of cases), but may also occur along the splenic vessels, in the gastrospelnic or splenorenal ligaments, within the pancreatic tail, in the wall of the stomach or bowel, in the greater omentum or the mesentery, or even in the pelvis and scrotum. Morphologically and functionally they are equal to the normal spleen. Notes, those accessory spleens can imitate

enlarged lymphatic nodules or tumors in the adrenal gland, pancreas, stomach or intestines.

16.6. Lymph node (nodus lymphaticus, lymphonodus)

16.6.1. Introduction into histology of the lymph nodes

Lymph nodes are small, bean-shaped encapsulated organs (size from about 1mm to bout 1 to 2 cm) located along the pathway of lymphatic vessels. The convex surface of a lymph node receives efferent lymphatic vessels, whereas the concave surface (the hilum) is the site where arterioles enter and venules and efferent lymphatic vessels exit. In average, 500 – 600 lymph nodes are found in the human body. They occur, often as clusters of lymph nodes, in strategic regions such as the neck (cervical and pericranial ring), thorax (tracheal nodes), mesenteries, axillae (axillar nodes), femoral regions (femoral nodes) and groin (inguinal nodes). They play important roles in circulating and filtering lymph, defending against microbial invasion, and providing a place for lymphocytes to meet antigens.

16.6.2. Functional overview of lymph nodes

The most important functions of lymph nodes are:

- *Filtration of lymph and site of phagocytosis* of bacteria, cell debris and foreign materials,
- *Initiation of immune response and antibodies production* by plasma cells. Lymph nodes are also the sites of antigenic recognition and antigenic activation of B- lymphocytes, which give rise to plasma cells and memory B- lymphocytes,
- *Recirculation and storage of lymphocytes*.

16.6.3. Microscopic structure of lymph nodes

Lymph nodes are covered by thick dense irregular connective tissue capsule with blood vessels and adipose cells that sends trabeculae into the substance of the node (Fig. 5). Lymph nodes possess a stroma composed of framework of fibroblastic reticular cells manufacturing reticular fibers – reticular connective tissue (mesodermal origin). The reticular

framework of lymph node contains several types of cells, e.g. antigen-presenting dendritic cells, follicular dendritic cells and macrophages (with both phagocytic and antigen-presenting functions). Lymph nodes are divided into a cortex and medulla, and cortex is subdivided into outer and inner cortex:

- *Outer cortex* – contains B- lymphocytes-rich lymphoid nodules. The lymphatic nodule consists of an outer dark part of small B- lymphocytes (mantle zone) and an inner pale part of immature B- lymphoblasts called germinal center (see chapter 15. 4. 2 Primary and secondary lymphatic nodules),
- *Inner cortex (deep cortex, paracortex)* – most T- lymphocytes are hosted here, it is also called the *thymus-dependent cortex*,
- *Medulla* – is surrounded by the cortex, except at the region of the hilum and consists of two major components:
  - *Medullary sinusoids* – are lymphatic channels lined by endothelial cells surrounded by reticular cells and macrophages,
  - *Medullary cords* – formed by reticular tissue with B- lymphocytes, macrophages, dendritic cells and plasma cells. This is a strategic place where plasma cells (after activating migrate from the cortex) can secrete antibodies directly into the medullary sinuses without leaving the lymph nodes.

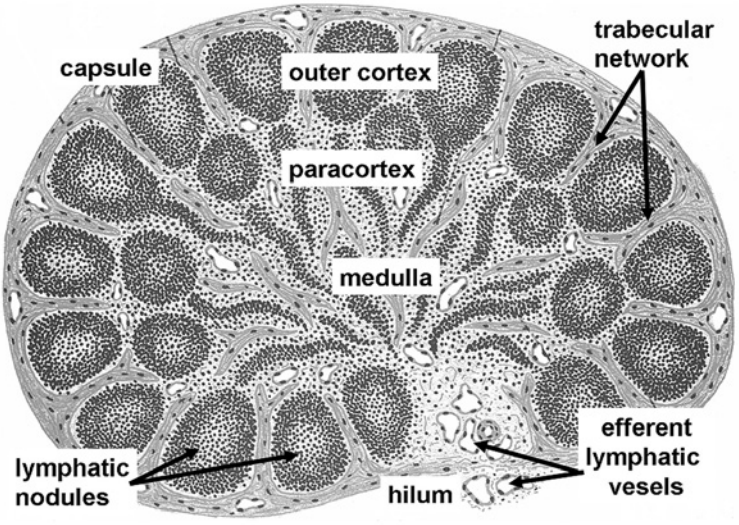

Fig. 5. Microscopic structure of lymph node

16.6.4. Blood and lymph circulation in lymph nodes

Arteries enter and veins leave the lymph nodes at the hilum. The region between cortex and medulla is supplied by special blood vessels called *post-capillary high-endothelial venules*. They are lined with simple cuboidal epithelium, and not simple squamous epithelium like other vessels. High-endothelial venules are specialized migratory pathways for lymphocytes, both B- and T- lymphocytes leave the bloodstream via the high-endothelial venules.

Filtration of lymph in the lymph node occurs within a network of interconnected lymphatic channels lined by endothelial cells called *sinuses*. Afferent lymphatic vessels reach lymph node via its convex surface. They have valves which allow lymph to move in one direction. Afferent vessels pierce the capsule and enter the lymph node. Lymph slowly diffuses through the meshwork of reticular cells and fibers with high number of macrophages and dendritic cells. Lymph is drained at first from *subcapsular sinuses*, to *paratrabecular cortical sinuses* and penetrates the cortex, then to *medullary sinuses*. Lymph leaves lymph node in the concave surface (the hilum) via efferent lymphatic vessels.

Clinical correlations

Lymph nodes can undergo hypertrophy and histologic change in response to many clinical conditions. Abnormal enlargement of lymph nodes, or *lymphadenopathy*, may be due to the increased number of lymphocytes and macrophages in the node during antigenic stimulation in a bacterial or viral infection. It may also by caused by metastasis, whereby neoplastic cells spread from local site of development to distant locations. Such cells are often carried by lymphatics to the nearest lymph node. Surgical biopsy and microscopic examination of a lymph node are used for diagnosis and staging of many malignancies and may provide useful prognostic information.

16.7. Thymus gland (thymus)

16.7.1. Introduction into histology of the thymus

Since the first description by Galen of Perganum (130-200AD), the thymus has unknown function throughout the 2000 years of history of

medicine. Examination of the thymic functions was unsuccessful until 1961. That year Australian physician Jacques Miller demonstrated the effect of the thymectomy on the immune system of the newborn mice and discovered the key role of thymus in development of cell mediated immunity.

From embryological point of view, the embryonic pharynx serves as the origin of the mammalian thymus. The thymic anlagen are formed from the dorsolateral portions of the third paired pharyngeal pouches. Thymic organogenesis depends on the interactions between the cells of all three embryonic germ layers: endoderm-derived epithelium of pharyngeal pouches, neuroectoderm-derived neural crest mesenchyme and mesoderm-derived hemopoietic cells. The paired epithelial primordia of thymus derived from the third pharyngeal pouches descend together with the third parathyroid primordia. The rapid growth of embryo causes the paired thymus primordia come close to each other and lay in front of the pericardium at the 7th and 8th week of development. They stay separated by a layer of connective tissue and never fuse together.

Human thymus is a lymphoepithelial organ and usually consists of two asymmetrical lobes divided by connective tissue. It is located in the superior mediastinum, anterior to the heart and great vessels but in newborns it caudally reaches to anterior mediastinum. Thymus undergoes its maximum development in the time of birth, when it also has the greatest relative weight (35-40 g). The highest immunological activity of the thymus is at the age of 6 months after birth, when the thymus contains the highest overall number of developing lymphocytes. After the 1st year of life, the thymus undergoes a progressive reduction in size. These age-related changes in thymic structure are called *age-related involution*. The thymus loses its cellularity by 3-5% per year until middle age, after which the process slows down to 1% per year. Even though, the remaining tissue is able to produce mature T- lymphocytes till a late age. It is therefore probable, that the thymus as an organ with an immune and endocrine function is important throughout lifetime.

16.7.2. Functional overview of the thymus

The thymus is a central (primary) lymphatic organ also with an important endocrine function. Thymus thus plays an essential role in securing the microenvironment for production of T- lymphocytes and in the development of the immunocompetence of children. The main role of thymus derived lymphocytes is the protection against the tumour cells, intracellular parasites and viruses; through cytokine secretion they activate other cells of the immune system. Immunological incompetent T- lymphocytes progenitors are first formed in red bone marrow from pluripotential hematopoietic stem cells and than they migrate into thymic cortex for further proliferation and differentiation to become mature, immunocompetent T- lymphocytes. The mature T- lymphocytes (which can distinguish between self and non-self antigens) leave the thymus and enter the blood stream through the walls of high endothelial venules. In next step, T- lymphocytes reach the thymus-dependent zones in the secondary lymphatic organs (mostly the periarterial lymphatic sheaths in spleen and inner cortex in lymph nodes), where they are further differentiated into functional T- lymphocytes which can carry out the cellular immune response. Epithelial cell network also secrete thymic hormones (e.g., thymopoietin, thymulin), which stimulates maturation and activity of lymphocytes and probably other parts of lymphatic system.

The thymus is also a crossroad between the immune and the neuroendocrine systems. Thymus in children is extremely sensitive to intrinsic and extrinsic negative stimuli (e.g., stress, malnutrition, chronic illness, infections, chemo- and radiotherapy, hormonal therapy), causing *stress-related thymic involution*. That means, some chronic diseases can begin in "head" (e.g. chronic stress), and via thymus can negatively influence the overall immunity.

16.7.3. Microscopic structure of the thymus

The general microscopic description of human thymus is very complicated, because the thymus is a very dynamic organ that rapidly changes under exogenous influence and it involutes with age. The thymus is a soft, encapsulated and lobulated organ. The *capsule and septa* (trabeculae) consist of dense irregular connective

tissue that supports blood and lymphatic vessels, and nerve fibers. The septa divide the parenchyma into incomplete *lobules*, each of which contains a cortical (*cortex thymi*) and a medullar (*medulla thymi*) part, although the medullae of adjacent lobules are confluent with each other (Fig. 6). The cortex is subdivided into subcapsular cortical region (a thin layer, 1 or 2 cells deep with mitotically active lymphoblasts) and inner cortical region. Individual thymic lobules are variable in shape, size, and orientation. Stained histological sections show a dark cortex coating a pale medulla. Both consist mostly of T- lymphocytes and thymic epithelial cells, but the rate between them is different. The cortex of the thymus lobes is darker because of the presence of numerous closely packed maturating T- lymphocytes (cortex is the region in which T- lymphocytes maturation occur). The thymus is the only organ that has *an epithelial stroma* (in contrast to the mesenchyme origin of the reticular cells and reticular tissue of other lymphatic organs). Within the thymus, only the capsule and septa are of mesenchyme origin, the thymic epithelial cells are derived from endoderm (or according to some studies they have dual, endodermal and ectodermal origin, too).

Between cortex and medulla, the cortico-medullary junction is recognized as an area rich in blood vessels, where connective-tissue septa reach the medulla region. Since 1849, when London physician Arthur Hill Hassall described acidophilic squamous spherical structures in the thymic medulla, these corpuscles have been thought to be specific for this organ. *Hassall's corpuscles* (*corpuscula thymi*) are "onion-like" structures, variable in number and size, often displaying degenerative changes in the central area, such as necrosis, cellular detritus, sometimes extensive calcification, cystic alterations, and foamy macrophages. Hassall's bodies have probably secretory function (secrete cytokines and growth factors), as well as function in communication between antigen presenting cells and T- lymphocytes. Their number, size and morphological features depend mostly on the age of the individual.

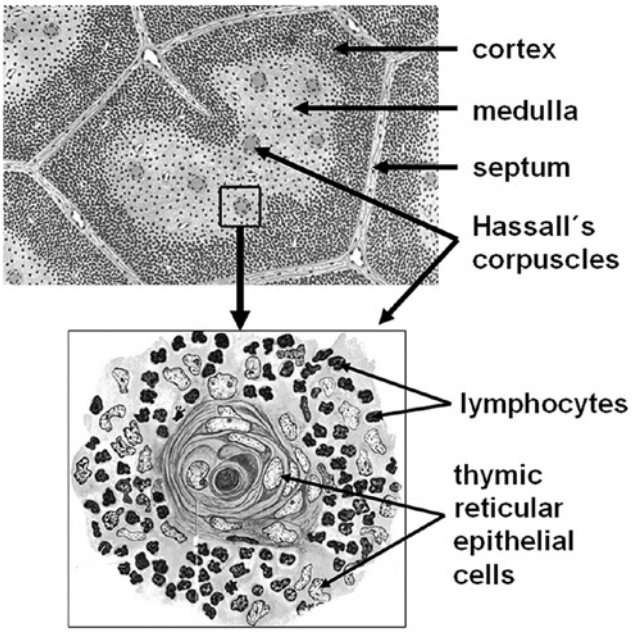

Fig. 6. Microscopic structure of thymus and detail view on Hassall's corpuscle

16.7.4. Thymic microenvironment

Thymic lymphocytes form the largest part of the cell population of the cortex. Population of developing T lymphocytes (in thymus also termed thymocytes) lies between processes of supporting thymic epithelial cells, and constitute up to 90% of the weight of the thymus. Mitotically active lymphoblasts (15%) are most abundant in the subcapsular area of the cortex. Small lymphocytes with reduced mitotic activity are found in the deeper cortex and they are rarely found in the medulla.

The thymic microenvironment is composed of a variety of stromal cells. The thymic microenvironment constitutes a unique environment for the differentiation, maturation and selection of T- lymphocytes. It is composed of:

- *Thymic reticular epithelial cells* – their numerous branched cytoplasmic processes forming supporting network for lymphocytes, macrophages and dendritic cells of cortex and medulla, too (discussed later),
- *Macrophages* - are situated mainly in the cortex and their function during T cell development is not completely known. It seems clear that macrophages participate in the removal of apoptotic T- lymphocytes.

More then 95-98% of developing lymphocytes die in the cortex and are phagocyted by macrophages (only 2-5% of lymphocytes survive and continue to develop). Macrophages also produce and secrete cytokines, which influence the proliferation, maturation and differentiation of T- lymphocytes. Macrophages are also one of the antigen-presenting cells for the lymphocytes during their terminal development,

- *Interdigitating dendritic cells* - are unique antigen-presenting cells originating from the bone marrow. In the thymus they are situated mainly in the cortico-medullary junction and medulla. They are about 20-30 μm in diameter and often surround other cells with their thin cytoplasmic processes, mainly lymphocytes. According to some authors, the cytoplasm of interdigitating cells contains the club-shaped Birbeck granules with unknown function (similarly to Langerhans cells of the skin).
- *Thymic myoid cells* – correspond to a muscle-like cell population present in the thymus medulla. Their origin and biological role is not yet clear. Myoid cells express muscle proteins and have surface receptors for acetylcholine. Thereby, it is presumable that this cell type can play an initial role in pathogenesis of the autoimmune neurological disease *myasthenia gravis,*
- *Other cells* of hemopoietic origin, such as eosinophilic and neutrophilic granulocytes, B lymphocytes, mast cells and red blood cells.

Both cortex and medulla of the thymus contain *thymic epithelial cells* that create a meshwork (*reticulum*). Therefore they are termed as *epithelial reticular cells* (or only thymic epithelial cells). The thymic epithelial cells have a stellate shape and send many cytoplasmic processes that form desmosomes with neighbouring cells to form a diffuse network. These cells contain a large, pale, and oval shaped nucleus with 1 or 2 nucleoli. The cytoplasm contains a poorly developed Golgi complex, rough endoplasmic reticulum, mitochondria and intermediary cytokeratin fibrils, demonstrating the epithelial nature of these cells in contrast to reticular tissue of other lymphatic organs.

There are six types of epithelial reticular cells, out of which, the first 3 occur mainly in the cortex and the remaining 3 in the medulla of the thymus:

- *Type 1*: called as subcapsullar or perivascular. Divides the cortex of the thymus from the connective tissue constituting the capsule, septa and the space around vessels. It participates in the *thymus-blood barrier* (discussed later),
- *Type 2:* is situated in the middle of the thymus cortex. Together with type 3, they constitute a dominant portion of the thymic epithelial network of the cortex. They have abundant cytoplasm that includes many mature lymphocytes. Hence they are termed as "*nurse cells*". Thymic nurse cells are defined as multicellular complexes of thymic epithelial cells with maturing T- lymphocytes. Nurse cells realize a specialized medium for maturation, differentiation, and selection of lymphocytes. Lot of data have been reported about the multi-function of thymic nurse cells, such as endocrine capability, and secretion of humoral factors,
- *Type 3:* is situated in the deeper portion of the cortex, in the area of the cortico-medullary junction. In their cytoplasm, rough endoplasmic reticulum forms dilated cisterns in the cytoplasm, which indicate active protein synthesis. Thymic epithelial cells of the type 2 and 3 express specific surface antigens, molecules of the major histocompatibility complex (MHC) I and II, which they present to developing lymphocytes, thereby they are ranked together with antigen presenting cells,
- *Type 4:* participates in the formation of the cortico-medullary junction and medulla,
- *Type 5:* termed as medullary, forms the meshwork of the thymic medulla,
- *Type 6:* forms more prominent component in the thymus medulla. These large pale cells pile up into a spindle shape, to form the Hassall's corpuscules. Hassall's corpuscles are unique, antigenically distinct, functionally active, multicellular components of the nonlymphocytic microenviroment of the thymic medulla and participate in the physiological activities of the thymus.

16.7.5. Blood-thymus barrier

It is the barrier between blood stream inside cortical capillaries and the immature T- lymphocytes in the cortex of the thymus. This barrier ensures that developing T- lymphocytes undergo selection in an

antigen-free environment. No such barrier is present in the medulla and mature T- lymphocytes exit the thymus via venules in this zone. Blood-thymus barrier is formed of:

- Continuous non-fenestrated endothelium of the cortical capillaries,
- Tight junctions between endothelial cells,
- Thick basal lamina, and occasional pericytes (also a part of the capillary wall),
- Surrounding perivascular connective tissue macrophages,
- Thymic epithelial cells with their occluding junctions and their basal lamina around capillaries.

16.7.6. Unique features of thymus

The thymus, in contrast to other lymphatic organs, has some unique morphological features:

- Its thymic reticular cells are of endodermal (and probably ectodermal) origin, and not mesodermal, and produce no reticular fibers,
- Thymic medulla contains characteristic onion-shaped Hassall's corpuscles,
- Postnatally undergoes age-related involution (atrophy) – decreasing the lymphopoiesis and infiltration by adipose cells,
- Under normal condition it has no lymphoid nodules, no plasma cells and no afferent lymphatic vessels (thymus does not constitute a lymph filter, as do lymph nodes).

Clinical correlations

The most frequent thymic disorders are *thymic hyperplasia*, *thymic cyst* and *thymic tumors* (thymoma, thymic carcinoma, lymphomas, Langerhans cell histiocytiosis, thymolipoma and thymic carcinoid). One of the most interesting phenomena in dynamic changes in morphology of thymus is the thymic enlargement after atrophy (during prolonged period of illness or after chemotherapy for malignancies). In some cases, the thymus after atrophy not only regrows, but occasionally overgrows or "rebounds" (regrowth 50% greater than baseline volume). When the thymus increases in size after chemotherapy and/or radiation therapy, it may be difficult to differentiate rebound thymic hyperplasia from recurrence of lymphoma. A biopsy for exact diagnosis may be necessary in some patients.

The disruption of embryonic thymic descent from pharynx into mediastinum can result in an *ectopic thymus* localized anywhere from the mandibular angle to the upper mediastinum. An ectopic thymus localized in the cervix is often called also *cervical thymus*. Various types of cervical thymus may be present as a neck mass, usually laterally, from the angle of the mandible to the manubrium. Cervical thymus can cause severe dyspnoe, stridor or dysphagia or can mimick thyroid gland enlargement.

The agenesis of thymus is a rare congenital anomaly and is often associated with cardiac defects, hypocalcaemia due to the agenesis of parathyroid glands and other congenital anomalies affecting neck and face. These congenital anomalies are grouped together and called as *DiGeorge syndrome*. DiGeorge syndrome is associated with chromosome 22 microdeletions, produced by a recombination error in meiosis. In case of patients with DiGeorge syndrome, selective T- lymphocytes lead to immunodeficiency with recurrent opportunistic infections.

Myasthenia gravis is an autoimmune disorder affecting neuromuscular junctions in which autoantibodies from blood directed to the acetylcholine receptors impair neuromuscular transmission. In this disease the thymus plays an important role in induction of anti-acetylcholine receptor antibodies, and total thymectomy is accepted therapy for this condition.

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17. Urinary system

Anatomy introduction

The urinary system maintains the composition and properties of the body fluid, which forms the internal environment of the body cells. The final product of the urinary system is urine, which is voided from the body during micturition. The production of urine is essential to the control of concentration of various substances in the body fluids (maintaining the electrolyte and water balance). The kidneys also have endocrine function and produce e.g. erythropoietin, renin, prostaglandins, etc.

The urinary system consists of: 1. two *kidneys* producing urine, 2. paired *ureters*, conveying the urine into urinary bladder, 3. *urinary bladder* for temporary storage of the urine, 4. *urethra* by which the bladder empties

Kidneys – bean-shaped organs localized behind the peritoneal cavity (retroperitoneal space) in the lumbar region on both side of the vertebral column. The *upper pole* extends as far as the 12th thoracic vertebra, the *lower pole* reaches down to the 3rd lumbar vertebra. The right kidney is about half a vertebra lower than the left. On the medial margin lies the *renal hilum* through which the blood and lymphatic vessels, nerves and the *renal pelvis* enter and leave. *Renal pelvis* collects the urine and continuos into the ureter. Kidney consists of *medulla* and *cortex.*

Ureters – are muscular tubes whose peristaltic contractions convey the urine to the urinary bladder. Ureter has *abdominal*, *pelvic* and *intramural part.* The total length is about 30 cm.

Urinary bladder – lies in the lesser pelvis beneath the peritoneum and behind the pubic bones. It has the *apex, body* and *fundus.*

Male urethra – is about 20 cm long and extends from an *internal urethral orifice* in the urinary blader to an *external urethral orifice* at the end of the penis. It has four parts: *intramural, prostatic, membranous* and *spongious part.*

Female urethra – is about 4 cm long. It begins at the *internal urethral orifice* in the urinary blader and ends at the *external urethral orifice* anterior to the opening of the vagina, about 2.5 cm behind the clitoris.

The urinary system consists of the two kidneys, which excrete urine, the excretory passages, which convey urine from the kidneys to the exterior of the body. Excretory passages include: the minor calyces, major calyces, renal pelvis (one for each kidney), the two ureters, the urinary bladder, the urethra.

Functions:

1. To clear the blood of nitrogenous and other waste metabolic products by *filtration* and *excretion*.
2. To balance the concentration of body fluids and electrolytes also by filtration and excretion.
3. To recover by *reabsorption* small molecules (amino acids, glucose, peptides), ions (Na^+, Cl^-, Ca^{2+}, P^{3-}) and water, in order to maintain blood *homeostasis*.
4. The kidney regulates *blood pressure* by producing the enzyme *renin*.
5. The kidney is also an *endocrine organ*. It produces hormone *erythropoietin*, a stimulant of red blood cells production in bone marrow. It also activates 25-hydroxycholecalciferol to 1.25-dihydroxycholecalciferol (calcitriol), an active form of vitamin D involved in the control of calcium metabolism.

17.1. Kidney

The kidneys are located in the retroperitoneal space of the posterior abdominal cavity. On the upper pole of each kidney lies an adrenal gland. The kidney is a bean-shaped organ with a lateral convex border and medial concave border called the hilum. The space between and around the structures within the hilum of the kidney is called renal sinus.

The essential composition of kidney is that of a gland with highly modified secretory units and highly specialized ducts.

The main function of the kidney is to *filter the blood* supplied by the renal arteries branching from the descending aorta. The kidneys receive about 20% of the cardiac output per minute and filter about 1.25 l of blood per minute. All the blood of the body passes through the kidneys every 5 minutes. Approximately 125 ml of filtrate is produced per minute, but 124 ml of this amount is reabsorbed. About 180 l of fluid ultrafiltrate is produced in 24 hours. Of this amount, 178.5 l is returned to the blood circulation, whereas only 1.5 l is excreted as *urine*. The final urine contains water and electrolytes as well as waste products, such as urea, uric acid, creatinine and breakdown products of various substances.

Histological structure of the kidney

The kidney surface is covered by a connective tissue capsule. Each kidney has *cortex* (subdivided into outer cortex and juxtamedullary cortex) and *medulla*.

The cortex consists of renal corpuscles and their associated tubules. The vertical striations, the *medullary rays* (of Ferrein) project into the cortex from the medulla. Each medullary ray is an aggregation of straight tubules and collecting ducts.

The medulla is formed by conical structures the *medullary pyramids*, with their bases located at the corticomedullary junction. Usually 8 to 18 pyramids may by present in the human kidney. The apices of the pyramids face the renal sinus. The apical portion of each pyramid, the *papilla*, projects into a minor calyx. The tip of the papilla, also known as the *area cribrosa*, is perforated by openings of the collecting ducts. Each pyramid is divided into an outer medulla (adjacent to the cortex) and an inner medulla. The outer medulla is subdivided into an inner stripe and an outer stripe. The medulla is characterized by straight tubules, collecting ducts and a special capillary network, the vasa recta. The cortical tissue surrounding the lateral portion of the pyramid is forming the *renal columns* (of Bertini). The renal columns represent cortical tissue within the medulla.

Each medullary pyramid and the associated cortical tissue at its base and sides (one half of each adjacent renal column) constitute a lobe of the kidney. The lobar organization of the kidney is conspicuous in the developing fetal kidney. The number of lobes in human kidney equals the number of medullary pyramids. Kidneys of some animals possess only one pyramid; these kidneys are classified as unilobar, in contrast to the multilobar kidney of the human.

The lobes of the kidney are subdivided into *lobules consisting of a central medullary ray and surrounding cortical tissue.* A lobule consists of a collecting duct and all the nephrons that it drains.

17.1.1. Nephron

The nephron is the structural and functional unit of the kidney. Each human kidney contains approximately 1 million nephrons. The nephron consists of the renal corpuscle and tubule system.

Renal corpuscle or Malpighian corpuscle

The renal corpuscle represents the beginning of the nephron. It is the filtering unit. It consists of *the glomerulus*, a tuft of capillaries composed of 10 to 20 capillary loops and the renal or *Bowman´s capsule*, a double-layered epithelial cup surrounds the glomerulus. The glomerular capillaries are supplied by an afferent arteriole and are drained by an efferent arteriole. The site where the afferent and efferent arterioles penetrate the renal corpuscle is called the *vascular pole*. Opposite this site is the *urinary pole* of the renal corpuscle, where the proximal convoluted tubule begins (Fig. 1).

Bowman's capsule

The *capsule of Bowman* has two layers:

The *visceral layer*, attached to the capillary walls of glomerulus. It is lined by epithelial cells called *podocytes*.

The *parietal layer*, associated with connective tissue stroma. It is covered by simple squamous epithelium and is continuous with simple cuboidal epithelium of the proximal convoluted tubule.

Urinary space or Bowman´s space or capsular space exists between the visceral and parietal layers of the capsule. It contains the plasma ultrafiltrate (primary urine). The urinary space is continuous with the lumen of the proximal convoluted tubule at the urinary pole.

The glomerulus

The *glomerular capillaries*, lined by fenestrated endothelial cells.

The *mesangium*, formed by *mesangial cells* embedded in the *mesangial matrix*. The mesangial cells are enclosed by the basal lamina of the glomerular capillaries. Same of *mesangial cells are located outside the corpuscle along the vascular pole*, where they are called as *lacis cells* and form part of the juxtaglomerular apparatus.

The functions of mesangial cells:

Phagocytosis - they remove trapped residues and aggregated proteins from the glomerular basement membrane. The mesangial cells proliferate in certain kidney diseases in which abnormal amounts of protein and protein complexes trapped in the basement membrane.

Structural support - they provide support for the podocytes in the areas where the epithelial basement membrane is absent or incomplete.
Secretion - they synthesize and secrete a variety of molecules such as interleukin and platelet-derived growth factor, which play a central role in response to glomerular injury.
Mesangial cells are contractile. They may also play a role in regulating glomerular blood flow. They *regulate glomerular filtration by controlling blood flow trough glomerular capillaries.* Mesangial and juxtaglomerular cells are derived from smooth muscle cell precursors.
The *podocytes* (cells of visceral layer of the capsule of Bowman), have long and branching cell processes that encircle the surface of the glomerular capillary. Numerous secondary processes called *pedicels* or *foot processes*. The podocytes and fenestrated endothelial cells and their corresponding basal laminae constitute *the glomerular filtration barrier*.

Components of the filtration apparatus (barrier)
The renal corpuscle contains the filtration apparatus of the kidney.
The filtration apparatus of the kidney consists of:
The *endothelium* of the glomerular capillaries. It is *fenestrated* and permeable to water, sodium, urea, glucose and small proteins.

The *basal lamina* (glomerular basement membrane), a product of endothelial cells and podocytes. It acts as a physical barrier and an ion-selective filter. The glomerular basement membrane consists of: the *lamina rara externa*, adjacent to the podocyte processes, the *lamina rara interna*, adjacent to the capillary endothelium, the *lamina densa*, the overlapping portion of the two basal laminae, sandwiched between the laminae rarae. It contains type 4 collagen, which acts as a physical filter. The laminin and other proteins present in the lamina rara interna and externa are involved in the attachment of the endothelial cells and podocytes to the glomerular basement membrane.

The pedicels are cell processes of podocytes covering the basal lamina. The space between adjacent pedicels is called the *filtration slit*. *A filtration slit diaphragm* links adjacent pedicels. The filtration apparatus may thus be described as a semipermeable barrier.

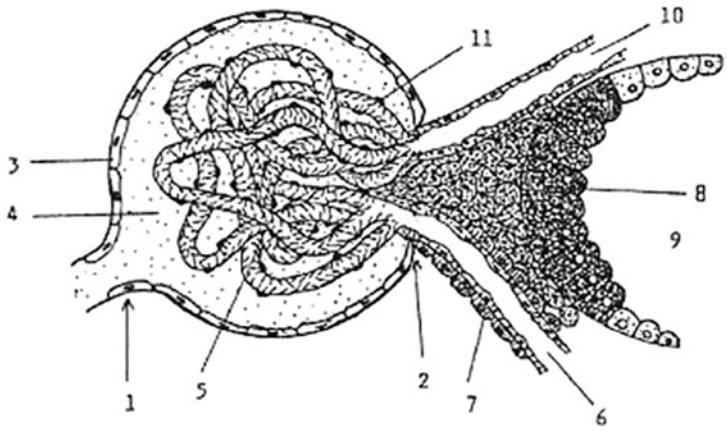

Fig. 1. The renal corpuscle

1-urinary pole, 2-vascular pole, 3-Bowman´s capsule – parietal layer, 4-urinary space, 5-glomerulus, 6-afferent arteriole, 7-juxtaglomerular cells, 8-macula densa of distal tubule, 9-distal tubule, 10-efferent arteriole, 11-podocyte

Juxtaglomerular apparatus

The juxtaglomerular apparatus includes *the macula densa, the juxtaglomerular cells*, and *the extraglomerular mesangial cells.*

The macula densa is an epithelial region found in the distal straight tubule at the vascular pole of the renal corpuscle. The macula densa faces the triangular area formed by the afferent and efferent arterioles of the same nephron. The cells of the macula densa are narrower and taller than other distal tubule cells. They are in contact with extraglomerular mesangial cells.

The juxtaglomerular cells. They are modified the smooth muscle cells of the afferent arteriole. These cells contain secretory granules, and their nuclei are spherical. The granules of the juxtaglomerular cells contain *renin*, which is synthesized, stored, and released into the bloodstream from the modified smooth muscle cells. The juxtaglomerular apparatus regulates blood pressure by activating the renin-angiotensin-aldosterone system. This system plays an important role in maintaining sodium homeostasis and renal hemodynamics.

The extraglomerular mesangial cells. They are located outside the corpuscle along the vascular pole (lacis cells).

Clinical correlations

Primary structural abnormalities of the glomerulus are known by the name *glomerulonephritis.* Abnormalities in the structure of the glomerular basement membrane are responsible for some important kidney diseases, which are characterized by an excessive loss of protein in urine *(proteinuria),* a low blood albumin *(hypoalbuminemia)* and *edema.* The combination of proteinuria, hypoalbuminemia and edema is called the *nephrotic syndrome.*

In some forms of imunological damage, there is a proliferation of mesangial cells which leads to compression of the glomerular capillaries (*mesangial glomerulonephritis*). It is often associated with the deposition of immune complexes within the mesangium. *Chronic essential hypertension***,** the most common form of hypertension, was somehow related to an abnormality in the renin-angiotensin-aldosterone system.

Tubular parts of nephron

The tubular segments of the nephron are named according to the course that they take (convoluted or straight), location (proximal or distal), and wall thickness (thick or thin). As the glomerular ultrafiltrate passes through the uriniferous and collecting tubules of the kidney, it undergoes changes. Certain substances within the ultrafiltrate are reabsorbed, some partially (water, sodium, and bicarbonate) and some completely (glucose). Other substances (creatinine and organic acids and bases) are added to the ultrafiltrate by secretory activity of the tubule cells.

