МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

# PART 4
# FUNCTIONAL BIOCHEMISTRY

**Self-Study Guide for Students of
General Medicine Faculty in Biochemistry**

# ЧАСТИНА 4
# ФУНКЦІОНАЛЬНА БІОХІМІЯ

**Методичні вказівки
ДЛЯ ПІДГОТОВКИ ДО ПРАКТИЧНИХ ЗАНЯТЬ
З БІОЛОГІЧНОЇ ХІМІЇ
(Для студентів медичних факультетів)**

Затверджено вченою радою ХНМУ.
Протокол № 4 від 27.04.2017 р.

Approved by the
Scientific Council of KhNMU.
Protocol 4 (April 27, 2017)

**Харків
ХНМУ
2017**

Self-study guide for students of general medicine faculty in biochemistry. Part 4. Functional biochemistry / comp. O. Nakonechna, S. Stetsenko, L. Popova, A. Tkachenko. – Kharkiv : KhNMU, 2017. – 80 p.

Методичні вказівки для підготовки до практичних занять з біологічної хімії (для студентів медичних факультетів). Частина 4. Функціональна біохімія / упоряд. О.А. Наконечна, С.О. Стеценко, Л.Д. Попова, А.С. Ткаченко. – Харків : ХНМУ, 2017. – 80 с.

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## SOURCES
## For preparing to practical classes in "Biological Chemistry"
### Basic Sources


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## ***CLASS 1 (4 hours)***

**TOPIC 1 (4 hours): Functions of the blood. Physical and chemical properties and chemical composition. Buffer systems, the mechanism of their action and role in maintaining acid-base balance of the organism. Plasma proteins and their role. Quantitative determination of total protein in serum.**

**IMPORTANCE.** Blood is a liquid tissue consisting of cells (formed elements) and extracellular fluid called plasma. The blood performs the transport, osmoregulatory, buffer, detoxificative, protective, regulatory, hemostatic and other functions. The composition of blood plasma is a "mirror" of metabolism because changes in the content of metabolites in the cells are shown on their concentration in blood plasma. Composition of the blood plasma varies in the changes in the permeability of cell membranes. In this regard, as well as the availability of blood samples for analysis, its research is widely used to diagnose diseases and to monitor the treatment effectiveness. In addition to specific nosological information, quantitative and qualitative study of plasma proteins gives an idea about the state of protein metabolism as a whole. The index of hydrogen ion concentration in the blood (pH) is one of the most importnant constants of the body. It reflects the state of metabolic processes and depends on the functioning of many organs and systems. Disorders of blood acid-base balance are observed in numerous pathological processes and serve as a cause of severe disorders of vital activity. Therefore, they are an essential component of therapeutic interventions to correct acid-base balance properly.

**AIM.** Familiarize yourself with the physical and chemical properties of the blood acid-base balance and its main characteristics. Learn blood buffer systems and the mechanism of their action; disorders of acid-base balance of the body (acidosis, alkalosis), their forms and types. Form an idea of the blood plasma protein composition. Characterize protein fractions and individual proteins, their role, disorders and methods for their determination. Familiarize yourself with the methods of quantitative determination of the total protein in the blood serum, of individual proteins fractions and their clinical and diagnostic significance.

## *THEORETICAL QUESTIONS*

1. Functions of the blood in the living organism.
2. Physical and chemical properties of blood, serum, lymph: pH, osmotic and oncotic pressure, relative density, viscosity.
3. Blood acid-base balance, its regulation. The main parameters reflecting its disorders. Modern methods for the determination of blood acid-base balance.
4. Blood buffer systems. Their role in maintaining the acid-base balance.
5. Acidosis: types, causes, mechanisms of development.
6. Alkalosis: types, causes, mechanisms of development.
7. Blood proteins: content, functions, changes in pathological states.
8. The main fractions of blood plasma proteins. Methods of investigation.
9. Albumins, physical and chemical properties, their role.
10. Globulins, physical and chemical properties, their role.
**11*.** Immunoglobulins of blood, structure, and functions.
12. Hyper-, hypo-, dys- and paraproteinemias; causes of their appearance.
**13*.** Acute phase proteins. Clinical and diagnostic significance of their determination.

