GENERAL
HISTOLOGY


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Donetsk
2011
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The textbook is designed for the students' self-learning within the module educational system and assembled according to the typical curriculum for the discipline "Histology, cytology and embryology" (Kiev, 2005.) for students of the highest school in medicine. It is based on the experience of conducting practical training by lecturers of the department of Histology, cytology and embryology of M. Gorky Donetsk national medical university. Advantage of this textbook is organisation of multilevel system of learning, use of visual methods, information about new achievements in medicine and application-specific data, development of objective assessment of students' work.

Approved and recommended by Academic Council of M.Gorky Donetsk national medical university
(document No 4; 20/05/2011)
LESSON 1

THEME: TISSUES. EPITHELIA

BACKGROUND. This lesson begins the new level of microscopic structure of the human body—tissue. There are 4 types of tissues in the human body: epithelial, tissues of internal environment, muscular and nervous. Organs are built from different tissues, which is why learning their histophysiology provides understanding of their structure, functions, ability to reactive changes and regeneration of organs. The most widespread tissues within the organism are epithelial tissues. They are seen in the skin, digestive, respiratory, urinary and reproductive organs, serosa, and glands. Knowing morphological and functional characteristic of epithelia, general features of their differentiation and regeneration depending on the different organization of the cells, helps to understand the essence of such pathological conditions as inflammation, malignancy etc, make prognosis for the disease development.

AIM OF STUDY: to be able to differentiate the basic principles of phylogenetic and functional classification of the tissues, different organization, main features; distinguish covering and glandular epithelium, main features of this tissue, which is necessary for the diagnostics of the pathological changes during clinical work.

THEORETICAL QUESTIONS
1. The term “tissue” as one of the levels of life organization. Morphological and histogenetic classification of tissues.
3. Features of tissues: determination, differentiation, changeability and regeneration.
4. Ability for reparation: physiological and reparational regeneration of the renewing, growing and stable tissues.
5. General functional and histogenetical characteristic of the epithelial tissues. Classification. Location.
7. Systemic qualities of epithelial tissues.
11. Characteristic of secretory portions and ducts of the glands.

Tissue is a complex of different histological elements (cells, symplast, postcellular structures, syncytium, or extracellular matrix), which are joined embryologically, structurally and functionally.

Despite its complexity, the human body is composed of only four basic types of tissue:
1. Epithelial
2. Tissues of internal environment
3. Muscular

This classification is based on three main principles:
- embryonic development,
- structure,
- functions.

Tissues exist not as isolated units but rather in association with one another and in variable proportions, forming different organs and systems of the body.
Each tissue consists of one or several differons. Differon is a complex of histogenetic sequence of cells with increased degree of differentiation and includes 3 components:

1. stem cell.
2. cells which undergo differentiation (progenitors and precursors).
3. mature cells.

Complete or incomplete structure of differon determines one of the main properties of tissues – regeneration (physiological and reparative). Ways of regeneration
- cellular (by mitosis)
- intracellular (by synthesis and organells renewing)

According to Leblon's classification in human organism there are 3 types of tissues:

1. Renewing tissues. Include full differon, have high ability for cellular regeneration, short lifespan of mature cells. Examples: covering epithelia, blood, connective tissues.
2. Growing tissues. Include precursors and mature cells. Because of absence of stem cells tissues have limited ability for cellular regeneration, but characterized by long lifespan and high intensity of intracellular regeneration. Examples: glands, speciliased epithelia, skeletal muscles.
3. Stable tissues. Include only mature highly specialized cells without ability to division. Cells have the longest lifespan and high intracellular regeneration. Examples: nerve cells, ardiomyocytes.

Epithelial tissues.

In the early development of the embryo the rapidly multiplying cells lie in layers. From each of these three germ layers a class of tissue develops that persists to maturity as a tightly packed layer of cells, contrasting with the connective tissues where the cells are spaced out in an extensive extracellular matrix.

Functional classification of epithelial tissues
1. covering - cells form cellular sheets that cover the surface of the body and line its cavities
2. glandular – cells are arranged as three-dimensional secretory units or other secretory active structures (follicles, islets, cords)
3. sensory epithelium – in special senses – inner ear and taste buds.

The principal functions of epithelial tissues are:
- barrier because of lining of surfaces (eg, skin, intestines),
- absorption (eg, intestines),
- secretion (eg, glands),
- sensation (eg, gustative and olfactory neuroepithelium), and
- contractility (eg, myoepithelial cells).

Structural characteristics:
1. Epithelial tissues are composed only of cells
2. No extracellular matrix.
3. Cells are connected to each other by intercellular junctions and form layer.
4. Layer of epithelial cells lies on basal membrane.
5. Epithelia never include vessels
6. The nutrition of epithelial cells is by indirect exchange through the basal membrane (basal lamina) and matrix substances with blood in the capillaries of loose connective tissue.
7. Epithelium rich in nerve endings
8. Epithelia have high ability to regeneration because of stem cells.
9. The majority of epithelial cells contain cytokeratin intermediate filaments, and this can be used to recognise an epithelial phenotype using immunohistochemistry

The basement membrane (BM)

Most epithelial cells are separated from the connective tissue by a sheet of extracellular material called the basal lamina. This structure is visible only with the electron microscope, where it appears as a dense layer, 20–100 nm thick, consisting of a delicate network of very fine fibrils. The principal epithelial grip is by cell-membrane integrin to laminin.

Seen with TEM, the basal lamina is subdivided into two or three layers:
- a pale lamina lucida next to the epithelium,
- a lamina densa, then the deeper
- a lamina fibroreticularis (less consistently visible) (fig. 1).

The main components of basal laminae are type IV collagen, the glycoproteins laminin and entactin, and proteoglycans (e.g., the heparan sulfate proteoglycan called perlecan). The precise molecular composition of these components varies between and within tissues. Basal laminae are attached to the underlying connective tissues by anchoring fibrils formed by type VII collagen. BMs differ by location, and experience various pathological changes - thickening, breaks, duplication, autoimmune attack, etc.

Basal laminae have many functions. In addition to simple structural functions supporting the cells, they provide a barrier that limits or regulates the exchange of macromolecules between connective tissue and cells of other tissues. The basal lamina is also able to influence cell polarity, regulate cell proliferation and differentiation by binding with growth factors, influence cell metabolism, and serve as pathways for cell migration. The basal lamina seems to contain the information necessary for certain cell-to-cell interactions, such as the reinnervation of denervated muscle cells. The presence of the basal lamina around a muscle cell is necessary for the establishment of new neuromuscular junctions.

INTERCELLULAR JUNCTIONS IN EPITHELIA (fig. 2)

1. Junctional complex of: the girdle-like zonula occludens and zonula adhaerens/belt desmosome, below which is a ring of maculae adhaerentes/ spot desmosomes. Filaments of the terminal web in each cell’s apical cytoplasm fasten to the complex. Something of the complex was seen as the terminal bar of LM.

2. Desmosome (the macula/spot/punctate kind of adhaerens attachment): disc-like structures scattered on cell’s surface; each is formed by the membranes of two cells; cytoplasmic tonofilaments (keratin intermediate filaments) converge on and insert into dense subplasmalemmal plaques. There are distinct plaque and desmosomal membrane proteins.

3. Hemi-desmosome: for better adhesion of the basal cell membrane to the basal lamina; includes a plaque and tonofilaments.

4. Gap junction/nexus: where two cells’ membranes come closely together with only a 2 nm gap bridged by “connexons” allowing ions, nucleotides, and amino acids to pass from cell to cell for coupling and coordination of many cells’ activities.

5. Tight junction (resembles a zonula occludens but is not always belt-like): outer parts of two cells’ membranes are fused together thereby occluding the intercellular cleft.

6. Plication/folding and interdigitation of the adjoining cells’ folded membranes.

Fig. 1. Basement membrane: A – scheme of microscopic structure; B – electron microgram, mag. × 80000.

1 – basement membrane; 2 – lamina densa; 3 – laminin; 4 – lamina lucida; 5 – reticular fibrils; 6 – microfibrils; 7 – hemidesmosomes; 8 – epithelial cell.
Attachments provide for:

- mechanical strength;
- barriers to the indiscriminate passage of materials, and thus the possibility of selective transport;
- formation of intercellular compartments, e.g., bile canaliculus;
- communication for migration, differentiation, function and exfoliation.

But, for repair and normal cell replacement, cells move to one another and must break their attachments. Also, white blood cells can pass between them.

**MORPHOLOGICAL VARIETIES OF COVERING EPITHELIA**

**Simple and stratified epithelia**

1. The primary classification is based upon the layering: one cell thick is *simple*, two or more cell layers thick constitutes *stratified/compound*. Cell shapes give the secondary classes.

2. *Simple epithelia*, in general, are adapted to absorptive and secretory roles, while *stratified epithelia* protect against damaging mechanical and chemical actions.

3. Stratified epithelia frequently, and simple sometimes, have several types of cell present. Cells lying basally on the BL are mitotically active and migrate upwards, differentiating to replace cells lost from the surface, or cells that have destroyed themselves by apoptosis.

4. Epithelia shed cells continually. Such cast-off or desquamated cells may be examined in smears of the appropriate fluid - sputum, gastric, uterine cervical - for signs of malignant change and/or chromosomal abnormality in their epithelium of origin: the technique of *exfoliative cytology*.

**Simple epithelia classification** (fig. 3)

1. *Squamous*
2. *Cuboidal* and
3. *Columnar*

- (a) Cell *shape* is indicated approximately by the name; most epithelial cells are really polyhedral with many sides or faces.

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Fig. 2. Scheme of intercellular junctions.

1 – zonula occludens (tight junctions);
2 – adhesive belt (zonula adherens);
3 – desmosome; 4 – infoldings of membrane;
5 – gap junction.
Fig. 3. Different types of simple epithelium. **Simple squamous** (mesothelium): A – scheme, B – histological specimen, mag. × 600; **simple cuboidal**: C – scheme, D – histological specimen, mag. × 600; **simple columnar**: E – scheme, F – histological specimen, mag. × 600; **simple columnar with brush border**: G – scheme, H – histological specimen, mag. × 600; **simple pseudostratified**: I – scheme, J – histological specimen, mag. × 600.

1 – basement membrane; 2 – nucleus; 3 – columnar ciliated cells; 4 – microvilli; 5 – goblet cell (single-cell endoepithelial gland); 6 – ciliated cell; 7 – cilia; 8 – basal cells; 9 – basal pole; 10 – apical pole; 11 – loose connective tissue; 12 – blood vessel; 13 – squamous cells; 14 – border between cells; 15 – cuboidal cells.
o (b) Cells stand one cell high, although their nuclei may lie at slightly different levels.

o (c) Cells are fastened and sealed at the top of their sides by encircling junctional complexes.

o (d) Cells have three surfaces: free/luminal, lateral and basal; each may have membrane specializations, e.g., cilia at the free, occluding junctions and desmosomes at the lateral, and infoldings at the basal surfaces.

o (e) Note the position and shape of the nucleus, and special locations of organelles and inclusions that also indicate the cell’s polarization.

3. Squamous/pavement

· (a) Very flattened cells presenting a minimal barrier to the passage of materials, e.g., oxygen, through them.

· (b) Cytoplasm is very hard to see with LM.

· (c) The very similar endothelium and mesothelium line blood and lymph vessels, and serous cavities, respectively.

4. Pseudostratified columnar

· (a) Nuclei lie at different levels suggesting stratification, but all cells are in contact with the BL.

· (b) Two or more cell types are present: short basal, tall columnar.

· (c) Lines respiratotry tree, clean air.

Key feature of all simple epithelia is POLARITY

In many types of epithelial cells the distribution of organelles and membrane proteins is different when comparing the basal and apical poles of the cell. This differential and stable organization of cell components is called polarity. This means that different parts of the cell may have different functions.

Because blood vessels do not normally penetrate an epithelium, all nutrients must pass out of the capillaries in the underlying lamina propria. These nutrients and precursors of products of the epithelial cells then diffuse across the basal lamina and are taken up through the BL (basal/luminal surface) and lateral surfaces (basolateral surface) of the cell, usually by an energy-dependent process. Receptors for chemical messengers (e.g., hormones, neurotransmitters) that influence the activity of epithelial cells are localized in the basolateral membranes. In absorptive epithelial cells, the apical cell membrane may contain, as integral membrane proteins, enzymes such as disaccharidases and peptidases, which complete the digestion of molecules to be absorbed.

The portion of the epithelial cells that faces the connective tissue is called the basal pole, whereas the opposite side, usually facing a space, is called the apical pole. The surface of the apical pole is also called the free surface, whereas the surfaces that are apposed to neighbor cells are called lateral surfaces.

The lateral membranes of many epithelial cells often exhibit several types of membrane modifications, the intercellular junctions. One type of junction provides a mechanism for communication between adjacent cells. Other junctions serve as sites of adhesion and as seals to prevent the flow of material through the space between epithelial cells. In several epithelia the various junctions are present in a definite order from the apex toward the base of the cell.

Tight junctions, or zonulae occludens (singular, zonula occludens), are the most apical of the junctions. In properly stained thin sections viewed in the transmission electron microscope, the outer leaflets of membranes of neighbor cells are seen to fuse, giving rise to a pentalaminar sheet. The principal function of the tight junction is to form a seal that prevents the flow of material between epithelial cells (called the paracellular pathway) in either direction (from apex to base or from base to apex).

The final type of junction is the desmosome (Gr. desmos, band, + soma, body), or macula adherens. The desmosome is a complex disk-shaped structure at the surface of one cell that is matched with an identical structure at the surface of the adjacent cell. The cell membranes in this region are very straight and are frequently somewhat farther apart (>30 nm) than the usual 20 nm. On the cytosolic side of the membrane of each cell and separated from it by a short distance is a circular plaque of material called an attachment plaque, made up of at least 12 different proteins. In epithelial cells, groups of intermediate cytokeratin filaments are inserted into the attachment plaque or make hairpin turns and return to the cytoplasm. Because intermediate filaments of the cytoskeleton are very strong, desmosomes provide a firm adhesion among the cells. In nonepithelial cells, the intermediate filaments attached to desmosomes are made not of cytokeratin but of other proteins, such as desmin or vimentin. Proteins of the cadherin family participate in the adhesion provided by desmosomes. In vitro this adhesiveness is abolished by the removal of Ca^{2+}. 
In the contact zone between certain epithelial cells and the basal lamina, hemidesmosomes (Gr. hemi, half, + desmos + soma) can often be observed. These structures take the form of half a desmosome and bind the epithelial cell to the subjacent basal lamina. However, in desmosomes the attachment plaques contain mainly cadherins, whereas in hemidesmosomes the plaques are made of integrins, a family of transmembrane proteins that is a receptor site for the extracellular macromolecules laminin and type IV collagen.

Apical zone of epithelial cells oftenly is specialized and has:
- microvilli
- ciliae.

STRATIFIED EPITHELIA CLASSIFICATION (fig. 4)

1. Stratified squamous non-keratinized
   · (a) Many cells thick.
   · (b) Surface cells are flat plates and flake off as squames.
   · (c) Basal-most cells are cuboidal or columnar and divide.
   · (d) Cells above the base become polyhedral and are held together by many desmosomes to resist the abrasive forces on this protective epithelium.
   · (e) Underside of the epithelium is indented by vascular papillae of connective tissue, except in the cornea.

Morphologically three distinct layers with special characteristics are seen:
1. stratum basale – with columnar cells which oval nuclei are dark and often in preparation for mitosis.
2. stratum spinosum includes polyhedral cells with round light nucleus and cytoplasm rich in organelles
3. stratum squamosum – with flattened cells

LOCATION - oral cavity, esophagus, vagina.

2. Stratified squamous keratinized epithelium
   · (a) Similar in its basal and middle layers to non-keratinized epithelium, but the uppermost epithelium has granular cells that contain keratin granules,
   · Ater the terminal differentiation these cells lost nuclei and become postcellular structures which form stratum lucidum and stratum corneum. These structures solidly packed together as a surface keratin layer for greater protection.

LOCATION – skin epidermis

9 Transitional epithelium
   · (a) Several cells thick, but the surface cells are large, rounded and sometimes binucleate, with spare cell membrane in vesicles.
   · (b) No connective tissue papillae indent the epithelium.
   · (c) basal layer also includes ctem cells for regeneration.

METAPLASIA

Metaplasia in any tissue is a change (usually abnormal) from one distinctive tissue to another, at a definite site after development is over. It implies a change in cell type - a transdifferentiation. Metaplasia is noted in epitelia, for example:
   · (a) a change from pseudostratified columnar to stratified squamous after repeated chemical or thermal insult, say, smoke in the larynx and trachea;
   · (b) from oesophageal stratified squamous to gastric or intestinal simple columnar (Barrett’s oesophagus), after repeated acid reflux;
   · (c) from columnar, cuboidal or transitional to cornified stratified squamous in severe vitamin A deficiency.
   · (d) from transitional to stratified squamous in bladders with a stone, or infected with worms.
Fig. 4. Structure of different types of stratified epithelia. **Stratified squamous nonkeratinised**
A – scheme, B – histological specimen, mag. × 80. **Stratified squamous keratinised**: C – scheme, mag. × 80,

1 – basement membrane; 2 – stratum basale; 3 – stratum spinosum; 4 – stratum squamosum;
5 – stratum granulosum; 6 – stratum lucidum; 7 – stratum corneum; 8 – stratum intermedium;
9 – surface layer; 10 – loose connective tissue; 11 – blood vessel.
SPECIAL ELEMENTS IN EPITHELIA

Non-epithelial structures sometimes occur within an epithelium:
1. *Capillaries* - very rarely; only in cochlear stria vascularis.
2. *Nerve axons* - common in skin, oral mucosa; less common elsewhere.
3. *Neural crest derivatives* - as melanocytes, and accessory glial-type cells associated with receptors.
4. *Lymphocytes* - common in gut and airway; less common elsewhere.
5. *Langerhans cells* - contributors to immune defence in stratified squamous epithelia.
6. *Globular leukocytes* - a special granular leukocyte of some epithelia

Innervation

Most epithelial tissues receive rich supply of sensory nerve endings from nerve plexuses in the lamina propria. The exquisite sensitivity of the cornea, the epithelium covering the anterior surface of the eye, is due to the great number of sensory nerve fibers that ramify between corneal epithelial cells.

Renewal of Epithelial Cells

In stratified and pseudostratified epithelial tissues, mitosis takes place within the germinal layer, closest to the basal lamina, which contains the stem cells.

GLANDULAR EPITHELIA

Glandular epithelia are formed by cells specialized to produce secretion. The molecules to be secreted are generally stored in the cells in small membrane-bound vesicles called **secretory granules**.

Glandular epithelial cells may synthesize, store, and secrete proteins (e.g., pancreas), lipids (e.g., adrenal, sebaceous glands), or complexes of carbohydrates and proteins (e.g., salivary glands). The mammary glands secrete all three substances. Less common are the cells of glands that have low synthesizing activity (e.g., sweat glands) and that secrete mostly substances transferred from the blood to the lumen of the gland.

Types of Glandular Epithelia

The epithelia that form the glands of the body can be classified according to various criteria. Unicellular glands consist of isolated glandular cells, and multicellular glands are composed of clusters of cells. An example of a unicellular gland is the **goblet cell** of the lining of the small intestine or of the respiratory tract (fig. 5). The term «gland,» however, is usually used to designate large, complex aggregates of glandular epithelial cells, such as in the salivary glands and the pancreas.

![Fig. 5. Goblet cell: A – electron micrograph, mag. × 7000; B – scheme of mucin secretion.](image)

1 – goblet cell; 2 – nucleus; 3 – granules of secret; 4 – striated columnar epithelial cell; 5 – blood vessel; 6 – basement membrane; 7 – ribosomes; 8 – endoplasmic reticulum; 9 – Golgi apparatus; 10 – exocytosis; 11 – aminoacids; 12 – monosaccharides; 13 – sulphates.
Glands arise during fetal life from covering epithelia by means of proliferation and invasion of the epithelial cells into the subjacent connective tissue, followed by further differentiation.

According to the mechanism of secretion, glands are subdivided into:

- **Exocrine** (Gr. exo, outside, + krinein, to separate) glands, which produce secret onto the surface of the epithelium from which they originated. This connection is transformed into tubular ducts lined with epithelial cells through which the glandular secretions pass to reach the surface.

- **Endocrine** (Gr. endon, within, + krinein) glands that lost their connection with the surface they originated from. These glands are therefore ductless, and their secretions are picked up and transported to their site of action by the bloodstream.

Exocrine glands have a **secretory portion**, which contains the cells responsible for the secretory process, and **ducts**, which transport the secretions (fig. 6).

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**Fig 6. Structure of exocrine glands:**

A – simple gland; B – compound gland.

1 – secretory portions: 1a – mucous; 1b – serose; 2 – secretory granules; 3 – ducts: 3a – intercalated duct; 3b – striated duct; 4 – blood vessel; 5 – connective tissue; 6 – basement membrane; 7 – covering epithelium; 8 – mucous cells; 9 – mixed secretory portions.

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**Simple glands** have only one unbranched duct, whereas **compound glands** have ducts that branch repeatedly.

The cellular organization of the secretory portion differentiates the glands further. **Simple glands** can have their secretory portion in the form of a **tubule**, a **coiled tubule**, a **branched tubule**, or an **acinus**, in which the cells organize as spherical or globular units. **Compound glands** can be **tubular**, **acinar**, or **tubuloacinar** (fig. 7).

According to chemistry of produced secret, secretory end-pieces of exocrine glands are subdivided into:

1. Serous – which produce proteins. They are basopholoc with round nuclei in centre of each secretory cell. Pancreas and parotid salivary glands are examples of serous cells. They are polyhedral or pyramidal, with central, rounded nuclei (fig. 8). Their polarity is well defined. In the basal region, serous cells exhibit an intense basophilia, which results from local accumulation of RNA present in polyribosomes apposed to abundant parallel arrays of cisternae of rough endoplasmic reticulum. Between the nucleus and the free surface lies a well-developed Golgi complex, several **immature secretory granules** derived from the Golgi complex, and **mature secretory granules** formed after water is removed from the immature granules. The mature secretory granules accumulate in the apical cytoplasm and can be seen as light-staining granules under the light microscope.

The granules stay in the apex until the cell is stimulated to secrete. The release of the secretory products happens by fusion of the membranes of secretory granules with the cell membrane, and the granule contents spill out of the cell, a process called **exocytosis**. The movement of secretory granules and of all other cytoplasmic...
structures is under the influence of cytoskeletal and motor proteins of the cytosol.

2. Mucous end-pieces – big light with flattened nuclei near basal membrane form small salivary glands and proper glands of esophagus (fig.9).

The cells of mucous end-piece have numerous large, lightly staining granules containing strongly hydrophilic glycoproteins called mucins. Secretory granules fill the extensive apical pole of the cell, and the nucleus is located in the cell base, which is rich in rough endoplasmic reticulum. The Golgi complex, located just above the nucleus, is exceptionally well developed, indicative of its important function in this cell. Data obtained by autoradiography suggest that proteins are synthesized in the cell base where most rough endoplasmic reticulum is located. Monosaccharides are added to the core protein by enzymes – glycosyltransferases—located in the endoplasmic reticulum and in the Golgi apparatus. When mucins are released from the cell, they become highly hydrated and form mucus, a viscous, elastic, protective lubricating gel.

3. Mixed end-pieces include 2 portins – the bigger mucous and serous demiluneum (fig.8).

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**Fig.7. Types of exocrine glands.**

1 – simple tubule with unbranched secretory portions; 2 – simple alveoli gland with unbranched secretory portion; 3 – simple tubule glands with branched secretory portion; 4 – simple alveoli-tubule glands with branched secretory portions; 5 – compound alveoli-tubule gland with branched secretory portion; 6 – compound alveoli gland with branched secretory portions.
Fig. 8. Submandibular salivatory gland. histological specimen, mag. × 600.
1 – mixed secretory portion; 2 – мукоциты; 3 – serose cells; 4 – serose secretory portion (end piece); 5 – myoepithelial cells; 6 – striated duct; 7 – intercalated duct; 8 – loose connective tissue; 9 – interlobular end piece.

Fig. 9. Sublingual salivatory gland. histological specimen, mag. × 600.
1 – loose connective tissue; 2 – serose end piece; 3 – duct; 4 – mixed end piece; 5 – serose demilune; 6 – mucous cells; 7 – interlobular duct.
LESSON 2

THEME: BLOOD. PLASMA. ERYTHROCYTES. PLATELETS

BACKGROUND. Tissues of internal environment form more than 50% of body weight, make the skeleton, dermis, participate in all organs and specialized tissues formation, provide the integrity of the body, its shape, trophics and protection. This type of tissue includes blood, lymph and connective tissues. Tissues of internal environment perform following functions: trophical, protectional, supporting and maintaining homeostasis. They also take part in pathological processes: inflammation, organisation, swelling, allergy etc.

AIM OF STUDY. To be able to distinguish general features of the tissues of internal environment, differentiate blood, interpret its functional state on basis of the state of its components, interpret blood changes under physiological and pathological conditions within different age groups.

YOU SHOULD BE ABLE TO:
1. Differentiate mesenchyme in the specimen.
2. Distinguish general features of the structure and importance of tissues of internal environment, principles of classification.
3. Study the blood components: interpret the chemical structure of plasma, functions of its main proteins.
4. Differentiate the elements of blood in blood specimens.
5. Learn the main features of microstructure of each element, understand the link between their quantity and functional state of the organism.

THEORETICAL QUESTIONS
1. Mesenchyme: embryonic development, structure, importance.
2. General characteristic of the tissues of internal environment. Classification.
3. Lymph components and its functions.
4. The components and qualities of blood.
5. Plasma, its chemical structure, role in homeostasis mainaining. Serum of the blood.
7. Erythrocyte: quantity, size, structure, functions, lifespan. Shape change in erythrocytes depending on hemoglobin and plasmalemme state.
8. Platelets: quantity, size, types, structure, functions, lifespan. Characteristic of platelets under different conditions of the organism.

Blood might be classified as a specialized tissue of internal environment because its cells are mesodermal (mesenchymal) in origin (fig. 1) and are separated by plasma.

Blood consists of formed elements and plasma (fig. 2).

Three are three types of formed elements of blood:
1. Erythrocytes (red blood cells) - about 5.2 million mm³ (man), 4-5 million (woman)- nucleusless element which main function is gases transport. Erythrocytes contain large amounts of oxygen-carrying haemoglobin, are primarily involved in the transport of oxygen and carbon dioxide, and function exclusively within the vascular system. The whole mass of red blood cells and their precursors in the bone marrow is called the erythron.
2. Platelets – fragments of red bone marrow cell – mecakaryocyte - 200000-400000 mm³. Platelets play a vital role in the control of bleeding (haemostasis) by plugging defects in blood vessel walls and contributing to the activation of the blood-clotting cascade.
3. Leukocytes (WBCs). The leucocytes constitute an important part of the defence and immune systems of the body and, as such, act mainly outside blood vessels in the tissues; thus the leucocytes found in circulating blood are merely in transit between their various sites of activity. 5000-9000 mm³ (healthy adult), which main function is defence.
Fig. 1. Mesenchyme of an embryo. Histological specimen, magn. × 1400.

1 - mesenchymal cells; 2 - syncytial junction; 3 - cytoplasmic processes; 4 - extracellular matrix; 5 - mesenchymal cells dividing by mitosis.

Fig. 2. Smear of human blood. Histological specimen, magn. × 900.