The sequential parts of the nephron consist of the following tubules:

Proximal convoluted tubule. It originates from the urinary pole of Bowman´s capsule. It follows a very convoluted and then enters the medullary ray.

Proximal straight tubule, (commonly referred to as the thick descending limb of the loop of Henle), descends into the medulla.

Thin descending limb of the loop of Henle. It makes a hairpin turn and returns toward the cortex.

Thin ascending limb of the loop of Henle is the continuation of the thin descending limb after its hairpin turn.

Distal straight tubule, (is also referred to as *the thick ascending limb of the loop of Henle*) ascends trough the medulla and enters the cortex in the

medullary ray. The distal straight tubule makes contact with the vascular pole of its parent renal corpuscle. At this point the epithelial cells of the tubule are modified to form the *macula densa*.
Distal convoluted tubule is less tortuous than the proximal convoluted tubule. The distal convoluted tubule empties into a collecting duct that lies in the medullary ray.

Proximal convoluted tubule

The proximal convoluted tubule is the initial and *major site of reabsorption.* It is lined by cuboidal cells with the apical microvillous *brush border* and abundant mitochondria within basolateral interdigitations. *Basal striations* consist of mitochondria concentrated in the basal processes and oriented vertically to the basal surface. The proximal convoluted tubule reabsorbs about 150 l of fluid per day or about 80 % of the ultrafiltrate. It also reabsorbs all the glucose and amino acids, the sodium chloride and water. Proteins and large peptides are reabsorbed in the proximal convoluted tubule.

Proximal straight tubule

The cells of the proximal straight tubule *are not as specialized for absorption as are the cells of proximal convoluted tubule.* They are shorter, with a less well developed brush border and less complex lateral and basal processes.

Thin segment of loop of Henle

The length of the thin segment varies with the location of the nephron in the cortex. Juxtamedullary nephrons have the longest limbs, cortical nephrons have the shortest. The thin descending and ascending limbs of the loop of Henle have *narrowed lumina and simple squamous epithelium.* The ultrafiltrate that enters the thin descending limb is isoosmotic whereas the ultrafiltrate leaving the thin ascending limb is hypoosmotic to plasma. This change is achieved by reabsorbing more salts than water. *The loop of Henle establishes a hypertonic medullary interstitium* by active and passive transport of sodium chloride and urea from the tubule into the medullary interstitium.

The distal straight tubule (The thick ascending limb loop of Henle)

In routine histological preparations, the large cuboidal cells of the distal straight tubule stain lightly with eosin. The nucleus is located in the apical portion of the cell. The distal straight tubule (thick ascending limb) transports ions from the tubular lumen to the interstitium. Approximately 15–25% of filtered NaCl is reabsorbed in the loop of Henle, mainly by the thick ascending limb. The descending thin limb is highly water-permeable. By contrast, water permeability is negligible in the ascending limb. The thick ascending limb loop of Henle has a critically important role in urinary concentrating ability by contributing to the generation of a hypertonic medullary interstitium in a process called *countercurrent multiplication.* It contributes to reabsorption of calcium and magnesium ions. In the thick ascending limb, there is a high level of active salt transport enabled by the $Na^+/K^+/2Cl^-$ co-transporter on the apical membrane in series with basolateral Cl^- channels and Na^+/K^+-ATPase. The $Na^+/K^+/2Cl^-$ co-transporter is the site of action for the most potent class of diuretic agents (loop diuretics, e.g. furosemide).

Distal convoluted tubule

The distal convoluted tubule is the last segment of the nephron. It is located in the cortical labyrinth, is only about one third as long as the proximal convoluted tubule. This tubule is lined by *simple cuboidal epithelium.* In the distal convoluted tubule *sodium is absorbed and potassium ions are secreted.* This is the site of the mechanism that controls the total salt and water in the body. The distal tubule also secretes hydrogen and ammonium ions into tubular urine. This activity is essential for maintenance of the acid-base balance in the blood. *Antidiuretic hormone (ADH*) acts on the last part of the distal convoluted tubule to increase its permeability, thus permitting the absorption of water to produce more concentrated urine.

In the permanent *absence of ADH,* as occurs in the disease *diabetes insipidus*, vast volumes of dilute urine are formed because of the failure of water reabsorption at the distal convoluted tubule.

In histological sections, the distinction between the proximal and distal convoluted tubules (both found in the cortex) is on the following characteristics:

Cells of proximal tubules are larger than the cells of distal tubules and they are more acidophilic.
The lumens of the distal tubules are larger, because distal tubule cells are flatter and smaller than those of proximal tubule. More cells and more nuclei are seen in the distal tubule (Fig. 2).

Types of nephrons

Cortical nephrons have their renal corpuscles located in the outer part of the cortex. They have *short loops of Henle,* extending only into the outer medulla. Their thin descending and ascending segments are short; therefore the *hairpin turn occurs in the distal straight tubule.* Cortical nephrons have very short descending thin limbs and no thin ascending limbs.

Juxtamedullary nephrons. They renal corpuscles occur to the base of a medullary pyramid. They have *long loops of Henle* and long ascending thin segments that extend into the inner region of the pyramid. These loops consist of a long thin descending and ascending limbs.

Intermediate (midcortical nephrons) have their renal corpuscles in the midregion of the cortex. Their loops of Henle are of intermediate length.

17.1.2. Collecting tubules and collecting ducts

Urine passes from the distal convoluted tubules to collecting tubules, which join each other, forming larger, straight collecting ducts, the *papillary ducts of Bellini*. They are open on the tips of the pyramids. The smaller collecting tubules are lined with cuboidal epithelium. As they penetrate deeper into the medulla, their cells become taller, they are columnar cells (Fig. 3). In the medulla, collecting ducts are a major component of the urine-concentration mechanism. Water permeability of the epithelium of the collecting ducts is regulated by antidiuretic hormone (ADH or vasopressin) a hormone produced in the hypothalamus. Antidiuretic hormone increases the permeability of the collecting duct to water, there by producing more concentrated urine. In the absence of antidiuretic hormone, copious, dilute urine is produced. This condition is called *diabetes insipidus*.

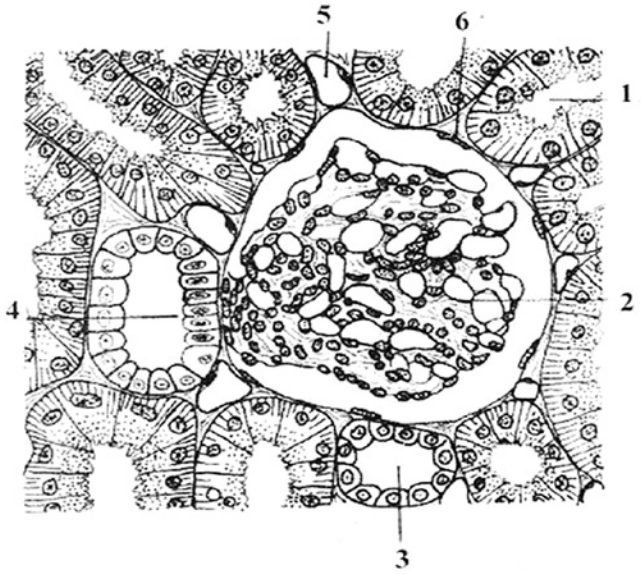

Fig. 2. Cortex of the kidney

1-proximal tubule, 2-glomerulus, 3-distal tubule, 4-macula densa, 5-blood capillary, 6- parietal layer of the Bowman´s capsule

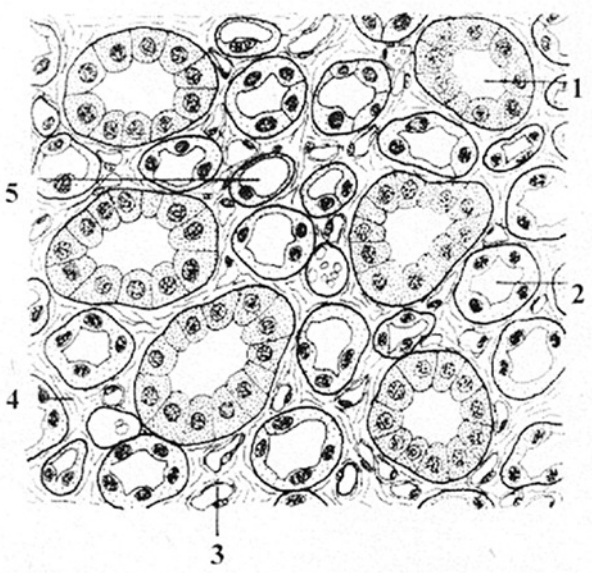

Fig. 3. Medulla of the kidney

1-collecting tubule, 2-thick limb of the loop of Henle, 3-blood capillary, 4-loose connective tissue, 5-thin limb of the loop of Henle

17.1.3. Blood supply

Each kidney receives a large branch from the abdominal aorta, called *renal artery*. The renal artery branches into the *lobar arteries* which form the *interlobar arteries* located between the renal pyramids. At the level of the corticomedullary junction, the interlobar arteries form the *arcuate arteries*. *Interlobular arteries* branch from the arcuate arteries ascend through the cortex toward the capsule. From the interlobular arteries arise the *afferent arterioles*, one to each glomerulus, which supply blood to the glomeruli. Blood passes from these capillaries into the *efferent arterioles*, which once branch again to form a *peritubular capillary network* that will nourish the proximal and distal tubules and carry away absorbed ions and low-molecular-weight materials. The efferent arterioles that are associated with juxtamedullary nephrons form long, thin capillary vessels *vasa recta*. They descend into the medulla alongside the loop of Henle. The capillaries of the outer cortex and the capsule of the kidney converge to form the *stellate veins*, which empty into the interlobular veins. Veins follow the same course as arteries. Blood from *interlobular veins* flows into *arcuate veins* and from there to the *interlobar veins*. Interlobar veins converge to form the *renal vein* through which blood leaves the kidney.

Lymphatic vessels

The kidneys contain two major networks of lymphatic vessels. One network is located in the outer regions of the cortex and drains into larger lymphatic vessels in the capsule. The other network is located more deeply in the substance of the kidney and drains into large lymphatic vessels in the renal sinus. There are numerous anastomoses between the two lymphatic networks.

Clinical correlations

Kidney failure. Decreased blood flow through the glomerular capillary system because of thickening of the arterial and arteriolar walls and the reduction in the lumina of these vessels *reduces glomerular filtration* rate and produces *chronic ischemia* of the tubular system. When these changes affect most of the glomeruli and their associated tubular systems, all of the functions of the kidney are impaired and the patient develops symptoms of *chronic renal failure.*

17.2. Urinary passages

On leaving the collecting ducts at the area cribrosa, the urine enters a series of structures that are specialized for its storage and passage to the exterior of the body. The urine flows to a *minor calyces*, *a major calyces* and *the renal pelvis* and leaves each kidney through the *ureters* to the *urinary bladder*, where it is stored. The urine is finally voided through the *urethra*.

17.2.1. Ureters

Two ureters conduct urine from the renal pelvis to the urinary bladder. It is approximately 24 to 34 cm long.

The wall of ureter consists of:

The mucosa layer. It consists of *transitional epithelium* and *a lamina propria* of loose connective tissue. The transitional epithelium (urothelium) lines the excretory passages leading from the kidney.

The muscularis. The smooth muscle is arranged in two layers: an inner longitudinal layer, an outer circular layer. At the distal end of the ureter are present three layers: an inner longitudinal, a middle circular and an outer longitudinal layer. This outer longitudinal layer continues into the wall of the urinary bladder to form a principal component of its wall. Peristaltic contractions of the smooth muscle move the urine from the minor calyces through the ureter to the bladder.

The adventitia. It is an external layer from loose connective tissue (Fig. 4).

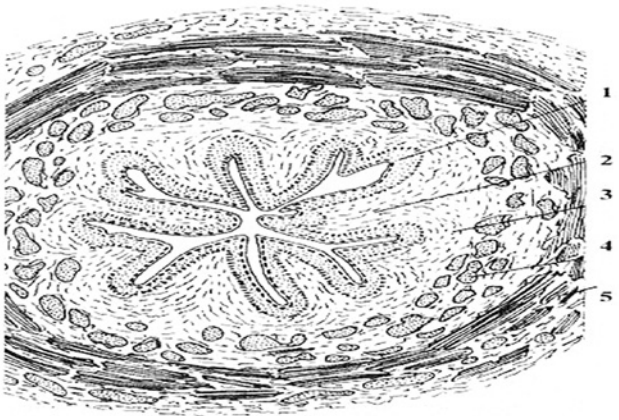

Fig. 4. The wall of ureter

1-transitional epithelium, 2-fold of mucosa layer, 3-lamina propria, 4-longitudinal layer of smooth muscle, 5-circular layer of smooth muscle

17.2.2. Urinary bladder

The bladder is a distensible reservoir for urine. Its size and shape change as it fills. It contains *three openings*, two for the ureters (ureteric orifices) and one for the urethra (internal urethral orifice). The trigone is the triangular region defined by these three openings.

The wall of the urinary bladder composed of *transitional epithelium, lamina propria*, three layers of *smooth muscle, adventitia and serosa*. In the bladder the epithelium varies in thickness according to the degree of distention of the organ. This stratified epithelium in the undistended state is 5-6 cells in thickness. The superficial cells are „dome-shaped"(rounded) cells, binucleate and bulge into the lumen. When the epithelium is stretched, as when the bladder is full of urine, the epithelium is only 3-4 cells in thickness and superficial cells become squamous. The muscle fibers of the bladder run in every direction without distinct layers. The smooth muscle forms the *detrusor muscle*. Contraction of the detrusor muscle of the bladder compresses the entire organ and forces the urine into the urethra. Toward the opening of the urethra, the muscle fibers form the *involuntary sphincter* of the urinary bladder. The urinary bladder is covered by the adventitia, except for the upper part of the bladder, which is covered by peritoneum (serosa).

17.2.3. Urethra

The urethra is the fibromuscular tube that conveys urine from the urinary bladder to the exterior through *external urethral orifice*. The size, structure, and functions of the urethra differ in males and females.

17.2.3.1. Male urethra

In the male, the urethra serves as the terminal duct for both the urinary and genital systems.

It is about 20 cm long and has three segments:

Prostatic urethra (3 to 4 cm). It passes through the prostate gland. It is lined with *transitional epithelium.* The ejaculatory ducts of the genital system enter the posterior wall of this segment and many small prostatic ducts also empty into this segment.

Membranous urethra (1 cm). It passes through the urogenital diaphragm. This segment is lined with *a stratified or pseudostratified columnar*

epithelium. Skeletal muscle of the urogenital diaphragm forms the *voluntary sphincter* of the urethra. The *Bulbourethral glands of Cowper* (mucous) are situated in the muscle near the distal part of the membranous urethra, but their ducts enter the spongy (cavernous) urethra.

Penile (cavernous, spongy) urethra (about 15 cm). This portion, lying in the penis, is the longest segment of the urethra and opens on the body surface at the glans penis. The penile urethra is surrounded by the corpus spongiosum. It is lined with *pseudostratified columnar epithelium except at its distal end,* where it is lined with *stratified squamous epithelium.* Ducts of the bulbourethral glands (Cowper´s glands) and of the mucus-secreting urethral glands (glands of Littré) empty into the penile urethra.

17.2.3.2. Female urethra

In the female, the urethra is short, measuring 3 to 5 cm in length. It is composed of an *epithelial lining, a connective tissue layer, and a muscular coat.* The epithelium of the proximal part is like that of the bladder. This type is replaced further down the tube *first by stratified columnar epithelium* and later, toward the *distal end, by stratified squamous epithelium*. The connective tissue layer contains elastic fibers and *rich plexus of veins,* which may be compared with the corpus spongiosum of the male urethra. The lumen of the organ is irregular, the connective tissue and epithelium are thrown into longitudinal rugae.

The muscularis consists of *two layers of smooth muscle*; *inner is longitudinal, outer circular*. Where the urethra penetrates the urogenital diaphragm, the striated muscle of this structure forms *the voluntary urethral sphincter*. Numerous small *urethral glands*, in the proximal part of the urethra, open into the urethral lumen. Other glands, the *paraurethral glands*, which are homologous to the prostate glands in the male, secrete into the common paraurethral ducts. These ducts open on each side of the external urethral orifice. They produce an alkaline secretion.

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18. Male reproductive system

Anatomy introduction

The male reproductive system produces spermatozoa and secrets male sex hormones, androgens. Androgens regulate spermatogenesis and the development and functioning of the secondary sex organs. The male reproductive system includes the *internal* and *external genital organs*. The internal genital organs consist of the *testes, epididymes, deferens ducts* and *prostate.* The external are *penis* and *scrotum.* Accessory glandular structures contain *seminal vesicles* and *bulbo-urethral glands.* The *spermatic cord* connects the testis to the abdominal cavity.

Testes – lie in the scrotum (externally). Testis is plum-shaped, 4-5.5 cm long. In the testis we distinguish an *upper* and *lower pole, medial* and *lateral surface* and *anterior* and *posterior margin.* Testes produce the sperm and male sex hormones.

Epididymes – are located on the posterior margin of testes. Epididymis has the *head, body* and the *tail.* Within the epididymis runs the *duct of the epididymis* which extends from the head to the tail of the epididymis and carries the sperm (spermatozoa).

Deferens ducts – paired structure which is a continuation of duct of the epididymis and serves for transport of sperm. It is 50-60 cm long and runs with nerves, blood and lymphatic vessels in the *spermatic cord* through the inguinal canal to the abdominal cavity. The deferens duct at the end dilates into the *ampulla of the deferens duct.*

Prostate – is a firm partly muscular, partly glandular body. It lies in the lesser pelvis under the urinary bladder, anterior to the rectal ampulla through which it can be palpated. Prostate surrounds the begining part of male urethra which runs through it (prostatic part of urethra).

Penis – consists of two erectile bodies *corpus cavernosum penis*, which start as two *crura of the penis* and serve only for erection, and unpaired *corpus spongiosum penis*, through which the urethra runs (spongious part). The penis has the *root of the penis, dorsum* and *glans penis* covered by *prepuce*.

Scrotum – the scrotum is the covering of testes and epididymes, it is formed by individual layers of abdominal wall (skin, fascias, muscles...) and regulates temperature in the testis.

Seminal vesicles – are paired, 5-10 cm long convoluted glands. They produce the alkaline fluid which runs through the *excretory duct*. Excretory ducts join the deferens ducts to form the *ejaculatory ducts*. The latter enter and open into the prostate (prostatic part of urethra).

Bulbo-urethral glands (glands of Cowper) – produce alkaline fluid which is part of semen. The glands are situated in the deep transverse perineal muscle and open directly into the urethra.

The male reproductive system or male gonads is a network of external and internal organs that produce, support, transport, and deliver viable sperm for reproduction. The male reproductive system includes:

1. *external genitalia*
   a) pair of testes within a scrotum
   b) the copulatory organ, the penis
2. *internal genitalia*
   a) extratesticular excretory ducts – epididymis, ductus deferens, and ejaculatory ducts
   b) accessory glands – seminal vesicles, the prostate and the bulbo-urethral glands

Functions:

The purpose of the organs of the male reproductive system is to perform the following functions:

a) to produce, maintain, and transport male mature reproductive cells – spermatozoa (sperm) and protective fluid (semen)
b) to facilitate fertilisation by introducing spermatozoa into the femal genital tract (copulation)
c) to produce and secrete male sex hormones (androgens) responsible for maintaining the male reproductive system

18.1. Testes and scrotum

The male gonads, testes, or testicles, begin their development high in the abdominal cavity near the kidneys. During the last two months before birth, or shortly after birth, they descend through the inguinal canal into the *scrotum,* a pouch that extends below the abdomen, posterior to the penis. The scrotum consists of skin and subcutaneous tissue. A vertical septum of subcutaneous tissue in the center divides it into two parts, each containing one testis. Smooth muscle fibers, called the dartos muscle,

in the subcutaneous tissue contract to give the scrotum its highly folded appearance. Another muscle, the cremaster muscle, consists of skeletal muscle fibers and controls the position of the scrotum and testes. The body temperature is very important for regulation of spermatogenesis which occurs only below temperature of 37ºC. Regulation of temperature is provide by blood vessels, evaporation of sweat from the scrotum and by relaxation or contraction of the dartos muscle and cremaster muscles.

Each testis is an oval structure about 4-5 cm long and 3 cm in diameter. A tough, white fibrous connective tissue capsule, *the tunica albuginea,* surrounds each testis and extends inward to form septa that divide the organ into lobules. Thicker posterior part of tunica albuginea forms *the mediastinum testis*. There are about 250 lobules in each testis. Each lobule contains 1 to 4 highly coiled tubules – *the seminiferous tubules* that converge to form a single *straight tubule*, which leads into the *rete testis*. Short *efferent ducts* exit the testes. *Interstitial cells (Leydig cells)*, which produce male sex hormones, are located between the seminiferous tubules within a lobule.

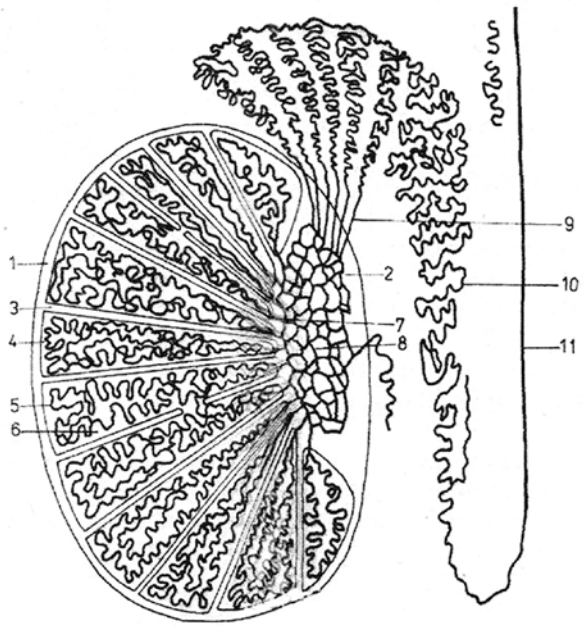

Fig. 1. Diagram of the testis, epididymis and excretory ducts of the male reproductive system

1-tunica albuginea, 2-mediastinum testis, 3-testicular septulus, 4-testicular lobulus, 5-seminiferous tubule, 6-interstitial connective tissue, 7-rete testis, 8-straight tubule, 9-ductulus efferentes, 10-epididymis, 11-ductus (vas) deferens

18.1.1. Seminiferous tubules

Each testicle has 250-1000 seminiferous tubules, which are 150 μm in diameter and 80cm in length. Total length of all of the seminiferous tubules is 300-900 m. They are coiled and have form the loops and their straight terminal portions, termed as straight tubules or tubuli recti enter into the system of anastomozing channels – *rete testis*. About ten or more ductuli *efferentes* drain the rete testis and form a major portion of the head of epidydimis. Each seminiferous tubule is line with epithelium termed as *germinal* or *seminiferous epithelium*. The lining epithelium is located on a well-defined basement membrane and layer of fibrous connective tissue, containing fibroblasts and spindle shaped myoid cells, which have properties of smooth muscle cells. Interstitial space between adjacent seminiferous tubules is filled by loose connective tissue containing rich blood and lymphatic supply and with supporting interstitial Leydig cells, secrete the androgen steroid *testosterone*.

The epithelium lining the seminiferous tubules is active dividing stratified epithelium consists of two types of cells: supporting *Sertoli cells* and germ cells undergoing meiosis and terminal maturation in the process of spermatogenesis. The germ cells form four to eight layers and vary with the appropriate stage of the maturation process. Morphologically distinct cells of the germ line occupy the germinal epithelium are the *spermatogonia, primary spermatocytes, secondary spermatocytes* and *spermatids*. The germinal epithelium produces mature haploid male gametes by a series of cell divisions. In process of *spermatocytogenesis* stem cells (spermatogonia) undergoes mitosis and following differentiation into primary spermatocytes. In subsequent process of first meiotic division are forming the secondary spermatocytes which undergo second meiotic division to produce haploid spermatids.

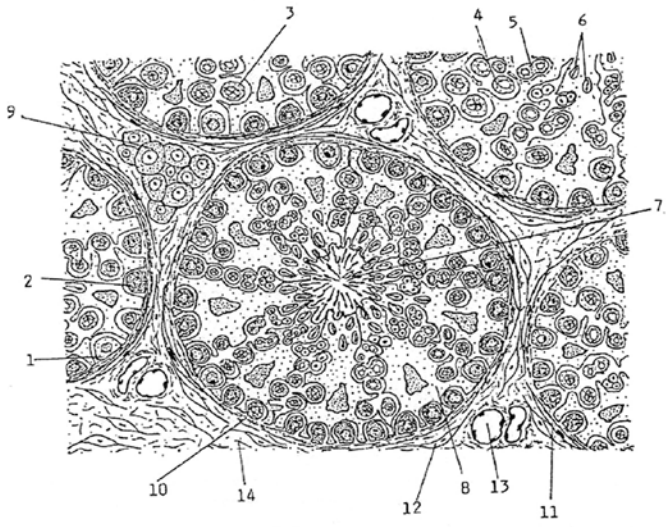

Fig. 2. Scheme of seminiferous tubules in testis

1-type B spermatogonia, 2-type A spermatogonia, 3-primary spermatocyte, 4-secondary spermatocyte, 5-early spermatide, 6-late spermatide, 7-spermatogonia (sperm), 8-Sertoli cell, 9-Leydig cells, 10-basement membrane, 11-myoid cell, 12-fibrocyte, 13-capillary, 14-interstitial connective tissue

18.1.2. Spermatocytogenesis and meiosis

In process of spermatocytogenesis the undifferentiated stem cells undergoes mitosis and following differentiation into primary spermatocytes. Initiation to mitotic division of stem cells, termed as spermatogonia appear during the puberty by stimulation of secretion of testosterone produced by Leydig cells. According to morphological patterns of their nuclei there are three subpopulations of spermatogonia: *type A dark spermatogonia (Ad)*, which are the type of stem cells, *type A pale spermatogonia (Ap)*, which arise from mitotic division of Ad cells. They mature into *type B spermatogonia*, the progenitor cells and differentiate into primary spermatocytes. The duration of spermatogenesis from spermatogonia to released immature spermatozoa is about 70 days.

The spermatogonia are small cells (about 12 μm in diameter) situated on basement membrane. They have poorly stained nucleus with fine chromatin. The type A spermatogonia are dome-shaped cells with ovoid nucleus. The nucleus of Ad spermatogonia stains more intensive than nucleus of Ap cells. The spermatogonia B have rounded nuclei with marginated

heterochromatin. These cells give rise to primary spermatocytes resides outwardly to spermatogonia.

Rounded shaped diploid primary spermatocytes have 46 (44+XY) chromosomes. There are the biggest cells in germinal epithelium and their nuclei contain chromatin in different phases of spiralisations according to appropriate stage of meiosis. The primary spermatocytes then pass through the prophase of first meiotic division. During this period, involves preleptotene, leptotene, zygotene, pachytene and diplotene phases, the nuclear chromatin undergo profound changes with formation of haploid secondary spermatocytes with only 23 chromosomes (22+X or Y). Secondary spermatocytes are smaller than primary and they have been seen only rarely because these cells are readily passes the second meiotic division. They enter into second meiotic division to produce haploid spermatids contain 23 chromosomes.

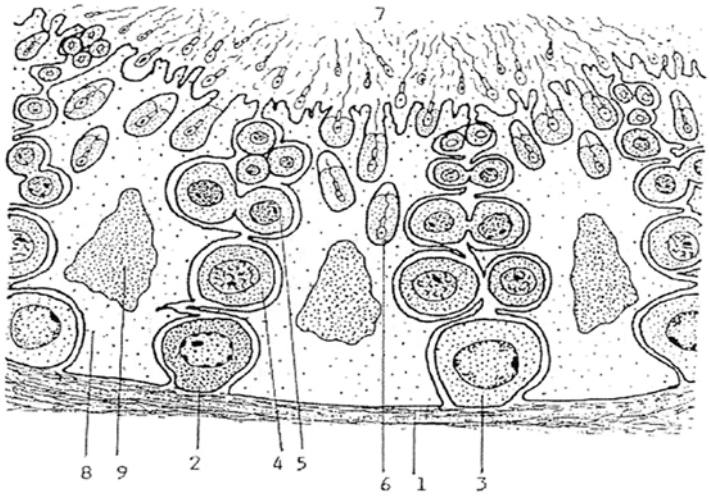

Fig. 3. Schematic view of spermiogenesis

1-basement membrane, 2-type A spermatogonia, 3-type B spermatogonia, 4-primary spermatocyte, 5-secondary spermatocyte, 6-spermatide, 7-spermatogonia (sperm), 8-Sertoli cell, 9-nucleus of Sertoli cell

18.1.3. Spermiogenesis

In process of *spermiogenesis* the haploid spermatids are transformed to spermatozoa. The spermatids are rounded cells resemble secondary spermatocytes but are smaller (5-8 μm in diameter). They undergo complex

process of differentiation involves chromatin condensation, nuclear elongation, deprivation of the cell's cytoplasm and organelles and formation of the acrosome and the flagellum. It can be divide into four phases:

a) *Golgi phase* – in prominent Golgi complex of spermatids are aggregates small PAS (periodic acid-Shiff stain) – positive granules – the proacrosomal granules. These fuse to form acrosomal vesicles bound to nuclear membrane on the anterior pole of the spermatozoon. The two centrioles migrate to the posterior pole of the spermatid and initiate formation of the axoneme, which forms the central core of the flagellum.

b) *Cap phase* – the acrosomal vesicles becomes flattened and spread anterior half of the nucleus as the acrosomal cap. The nuclear membrane becomes thicker and loses its pores.

c) *Acrosomal phase* – the nucleus increasingly flattens and elongates. It can be identify as anterior pole filled with acrosomal cap develop into acrosome and a posterior pole with constituting axoneme. Acrosome serve like specialized type of lysosome contain several hydrolytic enzymes that functioned in the process of fertilisation. The sheath of the microtubules extends from the posterior of the acrosomal cap towards the developing tail, called the manchette. The anterior pole of cell become pointed towards the base of the germinal epithelium and opposite the caudal pole together with developing flagellum extend towards the tubular lumen. The prominent mitochondria aggregate around the proximal part of newly forming flagellum and form the segment known as the middle piece. The mitochondria ended at a ring (annulus) demarcating the middle piece from the main parts of the tail (principal piece and end piece).

d) *Maturation phase* – is characterised by caudally displacement and rejection of cytoplasm as residual bodies and its phagocytosis by Sertoli cells. The immature spermatozoa are then disconnect from the Sertoli cells and releases in lumen of seminiferous tubule, to indicate the end of spermiogenesis.

18.1.4. Spermatozoon

The mature spermatozoon is composed of *a head* and *a tail*. Subsequently, the tail is subdivides into *neck, middle piece, principal piece and end piece*.

a) *Head* – is flattened about 4-5 μm long and 2.5-3.5 μm in width composed of the nucleus with condensed chromatin covered by acrosomal cap. The

acrosomal cap occupies most of the anterior part of nucleus containing numerous enzymes like protease, acid phosphatase, neuraminidase and hyaluronidase facilitate the penetration of the sperm into the ovum.

b) *Neck* – is short narrow region composed of the pair of centrioles and the connecting piece, which contains nine dense fibers of the flagellum surrounding the axoneme.

c) *Middle piece* – the center is composed of the axoneme surrouded by nine fibers from connective piece in the neck and an outer sheath of mitochondria. A distal part of the middle piece contains dense ring – annulus.

d) *Principal piece* – is the longest part of the sperm flagellum (40-45 μm long) and is composed of central axoneme (9+2) surrounded by seven outer dense fibers.

e) *End piece* – terminal part of the flagellum in which outer dense fibers terminates, therefore is composed of axoneme only.

18.1.5. Sertoli cells

Sertoli cells represent relatively constant type of cells of germinal epithelium. There are nondividing cells and main type of cells until puberty, after which their numbers decreases and again become predominant in senescence. Sertoli cells are elongated pyramidal cells and their bases sit on a basement membrane and apical site extending into lumen of seminiferous tubules. There are very hardly identifiable in light microscopy because of their irregular surface with numerous cytoplasmic extensions encloses the developing cells of germinal epithelium. Neighboring Sertoli cells form the tight junctions divide lining of seminiferous tubule into *basal* and *adluminal compartments*. Nucleus is irregular and euchromatic with prominent nucleolus. Cytoplasm is eosinophilic and contains abundant rough endoplasmic reticulum forming stacked cisternae (annulate lamellae) with ribosomes, prominent Golgi apparatus and numerous mitochondria. Sertoli cells cytoplasm is rich of lysosomes and lipid vacuoles.

The Sertoli cells are known as multifunctional cells having supporting, phagocytic, secretory function and play role in blood – testis barrier.

a) *Supportive function* – include exchange of nutrients and metabolites to developing germinal cells through the cytoplasmic processes of Sertoli cells. Sertoli cells serve also as barrier against the imunological attack.

b) *Phagocytic function* – they digest any residual cytoplasm (residual bodies) and organelles during the final stages of maturation of the spermatids to spermatozoa with final degradation by cell's lysosomes.

c) *Secretory function* – vary with stage of sexual maturity. During the embryonal life, Sertoli cells produce *Müllerian inhibitory substance (MIS)*, which causes regression of the developing Müllerian duct system. In mature testis secrete *androgen-binding protein (ABP)*, binds testosterone originated from the testicular interstitium and transport it into the lumen of seminiferous tubules and subsequently to the epidydimis. High levels of testosterone are need for normal germ cell maturation. Secretion of ABP is under control of follicle stimulating hormone (FSH) by the pituitary gland and enforce spermatogenesis. Another product of Sertoli cells, hormone inhibin inhibits secretion of FSH and act as negative feedback regulator of the rate spermatogenesis.

d) *Function in blood – testis barrier* – the existence of barrier is associated with fact, that there are only few substances from blood found in testicular fluid. This exclusion is form by occluding junctions located between processes of Sertoli cells. The junctions provide a barrier against the intercellular backflow to the interstitium of proteins from the new postmeiotic cells. Therefore, passages of large molecules occurs transcellularly through the Sertoli cell.

