

Histology of Normal Tissues

Ana-Maria Enciu^{1,2*#}, Cristina Mariana Niculite^{1,2#} and Ancuta Augustina Gheorghisean Galateanu^{1,3#}

¹Department of Biology, Carol Davila University of Medicine and Pharmacy, Romania

²Department of Biology, Victor Babes National Institute of Pathology, Romania

³Department of Morphological Sciences, CI Parhon National Institute of Endocrinology, Romania

#authors contributed equally to this work

***Corresponding author:** Ana-Maria Enciu, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, Tel: +4021-3192734, Email: ana.enciu@umf.ro

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INTRODUCTION

„Histology” is a word deriving from old greek (*histos- cloth, tissue* and *logos- word, speech*), meaning „science of tissues”. It provides the researcher with microscopic details of tissues and organs of the human body.

As a science, it started with Robert Hooke’s coining of the term „cell”, back in 1665 and continued with Antonie van Leeuwenhoek’s attempts towards developing staining techniques. It took almost a century of trial-and-error for stainings to become a serious research branch of organic chemistry, but by the end of the 1800s, Camillo Golgi and Ramon y Cajal, using specific protocols for silver staining, had reached another milestone in histology study by detailing the structure of the central nervous system, which was awarded the Nobel Prize for Medicine in 1906.

In the modern era, „histology is the tool for accessing a specific knowledge of the microscopic organization of the organs, microscopic anatomy, which is essential to understand the histopathology

for a possible diagnosis.”. The study of normal tissues reached a consensus widely accepted and published in reputed textbooks, which continue to update the information each year with more and more data from molecular biology and genetics. Discovery of adult stem cells, their niches and their rapidly dividing precursors changed the classical view of “tissues” as masses of similar or identical cells that perform the same function. However, the fundamental histological structure describing general organization of tissues changed very little during the last half of century, giving pathologists strong grounds for diagnosis and research.

Based on morphological and functional characteristics, classic histology describes only four types of fundamental tissues: epithelial, connective, muscle and nervous. These four classes respect the general pattern of organization of a tissue, a cellular compartment and an extracellular matrix, the latter comprising fibers and ground substance.

This chapter aims at offering basic knowledge on microscopy study of normal organization of fundamental tissues of the human body, which can be further used as a starting point for a better understanding of pathology.

EPITHELIAL TISSUE

Epithelial tissue is widely distributed in the human body, forming the covering layer of the skin, as well as covering layers of inner tubular organs, including blood vessels. It also forms glands with endocrine and exocrine secretion. It is characterized by a rapid turnover, rich innervation and the lack of vascularization. Cells are nourished via diffusion of metabolites and oxygen from the blood vessels of the underlying connective tissue.

The cellular compartment is composed of compact cell masses comprising cells with different forms, which are closely joined to each other by cell junctions. The epithelial cells show polarity, with an apical domain towards the free surface of the tissue and a basolateral domain, facing the extracellular matrix.

The extracellular compartment is weakly represented. All epithelia are attached to adjacent connective tissue by an extracellular supporting structure rich in glycoproteins and proteoglycans, produced by both epithelial cells and underlying connective tissue cells, called basement membrane.

Based on their function, epithelia are classified into covering epithelia, glandular epithelia and sensory epithelia. Furthermore, covering and glandular epithelia comprise several different types, depending on morphological characteristics.

Covering Epithelia

Covering epithelia are classified as simple or stratified epithelia according to the number of cell layers. The morphology of the cells may be squamous (flat), cuboidal and columnar. The nucleus of cells has also a different morphology, depending of the type of cell, being a strong diagnostic criterion: for squamous cells in cross section, the nucleus is flat, for cuboidal cells it is round and centrally located, and for columnar cells it is elongated, with the long axis parallel to the long axis of the cell.

For the stratified epithelium, the cells that form the top layer give the name of the epithelium.

Simple squamous epithelium is composed of a single row of flattened cells with flattened nuclei. This epithelium is found in the wall of blood and lymph vessels, where the epithelium is called endothelium, in serous membranes that line the body cavities (pleura, pericardia, peritoneum) where the epithelium is called mesothelium, in the parietal layer of Bowman's capsule and pulmonary alveoli of the lung.

Simple cuboidal epithelium is formed of a single row of cuboidal cells with round nuclei. This epithelium lines some uriniferous tubules and small ducts of exocrine glands.

Simple columnar epithelium contains a row of columnar cells with elongated nuclei. The cells of this type of epithelium may present microvilli or cilia. It is found in the stomach, small intestine (with microvilli forming the brush border), colon, gallbladder, and fallopian tubes (with cilia).

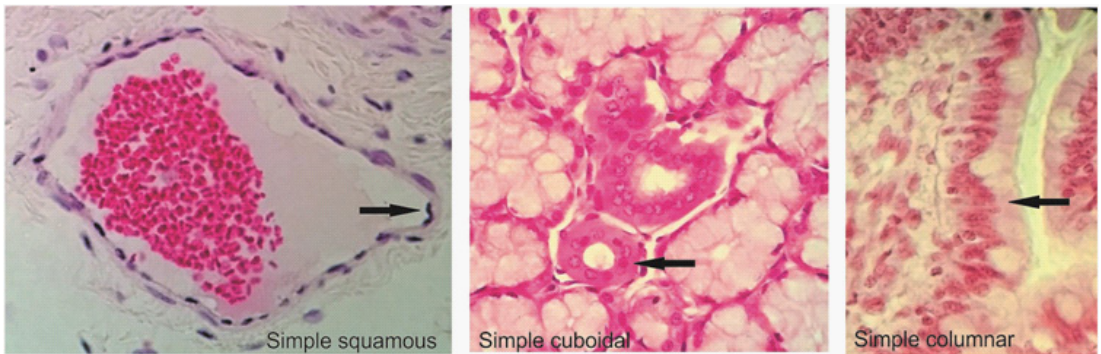


Figure 1: Simple epithelia.

Stratified squamous keratinized epithelium comprises several layers with cells of various forms: cuboidal or columnar in the basal layer, polygonal in the intermediate layer, and flat at the surface. Depending on the proportion of keratin filaments in the surface layer, this epithelium is classified into keratinized epithelium and non-keratinized. Non-keratinized epithelium presents nuclei in the superficial layer, while the keratinized epithelium does not. Non-keratinized epithelium lines the oral cavity, esophagus, and vagina. The keratinized epithelium is restricted to skin where it forms the epidermis.

Stratified cuboidal epithelium and stratified columnar epithelium are rarely met in the adult human body. Stratified cuboidal epithelium has usually two layers of cuboidal cells and is located in the excretory ducts of salivary glands and large sweat gland ducts. Stratified columnar epithelium has two layers, a basal one, with cuboidal cells and a superficial layer with columnar cells. It is found in larger ducts of exocrine glands and in the conjunctiva of the eyelid.

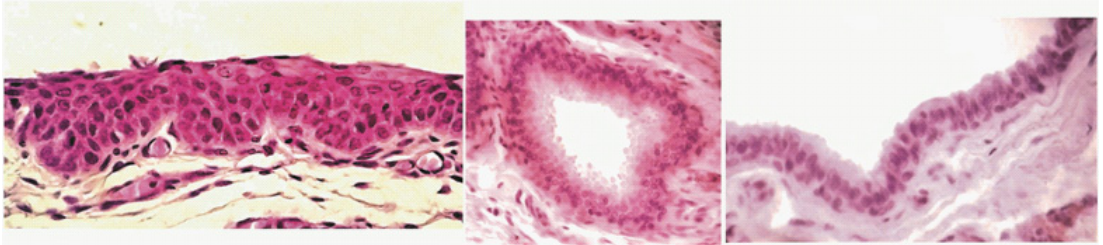


Figure 2: Stratified epithelia: squamous non-keratinized (left), stratified cuboidal (middle) stratified columnar (right) H&E 40x.

Pseudostratified epithelium is a particular type of covering epithelium where all cells are located on the basal lamina, but not all reach the epithelial surface. Nuclei are located at different heights. The cells of this epithelium may present cilia or stereocilia. The ciliated type is located in the trachea and bronchi (hence the name respiratory epithelium), whereas the stereocilia type is found in the male genital excretory pathway (epididymis and ductus deferens).

Transitional epithelium or urothelium is a form of stratified epithelium lining the urinary tract (calyx, renal pelvis, ureters, bladder, and proximal urethra). The number of cell layers and shape of cells of this epithelium varies according to the degree of distention of the organ. Transitional epithelium has three layers named basal, intermediate and superficial. The cells in the basal layer are cuboidal. The intermediate layer consists of racket-shaped cells with a widened apical pole organized in several rows. The surface cells are called umbrella cells, they are large and round and many contain two nuclei.

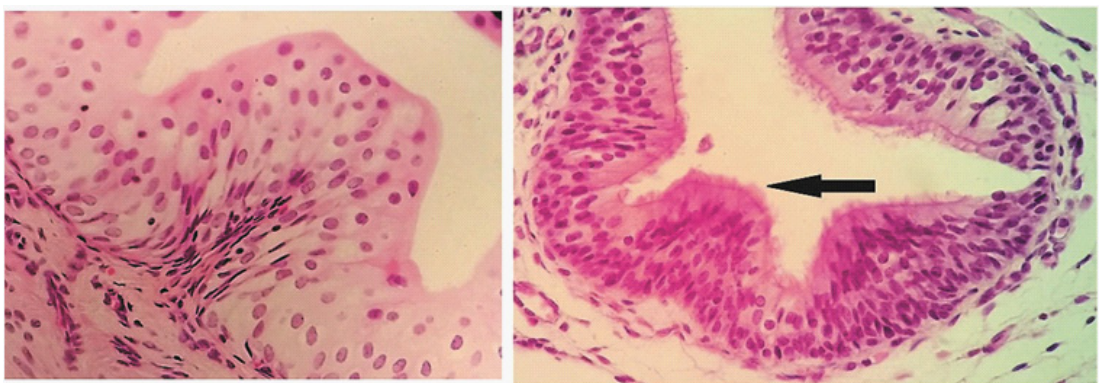


Figure 3: Transitional epithelium (left) and pseudostratified ciliated epithelium (right) H&E 40x.