1 - erythrocyte; 2a - stab neutrophilic granulocyte; 2b - segmented neutrophilic granulocyte; 3 - eosinophilic granulocyte; 4 - basophilic granulocyte; 5 - small and large lymphocytes; 6 - monocyte; 7 - platelets.
Main function of blood is transport of gases, nutrients, metabolic waste products, cells and hormones throughout the body.

Because the blood is composed not only of cells and molecules involved in transport processes but also cells and molecules in the process of being transported, laboratory analysis of blood plays a large role in diagnosis of disease.

A typical sample of plasma is composed of 90% water, 8% protein, 1% inorganic salts, 0.5% lipids and 0.1% sugar, the rest being made of lesser components. Salts are constantly exchanged with the extracellular fluid of body tissues. The three main groups of proteins in plasma are the blood coagulation proteins, albumin, and the globulins. The globulins can be divided into alpha globulins (proteases, antiproteases and transport proteins) beta globulins (transferrin, other transport proteins) and gamma globulins (mainly immunoglobulins). The plasma proteins are nearly all derived from synthesis in the liver, with the exception of the immunoglobulins which are synthesised by plasma cells.

MICROSCOPIC TECHNIQUES FOR BLOOD
1. A blood drop is smeared across a slide and
2. stained with a Romanowsky-type combined stain - a neutral combination of acidic (eosin) and basic (azure) stains.
3. In the stained smear, a differential count by eye or automated counter gives the proportions of the different varieties of leukocyte.
4. Absolute counts of blood, diluted by a known amount, in a counting chamber give the numbers of the formed elements:
5. EM study of WBCs and platelets in the buffy coat after its centrifugal separation from the RBCs.
6. Light and EM examination of cells in the lumens of blood vessels in sections of imbedded tissues.
7. Phase-microscopy and videorecording of leukocytes alive in fresh blood on a warmed slide under a sealed coverslip.
8. Tagged monoclonal antibodies to recognise cell-surface glycoproteins characteristic of particular subtypes of blood cell. This approach allows a specific cell population to be sorted for culture and study using automated flow cytometry.

ERYTHROCYTES (fig. 3)
The erythrocyte is highly adapted for its principal function of oxygen and carbon dioxide transport. During differentiation in the bone marrow, large quantities of the iron-containing respiratory pigment haemoglobin are synthesised. Before release into the blood circulation, the erythrocyte nucleus is extruded and, by maturity, all cytoplasmic organelles degenerate. The fully differentiated erythrocyte therefore simply consists of an outer plasma membrane enclosing haemoglobin and the limited number of enzymes necessary for maintenance of the cell.

Fig. 3. Human erythrocytes: A - Scanning electron micrograph of diskocyte, mag. × 7500; B - transmission electron micrograph, mag. × 12000.
1. **Biconcave discs**;
2. Close to 7.5 µm diameter in a smear.
3. Comprise a flexible *membrane* enclosing *haemoglobin* (iron-porphyrin-protein) in a closely packed state which, maintains the RBC’s optimal shape for gas exchanges involving the haemoglobin.

The erythrocyte plasma membrane is composed of a lipid bilayer incorporating various globular proteins conforming to the standard fluid mosaic model of membrane structure. Blood group substances are carried on the surface. Immediately beneath the plasma membrane is a meshwork of proteins forming a cytoskeleton anchored to the membrane by one or more membrane-incorporated proteins; the main skeletal protein is the long fibre-like protein, *spectrin*. The biconcave shape of erythrocytes is determined in part by the cytoskeleton and in part by its water content, the latter being related to the concentration of inorganic ions within the cell (fig. 4).

4. Mature RBCs have no nucleus, Golgi body, ER, ribosomes or mitochondria. **Osmolarity** of the plasma affects the shape of an RBC. Hypertonic solutions in vitro cause crenation and shrinkage; hypotonic - swelling and haemolysis.

5. *Globin* is acidophilic, and RBCs stain orange with eosin.

6. RBCs have glycolytic enzymes and substrates, and methaemoglobin reductase and carbonic anhydrase for their respiratory function:
   - (a) Oxygen binds to ferrous iron of haemoglobin (RBC) for transport: air —> lungs —> blood —> tissues
   - (b) Carbon dioxide leaves bicarbonate of the plasma and carbaminohaemoglobin (RBC) for transport: tissues —> blood —> lungs —> air

7. **Life** in circulation is estimated by $^{51}$Cr labelling at around 120 days, then the RBC is sequestered in the spleen, liver or bone marrow to be phagocytosed by macrophages. The spleen is most responsible.

   *Reticulocyte/polychromatophil erythrocyte*. Reticulocytes are immature red blood cells which have shed their nucleus, but still retain residual nuclear material. Reticulocytes are the immature form in which erythrocytes are released into the circulation from the bone marrow. They still contain sufficient mitochondria, ribosomes and Golgi elements to complete the cytoskeleton and the remaining 20% of haemoglobin synthesis. Final maturation into erythrocytes occurs within 24-48 hours of release. The rate of release of reticulocytes into the circulation generally equals the rate of removal of old erythrocytes by the spleen and liver. Since the lifespan of circulating erythrocytes is about 120 days, reticulocytes constitute slightly less than 1% of circulating red blood cells.

When severe erythrocyte loss occurs, such as after haemorrhage or haemolysis, the rate of erythrocyte
production in the bone marrow increases and the proportion of reticulocytes in circulating blood goes up (reticulocytosis). Clinically, the reticulocyte percentage is a useful indicator of erythropoiesis. In cases of anaemia, an elevated reticulocyte count indicates normal marrow function, while a decreased count may mean impaired erythropoiesis.

The volume of RBCs as a percentage of centrifuged whole blood - the haematocrit - is a quick, crude measure of the O₂-carrying quality.

Failure to maintain an adequate haemoglobin concentration is termed anaemia. There are several common causes of anaemia, including lack of factors required to synthesise haemoglobin, (e.g. iron, or vitamins B₁₂ and folic acid), excessive loss or inappropriate destruction of erythrocytes, or failure of bone marrow to manufacture enough cells. Erythrocyte morphology may be altered in certain types of anaemia. Lack of iron leads to cells that are smaller than normal (microcytes) while lack of B₁₂ and folate leads to cells that are larger than normal (macrocytes). Abnormally rounded and fragile erythrocytes (spherocytes) may be caused by mutations in genes coding for proteins in the red cell cytoskeleton.

PLATELETS (clotting and vessel-sealing) (fig, 5)

Platelets (thrombocytes) are small, non-nucleated cells formed in the bone marrow from the cytoplasm of cells called megakaryocytes. Their numbers in circulating blood range from 150 000 to 400 000/mL. Platelets have a variety of functions essential to the normal process of haemostasis. Firstly, they form plugs to occlude sites of vascular damage by adhering to collagenous tissue at the margin of a wound; later the platelet plug is reinforced by fibrin. Secondly, they promote clot formation by providing a surface for the assembly of coagulation protein complexes. Thirdly, platelets secrete factors that modulate coagulation and vascular repair.

Fig. 5. The structure of human blood platelets: A - scheme; B - transmission electron micrograph, mag. × 30000.

1 - circular bundles of microtubules; 2 - dense tubular system; 3 - marginal band of microtubules; 4 - α-granule; 5 – λ-granule; 6 - mitochondria; 7 - the inclusion of glycogen.

1. Rounded or ovoid parts of cells, 2-5 µm diameter.
2. Consist of cytoplasm, organelles and inclusions, bounded by a cell membrane, reflecting their formation as pseudopodia breaking off from extravascular cells - megakaryocytes.
3. The dense central granulomere (organelle zone) has mitochondria, dense bodies and alpha granules; the pale peripheral hyalomere (sol/gel region) is cytoplasm deficient in organelles, except for contractile filaments and a shape-giving ring of microtubules.
4. Platelets adhere to collagen, neutrophils and monocytes, and especially to each other; this platelet aggregation/agglutination is used to seal defects in blood-vessel walls.
5. Apart from several molecules for adhesion, the membrane supplies a phospholipoprotein: one of many factors in the cascade causing blood fibrinogen to form fibrin fibers in clotting. Platelets contract and cause a compacting of the fibrin to which they adhere - clot retraction. They also release several factors from their granules, e.g., serotonin and cytokines, having vasoconstrictive and other actions.
Platelets contain most of the cytoplasmic organelles of other cells including mitochondria, microtubules, glycogen granules, occasional Golgi elements and ribosomes as well as enzyme systems for both aerobic and anaerobic respiration. The most conspicuous organelles, as seen in micrograph (b), are the electron-dense granules which constitute about 20% of platelet volume and are of four types:

- **Alpha granules** are variable in size and shape and contain many proteins, both megakaryocyte-derived and derived by endocytosis from plasma. Granule membrane contains several membrane adhesion molecules. Important proteins include *glycoprotein (GP)Ib-IX-V complex, platelet integrin αIIbβ3 (GPIIb-IIIa), platelet factor 4, platelet-derived growth factor and beta thromboglobulin*; these granules also contain coagulation factors including fibrinogen, taken up from the plasma by endocytosis.

- **Dense granules** are very electron-dense and contain serotonin which is absorbed from the plasma having been produced by enterochromaffin cells of the gut. They also contain ADP.

- **Lysosomes** are membrane-bound vesicles containing usual lysosomal enzymes.

- **Microperoxisomes** are small in number and have peroxidase activity, probably catalase.

The platelet plasma membrane expresses cell adhesion molecules involved in platelet:platelet interactions, adhesion to extracellular matrix or binding of coagulation factors. Many of these proteins are held within granules until expressed on the surface following granule fusion with exocytosis.

Platelets contain a well-developed cytoskeleton. At the periphery of the cell is a **marginal band of microtubules** which depolymerise at the onset of platelet aggregation. The cytoplasm is rich in the contractile proteins actin and myosin which are involved in the functions of clot retraction and extrusion of granule contents as part of degranulation.

Located deep in the marginal band of microtubules and also scattered throughout the cytoplasm is the **dense tubular system (DTS)** consisting of narrow membranous tubules which contain a homogeneous electron-dense substance. This system is believed to be an intracellular store of calcium which is released into the platelet cytosol following signaling from platelet surface receptors and secondary messengers.

Platelets contain a system of interconnected membrane channels, the **surface-connected canalicular system (SCCS)**, which is in continuation with the external environment via external pits. Alpha granules fuse with the SCCS as part of secretion of their contents.

**Platelet disorders**

Lack of circulating platelets (*thrombocytopenia*) leads to a bleeding tendency while excess of platelets (*thrombocytosis*) leads to risk of excessive blood clotting (*thrombosis*). Some people have inherited problems with platelet function, for example defects in expression of cell adhesion proteins, or defects in the secretion of factors from platelet granules.
LESSON 3

THEME: BLOOD. LEUKOCYTES

BACKGROUND. Study of blood is one of the important subjects for future doctors. Understanding of the structure and functions of erythrocytes, leukocytes, platelets, plasma components, hemogramme, age and sex changes in blood are necessary for the successful study of preclinical and clinical subjects.

AIM OF STUDY. To be able to differentiate leukocytes, interpret their functional state and role in homeostasis.

YOU SHOULD BE ABLE TO:
1. Distinguish general features of blood.
2. Differentiate leukocytes in blood smear.
3. Interpret structure and functions of granulocytes.
4. Determine agranulocytes, their functional significance.

THEORETICAL QUESTIONS:
2. Granulocytes: microscopic and submicroscopic structure, functions (participation in inflammatory and allergic reactions), life span. Characteristic of granulocytes in different conditions.
3. Agranulocytes: microscopic and submicroscopic structure of monocytes, their functions, life span. Characteristic of agranulocytes in different conditions of the organism.
4. Lymphocytes, types, structure of T- and B-lymphocytes, functions.
5. Hemogramme, its clinical importance.

LEUKOCYTES (defence).
These are true cells, divided according to the granularity of their cytoplasm into two groups - granular and agranular.
Five types of leucocytes are normally present in the circulation. These are traditionally divided into two main groups based on their nuclear shape and cytoplasmic granules:

- **Granulocytes**
  - Neutrophils;
  - Eosinophils;
  - Basophils.
- **Mononuclear leukocytes**
  - Lymphocytes;
  - Monocytes.

The leukocytes have important roles as components of the body defense systems however this activity takes place in the tissues, not in the blood. All leucocytes carry surface proteins which are capable of binding to complementary receptors on endothelial cells in blood vessels. This binding then allows cells to actively migrate using amoeboid movement into the tissues. Neutrophils do not normally enter tissues in large numbers and a moderate number constantly circulate, generally only entering tissues in response to a disease stimulus in the process of acute inflammation. Eosinophils, basophils and monocytes constantly enter certain tissues in normal states and circulate in the blood in relatively low numbers. Lymphocytes constantly enter tissues from the blood in normal states and leave tissues via the lymphatic system, to reach the lymphoid system.

Functionally, neutrophils and monocytes are highly phagocytic and engulf microorganisms, cell debris and particulate matter in a non-specific manner; this activity may be enhanced and directed by immune responses to specific foreign agents. Lymphocytes play the key role in all immune responses and, in contrast to the other leukocytes, their activity is always directed against specific foreign agents.

In general, all the leucocytes perform their functions in the tissues and merely use the blood as a vehicle for transit between sites of formation, storage and activity. It follows, therefore, that increased demand for
particular leucocytes in various sites is reflected in increased numbers in the circulation. The absolute and differential white cell count is therefore a useful pointer to diagnosis and is an important laboratory investigation. Automated assays are generally used to perform white cell counts using advanced cytometric techniques that do not use microscopy.

- Raised neutrophil count (blood neutrophilia) indicates an acute inflammatory response and is especially seen in association with bacterial infections.
- Raised eosinophil count (blood eosinophilia) is seen in response to allergy and in infections with certain parasites.
- Malignant lymphocyte count (blood lymphocytosis) is seen in response to viral infections.

In these conditions it is common to find a very raised white cell count corresponding to circulating malignant cells.

- Reduction in white cells in the blood may indicate defective function of the bone marrow.

1. Granular leucocytes.

Granulocytes are so named for their prominent cytoplasmic secretory granules. Each of the three different types of granulocyte has type-specific granules, the names neutrophil, eosinophil and basophil being derived from the staining characteristics of these specific granules. The granulocytes have a single multilobed nucleus, which conveyed to early microscopists the erroneous impression that these cells were multinucleate and led to the confusing description of the other main group of leucocytes as mononuclear cells (see below). The multilobed nucleus may assume many morphological shapes leading to the use of the term polymorphonuclear leucocyte or polymorph as a synonym for the term granulocyte. To confuse matters further, the term polymorph is often used to refer to neutrophils since they exhibit the greatest degree of nuclear polymorphism and are by far the most prolific of the polymorphs. Granulocytes are also referred to as myeloid cells due to their exclusive origin from bone marrow; this should not, however, be taken to imply that they are the only white blood cells to be formed in the bone marrow. All kinds appear round in a smear with a diameter 10-14 µm.

1. Polymorphonuclear neutrophil (neutrophil/PMN/polymorph, for short) (fig. 1,2). Neutrophils are the most common type of leucocyte in blood and constitute 40-75% of circulating leucocytes. They generally only leave the circulation in large numbers in response to disease. Being highly motile and phagocytic, their principal function is in the acute inflammatory response to tissue injury where they secrete enzymes that degrade tissue components, ingest and destroy damaged tissue and kill invading microorganisms, particularly bacteria.

- (a) Nucleus has coarse, clumped, deeply staining chromatin, usually in two or more lobes or segments connected by thin chromatin strands. Unlobated band nuclei are in immature cells; older nuclei have several lobes.

- (b) Cytoplasm is granular from many, small, weakly staining (neutrophil) granules of two kinds:
  (i) non-specific azurophil granules that are lysosomes with destructive enzymes; These granules contain a number of microbicidal agents including myeloperoxidase and neutrophil defensins. They are believed to mainly contribute to the killing and degradation of engulfed microorganisms rather than secreting contents.
  (ii) numerous specific non-lysosomal granules holding a selectin-type glycoprotein for adhesion to endothelium and ECM, and lysozyme, and other bactericidal substances, gelatinase, collagenase, transcobalamin-1 and membrane proteins.
  (iii) Small tertiary granules, also termed gelatinase granules, mainly secrete enzymes to degrade tissue. They contain gelatinase which breaks down extracellular matrix and also insert adhesion molecules into the cell membrane.

- (c) This motile cell is attracted out of vessels into the tissues, where it attacks bacteria and phagocytoses them and immune complexes. The attack on bacteria is two-pronged:
  (i) with a respiratory burst that generates free radicals; also myeloperoxidase catalyzes the production of hypochlorous acid;
  (ii) by proteins, e.g., defensins, and bactericidal permeability-inducing protein (BPI), that damage bacterial cell walls.

Since the neutrophil has few organelles for protein synthesis, it has a limited capacity to regenerate
secreted proteins and specific enzymes which are depleted by phagocytic activity. The neutrophil is thus incapable of continuous function and degenerates after a single burst of activity. Defunct neutrophils are the main cellular constituent of pus and are therefore sometimes referred to as pus cells.

The paucity of mitochondria and the abundance of glycogen in neutrophils reflect the importance of the anaerobic mode of metabolism. Energy production via glycolysis permits neutrophils to function in the poorly oxygenated environment of damaged tissues whilst the hexose monophosphate pathway generates microbicidal oxidants.

Fig. 1. Granulocytes in human blood smear: A - segmented neutrophil granulocytes, histological specimen, mag. ×1400; B – sex chromatin in the nucleus of neutrophilic granulocytes, histological specimen, ×1400.

1 - segmented neutrophil granulocyte; 2 - Barr body (sex chromatin); 3 - erythrocyte.

Fig. 2. The structure of segmented neutrophil granulocytes: A - scheme B - transmission electron micrograph, mag. ×20000.

1 - segments of the nucleus; 2 - Golgi complex; 3 - primary (azurophilic) granules, 4 - secondary (specific) granules, 5 - microvilli.
Neutrophil functions.

Neutrophils in the circulation are attracted by chemotactic factors (chemotaxins) released from damaged tissue or generated by the interaction of antibodies with antigens on the surface of the microorganisms. Chemotaxins stimulate neutrophils and signal to fuse secretory granules with the cell surface, thereby expressing stored cell adhesion proteins that allow the neutrophil to stick to vascular endothelial cells and start to move into the tissues.

The coating of organisms with antibodies and complement enhances neutrophilic phagocytic activity, the phenomenon being known as opsonisation. Neutrophils have surface receptors that bind to opsonins and stimulate internalisation by phagocytosis.

As the first step in phagocytosis, an organism is surrounded by pseudopodia which then fuse to completely enclose it in an endocytotic vesicle called a phagosome. This then fuses with cytoplasmic granules, in particular the primary granules, which discharge their contents exposing the organism to a potent mixture of antimicrobial proteins. Killing is greatly enhanced by the generation of hydrogen peroxide and superoxide by enzymatic reduction of oxygen (respiratory burst oxidase).

Neutrophils also secrete granule contents into the extracellular environment by degranulation. The binding of a significant proportion of receptors on the neutrophil with substances such as products from damaged tissues, inflammation or bacteria activates intracellular secondary messengers to stimulate membrane fusion and exocytosis (degranulation). This happens first to the secretory granules, then to gelatinase granules and finally to specific granules. Primary granules are mainly involved in intracellular bacterial killing but can also be secreted into the extracellular space.

2. Eosinophil (fig. 3,4).

Eosinophils account for 1-6% of leucocytes in circulating blood; their numbers exhibit a diurnal variation, being greatest in the morning and least in the afternoon. The production of eosinophils by the bone marrow is stimulated by the cytokine interleukin 5 (IL-5), produced by activated lymphocytes, and to a lesser extent, interleukin 3 (IL-3) and granulocyte monocyte-colony stimulating factor (GM-CSF). Eosinophils circulate in the blood for about 8-12 hours and emigrate from capillaries to enter the tissues, where the majority of eosinophils reside. This migration of eosinophils is stimulated by chemotaxis. The cytokines eotaxin, IL-5 and eosinophil chemotactic factor of anaphylaxis (ECF-A) are able to stimulate recruitment of eosinophils into tissues from the circulation.

· (a) Nucleus is darkly staining and bilobed.
· (b) Cytoplasm has many large, eosinophil granules 0.5-1 µm diameter, and some smaller core-less granules.
· (c) Specific granules are a form of lysosome, which in EM have a crystalline core and a fine granular region. Defensive basic/cationic proteins, e.g., major basic protein, give the acidophil reaction. The enzymes differ somewhat from the neutrophil’s, e.g., generating antimicrobial O₂ metabolites differently.
· (d) Motile, and enter inflamed tissues, especially at sites of allergies and parasitic infestations. They attack helminths using the basic proteins and oxygen derivatives, and also may dampen mast cell-dependent reactions, e.g., by phagocytosing mast-cell granules.
· (e) They comprise 2-3 per cent of leukocytes (but rising for (d)).
· (f) In EM, the large granules, like a skunk, have a dark lengthwise central stripe, which helps in identifying the eosinophil.

Eosinophil function

The eosinophil plasma membrane has different immunoglobulin and complement receptors from other leucocytes. All eosinophils have receptors for IgE; this is not present on neutrophils. IgG and complement receptors are also present. Eosinophils are able to modulate inflammatory responses at several levels and have a central role in the induction and maintenance of inflammatory responses due to allergy, for example in allergic rhinitis (hay fever) and asthma.

Eosinophils can act as pro-inflammatory leucocytes. They have important roles in defence against helminthic parasites which is mediated by release of basic granule proteins as well as the production of leukotrienes. Degranulation occurs when eosinophils are exposed to mediators such as PAF (platelet activating factor) or antigen-antibody complexes. Increased numbers of circulating eosinophils (eosinophilia) has long been recognised as a diagnostic clue to the presence of helminthic infections.
Fig. 3. Eosinophilic granulocyte in a smear of human blood. Histological specimen, mag. × 1400.

1 - eosinophilic granulocyte; 2 - erythrocyte.

Fig. 4. The structure of eosinophilic granulocytes: A - scheme B - transmission electron micrograph, mag. × 21200.

1 - segments of the nucleus; 2 - Golgi complex; 3 - primary (azurophilic) granules; 4 - secondary (specific) granules, 5 - crystalloid structure of mature granules; 6 - microvilli.
Eosinophils are able to modulate local immune responses by production and release of cytokines including tumour necrosis factor (TNF), transforming growth factor (TGF), granulocyte monocyte-colony stimulating factor (GM-CSF) and interleukins 4, 5 and 8 (IL-4, IL-5, IL-8). Importantly, the production of factors such as TNF and leukotrienes may have adverse local effects in causing tissue damage. Eosinophils also have a minor role as antigen presenting cells.

1. Basophil (fig. 5, 6). Basophils are the least common leucocyte and constitute less than 1% of leucocytes in circulating blood. They are characterised by large intensely basophilic cytoplasmic granules and share many structural and functional similarities with tissue mast cells. The exact relationship between mast cells and basophils has been and remains controversial, but they do seem to share a common bone marrow precursor.

Basophils are formed in the bone marrow, sharing a common precursor with the other granulocytes up to the myeloblast stage; from here, development proceeds through analogous stages as for neutrophils and eosinophils. Interleukin 3 (IL-3) promotes basophil formation from bone marrow cells while IL-3 and stem cell factor (SCF) promote mast cell proliferation and maturation, and prevent death in tissues. Effete mast cells are removed from tissues by apoptotic cell death and are not believed to re-enter the blood again as basophils.

· (a) Nucleus is bilobed and sometimes twisted, but palely staining and often obscured by
· (b) basophilic cytoplasmic granules, containing sulphated proteoglycans, heparin and the vasodilator, histamine.
· (c) Basophils are reluctant to enter CTs, where there are mast cells holding the same materials. Thus, the function of basophils is in doubt, but they bind IgE and participate in various hypersensitivities.
· (d) They are rare; 0.5 per cent of the leukocytes.

Fig. 5. Basophilic granulocyte in a smear of human blood. Histological specimen, mag. × 1400.

1 - basophilic granulocyte; 2 - erythrocyte.

Basophil function
The cytoplasmic granules of basophils and mast cells contain proteoglycans consisting of sulphated glycosaminoglycans linked to a protein core; this accounts for their metachromatic staining property. The proteoglycans are a variable mixture of heparin and chondroitin sulphate. The granules also contain histamine and many other mediators of inflammatory processes such as slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor of anaphylaxis (ECF-A). Granules also contain a tryptase.

Basophil infiltration, mast cell proliferation and degranulation are features of a variety of immunological
and other disorders. Mast cells and basophils act as effector cells in allergic disorders mediated by IgE and T helper lymphocytes as well as in the immune responses to parasites.

Basophils and mast cells have high affinity membrane receptors specific for the Fc segment of IgE, a class of immunoglobulin which is produced by plasma cells in response to a variety of environmental antigens (allergens). Exposure to allergen results in the antigen forming bridges between adjacent IgE molecules which triggers rapid exocytosis of granule contents (degranulation). The release of histamine and other vasoactive mediators is thus responsible for the so-called **immediate hypersensitivity (anaphylactoid) reaction** characteristic of allergic rhinitis (hay fever), some forms of asthma, urticaria and anaphylactic shock. Nevertheless, there are other IgE-independent stimuli for mast cell degranulation.

Elevated serum levels of mast cell tryptase are detected in anaphylaxis.

Basophils may account for up to 15% of infiltrating cells in allergic dermatitis and skin allograft rejection, a phenomenon known as **cutaneous basophil hypersensitivity**; this is induced by sensitised lymphocytes and is thus a type of cell-mediated hypersensitivity. In this case degranulation is slow rather than rapid as in immediate hypersensitivity reactions.

Fig. 6. The structure of human basophilic granulocyte: A - scheme; B - transmission electron micrograph, mag. × 22000.

1 - segments of the nucleus; 2 - Golgi complex; 3 - primary granules; 4 - Secondary (specific) granules.

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2. Agranular leucocytes

1. **Lymphocyte** (fig. 7). Lymphocytes are the smallest cells in the white cell series, being only slightly larger than erythrocytes. They are the second most common leucocyte in circulating blood and make up 20-50% of the differential white cell count.

Lymphocytes play the central role in all immunological defense mechanisms. Lymphocytes circulate between various lymphoid tissues and all other tissues of the body via the blood and lymphatic vessels. There is a constant recirculation of lymphoid cells through tissues and back to the circulation as part of immune surveillance. Most of the lymphocytes in the blood are in a relatively inactive functional and metabolic state.

Lymphocytes are characterised by a round, densely stained nucleus and a relatively small amount of pale basophilic, non-granular cytoplasm (fig. 8,9). The amount of cytoplasm depends on the state of activity of the
lymphocyte, and in circulating blood there is a predominance of “small” inactive lymphocytes (6-9 µm in diameter). “Large” lymphocytes (9-15 µm in diameter) make up about 3% of lymphocytes in peripheral blood. Large lymphocytes represent activated B or T lymphocytes en route to the tissues where they will become antibody-secreting plasma cells; they also include natural killer cells.

Fig. 7. Lymphocytes in the smear of human blood. Histological specimen, mag. ×900.

1 - small lymphocyte; 2 - large lymphocyte; 3 - erythrocyte.

Fig. 8. Structure of human lymphocytes: A - scheme; B - transmission electron micrograph, mag. ×26000.

1 - nucleus; 2 - Golgi complex; 3 - lysosomes; 4 - mitochondria; 5 - microvilli.