18.1.6. Interstitial tissue and Leydig cells

The interstitial tissue filled the space between the seminiferous tubules. It represents connective tissue containing network of fibrocollagenous tissue, blood, lymphatic vessels and nerves. Cell population is represented by fibroblasts, macrophages, mast cells and interstitial or Leydig cells, which have been detected in puberty.

The Leydig cells have rounded or polygonal shape and have eosinophilic cytoplasm with numerous mitochondria, abundant smooth endoplasmic reticulum and contain enzymes, such as lipases, oxidative enzymes, esterases and steroid dehydrogenases. They have rounded nuclei and one or two nucleoli.

Typical property of Leydig cell is a presence of the Reinke crystalloids, which are eosinophilic rectangular or rhomboid mass. There are not present before puberty and increasing numbers have been occured when sexual maturity has been finished.

The Leydig cells secrete hormone testosterone promotes the development and maintenance of of male secondary sexual characteristics. Testosterone binds ABH within the seminiferous epithelium and is present in high levels in tubular fluid containing the sperm, transported to the excretory duct system.

Hormonal stimuli have a great influence on the activity and the number of Leydig cells. During a pregnancy, placental gonadotropic hormone passes from the maternal blood to the fetus and stimulate fetal interstitial cells to production of androgenic hormones and subsequent fully differentiation of the male genitalia. The Leydig cells remain quiescent troughout the last five months of pregnancy and prepubertal period when they began testosterone synthesis as response to the stimulus of luteinizing hormone (LH) from the pituitary gland.

18.1.7. Semen

Semen (ejaculum) is viscous whitish or yellowish fluid composed of sperm and seminal plasma secreted from the testis, epididymis, ductus deferens and mostly from the accessory genital glands. During the process of ejaculation, sperm passes through the ejaculatory ducts and mixes with fluids from the seminal vesicles, the prostate and the bulbourethral glands to form the semen. The seminal vesicles produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of human semen. The prostatic secretion, influenced by dihydrotestosterone, is a whitish thin fluid containing proteolytic enzymes, citric acid, acid phosphatase and lipids. The bulbourethral glands secrete a clear secretion into the lumen of the urethra to lubricate it.

Semen quality is a measure of the ability of male semen to accomplish fertilization. According the information published by WHO in 1992, normal human semen having a volume of 2 ml or greater, pH of 7.2 to 8.0, sperm concentration of 20×10^6 spermatozoa/ml or more, sperm count of 40×10^6 spermatozoa per ejaculate or more, and motility of 50% or more.

18.1.8. Intratesticular ducts

The intratesticular ducts carry the liquid produced by Sertoli cells and spermatozoa from seminiferous tubules to epidydimis and are represents by:

a) *Tubuli recti (straight tubules)* – are short terminal portions of the seminiferous tubules lined with simple cuboidal to low columnar epithelium with microvilli on their luminal surface.

b) *Rete testis* – is complex of highly anastomosing channels located at the mediastinum of the testis lined with simple cuboidal to low columnar epithelium with motile flagella projecting from the luminal surface.

c) *Ductuli efferentes or efferent ducts* – are 10-20 tubules extent from the rete testis lined with the mixed epithelium, composed of ciliated columnar cells and nonciliated cuboidal cells with microvilli on their apical surface. The cilia moves within the efferent duct and facilitate transport of sperm and fluid. The non-ciliated cells absorb some of the fluid, which transport the nonmotile immature spermatozoa. The efferent ducts are highly coiled and are surrouded by thin layer of circular smooth muscle cells. They finally fuse to form the ductus epidydimis.

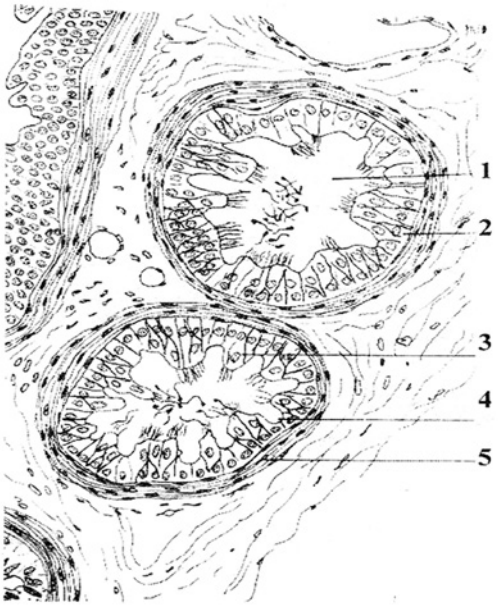

Fig. 4. Schematic section through the ductuli efferentes
1-stellate lumen of efferent ductulus, 2-epithelium, 3-columnar ciliated cells, 4-cuboidal nonciliated cells, 5-myoid cells

Clinical correlations

Cryptorchidism is the absence of one or both testes from the scrotum. It is the most common birth defect regarding male genitalia. About 3% of full-term and 30% of premature infant boys are born with at least one undescended testis. However, about 80% of cryptorchid testes descend

by the first year of life (the majority within three months), making the true incidence of cryptorchidism around 1% overall. Undescended testes are associated with reduced fertility, increased risk of testicular germ cell tumors and psychological problems when the boy is grown.

Testicular diseases can be classified as endocrine disorders or as disorders of the reproductive system. The most prominent diseases of testicles are:

a) *Testicular cancer and other neoplasms* – can be derive from any cell type found in the testicles, more than 95% of testicular cancers are germ cell tumors. Most of the remaining 5% are gonadal stromal tumours derived from Leydig cells or Sertoli cells.

b) *Swelling of a testicle*, cause by hydrocele testis is an accumulation of clear fluid in the tunica vaginalis, lead to enlargement in the scrotum.

c) Inflammation of the testicles, called orchitis – is an acute inflammatory reaction of the testis to infection. Most cases are associated with a viral infection (mumps), however other viruses and bacteria can cause orchitis.

18.2. Extratesticular ducts

The extratesticular ducts play role in transport of spermatozoa produced in testis toward accessory glands and finally to penis. The system consists of *epididymis, the vas deferens (ductus deferens), ejaculatory ducts and the urethra*.

18.2.1. Epididymis

The epididymis (ductus epididymis) is form by fused ductuli efferentes. The human epididymis is single highly coiled tube about 4-6 m long and is located on posterior surface of each testis. The organ consists of three anatomical parts: *a head (caput), a body (corpus)* and *a tail (cauda)*. The ductuli efferentes are fused in the head and forming a single tubule which continue into the body and the tail. The wall of epidydimis is composed of epithelium, thin tunica propria surrounded by smooth muscle layer. The muscle fibers are oriented circularly, but in the tail are present also inner longitudinal layer, which is internal to circular layer and outer longitudinal layer. The epididymis is lined with pseudostratified columnar epithelium composed of rounded basal cells and tall columnar cells with numerous long nonmotile stereocilia, which are a type of microvilli and are about 40-80 μm long.

A mass of sperm is located within the lumen and their movement is cause by peristaltic contraction of the tubular wall. The epidydimis plays important role in absorption of testicular fluid, phagocytosis and digestion of degenerate spermatozoa. Another important function is secretion of glycoproteins by epithelial cells in the epididymal lumen and subsequent absorption onto the sperm surface. Other secreted proteins are glycerylphosphorylcholine, sialic acid and carnitine. During the transition in epididymis, the spermatozoa become motile and capable of fertilizing ovum.

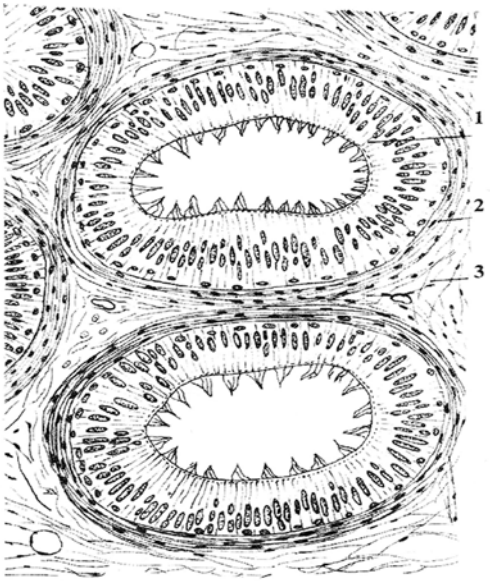

Fig. 5. Schematic view of epididymis
1-pseudostratified columnar epithelium with stereocilia, 2-circularly arranged smooth muscle layer, 3-interstitial loose connective tissue

18.2.2. Vas deferens

Vas deferens (ductus deferens) is straight and the most muscular tube of any excretory ducts with beginning behind the epididymis within the scrotal spermatic cord, which contain arteries, veins, lymphatic vessels, nerves and muscular and connective tissue bundles. The vas continues upwards passes through the inguinal canal, enters the pelvic cavity and terminates downwards to the base of urinary bladder, in dilatation named *ampulla*.

The wall of vas deferens consists of a mucosa, prominent muscularis and adventitia. Mucosa is forming longitudinal folds surround the narrow

lumen. The epithelium resemble to that in the epididymis, it is pseudostratified columnar epithelium with stereocilia. Underlying lamina propria is fibro-elastic and the thick muscularis (thickness: 1-1.5 mm) consists of smooth muscle arranged in the middle circular layer and inner and outer longitudinal layer. An advetitia of loose connective tissue surrounds the organ. For dilated ampulla is typical thicker mucosa because of the folds, and thinner muscularis. Peristaltic movement of muscular wall drives the spermatozoa forwads during ejaculation. Distal end of the ampulla is fused with a short duct from seminal vesicles.

18.2.3. Ejaculatory ducts

The paired ejaculatory ducts are 1 cm long formed by joining of the distal part of the vas deferens with the excretory duct of seminal vesicle. These ducts run straight course and enter the prostate. The duct's wall arrangement resembles the vas deferens, except of smooth muscle layer.

18.3. Accessory glands

The secretion from each of these glands is add to the testicular fluid and forms a substantial part of the semen. There are three major types of accessory glands: *the seminal vesicle, the prostate gland* and *the bulbourethral glands*.

18.3.1. Seminal vesicle

Seminal vesicles are paired highly coiled tubes about 15 cm long lie on the posterior part of urinary bladder apart to the ampullae of vasa deferentia. Mucosa of seminal vesicle is composed of fibroelastic lamina propria and is highly folded. The highly branching and anastomozing folds are covered with columnar or pseudostratified columnar nonciliated epithelium. The columnar cells have properties of secretory cells and contain large secretory vacuoles. The secret of seminal vesicle is the main component of the human ejaculate. It is viscid yellowish fluid contain carbohydrates (principle is fructose), prostaglandins, flavins, aminoacids and proteins, represents the components which activated the spermatozoa. The mucosa is surrouded by inner circular, outer longitudinal smooth muscle layer and external fibrocollagenous layer containing also elastic fibers.

18.3.2. Prostate

The prostate (prostate gland) is the largest of the male accessory glands composed of branched *tubuloalveolar* secretory glands. The ducts enter into the prostatic urethra, which crosses the organ. The prostate is located before the neck of urinary bladder and fills the first portion of male urethra. Thin fibrocollagenous capsula rich in smooth muscle and elastic fibers surrounds the organ from which septa extents into its body and divides it into lobes. Fibromuscular stroma contains numerous prostatic glands. The prostatic glands are arranged concentrically in three zones:

a) *inner periurethral zone* or *the transition zone* – occupies about 5% of the prostate, contain mucosal glands, group of 30-40 short glands which drain directly into the urethra,
b) *outer periurethral zone* or *the central zone* – occupies 25% of the gland, contain submucosal glands, which are situated more peripherally and drain larger ducts,
c) *peripheral zone* – occupies about 70% of the prostate volume, contain main glands, which are open into urethra via long ducts emptying on side of *seminal colliculus (verumontanum)* or lateral recesses of the prostatic urethra.

The glands are line with cuboidal or columnar pseudostratified epithelium. Columnar or cuboidal cells have pale stained foamy cytoplasm and lightly stained nuclei. Basal cells are small and flattened. Secretory products of these cells include acid phosphatase, amylase, fibrinolysin and zinc. Lumens of glands, predominantly in older men contain small spherical *corpora amylacea* or *prostatic concretions* composed of glycoprotein that may calcify. The ducts are lined with columnar epithelium become cuboidal and in prostatic urethra convert into transitional epithelium.

Prostatic epithelium is under influence of testosterone levels. Any variances manifest in changes of type epithelium from columnar to cuboidal and alterations of secretory activity increase with age.

Clinical correlations

The most common clinical condition of prostate in senescent age is *benign prostatic hyperplasia (BPH)* refers to the increase in size of the prostate. It is characterize by hyperplasia of prostatic stromal and

epithelial cells, resulting in the formation of large, fairly and discrete nodules in the periurethral region of the prostate.

Prostatic adenocarcinoma develops in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy, and eventually die of other causes. This is because cancer of the prostate is in 2/3 cases, slowly growing. Rates of detection of prostate cancers vary widely across the world, with South and East Asia detecting less frequently than in Europe, and especially the United States. Prostatic adenocarcinomas are composed of small glands with little or no intervening stroma. Prostate cancers may be detected by screening with a blood test for prostate specific antigen (PSA) produced almost exclusively in the epithelium of the prostate gland.

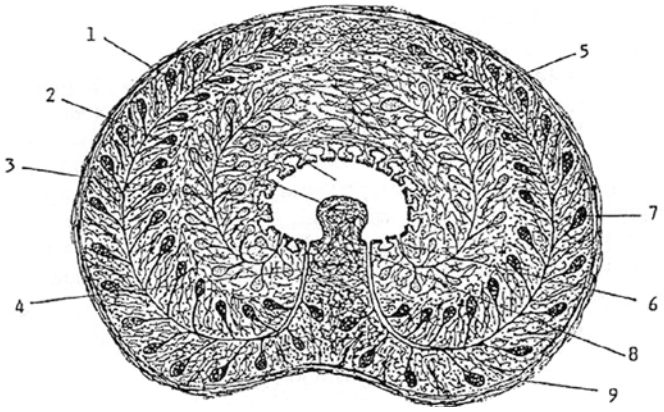

Fig. 6. Schematic diagram of prostate

1-prostatic urethra, 2-seminal colliculus, 3-mucosal glands, 4-fibromuscular stroma, 5-submucosal glands, 6-ducts of submucosal glands, 7-main glands, 8-ducts of main glands, 9-fibrocollagenous capsula rich in smooth muscle and elastic fibers

18.3.3. Bulbourethral glands

Bulbourethral glands known as *Cowper's glands* are paired small glands about 5 mm in diameter located in pelvic diaphragm surrounds the membranous urethra. The glands are compound and tubuloalveolar lined with simple cuboidal to columnar epithelium. Their mucous secret consists of fluid containing carbohydrates (mostly galactose), galactosamine, galacturonic acid and sialic acid. The secret is release through the ducts in the penile urethra (cavernous portion of urethrae) and acts like urethral lubricant.

18.4. Penis

This male copulatory organ is composed of three cylindrically arranged masses of erectile tissue. Dorsally are place two cylinders – *corpora cavernosa penis* and ventrally is located smaller *corpus spongiosum* or *corpus cavernosum urethrae* passes the penile urethra. The dilated terminal portion of the corpus spongiosum is termed as *glans penis*. The erectile tissue is surrounded with dense fibrocollagenous connective tissue, the *tunica albuginea*. Most of the penile urethra is line with pseudostratified columnar epithelium and in the glans penis, where the urethra dilates *(navicular fossa),* is replaced by stratified squamous non-keratinizing epithelium. The penile skin's fold *(prepuce)* contains connective tissue with smooth muscle and sebaceous glands. The prepuce allows retract the skin over the glans penis during sexual intercourse.

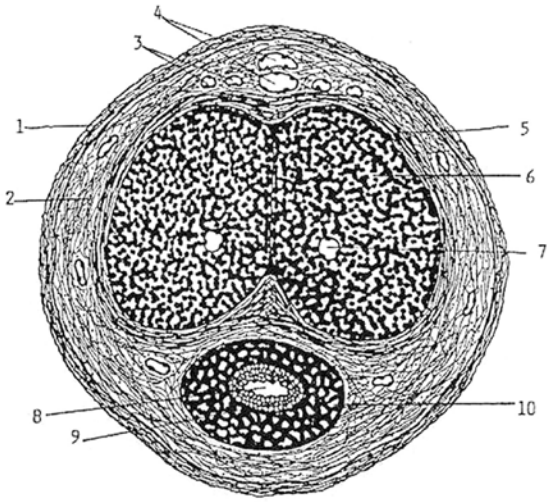

Fig. 7. Schematic diagram of penis

1-penile skin (prepuce), 2-subcutaneous tissue, 3-dorsal penis arteries, 4-deep dorsal penis veins, 5-tunica albuginea, 6-corpus cavernosum penis, 7-deep artery, 8-cavernous portion of urethrae (penile urethra), 9-epithelium, 10-corpus cavernosum urethrae (corpus spongiosum)

18.4.1. Erection, ejaculation and detumescence

Blood is brought to the penis by the *dorsal penis artery* and the paired *deep penis arteries*. The dorsal penis artery sends branches to

the tunica albuginea and to the large trabeculae of the cavernous bodies. Such branches break up into capilaries, from which blood passes into the lacunae of the erectile tissue (corpora cavernosa) and then to a plexus of veins in albuginea. The deep penis arteries run lengthwise, giving off branches that open into the cavernous spaces.

Erectile or cavernous tissue consists of large numbers of interconnective vascular spaces. These spaces are line with endothelium and separated trabeculae of connective tissue containing fibroelastic tissue and smooth muscle. In the flaccid condition of penis, the vascular spaces of the erectile tissue contain little blood. In this condition, much of the arterial blood flow is divert into arteriovenous anastomoses that connect the branches of the deep and dorsal penis arteries of the organ to veins that deliver their blood into the deep dorsal penis vein. Thus, the blood flow bypasses the vascular spaces of erectile tissue. In the erect penis, the arteriovenous anastomosis is constricted and blood flow into the vascular spaces of the erectile tissue is increase, causing the penis to become turgid with blood. Erection is controlled by the parasympathetic nervous system, it is result of sexual, tactile, olfactory, visual, auditory, and/or psychological stimulation. The parasymphatetic impulses trigger local release of nitric oxide, which causes relaxation of smooth muscles of the branches of the deep and dorsal arteries of the penis, increasing the blood flow into this organ.

Continued stimulation of the glans penis results in ejaculation, the forceful expulsion of semen from the male genital ducts. Ejaculation is regulated by the sympathetic nervous system.

Ejaculation is followed by the cessation of parasympathetic impulses to the vascular supply of the penis. The arteriovenous anastomosis is reopened. The vascular spaces of the erectile tissues are slowly emptied of blood by the venous drainage. As the blood leaves these vascular spaces, the penis undergoes detumescence and becomes flaccid.

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19. Female reproductive system

Anatomy introduction

The female reproductive system produces ova, secrets sex hormones, receives spermatozoa from the male, and provides sites for fertilization and implantation. The gestation is following by parturition. The female reproductive system consists of the *internal genital organs* and the *external genital organs.* The internal genital organs are situated within the lesser pelvis and include the *ovaries, uterine (Fallopian) tubes, uterus (womb)* and *vagina.* The external ones are the *labia majora* and *minora pudendi, clitoris, vestibule of the vagina*, *vestibular bulb* and *greater vestibular glands.* They are lying in front of and below the pubic arch.

Ovaries – are located in the lateral walls of the lesser pelvis *(ovarian fossa).* An ovary is 2.5 - 5 cm long and has two extremities, two surfaces and two margins. It consists of *cortex* and *medulla.* Ovaries produce eggs (ovum) and female sex hormones.

Uterine (Fallopian) tubes – lie on each side of the uterus. Uterine tube is about 10 cm long and opens medially by an *uterine ostium* into the uterine cavity and laterally by the *abdominal ostium* into the peritoneal cavity near the ovary. It has the following parts: *infundibulum, ampulla, isthmus* and *uterine part.*

Uterus – is a pear-shaped, hollow and thick-walled muscular organ. It is located in the lesser pelvis between the urinary bladder (in front the uterus) and rectum (behind the uterus). The uterine tubes open into its upper part, while caudally, it continues into the vagina. Uterus is divided into the *body of the uterus* and *cervix (neck);* the upper part of the body is called *fundus uteri.* The cervix has a *supravaginal portion* and a *vaginal portion.* The *cervical canal* opens at the *ostium uteri* into vagina.

Vagina – is an 8-10 cm long, thin-walled tube. It opens into the *vestibule of the vagina* at the *ostium of the vagina.*

Labia majora pudendi – are paired skin folds, which surround the pudendal cleft *rima pudendi*.

Labia minora pudendi – are thin skin folds, which form the borders of the *vestibule of the vagina.*

Clitoris – originates by two *crura of the clitoris,* which form the *body of the clitoris.* The body ends in the *clitoral glans.*

Vestibular bulb – is a paired cavernous body of the vestibule of vagina. Vestibular bulb consists of venous plexuses.

Function:

All organs of the female reproductive system function exclusively in *the sexual and reproductive capacity*. In general, female reproductive system has seven major functions:

- formation of female sex cells - gametes, the oocyte (ova, egg)
- acceptance of male gametes, the sperm (spermatozoa)
- providing of favourable conditions for fertilization of oocyte by sperm
- providing of suitable conditions for the development of embryo and fetus
- delivery of mature fetus to the external environment
- production of female sex hormones
- proper nutrition of newborn

These functions are controlled by precise co-operation of hormonal and nervous systems.

19.1. Ovaries

The surface of each ovary is covered by simple cuboidal epithelium derived from the peritoneum. This covering is called germinal epithelium, although it does not form any gametes. It is continuous with the simple squamous mesothelial covering of the mesovarium and uterus. Directly beneath this epithelium there is the tunica albuginea, a dense and irregular collagenous connective tissue capsule.

Each ovary is divided into a wide *cortex* and a central *medulla*, which are not sharply delineated. The ovarian cortex contains various developmental stages of follicles and connective tissue stroma consisting of cells that respond in unique ways to hormonal stimuli. The medulla consists of connective tissue with blood vesels, nerve fibers and lymphatic vessels.

19.1.1. Ovarian follicles

Primordial (resting) follicles

They are composed of a *primary oocyte (immature ovum)* enveloped by a single (unilaminar) layer of flat *follicular cells*. Primary oocyte has

a pale appearance and a large nucleus with prominent nucleolus. There are also many Golgi complexes, rough endoplasmic reticulum (RER) and mitochondria. The oocyte becomes arrested in prophase of meiosis I and remains in this stage until ovulation. Follicular cells are bound to one another by desmosomes and separated from the surrounding connective tissue (stroma) by a continuous basement membrane.

At the time of birth, the ovaries contain up to one million follicles. Only a few hundred of them become mature. The rest of these primordial follicles degenerate and become atretic. At menopause, only a few follicles remain in the ovary cortex.

Primary (growing) follicles

Primordial follicles give rise to primary follicles. They increase in size to about 50 μm. At this stage, follicular cells are called granulosa cells. As the follicular development continues, the glycoprotein *zona pellucida* emerges between the oocyte and the surrounding granulosa cells.

There are two types of primary follicles - *unilaminar and multilaminar*. The unilaminar primary follicles have a single layer of cuboidal cells surrounding the oocyte. The multilaminar primary follicles develop from the unilaminar follicles by proliferation of the cuboidal granulosa cells, which form several layers around the primary oocyte. Granulosa cells are separated from the surrounding stromal tissue by a basement membrane. The stromal tissue around these primary follicles starts to proliferate and differentiates into two layers, an *inner cellular layer* (theca folliculi interna) and an *outer fibrous layer* (theca folliculi externa). If the development of follicles continues, these theca folliculi cells develop the properties of steroid hormone – manufacturing cells. While growing, the follicles migrate to the deeper parts of the peripheral cortex.

Secondary (antral) follicles

Multilaminar primary follicles become secondary follicles as soon as a complete *antrum* is formed. The antrum is filled with follicular fluid (liquor folliculi) containing various hormones (e.g. estradiol, follistatin, activin, progesterone) and hyaluronic acid. The zona pellucida is distinct. The oocyte is eccentrically located on a small tuft called the cumulus

oophorus, where it is surrounded by granulosa cells. These cells are linked with zona pellucida by narrow processes. Granulosa cells keep in contact with each other via gap junctions. The cuboidal cells of vascular theca foliculi interna are steroid – producing secretory cells. These elements manufacture androgens, which diffuse into granulosa cells, where they are converted into estrogens.

Graafian (mature) follicles

Proliferation of the *granulosa cells* and increased volume of *fluid* in antrum folliculi result in the displacement of the oocyte to the periphery of the follicle. Mature follicle measures about 2.5 cm in diameter and bulges from the surface of the ovary. Granulosa cells of the cumulus oophorus form a single layer and these cells are known as corona radiata. Granulosa cells of the wall of the follicle form the membrane granulosa. Cells of the theca folliculi interna continue to produce androgens, which are then converted into estrogens. The theca folliculi externa contains many blood vessels, which provide nourishment to the theca interna.

19.1.2. Ovulation

It is the process of *releasing the secondary oocyte* from the graafian follicle to the peritoneal cavity.

Ovulation in a woman, who has a 28-day menstrual cycle, occurs 14 days after the onset of menstruation. By the 14th day, estrogen produced by both the graafian follicle and the secondary follicle causes elevation of blood estrogen. High levels of estrogen cause a negative feedback mechanism that inhibits the hypothalamic FSH (follicle – stimulating hormone) releasing factor and decreases the amount of FSH secreted by the anterior pituitary. High levels of estrogen also cause a sudden surge of LH (luteinizing hormone) by basophils of the anterior pituitary. LH controls the final maturation of the follicle, stimulates ovulation and influences the formation as well as maintenance of the corpus luteum.

During ovulation, the secondary oocyte and its corona radiata leave the ruptured follicle at the ovarian surface. Due to the fact that the ovary and uterine tube are intimately related, the ovum (oocyte), zona pellucida, corona radiata and liquor folliculi are all swept into the fimbriated end of the oviduct (uterine tube).

19.1.3. Corpus luteum

Corpus luteum is formed from the *remnants* of the mature follicle. The ovulated follicle is transformed into a yellow glandular structure, the yellow body. At ovulation, the majority of granulosa cells remain in the ovary and the remnants of the follicle undergo changes leading to the formation of the corpus luteum. The first of these changes is the formation of a clot of blood, coming from the vessels of the theca folliculi interna that ruptured at ovulation. The subsequent changes involve the alteration of the character of granulosa cells and the cells of the theca interna. The wall of the corpus luteum, membrane granulosa, is thrown into folds with a central clot. The wall of the corpus luteum contains granulosa lutein cells, which are derived from granulosa cells and theca lutein cells from the theca interna cells. Granulosa lutein cells are large, pale cells (up to 50 μm in diameter). They develop all of the organelles necessary for steroid production. These cells manufacture progesterone and convert androgens produced by the theca lutein cells into estrogens. The theca lutein cells are smaller, around 15 μm in diameter, and they are located mainly along the periphery of the corpus luteum. These cells produce progesterone, androgens and some estrogens.

19.1.4. Corpus albicans

If the ovum is not fertilized, the corpus luteum begins to degenerate about 14 days after ovulation. It becomes smaller and forms a structure known as corpus albicans. The corpus albicans consists of a *dense connective tissue scar*.

If the ovum is fertilized and becomes implanted in the uterine mucosa, the corpus luteum is rescued from degeneration by human chorionic gonadotropin (hCG) hormone produced by the placenta. The corpus luteum remains active during pregnancy for the first six months, after which it degenerates and transforms into corpus albicans. The formation of the corpus luteum is stimulated by the LH surge.

To sum up, the following elements and structures are present in the ovary, besides the connective tissue stroma:

- germinal epithelium

- interstitial cells
- primordial follicles
- primary follicles
- secondary follicles
- mature follicles
- atretic follicles
- blood clots
- corpora lutea
- scars

19.2. Uterus

The human uterus is a muscular organ consisting of a fundus, corpus and cervix.

Fundus and corpus

The uterine wall of the fundus and corpus consists of *three layers*: endometrium mucosa, myometrium muscularis and adventitia (or serosa).

19.2.1. Endometrium

The endometrium of the uterus is composed of *simple columnar epithelium* and *lamina propria*. The epithelium consists of both *non-ciliated secretory columnar cells* and *ciliated cells*. The lamina propria houses simple branched tubular glands. The uterine glands are lined with simple columnar epithelium without any ciliated cells. The lamina propria is composed of an irregular collagenous connective tissue. It is highly cellular and contains stellate cells, macrophages, leucocytes and an abundance of reticular fibers.

The endometrium consists of two layers:

- *The functionalis (functional layer)* - it is thick, superficial layer near the lumen and undergoes changes during menstrual cycle. During the menstrual phase, this layer sloughs off as a result of ischemia and necrosis caused by the contraction of coiled (spiral) arteries. The thickness of the functional layer is controlled by the ovarian hormones estrogen and progesterone, so this layer undergoes a cyclic

thickening and shedding each month. This occurs when fertilization does not take place and the corpus luteum atrophies, causing the levels of estrogen and progesterone to fall.

- *The basalis (basal layer)* - it is a deep, narrow layer of the endometrium that is preserved during menstruation (it does not undergo cyclic changes). It houses the basal portion of the endometrial glands that provide for the re-epithelialization of the endometrium after the functional layer is shed.

Blood supply of the endometrium

The *arcuate arteries* located in the middle layer of the myometrium give rise to *coiled helical arteries*. These arteries branch to form a rich capillary network that supplies the glands and stroma of the functional layer. Arcuate arteries also give rise to *straight arteries*, which supply the permanent basal layer of the endometrium.

19.2.2. Myometrium

The myometrium is a very thick layer of interwoven bundles of *smooth muscles separated by connective tissue*. Muscle cells can vary cyclically in length as well as in thickness and number. During pregnancy, smooth muscle cells grow by *hypertrophy* and increase in number by *hyperplasia.*

The myometrium is composed of the following *three layers*:

- an inner longitudinal layer,
- a thick middle layer, which is richly vascularized and contains mostly circularly arranged smooth muscle bundles with just a few oblique bundles, and
- an outer mostly longitudinal layer.

Both collagenous and elastic fibers are present throughout, but elastic fibers become increasingly abundant in the outer wall of the cervix.

19.2.3. Uterine serosa or adventitia

*Seros*a is present over the surface of the uterus bulging into the peritoneal cavity. It is covered with a layer of squamous mesothelial cells. *Adventitia* is present along the retroperitoneal surfaces of the uterus. It is a connective tissue with no epithelial covering.

19.2.4. Menstrual cycle

The endometrium undergoes *monthly cyclic changes* controlled by the ovarian hormones (estrogen and progesterone). The aim of these changes is to prepare the endometrium for the implantation and nourishment of the developing embryo. Normally, the average menstrual cycle lasts 28-days. It begins on the first day when bleeding appears. According to the complex changes in the endometrium, the menstrual cycle is divided into four phases.

Menstrual phase

Menstrual phase usually occupies 3 to 5 days of the cycle. It is characterized by *hemorrhagic discharge (menses, bleeding)* of the *functional layer* of the endometrium (approx. the upper three-fourths of the endometrium). Initially, there are contractions of the coiled arteries followed by their relaxations. This is caused by low levels of estrogen and progesterone, because fertilization does not occur and the corpus luteum atrophies. Vasoconstriction of these arteries results in ischemic changes of their wall and sudden vasodilatation leads to wall ruptures. Patches of blood-soaked tissue of the functional layer become detached. This exposes torn glands, stroma and vessels. Coiled arteries are shed more slowly than stroma and glands. They protrude from the denuded endometrium. The basal layer remains intact, since it is supplied by short straight arteries that do not undergo the above-mentioned changes. Basal layer is the source of reconstruction of the functional layer.

Proliferative phase (reparative, follicular, estrogenic)

This phase constitutes days 5 – 6 to 15 in the cycle. Its characteristic feature is the *renewal* of the entire functional layer up to 2 – 3 mm. The connective tissue, glands and surface epithelium is rebuilt by proliferation and differentiation of cells, which remain in the basal layer. At this stage, the surface of endometrium is smooth and new glands are straight with a narrow lumen and lined with simple columnar epithelium. Mitoses are frequent in the epithelium and stroma. The coiled arteries grow into regenerating tissue. Some mucoid secretion appears at the distal ends of the glandular cells. Changes of the endometrium in the proliferative

phase are driven by estrogens that are manufactured by the granulosa cells of the developing follicles.

Secretory phase (luteal, progravid)

Secretory phase constitutes days 16 to 28 in the cycle. It begins a day or two after ovulation. This phase is influenced by the progesterone produced by the corpus luteum. Histomorphological features of the endometrium are characteristic. At this stage, the endometrium becomes the *thickest (up to 6 – 7 mm)*. The uterine glands become tortuous and irregularly shaped in the middle part of the endometrium. The glandular cells secrete a mucoid fluid rich in glycogen. The coiled arteries become elongated and highly coiled and nearly reach the surface. The connective tissue stroma is edematous. By the day 20 of the menstrual cycle, the endometrium is maximally ready for the implantation of an embryo, if fertilization occurs. The glycogen-rich product of the glands will *nourish* the developing embryo before the formation of placenta.