Glandular Epithelia

Glandular epithelia consist of cells specialized for synthesis and secretion. The secretory products of cells are stored in the form of granules, or may be secreted continuously. These epithelia are often arranged into structures called glands.

Depending on the destination of the secretory product, glands can be classified in: exocrine glands, which release their contents on the body surface or inside cavities, endocrine glands, which release their contents in the internal environment, and mixed glands, which present both exocrine and endocrine parts.

Exocrine glands

Several criteria are used to classify exocrine glands, such as number of cells and morphology of the duct and the secretory unit.

Depending on the number of cells, exocrine glands are unicellular (goblet cell) and multicellular.

The multicellular exocrine glands are subclassified according to the duct as simple (their ducts are not branched) and compound (their ducts branch). According to the shape of the secretory unit (or adenomere), multicellular exocrine glands are tubular, acinar/alveolar and tubuloacinar/tubuloalveolar.

The term alveolar is often used synonymously with acinar.

The goblet cell is the only unicellular exocrine gland. It is a modified columnar cell, with a distended apical pole, shaped as a calyx/cup, with a basal nucleus. This cell synthesizes mucins (glycoproteins) and secretes mucus (hydrated mucins). In hematoxylin eosin (**H&E**) stained sections, the apical cytoplasm is pale, due to the presence of glycoprotein secretory granules, which are washed out during sample preparation. PAS method may be used to highlight the presence of the mucins. This cell is found in respiratory and intestinal epithelium.

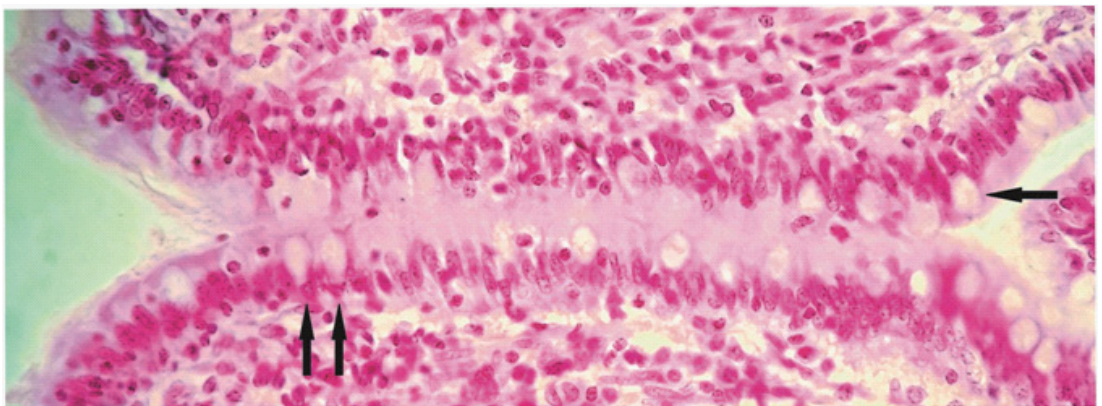


Figure 4: Goblet cells in intestinal epithelium (arrows) H&E, 40x.

Simple tubular glands have a single excretory duct and one tube-shaped adenomere. Examples of simple tubular glands include Lieberkühn glands of the small and large intestine.

The simple coiled tubular gland consists of a single excretory duct and one adenomere, shaped like a coiled tube. The secretory unit is lined by simple cuboidal epithelium and the excretory duct by bistratified cuboidal epithelium. The only example of simple coiled tubular gland is the sweat gland of the skin.

The simple branched tubular gland consists of a single excretory duct and several tubular secretory parts. This gland is found in the stomach (gastric glands that open at the base of gastric pits).

The simple branched acinar gland consists of a single excretory duct and several secretory acini. The cells of secretory acini contain a central nucleus and vacuolar cytoplasm. As the secretion product builds up, the nucleus becomes pyknotic and the cytoplasm more vacuolar. The secretory product consists of sebum and cellular debris that are eliminated following the lysis of cells. An example of simple branched acinar gland is the sebaceous gland of the skin.

The compound branched acinar gland consists of a branched ductal system and secretory units of acini with serous secretion. The parotid gland and the exocrine pancreas are examples of this type of gland.

Compound tubuloacinar/tubuloalveolar glands consist of a branched duct system and secretory units which are branched and have complex morphology of tubes ending with acini/alveolae, with either serous and/or mucous secretion. Examples of compound tubuloacinar/tubuloalveolar glands include sublingual glands, submandibular glands, the prostate gland and mammary glands.

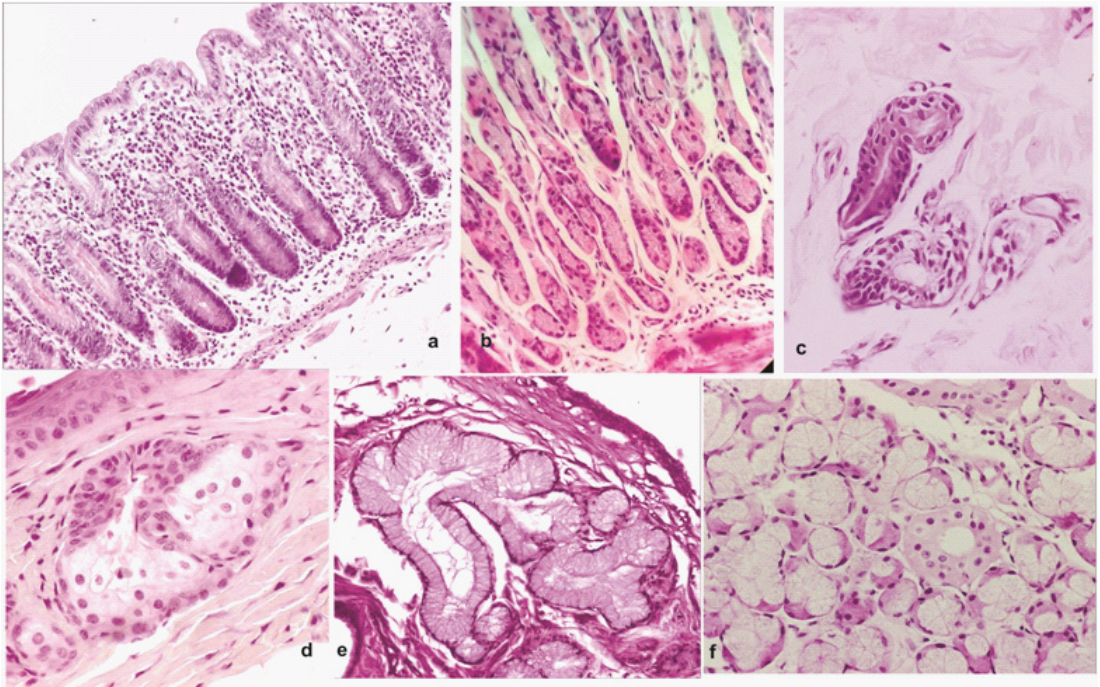


Figure 5: Types of exocrine glands: a) simple tubular; b) simple tubular branched; c) tubuloglomerular gland; e) simple acinar ramified; f) compound tubuloalveolar; g) compound acinar. H&E, 20x (a, b, e), 40x (c, d, f).

Classification of Exocrine Glands Based on The Nature of the Secretion Product

Exocrine glands may be subclassified according to the nature of the secretion product as serous glands, mucous glands, mixed glands (muco-serous or sero-mucous) and oily glands (such as fat, like sebaceous glands). Serous glands secrete a product rich in proteins. The serous cells have a basal round nucleus and tinctorial polarity: a basal basophilic pole (due to intense protein synthesis in rough endoplasmic reticulum) and apical acidophilic pole (due to the secretory granules).

Mucous glands secrete a product rich in glycoproteins. Mucous cells have flattened nuclei, located at the basal pole and a pale staining cytoplasm (due to the presence of carbohydrates). Additionally, mixed acini are present in some glands. In these glands, serous cells are organized in demilunes around mucous acini (serous demilunes are considered an artefact of sample preparation; normally there is an alternation of serous and mucous cells).

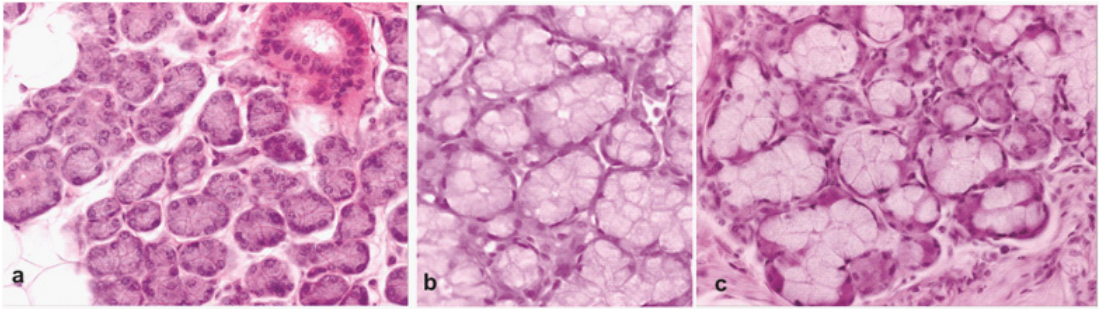


Figure 6: Acini serous (left), mucous (middle), mixed (right) H&E 40x.

Classification of Exocrine Glands Based on Mechanisms of Secretion

According to the mechanism of secretion, exocrine glands are classified as merocrine, holocrine, or apocrine.

Merocrine glands involve exocytosis of the secretory product, with no loss of cytoplasm.

Apocrine glands release membrane-bound vesicles along with the apex of the cell.

Holocrine glands breakdown and release the contents of the whole cell, along with the secretory product.

Endocrine glands

Endocrine glands have no excretory duct. They are well vascularized, because the secretion product is eliminated directly into the internal environment (usually blood). The cells are arranged in islands (endocrine pancreas), nests (adenohypophysis), cords (adrenal gland), and follicles (thyroid).