### *Recommendations for self-study of theoretical questions*

| *Question* | *Information* |
|---|---|
| *1. Blood immunoglobulins: structure, functions.* | *1.1. Immunoglobulins are proteins of tetrameric structure, consisting of four polypeptide chains: 2H (heavy) and 2L (light); Classes - IgA, IgM, IgG, IgE, IgD.*<br>*1.2. Site of synthesis is B-lymphocytes.*<br>*1.3. Blood plasma proteins of γ-globulin fraction.*<br>*1.4. Role: protective, effectors of humoral immunity.* |
| *2. Acute phase proteins. Clinical and diagnostical significance of their determination.* | *2.1. Acute phase proteins are a group of blood proteins with various functions, united by a common feature. Their concentration rapidly and significantly increase in inflammation, bacterial, viral or parasitic infections, trauma, toxic or autoimmune reactions, malignant tumors, acute diseases.*<br>*2.2. Characteristics of the major representatives: - C-reactive protein has a broad ligand specificity, activates the complement system, interacts with different types of cells; its role includes recognition of various substances present on the surface of cells in microorganisms or human tissues, activation of the corresponding functional systems and, as a consequence, elimination of pathogens, as well as necrotic cells from the body. C-reactive protein is determined in the blood inder the following circumstances: 1) diagnosis of bacterial infection and monitoring of response to antibiotic therapy; 2) diagnosis of the disease acute stage in systemic lupus* |

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| | *erythematosus, ulcerative colitis, etc.; 3) diagnosis of the disease acute stage and therapy monitoring in patients with rheumatoid arthritis; 4) early diagnosis of infectious and inflammatory complications in patients who underwent surgery; 5) diagnosis of infectious complications during bone marrow and kidney transplantation; 6) assessment of the risk of cardiovascular pathology, etc.*<br>*- α2-Macroglobulin is a universal inhibitor of proteinases; it participates in the regulation of tissue proteolytic systems, blood coagulation, fibrinolysis, immune processes, complement system, inflammatory reactions, and regulation of vascular tone. An increase in the content of α2-macroglobulin is observed in nephrotic syndrome, liver diseases, diabetes mellitus, bronchopneumonia, and congenital heart diseases (a decrease in its content is revealed in fibrinolysis, acute pancreatitis, gallstones or kidney stones, peptic ulcer and duodenal ulcer, and myocardial infarction).*<br>*- α1-Antitrypsin is an inhibitor of proteolytic enzymes of the kinin and complement systems, fibrinolytic system, proteases released by neutrophils. In the inflammatory process in the lung tissue it effectively suppresses the activity of elastase, preventing the degradation of the connective tissue protein elastin in the alveolar walls and the development of emphysema; it modulates the local immune response. α1-Antitrypsin has an antioxidant and antimicrobial effect. An increase in the content of α1-antitrypsin is observed in infectious and rheumatoid diseases, tissue necrosis, hepatitis.*<br>*- Haptoglobin is a specific protein that binds blood hemoglobin → a complex of haptoglobin-hemoglobin cannot pass through renal glomeruli, preventing hemoglobin excretion from the body; the main physiological role of haptoglobin is to retain iron: when the haptoglobin-hemoglobin complex is broken down, iron is released; it is transported by blood proteins to the red bone marrow, where red blood cells are formed; The haptoglobin-hemoglobin complex has an antioxidant effect. An increase in the content of haptoglobin is observed in infectious diseases, traumas, necrosis, burns, sepsis, systemic connective tissue diseases (rheumatism, systemic lupus erythematosus, rheumatoid arthritis), diabetes mellitus, ulcerative* |

| | |
|---|---|
| | *colitis, myocardial infarction, etc. (A decrease is revealed in hemolytic anemia) .*<br>*- Ceruloplasmin is responsible for the transport of copper into tissues, catalyzes iron oxidation (ferrooxidase), is involved in the oxidation of catecholamines and serotonin, has an antioxidant and anti-inflammatory effect (inhibits serum histaminase). An increase in the content of ceruloplasmin is observed in chronic inflammatory processes (especially after surgery) in acute infectious diseases, nonspecific pneumonia, pulmonary tuberculosis, rheumatism, malignant neoplasms, liver diseases (hepatitis, cirrhosis, mechanical jaundice), pernicious anemia, dysentery, melanoma, myocardial infarction, etc. (a decrease in its content is revealed in Wilson's disease).* |