Ischemic phase

If fertilization does not occur, corpus luteum degenerates and the progesterone level drops by the day 28. Changes of coiled arteries leading to ischemia start.

19.2.5. Cervix

The cervix (Latin cervix neck) is the *most caudal part* of the uterus. It protrudes into the upper vagina and this portion is called “exocervix”, “ectocervix” or “portio vaginalis”. It is covered with *stratified squamous nonkeratinized epithelium* similar to that of the vagina. The canal leading to the corpus of the uterus is called “endocervix”. It is lined with longitudinal mucosal ridges (plicae palmatae), which are composed mainly of a vascular and dense collagenous connective tissue interspersed with numerous elastic fibers and a small amount of smooth muscle cells. Endocervix is covered by *simple columnar (mucus-secreting) epithelium*. The squamocolumnar junction is an anatomic junction between squamous nonkeratinized epithelium and mucinous columnar epithelium. The mucus-secreting cells produce cervical mucus that is composed mostly of water,

but contains also glycoproteins, glucose and ions. At ovulation, cervical mucus is more hydrated and thus easier for spermatozoa to penetrate.

The cervix remodels continuously during life. During prenatal life and infancy, the columnar epithelium that is normally present within endocervix may extend onto the exocervix or vagina, a feature resulting in the original squamocolumnar junction being located on the exocervix. The columnar epithelium later regresses into the endocervical canal, where it is replaced by squamous epithelium. The area between the original squamocolumnar junction and the new squamocolumnar junction is termed *"transformation zone"*.

Clinical correlations

The majority of *cervical carcinomas* arise in the cervical transformation (transition) zone. Therefore, it is important for this zone to be sampled during screening with a Papanicolaou smear. Cervical cancer is one of the most common cancers in female population. When only the epithelium is affected, it is preinvasive lesion (carcinoma in situ). When cancer cells invade the basement membrane and spread to surrounding tissues or metastase via lymphatic vessels to regional lymph nodes (paracervical, hypogastric, and external iliac), it is diagnosed as invasive.

Fig. 1. Stratified squamous nonkeratinized epithelium of the cervix

Fig.2.Carcinomainsitu(preinvasive lesion)

19.3. Uterine tube (Fallopian tube, oviduct)

19.3.1. Introduction into histology of uterine tube

Over 300 years ago the Dutch anatomist, Regnier de Graaf (1641 – 1673), described microscopic findings of the ovaries and uterine tubes of

series of female rabbits at various intervals after mating. He commented that there was no trace of semen within the oviducts but realized that the eggs entered the oviducts after being released from the ovarian (Graafian) follicles, and eventually entered the uterine horns and developed into embryos. Within his description there is an implicit recognition that some semen should probably have entered the oviduct and "moistened" the eggs. These experiments and observations were conducted about 100 years after the oviduct was described anatomically by Italian anatomist Gabriele Fallopio (1523-1562), hence the common usage of name, the Fallopian tube, especially when referring to human anatomy.

The uterine tubes or Fallopian tubes are paired muscular tubes, 10 – 12 cm long, that conduct the secondary oocyte (or after fertilization the developing embryo) from ovary to the uterus. The uterine tubes also serve as the sites where capacitation of spermatozoa (discussed later) and fertilization take place.

Each tube opens proximally into the uterine cavity and distally into the peritoneal cavity nearby the ovary. The uterine tubes anatomically consist of four regions:

- *Infundibulum* – is flared open end of the uterine tube next to the ovary and has delicate finger like projections – *fimbriae*.
- *Ampulla* – is the longest segment of the uterine tube and has the largest diameter. This region is where fertilization occurs.
- *Isthmus* – is the narrow segment of the uterine tube between the ampulla and uterus.
- *Uterine part* – is the segment of the uterine tube contained within the wall of the uterus.

In general, the wall of the uterine tube consists of tunica mucosa, tunica muscularis, and tunica serosa. Sections of the uterine tube vary somewhat in appearance according to the region from which they are cut:

- *Mucosa* consists of lining epithelium and lamina propria and has longitudinal folds. In the infundibulum and ampulla these folds are high and branched. In the isthmus of the uterine tube the mucosal folds are low. The uterine tube is lined by *simple columnar epithelium* with two types of cells: ciliated cells and secretory non-ciliated (peg) cells (discussed later). The nuclei of the tubal epithelial cells

lie at different levels in the cells, thus simulating a pseudostratified epithelium, and the epithelial cells vary in height so that the luminal surface is irregular. The *lamina propria* is a thin layer of loose connective tissue containing collagen and reticular fibers, fibroblasts, mast cells, and lymphatic cells. In postmenopausal women the mucosal folds are thicker, the epithelium is lower, and the lamina propria is more abundant and denser than during reproductive life.

- *Tunica muscularis* consists of smooth muscle cells arranged in two indistinct layers, an inner circular and outer longitudinal. Its peristaltic contractions move the secondary oocyte / embryo toward the uterus. The muscularis layer is the thickest in the isthmus and uterine part of uterine tube.
- *Tunica serosa* consists of a thin layer of loose connective tissue covered by a single layer of squamous mesothelial cells. Just bellow the mesothelial cells of the uterine tube and adjacent board ligament there are often small benign clusters of epithelial cells called Walthard cell nests (carrying name of their discoverer Swiss gynecologist Max Walthard who provided a comprehensive description of them in 1903), with no clinical importance. Microscopically, they are composed of polygonal epithelium-like cells (with ovoid coffee-bean shaped nuclei) and may occasionally be cystic and reach 2 to 3 mm in size. These epithelial cell-clusters are probably derived from the mesothelium by invagination.

19.3.2 Structure of uterine tube epithelium and sperm capacitation

By light microscopy, the uterine tube is seen to have a single layer of columnar epithelium which often appears to be pseudostratified because of differently high cells crowding. The two major cell types are the ciliated cells and non-ciliated secretory cells (Fig. 3).

Ciliated cells are the most numerous in the infundibulum and ampulla and are found predominantly on the apex of the mucosal folds. They are about 30 μm high (at the time of ovulation), have very pale cytoplasm and large, pale, and oval nuclei located near the apexes of the cells. Ciliated cells posses many cilia which beat mostly toward the lumen of the uterus. Cilia are about 10 μm long and 0.25 μm in diameter. The

cilia possess a central bundle of microtubules, called the axoneme, in which nine outer doublet microtubules surround a central pair of single microtubules. At the base of each cilium is a basal body from which the cilium originates. The rate of ciliary beat is influenced by female sex hormones and assists in transport of oocyte or preimplantation embryo to the uterus.

Non-ciliated (peg) cells are *secretory cells* that produce the fluid that provides nutrient-rich medium for the oocyte and preimplantation embryo as they pass through the tubal lumen. Also their secrete aid in capacitation of spermatozoa. These secretory cells have granular cytoplasm and darkly stained nuclei which usually lie at the bases of the cells.

Sperm – uterine tube epithelium interactions (Fig. 3) that precede fertilization by hours or a few days are very important in processes of sperm capacitation ("activation of sperm"). Austin (1951) and Chang (1951) independently described changes that are prerequisite for mammalian spermatozoa to fertilize oocytes in vivo, described as the acquisition of "fertilizing capacity". This acquisition process, termed "capacitation", occurs only after spermatozoa spend a period of time in the female reproductive tract, mostly in uterine tubes. Capacitation involves changes in the plasma membrane, including shedding of proteins and cholesterol that prepare sperm to undergo the acrosome reaction and to fertilize oocytes. Capacitation also includes change in sperm flagellar beating that typically involves an increase in the flagellar bend amplitude.

As the rest of the female reproductive tract, the uterine tube undergoes cyclical changes in morphology of epithelial cells as well as ciliary activity under the influence of estrogen and progesterone. Epithelial cells are low in height during the menstrual phase of the cycle, increasing during the proliferative phase to reach their maximal height in the periovulatory period. At this time, both secretory and ciliated cells are of equal size. At the time of ovulation, the secretory cells reach peak of their activity and discharge their contents into the lumen of the tube, consequently reducing in height relatively to the ciliated cells. This results in greater prominence of the cilia and may enable them to move particulate material or viscous secretions more effectively. Subsequently, in the luteal phase, both cell types reduce their height.

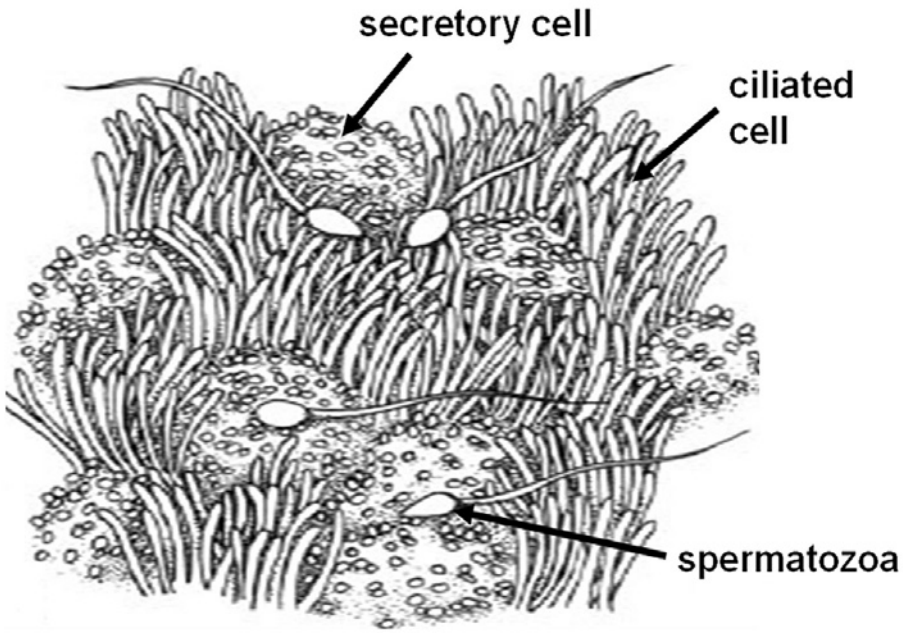

Fig. 3. Human sperm attached to the epithelium of uterine tube with two types of epithelial cells (according to scanning electron micrograph)

19.3.3. Functional overview of uterine tube

The uterine tube plays an essential role in tubal transport of both gametes and embryos and in early embryogenesis, and provides a site of fertilization (mostly in the ampulla). At ovulation, the fimbria comes into close contact with the surface of the ovary and moves back and forth over the ovulatory follicle, disrupting and removing the cumulus mass. Although the mechanism involved is unclear, the mesosalpingeal muscle seems to twist the tube and bring the fimbria into close apposition with the ovary.

Tubal transport is a precisely timed process, allowing propulsion of sperm in the opposite direction to oocyte and embryos, and supporting fertilization and early embryogenesis within the tubal lumen. There is no other tubular system in human body in which cells of such different dimensions are transported in opposite directions in a limited period of time. The oocyte or developing embryo remains in the uterine tube for about 3 days before it enters the uterine cavity. Tubal transit is influenced by contractions of the tubal musculature, ciliary activity and the flow

of tubal secretions. Although the relative importance of each of these mechanisms is still unclear, there is evidence that ciliary action may play the dominant role in transfer of gametes and embryos. Women with the "immotile cilia syndrome", or Kartagener's syndrome, suffer from subfertility.

According to some authors, the uterine tube has been suggested to serve as a potential sperm reservoir. It is very interesting that human sperm incubated with tubal epithelium in vitro remain viable longer than when they are incubated in medium alone. That means, uterine tubes provide a haven for sperm in vivo.

Clinical correlations

Acute and chronic *salpingitis* (inflammation of uterine tube) is a bacterial infection (most commonly Neisseria gonorrhea or Chlamydia trachomatis) of uterine tube that may lead to scarring of the uterine tube predisposing to ectopic pregnancy and infertility. Chlamydia trachomatis causes a direct cytotoxic effect on the mucosa of the human uterine tube, which results in loss of cilia and disruption of cell junctions, associated with rupture of the epithelial cells. Loss of tubal transport function may lead to *ectopic pregnancy* (occurrence of pregnancy outside the uterine cavity, the most common site is the uterine tube). The presenting symptoms reflect the physical expansion of the developing embryo within the limited space of the tube. Thus sudden offset of abdominal pain (may be confused with appendicitis in young women), with or without rupture of uterine tube, and heamoperitoneum are the most common presenting features. Ectopic tubal pregnancy is a medical emergency and should always be taken into consideration when cycling female suffers from abdominal pain!

The uterine tube epithelium is also a target of cigarette smoke. Women who smoke have a markedly increased risk of ectopic pregnancy and increased incidence of tubal infertility, too. Women, who smoke more than 20 cigarettes a day, have an almost four-fold risk of ectopic gestation compared to women who have never smoked.

The uterine tubes, like the uterus and upper part of vagina, is derived from the *paramesonephric Müllerian ducts* of the embryo. Small cystic remnants of the adjacent mesonephric Wolffian ducts (important in the

development of epididymises and ductus deferens in males) are often present in the serosa of the mature uterine tube or the adjacent broad ligament, especially between the uterine tube and ovary (*paraovarian cysts*).

19.4. Vagina

19.4.1. Introduction into functional histology of vagina

The vagina is a distensible fibromuscular tube (about 8 to 10 cm length) that connects the cervix of the uterus to the exterior of the body. It serves as the female organ of copulation and at final stages of pregnancy, the birth canal. In a virgin, the opening into the vagina may be surrounded by the *hymen*, folds of mucous membrane covered on both surfaces by nonkeratinized stratified squamous epithelium extending into the vaginal lumen. The function of hymen is not known and it is probably derived from the endodermal membrane that separates the developing vagina from the cavity of the definitive urogenital sinus in the human embryo.

The wall of the vagina includes *tunica mucosa* (epithelium and lamina propria), *tunica muscularis* and *tunica adventitia*. The mucosa creates transverse folds, or *rugae vaginales*, which are prominent in the relaxed vagina.

The vagina is lined by nonkeratinized *stratified squamous epithelium* (150 – 200 μm thick) like that covering the ectocervix of uterus. This epithelium is supported by a *lamina propria* of richly vascularized loose connective tissue with many elastic fibers. The lamina propria has numerous narrow papillae projecting into the epithelial layer. The deeper region of lamina propria contains many thin-walled veins that simulate erectile tissue during sexual arousal. The extensive vein and capillary plexuses in the lamina propria are the sources of much of lubricate fluid from blood plasma that seeps through the epithelium into the lumen during sexual stimulation. Vaginal mucosa contains few sensory nerve endings, only the lower third of the mucous membrane (near the entrance) is more plentiful in the sensory nerve endings. There are no glands in the vagina. The vaginal surface is moistened by mucus from the mucous glands of endocervix and from the vestibule contains paired tubuloalveolar greater vestibular *Bartholin´s glands* and numerous lesser vestibular glands.

Outside the lamina propria is a *tunica muscularis* composed of smooth muscle cells arranged, mainly, longitudinally (very few inner circularly arranged smooth muscle cells). A sphincter of skeletal muscle fibers (musculus sphincter urethrovaginalis as a part of musculus bulbospongiosus) encircles the vaginal entrance.

The *advetitia* is composed of dense irregular connective tissue (near the muscularis) and loose connective tissue (outer layer) with abundant elastic fibers and an extensive vascular and nerve supply. The advetitia connects the vagina to surrounding structures and is also often called paracolpium.

19.4.2. Vaginal epithelium

Under normal conditions, surface cells of the nonkeratinized stratified squamous epithelium of vagina retain their nuclei, and their cytoplasm appears to be washed out because the cells store variable amounts of glycogen. Near the time of ovulation, estrogen stimulates increased glycogen content. When the cells are shed, they discharge glycogen into vaginal lumen. In the next step the glycogen is fermented by Lactobacillus acidophilus Döderleini (normal bacterial microflora), which convert glycogen into lactic acid and cause the typically low pH (around pH 4) of the vagina. This inhibits the growth of pathogenic bacteria in the vaginal lumen.

The epithelium of the human vagina undergoes changes during the menstrual cycle, although these are less marked than those of the uterine mucosa. A vaginal smear (or vaginal exfoliate cytology stained with Pap method) may be used clinically to evaluate the hormonal status of a woman (historically often used before possibility of easier biochemical evaluation of hormone levels from peripheral blood). A vaginal smear contains three basic cell types and the gynecologist or histopathologist may count the maturation index based on the morphology of observed cells:

- Superficial acidophilic cells are flat with an irregular border and a light cytoplasm. These cells are formed under the influence of estrogen.
- Intermediate cells are flat like superficial cells, but are somewhat smaller and show a basophilic cytoplasm. These cells are formed under the influence of progesterone.

- Basal (or parabasal) cells are oval with large nucleus with prominent chromatin and basophilic cytoplasm. Basal cells in a vaginal smear imply the absence of estrogen or progesterone influence.

Because changes in vaginal epithelium are more marked in some mammals, vaginal smear examination can be an easy indicator of the time of ovulation for possible insemination of animals in veterinary medicine. Also, in human medicine the exfoliate cytology of the vagina together with cells from the endocervix provides a very important source of information for early detection of cervical or vaginal cancers.

Clinical correlations

Vaginitis is an infection most often caused by flagellated protozoan Trichomonas vaginalis, yeast Candida albicans or bacteria as Gardnerella vaginalis or Corynebacterium vaginale. The vaginal epithelium is resistant to bacterial, fungal, and protozoan invasion so that the pathogens remain within the lumen of the vagina. An alkaline environment in vaginal lumen can favor the growth of infectious agents.

19.5. Female external genitalia

The female external genitalia consist of the following parts, which are collectively referred to as *vulva*: mons pubis (prominence over the pubic symphysis formed by subcutaneous adipose tissue), labia majora and minora, clitoris and vestibule. These structures are richly innervated with Meissner's and Pacinian corpuscles along with free nerve endings.

19.5.1. Labia majora and minora

Homologous to the male scrotum, *labia majora* are two prominent skin folds covered by heavily pigmented epithelium. The folds are produced by abundant subcutaneous adipose tissue, and covered by hair on their outer surfaces. They contain a thin layer of smooth muscle that resembles the tunica dartos (smooth muscle layer) of the male scrotum. Many sebaceous glands and some apocrine sweat glands are present on both surfaces. Hair follicles development and adipose tissue accumulation

are regulated by sex hormones at the onset of sexual maturity (by the age of 10 to 13 year old).

The *labia minora* are paired hairless skin folds that possess core of connective tissue with many large veins (with function similar to erectile tissue during sexual stimulation), fine elastic fibers and bundles of smooth muscle cells. The epidermis of the labia minora is only slightly keratinized. Abundant melanin pigment is present in the deep cells of epithelium. Pigmentation of the epidermis of both labia minora and majora appears at the initiation of puberty. The labia minora contain many large sebaceous glands but no hair follicles and adipose tissue. From embryological point of view, the labia minora are homologous to the skin of the male penis.

19.5.2. Functional histology of clitoris

The *clitoris* corresponds to the penis in the male, is about 2 cm long and has two crura (covered by skin lacking hair follicles and glands) containing erectile tissue (corpora cavernosa) that end as a rudimentary glans of clitoris. Unlike penis, the clitoris lacks a corpus spongiosum. A dense connective tissue capsule with intervening, incomplete septum covers the crura. Erectile tissue of clitoris consists of a plexus of thin-walled venous channels that distend during sexual stimulation and the crura clitoridis may be larger seven-times (for comparison, the male penis change its size during erection only 2 - 2.5 times). Clitoris contains many sensory nerve fibers and special nerve endings (e.g., Meissner's and Pacinian corpuscles) which may be important in women orgasmic process.

The clitoridal orgasm is an interesting phenomenon related to evolutionary remodeling of pelvis which allows in humans (and also in chimpanzees) coitus "face to face" with more intensive stimulation of clitoris, other erectile structures and whole female genitalia in comparison with coital activity "from the back", which exist in all other mammals including majority of primates.

19.5.3. Vestibule and vestibular glands

The vestibule is the space at the orifice of the vagina and urethra lined by stratified squamous epithelium and includes two types of glands. Both

types secrete mucus during sexual stimulation. Numerous small mucous glands, the *lesser vestibular glands* (also called glandulae urethrales or Skene´s glands, according to American gynecologist Alexander Skene, 1838 – 1900), are present near the clitoris and around the external urethral orifice.

The large, paired *greater vestibular glands* (*Bartholin´s glands*, according to Dutch anatomist Caspar Bartholinus, 1655 - 1738) are compound tubuloalveolar mucous glands on the opposite site of the vestibule. They are embryologically analogous to the bulbouretral glands of Cowper in males. The terminal duct of each gland is lined by stratified squamous epithelium and opens at the vaginal orifice in the groove between the hymen and the labium minus. Bartholin´s glands are common sites of cysts and of abscesses secondary to infection of a cyst.

19.6. Placenta and umbilical cord

One of the most characteristic features of human prenatal development is the intimate relationship between embryo/fetus and the mother. To survive and grow during intrauterine life, the embryo must maintain an essentially parasitic relationship with the body of the mother for acquiring oxygen and nutrients and eliminating waste products. These exacting requirements are met by the placenta and extraembryonic membranes that surround the embryo and fetus and serve as the interface between the embryo and the mother.

The placenta is a transitory composite structure with both fetal (formed by the chorion) and maternal (formed by the decidua) components. The two parts are involved in physiologic exchange of substrates between the maternal and fetal circulation. The disc-shaped organ is 15 – 25 cm in diameter and 2 – 3 cm thick; it weights 400 – 600 g at term. When viewed on the maternal side, it has 15 – 20 lobules called *cotyledons*.

19.6.1. Formation of placenta

At the beginning, the fertilized ovum (*zygote*) undergoes several rounds of mitotic divisions without cell growth (*cleavage*). On 3rd day forms a cell mass covered by *zona pellucida* called *morula*. Morula

moves along the uterine tube toward the uterine cavity. At the 4th day after fertilization the embryo enters the uterine cavity and now it is called *blastocyst* because of a central cavity (blastocoele) formed inside. Blastocyst is composed of two types of cells:

- inner cells mass (*embryoblast*) – which will form the embryo and some extra-embryonal structures (amniotic cavity, yolk sac and umbilical cord),
- outer cell mass (*trophoblast*) – which will form the fetal part of placenta.

Two to three days after the blastocyst reaches the uterine cavity (around 6th day after fertilization), the embryo loss the zona pellucida (*hatching*) and a direct contact is made with the uterine wall by the trophoblastic cells. The trophoblast rapidly proliferates and begins to invade into the endometrium (in secretory phase of menstrual cycle) – the *implantation* (nidation) begins (Fig. 4). The trophoblast differentiates during implantation into:

- *Cytotrophoblast* – mitotically active inner layer of cuboidal cells (so-called Langhans cells, named after German anatomist Theodor Langhans, 1839 - 1915) with distinct cell membranes, clear cytoplasm containing abundant glycogen but few organelles, and round pale nuclei. The cytotrophoblast is arranged as solid anastomosing cords covered by the syncytiotrophoblast.
- *Syncytiotrophoblast* – a thick outer layer in which the cells have fused together to form a continuous mass of multinucleated cytoplasm without cell boundaries. The syncytiotrophoblast is more highly differentiated than the cytotrophoblast, from which it is derived, and is concerned both in the transport of nutrients from the maternal blood into the embryo/fetus and in the synthesis of several hormones (discussed later). The cytoplasm of the syncytiotrophoblast contains numerous mitochondria, abundant cisterns of rough endoplasmic reticulum, well-developed Golgi apparatus, and relatively large number of lipid droplets. The free surface of the syncytiotrophoblast has many microvilli, visible with the light microscope as a thin brush border.

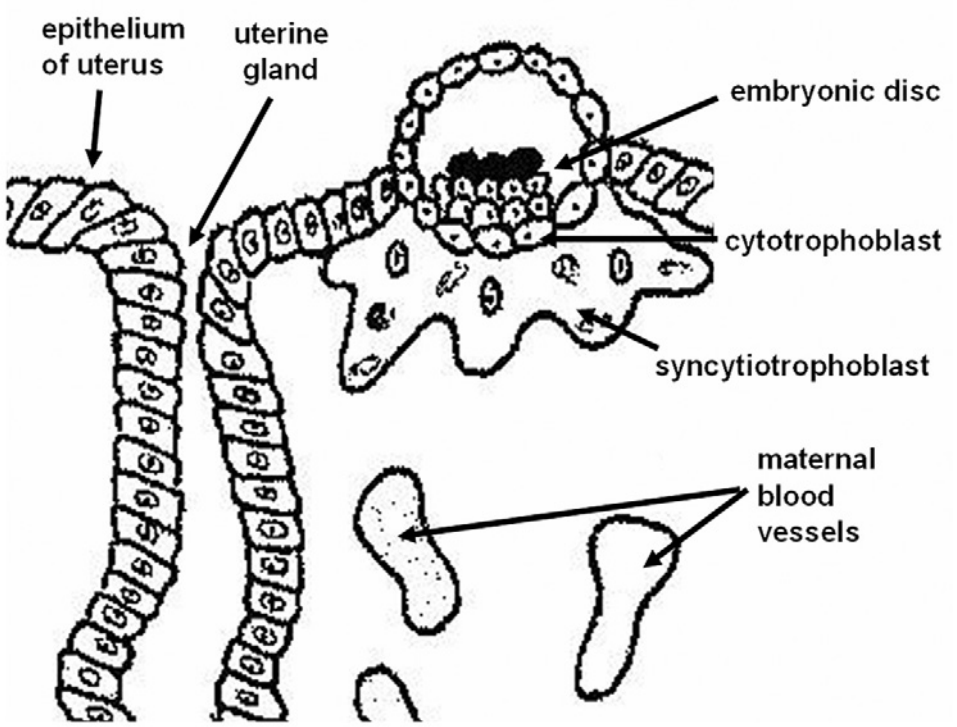

Fig. 4. Implantation of human embryo at the end of 1st week after fertilization

Trophoblast cells probably release anti-inflammatory cytokines to prevent an adverse reaction of the uterus to the implanted embryo. By the 13th day after fertilization, an extraembryonic space, the *chorionic cavity*, has been established (Fig. 5). The cell layers that form the outer boundary of this cavity are: syncytiotrophoblast, cytotrophoblast, and extraembryonic mesoderm. These layers are collectively referred to as the *chorion*:

- *Chorion frondosum* – bushy chorion, is the part of chorion under the developing embryo, where chorionic villi grow and expand – the future fetal part of placenta. The chorion frondosum consists of the *chorionic plate* and derived *chorionic villi*.
- *Chorion leave* – the remainder of the chorion ultimately become smooth, the chorionic villi degenerate (after the eight week of development).

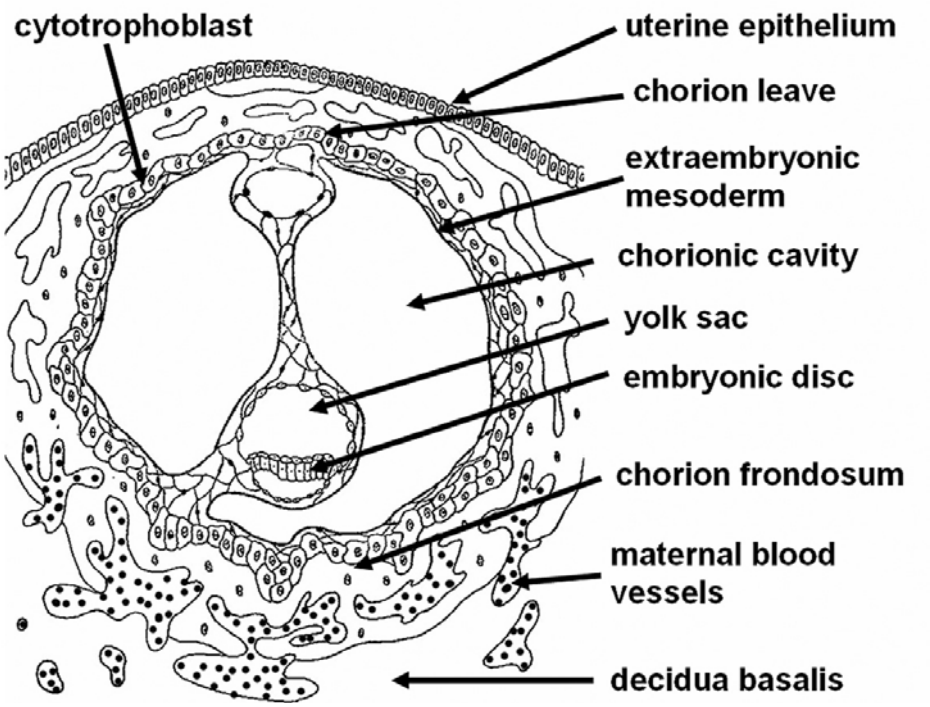

Fig. 5. Human embryo at the end of 2nd week after fertilization

The *chorionic villi* of forming placenta can be classified on the basis of their developmental stages:

- *Primary chorionic villi* - are newly formed villi at the beginning of the second week of development, and consist only of cords of cytotrophoblast covered by syncytiotrophoblast. Between these villi are irregular intervillous spaces, which are filled with maternal blood.
- *Secondary chorionic villi* – develop at the end of the second week when mesenchymal tissue grows into the villi and forms a mesenchymal core covered by cytotrophoblast and syncytiotrophoblast.
- *Tertiary chorionic villi* – develop during the third week, at that time the embryonic blood vessels are formed within the chorionic villi.

The mother's uterine endometrium goes through notable changes in the days following implantation of embryo. These changes are commonly

called *decidual reaction* and the endometrium of pregnant woman is called *decidua*. The decidual reaction consists of three important steps:

- The fibroblasts from the connective tissue of endometrium become enlarged and polygonal, more active in protein synthesis, and are now called *decidual cells*. Decidual cells provide the initial nutrients for embryonic development during implantation.
- The capillaries of the decidua (endometrium) become congested and dilated and form large, wide irregular *sinusoids*.
- Active glandular secretion – the glandular epithelial cells become irregular in size and shape with large, irregularly shaped, hyperchromatic nuclei (Arias – Stella reaction).

There are three regions of the decidua, named according to their relation to developing embryo / fetus:

- *decidua basalis* – is the region between embryo / fetus and myometrium, it forms the maternal component of the placenta,
- *decidua capsularis* – is the superficial layer covering the developing fetus and its chorionic cavity,
- *decidua parietalis* – is the rest of the decidua lining the cavity of the uterus not occupied by the fetus.

19.6.2. Microscopic structure of mature placenta

The placenta is fully formed during the fourth month. It consists of fetal and maternal parts ((Fig. 6). *The fetal part* is formed of the chorionic plate and chorionic villi developed from chorion frondosum. Early in pregnancy the villi are short, plump, finger-like sprouts. Later they become long, thin, and profusely branched. Some of the villi extend through the thickness of the placenta from its fetal part to the underlining endometrium and serve to anchor the placenta to the endometrium – *anchoring villi*. However, most of the villi project as branching finger into the intervillous space filled with maternal blood - *free villi*. Each villus is formed from a core of extraembryonic mesenchyme full of blood vessels and covered with a layer of cytotrophoblast and syncytiotrophoblast. The mesenchyme is composed of *mesenchymal cells*, which differentiate into fibroblasts, involved in the synthesis of various types of collagen and extracellular matrix and phagocytic *Hofbauer cells* (named after American gynecologist

Isfred Hofbauer, 1878 – 1961). Hofbauer cells might be involved in the processes of vasculogenesis and angiogenesis in the placenta, too. In mature placenta the cytotrophoblast layer disappears. *The maternal part* of the placenta is the basal plate - modified decidua basalis (endometrium in which embryo is implanted).

Deposits of fibrin from the maternal blood as small masses of homogenous pink-staining material especially in the last trimester of pregnancy are frequently seen on the villus surface.

The intervillous space between the maternal and fetal components contains circulating maternal blood (contain about 150 ml of maternal blood). Placental blood circulation has two characteristics:

- The maternal blood circulation is open (not bound by blood vessels). Oxygenated uterine arteries supply spiral arteries which enter the intervillous space. Deoxygenated blood leaves intervillous spaces by means of decidual veins and drain into uterine veins.
- The fetal blood circulation is closed within blood vessels. The deoxygenated blood reaches the chorionic villi via two umbilical arteries. Oxygenated blood now returns to the fetus via venules and veins which drain into the umbilical vein.