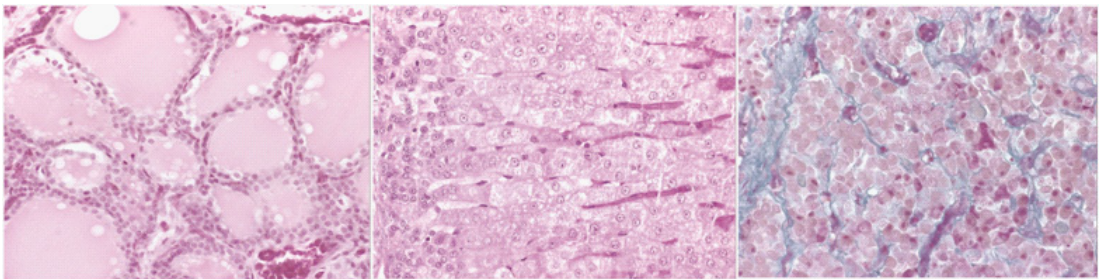


Figure 7: Examples of organization of endocrine cells: follicles (left, H&E 20x, thyroid), cords (H&E, 40x, adrenal gland), nests (right, Azan, hypophysis, 40x).

CONNECTIVE TISSUE

Connective tissues are composed of a variety of cell types, of mesenchymal or hematopoietic origin embedded in an extracellular matrix. The latter is composed of protein fibers (collagen, elastic and reticular fibers) and ground substance.

Connective Tissue Cells

Depending on their origin, the connective tissue cells can be classified into *resident cells*, differentiated from the mesenchymal stem cells (fibroblasts - fibrocytes, chondroblasts - chondrocytes, osteoblasts - osteocytes, adipocytes, myofibroblasts) and migrated cells from the blood stream, differentiated from hematopoietic stem cells (macrophages, mast cells, plasma cells, etc). Some tissues may contain pigment cells, derived from the neural crest.

The suffix “blast” describes a young, active cell, and the suffix “cyte”, a mature cell.

Resident Cells of Connective Tissue

The *mesenchymal* cell is the undifferentiated connective tissue stem cell, able to differentiate into all types of resident cells, as well as into endothelial cells and smooth muscle cells under certain circumstances.

It has a spindle-like body, with an oval, pale staining nucleus, with a conspicuous nucleolus. The mesenchymal cells show cytoplasm processes that communicate with neighboring cells through gap junctions, forming a cellular network.

The *fibroblast* is the most common cell within the connective tissue. It has an elongated cell body, with cell processes, a basophilic cytoplasm (rich in rough endoplasmic reticulum and polyribosomes), an oval, euchromatic nucleus with obvious nucleoli. Its main function is to synthesize the extracellular matrix components.

The *fibrocyte* is a less metabolically active cell compared to the fibroblast. It has a spindle shape, with fewer cytoplasmic projections, an acidophilic cytoplasm and an elongated heterochromatic nucleus.

The *white adipocyte* is a large cell, of round/oval shape (when isolated) or polygonal (when in groups). It contains one large lipid droplet (hence the name unilocular), not visible in routinely stained histological sections, because it is washed by organic solvents used during sample preparation. The cytoplasm is represented by a thin ring, pushed to the periphery, while the nucleus is eccentric and heterochromatic.

The brown adipocyte is a smaller cell than the white adipocyte, with many lipid droplets (hence the name multilocular), giving the cytoplasm a spongy appearance. The nucleus is round and located centrally

The myofibroblast is a spindle shaped connective tissue cell with contractile properties. It contains in the cytoplasm bundles of α smooth muscle actin that associates with non-muscle myosin. Unlike smooth muscle cells, it is found isolated, scattered in the connective tissue, occasionally connecting with other myofibroblasts via cell processes.

Migrated Cells of Connective Tissue

The macrophage differentiates from monocytes migrated in various tissues. It is a large, round cell, without any particular features, a cytoplasm with either granular or foamy appearance, depending on the type of ingested particles. The nucleus is oval or kidney-shaped, located peripherally or in the center of the cell. All tissue macrophages form the macrophage - mononuclear phagocyte system. Depending on location, macrophages have different names, such as dust cells (alveolar macrophages - the lungs), microglia (central nervous system), Kupffer cells (liver), Langerhans cells (skin), osteoclasts (bone tissue).

The mast cell is an oval cell, with a cytoplasm loaded with numerous ortho- and metachromatic granules (rich in glycosaminoglycans and proteoglycans, such as heparin). The nucleus is round, centrally located, euchromatic with obvious nucleolus.

The plasma cell is an activated B-lymphocyte, specialized for antibody synthesis. It is a round/oval shaped cell, with a basophilic cytoplasm (due to the high content of poly-ribosomes and RER). The nucleus is somewhat eccentric, with a specific pattern of alternating heterochromatin and euchromatin, giving it the appearance of a cartwheel or “clock-face. Around the nucleus there is a clear halo, where the Golgi apparatus is located.

The pigment cell (melanocyte) is a cell originating from the neural crests. It has a “snowflake” shape, with several processes, a cytoplasm filled with brown melanin granules, which makes it conspicuous on histological sections without any staining.

Connective Tissue Fibers

Connective tissue fibers are classified into three categories, depending on their chemical composition and synthesis mechanism: collagen fibers, elastic fibers and reticular fibers.

Collagen fibers are polymers of collagen protofibrils, biochemically composed of different types of collagen proteins. Depending on the type of protein, collagen protofibrils can associate into thick fibers, that do not branch or anastomose with each other, or they can form delicate fibrils that associate into networks. Collagen fibrils are thin; they are not visible in routine histological sections unless they form thick, wavy, acidophilic collagen fibers. These thick fibers can be highlighted in the matrix of connective tissues using trichrome stains, such as hematoxylin-eosin-methylene blue (intensely blue), Van Gieson (red) or Azan (blue, contrasting with the red nuclei).

Elastic fibers are thinner than collagen fibers, also acidophilic, therefore, more difficult to identify on routine sections. They branch and anastomose and are best visible in special stains such as orcein or Weigert's resorcin-fuchsin.

Reticular fibers are thin bundles of collagen III fibrils that anastomose into networks, to form a scaffold for myeloid and lymphoid tissues. They differ from collagen fibers by their greater carbohydrate content, which also makes them visible in silver stains.

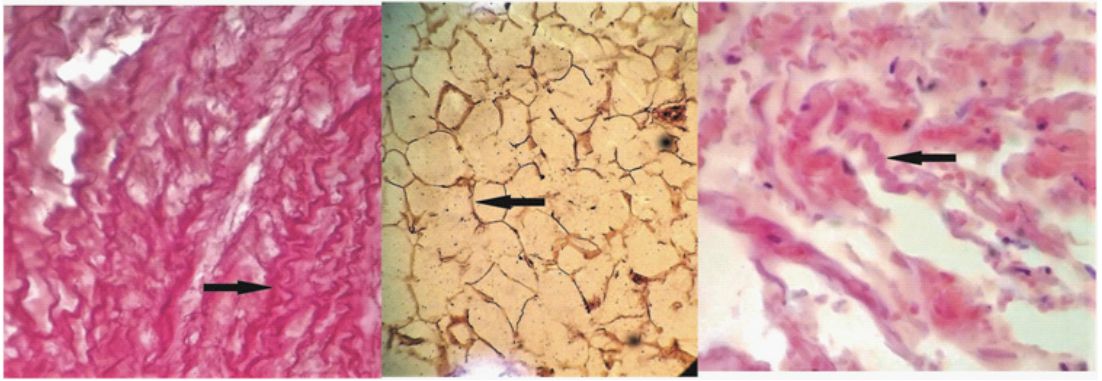


Figure 8: Connective tissue fibers: Elastic(left, orcein stain), reticulin (middle, silver stain) collagen (right, H&E) 40x.

Ground Substance

Ground substance is an amorphous, homogeneously-looking component of the extracellular matrix, located among cells and connective tissue fibers. In H&E stain it is pale acidophilic (due to the high content of glycosaminoglycans and glycoproteins).

Depending on the type of cells and fibers within a tissue, as well as proportions of different tissue components, one may classify connective tissues in *connective tissue proper* and *specialized connective tissue*.

Connective tissue proper can be further classified into several categories, based on the predominant type of fiber or cell:

- loose connective tissue: equal amounts of cells, fibers and ground substance
- dense irregular connective tissue - predominant collagen fibers with irregular distribution through the tissue
- dense regular connective tissue - mostly collagen fibers arranged in a parallel pattern
- reticular connective tissue - formed mostly of reticular cells and reticular fibers
- elastic tissue - predominant elastic fibers, produced by fibroblasts
- adipose tissue - composed mostly of adipocytes, either white, or brown
- mucous tissue - an embryonic type of tissue, composed mostly of mesenchymal cells

Specialized connective tissue: cartilage, bone, blood.

Loose connective tissue is the most common type of connective tissue. It contains the three major components of any connective tissue - the connective cells (various types, both resident and mobile), connective fibers and ground substance (rich in GAGs) - present in equal proportions.

It is widely spread, part of most mucosae of luminal organs, but also in the dermis of skin and part of muscles and peripheral nerves.

Dense irregular connective tissue is composed mostly of collagen fibers arranged in bundles, oriented in all directions, hence the name irregular connective tissue. It contains all types of cells: fibroblasts, fibrocytes, plasma cells, macrophages, mast cells, migrated leukocytes. It can be found in the deep dermis (reticular dermis), the capsule of parenchymal organs, dura mater, sclera, epineurium, epimysium, epitenon, submucosa of luminal organs.

Dense regular connective tissue consists mainly of bundles of collagen with parallel distribution. It forms aponeurosis, tendons and ligaments.

The *aponeurosis* ensures broad insertion of muscles to the bone. It is an inextensible structure, consisting of 2 to 5 layers of fibers and cells. The predominant feature is the presence of collagen fibers parallel to each other within the same layer and perpendicular to the underlying and overlying leaflets. Overall, the microscopic aspect is of a cloth. The cells are almost exclusively fibroblasts and fibrocytes.