## *TESTS FOR SELF-CONTROL*

**1.** Which of the pH values mentioned below corresponds to normal blood pH?

*A. 7.25-7.31* *B. 7.40-7.55* *C. 7.35-7.45*
*D. 6.59-7.0* *E. 4.8-5.7*

**2.** Which mechanisms provide blood pH stability?

*A. $CO_2$ removal by lungs* *B. Buffer systems*
*C. Hydrogen ion secretion by kidney* *D. Metabolism of substances*
*E. All options mentioned above are correct*

**3.** What is the cause of metabolic acidosis development?

*A. Increased production and decreased oxidation of ketone bodies*
*B. Increased production and decreased oxidation of lactate*
*C. Loss of basic equivalents*
*D. Ineffective hydrogen ions secretion, retention of acids*
*E. All options mentioned above are correct*

**4.** What is the cause of metabolic alkalosis development?

*A. Uncompensated loss of hydrogen ions* *B. Loss of potassium*
*C. Retention of alkalis* *D. Intake of alkalis*
*E. All options mentioned above are correct*

**5.** Considerable losses of gastric juice in prolonged vomiting provide the development of:

*A. Respiratory acidosis* *B. Metabolic alkalosis*
*C. Respiratory alkalosis* *D. Metabolic acidosis*

**6.** Considerable disturbances of blood circulation in response to shock provide the development of:

*A. Metabolic acidosis* *B. Respiratory acidosis*
*C. Respiratory alkalosis* *D. Metabolic alkalosis*

**7.** Inhibition of respiratory center in the brain by narcotic drugs results in:

*A. Respiratory acidosis* *B. Metabolic acidosis*
*C. Hyperglycemia* *D. Respiratory alkalosis* *E. Metabolic alkalosis*

**8.** Blood pH is 7,3 in a patient with diabetes mellitus. Which buffer system components are used for diagnostics of acid-base balance disturbances?

*A. Phosphate* *B. Bicarbonate* *C. Oxyhemoglobin*
*D. Hemoglobin* *E. Protein*

**9.** Embolism of respiratory tract by phlegm is observed in a patient. Which disorder of acid-base balance may be found in blood?

*A. Respiratory acidosis* *B. Metabolic acidosis*
*C. Acid-base balance is normal*
*D. Respiratory alkalosis* *E. Metabolic alkalosis*

**10.** Apparatus of artificial ventilation of lungs has been attached to a patient with severe trauma. Determination of acid-base balance indices show a decrease in the content of blood carbon dioxide and an increase in its removal. These changes are typical for:

*A. Respiratory alkalosis* *B. Respiratory acidosis*
*C. Metabolic alkalosis* *D. Metabolic acidosis*

**11.** Point out a blood buffer system, which is the most important in the regulation of acid-base balance:

*A. Phosphate* *B. Hemoglobin*
*C. System of blood plasma proteins* *D. Bicarbonate*

**12.** Which buffer system plays an important role in supporting pH of urine?

*A. Phosphate* *B. Hemoglobin*
*C. Bicarbonate* *D. Protein*

**13.** Which physical and chemical properties of blood are provided by electrolytes?

*A. Oncotic pressure* *B. Erythrocyte sedimentation rate*
*C. Osmotic pressure* *D. Viscosity*

**14.** Examination of a patient revealed hyperglycemia, glycosuria, hyperketonemia and ketonuria, polyuria. Which type of acid-base balance disorder is observed in this case?

*A. Respiratory alkalosis* *B. Metabolic acidosis* *C. Metabolic alkalosis*
*D. Respiratory acidosis* *E. Acid-base balance is not affected*

**15.** At rest a man makes himself to breathe frequently and deeply within 3-4 minutes. How it affects the acid-base balance of an organism?

*A. There is metabolic alkalosis.* *B. There is respiratory acidosis*
*C. There is respiratory alkalosis* *D. There is metabolic acidosis*
*E. Acid-base balance is not affected*

**16.** Which blood plasma protein binds and transports copper?

*A. Transferrin* *B .Bradykinin* *C. C–reactive protein*
*D. Kallikrein* *E. Ceruloplasmin*

**17.** The content of total protein in blood plasma is normal. Which of the below - mentioned parameters corresponds to physiological norms?