Fig. 9. Types of lymphocytes. Scanning electron micrographs of: A - microvilliferous (B-lymphocyte); B - adjusted (T-lymphocyte), mag. ×1400.
· (a) Small spheroid cell about 5-8 µm in diameter.
· (b) Large, spheroid, darkly staining nucleus leaves only a narrow rim of cytoplasm with a few small azurophil granules.
· (d) Motile to enter CT and epithelial tissue, but is not phagocytic.
· (e) Larger lymphocytes up to 12 µm diameter, with more abundant cytoplasm, may be seen in small numbers. The large granular lymphocyte is the natural killer cell.
· (f) Unlike granular leucocytes, small lymphocytes can be stimulated to enlarge and divide by antigens, cytokines, and some plant lectins.
· (g) The complex role of lymphocytes in immune defences.
· (h) Lymphocytes circulate in blood and lymph systems and migrate to CT and mucous membranes. Some lymphocytes have a lifespan of months or years.
· (i) They amount to 25-35 per cent of the leucocytes.

2. Monocyte (fig. 10).
· (a) Large, spheroid cell about 12-20 µm in diameter.
· (b) Nucleus has fine chromatin not densely stained, and is an indented sphere.
· (c) Golgi body and centrioles lie by the nuclear indentation.
· (d) Cytoplasm is abundant, with a few granules that are precursors of many larger lysosomes seen in EM when the cell is actively phagocytic.
· (e) Motile, to leave the vessels after only a day or so to become the phagocytic macrophages/histiocytes of CT, or other derivatives.
· (f) Macrophages/Monocytes spend months in CTs cooperating with lymphocytes in defensive responses. Macrophages, by releasing cytokines after activation, coordinate inflammatory and defensive reactions.
· (g) They comprise 3-10 per cent of the leukocytes.

Fig. 10. Monocyte in a smear of human blood. Histological specimen, mag. × 1400.

1 - monocyte; 2 - erythrocyte.

Monocyte-macrophage system

Monocytes migrate to peripheral tissues where they assume the role of macrophages. This has led to the concept of a single functional unit, the monocyte-macrophage system (mononuclear phagocyte system), consisting of circulating monocytes, their bone marrow precursors, and tissue macrophages both free and fixed.
(histiocytes). Included in the system are the Kupffer cells of the liver, microglia of the CNS, Langerhans cells of the skin, alveolar macrophages in lung, antigen-presenting cells of the lymphoid organs and the osteoclasts of bone.

Monocyte function.

Monocytes appear to have little function in circulating blood. They respond by chemotaxis to the presence of factors from damaged tissue, microorganisms and inflammation by migration into the tissues and differentiation into macrophages; with their capacity for phagocytosis and content of hydrolytic enzymes, they engulf and destroy tissue debris and foreign material as part of the process of healing.

Most monocytes that migrate into a tissue die by apoptosis. They can survive and proliferate as macrophages if they are stimulated by growth factors, such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-3.

Macrophages become active when exposed to interferon-gamma (IFN-γ) a cytokine produced by T-lymphocytes. They can then process antigen, presenting it to the immune system, and can secrete cytokines that are involved in tissue healing and repair.

Lipopolysaccharide (LPS) which is a component of Gram-negative bacteria also stimulates monocytes/macrophages to secrete the powerful cytokine, TNF-α. This is one mediator of so-called “septic shock”.

Fig. 11. Structure of human monocyte: A - scheme; B - transmission electron micrograph, × 20000.

1 - nucleus; 2 - Golgi complex, 3 - lysosomes, 4 - mitochondria; 5 - vacuoles.
LESSON 4

THEME: HEMATOPOIESIS

BACKGROUND. Hemopoiesis is the process of blood formation that has two periods: embryonic, which provides formation blood as a tissue, and postembryonic – physiological regeneration of blood components. Normally creation, development and degradation of components of blood takes place within the hemopoietic organs. That provides the constancy of blood structure. Learning the processes of hemopoietics regulation is one of the most important problems of the modern experimental and clinical hematology.

AIM OF STUDY. To be able to describe the main periods of postembryonic hemopoiesis according to the modern hemopoietic theories so as to be able to differentiate pathologic changes in hemopoietic organs during the further study.

THEORETICAL QUESTIONS.
1) Embryonic hemopoiesis. Hemopoietic organs during embryonic development. Features of hemopoiesis in the yolk sac, liver, thymus, spleen, lymphatic nodules and bone marrow.
2) Postembryonic hemopoiesis (physiological blood regeneration). Myeloid and lymphoid tissues.
3) Unitary theory of the hemopoiesis.
4) Characteristic of stem and half stem cells. Oligopotential cells. Functions of stem cells.
5) Regulation of the self-maintenance and commitment of the stem cell of blood. The role of microenvironment in hemopoiesis.
6) Regulation of self-maintenance and commitment of polypotent cells.
10) Postembryonic thrombocytopoiesis. Microscopic characteristic of the cells from megacaryocyte differon. Main regulators.


According to chronology there are 2 types of hemopoiesis:
1) Embryonic - histogenesis of blood;
2) Postembryonic - physiological regeneration of blood cells.

In the earliest stages of embryogenesis, blood cells arise from the yolk sac mesoderm. Embryonic: mesenchyme gives rise to:
· (a) blood islands in the yolk sac;
· (b) fetal liver haemopoietic tissue;
· (c) bone marrow in cavities of the developing bones;
· (d) marrow in the spleen and lymph nodes.

Hemopoiesis starts since 3d week of embryogenesis when mesenchymal cells are divided and make islets. Then cells in centre of islet are differentiated into hemopoietic stem cells and first blood cells – primary erythroblasts. Peripheral cells of islets are differentiated into angioblasts (future endotheliocytes). This type of hemopoiesis is known as intravascular blood formation.

Sometime later, the liver serves as temporary hemopoietic organ (since 5 week), than spleen and lymph nodes, but by the second month the clavicle has begun to ossify and begins to develop bone marrow in its core. As the prenatal ossification of the rest of the skeleton accelerates, the bone marrow becomes an increasingly important hemopoietic tissue.
After birth and in childhood, erythrocytes, granular leukocytes, monocytes, and platelets are derived from stem cells located in bone marrow. The origin and maturation of these cells are termed, respectively erythropoiesis (Gr. erythros, red, + poiesis), granulopoiesis, monocytopoiesis, and megakaryocytopoiesis. The bone marrow also produces cells that migrate to the lymphoid organs producing the various types of lymphocytes (table 1).

Hemopoietic Tissues – specialized CT derived from mesenchyme, responsible for production of new blood cells; 2 Types:
1) Myeloid Tissue (Red Bone Marrow) – responsible for production of most blood cell types (fig. 1);
2) Lymphatic Tissue (Thymus, etc.) – responsible for formation of T-lymphocytes (fig. 2), proliferation of B-lymphocytes, immune defenses (lymph nodes & nodules, spleen – generate lymphocytes upon antigen stimulus).

Mature blood cells have a relatively short life span, and consequently the population must be continuously replaced by the progeny of stem cells produced in the hematopoietic (Gr. haima, blood, + poiesis, a making) organs.

Before attaining maturity and being released into the circulation, the blood cells go through specific stages of differentiation and maturation. Because these processes are continuous, cells with characteristics that lie between the various stages are frequently encountered in smears of blood or bone marrow.

STEM CELLS, GROWTH FACTORS & DIFFERENTIATION.
Stem cells are pluripotential cells that can divide continuously and whose daughter cells form specific, irreversibly differentiated cell types. Stem cells play a central role in hematopoiesis and, because of their importance in biomedical research, will be considered in detail.

The study of stem cells in bone marrow is possible because of experimental techniques that permit analysis of hematopoiesis in vivo and in vitro.

In vivo techniques include injecting the bone marrow of normal donor mice into lethally irradiated mice whose hematopoietic cells have been destroyed. In these animals, the transplanted bone marrow cells develop colonies of hematopoietic cells in the spleen.

In vitro investigation of hematopoiesis is made possible through the use of a semisolid tissue culture medium made with a layer of cells derived from bone marrow stroma. This medium creates favorable microenvironmental conditions for hematopoiesis.

Data from an extensive series of experiments show that under these suitable microenvironmental conditions, stimulation by growth factors influences the development of the various types of blood cells.
Pluripotential and multipotential stem cells. It is thought that all blood cells arise from a single type of stem cell in the bone marrow. Because this cell can produce all blood cell types, it is called a pluripotential stem cell. These cells proliferate and form one cell lineage that will become lymphocytes (lymphoid cells), and another lineage that will form the myeloid cells that develop in bone marrow (granulocytes, monocytes, erythrocytes, and megakaryocytes). Both these types of stem cells are called multipotential stem cells. Early in their development, lymphoid cells migrate from the bone marrow to the lymph nodes, spleen, and thymus, where they complete their differentiation into lymphocytes.

Progenitor and precursor cells. The proliferating multipotential stem cells form daughter cells with reduced potentiality. These uni- or bipotential progenitor cells generate precursor cells (blasts) in which the morphologic characteristics differentiate for the first time (unlike these cells, stem and progenitor cells cannot be morphologically distinguished and resemble lymphocytes), suggesting the cell types they will become. Both pluri- and multipotential stem cells divide at a rate sufficient to maintain their relatively small population (in mouse bone marrow, only 0.1-0.3% of the cells are multipotential cells). Mitotic rate is accelerated in progenitor and precursor cells, producing large numbers of differentiated, mature cells (3 × 10⁹ erythrocytes and 0.85 × 10⁹ granulocytes/kg/day in human bone marrow). While progenitor cells can divide and produce both progenitor and precursor cells, precursor cells produce only mature blood cells.

Hematopoiesis is therefore the result of simultaneous, continuous proliferation and differentiation of cells derived from stem cells whose potentials are reduced as differentiation progresses. This process can be observed in the previously mentioned in vivo and in vitro studies, in which colonies of cells derived from stem cells with various potentialities appeared. Colonies derived from a multipotential myeloid stem cell can produce erythrocytes, granulocytes, monocytes, and megakaryocytes, all in the same colony. In these experiments, however, some colonies produced only red blood cells. Other colonies produce granulocytes and monocytes. Cells forming colonies of specific cell types are called colony-forming cells (CFC), or colony-forming units (CFU). The convention in naming these various cell colonies is to use the initial letter of the cell each colony produces. Thus, CFU-M denotes a monocyte-producing colony, CFU-E produces eosinophils, CFU-MG produces monocytes and granulocytes, and so on.

Hematopoiesis depends on the presence of suitable microenvironmental conditions and growth factors. The microenvironmental conditions are furnished by cells of the stroma of hematopoietic organs, which produce an essential extracellular matrix. A general view of hematopoiesis shows that as this process takes place, both the potential for differentiation and the self-renewing capacity of the initial cells decrease gradually. In contrast, the mitotic response to growth factors increases gradually, attaining its maximum at the middle of the process. From that point on, morphology and functional activity develop, and mature cells form. Once the necessary environmental conditions are present, the development of blood cells depends on factors that affect cell proliferation and differentiation. These substances are called growth factors, colony-stimulating factors (CSF), or hematopoietins (poietins). Growth factors, which have differing chemical compositions and complex, overlapping functions, act mainly by stimulating proliferation (mitogenic activity) of immature (mostly progenitor and precursor cells, supporting the differentiation of immature cells as they mature, and enhancing the functions of mature cells.

These three functions may be present in the same growth factor, but they may be expressed with different intensities in different growth factors. The isolation and cloning of genes for several growth factors permits both the mass production of growth factor and the study of their effects in vivo and in vitro.

Growth factors have been used clinically with some patients to increase marrow cellularity and blood cell counts. The use of growth factors to stimulate the proliferation of leukocytes is opening broad new applications for clinical therapy. Potential therapeutic uses of growth factors include

1. increasing the numbers of blood cells in diseases or induced conditions (eg. chemoiherapy, irradiation) that cause low blood counts;
2. increasing the efficiency of marrow transplant by enhancing cell proliferation;
3. host defenses in patients with malignancies and infectious and immunodetricent diseases: and enhancing the treatment of parasitic diseases.

Hematopoietic diseases rarely result from malfunctions of hematopoietic organ stroma. They are usually caused by suppression on enhancement of undifferentiated cell production, with a consequent reduction (or overproduction) of hematopoietic cells. In some diseases however, sequential or simultaneous suppressed and
enhanced proliferation of more than one type of stem cell can occur. There are, in such cases, reduced numbers of some cell types (eg. aplastic anemia, a disorder characterized by decreased production of hematopoietic cell) coinciding with increased numbers all others (eg. Leukemia, the abnormal proliferation of white blood cells).

The initial experiments with transplanted normal bone marrow to lethally irradiated mice established the basis for bone marrow transplantation, now routinely used to treat some hematopoietic-cell-growth disorders.

THEORIES OF HEMOPOIESIS.
1) Monophyletic Theory (accepted by majority) – all blood cells arise from a single common stem cell (hemocytoblast).
2) Diphyletic Theory – lymphocytes and monocytes derived from one stem cell (lymphoblast), granular leukocytes and RBCs from another stem cell (myeloblast).
3) Polyphyletic Theory – a separate stem cell exists for each blood cell type.
- Difficulty in determining which is correct lies in the fact that the earliest stages of blood cell formation involve progenitor cells that cannot be readily identified under the microscope.

Generalized Scheme of Blood Cell Formation (Monophyletic Theory).
Differentiating blood cells can be grouped into 6 general categories:

1) Pluripotential Stem Cells.
2) Commited semi-stem cells and polypotent cells CFU-GEMMg and CSF-Lph.
3) Oligo- and unipotent Progenitor Cells.
4) Blasts.
5) Differentiated precursor cells.
6) Functional Blood Cells.

Stem Cell – has capacity to give rise to several different lines of specialized cells
- Nucleus is undifferentiated (cells look like large lymphocyte) with dense accumulations of chromatin material; basophilic cytoplasm due to presence of ribosomes; capable of extensive proliferation by mitotic division, so can serve as lifelong source of potential blood cells.

Restricted Progenitor Cells – prospective fate of various progenitor cells becomes increasingly restricted: first to myeloid or lymphoid (lymphocytes & monocytes) and finally to progenitor cells specific for a certain blood cell.
- These cells are still relatively undifferentiated and cannot be identified under the microscope.
- Proliferation and differentiation of restricted progenitor cells is under control of regulating factors specific to each cell lineage.
- Eventually, progenitor cells become recognizable under microscope; at this stage they are termed blood cell precursors, which then mature into functional blood cells.

MATURATION OF ERYTHROCYTES (fig. 3).
A mature cell is one that has differentiated to the stage at which it has the capability of carrying out all its specific functions. The basic process in maturation is the synthesis of hemoglobin and the formation of enucleated, biconcave, small corpuscle, the erythrocyte. During maturation of the erythrocyte, several major changes occur. Cell volume decreases, and the nucleoli diminish in size until they become invisible under the light microscope. The nuclear diameter decreases, and the chromatin becomes increasingly more dense until the nucleus presents a pyknotic appearance and is finally extruded from the cell. There is a gradual decrease in the number of polyribosomes (basophilia), followed by a simultaneous increase in the amount of hemoglobin (acidophilia) within the cytoplasm, and the mitochondria gradually disappear.

There are from three to five intervening cell divisions between the proerythroblast and the mature erythrocyte. The development of an erythrocyte from the first recognizable cell of the series to the release of reticulocytes into the blood takes approximately 7 days. The hormone erythropoietin and substances such as iron, folic acid, and vitamin B₁₂ are essential for the production of erythrocytes. Erythropoietin is a glycoprotein produced in the kidneys that stimulates the mRNA for globin, the protein component of the hemoglobin molecule.
Fig. 3. Scheme of erythropoiesis.

1 – Stem cell of blood; 2 – polipotent cell of myelopoiesis (CFU-GEMMg); 3 – unipotent progenitor cell (BFU-E);
4 – unipotent progenitor cell (CFU-E); 5 – proerythroblast; 6 – basophilic erythroblast; 7 – polychromatophilic erythroblast; 8 – orthochromatophilic erythroblast; 9 – reticulocyte; 10 – erythrocyte.

Fig. 4. Structure of erythroid islet of Red Bone Marrow. A-scheme. B- transmission electron micrograph, mag. ×7000.

1 – macrophage; 2 – unipotent progenitor cell (CFU-E); 3 – proerythroblast; 4 – basophilic erythroblast; 5 – polychromatophilic erythroblast; 6 – orthochromatophilic erythroblast; 7 – reticulocyte; 8 – erythrocyte; 9 – erythropoietin; 10 – biosynthesis of protein; 11 – synthesis of the hemoglobin; 12 – expelling of the nucleus; 13 – endothelium of the capillary.
Differentiation

The differentiation and maturation of erythrocytes involve the formation (in order) of proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts, orthochromatophilic erythroblasts (normoblasts), reticulocytes, and erythrocytes.

The first recognizable cell in the erythroid series is the proerythroblast. It is a large cell with loose, lacy chromatin and clearly visible nucleoli; its cytoplasm is basophilic. The next stage is represented by the basophilic erythroblast, with a strongly basophilic cytoplasm and a condensed nucleus that presents no visible nucleolus. The basophilia of these two cell types is caused by the large number of polyribosomes involved in the synthesis of hemoglobin. During the next stage, polyribosomes decrease and areas of the cytoplasm begin to be filled with hemoglobin. Staining at this stage causes several colors to appear in the cell – the polychromatophilic (Gr. polys, many, + chroma, color, + philein, to love) erythroblast. In the next step, the nucleus continues to condense and no cytoplasmic basophilia is evident, resulting in a uniformly acidophilic cytoplasm – the orthochromatophilic (Gr. orthos, correct, + chroma, + philein) erythroblast. At a given moment, this cell puts forth a series of cytoplasmic protrusions and expels its nucleus, encased in a thin layer of cytoplasm. The remaining cell still has a small number of polyribosomes that, when treated with the supravital dye brilliant cresyl blue, aggregate to form a stained network. This cell is the reticulocyte, which soon loses its polyribosomes and becomes a mature red blood cell (erythrocyte).

MATURATION OF GRANULOCYTES (fig. 5, 6)

Myeloblast is the most immature recognizable cell in the myeloid series. It has a finely dispersed chromatin, and nucleoli can be seen. In the next stage, the promyelocyte (L. pro, before, + Gr. Myelos, marrow, + cytos, cell) is characterized by its basophilic cytoplasm and azurophilic granules. These granules contain lysosomal enzymes and myeloperoxidase. The promyelocyte gives rise to the three known granulocytes. The first sign of differentiation appears in the myelocytes where specific granules gradually increase in quantity and eventually occupy most of the cytoplasm. These neutrophilic, basophilic, and eosinophilic myelocytes mature with further condensation of the nucleus and a considerable increase in their specific granule content. The neutrophilic granulocyte presents an intermediate stage whose nucleus has the form of a curved rod (band cell). This cell appears in quantity in the blood with strong stimulation of hematopoiesis.

The appearance of large numbers of immature neutrophils (band cells) in the blood is called a shift to the left and is clinically significant, usually indicating bacterial infection.

KINETICS OF NEUTROPHIL PRODUCTION.

The total time taken for a myeloblast to emerge as a mature neutrophil in the circulation is about 11 days. Under normal circumstances, five mitotic divisions occur in the myeloblast, promyelocyte, and neutrophilic myelocyte stages of development.

Neutrophils pass through several functionally and anatomically defined compartments.

The medullary formation compartment can be subdivided into a mitotic compartment (~ 3 days) and a maturation compartment (~ 4 days).

A medullary storage compartment acts as a buffer system, capable of releasing large numbers of mature neutrophils upon demand. Neutrophils remain in this compartment for about 4 days.

The circulating compartment consists of neutrophils suspended in plasma and circulating in blood vessels. The marginating compartment is composed of neutrophils that are present in blood but do not circulate. These neutrophils are in capillaries and are temporarily excluded from the circulation by vasoconstriction, or – especially in the lungs – they may be at the periphery of vessels, adhering to the endothelium and not in the main bloodstream.

The marginating and circulating compartments have equal size, and there is a constant interchange of cells between them. The life-span of a neutrophil in these two compartments is 6-7 hours. The medullary formation and storage compartments together are about 10 times as large as the circulating and marginating compartments.
Fig. 5. Scheme of granulocytopoiesis.

1 – stem cell of blood; 2 – oligopotent progenitor cell of neutrophils and monocytes (CFU-GEMMg); 3 – oligopotent progenitor cell of neutrophils and monocytes (CFU-GM); 4 – unipotent progenitor cell of eosinophils (CFU-Eo); 5 – myeloblast; 6 – eosinophilic promyelocyte; 7 – eosinophilic myelocyte; 8 – eosinophilic metamyelocyte; 9 – eosinophilic band cell; 10 – eosinophilic segmented granulocyte; 11 – unipotent progenitor cell of neutrophils (CFU-GN); 12 – neutrophilic promyelocyte; 13 – neutrophilic myelocyte; 14 – neutrophilic metamyelocyte; 15 – neutrophilic band cell; 16 – neutrophilic segmented granulocyte; 17 – unipotent progenitor cell of basophils (CFU-B); 18 – basophilic promyelocyte; 19 – basophilic myelocyte; 20 – basophilic granulocyte.

Fig. 6. Differentiation of cells during the granulocytopoiesis. A – scheme; B – histological specimen, mag. × 600.

1 – myeloblast; 2 – promyelocyte; 3 – neutrophilic myelocyte; 4 – neutrophilic metamyelocyte; 5 – neutrophilic band cell; 6 – neutrophilic segmented granulocyte; 7 – erythrocyte.
Neutrophils and other granulocytes enter the connective tissues by passing through intercellular junctions found between endothelial cells of capillaries and postcapillary venules (diapedesis). The connective tissues form a fifth compartment for neutrophils, but its size is unknown. Neutrophils reside here for 1-4 days and then die, whether or not they have performed their major function of phagocytosis.

Changes in the number of neutrophils in the peripheral circulation must be evaluated by taking all these compartments into consideration. Thus, neutrophilia, an increase in the number of neutrophils in the circulation, does not necessarily imply an increase in neutrophil production. Intense muscular activity or the administration of epinephrine causes neutrophils in the marginating compartment to move into the circulating compartment, causing an apparent neutrophilia even though neutrophil production has not increased.

Neutrophilia may also result from liberation of greater numbers of neutrophils from the medullary storage compartment. This type of neutrophilia is transitory and is followed by a recovery period during which no neutrophils are released.

The neutrophilia that occurs during the course of bacterial infections is due to an increase in neutrophil production and a shorter stay of these cells in the medullary storage compartment. In such cases, immature forms such as band cells, neutrophilic metamyelocytes, and even myelocytes may appear in the blood-stream. The neutrophilia that occurs during infection is of longer duration than that which occurs as a result of intense muscular activity.

MATURATION OF LYMPHOCYTES & MONOCYTES (fig. 8)

Study of the precursor cells of lymphocytes and monocytes is difficult because these cells do not contain specific cytoplasmic granules or the nuclear lobulation that is present in granulocytes, both of which facilitate the distinction between young and mature forms. Lymphocytes and monocytes are distinguished mainly on the basis of size, chromatin structure, and the presence of nucleoli in smear preparations. As lymphocyte cells mature, their chromatin becomes more compact, nucleoli become less visible, and the cells decrease in size. In addition, subsets of the lymphocytic series acquire distinctive cell-surface receptors during differentiation that can be detected by immunocytochemical techniques.

Lymphocytes.

Circulating lymphocytes originate mainly in the thymus and the peripheral lymphoid organs (spleen, lymph nodes, tonsils, etc). It is probable, however, that all lymphocyte progenitor cells originate in the bone marrow. Some of these relatively undifferentiated lymphocytes migrate to the thymus, where they acquire the attributes of T lymphocytes. Subsequently, T lymphocytes populate specific regions of peripheral lymphoid organs. Other bone marrow lymphocytes remain in the marrow, differentiate into B lymphocytes, and then migrate to peripheral lymphoid organs where they inhabit and multiply in their own special compartments.

The first identifiable progenitor of lymphoid cells is the lymphoblast, a large cell capable of incorporating \(^{3}\text{H}\)-thymidine and dividing two or three times to form prolymphocytes. These latter cells are smaller and have relatively more condensed chromatin but none of the cell-surface antigens that mark prolymphocytes as T or B lymphocytes. In the thymus or bone marrow, these cells synthesize cell-surface receptors characteristic of their lineage, but they are not recognizable as distinct cell types using routine histologic procedures. The distinction is made by using immunocytochemical techniques.

Monocytes.

The monoblast is a committed progenitor cell that is virtually identical to the myeloblast in its morphology. Further differentiation leads to the promonocyte, a large cell (up to 18 mm in diameter) with a basophilic cytoplasm and a large, slightly indented nucleus. The chromatin is lacy, and nucleoli are evident. Promonocytes divide twice in the course of their development into monocytes. A large amount of rough endoplasmic reticulum is present, as is an extensive Golgi complex in which granule condensation can be seen to be taking place. These granules are primary lysosomes, which are observed as fine azurophilic granules in blood monocytes. Mature monocytes enter the bloodstream, circulate for about 8 hours, and then enter the connective tissues, where they mature into macrophages and function for several months. Abnormal bone marrow can produce diseases based on cells derived from that tissue. Leukemias are malignant clones of white blood cell precursors. They occur in lymphoid tissue (lymphocytic leukemias) and in bone marrow (myelogenous and monocytic leukemias).
Fig. 7. Neutrophilic myelocyte. Transmission electron micrograph, mag. × 15000.

1 – nucleus; 2 – Golgi complex; 3 – primary azurophilic granules; 4 – specific granules; 5 – microvilli.

Fig. 8. Scheme of monocytopoiesis.

1 – stem cell of blood; 2 – polypotent cell of myelopoiesis (CFU-GEMMg); 3 – oligopotent progenitor cell of neutrophils and monocytes (CFU-GM); 4 – unipotent progenitor cell of monocytes (CFU-M); 5 – monoblast; 6 – promonocyte; 7 – monocyte.

IL-3 CSF-GM CSF-M

Fig. 9. Scheme of lymphocytopoiesis.

1 – stem cell of blood; 2 – polypotent cell of lymphopoiesis; 3 – oligopotent progenitor cell; 4 – unipotent progenitor cell; 5 – lymphoblast; 6 – prolymphocyte; 7 – small lymphocyte; 8 – large lymphocyte.
In these diseases, there is usually a release of large numbers of immature cells into the blood. The symptoms of leukemias are a consequence of this shift in cell proliferation, with a lack of some cell types and excessive production of others (which are often abnormal in function). The patient usually exhibits anemia and is prone to infection.

A clinical technique helpful in the study of leukemias and other bone marrow disturbances is bone marrow aspiration. A needle is introduced through compact bone (usually the sternum) and a sample of marrow is withdrawn. The sample is spread on a microscope slide and stained. The use of labeled monoclonal antibodies specific to proteins in the membranes of precursor blood cells aids in identifying cell types derived from these stem cells and contributes to a more precise diagnosis of the various possible types of leukemia.

ORIGIN OF PLATELETS (fig. 10,11,12)

In adults, the platelets originate in the red bone marrow by fragmentation of the cytoplasm of mature megakaryocytes (Gr. megas, big, + karyon + kytos) These, in turn, arise by differentiation of the megakaryoblasts.

Megakaryoblasts.

The megakaryoblast is 15-50 mm in diameter and has a large ovoid or kidney-shaped nucleus with numerous nucleoli. The nucleus becomes highly polyploid (it contains up to 30 times as much DNA as a normal cell) before cytoplasmic differentiation begins. The cytoplasm of this cell is homogeneous and intensely basophilic.