The tendon is an inextensible structure that ensures insertion of long muscles on the bone. Also formed predominantly by collagen fibers, but in this case arranged parallel to each other, tightly wrapped in dense irregular connective tissue. The cells are called tenocytes and are located in the spaces between the collagen fibers. In longitudinal section tenocytes have an elongated appearance, and are arranged parallel to the direction of the fibers. In cross section, they extend processes surrounding the collagen fibers. The tendon presents connective tissue sheets that protect and join together fibers into a tensile-resistant structure. These sheets are:

- endotenon: loose connective tissue surrounding the primary and secondary fascicles
- epitenon: dense irregular connective tissue that surrounds the entire tendon

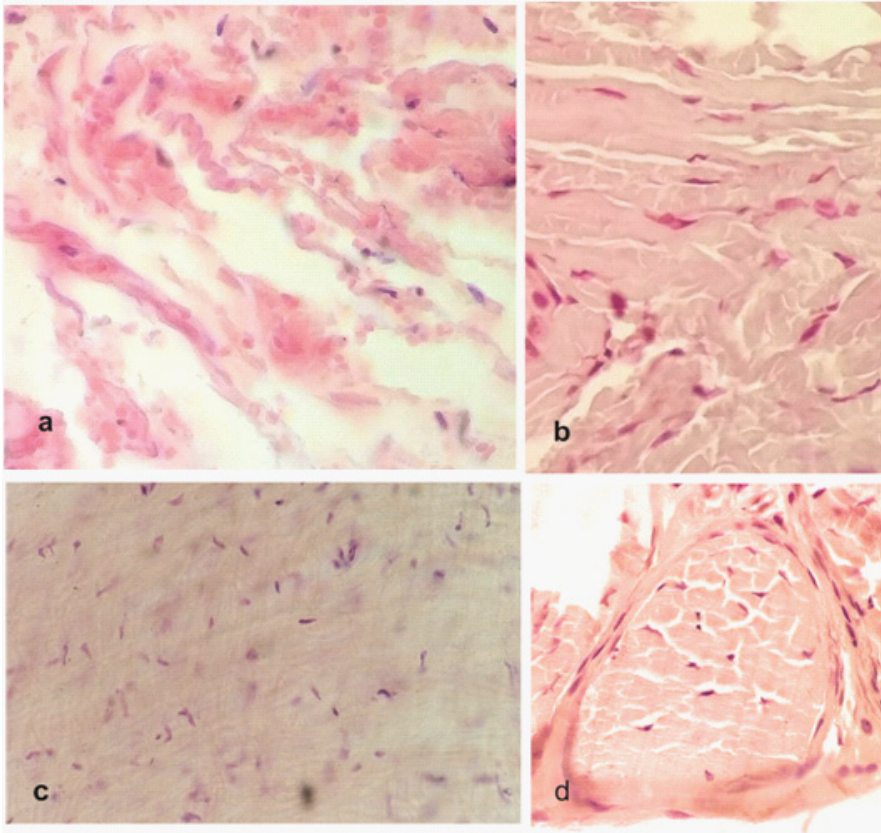


Figure 9: Proper connective tissues a - loose connective tissue, b - dense irregular connective tissue, c - aponeurosis, d - tendon in cross-section. H&E, 40x.

Reticular connective tissue acts as a scaffold for myeloid and lymphoid organs, therefore, two types are described accordingly: myeloid reticular connective tissue, located in the bone marrow and lymphoid reticular connective tissue, in the lymphoid organs: lymph nodes, white pulp of the spleen, tonsils. It is composed of reticular cells, stellate-shaped cell with long, thin processes and a round, pale nucleus, with a prominent nucleolus. The cytoplasm is scarce and acidophilic with extensions connecting them to each other, but also with reticular fibers to form a network. In addition, macrophages and antigen presenting cells may be present in high number, depending on the location of the tissue. The ground substance is located in the spaces left between cells and fibers.

White adipose tissue (unilocular) is composed mainly of white adipocytes, having few ground substance and few fibers, in particular reticular fibers, surrounding cells in a delicate meshwork.

Brown adipose tissue (multilocular) is formed of brown adipocytes, few ground substance and few reticular and collagen fibers. This type of tissue is not common in adulthood, but mostly found in new-born and infants, having a thermal protection role. The term “brown” comes from the

macroscopic brownish appearance, due to the numerous mitochondria (containing cytochromes) and a rich blood supply. It can be found retrosternally, in the groin, neck, in the subscapular area, mediastinum, and around the kidneys and adrenal glands.

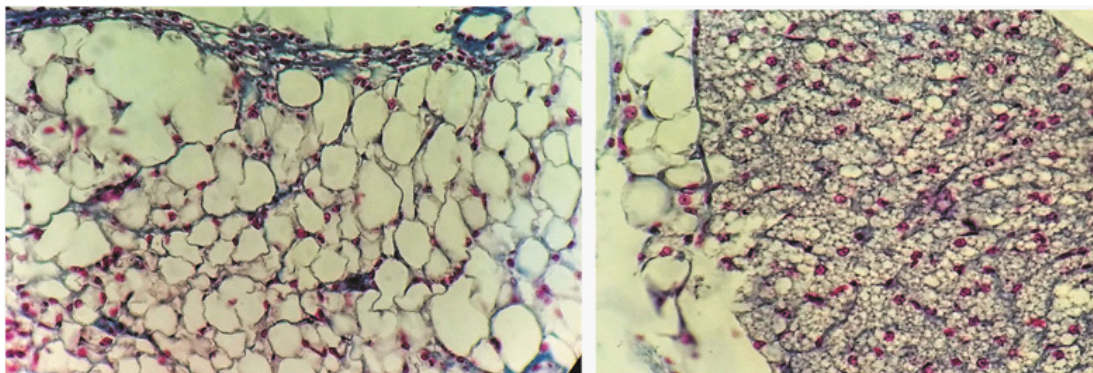


Figure 10: Adipose tissue (left-white adipocytes, right brown adipose cells) H&E-methylene blue, 40x.

Elastic connective tissue is a type of connective tissue in which the predominant component is the elastic fibers. It has little ground substance and few fibroblasts. It is located in the structure of elastic ligaments, the fibrous ligaments of joints and the suspensory ligament of the penis.

Mucous connective tissue is a type of tissue found mostly during the embryonic period. Its predominant feature is the prevalence of ground substance, with only few, inconspicuous collagen fibers. The cellular compartment is represented by mesenchymal cells.

Cartilage is a specialized type of connective tissue, composed of cells called chondroblasts and chondrocytes and an extracellular matrix capable of bearing weight and mechanical stress.

Chondroblasts are immature cells, active in extracellular matrix production; they possess a euchromatic nucleus and a basophilic cytoplasm, evidence of the presence of polyribosomes and rough endoplasmic reticulum, involved in protein synthesis. Chondrocytes are more mature cells, with an acidophilic cytoplasm, located inside cavities called lacunae. The extracellular matrix consists of collagen and elastic fibers and ground substance, which is very rich in glycosaminoglycans and proteoglycans. Cartilage is covered by perichondrium (except fibrous cartilage and articular cartilage), consisting of two layers: an outer fibrous layer and an inner cellular layer, involved in cartilage growth. Blood vessels from the perichondrium provide nutrients to the avascular cartilage; perichondrium also contains lymph vessels and nerves.

On the basis of matrix composition, cartilage has been classified into three types:

- Hyaline cartilage
- Elastic cartilage
- Fibrous cartilage

Hyaline cartilage contains chondroblasts, located at the periphery and chondrocytes arranged in isogenous groups (several cells arising by repeated cell division from the same mother-cell) in cavities of the matrix called lacunae. The extracellular matrix has a homogenous basophilic appearance; it consists of type II collagen fibers that do not form bundles, and glycosaminoglycans, proteoglycans and glycoproteins. The territorial matrix is a more intensely staining area around the isogenous groups and the interterritorial matrix occupies the spaces between the groups of chondrocytes; it has a less intense stain and a lower concentration of sulfated proteoglycans. Hyaline cartilage can be found in the nose, the larynx, trachea, bronchi, costal cartilage and articular cartilage.

Elastic cartilage has a high content of elastic fibers in the extracellular matrix, but contains type II collagen fibers as well. Elastin can be demonstrated with a special stain, like orcein. Elastic cartilage has a perichondrium, chondroblasts and chondrocytes, organized in smaller isogenous groups. It is located in the epiglottis, larynx, external acoustic meatus and the Eustachian tube.

Fibrous cartilage has fewer chondrocytes, arranged in rows or isogenous groups between parallel type I collagen fibers. The ground substance is reduced in quantity. Fibrous cartilage is associated with dense connective tissue and it is located in areas where resistance to compression and shearing forces is required; it can be found in intervertebral disks, pubic symphysis and certain ligaments. The intervertebral disks contain a peripheral annulus fibrosus (with concentric rings of dense connective tissue and fibrocartilage) and a central nucleus pulposus, with groups of cells surrounded by ground substance rich in hyaluronic acid.

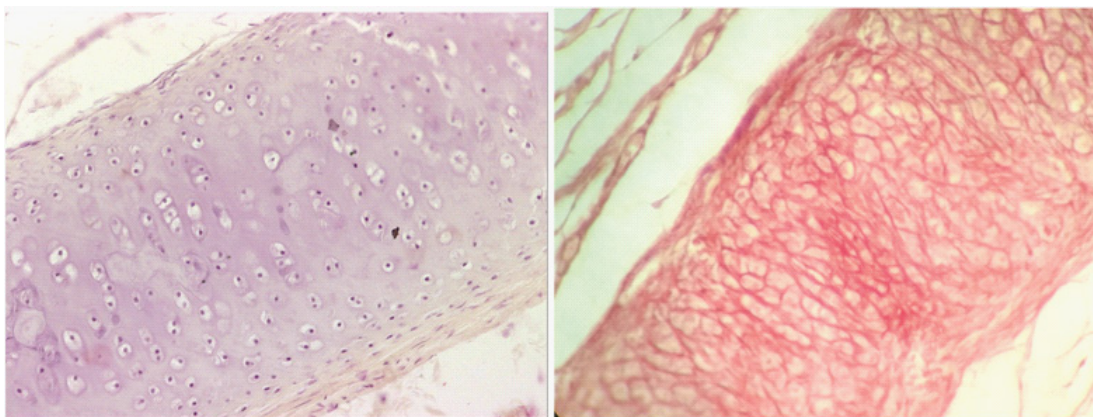


Figure 11: Left: hyalin cartilage, H&E 20x, right: elastic cartilage, orcein 40x.