*A. 33-45 g/L* *B. 50-60 g/L* *C. 55-70 g/L*
*D. 65-85 g/L* *E. 85-95 g/L*

**18.** Which fraction of blood globulins provides humoral immunity performing a function of antibodies?

*A. $\alpha_1$-Globulins* *B. β-Globulins* *C. γ-Globulins*
*D. Cryoglobulins* *E. $\alpha_1$-Macroglobulins*

**19.** Symptoms of liver cirrhosis with ascites and edema of lower extremities appeared in a patient who had hepatitis C and constantly consumed alcohol. Which changes in blood composition underlied edema development?

*A. Hypoglobulinemia* *B. Hypoalbulinemia* *C. Hypokaliemia*
*D. Hypoglycemia* *E. Hypocholesterolemia*

**20.** Which physical and chemical property of protein is the base of the method of electrochemical determination of blood protein spectrum?

*A. Viscosity* *B. Presence of charge* *C. Ability to be denaturated*
*D. Hydrophility and ability to swell* *E. Optical activity*

**21**. Edema rapidly develops in a patient. Which protein is reduced in blood serum in edema?

*A. $\alpha_1$-Globulins* *B. $\alpha_2$-Globulins* *C. Albumins*
*D. β-Globulins* *E. Fibrinogen*

**22**. There are several dozens of proteins in blood plasma of healthy individuals. New proteins may appear in blood during various diseases, in particular "acute phase proteins." One of the following proteins belongs to this group:

*A. Immunoglobulin A* *B. C-Reactive protein* *C. Prothrombin*
*D. Immunoglobulin G* *E. Transcobalamin*

**23**. A patient with acute pancreatitis had a threat of pancreatic necrosis, which was accompanied by the release of active pancreatic proteinases into the bloodstream and tissues and breakdown of tissue proteins. Which protective factors can inhibit such processes?

*A. Ceruloplasmin, transferrin* *B. Hemopexin, haptoglobin*
*C.Cryoglobulin, interferon* *D. Immunoglobulin*
*E. α2-Macroglobulin, α1-antitrypsin*

**24**. A patient has rheumatic fever in the active phase. Which blood serum parameter is of diagnostic significance in this pathology?

*A. C-reactive protein* *B. Uric acid* *C. Urea*
*D. Creatinine* *E. Transferrin*

**25**. In diabetes mellitus the activation of fatty acid oxidation leads to ketosis. Which disorders of acid-base balance can lead to excessive accumulation of ketone bodies in the blood?

*A. Metabolic alkalosis* *B. There will be no changes*
*C. Metabolic acidosis* *D. Respiratory acidosis* *E. Respiratory alkalosis*

**26**. A female complains of frequent chest and spine pain, fractures of ribs. A doctor suspected myeloma (plasmacytoma). Which of the laboratory parameters mentioned below will be of the greatest diagnostic significance?

*A. Hypoproteinemia* *B. Hyperalbuminemia* *C. Hypoalbuminemia*
*D. Proteinuria* *E. Paraproteinemia*

**27**. The activation of the inflammatory process, some autoimmune and infectious diseases leads to a sharp increase in the level of acute phase proteins in the blood

plasma. Which of the following proteins can form a gel when the serum is cooled?

*A. Haptoglobin* *B. Cryoglobulin* *C. C-reactive protein*
*D. α2-Macroglobulin* *E. Ceruloplasmin*

**28**. A patient was diagnosed with myeloma. The total blood protein level is 180 g/L. Such protein level was due to:

*A. Transferrin* *B. Albumins* *C. Paraproteins*
*D. Haptoglobin* *E. Immunoglobulin*

**29**. A patient had airway obstruction at the level of small and middle-sized bronchi. Which changes in the acid-base balance can develop in a patient?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**30**. Wilson disease (hepatocerebral dystrophy) is accompanied by low ceruloplasmin levels. What is the consequence of this transport protein insufficiency?

*A. Breakdown of tissue proteins*
*B. Complex formation of amino acids with copper*
*C. Decarboxylation of amino acids* *D. Urea synthesis*
*E. Transamination of amino acids*

**31**. It has been known that hemoglobin and oxyhemoglobin can act as components of blood buffer systems. What is the mechanism of their action as buffers?

*A. They are acids that form potassium salts*
*B. These are acids that form bicarbonates*
*C. They are strong alkalis*
*D. They are acids that form sodium salts*
*E. They are acids that form calcium salts*

**32.** A patient, hospitalized to the pulmonological department, was diagnosed with pulmonary emphysema, which resulted from the destruction of interalveolar septa by tissue trypsin. Which protein congenital insufficiency can caused the development of this disease?