Megakaryocytes.

The megakaryocyte is a giant cell (35-150 mm in diameter) with an irregularly lobulated nucleus, coarse chromatin, and no visible nucleoli. The cytoplasm contains numerous mitochondria, a well-developed rough endoplasmic reticulum, and an extensive Golgi complex. Alpha granules and vesicles containing lysosomal enzymes (lambda granules) develop from Golgi vesicles and cisternae. With maturation of the megakaryocyte, numerous invaginations of the plasma membrane ramify throughout the cytoplasm, forming the demarcation membranes. This system defines areas of the megakaryocyte’s cytoplasm that will be shed as platelets.

In certain forms of thrombocytopenic purpura, a disease in which the number of blood platelets is reduced, the platelets appear bound to the cytoplasm of the megakaryocytes, indicating a defect in the liberation mechanism of these corpuscles. The life span of these corpuscles was found to be approximately 10 days.

Fig. 10. Scheme of thrombopoiesis.

1 – Stem cell of blood; 2 – polipotent cell of myelopoiesis (CFU-GEMMg); 3 – unipotent progenitor cell (CFU-Mg); 4 – megakaryoblast; 5 – promegakaryocyte; 6 – megakaryocyte; 7 – platelets.
Fig. 11. Megakaryocyte in red bone marrow. Histological specimen, mag. × 600.

1 – megakaryocyte; 2 – lumen of vessel with erythrocytes; 3 – hemopoietic cells.

Fig. 12. Scheme of formation of platelets.

1 – megakaryocyte; 2 – areas of cytoplasm of megakaryocyte; 3 – platelets; 4 – lumen of the vessel.
### Table 1: Cells of Hemopoiesis

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<th>Stem cells</th>
<th>Progenitor cells</th>
<th>Precursor cells</th>
<th>Mature cells</th>
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**Stem Cells:**
- BFU-E (Bone Marrow Units for Early Erythroblasts)
- CFU-E (Colony Forming Units-Erythroid)
- CFU-G (Colony Forming Units-Granulocyte)
- CFU-Meg (Colony Forming Units-Megakaryocyte)
- CFU-Eosinophil
- CFU-Basophil

**Progenitor Cells:**
- Myeloblast
- Megakaryoblast
- Basophilic erythroblast
- Polychromatophilic erythroblast
- Orthochromatophilic erythroblast
- Erythroblast
- Promyelocyte
- Promonocyte
- Myeloblast
- Basophil
- Neutrophil
- Monocyte
- Eosinophil
- Basophil

**Precursor Cells:**
- Proerythroblast
- Basophilic erythroblast
- Polychromatophilic erythroblast
- Orthochromatophilic erythroblast
- Erythroblast
- Promyelocyte
- Promonocyte
- Myeloblast
- Basophil
- Neutrophil
- Monocyte
- Eosinophil
- Basophil

**Mature Cells:**
- Erythrocyte
- Reticulocyte
- Eosinophil
- Basophil
- Neutrophil
- Monocyte
LESSON 5

THEME: CONNECTIVE TISSUES. CELLS

BACKGROUND: connective tissues are the part of the tissues of internal environment and include: fibrous connective tissues, connective tissues with special properties, skeletal and hemopoietic tissues.

Loose fibrous connective tissue is the type of fibrous connective tissues. It forms the internal environment of the organism, provides homeostasis, takes part in different metabolic processes, protective reactions, performs the plastic function during the wound healing and adaptation to changing environment. This kind of specialization provides the leading part of connective tissue in realization of physiological processes, adaptation and compensation processes, which is why changes in it can cause changes in organism functioning.

GENERAL AIM OF STUDY: to be able to identify connective tissues, differentiate loose connective tissue.

THEORETICAL QUESTIONS:
2. Loose connective tissue: location, structure (cells, intercellular matrix) and functions.
3. General characteristic of the cells of loose CT: fibroblasts, macrofages, mast cells, plasmocytes, pigmentocytes, adipocytes, pericytes, leukocytes.
4. The role of cellular elements of connective tissue in the protective reactions of the organism. The idea of macrophage system of the organism.

Development. Connective tissues are derived from mesoderm or mesectoderm (for the head) of the embryo, via an intermediate stage called mesenchyme.

Mesenchyme is embryonic tissue, which consists of cells and jelly-like matrix. In later development, the cells and extracellular matrix (ECM) become specialized for various tasks, and the matrix comprises amorphous “ground substance” reinforced to greater or lesser extent by specialized fibers.

Mesenchymal cell:
- has a similar appearance to a small, young fibroblast with star-like shape
- has processes which are connected with other cells
- has high nucleo-cytoplasmic ratio

Structure. Connective tissues consist of cells and extracellular matrix which includes fibers and ground substance.

Functions of connective tissues
1. Mechanical and protective - supporting, restraining, binding, separating, directing and padding.
2. Transport of nutrients, metabolites, and signalling factors.
3. Storage of energy-rich lipids, water and electrolytes.
4. Defence against pathogenic organisms.
5. Repair of damage to itself, and organs supported or enclosed, by fibrosis - the formation of irregular collagenous scar tissue.
6. Thermogenesis (brown fat) and insulation (white fat).

TYPES OF CONNECTIVE TISSUES
Based upon: (a) the density and order of fiber packing; and (b) the predominant cell and fiber types; c) functions.
1. Fibrous (proper) CT
   - Loose fibrous (collagenous) tissue
   - Dense fibrous (collagenous) tissue
Two kinds:
... (a) Regular,
... (b) Irregular
2. CT with special properties
   - Adipose tissues – white and brown
   - Mucous/mucoid/primitive connective tissue
   - reticular
   - pigment
3. Hematopoietic (myeloid and lymphoid)
4. Skeletal
   - cartilage and bone.

LOOSE CONNECTIVE TISSUE
Widely distributed in human body.
Includes numerous and various cells and extracellular matrix
Matrix is composed of abundant ground substance and few thin fibers (fig. 1)
Rich in vessels and nerves
Main function – trophic, defence, regeneration.

Fig. 1. Scheme of loose fibrous connective tissue structure: A - a three-dimensional diagram; B - drawing from the histological specimen, mag. × 600.

1 - fibroblast; 2 - fibrocyte; 3 - macrophage; 4 - mast cell; 5 - lymphocyte; 6 - neutrophilic granulocyte; 7 - plasma cell; 8 - adipocyte; 9 - pericyte.

CELLS OF CONNECTIVE TISSUES
The cells of supporting tissue are derived from precursor cells in primitive supporting tissue (*mesenchyme*) and may be divided into several types, each with different functions. A dominant common function is synthesis and maintenance of extracellular matrix material (fig. 2).

Mechanocytes:
- The most common support cell is termed the *fibroblast* which is responsible for secreting the extracellular matrix in most tissues.
- *Chondrocytes* and *osteocytes* are responsible for secreting the extracellular matrix in cartilage and bone respectively.
- *Myofibroblasts* have a contractile function as well as a role in secretion of extracellular matrix.
A group of highly modified support cells are responsible for the storage and metabolism of fat. These are known as adipocytes and may collectively form adipose tissue.

Cells with defence and immune functions are commonly encountered in the support tissues. This includes:
- the mast cells,
- tissue macrophages,
- all types of white blood cells and
- antibodysecreting plasma cells. Some of these cells migrate into support tissues and remain static, performing their local function. Other immune cells migrate through support tissue and are en route to perform their function elsewhere.

**Fig. 2. Scheme of loose fibrous connective tissue structure.**

1 - fibroblast; 2 - macrophage; 3 - plasma cell; 4 - lymphocyte; 5 - mast cell; 6 - neutrophilic granulocyte; 7 - adipocyte; 8 - endothelial cell; 9 - pericyte; 10 - collagen fibers; 11 - elastic fibers.

**CHARACTERISTICS OF FIBROBLAST**
- Occurs in young active, and adult quiescent/less active forms.
- The cell is elongated, and often sends out processes to take on a more elongated or stellate form, fixed to fibers of matrix.
- Young has abundant, basophilic cytoplasm, with a well-developed Golgi complex and GER for protein and proteoglycan synthesis.
- Nucleus is ovoid, with weakly staining chromatin granules.
- Adult fibroblasts (fibrocytes) have smaller, darker nuclei, and very little cytoplasm. They remain fixed and squashed into a spindle/cigar form amongst the fibres that they formed (fig. 3).
Function of fibroblast:
- forming and remodelling collagen, reticular and elastic fibres, and the ground substance.

The remodelling requires the production of destructive enzymes, and inhibitors to help restrain their action. TIMPs - Tissue Inhibitors of MetalloProteinases - are an example.

In some sites, e.g., the periodontal ligament holding the teeth in place, the fibroblasts destroy fibers more aggressively, in the process of matrix turnover.

Young fibroblasts, aside from making fibers, may in some circumstances (e.g., wound repair) take on some smooth-muscle characteristics, and become contractile myofibroblasts, which contribute to the disabling contractures of some scar tissue.

Macrophage/histiocyte
- An ovoid or spheroid cell, which may change its shape while lying alongside fibers, or when extending pseudopodia to move and ingest materials (fig. 4).
- Nucleus is smaller and more condensed than that of the active fibroblast.
- Cytoplasm is pale with little GER, but has many lysosomes, when digesting phagocytosed material.

Functions of macrophage:
- Phagocytoses dead cells, cell debris, live and inert foreign bodies.
- Coordinates the inflammatory response and healing by means of signalling peptides and proteins - cytokines, e.g., IL-1, TGF-b (Chapter 8.F).
- Macrophages may fuse to become foreign-body giant cells with many nuclei, when faced with a large object for digestion.

Macrophage/reticuloendothelial/mononuclear phagocyte system (MPS)
1. Comprises cells related directly to blood monocytes, or derived from the same precursor in marrow.
2. A tentative division of the macrophage-system cells recognizes:
Phagocytic antigen-presenters
(a) Macrophages of connective tissues and serous cavities.
(b) Alveolar macrophages/lung dust cells.
3. The phagocytic group (i.e., the original reticulo-endothelial series) can be revealed by *vital injection* (into the living animal) of colloidal or particulate coloured matter, e.g., Trypan blue or India ink, which the phagocytic cells of the system preferentially accumulate in their cytoplasm, thereby identifying themselves. Nowadays, MPS cells are distinguished by their cell-surface glycoprotein profiles.

4. The molecules of II Class Major Histocompatibility Complex are expressed on the surface of macrophages to recognize antigen and make it possible to present it to lymphocytes.

3. Mast cell (fig. 5).
   - Spheroid or ovoid with a small central nucleus, and its cytoplasm packed with dense basophilic granules.
   - Granules give a *metachromatic* staining reaction with thionine or toluidine blue, i.e., a reddish-purple colour, because they contain a sulphated polysaccharide - *heparin*.
     - Heparin is an anticoagulant for blood, first obtained from the liver (hepar), but it also inhibits vascular smooth muscle proliferation and some immune complement reactions. As a polyanion, it can pack materials, e.g., the trypsin-like enzyme, tryptase, in the granules.
     - *Histamine*, increasing capillary permeability, is also present in the granules. The chemokines also released can then more easily attract white blood cells out of the vessels.
     - Many stimuli (e.g., antigens and agents released by lymphocytes during an immune response) activate a release of the granule contents, from this “mobile-pharmacy” cell, with its many chemical mediators.

(c) Macrophages of lymph nodes, spleen and bone marrow.
(d) Kupffer sinusoid-lining cells of liver.

*Weakly phagocytic antigen-presenters*
(e) Dendritic and interdigitating reticulum cells of lymphoid tissues.
(f) Langerhans cells of epidermis and other epithelia.

*Specialized* (Some not phagocytic? Some not antigen-presenters?)
(g) Foreign-body giant cells.
(h) Microglia cells of CNS.
(i) Synovial A cells lining joints.
(j) Osteoclasts resorbing bone.

---

3. The phagocytic group (i.e., the original reticulo-endothelial series) can be revealed by *vital injection* (into the living animal) of colloidal or particulate coloured matter, e.g., Trypan blue or India ink, which the phagocytic cells of the system preferentially accumulate in their cytoplasm, thereby identifying themselves. Nowadays, MPS cells are distinguished by their cell-surface glycoprotein profiles.

4. The molecules of II Class Major Histocompatibility Complex are expressed on the surface of macrophages to recognize antigen and make it possible to present it to lymphocytes.

3. Mast cell (fig. 5).
   - Spheroid or ovoid with a small central nucleus, and its cytoplasm packed with dense basophilic granules.
   - Granules give a *metachromatic* staining reaction with thionine or toluidine blue, i.e., a reddish-purple colour, because they contain a sulphated polysaccharide - *heparin*.
     - Heparin is an anticoagulant for blood, first obtained from the liver (hepar), but it also inhibits vascular smooth muscle proliferation and some immune complement reactions. As a polyanion, it can pack materials, e.g., the trypsin-like enzyme, tryptase, in the granules.
     - *Histamine*, increasing capillary permeability, is also present in the granules. The chemokines also released can then more easily attract white blood cells out of the vessels.
     - Many stimuli (e.g., antigens and agents released by lymphocytes during an immune response) activate a release of the granule contents, from this “mobile-pharmacy” cell, with its many chemical mediators.
- Mast cells favour positions in CT close to veins (MCt subtype), and at dermal and mucosal interfaces with the hostile environments of the skin, airway, and gut (MCtc subtype).

- The mast cell subtypes in human differ depending on the proteases that they contain:
  
  *MCt* cells have mast-cell tryptase and are involved directly with defence.
  
  *MCtc* cells contain chymase, cathepsin G, and other proteases, in addition to mast-cell tryptase, and are more involved in adaptation and remodelling responses of blood vessels and CT.

4. Plasma cell (fig. 6).

1. Arised from B-lymphocytes after antigen-dependent proliferation and differentiation
2. Many tissues, particularly those whose lining tracts open to the external environment, are not immunologically virgin, but have been exposed to foreign organisms that have provoked immune responses by local CT plasma cells and lymphocytes. A lamina propria may have many of both and some eosinophils, e.g., in the gut.

3. Plasma cells are ovoid, roughly 10 µm in length, with an *eccentrically* placed nucleus having its denser chromatin granules clumped regularly around the nuclear membrane (clock-face appearance).

4. Cytoplasm is deeply *basophilic* from the rich GER, except for a pale central region where the Golgi complex lies.

5. Proteins synthesized by plasma cells in lymphoid organs reach the plasma as *immunoglobulins/antibodies*, inactivating foreign invaders, e.g., viruses.

6. Plasma cells in CT make antibodies for local use, e.g., in the airway or gut, to counter toxins and control microbial populations.

Fig. 6. Plasma cells of lymph node. Histological specimens: A – mag. × 320, B – mag. × 900.

*1 - plasma cells; 2 - reticular cell; 3 - lymphocyte.*
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Representative Product or Activity</th>
<th>Representative Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast, chondroblast, osteoblast</td>
<td>Production of fibers and ground substance</td>
<td>Structural</td>
</tr>
<tr>
<td>Plasma cell</td>
<td>Production of antibodies</td>
<td>Immunological (defense)</td>
</tr>
<tr>
<td>Lymphocyte (several types)</td>
<td>Production of immunocompetent cells</td>
<td>Immunological (defense)</td>
</tr>
<tr>
<td>Eosinophilic leukocyte</td>
<td>Participation in allergic and vasoactive reactions, modulation of mast cell activities and the inflammatory process</td>
<td>Immunological (defense)</td>
</tr>
<tr>
<td>Neutrophilic leukocyte</td>
<td>Phagocytosis of foreign substances, bacteria</td>
<td>Defense</td>
</tr>
<tr>
<td>Macrophage</td>
<td>Secretion of cytokines and other molecules, phagocytosis of foreign substances and bacteria, antigen processing and presentation to other cells</td>
<td>Defense</td>
</tr>
<tr>
<td>Mast cell and basophilic leukocyte</td>
<td>Liberation of pharmacologically active molecules (eg, histamine)</td>
<td>Defense (participate in allergic reactions)</td>
</tr>
<tr>
<td>Adipose (fat) cell</td>
<td>Storage of neutral fats</td>
<td>Energy reservoir, heat production</td>
</tr>
</tbody>
</table>
LESSON 6

THEME: CONNECTIVE TISSUES. EXTRACELLULAR MATRIX. FIBERS OF CONNECTIVE TISSUES

THEORETICAL QUESTIONS
1. Intercellular matrix of loose connective tissue: structure, development, functions.
2. Collagen, elastic and reticular fibers.
3. Collagen fibers: structure, development, biochemical characteristic, properties, importance.
4. Ground substance of the loose CT: structure, histochemical characteristic, physical and chemical properties, functions.
5. Organ-depending features of the structure of loose CT.
6. Physiological and reparative regeneration of loose CT.
7. Age-related features of loose CT.

THERE ARE THREE TYPES OF FIBERS IN CT: COLLAGEN, ELASTIC AND RETICULAR.

Collagen is the main fiber type found in most supporting tissues and is the most abundant protein in the human body. Its most notable function is the provision of tensile strength. Collagen is secreted into the extracellular matrix in the form of tropocollagen which consists of three polypeptide chains (alpha chains) bound together to form a helical structure 300 nm long and 1.5 nm in diameter (fig. 1). In the extracellular matrix, the tropocollagen molecules polymerise to form collagen. At least 27 different types of collagen (designated by Roman numerals I-XXVII) have now been delineated on the basis of morphology, amino acid composition and physical properties.

Collagen types:
- Fibril-forming - I, II, III, V, XI
- Network-forming – IV, VIII, X
- Beaded filament-forming - VI
- Anchoring filament-forming - VII
- FACIT (Fibril-Associated Collagens with InterrupTed helices) - IX, XII, XIV, XVI, XIX
- Nonsecreted transmembrane – XIII, XVII*, XV, XVIII
- Basement membrane zone - XV, XVIII
- * transmembrane collagen XVII is a component of hemidesmosomes. An autoimmune reaction to it can cause poor epidermal adhesion and hence skin blistering in humans.

- **Type I collagen** is found in fibrous supporting tissue, the dermis of the skin, tendons, ligaments and bone, in a variable arrangement from loose to dense according to the mechanical support required. The tropocollagen molecules are aggregated to form fibrils strengthened by numerous intermolecular bonds. Parallel collagen fibrils are further arranged into strong bundles 2-10 µm in diameter which confer great tensile strength to the tissue; these bundles are visible with the light microscope.

- **Type II collagen** is found in hyaline cartilage and consists of fine fibrils which are dispersed in the ground substance.

- **Type III collagen** makes up the fiber type known as reticulin which was previously thought to represent a separate type of fiber because of its affinity for silver salts. Reticulin fibres form the delicate branched “reticular” supporting meshwork in highly cellular tissues such as the liver, bone marrow and lymphoid organs.

- **Type IV collagen** does not form fibrils but rather a meshlike structure and is an important constituent of basement membranes.

- **Type VII collagen** forms anchoring fibrils that link to basement membrane

Levels of molecular and structural organization of collagen

*Collagen molecule and fibril-formation*

- (a) The collagen molecule is made up of three intertwined and cross-linked helical chains of amino acids, with glycine, proline and hydroxyproline prominent amongst them. Dependent on the amino-acids, a chain may be a 1, types I or II, or a 2, etc. Triplet combinations of these and other chains furnish around 19 types of collagen.
· (b) Procollagen is made in the GER of the fibroblast, osteoblast, etc. and cleaved to 300 nm collagen at its release from the cell.

· (c) Fibril-formation takes place immediately outside the cell, by self-assembly followed by a crosslinking, a process that is crucial to strength.

· (d) Fibrils farther from the cells are thicker (30-300 nm), so presumably the initially thin fibrils can thicken by additions from soluble collagen to the orderly crystalline array.

· (e) Various enzymes, e.g., collagenase, break down collagen molecules.

· (f) Man can achieve man-made fibrous strength by repeated spinning, e.g., for hawsers to tow ships: cells can only spin inside themselves at the molecular level of trimers, thereafter strength comes solely from crosslinking the molecules and the diverse glycoprotein and FACIT-collagen binders.

Fig. 1. Stages of the collagen fibers synthesis.
Fibrils
· (a) Fibrils show a characteristic, complex cross-banding pattern regularly along their length with a periodicity of 67 nm (64 nm when shrunken).
· (b) Chemical dissolution of collagen followed by chemical manipulation results in a reforming of fibrils displaying a longer periodicity - a long-spacing collagen, believed to comprise rearranged collagen molecules.
· (c) The fundamental unit is a sequence of 234 amino acids, 67 nm long. These units in differently organised arrays account for the various banding patterns of artificially and naturally produced collagens, including the main natural collagen molecule with a length of 300 nm.
· (d) In the natural shorter-spacing fibril, these 300 nm-long units lie end to end, side by side, but organised in such a way so as to produce regions of greater and lesser density with the 67 nm periodicity.

Fibers (fig. 2)
· (a) Fibers are long, wavy or straight, and colourless.
· (b) They have great tensile strength and resistance to stretching, whilst retaining considerable flexibility.
· (c) Fibers are made up of finer fibrils packed together.

Fig. 2. Collagen fibers. Histological specimens: A - loose connective tissue, mag. × 320; B - dense connective tissue, mag. × 320.

1 - intercellular substance; 2 - bundles of collagen fibers; 3 - ground substance; 4 - cell nucleus; 5 - blood vessel.

Collagen staining
(a) Collagen (type I) often is present in bulk, and is stained selectively by: aniline blue in Mallory’s method, light green in Masson’s, or red acid fuchsin in van Gieson’s. (Eosin stains it orange.)
(b) Mallory’s, Masson’s and van Gieson’s trichrome methods distinguish collagen from muscle, and also react with the nuclei and cytoplasm of other cells.

DISEASES DUE TO DISORDERS OF COLLAGEN
There are several inherited diseases caused by mutations in genes coding collagen. The main effect is reduced tensile strength in supportive tissues leading to abnormal tissue laxity or susceptibility to injury.

Ehlers-Danlos syndromes are characterised by abnormal skin laxity and hypermobility of joints which can predispose to recurrent joint dislocations. There are several genetic subtypes of disease and six main forms have been described characterised by distinct clinical associations. In some individuals, disease is caused by mutation in a collagen gene or in an enzyme related to collagen metabolism.

In addition to these disorders, several diseases develop as a result of 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, causing hardening and functional impairment of the implicated organs. Keloid is a local swelling caused by abnormal amounts of collagen that
form in scars of the skin. Keloids, which occur most often in individuals of black African descent, can be a troublesome clinical problem to manage; not only can they be disfiguring, but excision is almost always followed by recurrence.

Vitamin C (ascorbic acid) deficiency leads to scurvy, a disease characterized by the degeneration of connective tissue. Without this vitamin, fibroblasts synthesize defective collagen, and the defective fibers are not replaced. This process leads to a general degeneration of connective tissue that becomes more pronounced in areas in which collagen renewal takes place at a faster rate. The periodontal ligament that holds teeth in their sockets has a relatively high collagen turnover; consequently, this ligament is markedly affected by scurvy, which leads to a loss of teeth. Ascorbic acid is a cofactor for proline hydroxylase, which is essential for the normal synthesis of collagen. Table 5–4 lists a few examples of the disorders caused by failure of collagen biosynthesis.

Collagen renewal is in general a very slow process. In some organs, such as tendons and ligaments, the collagen is very stable, whereas in others, as in the periodontal ligament, the turnover of collagen is very high. To be renewed, the collagen must first be degraded. Degradation is initiated by specific enzymes called collagenases that cut the collagen molecule into two parts that are susceptible to further degradation by nonspecific proteases (enzymes that degrade proteins).

**Reticular fibers**

1. Collagen fibres, running parallel to one another, do not join up with others running in different direction. Such an arrangement is seen, however, in reticular fibers, which form a network or reticulum.

2. Reticular fibers stain black with reduced silver methods, hence their other names - argyrophil or argentophil. H and E and some trichrome stains leave them unstained (fig. 3).

3. X-ray diffraction and EM show them to be like fine collagen fibres, having the same 67 nm-repeating crossbanding. Furthermore, they appear first at many sites, as in mesenchyme and healing wounds, where collagen fibres will later form. Thus reticular fibres are an immature, fine kind of collagen fibre, mostly of type III collagen.

4. They persist into the adult in several organs, where a fine fibrous support is needed that does not interfere with a close relation between fixed cells and blood or lymph, e.g., in endocrine glands.

5. Reticular fibers fasten to the underside of basal laminae of epithelia and endothelium, and bind and secure muscle and nerve fibers, using their external laminae.

**Elastic fibers** (fig. 4).

1. May be fine, single and branching in areolar CT, or thick and parallel in elastic ligaments. Walls of blood vessels have incomplete elastic membranes.

2. The elastic nature of the fibres is shown by the spiralling and kinking of their recoiled broken ends, in spread specimens.

3. Elastic fibres and membranes, if thick, stain pink with eosin, or red with Masson’s method; otherwise,

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**Table 1. Examples of Clinical Disorders Resulting from Defects in Collagen Synthesis.**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Defect</th>
<th>Symptoms</th>
</tr>
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<tbody>
<tr>
<td>Ehlers–Danlos type IV</td>
<td>Faulty transcription or translation of type III</td>
<td>Aortic and/or intestinal rupture</td>
</tr>
<tr>
<td>Ehlers–Danlos type VI</td>
<td>Faulty lysine hydroxylation</td>
<td>Augmented skin elasticity, rupture of eyeball</td>
</tr>
<tr>
<td>Ehlers–Danlos type VII</td>
<td>Decrease in procollagen peptidase activity</td>
<td>Increased articular mobility, frequent luxation</td>
</tr>
<tr>
<td>Scurvy</td>
<td>Lack of vitamin C (cofactor for proline hydroxylase)</td>
<td>Ulceration of gums, hemorrhages</td>
</tr>
<tr>
<td>Osteogenesis imperfecta</td>
<td>Change of one nucleotide in genes for collagen type I</td>
<td>Spontaneous fractures, cardiac insufficiency</td>
</tr>
</tbody>
</table>
they remain unseen, unless elastic stains, e.g., orcein or Verhoeff's, are used.

4. In bulk, unstained, they appear yellow to the naked eye.

5. Formation and nature - fibroblasts and vascular smooth muscle cells form and release two components: (a) fine protein microfibrils thought to orient (b) tropoelastin as it polymerizes into amorphous elastin. With little structure in EM, elastin is a network of long protein chains held in a springy arrangement crosslinked by desmosines, each derived from four lysines of the protein amino-acid chains.

Fig. 3. Section of an adrenal cortex, silver stained to show reticular fibers. This is a thick section made to emphasize the networks formed by these fibers, which consist of collagen type III. Nuclei are black, and cytoplasm is unstained. Medium magnification.

Fig. 4. Elastic fibers (A) and membranes (B). Histological specimens: A - staining by iron haematoxylin, mag. × 600; B – orcein stain, mag. × 280.

1 - elastic fibers; 2 - elastic membrane; 3 - the nucleus of cells.
GROUND SUBSTANCE

Ground substance takes its name from being an amorphous transparent material which has the properties of a semifluid gel. Tissue fluid is loosely associated with ground substance, thereby forming the medium for passage of molecules throughout supporting tissues and for the exchange of metabolites with the circulatory system (fig. 5).

Fig. 5. Intercellular matrix of loose fibrous connective tissue. Electron micrograph, mag. × 160000.

1 - collagen fibrils; 2 - elastic fibril; 3 - proteoglycans of ground substance.