Bone is a type of connective tissue with a mineralized extracellular matrix.

There are four types of bone cells:

- Osteoprogenitor cells - precursor cells, derived from mesenchymal stem cells
- Osteoblasts - involved in the synthesis of unmineralized bone; they have a euchromatic nucleus and a basophilic cytoplasm

- Osteocytes - mature bone cells, enclosed in lacunae; they have a heterochromatic, flattened nucleus and a slightly basophilic or acidophilic cytoplasm
- Osteoclasts - multinucleated phagocytic cells located in Howship's lacunae.

The extracellular matrix is composed of 35% organic material, mainly type I collagen fibers, as well as noncollagenous proteins, and 65% inorganic material, represented by hydroxyapatite crystals. The organic component can be studied in sections of demineralized bone, obtained by means of acidic solutions, while the architecture of bone and the mineral component can be observed in ground bone, which has been dried and ground down to a thickness that permits the light to be transmitted.

Bone tissue can be classified into:

- spongy (trabecular) bone - consisting of bone trabeculae, covered by endosteum and cavities containing bone marrow; in the immature bone, collagen fibers are randomly arranged, whereas in the mature bone, they are organized in orderly lamellae
- compact (lamellar) bone - collagen fibers have a compact arrangement, with different orientations in adjacent lamellae

Compact bone is organized in structural units called osteons or Haversian systems, consisting of 4-20 concentric bone lamellae surrounding a central Havers canal. Between the lamellae there are lacunae with osteocytes, as well as canaliculi containing the processes of osteocytes. Interstitial lamellae are found between the osteons. Transverse (Volkmann) canals perforate osteons and they contain blood vessels and nerves. The outer surface of bone is covered by periosteum, which contains an outer fibrous layer and an inner layer with osteoprogenitor cells. Bone cavities with marrow are lined by endosteum, a thin layer of connective tissue with osteoprogenitor cells. Beneath the periosteum and around the marrow cavity are located outer and inner circumferential bone lamellae.

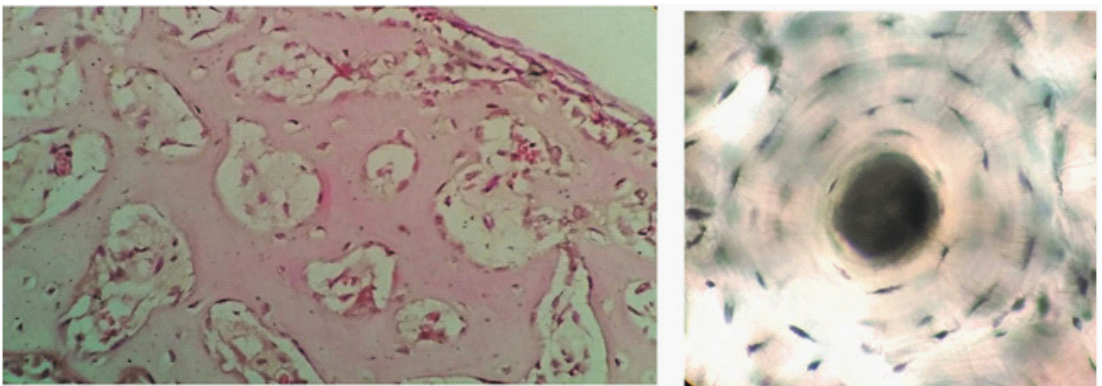


Figure 12: Spongy bone (demineralized) (left) osteon of compact bone (dried) (right).

Blood is a variety of connective tissue consisting of cells and plasma. Blood cells originate in the red bone marrow in the hematopoietic stem cell. Peripheral blood cells are also called formed elements and consist of: red blood cells (anucleated cells), white blood cells (nucleated cells, also called leukocytes) and platelets (fragments of cells found in the red bone marrow, called megakaryocytes). Blood smears require special staining, such as May-Grünwald-Giemsa (**MGG**) or Wright's stain.

Using MGG, the nucleus of leukocytes will appear basophilic (purple-blue) and the cytoplasm basophilic (bluish) for agranulocytes, acidophilic (pink) for granulocytes and intensely basophilic (purple-blue) for platelets. The nucleus of leukocytes may assume different forms, from a classic round nucleus to a polymorphic, polylobulated nucleus, classifying them into mononucleated leukocytes (monocytes, lymphocytes) and polymorphonucleated leukocytes (neutrophils, basophils and eosinophils).

Leukocytes contain in the cytoplasm both nonspecific granules (found in all leukocytes) and specific granules (found only in granular leukocytes).

Erythrocytes are small, anucleated cells with a diameter of 7 to 8 μm , an acidophilic cytoplasm, and a paler staining center (due to their 3D shape of biconcave disc). Pathologically, their size and shape may vary from smaller than normal (in iron deficiency) to very large (in megaloblastic anemia) or to peculiar shapes (in sickle cell anemia). On a blood smear, erythrocytes can be used as a standard for size and color.

White blood cells can be classified based on the aspect of their nucleus and the type of granules in the cytoplasm.

Depending on the aspect of the nucleus, leukocytes can be classified as mononuclear (lymphocytes, monocytes) and polymorphonuclear (PMN). The latter can be further classified, depending on the type of granules contained, in: neutrophilic PMN, acidophilic PMN and basophilic PMN.

Neutrophilic granulocytes are characterized by a nucleus with 2 to 5 segmented lobes, united by fine chromatin bridges; an acidophilic cytoplasm with small pink – purple granules. On the blood smear young neutrophils may be identified by their unsegmented nucleus (so-called band cells).

Acidophilic granulocytes are characterized by a typically-looking nucleus with 2 lobes, united by a chromatin bridge; an acidophilic cytoplasm with large, red orange granules.

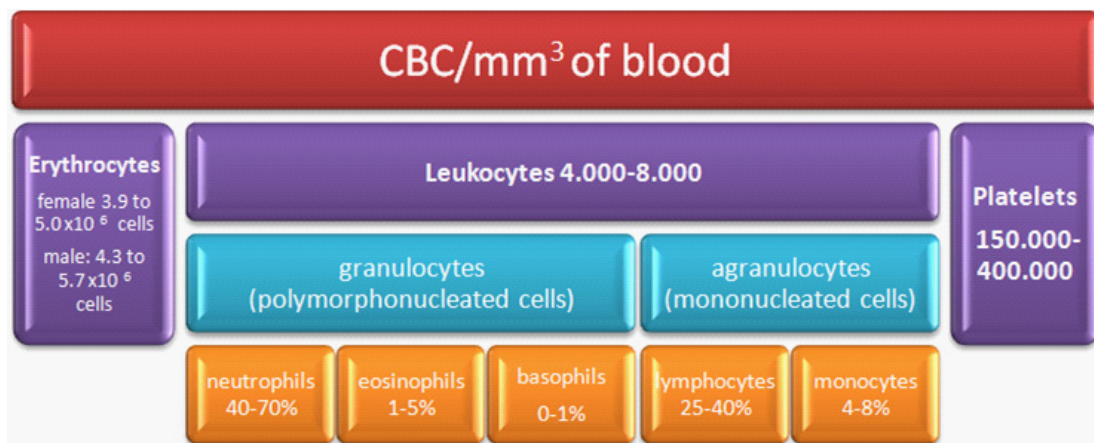
Basophilic granulocytes have a segmented nucleus, but it is usually masked by the specific, darkly staining granules. They have an acidophilic cytoplasm, with secondary granules of different sizes, stained dark blue/black. Being in low number in the blood, they are rarely encountered on the blood smear.

Lymphocytes have a diameter that may vary between 6 to 8 μm for small lymphocytes up to 18 μm for large lymphocytes. The nucleus is large, round, heterochromatic and the cytoplasm is reduced to a basophilic ring on the periphery of the cell. They contain only primary, non-specific granules.

Monocytes are the largest cells on the blood smear, with a big kidney-like /"horseshoe" nucleus, a weak basophilic cytoplasm with small primary, non-specific granules.

Platelets are the smallest elements on the blood smear (2-4 μm), intensely basophilic. They have no nucleus, as they are not true cells, but fragments of a large cell named megakaryocyte, found only in the red bone marrow.

The average number/mm³ of blood of each type of peripheral formed element falls within specific limits, making the complete blood count (CBC), a widely used test in clinical practice.



MUSCLE TISSUE

Muscle tissue is composed of aggregates of elongated muscle cells, also called muscle fibers and an underrepresented extracellular compartment, made up of varieties of connective tissue (loose, dense irregular) which hold together the muscle fibers. Vascularization is ensured by the supporting connective tissue. Muscles have both sensory and motor innervation. The former is achieved by encapsulated receptors (muscle spindles and Golgi tendon organs) and the latter by motor neurons from the spinal cord and brainstem, which form neuromuscular junctions named motor end plates.

The plasma membrane of muscle cells is called sarcolemma (Gr. sarkos - flesh), the cytoplasm is named sarcoplasm, and the smooth endoplasmic reticulum, involved in muscle contraction, is called sarcoplasmic reticulum. Muscle cells contain in their sarcoplasm specific organelles named myofibrils, organized by myofilaments (thick and thin, invisible by light microscopy), Thin myofilaments are composed of actin and actin associated proteins and thick myofilaments are composed of myosin. Based on the organization of the myofilaments, muscle tissue is classified in:

- striated muscle - skeletal and cardiac
- smooth muscle

Skeletal Muscle

Skeletal muscle fibers are cylindrical, multinucleated cells, parallel to each other. They can reach up to tens of cm in length and their diameter varies between 10 and 100 μm . Their nuclei are elongated, heterochromatic and located peripherally, under the sarcolemma. The multinucleation results from the fusion of precursor cells called myoblasts, during muscle development. In cross section, the cells are polygonal or round and 1 to 3 nuclei are visible/cell. In H&E stained sections, the cytoplasm is intensely acidophilic. In longitudinal section, it presents cross striations (due to cross-striations of the myofibrils) and in cross section, it has a dotted appearance due to the myofibrils (Conheim fields).