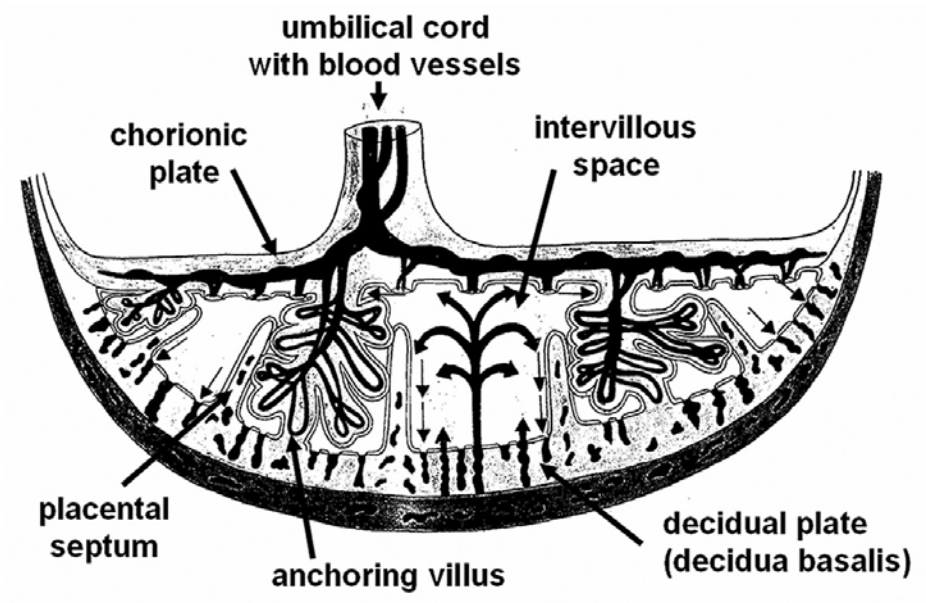

Fig. 6. The structure of placenta and blood circulation in placenta

19.6.3. Placental barrier

Separation of the fetal and maternal blood, referred to as the *placental barrier*, is maintained primarily by the layers of fetal tissue. Under normal conditions, there is no direct contact and mixture between maternal blood in the intervillous spaces and fetal blood in chorionic villi. Starting at the fourth month, these layers become very thin to facilitate the exchange of respiratory gases, nutrients, hormones, humoral antibodies (immunoglobulin IgG) and excretory products across the placental barrier. Also some harmful chemical agents (e.g., alcohol, organic mercury, thalidomide), bacteria (Treponema pallidum – syphilis), protozoa (Toxoplasma gondii) and viruses (rubella virus or cytomegalovirus) can pass through this barrier. These chemical and biological factors are commonly called teratogens (agents that produce birth defects) and can disrupt the normal development of embryo or fetus.

The placental barrier consists of the:

- endothelium lining of the fetal chorionic capillaries,
- basal lamina of the endothelium lining,
- embryonic connective tissue of the core of the villus with macrophages called Hofbauer cells (predominant in early pregnancy),
- basal lamina of the trophoblast,
- cytotrophoblast layer (after 4th month of development is this layer discontinuous and the cuboidal cells of cytotrophoblast are so-called Langhans cells),
- syncytiotrophoblast layer covering the villi. The apical surface of the syncytiotrophoblast contains numerous microvilli.

19.6.4. Functional overview of placenta

The major function of placenta is to provide for diffusion of foodstuffs (placenta acts as a digestive system) and oxygen (placenta acts as lungs) from the mother's blood into the fetus' blood and diffusion of excretory products (placenta acts as kidney) from the fetus back into the mother:

- *Diffusion of oxygen* through the placental membrane – the dissolved oxygen in the mother's blood passes into the fetal blood by simple diffusion. There are two specializations of fetal blood which help pass the oxygen through the placental barrier:

  - the hemoglobin of the fetus is mainly fetal hemoglobin, a type of hemoglobin synthesized in the fetus before birth, which can carry a greater quantity of oxygen than can carry adult hemoglobin in maternal blood,
  - the hemoglobin concentration of fetal blood is about 50 percent greater than that of the mother.
- *Diffusion of carbon dioxide* through the placental membrane – from fetal blood into the maternal blood.
- *Diffusion of nutrients* through the placental membrane – other metabolic substrates needed by the fetus diffuse into the fetal blood in the same manner as oxygen does. For instance, in the late stages of pregnancy, the fetus often uses as much glucose as the entire body of the mother uses.
- *Excretion of waste products* through the placental membrane – in the same manner that carbon dioxide diffuses from the fetal blood into the maternal blood, other excretory products formed in the fetus, especially nonprotein nitrogens such as urea, uric acid, and creatinine.
- *Hormonal production* – in pregnancy, the placenta forms especially large quantities of different hormones essential in the maintenance of pregnancy. As pregnancy proceeds, the placenta takes over the major role in the secretion of steroid hormones (estrogens and progesteron) from the corpus luteum of the ovary. Immunohistochemical studies indicate that the syncytiotrophoblast is the site of synthesis of most of placental hormones:
  - *Human chorionic gonadotropin* (HCG) – can be measured in the maternal blood (and later in urine, too) shortly after the blastocyst implants in the endometrium (used for making diagnosis of pregnancy). Then the rate of secretion rises rapidly to reach maximum at about 10^{th} to 12^{th} week of pregnancy. The structure and function of HCG is very similar to luteinizing hormone secreted by the pituitary gland. The HCG causes persistence of the corpus luteum of the ovary and prevents menstruation. Another effect of HCG is stimulation of testosterone secretion in males fetal testes.
  - *Human chorionic somatomammotropin* (HCS) – a more recently discovered placental hormone, firstly named human placental lactogen and believed to have similar functions to those of prolactin

(development of breast and causes lactation). The functions of HCS are uncertain, but it is secreted in quantities several times greater than all other pregnancy hormones combined. The new hypotheses showed that this hormone has weak actions similar to those of growth hormone (formation of tissues and growth promoting activity) and causes decreased insulin sensitivity and decreased utilization of glucose in mother, thereby making larger quantities of glucose available for fetus.

- *Estrogens and progesterone* – the placenta, like the corpus luteum, secretes both estrogens and progesterone. During pregnancy, the extreme quantities of estrogens cause enlargements of the mother's uterus and breast. The estrogens also relax the pelvic ligaments of the mother for easier passage of the fetus through the birth canal. Progesterone causes decidual cells to develop in the uterine endometrium (important role in the nutrition of the early embryo during implantation), decreases the contractility of the pregnant uterus (prevention from spontaneous abortion) and helps the estrogen to prepare mother's breast for lactation.
- *Relaxin* – is synthesized by decidual cells of placenta (and corpus luteum of the ovary, too). This hormone causes relaxation of the ligaments of the symphysis pubis and pelvic ligaments (function similar to estrogens) and softens the cervix of the pregnant woman at the time of delivery.
- *Leptin* – regulate maternal nutrient storage and is also involved in transporting nutrients across the placental barrier from maternal to the fetal blood.

19.6.5. Anomalies of the placenta

The premature separation of the normally implanted placenta is called *placental abruption*. Symptoms may include vaginal bleeding, uterine contractions and abdominal tenderness. Separation of the placenta from uterus impairs oxygenation of the fetus. Possible causes include trauma, maternal hypertension (preeclampsia), blood clotting abnormalities, smoking, and cocaine use by the mother.

An abnormal implantation site close to the internal opening of the cervical canal is called *placenta praevia*. It can be classified according to degree to which placenta covers cervical canal as complete, partial or

marginal. Placenta praevia is an obstetrics complication affecting 1 from 200 pregnancies. When the placenta (or its part) covers the cervical outlet of the uterine cavity, its presence is a mechanical obstacle in the birth canal. In addition, bleeding (hemorrhage) which can be fatal for the fetus of the mother is a common consequence of placenta praevia as a result of partial separation of the placenta from the lower portion of the uterus and cervix.

Placenta accreta is a rare disorder (1 from 2,500 pregnancies in which the chorionic villi are immediately adjacent to, or penetrate, the myometrium to a varying degree:

- placenta accreta – abnormal attachment of placenta to myometrium,
- placenta increta – invasion into myometrium,
- placenta percreta – penetration through myometrium.

Clinical correlations

The main clinical significance of these placental developmental anomalies is the risk of ante-partum bleeding, or also post-partum bleeding due to failure of placental separation from the uterine wall.

Choriocarcinomas are malignant tumors derived from embryonic cytotrophoblast and syncytiotrophoblast. These tumors are highly invasive into the maternal decidual tissues and blood vessels.

19.6.6. Microscopic structure of umbilical cord

The umbilical cord (funniculus umbilicalis) connects the fetus to the placenta and at term is an average as long, as is the length of full-term newborn (in average 50 cm). It is covered by a single layer of amniotic epithelium. Umbilical cord contains *two umbilical arteries* (lack elastic lamina, carry deoxygenated blood from the fetus to the placenta) and *one umbilical vein* (lack vasa vasorum, delivers oxygenated blood from placenta to the fetus) which are embedded in a gelatinous mass of embryonic connective tissue called *mucous connective tissue* or *Wharton´s jelly* (named after English physician Thoman Wharton, 1610 – 1676). This tissue, which is rich in proteoglycans, functions as a protective layer for the blood vessels. In some cases the center of umbilical cord contains remnants of embryonic allantois (a ventral outpocketing of the hidgut, it is just a vestige of the saclike structure which functioned as a repository of urinary wastes in subhuman animals).

Funisitis is inflammation of the umbilical cord that often accompanies inflammation of the fetal membranes (chorioamnionitis). Mechanical lesions of the umbilical cord include knots, rupture, torsion and stricture. In severe cases, obstruction of blood supply can result in the fetal death. A single umbilical artery is often accompanied by congenital fetal malformations.

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20. Nervous system

Anatomy introduction

The nervous system consists of structural components which orient the body, coordinate body activities, permit the assimilation of experiences, and program instinctual behavior. The nervous system divides into **central** and **peripheral.**

The central nervous system (CNS) consists of the *brain* and *spinal cord*. CNS is composed of nerve cells – *neurons* and their processes – *axons* and *dendrites*, which are supported by specialized tissue – *neuroglia.* The interior of CNS is organized into *gray* and *white mater.*

The spinal cord – is situated in the *vertebral canal* of the vertebral column. It begins superiorly at the foramen magnum and terminates in the adult at the level of the lower border of the first lumbar vertebra (L1). The spinal cord is surrounded by three meninges – *dura mater, arachnoid mater* and *pia mater*, next by *cerebrospinal fluid (CSF)* which surrounds the spinal cord in the *subarachnoid space.* We distinguish the *cervical, thoracic, lumbar, sacral* and *coccygeal parts (segments)* of the spinal cord.

The brain – lies in the cranial cavity (skull) and is continuous with the spinal cord through the foramen magnum. The brain is surrounded by three meninges which are continuous with the corresponding meninges of the spinal cord.

According to development we divide the brain into:

1. *Rhombencephalon* consists of: *myelencephalon (medulla oblongata)* and *metencephalon (pons, cerebellum).*
2. Mesencephalon (midbrain).
3. *Prosencephalon* consists of: *diencephalon (thalamus, epithalamus, metathalamus, subthalamus, hypothalamus)* and *telencephalon.*

   Brain stem – is very frequent term for medulla oblongata, pons and mesencephalon (midbrain).

The peripheral nervous system (PNS) includes the afferent nerve fibers connecting receptors to the CNS and efferent nerve fibers connecting the CNS to the effector apparatus. Within the PNS we distinguish the *spinal nerves*, *cranial nerves* and *autonomic nervous system*, which will be describe in a separate part.

Spinal nerves – are grouped in 31 pairs which start from the spinal cord. They arise from a ventral (anterior) grey column as a *ventral nerve roots (motor)* and from dorsal (posterior) grey column as a *dorsal nerve roots (sensory).*

We have 8 pairs of cervical spinal nerves (C1-C8), 12 pairs of thoracic (Th1-Th12), 5 pairs of lumbar (L1-L5), 5 pairs of sacral (S1-S5) and 1 pair of coccygeal (Co1) spinal nerve.

Cranial nerves – there are 12 pairs of cranial nerves:

I Olfactory, II Optic, III Oculomotor, IV Trochlear, V Trigeminal, VI Abducens, VII Facial, VIII Vestibulocochlear, IX Glossopharyngeal, X Vagus, XI Accessory, XII Hypoglossal.

I. Olfactory nerve – serving the sense of smell.

II. Optic nerve – mediating vision, is distributed to the eyeball.

III. Oculomotor nerve – supplies all the extraocular muscles except the *obliquus superior* and *rectus lateralis*, it also supplies the *sphincter pupillae* and *ciliary muscles.*

IV. Trochlear nerve – the thinnest cranial nerve, supplies the *obliquus superior muscle*.

V. Trigeminal nerve – the largest cranial nerve, sensory supplies the face, the greater part of the scalp, the teeth, the oral and nasal cavities, dura mater and gives the motor supply to the masticatory muscles.

VI. Abducens nerve – supplies the *rectus lateralis muscle.*

VII. Facial nerve – the motor root supplies the facial (mimetic) muscles, stapedius, stylohyoid and posterior belly of digastric mm., the sensory root conveys the gustatory fibers from the tongue (anterior 2/3), it also carries parasympathetic fibers for salivary (submandibular, sublingual) and lacrimal glands.

VIII. Vestibulocochlear nerve – transmits the impulses from the inner ear to the brain. The vestibular division indicates the position of the head in space, whereas the cochlear division carries auditory information.

IX. Glossopharyngeal nerve – its motor fibers supply the stylopharyngeus muscle, parasympathetic fibers supply the parotid gland and sensory fibers innervate the tympanic cavity. It carries also gustatory fibers from posterior 1/3 of the tongue.

X. Vagus nerve – has a more extensive distribution than any other cranial nerve, traversing the neck, thorax and abdomen. It supplies parasympathetically organs in the thoracic cavity (heart, lungs, esophagus) also organs in the abominal cavity (digestive system up to the left colic flexure, pancreas, liver, kidneys, ovaries and testes). Its motor fibers innervate the striated muscles of the pharynx, larynx, soft palate and cranial part of esofphagus. It has also gustatory fibers from the epiglotic area.

XI. Accessory nerve – its spinal root innervates the sternocleidomastoid and trapezius muscles.

XII. Hypoglossal nerve – is motor to all the muscles of the tongue.

The autonomic nervous system (ANS) is the part of the nervous system concerned with the innervation of involuntary structures, such as the heart, smooth muscles,

and glands within the body. It may be divided into two parts: the *sympathetic* and the *parasympathetic.*

The activities of the sympathetic part prepare the body for an emergency. It accelerates the heart rate, causes vasoconstriction (peripheral blood vessels), and raises the blood pressure. It redistributes the blood, and blood subsequently leaves the areas of the skin and intestine and becomes available to the brain, heart and skeletal muscles. It also inhibits peristalsis and closes the sphincters of digestive system.

The sympathetic system consists of the efferent outflow from the spinal cord (sympathetic intermediolateral nuclei –IML), two ganglionated *sympathetic trunks,* branches, plexuses and regional ganglia.

The activities of the parasympathetic part are concentrated on the conserving and restoring energy. It slows the heart rate, increases peristalsis of the intestine, increases glandular activities (e.g. sweat glands, salivary glands), and opens the sphincters of the intestinal tract. The nuclei of parasympathetic part are located in the brainstem and the sacral segments of spinal cord (S2-S4).

20.1. General morphological and functional features of the nervous system

The nervous system (NS), in coordination with the endocrine system, provides the means by which cell and tissue functions are integrated into an autonomous, surviving organism. It controls skeletal muscle movement and helps to regulate cardiac and visceral smooth muscle activity. The NS enables the reception, integration, and perception of sensory information; it provides the substratum necessary for intelligence, anticipation, and judgment; and it facilitates adaptation to an ever-changing external environment.

The NS controls and coordinates all the body activities using billions of nerve cells - *neurons* and supporting nerve cells – *neuroglial cells.* Neurons are specialized for *irritability* and *conductivity*, neuroglial cells support and protect neurons, and participate in neural activity, neural nutrition, and defence of cells in the NS. Nerve tissue is distributed throughout the body as an integrated communication network.

Anatomists divide the nervous system into the following:

a) *Central nervous system* (CNS), consisting of the *brain* and *spinal cord*

b) *Peripheral nervous system* (PNS), composed of the cranial, spinal, and peripheral *nerves, ganglia*, and *nerve endings* (Table 1)

Table 1. Structural divisions of the nervous system

Organization/ Structural components	Components	General description
Central nervous system - CNS	Brain (cerebrum, cerebellum) Spinal cord	Overall "command center", processing and integrating information
Peripheral nervous system - PNS	Nerves Ganglia Nerve endings	Receives and projects information to and from the CNS, mediates some reflexes

20.2. Central nervous system

The organs of CNS (brain and spinal cord) are composed of *two distinct substances – gray* and *white matter*. Gray matter consist of the abundant neuronal cell bodies (perikarya) and supporting glial cells (protoplasmic astrocytes and microglial cells), plus the intervening delicate interwoven meshwork of neuronal and glial cell processes that is referred to as neuropil. The other important components of gray matter are the initial portions of unmyelinated axons. This is also the region where synapses occur.

The characteristic colour of white matter comes from myelin of myelinated axons that predominate here. There are no nerve cell bodies, but myelin-producing oligodendrocytes, fibrous astrocytes and microglial cells are abundant here. Gray matter is more vascular than white matter. Nerve tissue of the CNS does not contain connective tissue other than that in the meninges and in the walls of large blood vessels. Because of the absence of connective tissue, fresh CNS tissue has a very soft, somewhat jelly-like consistency.

Within the CNS, specific terms are used to describe arrangements of nerve cells and their connections:

- *cortex* - arrangement of neurons over the surface of the brain and cerebellum
- *nuclei* - clusters of neuron bodies forming islands of gray matter embedded in white matter
- *column* - arrangement of gray matter running along the spinal cord

- *funiculus or fascicle* - bundle of axons running in white matter

20.2.1. Spinal cord

The spinal cord is a cylindrical structure approximately 40 – 45 cm long and 1.5 cm wide, situated in the vertebral canal but not reaching up to its end. Spinal cord plays a vital role in the movement of various parts of the body and controls several body functions. Primarily, spinal cord functions include *transmission of information* and *initiation of reflexes*. Spinal cord facilitates the transmission of sensory and motor nerve impulses, into and from the brain. The spinal cord is enveloped by the *spinal dura mater* that is separated from the periosteum of the vertebrae by epidural space. *Arachnoid trabeculae* from the arachnoid insert into *pia mater*. Lateral extensions of the latter form *denticulate ligaments* that fix the spinal cord to the dura mater. The *subarachnoid space* lies between the arachnoid and the pia mater.

20.2.1.1. Structure of the spinal cord

The *gray matter* is centrally situated in the spinal cord and contains perikarya of nerve cells. The gray matter has a butterfly shape or shape of H (in cross section). In the central portion is a small opening, *the central canal*, which is lined by *ependymal cells*. The gray matter is divided into *anterior, posterior,* and *lateral columns* (also called as *dorsal, ventral*, and *lateral horns*), all three joined to the contralateral gray matter by *central gray commisure* in which central canal is situated. Axons of somatomotor neurons grouped into *anterior root fibers* leave from the anterior column, whereas *dorsal root fibers* enter into the posterior column. In the gray matter, groups of not always clearly delimited neurons called the *nuclei* are located. Motor neurons, subdivided into the *nucleus anteromedialis, anterolateralis and nucleus posteromedialis, posterolateralis and centralis* are located in the *anterior column*. In the *lateral column* the *nucleus intermediolateralis* and *nucleus intermediomedialis* are present. Perikarya of intercalated neurons, which constitute the *nucleus proprius columnae posterioris*, are situated in the *substantia gelatinosa* of Rolandi. The *nucleus thoracicus* or thoracic nucleus of Stilling-Clarke, is situated at the base of the *posterior column*. The *nucleus apicalis* as well as the

zone of Lissauer are situated at the apical portion of *posterior column* of spinal gray matter (Fig. 1).

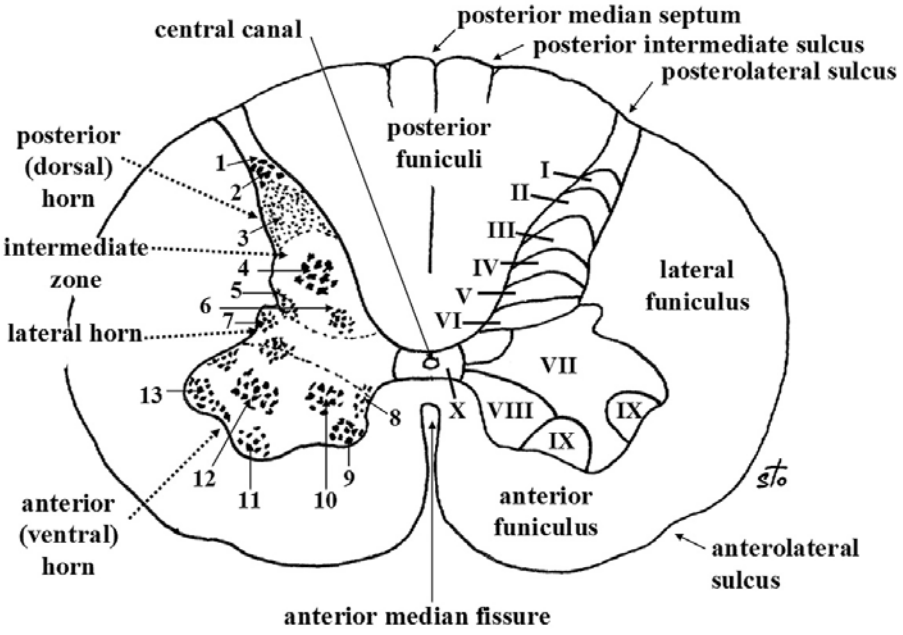

Fig. 1. Section through the cervical level of spinal cord illustrates some subdividions of the gray and white matter. The white matter is composed of three funiculi. The gray matter is divided into two horns and an intermediate zone. Division of the gray matter into ten Rexed's laminae is shown on the right. On the left: 1-zone of Lissauer, 2-nucleus apicalis, 3-substantia gelatinosa, 4-nucleus proprius columnae posterioris, 5-formatio reticularis, 6-nucleus thoracicus, 7-nucleus intermediolateralis, 8-nucleus intermediomedialis, 9-nucleus anteromedialis, 10-nucleus posteromedialis, 11-nucleus anterolateralis, 12-nucleus centralis, 13-nucleus posterolateralis.

20.2.1.2. Laminae of Rexed

Cytoarchitecture of the spinal gray matter reveals a complex structure, characterized by successive layers of cells from dorsal to ventral horns. Rexed divided the spinal gray matter into 10 regions on the basis of cytoarchitecture as seen in transverse senctions (Fig. 1 & Table 2). The first nine laminae are arranged from dorsal to ventral horns. The tenth is merely the circle of cells surrounding the central canal. Laminae I-IVare

the main cutaneous receptive regions. Lamina V receives afferents from the viscera, skin and muscles. Lamina VI receives mostly proprioceptive impulses.

Table 2. Rexed's laminae

Rexed terminology	Corresponding nuclei
Lamina I	Posteromarginal (apical) nucleus
Lamina II	*Substantia gelatinosa* (Rolandi)
Lamina III, IV	Proper sensory nucleus (*Nucleus proprius columnae posterioris*)
Lamina V	Neck of posterior (dorsal) horn Zone anterior to lamina IV
Lamina VI	Base of posterior (dorsal) horn *Nucleus thoracicus* (Stilling-Clark)
Lamina VII	Intermediate zone, Intermediolateral horn Intermediomedial, Intermediolateral, Dorsal nucleus, and Sacral autonomic nuclei
Lamina VIII	Commissural nucleus Motor neurons of the anterior horn
Lamina IX	Ventral horn Medial nuclear column and lateral nuclear column Motor neurons which also contain the Onuf's nucleus in the sacral region
Lamina X	*Substantia grisea intermedia* (around the central canal of spinal cord)

The *white matter* consists of ascending and descending nerve fibers grouped into the *anterior, lateral,* and *posterior funiculi*. The posterior funiculi are separated from each other by the *posterior median septum* and the anterior funiculi by the *anterior median fissure*.

20.2.1.3. Neurons of the spinal cord

Nerve cells of the spinal cord can be divided into:

a) Radicular neurons – (motor neurons)
b) Interneurons – (connecting)
c) Funicular neurons

a) Radicular neurons (Golgi type I) are *efferent neurons* whose long axons leave the spinal cord and form the anterior root of a peripheral nerve. They include *somatomotor, sympathetic and parasympathetic neurons. Somatomotor (motor) neurons are*

large multipolar neurons, with axons terminating in motor end plates of skeletal muscle fibers or in muscle spindles (Fig. 2). *Sympathetic neurons are small multipolar neurons* situated in the *nucleus intermediolateralis* and *nucleus intermediomedialis* (in the cervical and lumbar segments of the spinal cord). *Parasympathetic neurons* are also *small multipolar neurons* situated in the above mentioned two nuclei, but in the sacral segments. Sympathetic and parasympathetic neurons provide the autonomic innervations of organs.

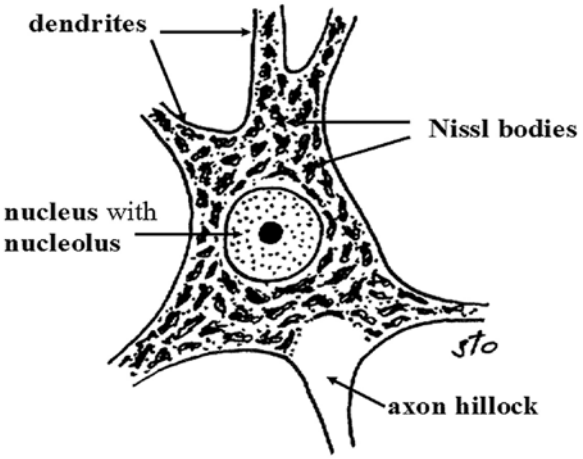

Fig. 2. Large multipolar motor neuron in the ventral horn.

b) Interneurons are neurons whose axons remain in the spinal cord. They include *intercalated, commissural*, and *association interneurons*. They are also called as *connecting interneurons* with short axon *(Golgi type II). Intercalated interneurons* have perikarya situated in the *substantia gelatinosa* and axons that synapse with somatomotor neurons. *Commissural interneurons* have axons that cross the midline of the gray commissure. *Association interneurons* of the *nucleus dorsalis* of Stilling-Clarke have axons that form the *tractus spinocerebellaris anterior* and *posterior.*

c) The last types of neurons are *funicular neurons* (*cellulae funiculares*). They are sensitive, afferent neurons with long axon *(Golgi*

type I) which create the ascending tracts of spinal cord. The funicular neurons of the dorsal horn whose axons join the tracts of funiculi, thus forming most of the white matter of spinal cord (*tractus spinobulbaris*).

20.2.2. Cerebellum

The cerebellum is part of CNS, consisting of gray matter, the *cerebellar cortex,* and a central core of white matter or *cerebellar medulla* containing four pairs of intracerebellar nuclei. These nuclei are relay stations in the efferent connections of the cerebellar cortex —that is, the majority of the Purkinje cell axons terminate in the intracerebellar nuclei. The cerebellar cortex forms long and narrow folds, the *folia,* which are separated by *sulci.* If the cerebellum were unfolded completely in the anteroposterior direction, it would measure 2 m! The cerebellum is enveloped by the *pia mater.* The cerebellum *coordinates muscular activity, maintains posture* and *equilibrium.* The cerebellum is involved in the *control of movement*, particularly those linked to the voluntary nervous system and movements where timing is an important aspect. It coordinates the different muscle groups so that muscle exerts movements fluently and precisely. The cerebellum receives continual feedback information about intended movement and actual movement.

20.2.2.1. Structure of the cerebellum

The *cerebellar cortex* is a simple layered structure. There are three layers: 1. an outer *molecular layer*, 2. a central *Purkinje cell layer* (ganglionic layer), and 3. an inner *granular layer* (Fig. 3).

The *molecular layer* contains mainly cell processes and only a *few neurons* (inhibitory interneurons). One main type of interneuron is the *stellate cell*, located in the superficial part of the molecular layer. Another kind of interneuron, the *basket cell*, is located close to the Purkinje cell layer.

The *ganglionic layer* is made up of *large Purkinje cells*, whose extensive dendritic arborisation is always oriented perpendicularly to the axis of the folium and situated in the molecular layer. Thus, the Purkinje cells appear in a longitudinal section of a folium as thin, flask or pear-like cells with moderate vertical dendritic branching. Purkinje cells have a

large pale nucleus containing a voluminous and distinct nucleolus. The cytoplasm contains Nissl bodies. The Purkinje cells contain *γ-aminobutyric acid* (GABA), and they inhibit their target cells. *Bergmann's neuroglial cells* (radial astrocytes) are situated at the level of Purkinje cells and furnish them with a *neuroglial sheath* which envelops the perikaryon and the stalks of dendritic spines. *Fañanás neuroglial cells* (feathered astrocytes) are small glial cells which send fine and branched processes toward the molecular layer.

The *granular layer* consists of *small neurons*, among which so-called *cerebellar glomeruli (glomerulli cerebellares)* are found (Fig. 4). The granular layer is wide layer of densely packed small neurons called *granule cells* and *Golgi* or *large granule cells*. Golgi cells are scattered in the superficial part of the granular layer. The granule cells have an excitatory action on the Purkinje cells, releasing *glutamate*.

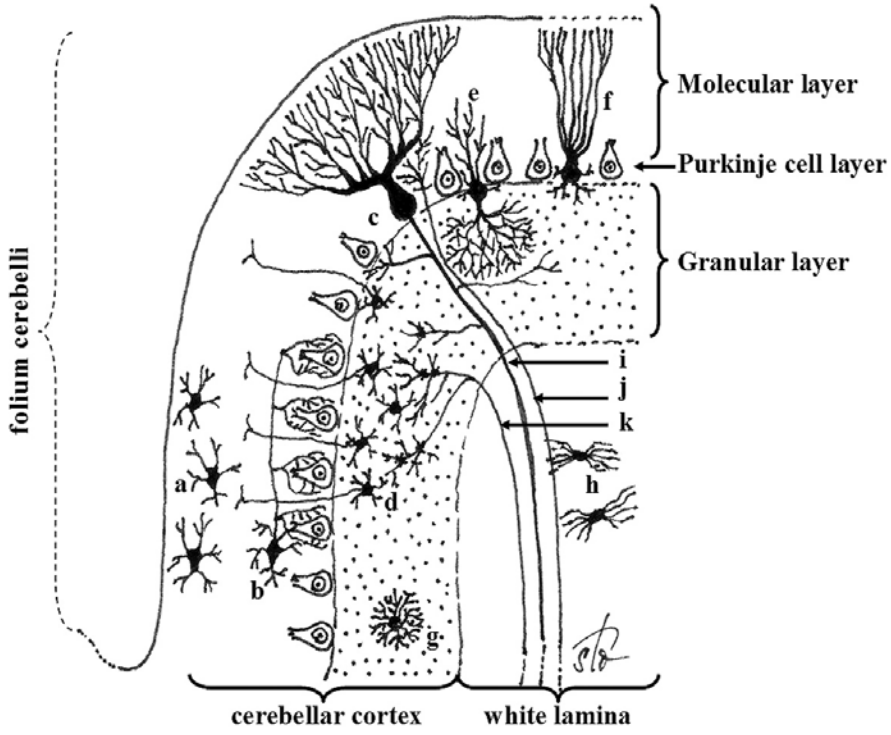

Fig. 3. Cerebellum - histological structure. Types of nerve cells and nerve fibers in cerebellar cortex and adjacent cerebellar medulla: a-stellate cells,

b-basket cell, c-Purkinje cells, d-granule cells, e-Golgi cell, f-radial astrocyte, g-protoplasmic astrocyte, h-fibrous astrocyte, i-axon of Purkinje cell, j-climbing fiber, k-mossy fiber.

The *cerebellar medulla* consists of *afferent* and *efferent nerve fibers*. *Myelinated axons of Purkinje cells* represent the only one efferent nerve fibers of cerebellum. *Afferent nerve fibers* (coming from the spinal cord or brain stem) are *climbing* and *mossy fibers*. *Climbing fibers* are nonbranched afferent fibers that climb along the axons and form synapses with dendrites of Purkinje cells. *Mossy fibers* are branched afferent fibers that end as a much ramified rosette filled with synaptic vesicles. Dendrites of granule cells and axons of Golgi cells come into synaptic contact with mossy fibers. This highly complex synaptic structure is surrounded by the cytoplasmic processes of special type of astrocytes, the whole constituting a *cerebellar glomerulus*. The astrocyte cytoplasmic processes prevent diffusion of neurotransmitters to adjacent synapses (Fig. 5).

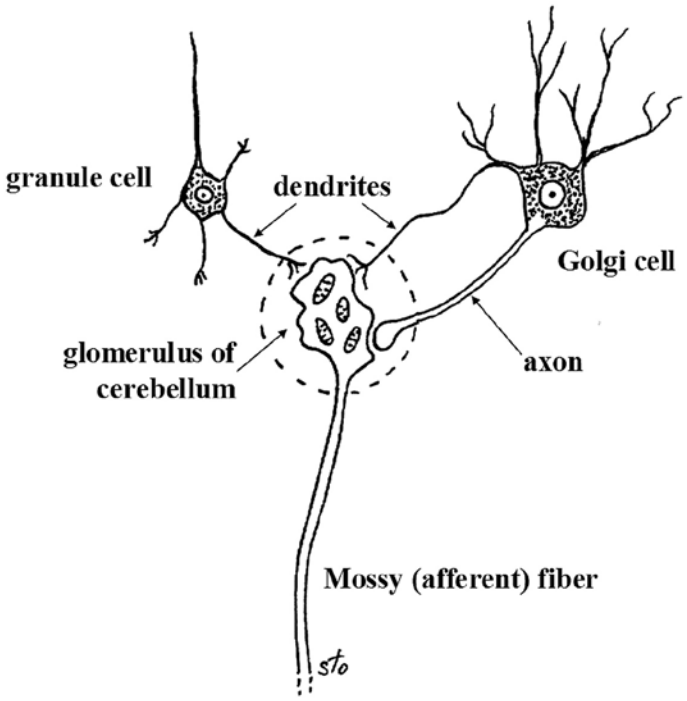

Fig. 4. Glomerulus cerebellaris - diagram showing the different sources of converging fibers.