Ground substance consists of a mixture of long, unbranched polysaccharide chains of seven different types, each composed of repeating disaccharide units. One of the disaccharide units is usually a uronic acid and the other an amino sugar (either N-acetyl glucosamine or N-acetyl galactosamine) thus giving rise to the modern term glycosaminoglycans (GAGs); these were formerly called mucopolysaccharides. The glycosaminoglycans are acidic (negatively charged) due to the presence of hydroxyl, carboxyl and sulphate side groups on the disaccharide units.

Hyaluronic acid is the predominant GAG in the loose supporting tissues and is the only one without sulphate side groups; the other GAGs (chondroitin4sulphate, chondroitin6sulphate, dermatan sulphate, heparan sulphate, heparin sulphate and keratan sulphate) differ from hyaluronic acid in that they are covalently linked to a variety of protein molecules to form proteoglycans (formerly known as mucoproteins); these proteoglycans are huge molecules consisting of 90-95% carbohydrate. Further, the proteoglycans may form noncovalent links with hyaluronic acid chains to form even larger molecular complexes.

Proteoglycan chemistry - from a long protein backbone molecule, many long sugar side chains stick out, because negative charges along each chain repel adjacent chains and each other. The chains are composed of repeating pairs of sugar/saccharide units. Each pair has an hexosamine and a uronic acid. The loss of hydrogen ions from the many acids in the chain of glycosaminoglycans (GAGs) leaves negative charges, only some of which are neutralized by counterions such as Na⁺.

Nomenclature - the many linked sugars of the side-chains are polysaccharides, hence with the protein backbone the general name - “protein-polysaccharide”. However, this also describes glycoproteins, for example, mucoproteins and mucopolysaccharides. Proteoglycans differ from glycoproteins in: their core proteins; the use of fewer species of sugar; lack of branching of the sugar chains; and usually their longer sugar chains, and more acidic/negative character

Proteoglycan varieties - dependent on the specific sugars, and the sites of sulphation, if any:
- Hyaluronate - soft connective tissues; synovial fluid; vitreous humour;
Dermatan sulphate (chondroitin sulphate B) - skin and corneal CT; 
Keratan sulphate - cartilage matrix; 
Chondroitin-4-sulphate (A) - cartilage matrix; 
Chondroitin-6-sulphate (C) - cartilage matrix; 
Heparin (also sulphated) - granules of mast cell and basophil.

**Staining** - the failure of counterions to neutralize all anions leaves regions of **high negative charge density**. If the proteoglycan is prevented from dissolving out, its reactions are:

- (a) **basophilic** with basic stains, e.g. in hyaline cartilage;
- (b) positive with **Alcian blue** and Hale’s iron;
- (c) **metachromatic**, e.g., with toluidine blue, where the blue molecules, in binding to the closely spaced anions, interfere with each other, so that their effect on light becomes a **red** one, e.g., in mast cell granules.

**Physical properties** - the high negative charge:

- (a) attracts cations, and restricts the movement of water, ions and molecules (and bacteria), thus controlling transport through the CT to the cells and other tissues;
- (b) structures the matrix, making it gel-like, or even firmer in cartilage, where the proteoglycans are themselves strung along a hyaluronic acid backbone;
- (c) binds CT fibers to one another, influencing their strength and functioning;
- (d) acts as a reservoir for growth factors and other agents controlling cell behaviour.

In summary, ground substance is basically composed of glycosaminoglycans in the form of hyaluronic acid and proteoglycans. These huge molecules are entangled and electrostatically linked to one another and their water of hydration, to form a flexible gel through which metabolites may diffuse. The size of the spaces between the GAG molecules and the nature of electrostatic changes determine the permeability characteristics of every particular supporting tissue, a fact of particular significance in the structure of basement membranes. The mechanical properties of ground substance are reinforced by the fibrous proteins of the extracellular tissue to which the components of ground substance are also bound.

**Overview of proteoglycans (PGs) and glycoproteins in connective tissues**

1. The large PG monomer molecules may be aggregated by being strung along a **hyaluronate backbone**, by means of a **link protein** for the core protein-HA attachment.

   PG aggregation produces huge molecules extending over micrometres, and visible with conventional TEM. Proteoglycans amenable to such assembly are **aggrecans**, susceptible to breakdown by aggrecanase. However, the chemical nature and heterogeneity of monomers and their aggregates make study of these important matrix constituents difficult.

   Note that proteoglycans are also kept **within** some cells to work with other molecules.

2. The glycosaminoglycan side chains of proteoglycans vary in number, nature and length. Combinations of **sulphated** and **non-sulphated** hexosamines, and relatively tissue-specific core proteins, yield a diversity of PGs, crudely classifiable by molecular size into large and small:

   **LARGE**
   - Chondroitin-6-sulphate, skeletal keratan sulphates - Cartilage
   - Versican/Fibroblast PG - Soft CTs
   - Cell-surface-associated, e.g., the membrane-attached PGs **syndecans**, with heparan-sulphate and chondroitin-sulphate chains, and the HSPGs - **glypicans** - on epithelial and other cells
   - Basement-membrane heparan-sulphate **PGs** - basement membranes, e.g., **perlecan**

   **SMALL**
   - Decorin/PGII (chondroitin/dermatan sulphates) - extracellular matrix
   - Biglycan/PG-S1 («) - associated with a variety of cells including non-CT ones
   - Fibromodulin (keratan sulphate)
   - Dermatan sulphate
   - Small bone proteoglycans **I & II**
**Non-collagenous GLYCOPROTEINS of connective tissues** include: Fibronectin, Tenascin, Thrombospondin, Bone sialoprotein/BSPII, Osteopontin/BSPI, Osteonectin/Bone Gla protein, Cartilage-matrix protein, Alkaline phosphatase, Chondronectin, and Fibrillin.

They interact with other macromolecules and influence cell behaviour.

One clinical aspect is their use as urinary or serum *markers* of excessive turnover, e.g., Gla protein for bone disease.

*Fibrillin* is a crucial component of elastic fibres and other structures in CTs; and genetic defects in its formation result in the weak arterial walls, poorly suspended eye lens, lax ligaments, etc. of Marfan’s syndrome.

**Fibronectin** and **Tenascin**

- (a) Forms of the glycoprotein, fibronectin, occur in CT matrixes, basal laminae and blood plasma.
- (b) Fibronectin is a multiple *adhesive*, since various domains of the molecule bind glycosaminoglycans, collagen, fibrin, and some cells.
- (c) Made by fibroblasts and available from blood, it helps in the *scaffold-building*, and *cellular migrations* and *attachments*, which give tissues their microarchitecture during embryogenesis and wound repair.
- (d) *Tenascin* shares some structure with fibronectin, but plays its part more during *development*, e.g., at sites of epithelial-mesenchymal interaction. It reappears in malignant tumours.

*Anti-adhesive* macromolecules provide another control of cell interactions with the ECM. Tenascin and decorin are respectively glycoprotein and proteoglycan examples.

**Healing and repair**

Following damage to cells and tissues there is an inflammatory response which is responsible for eliminating the damaging agents and clearing away dead tissues.

Repair to damaged tissues is mainly delivered by supportive cells from connective tissues. Briefly, there is a local proliferation of mesenchymal cells from the margins of residual normal tissue to form fibroblasts and myofibroblasts. These grow out to replace the area of tissue damage. This proliferation is also associated with growth of new capillary blood vessels to supply nutrients. The fibroblasts and myofibroblasts secrete extracellular matrix material, ground substance and collagen, to replace the damaged area by fibrocollagenous material. This is the basis of the formation of a collagenous scar. Over time there is remodelling of collagen to maximise strength of collagen and link it to adjacent tissues.

This process is termed **healing by repair**. Specialised tissues damaged by disease, such as muscle or lung, are replaced by strong but nonfunctional **collagenous scar**.
LESSON 7

THEME: DENSE CONNECTIVE TISSUES. CONNECTIVE TISSUES WITH SPECIAL PROPERTIES

BACKGROUND. Connective tissues are widely distributed in human organism, form tendons, inner organ capsule, microenvironment for hematopoietic cells, participate in nutrients and pigments metabolism and termoregulation. Understanding of structure and functions of different connective tissues structure is necessary for interpreting mechanisms of pathological processes in diabetes, obesity etc.

AIM OF STUDY: To interpret structure and functions of dense connective tissues and tissues with special properties.

THEORETICAL QUESTIONS:
1. Dense connective tissues. Classification, location, structure and functions.
2. Tendon histophysiology.
3. Reticular tissue.
7. Mucous tissue.
8. Pigment tissue.

Instruction of self-training

- Dense fibrous (collagenous) tissue
  (a) Regular, e.g., tendon, ligament, aponeurosis, fascia, with collagen fibres oriented to take stress principally in one direction.
  (b) Irregular, e.g., dermis, organ capsules, periosteum, perichondrium, epitendineum, with irregular, interwoven bundles of collagen. Dense CT – regular and irregular (fig. 1)

Fig 1. Structure of different types of dense fibrous connective tissue: A – regular, mag. × 480; B – irregular, mag. × 600.

1 – bundles of collagen fibers in longitudinal section; 2 – bundles of collagen fibers in transversal section; 3 – layers of ground substance; 4 – nuclei of fibrocytes; 5 – nuclei of fibroblasts.
Dense connective tissue is adapted to provide resistance and protection. It consists of the same components found in loose connective tissue, but there are fewer cells and a clear predominance of collagen fibers. Dense connective tissue is less flexible and far more resistant to stress than is loose connective tissue. It is known as dense irregular connective tissue when the collagen fibers are arranged in bundles without a definite orientation. The collagen fibers form a three-dimensional network in dense irregular tissue and provide resistance to stress from all directions. This type of tissue is seen in areas such as dermis.

The collagen bundles of dense regular connective tissue are arranged according to a definite pattern. The collagen fibers of this tissue are aligned with the linear orientation of fibroblasts in response to prolonged stresses exerted in the same direction; consequently they offer great resistance to traction forces.

Tendons are the most common example of dense regular connective tissue. These elongated cylindrical structures attach striated muscle to bone; due to their richness in collagen fibers, they are white and inextensible. They have parallel, closely packed bundles of collagen separated by a small quantity of intercellular ground substance. Their fibrocytes contain elongated nuclei parallel to the fibers and sparse cytoplasmic folds that envelop portions of the collagen bundles. The cytoplasm of these fibrocytes is rarely revealed in H&E stains, not only because it is sparse but also because it stains the same color as the fibers.

Most supporting tissues contain cells which are adapted for the storage of fat; these cells, called adipocytes, are derived from primitive mesenchyme where they develop as lipoblasts. Adipocytes are found in isolation or in clumps throughout loose supporting tissues or may constitute the main cell type as in adipose tissue.

There are two main types of adipose tissue:

· White adipose tissue. This type of adipose tissue comprises up to 20% of total body weight in normal, wellnourished male adults and up to 25% in females. It is distributed throughout the body particularly in the deep layers of the skin. In addition to being an important energy store, white adipose tissue acts as a thermal insulator under the skin and functions as a cushion against mechanical shock in such sites as around the kidneys (fig. 2).

· Brown adipose tissue. This highly specialised type of adipose tissue is found in newborn mammals and some hibernating animals, where it plays an important part in body temperature regulation. Only small amounts of brown adipose tissue are found in human adults (fig. 3).
Fat cell/adipocyte (fig. 4).
1. A genuinely fattened cell, initially resembling a fibroblast with a few droplets in the cytoplasm. Fat cells are enclosed in basal lamina, and held on a framework of reticular fibres in association with many blood capillaries.
2. For the white or yellow unilocular fat seen in adult man, the droplets (mainly glycerides of fatty acids) coalesce and more fat is added,
3. Until the nucleus is bulged to one side of a spheroid cell up to 200 µm in diameter, distended by a huge droplet.
4. Cytoplasm, with a Golgi complex, ER and mitochondria, is present as an attenuated peripheral shell.
5. The cell is static, but its content is not. The stored fat is participating in the body’s carbohydrate and fat metabolism.
6. Fat in the usual wax-embedded section is dissolved out, but with osmium tetroxide fixation it remains and is black. Some dyes colour it, if it is preserved by frozen sectioning.
7. Besides a number of adipocyte-specific enzymes for fat metabolism, fat cells secrete leptin, which helps to control energy balance and body fat mass.

Fig. 4. Ultramicroscopic structure of different types of lipocytes. Electron microgram: A – white, mag. × 15000; B – brown, mag. × 35000.
1 – cytoplasm; 2 – drops of lipids; 3 – mitochondria.

Stored fat within adipocytes is derived from three main sources: dietary fat circulating in the bloodstream as chylomicrons; triglycerides synthesised in the liver and transported to blood; and triglycerides synthesised from glucose within adipocytes. Adipose tissue is often regarded as an inactive energy store, however it is an extremely important participant in general metabolic processes where it acts as a temporary store of substances for the energyderiving processes of almost all tissues. Adipose tissue, therefore, generally has a rich blood supply. The rate of fat deposition and utilisation within adipose tissue is largely determined by dietary intake and energy expenditure, but a number of hormones and the sympathetic nervous system profoundly influence the fat metabolism of adipocytes.

In addition to their energystorage role adipocytes have an important endocrine role. Through secretion of several proteins adipocytes modulate energy metabolism and influence general metabolism in coordination with hormones such as insulin to regulate body mass. Adipose tissue is responsible for the secretion of several proteins, collectively known as adipocytokines. These include leptin, adipsin, resistin, adiponectin, tumor necrosis
factor alpha, and plasminogen activator inhibitor type 1.

Adipocytes have receptors for insulin, glucocorticoids, growth hormone and noradrenalin (norepinephrine) that modulate uptake and release of fat. Adipocytes secrete the hormone leptin that is involved in regulation of appetite.

**BROWN ADIPOSE TISSUE**

1. Seen in the human newborn; in adults BAT is detectable after adrenergic stimulation. Brown fat might dissipate surplus energy from overeating. Found around the thorax and kidneys of animals naturally exposed to severe cold, particularly hibernators.

2. Cells have many separate (multilocular) fat droplets, relatively more cytoplasm, and are smaller than white fat cells. The cytoplasm of brown adipocytes is crammed with mitochondria which have numerous, closely packed cristae. These mitochondria are extremely rich in cytochromes, part of the electron transport chain involved in oxidative energy production; this accounts for the brown colour of brown adipose tissue when examined macroscopically.

3. Brown adipose tissue is involved in nonshivering thermogenesis, an increase in metabolic activity induced by cold stress. Brown fat is a thermogenic organ providing a prompt and direct source of heat to maintain the temperature of vital organs.

4. Brown adipose tissue is characterised by expression of a unique uncoupling protein called UCP1 (thermogenin). Uncoupling protein 1 lets mitochondria divert energy in this otherwise unwanted thermal way by uncoupling respiration from ATP formation.

5. Unlike the metabolism of other tissues, in brown adipose cells the process of electron transport is readily uncoupled from the phosphorylation of ADP to form ATP. The energy derived from oxidation of lipids, and energy released by electron transport in the uncoupled state, is dissipated as heat which is rapidly conducted to the rest of the body by the rich vascular network of brown adipose tissue. Note the intimate association of capillaries with the brown adipocyte.

6. Using these metabolic processes, neonatal humans and other mammals utilise brown adipose tissue to generate body heat during the vulnerable period after birth. Brown adipose tissue undergoes involution in early infancy and in adult humans is found only in restricted sites such as around the adrenal gland and great vessels in retroperitoneal fat. The production of heat by brown adipose tissue is controlled directly by the sympathetic nervous system.

**OBESITY**

Among affluent societies there is concern about the rapid increase in prevalence of obesity - the so-called “obesity epidemic”. A syndrome has been characterised termed “the metabolic syndrome” which comprises abdominal obesity, lipid changes in blood, high blood pressure, insulin resistance, and a proinflammatory/prothrombotic state. The obesity epidemic has been attributed to the rising prevalence of metabolic syndrome which in turn is a major contribution to the development of cardiovascular diseases such as atheroma. Adipocytes are not merely fat storage cells and have complex metabolic roles including the ability to secrete a variety of wideacting cytokines. Factors which increase the mass of adipocytes are believed to contribute to the development of the metabolic syndrome. Obesity has been mainly linked to environmental factors such as overeating and physical inactivity. Obesity has also been linked to genetic factors. In rare familial cases obesity has been caused by mutations in leptin, leptin receptor, pros hormone convertase, proopiomelanocortin or melanocortin4 receptor. Sporadic human obesity has been linked to several genetic loci.

**RETICULAR TISSUE**

The very delicate reticular tissue forms three-dimensional networks that support cells. Reticular tissue is a specialized loose connective tissue consisting of reticular fibers intimately associated with specialized fibroblasts called reticular cells. Reticular tissue provides the architectural framework that creates a special microenvironment for hematopoietic organs and lymphoid organs (bone marrow, lymph nodules and nodes, and spleen). The reticular cells are dispersed around this framework and partially cover the reticular fibers and ground substance with cytoplasmic processes. The resulting cell-lined trabecular system creates a spongelike structure within which cells and fluids are freely mobile.

The fibres are made by some of the stellate reticular cells acting as fibroblasts. Certain highly cellular
tissues, such as lymph nodes and the haemopoietic cords of bone marrow, have a supporting framework of reticulin fibres upon which are draped cells with long cytoplasmic processes morphologically similar to primitive mesenchymal cells; these cells are traditionally described as reticulum or reticular cells (fig. 5).

Some if not all of these cells are probably responsible for synthesis of the reticulin framework, being thus similar to fibroblasts; many of these cells, if not all, may also exhibit considerable phagocytic activity. Immunocytochemistry, EM, and enzymatic analysis distinguish at least three kinds of reticular cell: fibroblastic, and two phagocytic kinds - interdigitating (T-zone:) and dendritic (B-zone: antigen-presenting). Because of the close structural and functional association of these cell types with the haemopoietic, macrophagemonocyte and immune systems, they were thought to represent a single functional system.

**Mucous/mucoid/primitive connective tissue**

Mucous tissue very rich in proteoglycans and water, has some fine collagen fibres and widely separated young fibroblasts. As Wharton’s jelly of the umbilical cord it encloses and cushions the vessels; the ocular vitreous and young dental pulp also fit tolerably well in this class (fig. 6).

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![Fig. 5. Arrangement of reticular cells and fibers: A – light microscopy, B – ultrastructure.](image)

1 – reticular cells; 2 – processes of reticular cells; 3 – network of reticular fibers.

![Fig. 6. Mucous tissue of umbilical cord.](image)

1 – nuclei of fibroblasts; 2 – extracellular matrix.
LESSON 8

THEME: SKELETAL TISSUES. CARTILAGE

BACKGROUND: Skeletal tissues (cartilage and bone) are the types of connective tissue. Cartilage tissues form respiratory system, joints and intervertebral discs, etc. They consist of cells from chondrocytes differon and a large amount of intercellular substance, which is characterized by high hydrophilicity, turgor and elasticity. Blood vessels, large protein molecules, including antigens cannot penetrate into the cartilage, which prevent the development of cartilage transplant rejection. Study of the structure and function of skeletal tissues, their characteristics in different periods of ontogeny are necessary to detect pathologic changes that underlie the pathogenesis of the locomotor apparatus.

AIM OF STUDY: To be able to determine the types of cartilage in histological specimens, interpret their structural and functional features.

TO ACHIEVE THIS AIM ONE HAS TO (practical procedures):
1. Identify the source of cartilage.
2. Know the general features of the structural organization of cartilage, classification.
3. Distinguish in histological specimen variety of cartilage, tissue elements, interpret the structural features of tissue elements of cartilage in relationship to the nature of biomechanical loads.
4. Distinguish the basic structural components of cartilage as organs of locomotion apparatus.
5. Interpret patterns of growth, regeneration, age-related changes of cartilage.

TO ACHIEVE THE AIM IT IS NECESSARY TO CENTRE AROUND THE FOLLOWING POINTS:
1. Sources and stages of development, the general morpho-functional characteristics, the classification of skeletal tissues.
3. Differon of cartilage cells.
5. Cartilage as an organ: perichondrium, areas of young and mature cartilage. Age-related changes in cartilage.

WHAT MUST YOU KNOW? (INSTRUCTION FOR YOUR SELF-LEARNING)

CARTILAGE

A specialized CT to resist compression, and provide modest rigidity with flexibility, by having its cells, chondrocytes, produce a firm resilient matrix of ground substances, and fibres or fibrils. The rapid growth of cartilage assists the growth of bones and the repair of fractures.

Key morphological features:
- consist of cells and extracellular matrix, the extracellular matrix predominates and determines cartilage’s mechanical properties (type II collagen is a characteristic cartilage matrix component),
- the cells are embedded in the matrix cavities (lacunae) singly or in isogenous groups of 2-8 cells derived from one parent cell,
- vascular supply: most cartilage is enveloped by a layer of connective tissue - the perichondrium.

Classification of cartilage.

Depending on the composition of the matrix, three kinds are distinguished:
- hyaline,
- elastic,
- fibrocartilage.

DISTRIBUTION OF THE THREE CARTILAGE VARIETIES.
1. Hyaline - articular surface of most synovial joints; costal cartilages; nasal and respiratory tract cartilages; basis of most of the fetal skeleton; fracture callus.
2. Elastic - external ear, pharyngotympanic tube, epiglottis, and some laryngeal and bronchiolar cartilages.
3. Fibrocartilage - intervertebral disc’s annulus fibrosus (around a nucleus pulposus of notochordal
HYALINE CARTILAGE

1. **Occurs** fused with bone or as discrete pieces, looking hyaline/translucent (glass-like) to the unaided eye. Most surfaces, except joint/articular ones, are covered by a nutritive CT *perichondrium/capsule* with collagen and elastic fibres, fibroblasts and blood vessels. It merges gradually via a chondrogenic zone with the cartilage proper.

2. **Matrix**, apparently amorphous with HE staining in LM, contains:
   - (a) Ground substances rich in soluble collagens and the proteoglycan - *aggrecan*: a core protein with chondroitin and keratan sulphates side chains, and a link protein for attachment to hyaluronic acid. The aggregated proteoglycans impart basophilic and metachromatic staining properties, and *bind water* and confer resilience.
   - (b) Collagen fibrils visible in EM; and LM after silver impregnation, digestion of the ground substance, or in polarized light. The reinforcing fibrils are oriented in some relation to stresses experienced by the cartilage.

3. **Chondrocytes** or cartilage cells are large and rounded, each lying in a space *- lacuna -* enclosed by matrix. Cells often are grouped in nests of 2, 4, or 6 as a result of mitoses and restricted cellular movement. EM reveals cells to have short stubby processes, fat droplets, glycogen and the GER and Golgi complex appropriate for secretion of the matrix components: proteoglycans, *type II collagen* [with the homotrimeric molecule a1(II)3], and glycoproteins (fig. 1).

Fig. 1. The structure of hyaline cartilage: A - scheme; B - histological specimen, hematoxylin and eosin staining, mag. × 600.

1 - *perichondrium*; 2 - *hyaline cartilage*; 3 - fibrous layer; 4 - chondrogenic layer; 5 - chondroblast; 6 - young chondrocytes; 7 - mature cartilage cells; 8 - isogenic groups; 9 - intercellular matrix.

CARTILAGE AS ORGAN includes 3 ZONES:

1) Perichondrium – a layer of dense connective tissue (although cells in the inner layer of the perichondrium resemble fibroblasts, they are chondroblasts and easily differentiate into chondrocytes).

2) Growing zone – single chondroblasts has elliptic shape, parallel to the surface.

3) Mature cartilage - chondrocytes are embedded in the matrix cavities (lacunae) singly or in isogenous groups of 2-8 cells; the matrix immediately surrounding the chondrocytes, called the territorial matrix, is the more basophilic than the interterritorial matrix.
Growth occurs in two ways:

· (a) **Appositional/perichondral** by the recruitment of fresh cells, chondroblasts, from perichondral stem cells, and the addition of new matrix to the surface;

· (b) **Interstitial** by the mitotic division of, and deposition of more matrix around, chondrocytes already established in the cartilage.

Growth is vulnerable to X-rays, poor nutrition, and disturbed blood supply, for example, from fractures at the growth plate.

**Territories** of mature cartilage.
Most noticeably in articular cartilage there are:
1) the **chondron** - the chondrocyte and the pericellular matrix immediately around it;
2) proteoglycan-rich **territorial matrix** outside the chondron;
3) **interterritorial matrix**, lying between the territorial matrices.

The matrix of the chondron has its own profile of special collagens, proteoglycans, and cartilage glycoproteins, whereas the differences between territorial and interterritorial matrices are more quantitative, and related to collagen fibril thickness and orientation (fig. 2).

**Nutrition** - cartilage is **avascular** and no blood vessels serve the matrix directly, but cartilage canals may carry vessels through the matrix to non-cartilaginous regions, e.g., secondary ossification centres. Therefore, nutriment and wastes must **diffuse** through the matrix for the cells to stay alive and perform their slow turnover of the matrix macromolecules. The diffusion may break down and various degenerations then occur, e.g., calcification. This last is prompted, organized and made use of in the process of endochondral ossification.

**Elastic Cartilage**
Is more opaque and flexible than the hyaline kind, but the cells are similar in appearance and distribution; and it occurs as separate pieces with a perichondrium.

Matrix is permeated by many **elastic fibres** that can be selectively stained by stains such as orcein or Verhoeff’s. The matrix is not prone to degeneration and calcification (fig. 3).

**Fibrocartilage**
In the intervertebral (IV) disc, fibrocartilage at first appears to have a rather disorderly matrix with many thick collagen fibres, amongst which are dispersed only a few chondrocytes in lacunae. However, the fibres are orderly in their alternating orientations and layering, like the burst-resisting fibres of an old-style bias-ply car tyre.
The matrix gives the staining reaction of collagen, mostly type I, except for close around the cells where proteoglycans are abundant.

Lacks a perichondrium and is not seen as discrete pieces; rather it is a strong tension-resistant, but flexible transitional tissue located between tendon and bone, bone and bone, hyaline cartilage and hyaline cartilage (fig. 4).

In the IV disc, the enclosed central nucleus pulposus is not cartilage, but nevertheless has collagen type II, which diminishes in the innermost layers of the annulus fibrosus as it is replaced by type I.
LESSON 9

THME: SKELETAL TISSUES. BONE

BACKGROUND: Bone tissue is a type of connective tissue. It consists of the cells and extracellular matrix, 70% of which are inorganic compounds (mostly calcium phosphate). Bone tissue, which forms the skeleton bones, performs support-mechanical function. Bone tissue is the depot of calcium and phosphorus. Morphological and functional properties of bone vary with age and physical activity of a person depending on different conditions of nutrition, the influence of endocrine glands, innervation, etc. Study of the structure and function of skeletal tissues, their characteristics in different periods of ontogeny are necessary for detecting pathologic changes of the locomotor apparatus.

AIM OF STUDY: to be able to determine the types of bone tissue in histological preparations, interpret their structural and functional features.

TO ACHIEVE THIS AIM ONE HAS TO (practical procedures):
1. Identify the source of embryologic development of bone tissue.
2. Learn the general features of the structural organization of bone tissues, and their classification.
3. Distinguish variety of bone tissues in histological specimens, their tissue elements, interpret the structural features of tissue elements of bone tissue in relationship to the nature of biomechanical loads.
4. Learn the basic structural components of bones as organs of locomotion apparatus.
5. Interpret patterns of growth, regeneration, age-related changes of bone tissues.