Myofibrils present a repetitive patterns of dark (anisotropic or A bands) and light (isotropic or I bands) bands, responsible for the striated appearance. This repetitive pattern is detailed in electron microscopy, which revealed that the smallest repetitive subunit of the contractile apparatus is the sarcomere, the part of the myofibril between two adjacent Z lines, on which actin filaments attach. Each sarcomere is composed of one central A band, and one half of an I band on each side. During contraction, actin and myosin fibers slide through each other, pulling the Z-lines closer and shortening the sarcomere.

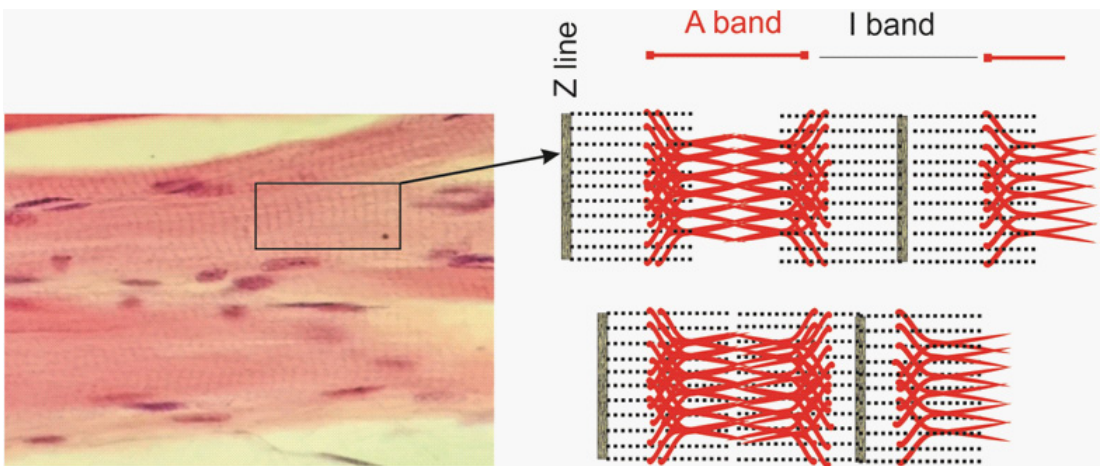


Figure 13: Pattern of organization of striated muscle. Left: striated skeletal muscle, H&E, 40x, right up: sarcomere, relaxed, down: sarcomere in contraction.

Skeletal muscle fibers are surrounded by connective tissue sheaths that provide support and nutrition. The endomysium is a thin layer of loose connective tissue that wraps around each muscle fiber. Several muscle cells form a bundle, surrounded by a thicker loose connective tissue layer, called the perimysium. Several bundles of muscle fibers form a muscle, which is covered by

a layer of dense connective tissue, the epimysium. All these connective tissue sheaths have a rich supply of blood vessels and nerves.

Tendons attach muscles to bone. At the site of attachment (tendon-muscle junction), muscle fibers show finger-like extensions; the collagen fibers from the tendon attach to the external lamina of muscle cells.

Skeletal muscle fibers can be classified into red (I), intermediate (IIa) and white fibers (IIb), based on their myoglobin and mitochondria content, as well as the type of metabolism used during contraction:

- red fibers have a high content of myoglobin, numerous mitochondria, and an aerobic (oxidative) metabolism; their contraction is slow, resistant to fatigue

- intermediate fibers have a relatively high concentration of myoglobin and mitochondria, but they also possess considerable amounts of glycogen; their metabolism is oxidative-glycolytic, their contractions are rapid, but resistant to fatigue

- white fibers have a low content of myoglobin, few mitochondria and an anaerobic (glycolytic) metabolism; they are adapted for rapid contractions, but are prone to fatigue.

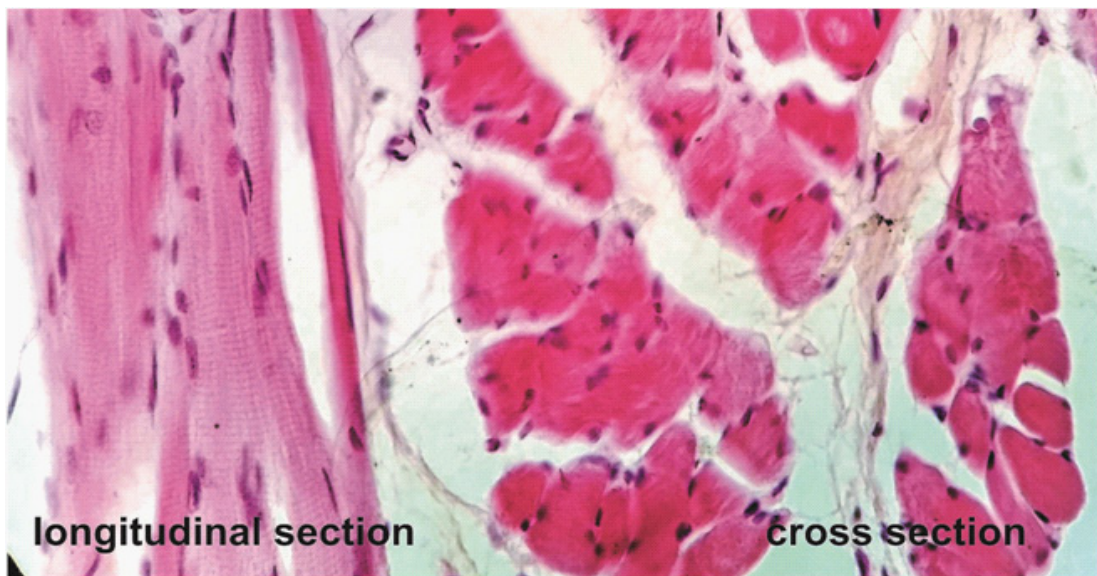


Figure 14: Skeletal striated muscle. H&E, 40x.

Cardiac Muscle

Examined in an H&E section, cardiac muscle cells have an elongated, branched appearance, with cross striations in the cytoplasm, absent in the perinuclear region. They exhibit dense staining cross bands, which are specialized junctions. Cardiac muscle fibers can reach up to 85-100 μm in length and 15 μm in diameter, they have one euchromatic nucleus (rarely two), located centrally, with an obvious nucleolus. Inclusions of lipofuscin are visible in the cytoplasm.

In cross section, the cells appear polygonal and the nucleus isn't always visible, depending on the area in which the section was made.

Sarcomeres from cardiac muscle fibers have a similar structure to those from skeletal muscle fibers, but are not as regularly aligned.

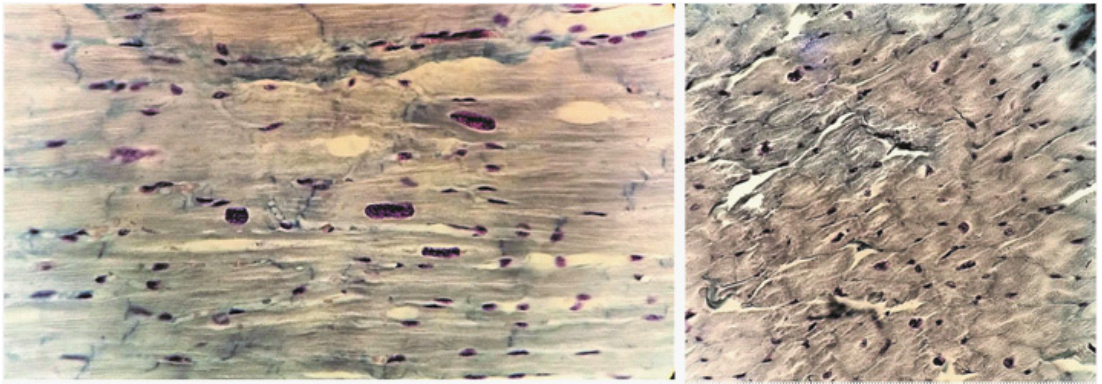


Figure 15: Cardiac muscle in longitudinal section (left) and cross section (right) in H&E methylene blue stain.

Smooth Muscle

Smooth muscle cells are spindle-shaped, with tapered ends. The cytoplasm is acidophilic, but lacks cross striations, the nucleus is elliptic, located in the center of the cell and during contraction it has a corkscrew appearance. Smooth muscle fiber length can vary from 20 to 500 μm and their diameter ranges from 4 to 10 μm . In cross section, the cells are polygonal or round and the nucleus may not be visible, depending on the area in which the section was made.

There are no sarcomeres in smooth muscle fibers and myofilaments are scattered in the cytoplasm. The contractile apparatus contains thin (actin, tropomyosin) and thick filaments (myosin II), as well as intermediate filaments of desmin or vimentin. Dense bodies are attachment sites for thin and intermediate filaments throughout the cytoplasm; they are the equivalent of Z lines.

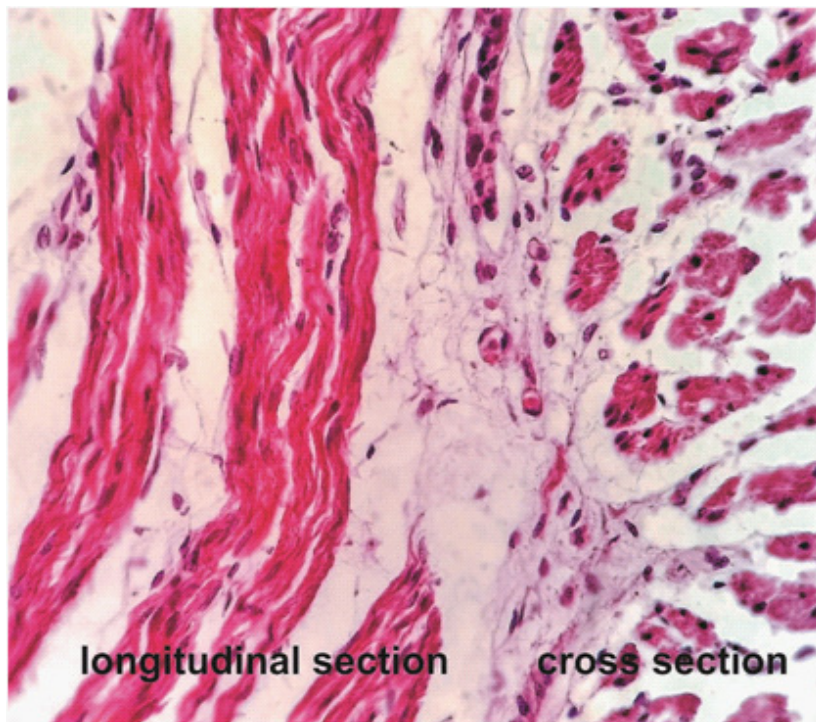


Figure 16: Smooth muscle H&E, 40x.