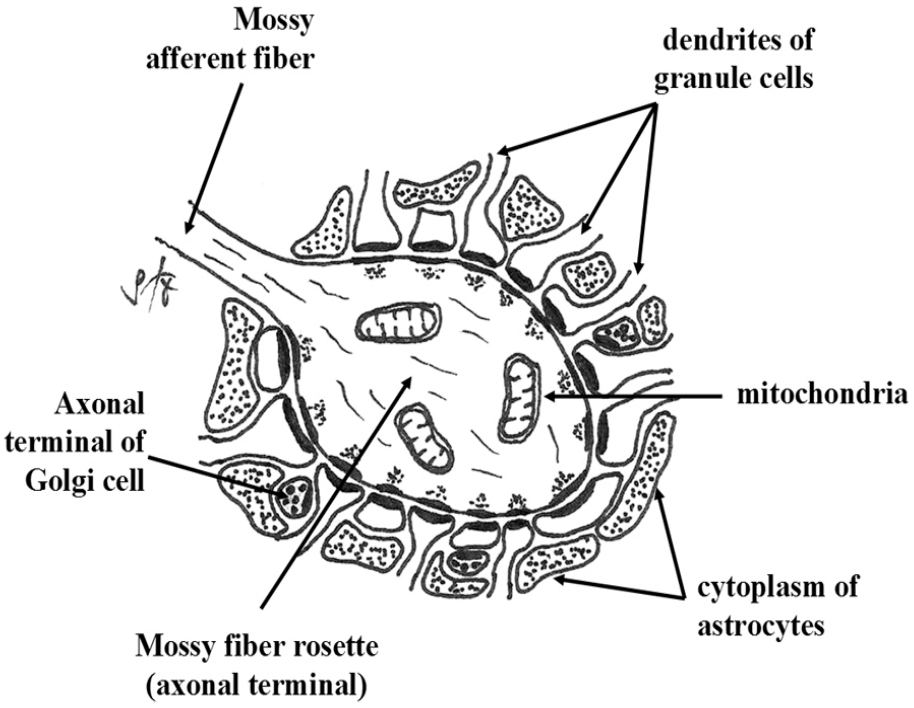

Fig. 5. Ultrastructure of synaptic cerebellar glomerulus in the granule cell layer.

20.2.3. Brain

Morphologically and functionally the brain is the most complex structure in the human body. It controls ability to think, awareness of things around us, and interactions with the outside environment.

The brain consists of two large symmetrically arranged lobes or *hemispheres* which are connected by a bridge of *white matter*, the *corpus callosum*. Each hemisphere contains a central mass of *white matter*, the *medulla*, in which aggregations of neurons known as the *basal ganglia* can be found and a superficial covering *gray matter* known as the *cerebral cortex*. The thickness of cerebral cortex varies from 1.5 to 4.5 mm, with an average thickness of 2.5 mm. The cerebral cortex is irregularly convoluted forming *gyri* separated by *sulci* or *fissures*.

On the basis of phylogenetic development and microscopic structure, the following two types of cortices are recognized – *Allocortex* and *Neocortex*.

Allocortex

This cortex is phylogenetically older and morphologically simple and usually three (or four) layered part of the cerebral cortex. The allocortex can be found in *paleocortex* and *archicortex. Paleocortex* comprises approximately 0.9 % of the human cerebral cortex and contains *bulbus olfactorius*, *tractus olfactorius*, *trigonum olfactorium*, *stria olfactoria medialis et lateralis*, and anterolateral part of *uncus gyri hippocamp*i. *Archicortex* comprises approximately 3.5 % of human cerebral cortex and contains *indusium griseum*, *striae longitudinales corpori callosi*, *hippocampus*, *subiculum* and *gyrus dentatus*. These anatomical structures of archicortex are together called as hippocampal formation.

Neocortex or isocortex

This cortex is six layered and of recent phylogenetic development, and comprises 95% of the cerebral cortex in humans. Isocortex in which six layers are clearly evident (such as the primary sensory cortex) is termed *homotypical cortex*. Isocortex in which some of the six layers are obscured (such as the motor or visual cortex) is termed *heterotypical cortex*.

20.2.3.1. Types and cytoarchitecture of neurons in the cerebral cortex

The cerebral cortex is composed of over 20 billion neurons, nerve fibers, neuroglia, and blood vessels. It constitutes more than half of all gray matter of the CNS. The cortical neurons are of two *functional categories*: (1) Principal neurons and (2) Interneurons

Two types of cortical neurons belong to the principal category. They are *pyramidal* and *fusiform cells.* Several types of cortical interneurons are recognized on the basis of dendritic arborization. They include the *stellate cells*, the *basket cells*, the *horizontal cells of Cajal*, and the *cells of Martinotti* (Fig. 6).

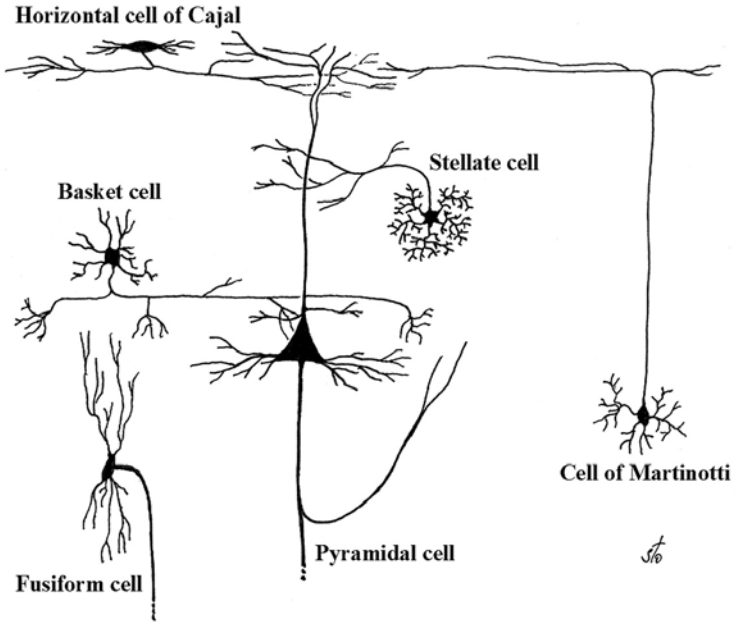

Fig. 6. Schematic diagram of the various types of cortical neurons.

The principal neurons (Golgi type I neurons with long axons) are ***pyramidal cells***. Their cell bodies range in height from 10 to 50 μm. Giant pyramidal cells, also known as Betz cells, have cell bodies up to 100 μm. Each pyramidal cell has conspicuous apical and basal or lateral dendrites, with dendritic spines. The axon emerges from the base of the pyramid or from one of the largest dendrites and gives off many collateral branches before it enters the subcortical white matter. Thus, they are the projection neurons of the cortex, which most likely constitute more than two-thirds of all cortical neurons. The pyramidal cells are glutamatergic and their neurotransmitter is *glutamate*. ***Fusiform cells*** which are located in the deepest layer of cerebral cortex, are *atypical principal cells* with irregularly elliptical cell bodies. The ***spindle cells of von Economo*** are special fusiform cells in the fifth cortical layer (anterior cingulate, anterior insulate and orbitofrontal cortices). They are large, bipolar neurons that are characterized by a large spindle-shaped cell body, gradually tapering into a single apical axon in one direction, with only a single basal dendrite facing opposite.

Stellate cells, which have dendritic spines are, occur mainly in the fourth cortical layer. They are excitatory, and the neurotransmitter is probably *glutamate*. All the other types of interneurons are inhibitory, and probably secrete *γ-aminobutyric acid* at their synapses (GABAergic neurons). Others are called **basket cells** because their axonal branches form a wickerwork around the cell bodies of pyramidal cells. The **horizontal Cajal cells** (so called Retzius-Cajal cells) are confined to the most superficial cortical layer, and the **cells of Martinotti** are more deeply placed, with axons that project to the pial surface.

20.2.3.2. Layers of the isocortex

Morphologically, the isocortex displays the characteristic stratification. The division of the isocortex into layers has been the outcome of extensive *cytoarchitectonic studies* (based on the studies of stained cells, by the *Nissl* or *Golgi neurohistological techniques*) and *myeloarchitectonic studies* (based on the studies of myelinated fiber preparations, by the *Weigert method* for myelin sheath). Such *cytoarchitectonic differences* (i.e., differences in size, shape, and packing density of the neuronal cell bodies) form the basis of a parcellation of the cerebral cortex of each hemisphere into approximately **50 *cortical areas*** (***areae***). Although several histological studies are available, the most widely used are the cytoarchitectonic classification of Brodmann and the myeloarchitectonic classification of Vogts. According these two classifications, the isocortex is divided into *six histological layers* (Table 3 & Fig. 7). The cerebral cortex has its full complement of neurons by the 18th week of intrauterine life, and six layers, which differ in the density of cell population and in the size and shape of constituent neurons, can be recognized by about the 7th month.

Table 3. Layers of isocortex

Layer	Cytoarchitectonic name	Myeoloarchitectonic name
I	Molecular	Stria Exneri
II	External granular	
III	External pyramidal	Stria Kaes-Bechterevi
IV	Internal granular	Stria Baillargeri externa
V	Internal pyramidal	Stria Baillargeri interna
VI	Multiform	Stria Meynerti

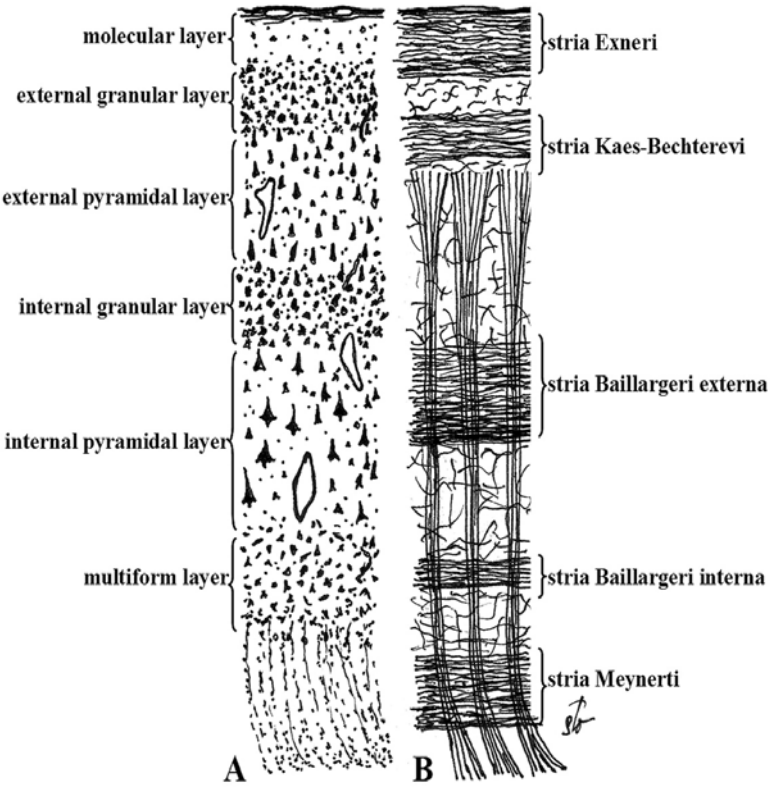

Fig. 7. Cortical histology, as revealed by two different histological staining methods. (A) Golgi method (cytoarchitecture, cellular organization); (B) Weigert method for myelin (myeloarchitecture, myelinated nerve fibers organization).

I. Molecular or plexiform layer

The superficial layer consists predominantly of terminal braches of dendrites and axons. Most of the dendritic branches come from *pyramidal cells. Cells of Martinotti* in any deeper layers also send axons to this layer. The *horizontal cells of Cajal* are situated between some axons and dendrites. In this layer, cell processes of horizontal Cajal cells, pyramidal cells, and Martinotti cells form the – *stria Exneri* of nerve fibers. The molecular layer is essentially a synaptic field of the isocortex.

II. External granular layer

This layer contains many *small* and *medium pyramidal cells* and *interneurons* (mainly *stellate cells*). The dendrites of pyramidal cells in

this layer project to the first layer, while their axons project to deeper layers. This layer contributes to the complexity of intracortical circuitry.

III. External pyramidal layer

The neurons are typical *pyramidal cells* that increase in size from the external to the internal borders of the layer. *Basket cells*, situated at the borders between layers III. and IV. are horizontal inhibitory neurons acting on perikarya of pyramidal cells. The dendrites of pyramidal cells in this layer extend to the I. layer, while the axons project to other layers within the same and contralateral hemisphere (association and commissural fibers) or leave the hemisphere as long projecting fibers to more distant extracortical sites (tractus corticospinalis – main motor pathway). This layer contains stripes of nerve fibers – *stria Kaes-Bechterevi*.

IV. Internal granular layer

This layer is dominated by *stellate cells*, although smaller number of other *interneurons* and *pyramidal cells* are present here. The cell concentration in the layer IV. is the greatest of all cortical layers. Few of the *larger stellate cells* in this layer project their axons to deeper cortical layers. Layer IV. is especially well developed in primary sensory cortical areas. In the primary visual cortex, this layer is traversed by a dense band of horizontally oriented thalamocortical nerve fibers known as the *stria Baillargeri externa* or the strip of Gennari. The internal granular layer is the major recipient of thalamocortical fibers from modality-specific sensory relay nuclei (visual, auditory, and primary sensory radiation).

V. Internal pyramidal, ganglionic layer

This layer contains *large* and *medium-sized pyramidal cells, stellate cells*, and *cells of Martinotti*. The cell concentration in this layer is the lowest of all cortical layers. The *giant pyramidal cells* in the cerebral cortex – *cells of Betz* are found in the primary motor area. The axons of Betz cells contribute to the formation of tractus corticospinalis or pyramidal tract. The number of Betz cells, however, is much too low to account for the number of axons in the pyramidal tract (about 1 million in humans). This cortical layer contains the *spindle cells of*

von Economo. Dendrites of neurons in this layer project to the more superficial layers. This layer also contains a horizontal fiber arrangement called *stria Baillargeri interna*.

VI. Multiform layer

Although *fusiform cells* are typical of this layer, there are also *pyramidal cells* and small polymorphic *interneurons* of various shape. Their dendritic organization is similar to the one described for the pyramidal cells in layer V. Axons of fusiform cells wrap around the dendrites of large pyramidal cells. This layer contains a delicate strip - *stria Meynerti*.

The brain and the spinal cord are covered on the surface by connective tissue envelopes - *meninges*.

20.2.4. Meninges

The skull and the vertebral column protect the organs of CNS. Between the bone and nerve tissue are membranes of connective tissue called the meninges. Three *meningial layers* are distinguished – dura mater, arachnoid and pia mater (Fig. 8).

A. Dura mater

The dura mater is a thick external layer, consisting of dense irregular connective tissue with blood vessels. There is a tight junction between the dura matter and periosteum of the skull. The dura mater that envelops the spinal cord is separated from the periosteum of the vertebrae by the epidural space filled by adipose and loose connective tissue with thin veins.

B. Arachnoid

The arachnoid has two components: (a) a sheet of connective tissue in contact with the dura mater, and (b) a system of loosely arranged *arachnoid trabeculae* containing fibroblasts and collagen fibers. This trabecular system is continuous with deeper pia mater. The connective tissue of arachnoid is said to be avascular because it lacks nutritive capillaries, but larger blood vessels run through it. The arachnoid and the pia mater are intimately associated and are often considered a single membrane the *pia-aranchnoid*.

C. Pia mater

The innermost pia mater is thin vascular membrane adherent to the neuroglial membrane – *limiting membrane of superficial glia*, delimiting the underlying nerve tissue. This layer does not directly contact nerve cells or fibers. Together the pia matter and neuroglial membrane form a physical barrier on the surface of the CNS.

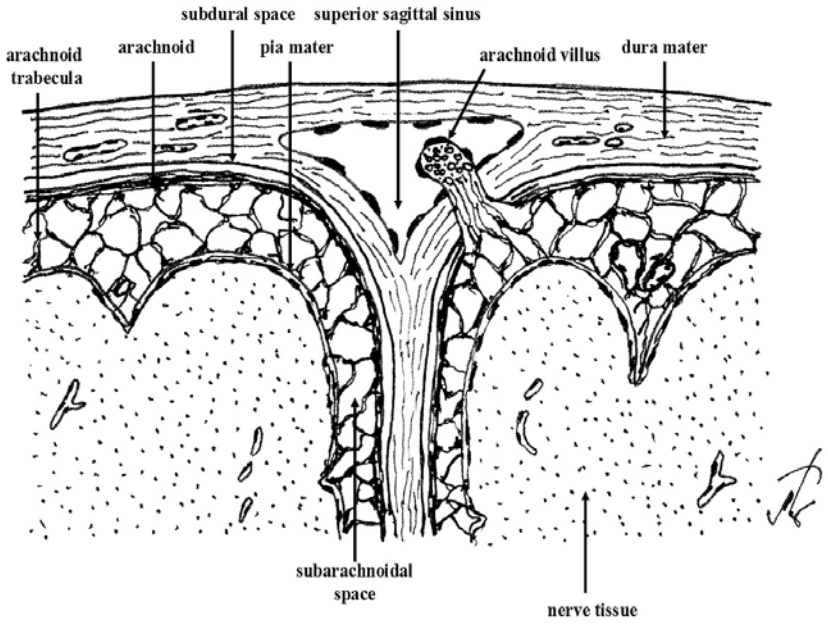

Fig. 8. Meninges – topography and general histological structure

Meninges are separated by the *subdural* and *subarachnoidal spaces* (the latter filled with cerebrospinal fluid). The subarachnoidal space communicates with the ventricles of the brain.

20.2.5. Blood-Brain Barrier

The blood-brain barrier (BBB) or **neurovascular unit** serves as a functional barrier between the blood and nerve tissue of CNS, protecting the nature of neuronal microenvironment.

The BBB is composed of three parts (Fig. 9):

1) nonfenestrated endothelial cells with tight junctions
2) 30 – 40 nm thick and continuous basement membrane with pericytes

3) limiting membrane of perivascular glia (formed by astrocytic end feet)

The BBB allows the stable composition and constant balance of ions in the interstitial fluid surrounding neurons and neuroglial cells that is required for their function and protects these cells from potential toxins, drugs such as antibiotics and infectious agents. The areas of brain that no BBB include the *subfornical organ*, *vascular organ of the lamina terminalis*, *area postrema*, *recessus pinealis* and *neurohypophysis*. There are present the fenestrated blood capillaries.

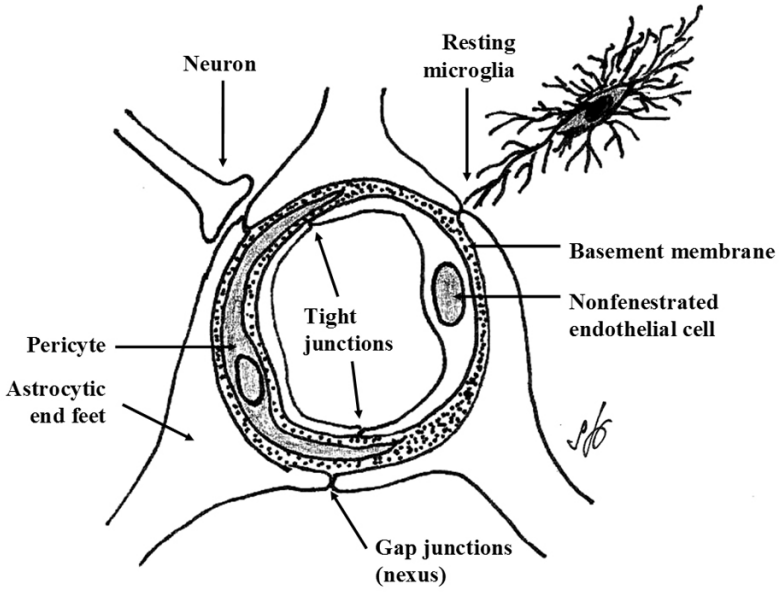

Fig. 9. The blood–brain barrier or neurovascular unit. Schematic drawing showing the main histological features. Important components are **tight junctions (***zonulae occludentes***)** between the **nonfenestrated endothelial cells** and a continuous layer of **perivascular astrocytic end feet.** There are very **few endocytotic (pinocytotic) vesicles** in the cytoplasm of endothelial cells in the CNS. **Gap junctions** establish low-resistance connections between the astrocytes. The **basement membrane** also contributes to the barrier properties of the neurovascular unit. **Pericytes** are found within the endothelial basement membrane and occasionally the astrocytic end feet coverage is interrupted to allow contact of resting **microglia** and **neurons** with basement membrane. The pericytes surrounding capillaries are contractile, and may contribute to the change

of blood flow induced by synaptic activity. Together the whole morphological framework is named the **"neurovascular unit".**

20.2.6. Choroid plexus

The choroid plexus is a *highly specialized tissue* that projects as elaborate folds with many villi into the ventricles of the brain. Each villus of the choroid plexus contains a thin layer of highly *vascularized pia mater* covered by a layer of *cuboidal* or *low columnar epithelium* of *ependymal cells* attached to the basement membrane (Fig. 10). Remainder ependymal cells that form a single layer lining the fluid-filled ventricles of cerebrum and the central canal of the spinal cord do not form the epithelium. Apical surface of ependymal epithelial cells contains bulbous *microvilli* and basal surface a few cell membrane *infoldings.* Both microvilli and membrane infoldings are structures associated with intensive fluid transport. The main function of choroid plexus is production of *cerebrospinal fluid*. *Blood-cerebrospinal fluid barrier* consists of the tight junctions between the choroid plexus epithelial cells, it is permeable to some circulating peptides (e.g. insulin) and plasma proteins (e.g. prealbumin).

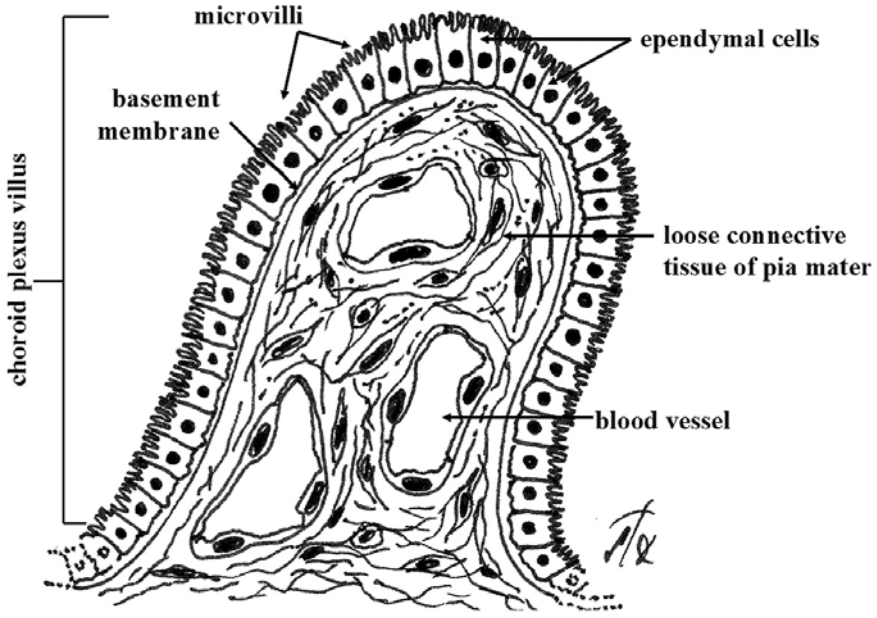

Fig. 10. Villus of the choroid plexus.

20.3. Peripheral nervous system

The main components of the peripheral nervous system (PNS) are the *nerves, ganglia*, and *nerve endings*.

Nerves are bundles of nerve fibers surrounded by connective tissue sheaths.

Ganglia are aggregations of neurons outside the CNS.

Nerve endings and *receptors* are highly specialized terminal portions of axons or dendrites.

20.3.1. Nerves

In the peripheral nervous system, the nerve fibers are grouped in bundles and fascicles to form the nerves. The nerves establish communication between CNS (brain and spinal cord) and receptors (sensory organs) or effectors (e.g. glands, muscles). A nerve is simply a bundle, or more frequently several bundles of nerve fibers enclosed in a connective tissue sheath. Except for a few very thin nerves made up of unmyelinated fibers, nerves have a whitish, homogeneous, glistening appearance because of their myelin and collagen content.

Histologically, the *peripheral nerves* are surrounded by an *epineurium*, individual fascicles are enclosed by a *perineurium*, and *endoneurium* is a delicate sheath around each nerve fiber (Fig. 11). Nerves are richly supplied by blood vessels, which form numerous anastomoses. Arteries pass into the epineurium, form arteriolar networks beneath the perineurium and form the capillaries within the endoneurium.

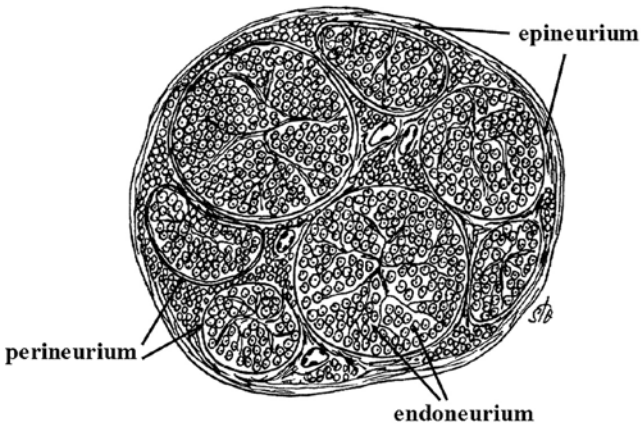

Fig. 11. Peripheral nerve – cross section.

Nerves have an external supporting and protective coat of dense connective tissue called *epineurium*, which also fills the space between the bundles of nerve fibers. This connective tissue contains variable amounts of adipose tissue, which seems to have a protecting role. Within the nerve, the *perineurium* divides nerve fibers into individual *fascicles - bundles*. Each bundle is surrounded by the perineurium, a sleeve formed by layers of flattened cells (Fig. 12). The cells of each layer of the perineurial sleeve are joined at their edges by tight junctions, an arrangement that makes the perineurium a protective barrier to the passage of most macromolecules and has the important function of protecting the nerve fibers from aggression. The cell layers are separated by spaces containing connective tissue rich in collagen and elastic fibers and longitudinally oriented blood capillaries. Within the perineurial sheath run the Schwann cell-sheathed axons and their enveloping connective tissue, the *endoneurium*. The endoneurium surrounding individual axons consists of a thin layer of fine loose connective tissue with reticular fibers, produced by Schwann cells and few fibroblasts. Fibrocytes, macrophages and mast cells are also present in the endoneurium. Additional structural components of the endoneurium are the *endoneurial capillaries*. Endoneurial capillaries derive from the *vasa nervorum* and are lined by continuous endothelial cells joined by tight junctions.

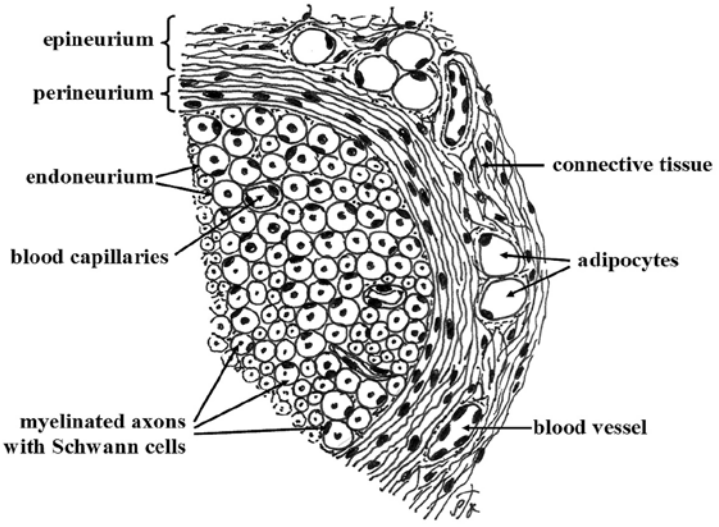

Fig. 12. Peripheral nerve – detail.

20.3.1.1. Types of nerve fibers in peripheral nerves

- *Type A fibers* (myelinated) are 4 - 20 μm in diameter and conduct impulses at high velocities (15 - 120 m per second). Examples: motor fibers, which innervate skeletal muscles, and sensory fibers.
- *Type B fibers* (myelinated) are 1 - 4 μm in diameter and conduct impulses with a velocity of 3 - 14 m per second. Example: preganglionic autonomic fibers.
- *Type C fibers* (unmyelinated) are 0.2 - 1 μm thick and conduct impulses at velocities ranging from 0.2 to 2 m per second. Examples: autonomic and sensory fibers.

20.3.1.2. Classification of nerve fibers in relation to the CNS

The nerves establish communication between brain and spinal cord and the sensory organs and effectors (muscles, glands, etc). They possess afferent and efferent fibers running to and from the CNS. *Afferent* fibers carry the information obtained from the interior of the body and the environment to the CNS. *Efferent* fibers carry impulses from the CNS to the effector organs.

20.3.1.3. Classification of peripheral nerves

Nerves possessing only sensory (afferent) fibers are called *sensory nerves;* those composed only of motor (efferent) fibers carrying impulses to the effectors are called *motor nerves.* Most nerves have both sensory and motor fibers and are called *mixed nerves;* these nerves have both myelinated and unmyelinated axons.

The PNS may be further divided, on a purely functional basis, into the *somatic nervous system* and *autonomic nervous system*. The somatic nervous system is composed of afferent (sensory nerves) part and an efferent (motor nerves) part. The autonomic nervous system has three parts: the *sympathetic system*, the *parasympathetic system*, and the *enteric nervous system*.

20.3.2. Ganglia

Ganglia are ovoid structures containing neuronal cell bodies and glial cells supported by connective tissue. Because they serve as relay stations to transmit nerve impulses, one nerve enters and another leaves each ganglion. The direction of the nerve impulse

determines whether the ganglion will be a *sensory*, an *autonomic* or an *enteric ganglion*.

20.3.2.1. Sensory ganglia

Sensory ganglia receive afferent impulses that go to the CNS. Two types of sensory ganglia exist. Some are associated with cranial nerves - *cranial ganglia*; others are associated with the dorsal root of the spinal nerves and are called *spinal ganglia*. The latter comprise large round neuronal cell bodies with prominent and fine Nissl substance. They are surrounded by abundant small glial cells called *satellite cells*. A connective tissue framework and capsule support the ganglion cells. The neurons of these ganglia are *pseudounipolar* and relay information from the ganglion's nerve endings to the gray matter of the spinal cord via synapses with local neurons. The neuronal cell bodies predominate in the peripheral part of the spinal ganglion. The central part shows a great predominance of myelinated axons (Fig. 13). These neurons have only one nerve process that is branched like letter "T". Thinner branch serves as an axon and run centrally into the dorsal horns of the spinal cord. The thicker process is functionally dendrite, but morphologically has the same characteristic like the axon and caries information impulse from the periphery into the CNS.

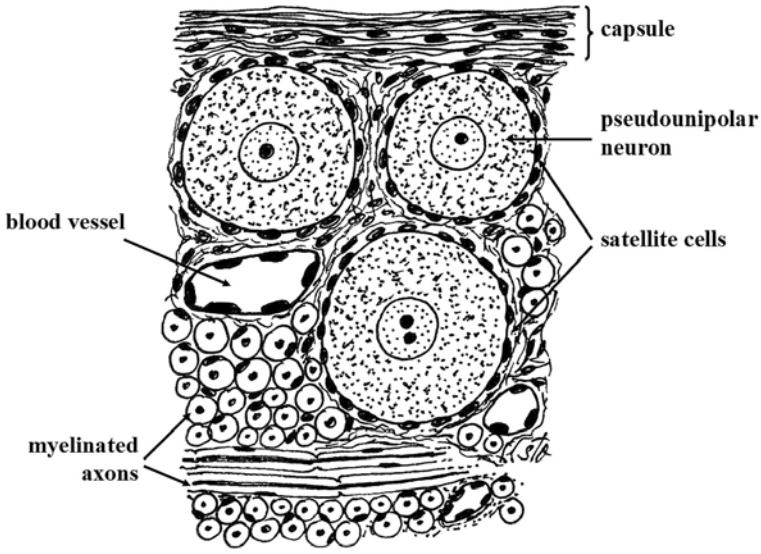

Fig. 13. Spinal ganglion – detail of the peripheral part.

20.3.2.2. Autonomic ganglia

Autonomic ganglia are associated with sympathetic and parasympathetic parts of PNS. They appear as bulbous dilatations of the autonomic nerves. Some are located within certain organs, especially in the walls of the viscera (hollow organs), where they constitute the *intramural ganglia*. These ganglia are devoid of connective tissue capsules, and their cells are supported by the stroma of the organ in which they are found. Autonomic ganglia usually have *multipolar neurons*. The neurons are not arranged in definite groups as in sensory ganglia, but are scattered throughout the ganglion. As with craniospinal ganglia, autonomic ganglia have neuronal perikarya with fine Nissl bodies. A layer of satellite cells frequently envelops the neurons of autonomic ganglia. In intramural ganglia, only a few satellite cells are seen around each neuron. The nerve fibers are unmyelinated and thinner. They are, therefore, much less conspicuous than in sensory ganglia.