TO ACHIEVE THE AIM IT IS NECESSARY TO CENTRE AROUND THE FOLLOWING POINTS:
1. Bone tissue: classification, location, morpho-functional characteristics.
2. Differon of osteocytes and osteoclasts. Structure, function.
3. Extracellular matrix of bone tissue. Physical and chemical characteristics.
4. Lacunae-canicular system of bone tissue.
5. Woven and lamellar bone tissue: location and morphological features. Osteon - structural and functional unit of bone.
6. Bone as an organ: the periosteum (periosteum), compact and spongy zone, endosteum: structure, role in the trophic, growth and regeneration. Comparative characteristics of flat and long bones.

WHAT MUST YOU KNOW? (INSTRUCTION FOR YOUR SELF-LEARNING)

Bone is a hard CT with cells, osteocytes, distributed in the abundance of matrix. It serves for support, attachment, leverage, protection and mineral storage.

To obtain great strength and rigidity with some elasticity, the matrix is composed of densely packed collagen fibrils infiltrated with bone mineral as fine crystals of calcium salts resembling hydroxyapatite crystals. Mineral constitutes about 65 per cent of the dry weight of bone. The densely packed collagen fibrils are primarily type I. There are small amounts of distinctive non-collagenous proteins, e.g., calcium-binding osteocalcin and bone sialoproteins.

Matrix is strong but dense, thus nutritive fluids cannot diffuse freely through it. Osteocytes therefore have to differ from chondrocytes in having many long processes extending through canaliculi (narrow passages) and making contact with one another and, indirectly, with blood vessels. The cell body lies in a cavity, a lacuna, in the matrix.

Throughout life, because of maintaining mineral homeostasis, and its features of growth, bone remains in an unending turnover, with selective destruction and replacement - the remodelling process.

CLASSIFICATIONS OF BONE
1. Based on the size of the spaces within the bone, and its trabecular (lattice-like) or dense nature:
   (a) Cancellous/spongy/trabecular
   (b) Compact/dense
2. Based on the presence or absence of lamellae (layers) and osteons/Haversian systems:
   (a) Woven/primitive
   (b) Lamellar/Haversian

Woven bone's matrix has disorderly fibrils, whereas in lamellar bone the fibrils of a lamella share a predominant orientation. Note that a particular bone will have areas of woven and lamellar bone, depending on how far remodelling has involved all regions (fig. 1).

Fig. 1. The general principle of structural organization of bone tissue: A - woven/primitive; B - lamellar/Haversian.

1 - interlacing bundles of collagen fibers; 2 - osteocytes; 3 - contacts of osteocytes processes; 4 - bony lamellae; 5 - parallel bundles of collagen fibers.

HAVERSIAN SYSTEM OF BONE

A Haversian system is roughly cylindrical and arranged around one or two small vessels in a central Haversian canal.

Osteocytes and bone lamellae making up the system are disposed in 4-20 concentric rings centred on the canal.

A lamella is the territory formed and maintained by the osteocytes lying in a ring when seen in a cross-section. From the orderliness of the fibrils, lamellae can be distinguished in polarized light, but it is only in a smaller unit, the domain, that SEM reveals the fibrils to be aligned in the same direction.

Haversian canals branch and join up with others. Their vessels originally entered the bone from the periosteum or marrow via Volkmann's canals, around which osteocytes are not especially ordered.

MATURE HUMAN BONE (fig. 2)

Studied from the outside working inwards has:
1. Periosteum of dense CT is divided into:
   · (a) an external fibrous layer of collagen and elastic fibres, fibroblasts, other cells, vessels and nerves; and
· (b) an inner cambial layer of bone cells, mostly resting osteoblasts.

2. **Dense cortical bone**. In the wide parts, e.g., femoral shaft, this layering is often present:
   · (a) *external circumferential/basic lamellae* lie outside;
   · (b) the main thickness with many osteons of various generations (primary, secondary, etc); *interstitial lamellae* fill the chinks between osteons and are lamellae of earlier osteons that have been spared form total erosion;
   · (d) *endosteal/internal circumferential lamellae* lie to the inside, with their osteocyte bodies lying parallel with the inner surface.

   In practice, some areas of dense bone remain woven or primary and are not replaced by this classic lamellar architecture.

3. **Cancellous medullary bone** whose trabeculae are lined by a thin cellular *endosteum* and have some lamellae, but can be sustained by marrow blood vessels without the need for Haversian canals.

4. **Marrow cavities** lie between trabeculae, inside the tubular shaft, or in the diploic spaces of flat skull bones.

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**Fig. 2.** Scheme of a long bone diaphysis structure.

1 - periosteum; 2 - external circumferential/basic lamellae; 3 - osteons; 4 - intercalated plates; 5 - endosteal/internal circumferential lamellae; 6 - endosteum; 7 - channel with a blood vessel; 8 - bone lamellae; 9 - bone lacune; 10 - bone canaliculi.

**BONE CELLS**

1. **Osteoblast** (fig. 3 A)
   - Lies on the surfaces of bone, in a one-cell thick layer, as most of the endosteum and inner periosteum.
   - May be in two states:
     · (a) *active*, forming bone matrix, with a large Golgi complex and much GER in a plump cell, appearing in LM to have a pale juxtanuclear vacuole (Golgi) in the basophilic cytoplasm;
· (b) *resting* or bone-maintaining; small cell with a dark nucleus and flattened against the bone.
- Forms the collagen, glycoproteins, and proteoglycans of the matrix, and controls the deposition of mineral crystals on the fibrils.

2. **Osteocyte** (fig. 3 B)
- Osteoblast becomes an osteocyte by forming matrix around itself and becoming buried or immured.
- Young osteocyte thus resembles an active osteoblast; older ones have smaller, flattened bodies.
- Processes extending from the body down the canaliculi are not visible by LM; but EM shows that osteocytes, like osteoblasts, remain connected by gap junctions.
- The mature osteocyte is involved in maintaining the matrix of its territory. SEM evidence puts into doubt the proposal that osteocytes can resorb bone by osteolysis. Lacunae empty of osteocytes indicate dead bone.

![Fig. 3. Scheme of the osteoblast (A) and osteocytes (B) ultramicroscopic structure.](image)

1 - nucleus; 2 - endoplasmic reticulum; 3 - Golgi complex; 4 - mitochondria; 5 - cytolemma; 6 - cytoplasmic processes; 7 - osteoid tissue; 8 - intercellular matrix of bone tissue.

3. **Osteoclast** (fig. 4)
- Large, multinuclear cell, with a pale acidophilic cytoplasm.
- Lies on the surface of bone, often in an eaten-out hollow - *Howship’s lacuna*.
- Cell surface is attached to the bone by podosomes to create a sealed compartment against the bone, in which the moving long cell processes of the *ruffled border* can agitate the resorbing - bone-destroying - materials.
- Cytoplasm has vacuoles and lysosomes, since the mechanism of bone resorption is partly an *enzymatic digestion*, by cathepsins and collagenase, and also from *acid* made by an osteoclastic proton pump.
- In dense bone, many osteoclasts act together to erode *resorption tunnels*, which are later partially filled in with lamellar bone to become osteons.

4. **Bone cell dynamics**

Skeletal growth, changes of shape, and the physiological responses of bone need changes in the populations of “blasts and “clasts.

These rely on a proliferation of osteoblasts or a precursor, while osteoclasts come from the fusion of blood-derived monocytes, which also participate indirectly in the bone resorption as macrofages.

The osteoprogenitor cell is a small, organelle-poor cell on the surface or lying just behind the osteoblasts.

It might be just an inactive osteoblast: that it is more of a stem cell is proved by the fact that it occasionally becomes chondroblastic, e.g., in tumours and fracture repair.
JOINTS

1. **Synarthroses** (poorly movable)
   1. **Syndesmosis**. Bones linked by dense fibrous CT, e.g., a skull suture, which may be replaced by bone with increasing age to become a *synostosis*.
   2. **Synchondrosis**. Bones linked by cartilage, e.g., pubic symphysis.

2. **Diarthrosis** (movable)
   1. **Articular cartilage**, usually hyaline, covers the moving bone ends, and is nourished and lubricated by *synovial fluid*.
   2. **Joint capsule** of dense irregular fibrous CT, continuous with the periostea, encloses a joint space for synovial fluid.
   3. **Nervous joint receptors** for proprioception are in the capsule.
   4. **Synovial membrane**: lines the capsule; a cellular layer, with macrophage (A/M) and fibroblastic (B/F) cells, lies on a loose vascular CT, sometimes thrown up into folds, *synovial villi*. The cells make lubricating hyaluronic acid and glycoproteins, and determine the nature of the cartilage-sustaining synovial fluid.
   5. **Articular cartilage layers**: although the cartilage is not thick, variation in the amounts and arrangements of proteoglycans and collagen with depth distinguishes these layers:
      - superficial/tangential
      - intermediate/transitional
      - deep/radial
      - calcified/mineralized, attached to the “subchondral” bone.

   A lamina splendens is at the free surface of the superficial layer: below it the collagen fibrils are better organized, in a packed series of “leaves” that curve up from the radial layer, run parallel with the surface superficially, then descend to the radial layer.


![Fig. 4. Scheme of the osteoclasts ultramicroscopic structure.](image)
Lesson 10

Theme: Bone Formation

Background: Bone tissue is a constituent part of the locomotor apparatus. The processes of remodeling, resorption and formation of the osteons vary with age and depend on embryonic development. Knowledge of histogenesis, histophysiology and regeneration of bone, their characteristics in different periods of ontogeny are important and necessary for traumatologists to understand the pathogenesis and treat pathologic changes in bone.

Aim of Study: Able to determine the features of direct and indirect osteogenesis, interpret mechanisms of development of flat and long bones.

To achieve this aim one has to (Practical procedures):
1. Identify the way of development of bone tissue.
2. Explain the general features of the direct osteogenesis – formation of the flat bones of skull from mesenchyme.
3. Distinguish the bone structures developed from mesenchyme in histological specimens.
4. Identify the general features of the indirect osteogenesis – formation of the long bones of the axial skeleton on the place of hyaline cartilage model.
5. Distinguish different stages of endochondral ossification – formation of the primary and secondary centers of ossification in histological specimens.

To achieve the aim it is necessary to centre around the following points:
1. Embryonic and postembryonic osteogenesis.
2. Direct osteogenesis.
3. Indirect osteogenesis.
4. Hyaline cartilage model formation.
5. The primary center of ossification formation.
6. The secondary center of ossification formation in the diaphyses.
7. The general features of secondary center of ossification formation in epiphyses.
8. Regulation of osteogenesis.

What must you know? (Instruction for your self-learning)

The development of a bone is traditionally classified as endochondral (indirect) or intramembranous (direct).

The bones of the extremities and those parts of the axial skeleton that bear weight (e.g., vertebrae) develop by endochondral ossification. The flat bones of the skull and face, the mandible, and the clavicle develop by intramembranous ossification.

Direct bone formation involves the following events:
1. Mesenchymal cells, condensed into primary ossification centers, differentiate into osteoblasts, which begin secreting osteoid.
2. As calcification occurs, osteoblasts are trapped in their own matrix and become osteocytes. These centers of developing bone are called trabeculae (fused spicules).
3. Fusion of the bony trabeculae produces spongy bone as blood vessels invade the area and other undifferentiated mesenchymal cells give rise to the bone marrow.
4. The periosteum and endosteum develop from portions of the mesenchymal layer that do not undergo ossification.
5. Mitotic activity of the mesenchymal cells gives rise to osteoprogenitor cells, which undergo cell division and form more osteoprogenitor cells or differentiate into osteoblasts within the inner layer of the developing periosteum.

In intramembranous ossification, bone is formed by differentiation of mesenchymal cells into osteoblasts (fig. 1, 2).
Fig. 1. Direct osteogenesis: A – scheme; B – histological specimen, mag. × 56.

1 – mesenchymal cells; 2 – blood vessels; 3 – ossification centre; 4 – osteoblasts; 5 – osteocytes; 6 – calcified extracellular matrix; 7 – trabecules.

Fig. 2. Structure of bone trabecule formed by direct osteogenesis: A – scheme, B – histological specimen, mag. × 400.

1 – trabecule of reticulofibrosis bone tissue; 2 – mesenchymal cells; 3 – blood vessel; 4 – osteoblasts; 5 – osteocytes in lacunae; 6 – osteoid; 7 – calcified extracellular matrix; 8 – osteoclast; 9 – osteogenic islet.
The first evidence of intramembranous ossification occurs around the eighth week of gestation in humans. Some of the pale-staining, elongate mesenchymal cells within the mesenchyme migrate and aggregate in specific areas, the sites where bone is destined to form. This condensation of cells within the mesenchymal tissue is the membrane referred to in the term intramembranous ossification. As the process continues, the newly organized tissue at the presumptive bone site becomes more vascularized, and the aggregated mesenchymal cells become larger and rounded. The cytoplasm of these cells changes from eosinophilic to basophilic, and a clear Golgi area becomes evident. These cytologic changes result in the differentiated osteoblast, which then secretes the collagens and other components of the bone matrix (osteoid). The osteoblasts within the bone matrix become increasingly separated from one another as the matrix is produced, but they remain attached by thin cytoplasmic processes. Because of the abundant collagen content, the bone matrix appears denser than the surrounding mesenchyme, in which the intercellular spaces reveal only delicate connective tissue fibers.

Newly formed bone matrix appears in histologic sections as small, irregularly shaped spicules and trabeculae. With time, the matrix becomes calcified, and the interconnecting cytoplasmic processes of the bone-forming cells, now termed osteocytes, are contained within canaliculi. Concomitantly, more of the surrounding mesenchymal cells in the membrane proliferate, giving rise to a population of osteoprogenitor cells. Some of the osteoprogenitor cells come into apposition with the initially formed spicules, become osteoblasts, and add more matrix. By this process, called appositional growth, the spicules enlarge and become joined in a trabecular network with the general shape of the developing bone. Through continued mitotic activity, the osteoprogenitor cells maintain their numbers and thus provide a constant source of osteoblasts for growth of the bone spicules. The new osteoblasts, in turn, lay down bone matrix in successive layers, giving rise to woven bone. This immature bone is characterized internally by interconnecting spaces occupied by connective tissue and blood vessels. Bone tissue formed by the process just described is referred to as membrane bone or intramembranous bone.

Endochondral bone formation begins in a segment of hyaline cartilage that serves as a small model for the bone (fig. 3,4,5). The two stages of endochondral bone formation involve the development of primary and secondary centers of ossification.

a. The primary center of ossification develops at the midriff of the diaphysis of the hyaline cartilage model by the following sequence of events:
   (1) Vascularization of the perichondrium at this site causes the transformation of chondrogenic cells to osteoprogenitor cells, which differentiate into osteoblasts. This region of the perichondrium is now called the periosteum.
   (2) Osteoblasts elaborate matrix deep to the periosteum, and via intramembranous bone formation, form the subperiosteal bone collar.
   (3) Chondrocytes within the core of the cartilaginous model undergo hypertrophy and degenerate, and their lacunae become confluent, forming large cavities (eventual marrow spaces).
   (4) Osteoclasts create perforations in the bone collar that permit the periosteal bud (blood vessels, osteoprogenitor cells, and mesenchymal cells) to enter the newly formed spaces in the cartilaginous model. The cartilage that constitutes the walls of these spaces then becomes calcified.
   (5) Newly developed osteoblasts elaborate bone matrix that becomes calcified on the surface of the calcified cartilage, forming a calcified cartilage-calcified bone complex. In histologic sections the calcified cartilage stains basophilic, whereas the calcified bone stains acidophilic.
   (6) The subperiosteal bone collar becomes thicker and elongates toward the epiphysis.
   (7) Osteoclasts begin to resorb the calcified cartilage-calcified bone complex, thus enlarging the primitive marrow cavity.
   (8) Repetition of this sequence of events results in bone formation spreading toward the epiphyses.

b. Secondary centers of ossification develop at the epiphyses in a sequence of events similar to that described for the primary center, except a bone collar is not formed.
   (1) Development of these centers begins when osteoprogenitor cells invade the epiphysis and differentiate into osteoblasts, which elaborate bone matrix to replace the disintegrating cartilage. When the epiphyses are filled with bone tissue, cartilage remains in two areas, the articular surfaces and the epiphyseal plates.
   (2) Articular cartilage persists and does not contribute to bone formation.
Fig. 3. Scheme of indirect osteogenesis.

1 – cartilage model of bone; 2 – blood vessel; 3 – bone collar; 4 – perichondral ossification; 5 – endochondral ossification; 6 – epiphysial plate; 7 – epiphysial ossification; 8 – diaphysis; 9 – epiphysis.
Fig. 4. Endochondral ossification. Histological specimen, mag. 140.
1 – hyaline cartilage; 2 – perichondrium; 3 – epiphyseal plate;
4 – zone of hypertrophic cartilage cells;
5 – primary centre of ossification (perichondral);
6 – secondary centre of ossification (endochondral);
7 – calcified intercellular matrix; 8 – periosteum;
9 – epiphysis; 10 – diaphysis.

Fig. 5. Perichondral ossification: A – scheme;
B – histological specimen, mag. 600.
1 – perichondral bone; 2 – osteoblasts; 3 – osteocytes;
4 – calcified intercellular matrix; 5 – osteoclast;
6 – periosteum.
(3) **Epiphyseal plates** continue to grow by adding new cartilage at the epiphyseal end while it is being replaced at the diaphyseal end (lengthening the bone).

(4) **Ossification of the epiphyseal plates** and cessation of growth occurs at about age 20.

**Calcification** begins with the deposition of calcium phosphate on collagen fibrils and is stimulated by certain proteoglycans and **osteonectin**, a Ca\(^{2+}\)-binding glycoprotein.

**Endochondral ossification** also begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. However, the mesenchymal cells differentiate into chondroblasts that, in turn, produce cartilage matrix. Initially, a hyaline cartilage model with the general shape of the bone is formed. Once established, the cartilage model (a miniature version of the future definitive bone) grows by interstitial and appositional growth. The increase in the length of the cartilage model is attributed to interstitial growth. The increase in its width is largely due to the addition of cartilage matrix produced by new chondrocytes that differentiate from the chondrogenic layer of the perichondrium surrounding the cartilage mass. The first sign of ossification is the appearance of a cuff of bone around the cartilage model.

At this stage, the perichondrial cells in the midregion of the cartilage model no longer give rise to chondrocytes. Instead, bone-forming cells or osteoblasts are produced. Thus, the connective tissue surrounding this portion of the cartilage is no longer functionally a perichondrium; rather, because of its altered role, it is now called periosteum. Moreover, because the cells within this layer are differentiating into osteoblasts, an osteogenic layer can now be identified within the periosteum. As a result of these changes, a layer of bone is formed around the cartilage model. This bone can be classified as either periosteal bone, because of its location, or intramembranous bone, because of its method of development. In the case of a long bone, a distinctive cuff of periosteal bone, the bony collar, is established around the cartilage model in the diaphyseal portion of the developing bone. With the establishment of the periosteal bony collar, the chondrocytes in the midregion of the cartilage model become hypertrophic. As the chondrocytes enlarge, their surrounding cartilage matrix is resorbed, forming thin irregular cartilage plates between the hypertrophic cells. The hypertrophic cells begin to synthesize alkaline phosphatase, and concomitantly, the surrounding cartilage matrix undergoes calcification. The calcification of the cartilage matrix should not be confused with calcification that occurs in bone tissue.

The calcified cartilage matrix inhibits diffusion of nutrients, causing death of the chondrocytes in the cartilage model. With the death of the chondrocytes, much of the matrix breaks down, and neighboring lacunae become confluent, producing an increasingly large cavity. While these events are occurring, one or several blood vessels grow through the thin diaphyseal bony collar to vascularize the cavity. Periosteal cells migrate into the cavity along with growing blood vessels. Cells from the periosteum migrate with the penetrating blood vessels and some of the primitive periosteal cells to become osteoprogenitor cells in the cavity. Other primitive cells also gain access to the cavity via the new vasculature, leaving the circulation to give rise to the marrow. As the calcified cartilage breaks down and is partially removed, some remains as irregular spicules. When the osteoprogenitor cells come in apposition to the remaining calcified cartilage spicules, they become osteoblasts and begin to lay down bone (osteoid) on the spicule framework. Thus, the bone formed in this manner may be described as endochondral bone. The combination of bone, which is initially only a thin layer, and the underlying calcified cartilage is described as a mixed spicule.

Histologically, mixed spicules can be recognized by their staining characteristics. Calcified cartilage tends to be basophilic, whereas bone is distinctly eosinophilic. Also, calcified cartilage no longer contains cells, whereas the newly produced bone may reveal osteocytes in the bone matrix. Such spicules persist for a short time before the calcified cartilage component is removed. The remaining bone component of the spicule may continue to grow by appositional growth, thus becoming larger and stronger, or it may undergo resorption as new spicules are formed.

Growth of long bones depends on the presence of epiphyseal cartilage throughout the growth period. As the diaphyseal marrow cavity enlarges, a distinct zonation can be recognized in the cartilage at both ends of the cavity. This remaining cartilage, referred to as epiphyseal cartilage, exhibits distinct zones. The zones in the epiphyseal cartilage, beginning with that most distal to the diaphyseal center of ossification and proceeding toward that center, are

1. Zone of reserved cartilage, which exhibits no cellular proliferation or active matrix production.
2. Zone of proliferation, which is adjacent to the zone of reserve cartilage in the direction of the diaphysis.

In this zone, the cartilage cells undergo division and organize into distinct columns. These cells are larger than
Those in the reserve zone and actively produce matrix.

3. Zone of hypertrophy, which contains greatly enlarged cartilage cells. The cytoplasm of these cells is clear, which is a reflection of the glycogen that they normally accumulate (and that is lost during fixation). The matrix is compressed into linear bands between the columns of hypertrophied cartilage cells.

4. Zone of calcified cartilage, in which the enlarged cells begin to degenerate and the matrix becomes calcified.

5. Zone of resorption, which is the zone nearest the diaphysis. The calcified cartilage here is in direct contact with the connective tissue of the marrow cavity.

In the zone of resorption, small blood vessels and accompanying connective tissue invade the region occupied by the dying chondrocytes. They form a series of spearheads, leaving the calcified cartilage as longitudinal spicules, at least as seen in longitudinal sections of bone. Actually, in a cross section of the bone, the cartilage appears as a honeycomb because the invading vessels and connective tissue have migrated into the sites previously occupied by the cartilage cells.

As bone is laid down on the calcified spicules, the cartilage is resorbed, ultimately leaving a primary spongy bone. This spongy bone undergoes reorganization through osteoclastic activity and addition of new bone tissue, thus accommodating the continued growth and physical stresses placed on the bone.

Shortly after birth, a secondary ossification center develops in the upper epiphysis. The cartilage cells undergo hypertrophy and degenerate. As in the diaphysis, calcification of the matrix occurs, and blood vessels and osteogenic cells from the perichondrium invade the region, creating a new marrow cavity. Later, a similar epiphyseal ossification center forms at the lower end of the bone. This center is also regarded as a secondary ossification center, although it develops later. With the development of the secondary ossification centers, the only cartilage that remains from the original model is the articular cartilage at the ends of the bone and a transverse disc of cartilage, known as the epiphyseal plate, which separates the epiphyseal and diaphyseal cavities.

For a bone to retain proper proportions and its unique shape, both external and internal remodeling must occur as the bone grows in length. The proliferative zone of the epiphyseal plate gives rise to the cartilage on
which bone is later laid down.

- The thickness of the epiphyseal plate remains relatively constant during growth.
- The amount of new cartilage produced (zone of proliferation) equals the amount resorbed (zone of resorption).
- The resorbed cartilage is, of course, replaced by spongy bone.
- Actual lengthening of the bone occurs when new cartilage matrix is produced at the epiphyseal plate. Production of new cartilage matrix pushes the epiphysis away from the diaphysis, elongating the bone. The events that follow this incremental growth, namely, hypertrophy, calcification, resorption, and ossification, simply involve the mechanism by which the newly formed cartilage is replaced by bone tissue during development.
- Bone increases in width or diameter when appositional growth of new bone occurs between the cortical lamellae and the periosteum. The marrow cavity then enlarges by resorption of bone on the endosteal surface of the cortex of the bone.

![Fig. 7. New osteon formation under regeneration of the bone. A - scheme; B - histological specimen, mag. 480.](image)

**Repair of a bone fracture** (fig. 7, 9). A bone fracture damages the matrix, bone cells, and blood vessels in the region and is accompanied by localized hemorrhaging and blood clot formation.

1. **Proliferation of osteoprogenitor cells** occurs in the periosteum and endosteum in the vicinity of the fracture. As a result of this proliferation, a cellular tissue surrounds the fracture and penetrates between the ends of the damaged bone.
   a. Fibrous connective tissue and hyaline cartilage are formed in the fracture zone.
   b. Endochondral bone formation replaces the cartilage with primary bone.
   c. Intramembranous bone formation also produces primary bone in the area.
   d. The irregularly arranged trabeculae of primary bone join the ends of the fractured bone, forming a bony callus.
   e. The primary bone is resorbed and replaced with secondary bone as the fracture heals.

**Bone remodeling** (fig. 8). Bone is constantly being remodeled as necessary for growth and to alter its structural makeup to adapt to changing stresses in the environment throughout life. Several factors, including calcitonin and PTH, are responsible for this phenomenon.

1. Early on, bone development outpaces bone resorption as new haversian systems are added and fewer are resorbed.
2. Later when the epiphyseal plates are closed, ending bone growth, there is a balance between bone development and resorption.
Role of hormones in bone formation

1. **Parathyroid hormone (PTH)** activates osteoblasts to secrete osteoclast-stimulating factor, which then activates osteoclasts to resorb bone, thus elevating blood calcium levels. Excess PTH (hyperparathyroidism) renders bone more susceptible to fracture and subsequent deposition of calcium in arterial walls and certain organs such as the kidney.

2. **Calcitonin** is produced by parafollicular cells of the thyroid gland. It eliminates the ruffled border of osteoclasts and inhibits bone-matrix resorption, thus preventing the release of calcium.

3. **Pituitary growth hormone** is produced in the pars distal is of the pituitary gland and stimulates overall growth, especially that of epiphyseal plates. Excess during growing years causes pituitary gigantism and in adults causes acromegaly. Deficiency during growing years causes dwarfism.

Clinical Considerations

**Osteoporosis** is a disease characterized by low bone mass (bone mineral density) and structural deterioration of bone tissue, making the bone more fragile and susceptible to fracture. Osteoporosis is associated with an abnormal ratio of mineral to matrix.

1. It results from increased bone resorption, decreased bone formation, or both.
2. This disease is most common in postmenopausal women because of diminished estrogen secretion, and in immobile patients because of lack of physical stress on the bone.
3. Preventive measures include a balanced diet rich in calcium and vitamin D, and weight-bearing exercises.

**Rickets** occurs in children deficient in vitamin D, which results in calcium deficiency. It is characterized by deficient calcification in newly formed bone and is generally accompanied by deformation of the bone spicules in epiphyseal plates; as a result, bones grow more slowly than normal and are deformed.

**Osteomalacia** (rickets of adults) results from calcium deficiency in adults.

1. It is characterized by deficient calcification in newly formed bone and decalcification of already calcified bone.
2. This disease may be severe during pregnancy because the calcium requirements of the fetus may lead to calcium loss from the mother.

**Acromegaly** results from an excess of pituitary growth hormone in adults. It is characterized by very thick bones in the extremities and in portions of the facial skeleton.
Fig. 9. Scheme of the repair after long bone fracture: A – on the second day; B – after 7 days; C – after 2 weeks.