NERVOUS TISSUE AND NERVOUS SYSTEM

Nervous tissue is a type of tissue enriched in cells. The cellular compartment is formed by two distinct types of cells: neurons and supporting cells, also known as glial cells. The latter comprise several subtypes which are different in central nervous system (CNS: brain, spinal cord) and peripheral nervous system (PNS: peripheral nerves, nerve ganglia).

Extracellular matrix is composed of glycosaminoglycans and proteoglycans, with roles in support and attachment of cells. No fibrils (e.g. collagen) are visible in the extracellular matrix in H&E sections, but it has a rather homogenous aspect.

Neurons are large cells, comprised of a body and two type of processes: dendrites (usually several) and one axon.

The neuronal body (also called a soma or perykarion - "around the core"), contains a large nucleus, centrally located, euchromatic, with visible nucleolus. The aspect is quite peculiar, typical for neurons, and classically described as "owl eye". Within the cytoplasm, typically for neurons, basophilic granules, or Nissl bodies, can be found. They are formed of rough endoplasmic reticulum with attached ribosomes and can be highlighted using the Nissl stain. Nissl bodies are located only in the perykarion and at the base of dendrites. The cell body is traversed in all directions

by cytoskeleton elements (neurofilaments and microtubuli) visible only in silver staining. These extend also in the neuron processes and are involved in vesicular and organelle transport, as well as in cell stiffness. Also, quite typical for long-lived cells, granules of lipofuscin (a brown pigment, also called ageing pigment) can be found scattered in the cytoplasm of neurons, harvested from aged specimens.

Neuronal processes are permanent cell extensions, either short and thin (dendrites), or long and thick (axon).

Dendrites are several to many, taper towards the end and present dendritic spines - small buttons with an actin core, involved in synapse formation.

The axon emerges from the soma at the axon hillock, it has a longer or shorter trajectory, depending on the type of neuron, and ends in a terminal arborization with synaptic boutons. Axons are described as myelinated or non-myelinated, depending on the presence or absence of a myelin sheath. The myelin sheath is a lipoproteic structure formed by a membrane cell extension of glial cells (oligodendrocyte in the CNS and Schwann cell in the PNS) that wraps itself several times around the axon. Being mostly lipidic, it is not obvious in H&E staining. If fixed with osmium tetroxide, it will appear black.

Nervous tissue can be studied in H&E stain, as any other type of tissue, but also using special stains: silver stain, which highlights the neurofilaments that span throughout the cell body; toluidine blue, which highlights the Nissl bodies; osmium tetroxide, for the myelin sheath.

Neurons can be classified according to several criteria:

- Shape of cell body: pyramidal, star-shaped, granular, spherical, pyriform
- Size: large > 100µm, small 5µm
- Number of processes: unipolar, bipolar, pseudounipolar, multipolar
- Length of axon: Golgi type I (with a long axon) and Golgi type II (with a short axon)
- Function: motor, sensory and interneurons

Glial cells are supportive cells of nervous tissue that nourish and protect neurons. There are several different types of glial cells: oligodendrocytes, astrocytes, microglia, Schwann cells, satellite cells, each with their own characteristics and functions. On histological sections of nervous tissue, stained H&E or Nissl, different types of glial cells cannot be easily differentiated from each other, as only the nucleus is usually visible.

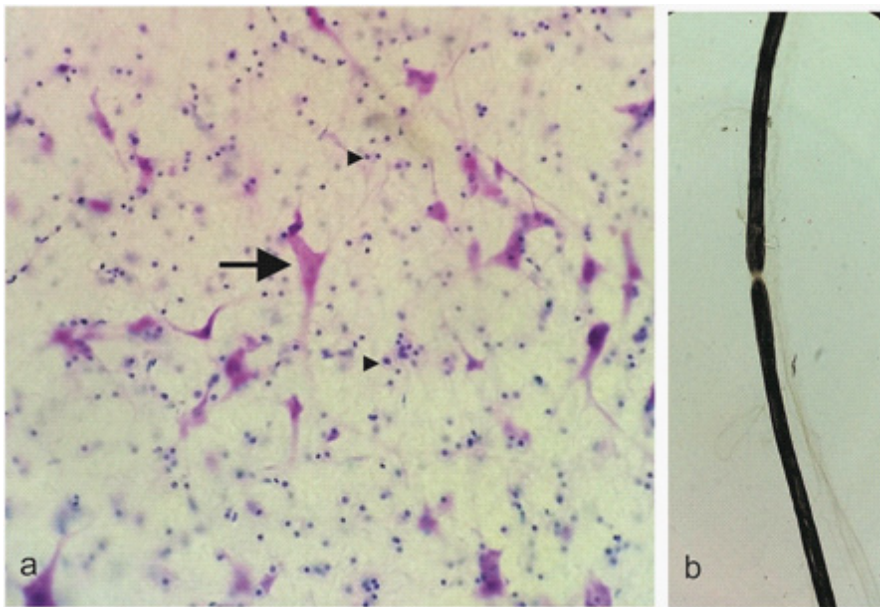


Figure 17: (a) Nervous tissue section: arrow – neuron, arrowhead – glial cell nuclei; cresyl violet, 40x (b) isolated myelinated nerve fiber, osmium tetroxide, 40x.

Oligodendrocytes are found in both white and gray matter of CNS, forming in white matter the myelin sheath of axons. They can be identified in usual stains by their small, round nuclei, and cell bodies with processes surrounding several axons.

Astrocytes are star-shaped cells with many processes that link neurons to capillaries, but also participate in separation of brain from the circulatory system. They extend end-feet that cover capillaries, participating in traffic of substances from the blood toward the brain (such as glucose), but they are also considered as a part of the blood-brain barrier, along with the endothelium and pericytes of brain capillaries. There are two histologic types described: protoplasmic, found mostly in gray matter, with numerous short and ramified processes, and fibrous, located mostly in white matter, with fewer and longer processes. Astrocytes form a network connected by gap junctions, which can send calcium-dependant signals over long distances.

Microglia, also known as the macrophages of the brain, are found in both white and gray matter. In an inactive state, they are small, elongated cells, with short, irregular processes, but they grow in size and assume a round shape with fewer/ no processes upon activation.

Schwann cells are peripheral glial cells, which form the myelin sheath in PNS, several Schwann cells surrounding different segments of the same axon. The cell body is rather small, with an elongated nucleus, similar in appearance in usual stains with fibrocytes.

Satellite cells are glial cells found in PNS. They are small cells surrounding neuronal bodies in nervous ganglia.

Nervous tissue forms the Nervous System, composed of **CENTRAL NERVOUS SYSTEM** (Spinal cord and Brain) and **PERIPHERAL NERVOUS SYSTEM** (Nerves and nervous ganglia).

In the central nervous system, the bodies of the neurons are grouped to form layers (named cortex, such as cerebral or cerebellum cortex) or nuclei (groups of neurons surrounded by fibers). Myelinated processes form the white matter.

The spinal cord is a cylindrical organ, sheltered in the spinal canal. In cross section, it exhibits a typical butterfly-shaped structure, with the white matter on the outside and the H-shaped gray matter on the inside. The gray matter is divided into large anterior and smaller posterior horns. The positive diagnostic element is the presence of star-shaped, multipolar motor neurons in the anterior horns.

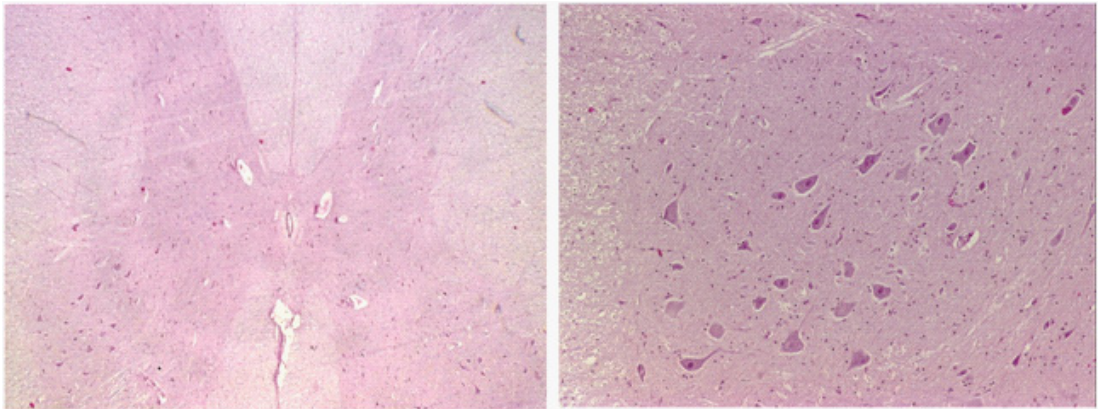


Figure 18: Spinal cord (a) - general aspect; (b) mononeuron from the anterior horn, H&E, 40x.

The cerebellum is characterized by a macroscopic foliated appearance. It has a cortex (grey matter) on the outside and the white matter on the inside. Within the white matter are located deep cerebellar nuclei that receive inputs from both cerebellum cortex and outside sources. Cerebellum cortex presents 3 layers:

1. *Molecular layer*- formed mostly by stellate and basket cells, small interneurons
2. *Purkinje cells layer* -formed of Purkinje neurons: large cells, with dendritic arborizations in the molecular layer. Their axon is the main output of cerebellum
3. *Granular layer*- small neurons (5 μm) that extend their axons toward the molecular layer, where it splits in two horizontal arms, forming a T-shaped structure. The horizontal arms run parallel to other similarly split axons to form the parallel fibers. The parallel fibers establish many excitatory synapses with Purkinje cell dendrites.