20.3.2.3. Enteric ganglia

The enteric nervous system (ENS) of the intestines consist of many small ganglia of *myenteric (Auerbach's)* and *submucosal (Meissner's) nerve plexus*. Each plexus consists of neurons and associated cells, and bundles of nerve fibers passing between plexuses. These ganglia contain a population of *stellate, myoid-type cells* known as the *interstitial cells of Cajal*. These specialized neurons regulate the frequency and propagation of peristaltic contractions in the wall of intestine.

20.3.3. Nerve endings

The nerve endings include *sensory endings* or *receptors*, which detect changes in the internal and external environments, and *effector endings*, which control the contraction of muscles and the activity of exocrine glands. Sensory nerve endings respond to mechanical, thermal or chemical stimulation, and like afferent nerve fibers conduct action potentials to the CNS. Effector nerve endings contact muscles or secretory epithelial cells of glands.

The *motor end-plate* or *neuromuscular junction* is a specialized effector nerve ending in the skeletal muscle tissue.

20.3.3.1. Sensory endings - receptors

Sensory (afferent) receptors are specialized structures located at the distal tips of the peripheral processes of sensory neurons. The sensory system is broadly divided into the *general* and *special senses*. The special senses - vision, hearing, balance, olfaction and taste are considered elsewhere.

There are three main types of *general sensory endings*:

A. Exteroreceptors – these occur superficially in the skin and respond to nociceptive (painful) stimuli, temperature, touch and pressure.
B. Proprioreceptors – these occur in muscles, joints and tendons and provide awareness of posture and movement.
C. Interoreceptors – these occur in viscera and inform about the changes in the visceral organs.

A. Exteroreceptors

The exteroreceptors are *sensory nerve endings* and may be either *unencapsulated* or *encapsulated* (Fig. 14).

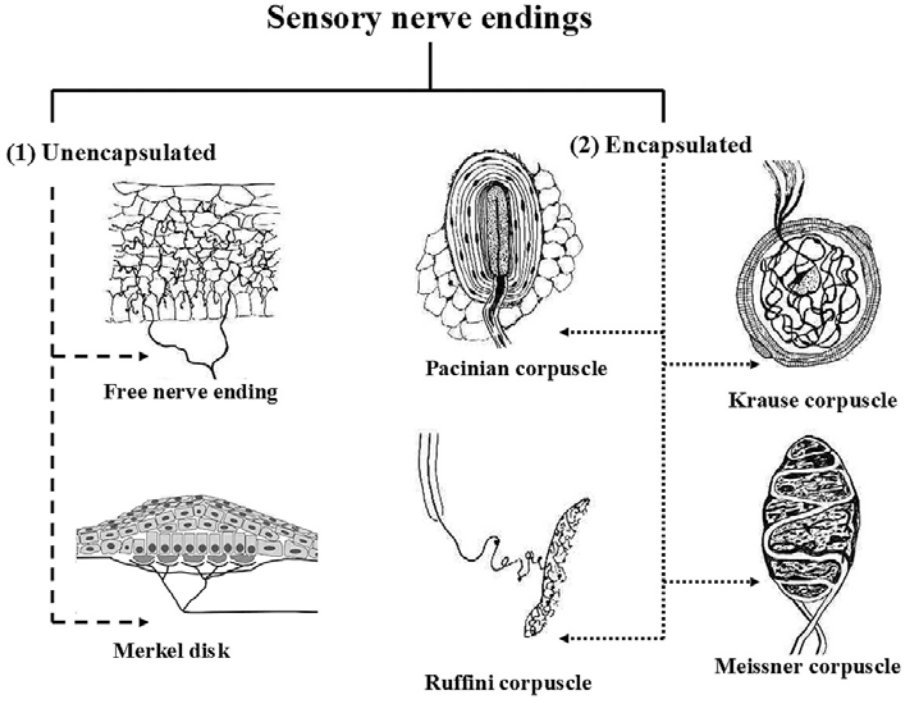

Fig. 14. Sensory nerve endings.

Unencapsulated or *free nerve endings* - consist of terminal branches of sensory (afferent) nerve fibers lying freely in the innervated tissue. They appear to mediate thermal and painful sensation. The *free intraepithelial nerve endings* are located in the epidermis of the skin and the cornea of the eye. They transmit sensation of touch and pain. *Merkel disks* (or tactile menisci) are small disc-like structures seen in relation to specialized epithelial cells (Merkel cells) present in the *stratum spinosus* of epidermis. *Peritrichial nerve endings* are cagelike formation of 2 up to 20 axons that surround hair follicle in the dermis. The axons approach the follicle deep to its sebaceous gland and branch in the connective tissue outside the outer root sheath. They are mechanoreceptors sensitive to pressure.
Encapsulated nerve endings - are surrounded by connective tissue capsule of non-nerve tissue. The combination of nerve ending and its encapsulation often being referred to as a *corpuscle.*
Pacinian corpuscles – largest lamellar corpuscles found in skin, nipples, genitals, in deep tissues, e.g. surrounding joints and in mesentery. They respond to mechanical distortion, vibration and pressure.
Meissner's corpuscles – are small oval, elongated in shape and found in the skin. They are particularly numerous in the dermal papillae of the finger-tips. They respond with great sensitivity to touch and are thought to be responsible for fine, or discriminative, touch.
Ruffini corpuscles – are spindle shaped structures present in the dermis of hairy skin. Some are also found in non-hairy skin, tongue, and external genitalia. They are thermoreceptors and mechanoreceptors in dermis.
Krause corpuscles – are usually spherical structures present in skin, oral cavity and external genitalia. Their significance is controversial. Some authors regard them to be degenerating or regenerating terminals of nerve fibers rather than specialised endings (cold thermoreceptor).

B. Proprioreceptors

They inform the CNS about the sense of position and state of motion of muscles, ligaments, joints and tendons. The term proprioreceptor is used to include reception of sensation in the musculo-skeletal system, i.e. deep sensation. The proprioreceptors or sensory receptors associated with skeletal muscles are the *muscle spindles* (stretch receptors) and the *Golgi tendon organs* (Fig. 15).

Muscle spindle – have an elongated spindle shape with a thicker midsection. Each spindle contains specialized modified skeletal muscle fibers called as *intrafusal fibers* that are oriented parallel to the regular, power-producing *extrafusal fibers*. The middle part of a spindle is covered by a capsule of connective tissue. Spindles contain two types of intrafusal fibers: *nuclear bag fibers* and *nuclear chain fibers*. Two types of sensory endings can be found in spindle – primary and secondary spindle endings. They are axons of specialized groups of afferent nerve fibers. At both ends, intrafusal fibers are connected to either extrafusal fibers or tendinous ligaments. So, when extrafusal fibers change length, intrafusal fibers are stretched or shortened correspondingly.

Golgi tendon organ – is an elongated encapsulated structure located at the junction between muscle and tendon – the *musculotendinous junction*. It has a very simple structure, and is supplied by only one type of afferent nerve fibers. Functionally, the Golgi tendon organ informs the CNS about muscle tension, whereas the muscle spindle informs primarily about muscle length.

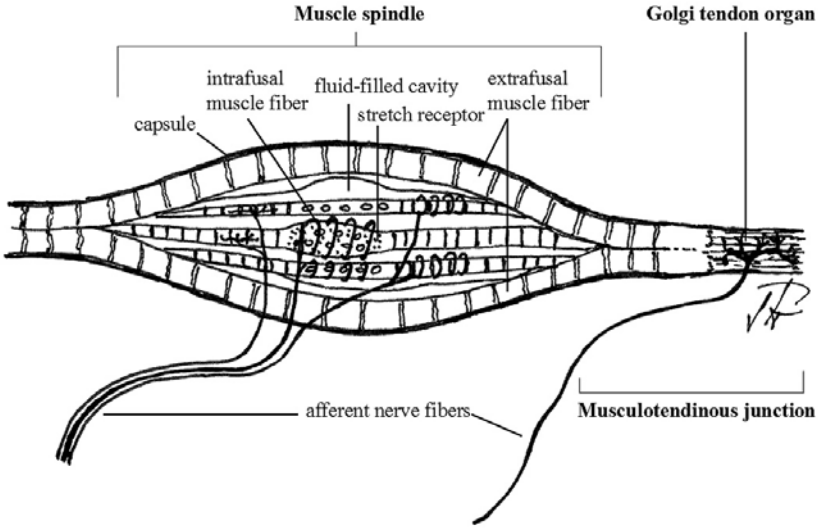

Fig. 15. Sensory receptors associated with skeletal muscle.

C. Interoreceptors

Interoreceptors receive sensory information from the viscera of the body and detect the internal body sensations – such as stomach pain, or inflammatory processes in the deep layers of the skin.

Clinical correlations

The *large multipolar motor neurons* of the spinal cord may be selectively affected by two diseases – *acute anterior poliomyelitis* and *motor neuron disease*.

Acute Anterior Poliomyelitis is the *viral infection* of motor neurons leading to rapid paralysis and wasting of the limb and respiratory muscles. Histopathological changes in the *large multipolar motor neurons,* varying in severity from *mild* to *severe degeneration* in the ventral horn of the spinal cord. The lumbar and cervical enlargements of the spinal cord are most affected. Microglia are increased early, later remaining neuroglia partake in *glial scar* formation. The disability is often asymmetrical and frequently affects the legs. Recovery occurs but may be incomplete.

Motor Neuron Disease is a severe condition characterized by *progressive degeneration of motor neurons* of the *brainstem* and *spinal cord. Degeneration* of ventral horn *motor neurons* causes weakness, hypotonia and fasciculation of the limb skeletal muscles *(progressive muscular atrophy)*. Degeneration of descending pathways leads to weakness and spasticity of the limb muscles, leading to death in about 3 years *(amyotrophic lateral sclerosis)*. Lateral sclerosis is caused by an increased number of astrocytes *(astrocytic gliosis)* following the degeneration and loss of motor neurons.

- the term *amyotrophic* refers to *muscle atrophy*
- the term *lateral* sclerosis refers to the *hardness to distinguish* of the *lateral columns* of the *spinal cord* in autopsy specimens

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21. Special senses

There are the eye and the ear. The eye perceives light. The ear detects sound.

21.1. Eye

Eyes are photosensitive organs which are located within bony orbits. Light is focused on the photosensitive portion of the retina. The visual information is transmitted by the optic nerve to the brain for processing. The eye develops from following parts:

a) The neuroectoderm of forebrain gives rise to the retina, the posterior layer of the iris and the optic nerve.
b) The surface of head gives rise to the lens and corneal epithelium.
c) The mesoderm between the neuroectoderm and surface ectoderm gives rise to fibrous and vascular tunics of the eye.
d) The neural crest differentiate into the choroid, the sclera and the corneal epithelium.

The eyes develop at about the 4th week of embryonic development.

The eyeball is composed of the three concentric layers:

1. The fibrous - external layer, which includes the sclera and the cornea.
2. The middle - vascular layer consisting of the choroid, the ciliary body and the iris.
3. The inner - neural layer, which includes photosensitive and not photosensitive part of the retina (Fig. 1).

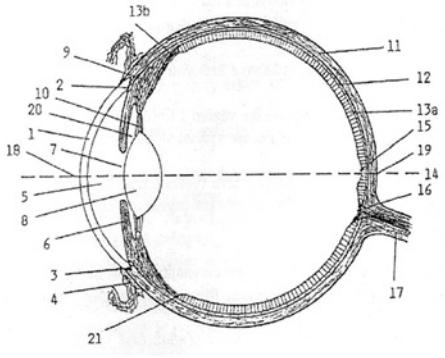


Fig. 1. Schema of the eyeball showing

1-the cornea, 2-the canal of Schlemm, 3-the corneoscleral junction, 4-the conjunctiva, 5-the anterior chamber, 6- the iris, 7-the pupil, 8-the lens, 9-the ciliary body, 10-the zonular fibers, 11-the sclera, 12-the choroid, 13a-the photosensitive region of the retina, 13b-the not-photosensitive region of the retina, 14-the axis of eye, 15-the fovea centralis, 16-the optic papilla, 17-the optic nerve, 18-the anterior pole, 19-the posterior pole, 20-the posterior chamber, 21-the ora serrata

21.1.1. Tunica fibrosa

The cornea is the anterior one sixth of the fibrous layer. The light enters the eye through this part. It is the chief refractive structure. The cornea is continuous with the sclera. It is thicker than the sclera forming avascular, transparent part of the tunica fibrosa.

The junction of the cornea and the sclera is the corneoscleral limbus-the highly vascularized ring-like structure. The corneoscleral limbus, specifically, the iridocorneal angle, contains endothelium-lined channels called trabecular meshwork (spaces of Fontana) merge to form the scleral venous sinus (canal of Schlemm), which drains fluid from the anterior chamber and communicates externally with the venous system.

The corneoscleral limbus is also important in maintaining the population of the stem cells.

The cornea is composed of five distinct following layers:

1. *The corneal epithelium* is stratified, squamous, non-keratinized epithelium formed of 5-6 layers of cells. Damage to the cornea is regenerated rapidly as cells migrate to the defect. Drying of the corneal surface may cause ulceration. The corneal epithelium plays role in transferring water and ions from stroma into the conjunctiva sac. The corneal epithelium is rich in free nerve endings which provide it with extreme sensitivity to touch. Stimulation of these nerve endings elicits blinking of the eyelid and the flowing of tears. The corneal epithelium is covered by layer of lipid and glycoprotein which act as a protective barrier. The corneal epithelium is continuous with the conjunctive epithelium overlying the adjacent sclera. If the external surface of the cornea is allowed to become dry, the cornea may ulcerate.
2. *The anterior limiting membrane (Bowman's membrane)* is 8-10 μm thick layer composed of type I collagen fibers and ground substance.

It is homogeneous and acts as a barrier to the spread of bacterial infections. It is resistant to trauma. It does not regenerate and changes in Bowman‘s membrane are associated with recurrent corneal erosions.

3. *The corneal stroma* is the thickest layer of the cornea. It is formed of many layers of type I collagen fibres interspersed with thin elastic fibers, flattened fibroblasts and ground substance containing chondroitin sulfate and keratan sulfate. The fibres form lamellae, which are layered parallel over the corneal surface. Lamellae are between 1-6 μm thick. Blood vessels absent.
4. *The posterior limiting membrane (Descement‘s membrane)* is a thin basement membrane between the stroma and the corneal endothelium and peripherally beneath the sclera as pectinate ligament penetrates the ciliary muscle and the sclera. It is produced by endothelial cells that cover the posterior surface of the cornea.
5. *The corneal endothelium is* simple squamous epithelium that covers the posterior surface of the cornea. It is responsible for synthesis of proteins and provides for metabolic exchange between the cornea and the aqueous humor. This epithelium has very limited proliferative potential, so serve damage to this epithelium can only be repaired by transplantation.

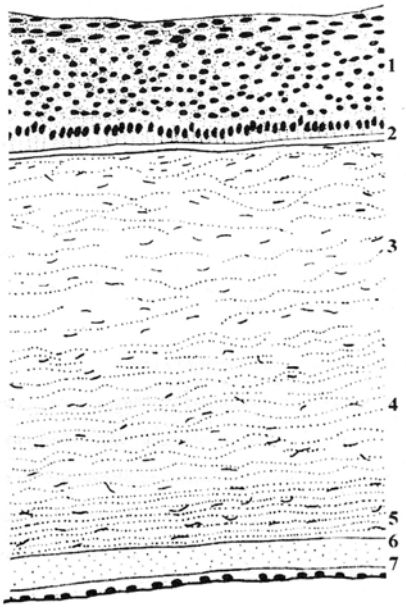


Fig. 2. Layers of the cornea

1-the corneal epithelium, 2-the anterior limiting membrane, 3-the corneal stroma, 4-the nucleus of fibroblast, 5-the collagen fibers, 6-the posterior limiting membrane, 7-the corneal endothelium

The sclera is the posterior five sixth of the fibrous layer. In humans, it forms a segment of a sphere 22 mm in diameter. The sclera is relative avascular. It is formed by connective tissue with collagen and elastic fibers. The fibers are less regularly arranged than in the stroma of the cornea. Elongated fibroblasts are found between the fibers. Melanocytes are located in deeper region.

The sclera is divided into following layers:
The episcleral layer consisting of loose connective tissue and thin collagen fibers near to the periorbital adipose tissue.
The substancia propria of the sclera consists of dense connective tissue. The extraocular muscle insertions, which are responsible for movements of the eyes, attach to this part of the sclera.
The suprachoroidal lamina is the inner, thin part of the sclera located adjacent to the choroid, containing collagen and elastic fibers, fibroblasts, melanocytes, macrophages.

21.1.2. Tunica vasculosa

It is the middle vascular pigmented layer consists of the choroid, the ciliary body and the iris. These parts are called the uveal tract.

The choroid is posterior part of the middle layer, situated between the retina and the sclera. Its function is to provide nourishment to the outer layers of the retina.
It is well vascularized loose connective tissue containing fibroblasts, macrophages, mast cells, plasma cells, melanocytes and blood vessels. The black color is due to the present of the melanocytes in it.

The choroid consists of following layers:

a) *A thin amorphous membrane (Bruch's membrane)* separates the choriocapillary layer from the retina. In its central part is a network of elastic fibers, which is lined on its surfaces with collagen fibers that are covered on one side by basal lamina of the capillaries of the

choriocapillary layer and on the other side by pigment epithelium of the retina.

b) *The choroidal stroma* contains collagen and elastic fibers, fibroblasts, arteries, veins and melanocytes.

c) *The choriocapillary lamina is* the layer of connective tissue with fenestrated capillaries. It has an important function in nutrition of the retina. It is found close to the pigment epithelium of the retina and ends at the ora serrata.

The ciliary body occupies the space between the ora serrata of the retina and the iris.

The cilliary body is 5 mm wide ring of muscle and vascular tissue. In cross section, it forms a triangle.

It is made up of loose connective tissue with elastic fibers, blood vessels and melanocytes.

Its inner surface is lined by the pars ciliaris of the retina, which is composed of two cubical cell layers. An inner layer is formed by non-pigmented ciliary epithelial cells-facing the posterior chamber. An outer layer is formed by pigmented ciliary cells, which have contact with stroma of ciliary body.

The bulk is composed of the ciliary muscle formed by three bundles of smooth muscle cells. Smooth muscle cells are classified into three portions:

a) The meridional fibres, which may help open the iridocorneal angle and facilitates drainage of the aqueous humor.

b) The radial fibres, which its contraction causes the lens to flatten and thus focus for distant vision.

c) The circular fibres, which reduces the tension on the lens.

The anterior one third of the ciliary body has about 60 finger-like projections called *the ciliary processes*. They have a loose connective tissue with fenestrated capillaries covered with two layers of columnar or cubical epithelial cells. The epithelial cells of the ciliary processes secrete aqueous humor. This fluid has an inorganic ion composition similar to that of plasma, but has only 0.1%protein. *The zonular fibers* radiate from the ciliary processes to insert into the lens capsule, forming the suspensory ligaments of the lens.

The iris is a thin, pigmented, vascular diaphragm with circular, central aperture called the pupil, which regulates the amount of light passing through to the retina. In the middle is thickest part of the iris.

The iris is responsible for controlling the diameter and size of the pupil. In response to the amount of light entering the eye, muscle attached to the iris expand or contract the aperture- pupil.

In dark conditions the pupil expands-or dilates to allow as much light as possible pass through.

In bright conditions the pupil shrinks, limiting the amount of light passing through.

The iris is divided into following parts:

a) *The papillary zone*, inner part whose edge forms the boundary of the pupil.

b) *The ciliary zone* is the rest of the iris that extends to its origin at the ciliary body.

The eye color is determined by the amount of melanin in the cells of the iris. Eye color is color of iris which can be green, blue or brown.

Color of the iris is due to lack of pigmentation, as in the pinkish-white of oculo-cutaneus albinism. The pink of their irises is due to the reflection of incident light from the blood vessels which are found in the stroma of the iris.

Most human irises also show a condensation of the brownish melanin in the thin anterior border layer.

Heterochromasia is an ocular condition in which one iris is a different color from the other iris.

The iris consists of:

a) *The anterior surface* of iris is irregular with ridges and grooves. Its epithelium is discontinued or absent.

b) *The stroma* is formed by vascularized connective tissue with fibroblasts and melanocytes. Melanocytes play role in the color of the eyes. The number of melanocytes is high in dark eyes and low in blue eyes.

c) *Dilator pupilae muscle* is formed by modified myoepithelial cells that form a circular band of smooth muscle which are stimulated by sympathetic impulses. Dilated fixed pupil occurs as positive sign of death. Certain drugs may also cause dilatation.

d) *Sphincter pupilae muscle* is located as concentric ring around the margin of the pupil and its contractions alter the diameter of the pupil. This occurs in bright light and during accommodation. The diameter changes with the amount if light entering it.

e) *The posterior surface* is smooth covered by two layers of cubical epithelium. The inner epithelium which lined the posterior chamber is very rich in melanin granules.

The iris and ciliary body together are known as the anterior uvea.

The lens

It is transparent, avascular biconvex disk. The nutrition of the lens derives from the aqueous humor. The lens is very difficult to section so it is frequently damaged during preparation of microscope slides.

The lens is located behind the iris and in front of the vitreous body. The lens is partially covered with the iris. The lens focuses light rays on the retina. The automatic focusing of the lens is a reflex response and is not controlled by the brain. The narrow space between the lens and the iris is posterior chamber. The lens is held in place by radial oriented zonular fibres which are important in the process know as accommodation.

The lens has following parts:

1. *The capsule of the lens* is 10-20 μm thick, homogeneous coating. It contains glycoproteins and collagen IV.
2. *The epithelium of the lens* is found on the anterior surface and is formed by single layer of cubical cells. At the equator these cells are high columnar cells. The cells exhibit many interdigitations with the lens fibers.
3. *The lens fibers* which derived from the subcapsular epithelium are very thin and elongated highly differentiated hexagonal cells, 7-10 μm long and about 2 μm thick. They form the main mass of the lens. The cells and the fibers of the lens contains proteins know as crystallins.

Vitreous body

It is a transparent gel that fills vitreous cavity behind the lens in the posterior segment of the eye. The vitreous body is bounded by the lens,

the zonular fibers and the ciliary body anterior and by the retina posterior. It is attached to retinal surface, especially at the optic nerve, the fovea centralis and ora serrata. It is composed of 99% water, electrolytes, collagen fibers, small population of macrophages and hyalocytes (these cells synthesis of collagen fibres and hyaluronic acid). The hyaloid canal runs through the vitreous body and was occupied in the fetus by the hyaloid artery. This artery later degenerated.

21.1.3. Tunica nervosa

The retina is innermost layer responsible for photoreception. The human retina is a delicate organisation of neurons, glia and nourishing blood vessels that lines the inside of the eye. When light enters the eye, the retina changes the light into nerve signals and sends visual messages through the optic nerve to the brain. Without the retina the eye cannot communicate with brain.

The retina consists of two parts:

1. *Photosensitive* – optic part of retina.
2. *The part of retina* which covered the ciliary body and the posterior part of the iris.

The photosensitive retina (optic part of retina) lines the inner surface of the choroid between the optic disc and ora serrata. This part consists of several layers of neurons interconnected by synapses. The photoreceptor cells such as the cones and the rods are only directly sensitive to light. Rods provide black and white vision. Cones support daytime vision and the perception of color.

Photosensitive region of the retina consists of 10 distinct layers (Fig. 3):

1. *the pigment epithelium,*
2. *the layer of rods and cones,*
3. *the outer limiting layer,*
4. *the outer nuclear layer,*
5. *the outer plexiform layer,*
6. *the inner nuclear layer,*
7. *the inner plexiform layer,*
8. *the ganglionic layer,*

9. *layer of nerve fibers,*
10. *the inner limiting layer.*

1. *The pigment epithelium* is formed by single layer of cubical cells. The basal part of cells attached to Bruch‘s membrane. The apical part of cells contains large microvilli that extend from the surface toward the outer segments the rods and the cones. Melanin granules are in apical portions of these cells. These cells absorb light and storage and release vitamin A. Tight junctions between retinal pigment epithelium also establish a blood-retina barrier. This epithelium is responsible for phagocytosis of membranous fragments from rods and cones. These cells are active in ion transport.
2. *The layer of rods and cones* is formed by two types of cells: rods and cones. Rods and cones are modified neurons with outer segment, inner segment, nuclear region and synaptic part.
   Outer segment is related to microvilli projecting from the adjacent pigment epithelial, cells.
   Inner segment contains organelles associated with synthesizing of proteins. Synaptic region forms synapses with the underlying cells.
   The rods are specialized for perceiving dim light. The rods contain the visual pigment rhodopsin. Rods are elongated cells. The retina contains approximately 120 million rods.
   The cones are specialized cells for perceiving of light and color. The cones contain photopigment iodopsin, which is the principal pigment. The cones are less abundant. The retina has 7milions cones. The cones are concentrated in the fovea centralis.
3. *The outer limiting layer* is formed by zonulae adherentes of neuroglial cells (Müller‘s cells) and the photoreceptors (rods and cones). It is not a true membrane.
4. *The outer nuclear layer* is formed by nuclei and cell bodies of rods and cones. The cone nuclei are larger and more oval than the rod nuclei. Many photoreceptors converge onto one bipolar cell.
5. *The outer plexiform layer* is region of axodendritic synapses between cones, rods and horizontal, amacrine and bipolar neurones.
6. *The inner nuclear layer* consists of the nuclei of three types of neurones: bipolar, horizontal and amacrine. In this layer also are

nuclei of supporting cells. Bipolar neurones are interposing between fotoreceptors and gangion cells. The axons of the bipolar neurones synapse with dendrites of the ganglion cells.

7. *The inner plexiform laye*r is formed by axodendritic synapses between axons of bipolar neurones and dendrites of the gangion cells.
8. *The ganglionic layer* contains multipolar nerve cells. Cell bodies up to 30 μm.
9. *The layer of nerve fibers* is formed by unmyelinated axons of the ganglion cells. These axons run paralel to the retinal surface. The axons of ganglion cells pass to various visual centers of the brain. The axons of the optic nerve are myelinated only after transversing the lamina cribrosa. The lamina cribrosa is a continuation of the sclera.
10. *The inner limiting layer* consists of terminal expansions of Müller cells processes.

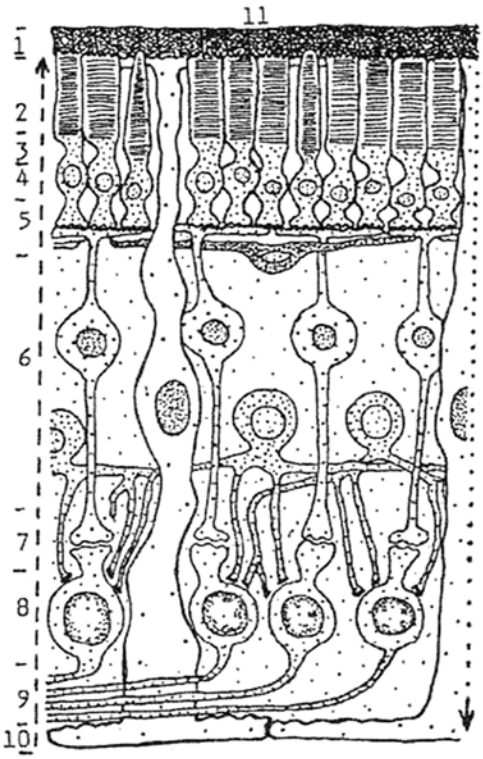


Fig. 3. Schematic drawing of the retina

1-the pigment epithelium, 2-the layer of rods and cones, 3-the external limiting membrane, 4-the outer nuclear layer, 5-the outer plexiform layer, 6-the inner

nuclear layer, 7-the inner plexiform layer, 8- the ganglionic layer, 9-the layer of nerve fibers, 10-the internal limiting membrane, 11-the choroid, dotted line -the nerve impulse, dashed line -the light rays

Specialized regions of the retina

Fovea centralis

It is the part of the retina containing cones that are longer and slender. This area is devoid of blood vessels and rods. It is most sensitive to light and is responsible for sharp central vision.

Macula lutea

Macula has carotenoids and it is yellow in the living state. Retinal blood vessels are no present.

Optic disc (optic papilla)

It is the part where the optic nerve converges at the back of the eye to exit the eye ball. This part is devoid of photoreceptor cells and forming a blind spot in the visual field. The optic disk is also the site of entry of the central retinal artery and vein.

Vessels of the retina

The outer retina is vascularized by the choriocapillary layer of the choroid. The inner retina receives its blood supply from the central artery. The central artery branches into upper and lower branches and these divides again. The branches of the central retinal artery do not anastomose. Evaluation of the retinal vessels and optic disc provides important information on the state of the eye and also provides early clinical signs.

21.1.4. Chambers of eye

Within the eye are three chambers.

The anterior chamber is space between the cornea and the iris and the ciliary body. The lateral border is formed by the limbus with trabecular network.

The posterior chamber is space between the posterior surface of iris and the anterior surface of the lens.

Both chambers contain *aqueous humor*, fluid that is secreted by ciliary epithelium and capillaries of ciliary processes and ciliary body. This fluid has similar composition as plasma but contains less protein. It is drain

through trabecular tissue into the canal of Schlemm, which is lined with endothelium and empties into the veins in sclera.
Aqueous humor plays an important role in providing nutrients to the lens and cornea. Obstruction in the flow of aqueous humor is in glaucoma with an increase of intraocular pressure.

The vitreous chamber is space between the posterior surface of the lens and neural retina. The vitreous humor is fluid component of the vitreous body. Vitreous body helps keep the neural retina in contact with retinal pigment epithelium.

As light rays pass through the components of the eye they are refracted. Refractive media of eye are the cornea, aqueous humor, the lens, the vitreous body.
The cornea is chief and the lens second refractive part of the eye. The aqueous humor and vitreous body have only minor roles in refraction.

21.1.5. Accessory structures of the eye

Accessory structures of the eye include: *the conjunctiva, the eyelid, the lacrimal apparatus.*

21.1.5.1. Conjunctiva

It is mucous membrane that lines the space between the inner surface of the eyelids and anterior surface of the eye lateral to the cornea.

The mucous membrane is formed by epithelium and lamina propria of loose connective tissue
The conjunctiva consists of: bulbar and palpebral parts

1. *The bulbar conjunctiva* covers the sclera and has stratified squamous epithelium.
2. *The palpebral conjunctiva* covers the inner surface of the eyelid. It has stratified columnar epithelium containing goblet cells and lamina propria composed of loose connective tissue. The secretion of goblet cells is component of the tears.

21.1.5.2. Eyelid

The eyelids are skin folds, which can be actively moved. The skin of the eyelids contains many melanocytes. This explains the dark

pigmentation. Act as a protective barrier for the anterior surface of the eye and help adjust the amount of incoming light. In addition, blinking the eyelid helps spread a liquid film of tears over the cornea. Tears also contain antibacterial proteins that helps protect the cornea and the conjunctiva from infection.

The eyelid is composed of:

1. *The folds of skin with hair follicles*. At the tip of lid margin are 3-4 rows of long hairs. The eyelashes are short, curved hairs with different lengths and diameters.
2. *The core,* which are formed by the orbicularis oculi muscle and the tarsal plate. The orbicularis muscle consisting of circularly oriented skeletal muscle fibres overlying the tarsal plate consisting of dense connective tissue.
3. Attached to the tarsal plate are *two muscles* that serve to elevate the eyelid. These muscles are: *levator palpebrae superioris*, which is skeletal muscle and *superior tarsal muscle* which is smooth muscle, innervated by sympathetic nerves.

The eyelid contains four types of glands:

1. Tarsal sebaceous glands are modified sebaceous glands, which are located in the tarsal plate. Twenty-five glands are found in the upper eyelid and 20 are present in the lower eyelid. These glands are simple, branched alveolar gland and produces oily fluid and their secretion empty into ducts onto the eyelids. Secretion serves to lubricate the eyelid surface.
2. Ciliary sebaceous glands are smaller glands that empty their secretion into the follicles of the eyelashes.
3. Palpebral sweat glands are modified apocrine sweat glands located toward the margin of the eyelid.
4. Accessory lacrimal glands - compound serous tubuloalveolar glands, which are found on the inner surface of the upper eyelids and in the fornix of the lacrimal sac. Glands of eyelid are inervated by autonomic nerve system.

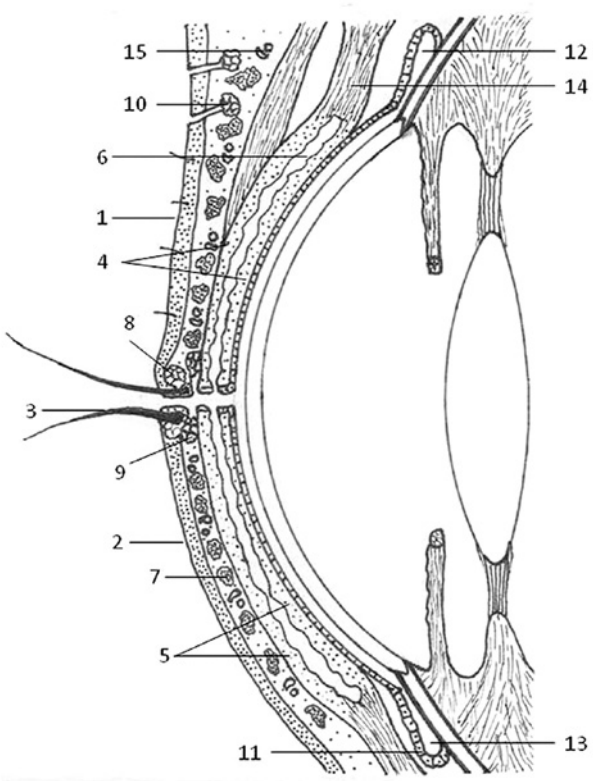


Fig. 4. Structure of eyelid showing

1- the superior palpebra, 2-the inferior palpebra, 3-the eyelash, 4-the tarsus superior 5-the tarsus inferior, 6-the tarsal sebaceous gland, 7-the orbicularis oculi muscle, 8-the ciliary sebaceous gland, 9-the palpebral sweat gland, 10-the sebaceous gland of skin, 11-the palpebral conjunctiva, 12-the superior fornix of conjunctiva, 13-the inferior fornix of conjunctiva, 14-the superior tarsus muscle, 15-blood vessels

21.1.5.3. Lacrimal apparatus

Lacrimal apparatus prevents dehydration of the cornea.