1 – zone of periostal proliferation; 2 – periosteum; 3 – compact bone; 4 – internal callus; 5 – external callus; 6 – endosteum; 7 – zone of endostal proliferation; 8 – chondral callus; 9 – primary (woven) bone tissue; 10 – secondary (lamellar) bone tissue.
LESSON 11

THEME: MUSCLE TISSUES

BACKGROUND. Muscle tissues are specialized tissues. They form up to 40% of body weight; provide movement of the whole body and its parts (organs). Muscle contractions provide the flow of blood and lymph, movements of internal cavity organs, heart beating. As a result, some part of the ATP energy is transferred into heat, which plays an important role in thermogenesis.

Learning the structure and histochemical features of muscles helps us to understand histophysiology of organs, as well as pathophysiology of certain diseases related to impairment of muscle contraction (myocardial infarction, muscular dystrophies etc).

Aim of Study: To be able to differentiate different types of muscle tissues, interpret their qualities on basis of the structure of muscle fiber functioning apparatus, understand the mechanism of regeneration of muscle tissues.

YOU SHOULD BE ABLE TO:
1. Differentiate the key morphological and functional features of the muscle tissues.
2. Interpret embryonic sources and mechanisms of development, regeneration and location of different types of muscle tissues.
3. Differentiate different types of muscle tissues in specimen, their structural elements and key features.
4. Interpret morphological and physiological features of different types of muscle tissues.
5. Interpret structural changes within the contraction and relaxation of skeletal muscle fibre and smooth myocyte.

TO ACHIEVE THE AIM IT IS NECESSARY TO CENTRE AROUND THE FOLLOWING POINTS:
1. General characteristics of muscle tissues.
2. Embryonic sources of muscle tissues development
3. Classification of muscle tissues.
4. Muscle fibre as structural and functional unit of muscle tissue.
5. Skeletal muscles: key morphological features.
7. Myofibrille structure.
8. Molecular organization of actin and myosin filaments.
10. Cardiac muscle tissue.
11. Smooth muscle tissue.

WHAT MUST YOU KNOW? (INSTRUCTION FOR YOUR SELF-LEARNING)

GENERAL CHARACTERISTICS OF MUSCLE TISSUES:
1. EMBRYONIC SOURCES: Most muscular tissue is derived from mesoderm, by a modification of the cells into elongated muscle fibres.
2. STRUCTURE: the structural unit of muscle tissues is muscle fibre. It can be cell, chain of cells or symplast.
   Each fibre has a special cytoplasm (sarcoplasm), in which contractile filaments lie, which can contract the fibre or cell along its long axis.
3. Although all cells are capable of some sort of movement, the dominant function of muscle fibres is to generate motile forces through contraction. In these specialised contractile cells, motile forces are generated by the interaction of the proteins actin and myosin (contractile proteins).

Classification of muscle tissues.
According to structure MT are subdivided into two types:
1) striated:
   a) skeletal;
   b) cardiac.
2) smooth.

Skeletal muscle is responsible for the movement of the skeleton and organs such as the globe of the eye and the tongue. Skeletal muscle is often referred to as voluntary muscle since it is capable of voluntary (conscious) control. The arrangement of the contractile proteins gives rise to the appearance of prominent cross-striations in some histological preparations and so the name striated muscle is often applied to skeletal muscle. The highly developed functions of the cytoplasmic organelles of muscle cells has led to the use of a special terminology for some muscle cell components: plasma membrane or plasmalemma = sarcolemma; cytoplasm = sarcoplasm; endoplasmic reticulum = sarcoplasmic reticulum.

Smooth muscle is so called because, unlike other forms of muscle, the arrangement of contractile proteins does not give the histological appearance of cross-striations. This type of muscle forms the muscular component of visceral structures such as blood vessels, the gastrointestinal tract, the uterus and the urinary bladder, which explains the alternative name the visceral muscle. Since smooth muscle is under inherent autonomic and hormonal control, it is also described as involuntary muscle.

Cardiac muscle has many structural and functional characteristics intermediate between those of skeletal and smooth muscle and provides for the continuous, rhythmic contractility of the heart. Although striated in appearance, cardiac muscle is readily distinguishable from skeletal muscle and should not be referred to by the term “striated muscle”

According to embryonic development there are 5 types of muscles.
- somatic – skeletal muscle – derived from myoptomes of somits;
- mesenchymal – smooth muscle – arised from mesenchyme of splanchnotome;
- from myoepicardial plate – cardiac;
- ectodermal origin - myoepithelial cells are an important component of certain secretory glands, where they function to expel secretions from glandular acini;
- neural origin – smooth muscles of eye (in iris and ciliary body).

Additionally there are several cell types, able to contraction: Pericytes are smooth muscle-like cells that surround blood vessels. Myofibroblasts are cells that have a contractile role in addition to being able to secrete collagen. This type of cell is generally inconspicuous in normal tissues but comes to be a dominant cell type when tissues undergo repair after damage in the formation of a scar.

SKELETAL MUSCLE
Skeletal muscle includes not only Striated skeletal muscle tissue but also connective tissue:
   Connective tissue (CT) sheaths form:
   - CT epimysium encloses the whole muscle;
   - CT perimysium encloses each fasciculus (bundle) of fibres;
   - CT endomysium encloses each muscle fibre.

Connective tissue carries blood vessels, lymphatics and nerves, and serves to harness and direct the force developed by contraction to the attached tendons.

SEPARATE SKELETAL MUSCLE FIBRE
In skeletal MT fibre is large and cylindrical, with a diameter between 10 and 100 µm and a length between 1 and 40 mm. Regularly along the length of the fibre a cross-bandng of light and dark lines is seen. Fainter longitudinal lines, the myofibrils, are also visible (fig. 1).

Skeletal muscle fibre is symplast – multinuclear structure with big volume of cytoplasm. It forms during embryogenesis and includes several steps of myogenesis (fig. 2):
1. Mesodermal cells of the myotome become elongated premyoblasts.
2. These cells multiply, acquire more cytoplasm and elongate further, becoming granular, with many mitochondria and ribosomes.
3. Filaments and microtubules appear in the cytoplasm of the myoblasts. New myoblasts fuse with the more mature ones accumulating myofilaments to build long, multinucleated cells.
4. Filaments aggregate into myofibrils near the sarcolemma, leaving a paler central core, with a row of nuclei (myotube stage).

5. The fibrils develop prominent striations; nuclei move to the periphery of the fibre; and mitochondria and SR arrange themselves in relation to the myofibrils.

6. Some cells stay in a peripheral position to lie within the basal lamina as a regenerative reserve of satellite cells.

Outside fibres a connective tissue called endomysium lies with some fibroblasts, collagen fibrils, and capillaries. Cell membrane in muscle fibre is the sarcolemma. Directly under the sarcolemma, i.e., peripherally, lie elongated numerous nuclei. In one place, the sarcolemma is modified to take a nerve fibre’s terminal motor-end-plate/myoneural junction. The interior of the fibre has sarcoplasm with orderly myofibrils.

Functional apparatus of skeletal muscle fibre:
1) contractile - myofibrils with contractile filaments
2) supporting apparatus -
3) transmission of irritation out-inside – T- and L-system
4) trophic and energy supply – *mitochondriae* and *inclusions of glycogen, lipids and myoglobin*
5) genetic control - nuclei
6) intracellular regenetarion – lysosomes and ribosomes

**MYOFIBRILL**

The fibre is cross-banded because the many constituent myofibrils are banded, and lie side by side with their dark areas in register. High power light microscopy reveals the repetitive sequence along the myofibril. There are light (isotropic) and dark (anisotropic) bands – IB and AB.

The myofibrils, which have a diameter of 1 - 2 mkm and run parallel to the long axis of the muscle fiber, consist of an end-to-end chain like arrangement of sarcomeres (fig. 3).

The lateral registration of sarcomeres in adjacent myofibrils causes the entire muscle fiber to exhibit a characteristic pattern of transverse striations.

Studies with the electron microscope reveal that this sarcomere pattern is due mainly to the presence of two types of filaments, thick and thin, that lie parallel to the long axis of the myofibrils in a symmetric pattern.

The thin filaments run between and parallel to the thick filaments and have one end attached to the Z line. Thin filaments are 1.0 mkm long and 8 nm wide. As a result of this arrangement, the I bands consist of the portions of the thin filaments that do not overlap the thick filaments.

The A bands are composed mainly of thick filaments in addition to portions of overlapping thin filaments. Close observation of the A band shows the presence of a lighter zone in its center, the H band, that corresponds to a region consisting only of the rodlike portions of the myosin molecule. Bisecting the H band is the M line, a region at which lateral connections are made between adjacent thick filaments. The major protein of the M line is creatine kinase. Creatine kinase catalyzes the transfer of a phosphate group from phosphocreatine (a storage form of high-energy phosphate groups) to adenosine diphosphate (ADP), thus supplying adenosine triphosphate (ATP) for muscle contraction.

**MOLECULAR ORGANIZATION OF FILAMENTS**

Thin (actin filaments) includes the three main proteins: *actin, tropomyosin, troponin*. Thick filaments includes *myosin* (fig. 4). Myosin and actin together represent 55% of the total protein of striated muscle.

Actin is present as long filamentous (F-actin) polymers consisting of two strands of globular (G-actin) monomers, twisted around each other in a double helical formation. G-actin molecules are structurally asymmetric and contain a binding site for myosin. When G-actin molecules polymerize to form F-actin, they bind back to front, producing a filament with distinguishable polarity. Actin filaments anchor perpendicularly on the Z line, that includes actinin and desmin (an intermediate filament protein) which are believed to tie adjacent sarcomeres together, thus keeping the myofibrils in register.

Tropomyosin, a long, thin molecule about 40 nm in length, contains two polypeptide chains. These molecules are bound head to tail, forming filaments that run over the actin subunits alongside the outer edges of the groove between the two twisted actin strands.
Troponin is a complex of three subunits: TnT, which strongly attaches to tropomyosin; TnC, which binds calcium ions; and TnI, which inhibits the actin-myosin interaction. A troponin complex is attached at one specific site on each tropomyosin molecule.

In thin filaments, each tropomyosin molecule spans seven G-actin molecules and has one troponin complex bound to its surface.

Myosin, a much larger complex (molecular mass 500 kDa), can be dissociated into two identical heavy chains and two pairs of light chains. Myosin heavy chains are thin, rodlike molecules (150 nm long and 2-3 nm thick) made up of two heavy chains twisted together. Small globular projections at one end of each heavy chain form the heads, which have ATP-binding sites as well as the enzymatic capacity to hydrolyze ATP (ATPase activity) and the ability to bind to actin. The four light chains are associated with the head. Several hundred myosin molecules are arranged within each thick filament with their rodlike portions overlapping and their globular heads directed toward either end.

**Fig 4. Molecular organization of the contractile miofilaments: A – myosin, B – actin.**

1 - myosin molecules; 2 - myosin heads; 3 - actin molecules; 4 - tropomyosin; 5 - troponin molecule.

**T- AND L-SYSTEM - TRANSVERSE TUBULE SYSTEM AND SARCOPLASMIC RETICULUM**

The depolarization of the sarcoplasmic reticulum membrane, which results in the release of Ca\(^{2+}\) ions, is initiated at a specialized myoneural junction on the surface of the muscle cell. Surface-initiated depolarization signals would have to diffuse throughout the cell to effect the release of Ca\(^{2+}\) from internal sarcoplasmic reticulum cisternae. In larger muscle cells, the diffusion of the depolarization signal would lead to a wave of contraction, with peripheral myofibrils contracting before more centrally positioned myofibrils. To provide for a uniform contraction, skeletal muscle possesses a system of transverse (T) tubules. These fingerlike invaginations of the sarcolemma form a complex anastomosing network of tubules that encircles the boundaries of the A-I bands of each

Adjacent to opposite sides of each T tubule are expanded terminal cisternae of the sarcoplasmic reticulum. This specialized complex, consisting of a T tubule with two lateral portions of sarcoplasmic reticulum, is known as the triad. At the triad, depolarization of the sarcolemma-derived T tubules is transmitted to the sarcoplasmic reticulum membrane (fig. 5). As described above, muscle contraction depends on the availability of Ca\(^{2+}\) ions, and muscle relaxation is related to an absence of Ca\(^{2+}\). The sarcoplasmic reticulum specifically regulates calcium flow, which is necessary for rapid contraction and relaxation cycles. The sarcoplasmic reticulum system consists of a branching network of smooth endoplasmic reticulum cisternae surrounding each myofibril.

After a neurally mediated depolarization of the sarcoplasmic reticulum membrane, Ca\(^{2+}\) ions concentrated within the sarcoplasmic reticulum cisternae are passively released into the vicinity of the overlapping thick and thin filaments, whereupon they bind to troponin and allow bridging between actin and myosin. When the membrane depolarization ends, the sarcoplasmic reticulum acts as a calcium sink and actively transports the Ca\(^{2+}\) back into the cisternae, resulting in the cessation of contractile activity.
SOME EVENTS AND CHEMISTRY OF CONTRACTION.

· **Excitation.** The ion permeability of the sarcolemma is altered by acetylcholine to the point where it propagates an action potential. This depolarization extends down the T-tubules and influences adjacent cisternae of the sarcoplasmic reticulum (SR), which extend feet to receive the stimulation. 

· The agent coupling excitation to contraction is ionic calcium, released by the stimulated SR to interact with one of two regulator proteins attached to the contractile protein - *actin*. The binding of Ca$^{2+}$ to *troponin* causes *tropomyosin* to stop interfering with the link between actin and myosin. 

· **Contraction** occurs because the myosin head, linked to the actin, somehow generates the force moving the thin filaments deeper between the thick ones. At the same time, the actomyosin complex acts enzymatically to release energy from *ATP* bound to the myosin. The actomyosin bond is between actin and heavy meromyosin that protrudes as many cross-bridges, or lateral projections (seen in EM), from the thick filament of light meromyosin. 

· **Relaxation** happens when Ca$^{2+}$ is actively taken up by the SR. Troponin then permits tropomyosin again to inhibit actomyosin binding, thus unfastening the cross-bridges (fig. 6).

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**Fig 5.** Scheme of the structure and interactions of transverse tubules (T-systems) and terminal cisterns (L-systems) in the striated skeletal muscle fibers. 

1 - sarcolemma; 2 - T-tubules; 3 - terminal cisterns; 4 - a network of the L-system cisterns; 5 - myofibrils.

**Fig 6.** Scheme of interaction between actin and myosin filaments: during contraction (A) and relaxation (B). 

1 - core of thick myosin filament; 2 - head of myosin; 3 - tropomyosin; 4 - molecule of G-actin; 5 - center of actin, binds to myosin; 6 - T-troponin, binds to tropomyosin; 7 - C-troponin, binds calcium; 8 - I-troponin, which prevents the interaction of actin-myosin.
SOME OTHER MUSCLE MOLECULES
a) $\alpha$-actinin is a non-contractile Z-line actin-attachment protein.
b) Nebulin is a giant ruler-like molecule to align, and establish the length of, the actin filaments.
c) The ryanodine receptor - a calcium channel - is the principal protein of the SR feet, linking the T-tubule to the sarcoplasmic reticulum. Other proteins are at the complex.
d) Desmin is the intermediate filament of muscle.
e) Dystrophin lies just under the sarcolemma to help attach it to the cytoskeleton. Its lack causes one type of muscular dystrophy.
f) Titin is another huge, long molecule, connecting the Z disc with the material of the M-line. It provides an elastic framework for filament movement, and helps actinin anchor the actin filaments in the Z disc.
g) The contractile and regulatory proteins exist as isoforms, characteristic of slow, fast, and cardiac muscles. The isoforms change during development and in disease, e.g., muscular dystrophy, cardiac hypertrophy.
h) Caveolin is associated with the inwardly budding sarcolemmal protrusions - caveolae - of cardiac and smooth muscle that are part of the signal transduction machinery for contraction.

RED AND WHITE FIBRES
Groups of only one kind of fibre can be identified by colour with the naked eye in some fresh, unstained muscles. They differ physiologically with red, richer in myoglobin and mitochondria, providing slower responses, but being less prone to fatigue. Histochemistry reveals further subtypes: white, intermediate, red (fast-twitch), and red (slow).

The classification of muscle fibre types is used in assessing muscular disease, but the classification by roman numeral can be based on the profiles of contractile proteins, or on the metabolic behaviour of the fibre, so one needs to ask the particular criteria for any types encountered.

MUSCULAR DYSTROPHY
In muscular dystrophy there is weakness and wasting of muscles due to disease caused by a defective protein involved in muscle function. One of the important groups of protein involved in muscle function is that associated with the cell membrane of skeletal muscle. A complex of large proteins act to link the contractile proteins within the cell through the cell membrane with structural proteins in the external lamina. Thus contraction forces within each muscle fibre are transmitted to the collagenous support tissues to bring about movement.

One of the most important of these link proteins is called dystrophin. Immunostaining for this protein, seen here as a brown stain, shows it closely associated with the muscle cell membrane. Some people have a mutation in the gene for dystrophin that results in inefficient linking of contractile forces to the support tissues in muscle. Muscle does not function correctly and fibres undergo progressive damage with repeated contraction, ultimately leading to death of muscle cells. The disease is called Duchenne muscular dystrophy.

Mutation in genes coding for proteins in the link complex is an important cause of the group of muscular dystrophies. Other genes coding for contractile and other structural proteins also cause muscular dystrophy

CARDIAC MUSCLE COMPARED WITH SKELETAL
1. Muscle fibre consists of chain of cells (cardiomyocytes)
2. Fibres are narrower, around 9-22 µm in diameter.
3. Fibres branch and anastomose and, until intercalated discs were discerned using EM, the muscle was believed to be syncytial - one huge cell.
4. Each cell has only one or two nuclei lying centrally, elongated, but with blunt ends.
5. Cross-banded, with the same repetitive sequence, but the banding is weaker.
6. Adjacent cardiomyocytes are connected end-to-end by Intercalated discs which include a complex of intercellular junctions – desmosomes, interdigitations and nexus.
7. Sometimes a Z line’s place is taken by a dark line across the width of the fibre - intercalated disc.
8. Mitochondria are more numerous.
9. There is less CT (fig. 7).
SMOOTH MUSCLE

Most smooth muscle is present in the walls of hollow viscera (e.g. gut, ureter, Fallopian tube) where it is arranged in sheets with cells aligned circumferentially or longitudinally, with contraction resulting in reduction of the lumen diameter.

The fibres are spindle-shaped (fusiform) with one, central, cigar-shaped nucleus, and are usually 200 µm or less long, but in the hypertrophic uterus they may reach 0.5 mm. Width is around 6 µm.

Fibres show no cross-banding, but have many fine filaments (fig. 8).

Cells are firmly attached by gap junctions, and elsewhere by glycoprotein external laminae (like basal lamina). Diverse patterns of attachment and contraction occur in gut, vessel walls, genital organs, etc.

Fibres are usually packed to form a sheet or bundle. Reticular fibres enfold the muscle fibres, assist in holding them together and carry blood vessels, and fine autonomic nerve fibres going to inconspicuous myoneural junctions.

The nuclei may be wrinkled in the contracted state.

EM shows thin and thick filaments, but the thick are labile and not easily preserved. These filaments connect with Z line-like densities in the cytoplasm, or at the cell membrane (fig. 9).
Tension generated by contraction is transmitted through anchoring densities in the cell membrane to the surrounding external lamina, thus allowing a mass of smooth muscle cells to function as one unit. The intermediate filaments of smooth muscle, desmin, are also inserted into the focal densities. Desmin intermediate filaments help to structure the filamentous arrays.

By the nucleus mitochondria and the Golgi body lie.

Peripheral vesicles are part of a vesicular and tubular Ca\(^{2+}\)-holding sarcoplasmic reticulum. These organelles, and inward protrusions of cell membrane, caveolae, function similarly to the better-defined SR and T-tubules of striated muscle.

Contraction is triggered by a Ca\(^{2+}\)-dependent phosphorylation of myosin light chain by smooth-muscle MLC kinase. This is the primary control, fine-tuned by the calcium-mediated binding of caldesmon and calponin to actin in ways which interfere with actomyosin force-generation and ATPase activities.

Vascular smooth muscle cells also can make elastin and collagen during development.

The contraction mechanism of smooth muscle differs from that for striated muscle. Because the contractile proteins are arranged in a criss-cross lattice inserted around the cell membrane, contraction results in shortening of the cell, which assumes a globular shape in contrast to its elongated shape in the relaxed state.

The mechanism of smooth muscle contraction is as follows:

- Thin filaments of actin are associated with troponyosin.
- Thick filaments composed of myosin only bind to actin if one chain is phosphorylated.
- Ca\(^{2+}\) ions in the cytosol of smooth muscle cells cause contraction as in striated muscle, but the control of Ca\(^{2+}\) ion movements is different. In relaxed smooth muscle, free Ca\(^{2+}\) ions are normally sequestered in sarcoplasmic reticulum throughout the cell. On membrane excitation, free Ca\(^{2+}\) ions are released into the cytoplasm and bind to a protein called calmodulin (a calcium-binding protein). The calcium-calmodulin complex then activates an enzyme called myosin light-chain kinase, which phosphorylates myosin and permits it to bind to actin. Actin and myosin subsequently interact by filament sliding to produce contraction in a similar way to that for skeletal muscle.
- Contraction of smooth muscle can be modulated by surface receptors activating internal second messenger systems. Expression of different receptors allows smooth muscle in different sites to respond to several different hormones.
- Compared with skeletal muscle, smooth muscle is able to maintain a high force of contraction for very little ATP usage.
TENDON

Musculo-tendinous junction entails *no continuity* between myofibrils and collagen fibres: the sarcolemma intervenes.

Myofibrils pull on the tapering sarcolemma at the muscle fibre’s extremity, and its contraction is conveyed by the muscle’s CT to the tendon with which the muscle CT merges.

Tendon is composed of:

· (a) many bundles of dense, regular, *collagen fibres* with
· (b) flattened *tendon cells* (fibroblasts) between them;
· (c) each bundle is loosely bound in a CT sheet - *endotendineum*;
· (d) *peritendinial* CT, bearing vessels and nerves, encloses several primary units as one fasciculus; and
· (e) a thick sheath - *epitendineum* - wraps around the whole tendon of several fasciculi.

Freedom of movement is provided for some tendons by enclosing them in lubricated *synovial sheaths*, or interposing a *synovial bursa* between the tendon and a bony prominence or ligament.

Tendons and skeletal muscle have nervous *proprioceptors* - Golgi tendon organs and muscle spindles.
LESSON 12

THEME: NERVE TISSUE. NEURONS. GLIAL CELLS

BACKGROUND: Nerve tissue is a highly specialized tissue composed of nerve cells (neurons) and glial cells (neuroglia). Implementation of specific functions of the nervous tissue is provided by neurons, which perceive the stimulation, transformed into a state of excitation, generate and transmit nerve impulses, regulating the work of cells, tissues and organs-targets.

Neuroglia – auxiliary component of nerve tissue that performs the supporting, isolating, trophic, secretory, and protective functions. Nerve tissue forms the central and peripheral nervous system. Knowledge of the laws governing the development, structure and function of neurons and neuroglia is necessary for understanding histophysiology of the nervous system - one of the governing body’s systems, ensuring its integrity and adaptation to environmental conditions, as well as for predicting the possible consequences of its structural changes during the development of pathological process.

AIM OF STUDY: to be able to identify the elements of nerve tissue, interprete structure and functions of neurons and glial cells.

TO ACHIEVE THIS AIM ONE HAS TO (practical procedures):
1. Identify the sources of nerve tissue development.
2. Identify the nerve tissue in the histological specimens.
3. Differentiate neurons and glial cells.
4. Differentiate special organelles of neurons and interpret their functional significance.
5. Interpret features of neuroglia.
6. Interpret neuro-glio-vascular interrelation; learn the possibilities of regeneration of tissue elements of nervous tissue.

TO ACHIEVE THE AIM IT IS NECESSARY TO CENTRE AROUND THE FOLLOWING POINTS:
1. General morpho-functional characteristics of nerve tissue. Sources of development, histogenesis.
3. The structure of the neuron body. Micro-and submicroscopic characteristics of the functions of organelles with general and special significance.
5. The general morphofunctional characteristics of the neuroglia. Classification.
6. Macroglia. Types of glial cells: glial cells of the central and peripheral nervous systems. Their structure and significance.
7. Microglia. The origin, structure and function.

WHAT MUST YOU KNOW? (INSTRUCTION FOR YOUR SELF-LEARNING)
1. ORIGIN. Nerve tissue is developed from neuroectoderm which forms the following elements during embryogenesis:
   - nerve tube
   - nerve crest
   - placodes

   CELL LINEAGES OF THE NERVOUS SYSTEM:
1. In the PNS:
   (a) the large number of neural-crest-derived cell types, including many non-neural ones (mostly because the crest is the major constructor of the head);
   (b) the evidence is chiefly from birds;
   (c) some head structures, e.g., receptors and ganglion neurons for hearing and balance come from ectodermal placodes.
2. In the CNS there is a multipotent *neural stem cell* giving rise to a self-propagating *progenitor* pool. From this pool, self-sustaining populations of *neuroblasts* and *glioblasts* derive. Further specifications, under the control of neural “growth factors”, are for transmitter type, shape, and axon length, and for glioblast derivatives, whether to be type 1 or 2 astrocytes, or oligodendrocytes. Microglia are regarded as invaders of haematopoietic origin, but is this always true for all of them? Other questions are: do neural progenitors live on in the adult CNS? (They are present in olfactory mucosa.) And how does the astrocyte 2 correspond to the fibrous astrocyte, and the 1 to the protoplasmic?

**Neural and other cell derivatives of neural tube and crest:**

**NEURAL TUBE**

*CNS*: Neurons, Astrocytes, Oligodendrocytes, Ependymal cells, Special central glia (fig. 1)

![Image](image-url)

Fig.1. Histogenetic classification of the elements of nerve tissue.

1 – nerve tube; 2 – neuroblast; 3 – free spongioblast; 4 – spongioblast of ependyma; 5 – neurons; 6 – ependymocytes; 7 – astroblast; 8 – oligodendrocyte; 9 – protoplasmic astrocyte; 10 – fibrous astrocyte.

**NEURAL CRESC**

*PNS*: Sensory- and autonomic-ganglion neurons, Adrenal neurons, Satellite cells, Schwann cells, Enteric glia

*OTHERS*: Chromaffin cells, C-cells, Melanocytes, some Cardiac (outflow tract) & Carotid-body cells

**NEURAL CRESC** via Mesectoderm:

*ANTERIOR CRANIAL SKELETAL TISSUES*: Osteoblasts, Chondroblasts;

*DENTAL TISSUES*: Odontoblasts, Cementoblasts, Ligament fibroblasts;

*HEAD MUSCLES & CONNECTIVE TISSUES*: Smooth & skeletal muscle cells, Fibroblasts, Adipocytes?

Meningeal cells.

2. **STRUCTURE**: nerve tissue consists of two types of cells:

- neurons (nerve cells)
- glial cells.

3. FUNCTIONAL SIGNIFICANCE

Nerve tissue form the main components of the nervous system, which provides control and coordination of all the body’s activities. It spreads out widely from central nervous organs to all organs, providing a multitude of finely graded responses to changes in the external and internal states.