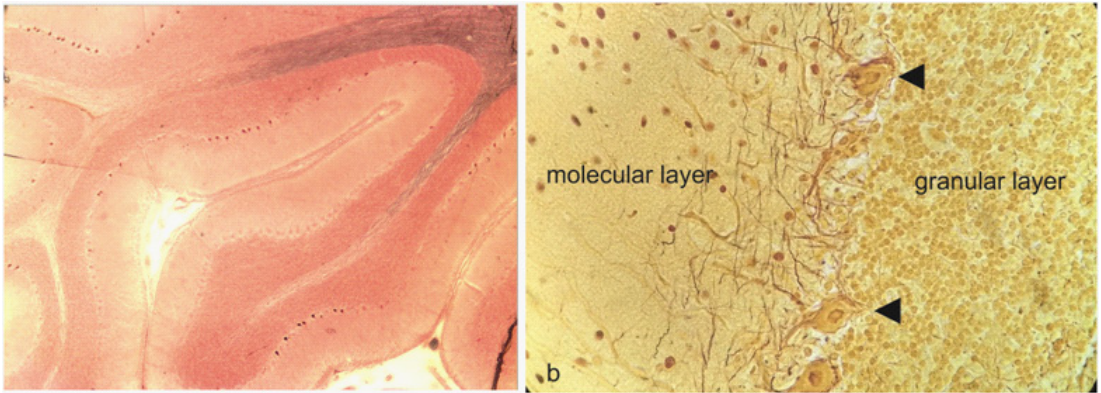


Figure 19: Cerebellum (a) general aspect; (b) layers of cerebellum in silver stain; arrowheads: Purkinje neuron cell bodies.

The *cerebrum* is organized, same as cerebellum, of grey matter (located on the outside as cerebrum cortex or forming deep nuclei) and white matter on the inside. Grey matter, is composed of neurons, with their cell processes and central nervous system glia. However, unlike cerebellum, the variety of neurons in the cerebrum cortex is far greater, both terms of morphology as well as neurotransmitter type and function. Within the regions of the cortex, complexity and number of layers increases from the allocortex (e.g. entorhinal cortex, hippocampus) to neocortex (e.g. frontal motor cortex). The latter is best described and illustrated in histological studies, composed of 6 layers, poorly defined and named after the shape of their neurons as: 1. Outer molecular layer 2. External granular layer 3. External pyramidal layer 4. Internal granular layer 5. Internal pyramidal layer - formed of large motor neurons, also named Betz neurons, the upper motor neurons of the primary motor cortex. 6. Polymorphic layer.

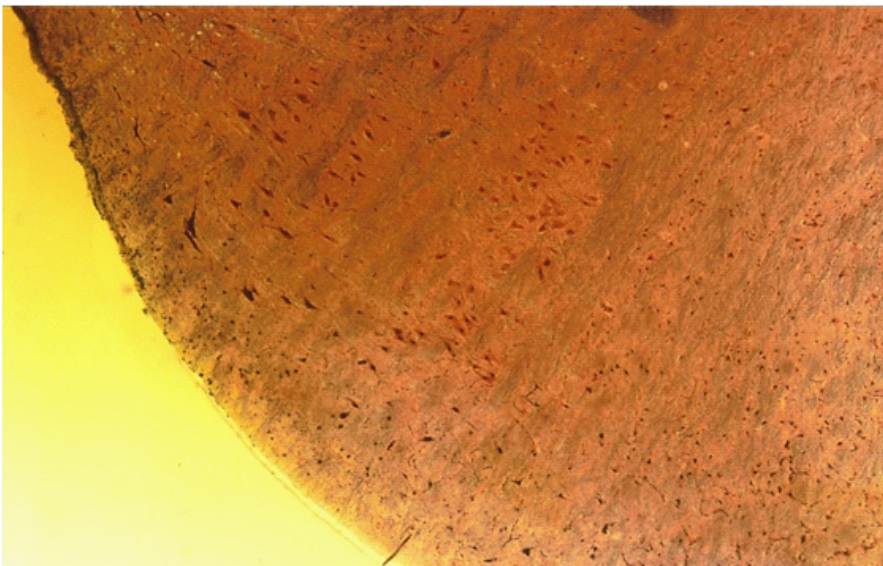


Figure 20: Cerebral cortex, silver stain 20x.

The central nervous system organs are protected by *meninges*, formed of three layers:

- Dura mater, formed of dense connective tissue attached firmly to the periosteum of the inner surface of the skull, but not to the vertebral canal
- Arachnoid - forms of an outer layer of simple squamous epithelium connected to dura mater and an inner layer of fibroblasts with processes(trabeculae) that come in contact with the inner most layer of the meninges, the pia mater. The space between the trabeculae of arachnoid is filled with cerebrospinal fluid of the subarachnoidial space.
- Pia mater - loose connective tissue that attaches intimately to the surface of the brain and spinal cord and lines the vessels that penetrate the fissures.

PERIPHERAL NERVOUS SYSTEM consists of nerves and nerve ganglia.

Nerves are bundles of nervous fibers and associated connective tissue. In the peripheral nervous system, a nerve fiber is formed by the axon and the Schwann sheath, formed by cell bodies of Schwann cells

Peripheral nerve- associated connective tissue is organized in three types of protective layers: endoneurium, perineurium and epineurium. Endoneurium is loose connective tissue surrounding each nerve fiber. Nerve fibers group in fascicles surrounded by perineurium, a special type of connective tissue. Epineurium is dense irregular connective tissue that enwraps and isolates the entire nerve from the outer environment.

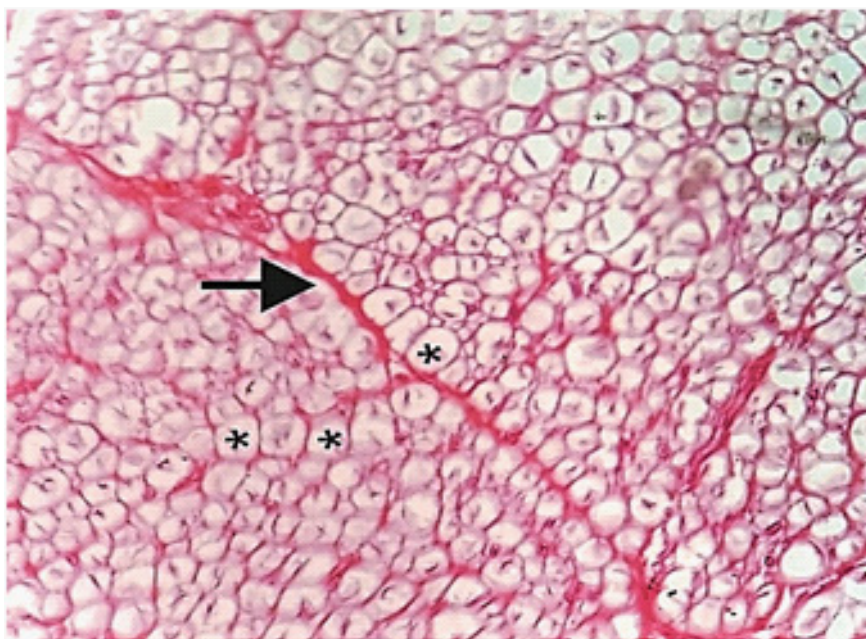


Figure 21: Cross section through a peripheral nerve with myelinated fibers. * - myelinated fiber, centered by the axon; arrow - perineurium. H&E, 40x.

Nerve ganglia are small, ovoid structures, located on the trajectories of nerves, formed of nerve cells and fibers. Depending on their locations, two types of ganglia, with different histological organization, were described: somatic ganglia like spinal ganglia, located on the sensory root of spinal nerves and autonomic ganglia, sympathetic or parasympathetic.

Spinal ganglia are structured into a cortex and a medulla. The cortex contains the cell body of pseudounipolar neurons and satellite cells that surround every perykarion. The medulla is formed by nerve fibers.

Autonomic ganglia show no distinct areas. They contain uni- and binucleate multipolar neurons incompletely surrounded by satellite cells as well as bundles of nerve fibers.

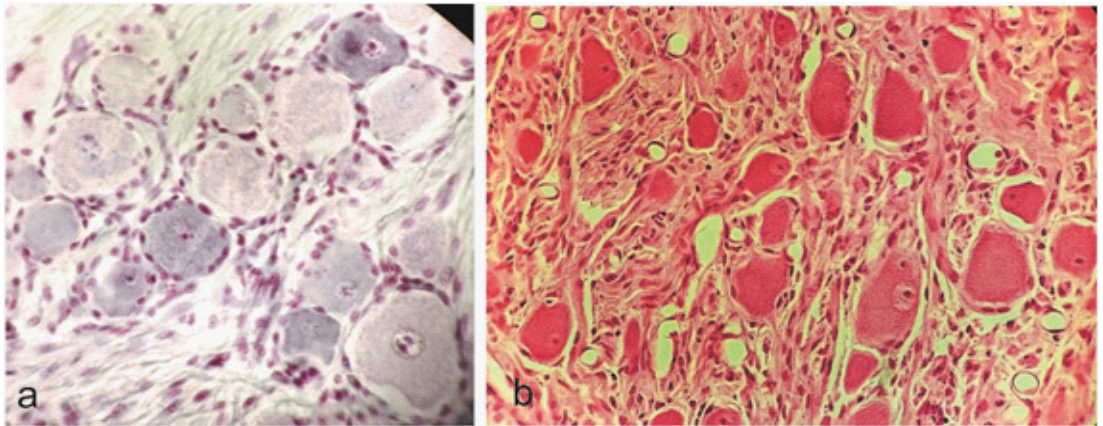


Figure 22: Details of nerve ganglia. (a) pseudounipolar neurons surrounded by satellite cells (H&E with methylene blue, 40x); (b) multipolar neurons in autonomous ganglia, separated by nerve fibers H&E 40x.

CONCLUSION

Tissues are an association of cells and extracellular matrix. Depending on the characteristics of cells, four fundamental types of tissues are described: epithelial, connective, muscle and nervous. Each of these types are further classified into different subtypes, according to morphological and functional features of their cells, increasing the diversity of histological structures visible in the normal, healthy, human organism. A good knowledge of normal tissues morphological features represents the grounds for correct pathological diagnosis.

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