It consists of:

1. The lacrimal glands

It lies outside the conjunctival sac, found in the superolateral side of the orbit.

Each lacrimal gland consists of several separate lobes. It is compound tuboacinar, serous gland. The acinus is lined with simple columnar

epithelium with myoepithelial cells, which are found between epithelial cells and the basal lamina. The lacrimal gland produces tears. It is sterile lacrimal fluid containing water, proteins, lipids with antibacterial lysozyme (hydrolyzes the wall of bacteria) and moisten the eye and the inner surface of the eyelids by passing through the conjunctiva.

The lacrimal fluid enters the lacrimal punctum located in each of medial margins of the upper and lower eyelid. The punctum leads directly to lacrimal canaliculus.

The film of tears covering the corneal surface is a mixture of products secreted by the lacrimal glands, the accessory lacrimal glands, goblet cells of the conjuctiva and tarsal glands of eyelid. Drying of the corneal surface may cause ulceration.

2. The lacrimal canaliculi

   They carry the lacrimal fluid are lined by stratified squamous epithelium. They have small openings called lacrimal puncta, which are located at the medial angle. Superior canaliculus and inferior canaliculus join to form the common lacrimal canaliculus that emptys into the lacrimal sac.

3. The lacrimal sac

   It is dilated part of the nasolacrimal duct, which is lined by pseudostratified columnar, ciliated epithelium.

4. The nasolacrimal duct

   It carries the lacrimal fluid into the inferior meatus of the floor of the nasal cavity.

The muscles of the eyeball

The eye is moved by extraocular muscles. There are six muscle of the eyeball: *the medial rectus muscle, the lateral rectus muscle, the superior rectus muscle, the inferior rectus muscle, the superior oblique muscle, the inferior oblique muscle.*

The combined action of these muscles allows vertical, lateral and rotational movement of the eye.

Clinical correlations

Conjunctivitis is inflammation of the conjunctiva and it may be caused for examples with bacteria, viruses, allergens.

Ptosis – lesions affecting either the occulomotor nerve or sympathetic inervation will result in a noticeable drooping of the eyelid.

Changes in the lens are associated with aging because the lens loses elasticity and ability to accommodate. This condition is normal aging process called *presbyopia*. When the lens is opaque, the condition is termed *cataract.*

Macular degeneration is characterized by loss of central vision because are present dead cells in the macula and is a common retinal problem of the aging eye.

Glaucoma is also common problem in aging, where the pressure within the eye becomes elevated, which can damage the eye‘s optic nerve.

Retinoblastoma is a cancer of retina.

Retinitis pigmentosa – is a group of genetic diseases that affect the retina and causes the loose of night vision and peripheral vision.

Retinal separation – the retina detaches from the back of the eyeball.

Retinal detachment – the neural retina separates from the pigment epithelium.

21.2. Ear – Vestibulocochlear apparatus

The ear develops from the surface ectoderm and components of the first and second pharyngeal arch. The surface ectoderm invaginates on each side of the myelencephalon, thus forming the *otic vesicle (otocyst)*. The otic vesicle serves for the development of the epithelia that line the membranous labyrinth of the internal ear. The sensory epithelia of the membranous labyrinth that also originate from the otic vesicle link with cranial nerve VIII, which is an outgrowth of the central nervous system. The bony, cartilaginous and muscular structures of the ear develop from the mesenchyme surrounding these epithelia.

The ear, as the organ for balance and hearing, is composed of the following three parts:

1. *the outer (external) ear* – receives sound waves;
2. *the middle ear* - sound waves are translated into mechanical vibrations by the tympanic
   membrane and transferred to the internal ear;

3. *the inner (internal) ear* - the vibrations are transformed to specific nerve impulses that pass via the acoustic nerve to the central nervous system.

21.2.1. Inner ear

The inner ear comprises of:

- *the vestibular portion* – for the perception of head and body motion (balance) and
- *the cochlear portion* – for the perception of sound (mechanism of hearing).

Both the vestibular and cochlear portion of the internal ear consist of two labyrinths – *bony labyrinth* and *membranous labyrinth*, one contained within the other.

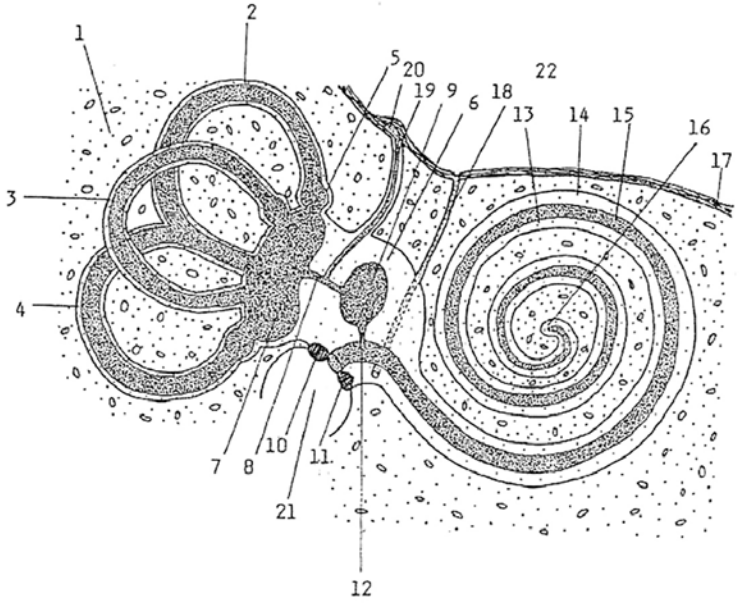


Fig. 5. Diagram of the vestibular (left) and cochlear (right) organ of the inner ear

1-pars petrosa, 2, 3, 4-semicircular canals and ducts-posterior, anterior and lateralis, 5-ampullae, 6-vestibule, 7-utricle, 8-ductus utriculosaccularis, 9-saccule, 10-oval window, 11-round window, 12-ductus reuniens, 13-scala vestibuli, 14-scala tympani, 15-scala media, 16-helicotrema, 17-periosteum, 18-perilymphatic duct, 19-endolymphatic duct, 20-endolymphatic sac, 21-tympanic cavity

21.2.1.1. Bony labyrinth

The bony labyrinth is composed of cavities and canals located within the petrous part of the temporal bone. These cavities are lined with endosteum and filled with *perilymph.* The perilymph is similar in composition to the extracellular fluid, i.e. it has a low K^+ concentration and a high Na^+ concentration. The bony labyrinth comprises a *vestibule, three semicircular canals and the cochlea*.

- *The vestibule* is the central portion of the bony labyrinth between the anteriorly located cochlea and the posteriorly located semicircular canals. The vestibule houses membranous labyrinths - *utriculus* and *sacculus,* that display a sensory epithelium called *macula.* The macula contains hair cells and supporting cells. Stereocilia of hair cells are covered by gelatinous glycoprotein mass – *otolithic membrane.* Surface of this membrane contains *otoliths.* Small ductules from the utricle and saccule join to form *endolymphatic duct* ending in the endolymphatic sac.
- *Semicircular canals (superior, posterior, and horizontal).* The three semicircular canals are oriented at right angles to each other and occupy three planes in space - sagital, frontal and horizontal. All three semicircular canals arise and return to the vestibule. One end of each canal is enlarged; this expanded region is called the *ampulla.* Within the canals there is a suspended membranous labyrinth - *semicircular ducts*.
- *The cochlea* arises as a hollow bony spiral canal, about 35 mm in total length, makes 2,5 turns around the central column of the spongy bone, known as *modiolus*. The modiolus contains blood vessels and bipolar nerve cells – *spiral ganglion* which is the cochlear portion of the vestibulocochlear nerve. In the basal region from the modiolus a thin bony ridge extends laterally into the cochlea, called the *osseous spiral lamina*.

21.2.1.2. Membranous labyrinth

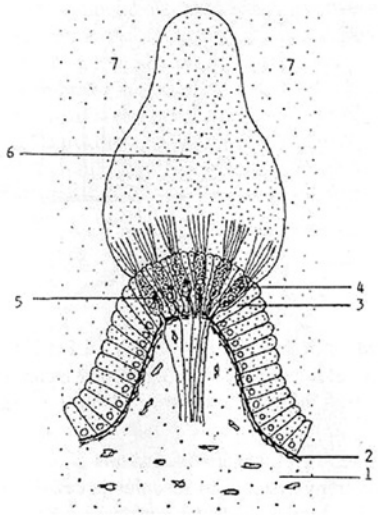
The membranous labyrinth, located within the bony labyrinth, is composed of a very thin connective tissue sheath lined with simple epithelium. The membranous labyrinth is bound to the periosteum of the bony labyrinth by strands of connective tissue that contain blood vessels

nourishing the epithelium of the membranous labyrinth and separate the perilymphatic spaces. The membranous labyrinth is filled with *endolymph*, a fluid with low sodium content, but high potassium content and low protein concentration. The membranous labyrinth contains small specialized areas of neuroepithelial cells that are innervated by branches of the vestibulocochlear nerve, which possess the following specialized sensory areas: the saccule and utricle, three semicircular ducts and the cochlear duct.

The membranous labyrinth is composed of two divisions: the *vestibular labyrinth*, which senses position of the head and linear movement, and the *cochlear labyrinth* with the mechanism of hearing.

1. *The vestibular membranous labyrinth* consists of two sacs:

- *The saccule and utricle* are connected to each other by the ductus utriculosacularis. The saccule and utricule are composed of a vascular layer of connective tissue and an inner layer of simple squamous epithelium. In the epithelium of the saccule and utricle there are specialized regions, which consist of a thickening neuroepithelium and posses two types of cells – receptor cells and supporting cells. These specialized regions of the membranous labyrinth called *maculae* sense the position of the head and its linear movement. Maculae consist of a sensory epithelium (hair cells and supporting cells) topped by the *otolithic membrane*, a gelatinous glycoprotein layer.
- Each of the three semicircular ducts lies within the semicircular canals. Semicircular ducts are dilated at their lateral end (near the utricule). These expanded regions called *ampullae* contain the so-called *cristae ampullares* - specialized receptor areas, which sense the linear and angular movement. The crista ampullaris consists of a sensory epithelium covered by a gelatinous glycoprotein layer called a *cupulla*. The sensory epithelium attached to the basal lamina consists of two cell types - *hair cells* and *supporting cells*. The cristae ampullares are structurally similar to the maculae, but their glycoprotein layer is thicker.



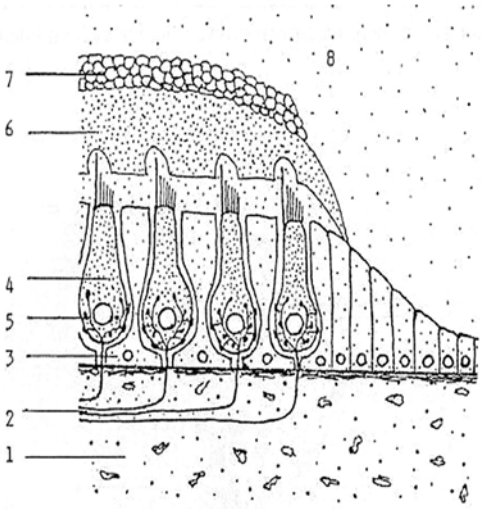


Fig. 6. **Fig. 7.**

Fig. 6. Crista ampullaris in the semicircular canal
1-bone, 2-endosteum, 3-supporting cells, 4, 5-receptor cells, 6-cupula, 7-endolymph

Fig. 7. Diagram of hair cells and supporting cells in the macula of the utricle
1-bone, 2-nerve fibres, 3-supporting cell, 4-receptor cell, 5-synapses, 6-otolithic membrane, 7-otoliths, 8-endolymph

2. *The cochlear membranous labyrinth (auditory system)* contains:
- *The cochlear duct* – a membranous-coiled duct arising as a diverticulum of the saccule, inserted in the bony cochlea. The cochlear duct represents the central chamber, contains the endolymph and is surrounded by the perilymphatic spaces (scala vestibule - above and scala tympani - below). The cochlear duct is responsible for the mechanism of hearing, since it contains specialized auditory receptor cells that form the *organ of Corti.*

The cochlear duct divides the cochlear canal into three parallel compartments or scalae:
- *scala media* (cochlear duct) - the middle compartment, contains the spiral organ of Corti,

- *scala vestibuli* - above the scala media, starting at the oval window,
- *scala tympani* - below the scala media, ending at the round window.

The scala vestibuli and the scala tympani contain the perilymph and communicate with each other at the apex of the cochlea through a small channel called the *helicotrema.*

Histological structure of the cochlear duct (scala media)

The cochlear duct is a triangular space with its acute angle attached to the *modiolus* - a spiraling bony core of the cochlea, which houses the *spiral ganglion.* The boundaries of the cochlear duct are: the *vestibular (Reissner´s) membrane* above, the *basilar membrane* below and the *stria vascularis* externally. On the internal side, the *spiral osseus lamina* projects outward from the modiolus to join the basilar membrane. On the external side, the basilar membrane is continuous with the *spiral ligament.* The vestibular membrane is composed of two layers of squamous epithelium separated from each other by a basal lamina. The stria vascularis is a pseudostratified epithelium and, unlike most epithelia, it is a vascularized epithelium. The stria vascularis is composed of three cell types – basal, intermediate and marginal cells. The *basilar membrane,* extending from the spiral lamina at the modiolus to the lateral wall, supports the organ of Corti. The basilar membrane is composed of two zones: the *zona arcuata*, which is thinner and lies more medial, and the *zona pectinata*, a fibrous meshwork containing a few fibroblasts. At the narrowest portion of the cochlear duct, where the vestibular and basilar membranes meet, periosteum covering the spiral lamina bulges out into the scala media, forming the *limbus of the spiral lamina*. The *interdental cells* located within the spiral limbus secrete the *tectorial membrane*, a proteoglycan gelatinous mass that overlies the organ of Corti.

The organ of Corti

The organ of Corti, a receptor organ for hearing, is located in the scala media, extends beyond the full length of the basilar membrane and is composed of *neuroepithelial hair cells* and several types of *supporting cells*. Although the supporting cells have different characteristics, they are all attached to the basilar membrane. The organ of Corti contains two types of neuroepithelial hair cells: *inner* and *outer hair cells,* separated

from each other by the *inner tunnel,* limited by *inner and outer pillar cells.* The *inner hair cells* are arranged in a single row and supported by the inner phalangeal cells. Their apical surface contains 50 to 60 stereocilia arranged in a "V" shape. The *outer hair cells* are supported by the outer phalangeal cells and arranged in three to four rows along the entire length of the cochlea. Their apical surface contain as many as 100 stereocilia variable in length, organized in "W" shape. The stereocilia of hair cells are embedded in the tectorial membrane that extends over the sensory epithelium from the *spiral limbus.* Only the outer hair cells are in direct contact with the *tectorial membrane.*

The supporting cells of the organ of Corti are the inner and outer pillar cells, the inner and outer phalangeal cells, the border cells and the Hensen's cells. The *inner* and *outer pillar cells* are tall cells with wide bases and apical ends attached to the basilar membrane. The pillar cells contain a large number of microtubules. The inner and outer pillar cells form a triangular space called the *inner tunnel*.

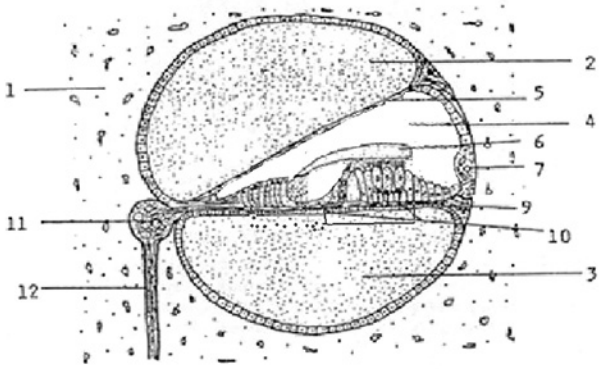


Fig. 8. Diagram of the cochlea

1-pars petrosa of the temporal bone, 2-scala vestibuli, 3-scala tympani, 4-scala media, 5-vestibular membrane, 6-tectorial membrane, 7-stria vascularis, 8-spiral ligament, 9-basilar membrane, 10-organ of Corti, 11-spiral ganglion, 12-canalis spiralis modioli

Clinical correlations

The sense of rotation without equilibrium signifies a dysfunctional vestibular system. Causes of vertigo and hearing loss include viral

infections, certain drugs and tumors, such as acoustic neuroma. Also, vertigo can be produced normally in individuals by excessive stimulation of the semicircular ducts. Excessive stimulation of the utricle can produce motion sickness (e.g. seasickness, carsickness).
Some diseases of the internal ear can affect the auditory and vestibular system, resulting in deafness, dizziness (vertigo) or both. For example, people with Meniere's syndrome (the increase in the volume of endolymph) initially complain of episodes of dizziness, nausea, vomiting and tinnitus (ringing) and later develop a low-frequency hearing loss. The causes of Meniere's syndrome are related to the blockage of the cochlear aqueduct, which drains the excess endolymph from the membranous labyrinth causing the distension of the membranous labyrinth.
Auditory disorders classified as *conductive hearing loss* involve various problems in the middle ear. A common example is otosclerosis, an infection of the middle ear (otitis media), which is common in young children, and infections of the membranous labyrinth. *Sensorineural deafness* involves defects in any of the structures or cells of the cochlea and can cause loss of hair cells or nerve degeneration.

21.3. Sense of smell (olfaction, olfactory perception)

The sense of smell is a primal sense for humans as well as animals and one of the most ancient human senses. Smell depends on sensory receptors – chemoreceptors, specialized sensory cells carry the molecules of chemicals – odorants. The odorants activated the olfactory system at very low concentrations. The olfactory system allows us to identify food, influences social and sexual behavior and provides both sensual pleasures (the odor of flowers and perfume). The odorants are carried by inhaled air to the olfactory epithelium (Regio olfactoria) located in the roof of the two nasal cavities of the human nose (nasal conchae). The specialized olfactory epithelial cells give rise to the processes known as olfactory vesicles serve as sites of stimulus of olfactory sense. Regio olfactoria is a small area about 10 cm^2 containing approximately 100 million primary sensory receptor cells. The olfactory epithelial cells characterize the only group of neurons capable of regeneration.

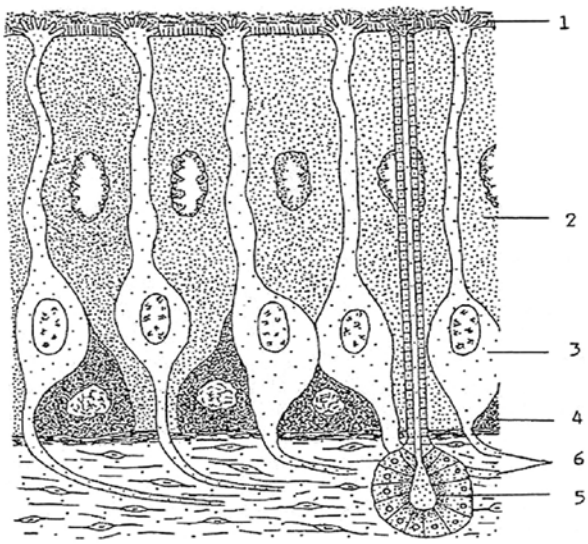


Fig. 9. Scheme of olfactory epithelium

1-mucous layer, 2-supporting cells, 3-olfactory receptor cells, 4-basal cells, 5-Bowman's gland, 6-axons of olfactory receptor cells

The olfactory epithelium is a type of pseudostratified columnar epithelium consists of 3 cell types: basal cells, supporting cells and olfactory receptor cells.

1. Basal cells – are stem cells located in the lowest cellular layer of the olfactory epithelium and are capable of mitotic cell division to form mature olfactory receptor cells.
2. Supporting cells – are columnar cells located among the olfactory receptor cells and have numerous microvilli and secretory granules, which release their contents onto the mucous layer. The mucous layer is produced by the Bowman's glands reside in the olfactory epithelium. This layer is a lipid-rich secretion and assists in transporting the odorant molecules that are soluble in the mucous and produce the signals that the brain interprets as odor. Supporting cells also contains granules with light yellow pigment. The depth of color seems to be correlated with olfactory sensitivity.
3. Olfactory receptor cells – are bipolar neurons located between the supporting cells. Each receptor cell possesses a dendrite on apical site that contains specialized cilia. Each olfactory receptor neuron

has 8-20 nonmotile cilia extending from the olfactory vesicle. The olfactory cilia are the sites where the reception of odorant occurs and starts the sensory transduction. Long central process on the basal site represents the bundles of axons that form the fila olfactoria. After reaching the olfactory bulb of the brain the olfactory signal from fila olfactoria is sent directly to the higher levels of the central nervous system.

Clinical correlations

Several disorders manifest some type of olfactory dysfunction, causes of which include head trauma, upper respiratory infections, tumors and exposure to toxic chemicals or infections.

Olfactory dysfunction includes syndromes such as Kallmann syndrome (i.e. hypogonadism with anosmia) and Foster Kennedy syndrome (i.e. papilledema, unilateral anosmia, and optic atrophy usually associated with an olfactory groove meningioma).

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22. Apoptosis

The principle of apoptosis was firstly described by German scientist Karl Christoph Vogt in 1842. Later on, in 1885, German anatomist and histologist Walter Flemming more precisely delivered morphological description of programmed cell death. Interestingly, this topic was resurrected not until 1965. While studying electron microscopy structure of tissues, John Foxton Ross Kerr at University of Queensland distinguished apoptotic morphology from traumatic cell death. Kerr published this phenomenon and based on it, he was invited to join Alastair Robert Currie, famous pathologist, and his graduate student Andrew Wyllie at University of Aberdeen. The trio published seminal article "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics" in British Journal of Cancer (1972). In the article, natural cell death was called apoptosis. In Greek language, apoptosis translates to the "falling off" of leves from a tree. For many years, the terms "apoptosis" or "programmed cell death" were not highly cited.

James Cormack, a professor of Greek language at University of Aberdeen, reintroduced the term "apoptosis" for medical use.

Kerr received the Paul Ehrlich and Ludwig Darmstaedter Prize in 2000 for his description of apoptosis, he shared the prize with American biologist Howard Robert Horvitz.

H. Robert Horvitz with Sydney Brenner and John Edward Sulston won the Nobel Prize for their discoveries about how genes regulate apoptosis in 2002.

Apoptosis, or programmed cell death, is the physiological mechanism of cell death. Apoptosis plays an essential role in the maintenance of normal tissue homeostasis and requires a precisely regulated balance between cell proliferation and death. It means, that apoptosis is an important counterpart to mitotic activity for the regulation of cell numbers during development, in homeostatic cell turnover in the adult, and in many other processes. Characteristic features of apoptosis are distinct biochemical and morphological changes in affected cells, such

as chromatin condensation, fragmentation of DNA, cell shrinkage, and blebbing of cell membrane.

Then, apoptosis is the physiological process and is different from the necrosis which represents the accidental form of cell death. In necrosis, plasma membranes of cells loss their integrity and an inflammatory reaction is found around necrotic area. In apoptosis, plasma membrane integrity is preserved, it is not accompanied by inflammatory response and the apoptotic cells are phagocytosed by neighbouring cells and macrophages. That is to say, apoptotic cells fragment into a number of small apoptotic bodies which are wrapped by protective protein membrane preventing the leakage of harmful substances and proinflammatory molecules into surrounding tissue. In case of necrosis, the result of cell and organelle oedema is rupture of organelles and plasma membrane. The intracellular content is released into surrounding tissue with subsequent inflammatory response.

Apoptosis can be induced by either specific extracellular signals or internal stimuli. Wide variety of these signals and stimuli are both physiological and pathological. Usually, the type and / or the degree of stimuli determine if cell undergoes apoptosis or necrosis. The variety of harmful stimuli can result in apoptosis at low doses (e.g. hypoxia, radiation, heat, cytotoxic anticancer drugs). On the other hand, these same stimuli can induce necrosis at higher doses. Necrosis is a passive phenomenon. Apoptosis is gene-directed, energy-dependent, usually requiring protein synthesis.

22.1. Morphology of apoptosis

Light microscope

The remarkable feature of apoptosis is its stereotyped morphology. During the early phase of apoptotic process, cells shrinkage. The water content in cytoplasm is reduced via plasma membrane channels. They become smaller in size, the cytoplasm is dense and the organelles are tightly packed. Mitochondria remain functional late into the apoptotic process. One of the most characteristic feature of apoptosis is chromatin condensation (pyknosis). Cell surface specializations are lost, e.g. microvilli and cell junctions. The cell outlines are irregular and then plasma membrane blebbing and

"budding" occur, what is followed by karyorrhexis and fragmentation of cell into apoptotic bodies. These bodies consist of cytoplasm, inside tightly packed organelles with or without nuclear remnants. Apoptotic bodies are enclosed within an intact plasma membrane. Smaller cells do not usually fragment, e.g. lymphocytes. The integrity of organelles is maintained. Apoptotic cells before fragmentation and apoptotic bodies, as well are located within an unstained "halo". Therefore, they are very easily identifiable by light microscope. Apoptotic bodies are quickly engulfed by surrounding parenchymal cells, macrophages, and in case of tumor by neoplastic elements and digested within phagolysosomes.

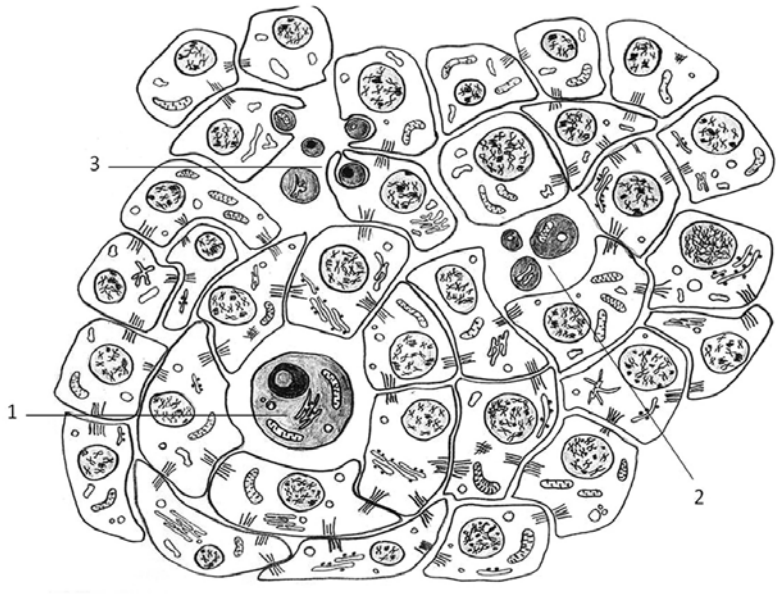

Fig. 1. Schematic diagram of apoptotic process
1-apoptotic cell is shrunken, out of contact with neighbouring cells, located in unstained "halo", 2-numerous apoptotic bodies, 3-engulfment of apoptotic bodies by neighbouring cells

22.2. Mechanisms of apoptosis

Mechanisms of apoptotic process are highly complex. They involve energy-dependent sequence of molecular events. Basically, apoptosis regulates the balance between cell proliferation and death. Apoptosis

can be induced by either extracellular signals (extrinsic inducers) or intracellular stimuli (intrinsic inducers).

The stimuli such as DNA damage, oxidative stress, hypoxia, cell detachment, growth factor deprivation, etc., are mediated via the intrinsic or extrinsic pathway.

22.2.1. Intrinsic or mitochondrial pathway of apoptosis

The stimuli which initiate the intrinsic pathway produce intracellular signals that may act either by positive or by negative way. Negative signals, there is the withdrawal of any factors causing loss of apoptotic inhibition, and subsequent activation of apoptotic cascade. Positive stimuli include hypoxia, radiation, viral infection, toxins, free radicals, and hyperthermia. All of these stimuli cause changes in the inner mitochondrial membrane. Several proteins from the intermembrane space are released. In normal healthy cells, antiapoptotic protein Bcl-2 is present on the outer mitochondrial membrane. Internal damage of the inner membrane results in migration of protein Bax to the surface of mitochondrial membrane to inhibit protective activity of Bcl-2. Moreover, Bax protein is involved in opening of mitochondrial permeability transition (MPT) pores and proapoptotic proteins cytochrome c, Smac/DIABLO and HtrA2/Omi are released from intermembranous space into cytosol. These proteins activate the cascade of caspase-dependent mitochondrial pathway. One of them, cytochrome c, is considered critical for intrinsic pathway. Cytochrome c induces the formation of a large multimetric complex “apoptosome”, which consists of cytochrome c, the adapter protein Apaf-1, adenosine triphosphate (ATP) and procaspase-9. This complex recruits and mediates the activation of the initiator caspase, caspase-9, which continues to activate caspase-3 and caspase-7. The activation of these “executioner” caspases trigger proteolytic activity which causes degradation of chromosomal DNA and digestion of some structural proteins in the cytoplasm. Proteins Smac/DIABLO and HtrA2/Omi act as inhibitors of IAP (inhibitors of apoptosis proteins).

Later in apoptotic process, other proapoptotic proteins are released from mitochondria - AIF (Apoptosis-Inducing Factor) endonuclease G and CAD (caspase-activated DNase). They cause DNA fragmentation and chromatin condensation.

22.2.2. Extrinsic or death receptor pathway

The extrinsic death receptor pathway is based on the activation of cell-surface death receptors. Members of the death receptor family include mainly Fas receptor (Apo-1/CD95) and TNF receptor (tumor necrosis factor). The receptors of this family contain cytoplasmic tail, termed as the death domain (DD). DD causes recruitment of adaptor molecules, which activate caspases. In extrinsic pathway, caspase-8 plays key role in most cases. E.g., binding of complementary death activator Fas ligand (fibroblast associated ligand) to Fas receptor forms a large death-inducing signaling complex (DISC) at plasma membrane. One adapter protein of DISC, the Fas-associated death domain (FADD) is able to activate the initiator caspase, procaspase-8. The active caspase-8 cleaves and activates effector caspases-3 and -7, which are directly or indirectly responsible for the cleavage and degradation of several crucial cellular proteins, as well as for the execution of cell death.

22.3. Caspases

So far, at least 14 different caspases are known in humans, they function in apoptosis and in inflammatory processes. Caspases are proteases that exist as inactive zymogens and are activated by proteolytic cleavage.

The initiation phases both the extrinsic and intrinsic apoptotic pathways lead to the activation of initiator caspases, such as caspase -8, -9, and -10. These type of caspases subsequently activate effector (executioner) caspases: caspase -3, -6, -7. Effector caspases can cleave a variety of cellular substrates, including the cytokeratins, the nuclear proteins, the plasma membrane proteins, etc. On the other hand, some substrates are activated by caspases, e.g. caspase-activated DNase (CAD).

22.4. Phagocytosis of apoptotic cells and bodies

The removal of apoptotic bodies or cells is the last step of apoptotic process. The hallmark of this final stage is phospholipid asymmetry including the presence of phosphatidylserine on apoptotic cell surface. Phosphatidylserine is normally located on the inner surface of the plasma membrane. During apoptosis, it is redistributed to the outer surface of cell.

These molecules mark apoptotic cells or bodies for phagocytosis by neighboring cells or by macrophages, which possess the appropriate receptors. The removal of apoptotic fragments is early and efficient with no release of cellular constituents resulting in no inflammatory response.

Clinical correlations

Humans acquired numerous defence systems against tumor development. The loss of homeostatic cell control can lead to activation of apoptotic pathways to delete these elements before formation of cancer. The abnormalities in regulation of apoptosis can become a significant component of malignant disease development. The apoptosis plays also important role in many other diseases, such as autoimmune syndromes, ischemia, AIDS, and neurodegenerative diseases (e.g. Alzheimer´s disease, Parkinson´s disease, etc.). Some of these conditions express insufficient apoptosis whereas other show excessive apoptosis.

Cancers are characteristic examples where normal regulation of cell cycle is dysfunctional. There is either an overproliferation of cells and/or decreased their removal. The inhibition of apoptosis during cancer development seems to play a crucial role. Apoptosis can be suppressed by a variety of molecular mechanisms.

Apoptosis is an important mechanism in limb development, for example in the formation of notches between the digital rays. Involution occurs during the early stages of limb development. By the end of the sixth week of development, mesenchymal tissue in the hand plates condense and form digital rays. The mesenchymal condensations are called finger buds and they outline the pattern of the fingers. During the seventh week, toe buds in the foot plates are formed. An apical ectodermal ridge (AER) forms at the apex of each limb bud. The AER is a multilayered epithelial structure that promotes the outgrowth of the bud. Between the digital rays, there is loose mesenchyme. Mesenchymal regions soon start breaking down, and form notches between the digital rays. Tissue starts to break down and separate digits are formed by the end of the eighth week. Apoptosis is responsible for the tissue breakdown in the interdigital region.

22.5. References

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