It makes use of millions of nerve cells having the special properties of excitability and conductivity. Information is conducted along the long nerve cell processes as an electrical excitation generated across the cell membrane. As in all cells, the resting neurone maintains an ionic gradient across its plasma membrane thereby creating an electrical potential. Excitability involves a change in membrane permeability in response to appropriate stimuli so that the ionic gradient is reversed and the plasma membrane becomes depolarised; a wave of depolarisation, known as an action potential, then spreads along the plasma membrane. This is followed by the process of repolarisation in which the membrane rapidly re-establishes its resting potential.

CHARACTERISTICS OF NEURONS

Parts of neuron (fig. 2)

Fig.2. Scheme of structure of neuron (A) and ultrastructure of the body of neuron (B).

1 – body(pericaryon); 2 – nucleus; 3 – nucleolus; 4 – axon; 5 – terminal endings of axon(presynaptic parts); 6 – dendrites; 7 – myelin sheath; 8 – oligodendrocyte; 9 – Nissl bodies; 10 – Golgi apparatus; 11 – microtubules; 12 – mitochondria; 13 – lysosomes; 14 – synapse.

Neurons are characterized by having long processes extending from a cell body/soma. That’s why in neuron we will discuss three parts:
- body/soma – main part of cell with nucleus and synthetic apparatus.
- Processes, one of these is the axon transmitting information; the others are receptive dendrites. Note that the dendrites branch repeatedly, becoming finer. The axon retains its diameter along most of its length. The axon, though, may give off side branches or collaterals, and will usually divide into many fine branches, telodendria or the preterminal axonal arborization, near to its terminal structures.
- Endings of processes which according to function classified into three types: sensory (afferent), motor (efferent) and synapses.
Neurons are derived from primitive neuroblasts during embryogenesis. Neurones are terminally differentiated cells that, for all practical purposes, do not regenerate in the event of cell death. Studies have shown cell division in neurones in the adult brain, although the biological significance of this remains uncertain. However, regeneration of axons and dendrites can occur in the event of damage, provided the neurone cell body remains viable. This is the basis of nerve grafting used to treat peripheral nerve injuries.

Classificaions of neurons are based on structure, functions and biochemistry

According to the number of processes (fig. 3) and shape of perikaryon neurons can be:

1. **Unipolar** have one process, e.g., neuroblast.

2. **Pseudounipolar** have one process branching into two a short way from the cell soma, e.g., dorsal-root ganglion cell.

3. **Bipolar** have two processes, e.g., bipolar cell of the retina.

4. **Multipolar** have many processes.

Shapes include:

(a) *stellate* or star-like,
(b) *pyramidal* with apical and basal dendrites, or
(c) *Purkinje* with a plump body tapering to an espalier-oriented dendritic tree.

According to the role and position in reflexory arc neurons can be:

1. Afferent (sensory)
2. associative (interneuron)
3. efferent (motor)

Nerve cell structure

1. **Soma** contains a large central nucleus with much sap, but little visible chromatin. The nucleolus is prominent because the neuron has to synthesize organelles and much cytoplasm to fill its long processes.

2. Around the nucleus there is the **perikaryon** with:
   (a) **Nissl bodies/granules** (fig. 4) - basophilic, cytoplasmic structures which are the concentrations of rough ER and ribosomes (polysomes).
   (b) **Neurofilaments** (fig. 5) - a variety of intermediate filaments - are aggregated into neurofibrils visible in the cytoplasm after silver impregnations.
   (c) Around the nucleus there are the elements of the Golgi apparatus, mitochondria, lysosomes, and microtubules. Actin filaments move vesicles into the zone directly under the neuron’s plasmalemma.
   (d) Pigment is sometimes present, e.g., melanin in the neurons of substantia nigra, and lipofuscin in old neurons.
   (e) Cell membrane has specialized receptive areas, the **subsynaptic membranes** of synapses.

2. **Dendrites** - Dendrites are highly branched, tapering processes which either end in specialised sensory receptors (as in primary sensory neurones) or form synapses with neighbouring neurones from which they receive stimuli. In general, dendrites function as the major sites of information input into the neurone.
   (a) Contain mitochondria, microtubules, and granular ER.
   (b) Membranes have receptive subsynaptic membrane areas.
   (c) Some dendrites have spine-like side processes, also receptive,
   (d) Dendrites integrate the excitatory influences along them, and modify their responses and morphology in learning.
Fig. 4. Nissl bodies in neurons. Histological specimen, Nissl staining: A – motor neuron, B – sensory neuron, mag. × 900.

1 – multipolar neuron; 2 – nucleus; 3 – nucleolus; 4 – Nissl bodies; 5 – axon; 6 – dendrite; 7 – glia nuclei; 8 – pseudounipolar neurons.

Fig. 5. Neurofibrils in multipolar neurons: A – scheme; B – histological specimen, silver impregnation, mag. × 600.

1 – body of neuron; 2 – processes; 3 – nucleus; 4 – neurofibrils; 5 – nucleolus.
3. Axon. Each neurone has a single axon arising from a cone-shaped portion of the cell body called the \textit{axon hillock}. The axon is a cylindrical process up to 1 metre in length terminating on other neurones or effector organs by way of a variable number of small branches which end in small swellings called \textit{terminal boutons}.

· (a) Contains \textit{axoplasm} flowing centrifugally from the somatic starting-point of the axon - the \textit{axon hillock}.

· (b) Has mitochondria, neurofilaments, microtubules, travelling vesicles, and, in some neurons, secretion droplets, in the axoplasm.

· (c) Membrane of the tube is the \textit{axolemma}, swelling out into a bag at its terminal/synapse which holds vesicles/microvesicles. (The axon is also termed the axis cylinder.)

· Axon may or may not be \textit{myelinated}. It may or may not give off \textit{collaterals}, sometimes recurrent back to near the soma.

· Final part of the axon branches to give \textit{preterminal fibres}, often connecting to a great number of nerve cells.

· All around the processes of the nerve cells, the space is almost fully taken by the \textit{glial cells’ processes}, unseen except in EM or after special staining.

Action potentials arise in the cell body as a result of integration of afferent (incoming) stimuli; action potentials are then conducted along the axon to influence other neurones or effector organs.

**GLIAL CELLS**

**Gliaal functions**

1. \textit{Myelination} of myelinated axons (oligodendrocytes).

2. Augmenting the \textit{extracellular space}, e.g., being an active compartment for ionic buffering by taking up and redistributing K~, and metabolizing transmitters (astrocytes). The CNS has little true tissue space and no lymphatics.

3. Helping to induce endothelial cells to create the \textit{blood-brain barrier} (astrocytes).

4. \textit{Insulating} chemical and electrical events from nearby sensitive structures (astrocytes and oligodendrocytes).

5. \textit{Storing glycogen} and passing on raw materials for the energetic and synthetic processes of the neuron (astrocytes).

6. \textit{Acting as macrophages} to remove degenerating nerve cell components (microglia).

7. \textit{Protecting} neurons by metabolising excess ammonia during liver disease (astrocytes).

8. \textit{Mechanically supporting} the neuronal elements and keeping them properly spaced (astrocytes and oligodendrocytes).

9. Transient radial glia \textit{guide} the migration of developing neurons.

**Gliaal cell types** (fig. 6). All glial cells are subdivided into two types

1. **Macroglia**, derived from neural tube or nerve crest and present in CNS:
   
   1. \textit{Oligodendrocytes}/oligodendroglia: plump cell body with fairly dense cytoplasm and a darker nucleus and fewer, shorter processes than an astrocyte; common in white matter, but some are perineuronal.
   
   2. Astrocytes:
      
      2.1. \textit{Protoplasmic astrocytes}: large, star-shaped with many processes, some of which attach pedicels/pedicles/sucker-feet to blood vessels or the basal lamina under the pia mater; have cytoplasmic filaments and microtubules; are common in grey matter.
      
      2.2 \textit{Fibrous astrocytes}: similar to protoplasmic astrocytes, but have more filaments and glycogen, and lie in the white matter.
   
   3. \textit{Ependymal cells}: lining ventricles, and covering the choroid plexus.

Peripheral glia: satellite cells and Schwann cells may be roughly equated with oligodendrocytes by function. Peripheral glia in the gut autonomic system - enteric glia - are more like astrocytes.

2. **Microglia** – (a) derived from mesenchyme via bone marrow;

(b) potentially phagocytic;

(c) dispersed throughout the brain;

(d) a small elongated cell with many short processes and a dark nucleus.

This is the ramified or resting microglial cell, which becomes round and phagocytic as a \textit{reactive microglial cell} (Gitter cell), when responding to damage.
Some evidence for cell types performing these functions
1. Oligodendroglia contain myelin basic protein. Their membranes are connected with myelin lamella that they form.
2. Excluding myelin, insulation is a task of astrocytes whose processes enfold synapses and neural membranes.
3. Astrocyte cytoplasm also could serve as a nutritive pathway via its pedicles and processes from the blood capillary wall to the neuron, and can transfer ions and inactivated transmitters in the reverse direction.
4. Fibrous astrocytes have long processes, firm connections with one another and very little content in their cytoplasm apart from filaments and glycogen. They would seem to be fitted for the role of mechanical support.

Neurones have a very high metabolic demand and are especially vulnerable to deprivation of oxygen or nutrients by obstructing the blood supply. Relatively short periods of deprivation of nutrients lead to nerve cell death.

Neurodegenerative diseases
A series of diseases mainly seen in old age characterised by progressive degeneration and death of nerve cells, often limited to specific neuronal systems. Alzheimer’s disease, motor neurone disease and Parkinson’s disease fall in this group.

Demyelinating diseases
The transmission of signals in the nervous system is largely performed by long nerve cell processes which are insulated by cells making a substance called myelin. These specialised myelin-forming cells can be the target of specific diseases, leading to loss of function of effective signalling between nerve cells. Multiple sclerosis is the main disease in this group.

Stroke
The vascular supply to the brain is vital in maintaining its function. If blood vessels become blocked or bleed there is corresponding damage to the functioning nerve cells. Stroke is one of the major causes of morbidity and death in affluent societies.

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Fig.6. Different types of glial cells.

1 – oligodendrocytes; 2 – protoplasmic astrocyte; 3 – fibrous astrocyte; 4 – ependymocytes.
**Drugs and the blood-brain barrier**

There is a highly specialised barrier to the diffusion of substances from the blood into the brain which functionally relates to the arrangement of support cells and basement membrane around capillaries in the CNS. Certain drugs cannot penetrate the brain because of this barrier (fig. 7).

![Diagram](image)

Fig. 7. Scheme of interaction between astrocyte, neuron and blood vessel.

1 – pericaryon of neuron; 2 – nucleus of neuron; 3 – astrocyte; 4 – processes of astrocyte forming perivascular glial membrane; 5 – synapses; 6 – blood vessel.
Lesson 13

Theme: Nerve Tissue 2. Nerve Fibres and Nerve Endings

Background: The basis of the functional activity of nerve tissue is complex functions of different neurons (the reflex arch), representing a dynamic system. Association of neurons in a certain system is ensured by intercellular junctions, which are formed by processes and endings of nerve cells. Processes of nerve cells with oligodendrocytes (neurolemmocytes) form the nerve fibers and transmit nerve impulses to the body of a neuron or target cell, which are located at great distance from the cell body, provide transport of substances and organelles, providing neurotrophic effect.

Aim of Study: Able to distinguish in histological specimens of nerve fibers, receptor and motor nerve endings of interneuronal synapses, interpret the characteristics of their structure in order to understand histophysiology of the nervous system, regeneration of its elements, that is the basis for mastery of basic and clinical disciplines (private histology, normal and pathological physiology, pathological anatomy, pharmacology, neurology, neurosurgery, psychiatry).

To achieve this aim one has to (practical procedures):
1. Identify objects in the morphological nerve fibers and endings.
2. Differentiate different types of nerve fibers, interpret the principles of their classification.
3. Identify the different types of nerve endings and interpret their function.
4. Interpret the structure of chemical and electrical synapses, the principles of classification.
5. Interpret the nature and mechanisms of regeneration of nerve fibers and endings.
6. Interpret simple and complex organization of nerve reflectory arcs, the role of synapses in their formation.

To achieve the aim it is necessary to centre around the following points:
1. Nerve fibers: general morphofunctional characteristics, classification.
2. Microscopic and submicroscopic structure of myelinated nerve fibers.
3. Structure of unmyelinate nerve fibers. Functional parameters of nerve fibers associated with the peculiarities of their structure.
4. Stages of myelination of peripheral and central nerve fibers. The concept of demyelination.
6. Receptor nerve endings: classification, localization, microscopic and submicroscopic structure and functions.
7. Synapses. Classification. Microscopic and submicroscopic structure of the chemical and electrical synapses. The concept of a mediator.
8. Morphological substrate of the reflex activity of the nervous system. The concept of simple and complex reflex arcs.
9. Regeneration of nerve fibers: the nature, stages, the role of glial cells, the effect of endogenous and exogenous factors.
10. Age-related changes of elements of nervous tissue. Changes that occur during individual development. Effect of endogenous and exogenous factors on the structure of the elements of nervous tissue.

What must you know? (Instruction for your self-learning)

Nerve fibre includes:
1. Nerve cell process is also termed the axis cylinder.
2. Glial sheath.
   - Membrane of the neuron processes is the axolemma, swelling out into a bag at its terminal/synapse which holds vesicles/microvesicles.
   - Myelin sheath of lipoprotein around the axolemma is interrupted at regular intervals to leave the axolemma “bare” at nodes of Ranvier. The membrane propagates the action potential.
   - EM reveals myelin to have lamellae with alternate dark (major dense) and light (intraperiod) lines, apparently concentric around the axon.
(f) Closer examination reveals a *mesaxon* from the innermost lamella by the axolemma, and an outer mesaxon from the outermost lamella to join the enclosing Schwann or glial cells’ plasmalemma.

To control the neuron’s ionic and transmitter environment, enclosure of the axon (and myelin) by Schwann or glial cells is complete, including nodes of Ranvier and the terminal bags (synapses). At intervals along the myelin, Schwann cell cytoplasm penetrates into conically oriented separations of the myelin lamellae, constituting the incisures of Schmidt-Lantermann, perhaps giving the myelin some flexibility and aiding molecular turnover.

**Myelination process** (fig. 1)

1. Many axons remain unmyelinated throughout their existence. However, for rapid *saltatory* (jumping) nerve conduction a myelin sheath interrupted by *nodes* is necessary. This sheath is a modified lipoprotein membrane, rich in cerebrosides and other special lipids and proteins.

2. The process of myelination in *peripheral* fibres is by an apparent “rotation” of the *Schwann cell* in relation to the axon that it has enfolded, thus enclosing the axon in many layers of Schwann-cell membrane. These membranes fuse together, but the lamellar structure remains visible in EM, and an outer *mesaxon* connects the last wrapping to the Schwann cell’s own plasmalemma. One Schwann cell myelinates a given length of axon, which is separated by an unmyelinated *node of Ranvier* from the next myelinated segment. Outside the Schwann-cell or neurolemmal sheath lies a *basal lamina*, beyond which the collagen fibrils and fibroblasts of the *endoneurium* are found.

3. In the CNS, the oligodendrocyte incrementally adds membranes to several axons, and to more than one segment per axon. This myelin configuration is compatible with “spiralling” membrane synthesis, but not actual rotation. Nodes are present, but not as distinct as in the PNS.

4. Myelination takes place in different tracts of the brain at different times during development. The time of myelination correlates fairly well with the development of the ability to function in that system.

5. *Remyelination* (successful or attempted) is involved in the mature nervous system in two circumstances - the regeneration of peripheral nerve fibres, and demyelinating diseases in the CNS and peripheral NS.

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**MYELINATED AND NON-MYELINATED NERVE FIBRES**

In the peripheral nervous system, all axons are enveloped by highly specialised cells called *Schwann cells* which provide both structural and metabolic support. In general, small diameter axons (e.g. those of the autonomic nervous system and small pain fibres) are simply enveloped by the cytoplasm of Schwann cells; these nerve fibres are said to be *non-myelinated*. Large diameter fibres are wrapped by a variable number of concentric layers of the Schwann cell plasma membrane forming a *myelin sheath*; such nerve fibres are called *myelinated*. Within the central nervous system, myelination is similar to that in the peripheral nervous system except that the myelin sheaths are formed by cells called *oligodendrocytes*. There are however distinct chemical differences between central and peripheral myelin. In all nerve fibres, the rate of conduction of action potentials is proportional to the diameter of the axon; myelination greatly increases axon conduction velocity compared...
with that of a non-myelinated fibre of the same diameter.

**Non-myelinated nerve fibres** (fig. 2, 3)

One or more axons become longitudinally invaginated into the Schwann cell so that each axon is embedded in a channel, invested by the Schwann cell plasma membrane and cytoplasm. The Schwann cell plasma membrane becomes apposed to itself along the opening of the channel, thus effectively sealing the axon within an extracellular compartment bounded by the Schwann cell. The zone of apposition of the Schwann cell membrane is called the *mesaxon*. Note that more than one axon may occupy a single channel within the Schwann cell. Each Schwann cell extends for only a short distance along the nerve tract and at its termination the ensheathment is continued by another Schwann cell with which it interdigitates closely end to end.

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**Myelinated nerve fibre** (fig. 4)

In peripheral nerves, myelination begins with the invagination of a single nerve axon into a Schwann cell; a mesaxon is then formed. As myelination proceeds, the mesaxon rotates around the axon thereby enveloping the axon in concentric layers of Schwann cell cytoplasm and plasma membrane. The cytoplasm is then excluded so that the inner leaflets of plasma membrane fuse with each other and the axon becomes surrounded by multiple layers of membrane which together constitute the myelin sheath. The single segment of myelin produced by each Schwann cell is termed an *internode*; this ensheaths the axon between one node of Ranvier and the next.
In the CNS, oligodendrocytes are responsible for myelination; a single oligodendrocyte, however, forms multiple myelin internodes, which contribute to the ensheathment of as many as 50 individual axons.

The layers of cell membrane that form myelin are bound together by special proteins that differ between CNS and PNS. In the CNS **proteolipid protein** links the exoplasmic surfaces, while cytoplasmic surfaces are linked by **myelin basic protein**. In the PNS **P0 protein** associates with **myelin basic protein** to form the major dense line. PNS myelin also contains **peripheral myelin protein-22**.

**DISORDERS OF MYELIN**

Several diseases specifically affect formation of myelin, either in the CNS, the PNS or both.

- **In multiple sclerosis** there is immune-mediated destruction of myelin confined to the CNS. This leads to slowing of axonal conduction and neurological dysfunction. Signs and symptoms relate to the location of affected white matter. Histological examination of an affected area shows loss of myelin staining in areas called **plaques of demyelination**.

- **In Guillain-Barré syndrome** there is immune-mediated destruction of myelin in the peripheral nervous system. Illness is often triggered by an infectious condition. Patients develop rapidly progressive weakness of limbs and weakness of respiratory muscles. Histological examination of affected nerve shows loss of myelin with preservation of axons. Conduction velocity in affected nerves is greatly slowed.

Mutation in genes coding for myelin proteins is the basis of several inherited disorders of the nervous system.
SYNAPSES

The sites of intercommunication between neurones are termed synapses. Depolarisation of one neurone causes it to release chemical transmitter substances, neurotransmitters, which initiate an action potential in the adjacent neurone. Within the nervous system, neurones are arranged to form pathways for the conduction of action potentials from receptors to effector organs via integrating neurones. Neurotransmitters not only mediate neurone-to-neurone transmission but also act as chemical intermediates between the nervous system and effector organs which also exhibit the property of excitability (fig. 5).

Synapses are specialized neuron-to-neuron cell contacts, firmly attached and functionally polarized to transfer excitation one way (except for 7).

Types of synapse
1. Axosomatic: to the neuron’s body.
2. Axodendritic: e.g., from climbing fibers to Purkinje cells’ dendrites.
3. Axodendritic to spines, e.g., from parallel fibers to Purkinje cells’ dendritic spines. (The presence of spines on dendrites is used to subclassify neurons in many brain regions.)
4. Glomerular: a rounded structure serving several dendrites, e.g., from mossy fibers to cerebellar granule neurons.
5. En passant: made “in passing” on the way to other synapses.
6. Axo-axonic: synapse onto another synapse or the axon’s initial segment (for presynaptic inhibition).
7. Reciprocal dendro-dendritic: e.g., in retina and olfactory bulb.

Synapses also differ in the number, size and density of their vesicles, in the transmitter and neuromodulator substances that these hold, in the organelles present, and in the cleft material and membrane densities.

Chemical neuroanatomy involves mapping which connections of the CNS employ particular neurotransmitters, e.g., serotonin, acetylcholine, dopamine, etc.

Synaptic loss and Alzheimer’s disease

In Alzheimer’s disease, the commonest cause of dementia, an early pathological feature is loss of synapses from the hippocampus and the cerebral cortex. The synapses mediating neurotransmission by acetylcholine (cholinergic system) are particularly affected. The identification of this transmitter deficit has led to development of drugs to maximise the concentration of acetylcholine in the remaining synapses. Acetylcholine is rapidly destroyed once secreted into the synaptic cleft by the action of cholinesterases. Cholinesterase inhibitor drugs are now given to patients with Alzheimer’s disease to compensate for the synaptic loss by maximising the impact of remaining cholinergic synaptic activity.

RECEPTORS AND SENSORY FIBERS

Sensory fibres (except for those of cranial nerves) are derived from the dorsal root/spinal ganglion cells lying just outside the spinal cord. The fiber is T-shaped with one branch entering the cord as an element of the dorsal root, and the other coming from a sensory receptor of one of the following kinds *

1. Skin and some mucous membranes (exteroceptors)
   1. *Meissner’s corpuscles* - common in dermal papillae of fingers, palms, nipple, etc (fig. 6 A).
   2. *Krause’s end-bulbs* and *Ruffini’s end-organs* - in external genitalia, dermis, tongue, joints, etc (fig. 6 B).
   3. *Pacinian corpuscles* - large, lamellated bodies, in external genitalia, also lie more deeply under the skin, in tendons, mesentery, joints, etc (fig. 7).
Receptors/endings 1 to 3 are definitely encapsulated.
5. *Free nerve endings* - also intra-epithelial (fig. 8).
6. *Palisade/peritrichal* endings around a hair follicle.

Fig. 6. Scheme of structure of sensory nerve endings: A – Meissner’s corpuscle; B – Ruffini’s end-organ.

1 – capsule; 2 – collagen fibers; 3 – myelinated nerve fibers; 4 – нервные термины; 5 – epithelial cells; 6 – auxiliary cells; 9 – internal capsule; 10 – space inside the capsule.

Fig. 7. Pacinian corpuscle. Histological specimen. Mag. × 200.

1 – encapsulated nerve ending; 2 – internal capsule; 3 – external capsule; 4 – nuclei of glial cells; 5 – connective tissue.

Fig. 8. Free nerve endings in stratified squamous keratinised epithelium. Mag. × 120.

1 – epidermis; 2 – free nerve endings; 3 – nerve fibers; 4 – connective tissue of dermis.
How far the morphology of a receptor can be related to a special sensitivity to a particular modality, e.g., pain, touch, cold, is disputed.

All receptor axon terminals lack myelin and contain vesicles, mitochondria, and filaments.

2. Muscle, joint and tendon (proprioceptors)

1. *Golgi tendon organ* - branching nerve fibres with thickenings between a tendon’s collagen fibers. Joint and ligament receptors are similar, but some take more specialized forms, e.g., Pacinian (fig. 9 A).

2. *Muscle spindle* (fig. 9 B)
   
   - (a) A spindle-shaped C.T. capsule encloses several thin specialized *intrafusal* skeletal muscle fibers. Away from the muscle fiber’s central region with clustered or linear nuclei (nuclear-bag and nuclear-chain fibers) are plate or trail motor endings served by mostly gamma (fusimotor) nerve fibres.
   
   - (b) Two types of sensory ending on the fibers - primary annulospiral and secondary flower-spray - increase the combinations of innervations.
   
   - (c) The larger *extrafusal* muscle fibers stimulate the attached spindle by their movements, while its sensitivity is varied by its own alpha and gamma motor control.
   
   - (d) The spindle informs on the complexities of muscle contraction, movement and stretch, and gives a “muscle sense”.

3. (Mechano-receptors of the vestibular apparatus (Chapter 14.C.) inform the CNS independently of the results of the muscles’ actions in terms of changed position and movement of the head. Skin receptors likewise contribute to “proprioception” in the lax sense.

3. Viscera (interoceptors)

1. *Carotid body - chemoreceptor* for blood O₂ tension; has sinusoids with blood passing in close relation to glomus/Type I cells. Clusters of these cells, with their cored vesicles, are innervated by axons of the glossopharyngeal nerve. The intermixed sustentacular/Type II cells are glial, and have no known role in signal transduction. The aortic body is similar in structure and function, and connects with the vagus nerve.

2. *Carotid sinus and aortic arch - pressoreceptors/baroreceptors* (for blood pressure) set within the vessel’s wall.

3. *In lung, gut, bladder, and other viscera - measuring distension, motility and chemical irritation.
4. Brain (other intero-chemoreceptors)
1. In hypothalamus: for blood osmolarity, glucose, hormones (and for temperature).
2. In medulla: for CO₂ tension of the blood.

MOTOR ENDINGS AND MOTOR FIBERS (fig. 10)
1. Skeletal-muscle motor fibres are derived from motor neurons/motoneurons within the CNS, either in the ventral-horn grey matter of the spinal cord or in motor nuclei of cranial nerves.

Light microscopic view (after-gold-chloride impregnation). Nerve fiber branches to serve several skeletal muscle fibers, terminating on each as an irregular branching net lying on a small area of the muscle cell, its sole plate.
2. *End-plate or neuromuscular/myoneural junction* (EM morphology)
   · (a) The sarcolemma is depressed into a trough.
   · (b) The *swollen ending* of the axon lies in the trough.
   · (c) The sarcolemma lining the trough invaginates into many small *junctional folds/secondary synaptic clefts*. It has *ACh receptors*.
   · (d) The space between the sarcolemma and axolemma is filled by a *basal lamina*-like material.
   · (e) The axon terminal has mitochondria, and vesicles containing the chemical transmitter substance, *acetylcholine*, which, when released by electrical activity in the axon, indirectly causes the muscle fibre to contract.
   · (f) *Schwann cells* cover the axon and its terminal bag.
3. A *motor unit* comprises a motoneuron and all muscle fibres on which it has end-plates.

2. **Postganglionic autonomic nerve fibre terminals**
   1. Control smooth muscle contraction and excocrine glandular secretion, or go to the heart muscle and adrenal medullary cells.
   2. Axons lie against, or sometimes within, invaginations of the muscle fibres or glandular cells, making mostly *en passant* contacts.
   3. but specialized sarcolemmal structures comparable with a motor end-plate’s are not present.
   4. The nerve fibres are, however, widely dispersed as a *plexus* between the smooth muscle fibres, and contain many *vesicles* concentrated periodically.
   5. These vesicles may contain one of the two principal transmitter substances - *acetylcholine* (ACh) and *norepinephrine* (*Ne*)/noradrenaline, along with other chemicals, e.g., peptides. Some neurons and fibres are neither cholinergic nor adrenergic. A chemical mapping of the PNS (crucial to pharmacology) is under way, including the sensory pathways to autonomic ganglia.

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**Fig. 12. Scheme of structure of simple reflectory arc.**

1 – receptor inside the skin; 2 – dendrite of sensory neuron; 3 – body of sensory neuron; 4 – spinal ganglion; 5 – axon of sensory neuron; 6 – spinal cord; 7 – body of motor neuron; 8 – axon of motor neuron; 9 – peripheral nerve trunk; 10 – motor plate; 11 – skeletal muscle fiber.
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