*A. Transferrin* *B. α2-Macroglobulin* *C. Cryoglobulin*
*D. α1-Proteinase inhibitor* *E. Haptoglobin*

**33**. A breastfed child suffers from diarrhea due to improper feeding. One of its main consequences is the excretion of large amounts of sodium bicarbonate. Which form of acid-base disorder is observed in this case?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**34**. A person at rest was forced to breathe frequently and deeply for 3-4 minutes. How will this affect the acid-base balance?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**35**. Which protein binds to hemoglobin in order to transport it to the reticuloendothelial system of the liver?

*A. Haptoglobin* *B. Albumin* *C. Ferritin*
*D. Transferrin* *E. Ceruloplasmin*

**36**. A child was diagnosed with Bruton's disease, which manifested by the severe course of bacterial infections, the absence of B-lymphocytes and plasma cells. Which changes in the content of immunoglobulins were observed in the serum of this child?

*A. An increase in IgD, IgE* *B. An increase in IgA, IgM*
*C. A decrease in IgA, IgM* *D. A decrease in IgD, IgE*
*E. There will be no changes*

**37**. The amount of plasma proteins changed in a person after physical exercise under high temperature. What is the cause of such changes?

*A. Absolute hyperproteinemia* *B. Dysproteinemia*
*C. Absolute hypoproteinemia* *D. Relative hyperproteinemia*
*E. Paraproteinemia*

**38**. A patient with diabetes mellitus has hyperglycemia, ketonuria, glucosuria, hypersthenuria, and polyuria. Which form of acid-base balance disorders occurs in this situation?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. Excretory alkalosis*

**39**. To study blood serum proteins, it is possible to use different physical and physicochemical methods. In particular, blood serum albumins and globulins can be separated using the method of:

*A. Polarography* *B. Dialysis* *C. Spectrography*
*D. Electrophoresis* *E. Refractometry*

**40**. A patient shows signs of mountain sickness: dizziness, dyspnea, tachycardia. Blood pH is 7.5, pCO2 is 30 mm Hg, the buffer base shift is +4 mmol/L. Which acid-base disorder developed?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. Excretory acidosis*

**41**. One of the major complications of diabetes mellitus is the development of ketoacidosis due to the accumulation of ketone bodies in the blood serum. Which form of acid-base balance disorders occurs in this case?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis* *E. -*

**42**. The toxic damage to the liver cells with their impaired functions led to the development of edema. Which changes in the blood plasma composition are the main causes of edema in this case?

*A. An increase in the content of globulins* *B. Reduction of fibrinogen content*
*C. An increase in albumin content*
*D. Reduction of the content of globulins* *E. Reduction of albumin content*

**43**. A patient has high levels of hydroxyproline, sialic acids, and C-reactive protein in the blood. Which pathology is exacerbated?

*A. Rheumatic fever* *B. Enterocolitis* *C. Hepatitis*
*D. Bronchitis* *E. Pancreatitis*

**44**. A patient has low blood pH values and hydrocarbonate ions (decreased alkaline reserve of blood), increased levels of lactic and pyruvic acids in blood and urine. Which type of acid-base balance disorder is observed?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis* *E. -*

**45**. A child with signs of prolonged protein starvation was hospitalized: growth retardation, anemia, edema, and mental retardation. The reason for the development of edema in this child is a decrease in the synthesis of:

*A. Hemoglobin* *B. Globulins* *C. Albumins*
*D. Lipoproteins* *E. Glycoproteins*

**46**. A patient who is being treated for hepatitis B shows signs of liver failure. Which blood changes that indicate abnormal protein metabolism are most likely observed in this case?

*A. Absolute hyperglobulinemia* *B. Blood protein spectrum is not affected*
*C. Absolute hyperproteinemia* *D. Absolute hypoproteinemia*
*E. Absolute hyperfibrinogenemia*

**47**. The prolonged action of a number of antibiotics and sulfonamides is caused by the fact that they circulate in the blood for a long time in a complex with:

*A. Hemoglobin* *B. Albumin* *C. Haptoglobin*
*D. Transferrin* *E. Hemopexin*

**48**. Wilson disease is associated with a decrease in the plasma content of the protein that transports copper ions. Select this protein.

*A. Ceruloplasmin* *B. Transferrin* *C. Haptoglobin*
*D. Fibronectin* *E. C-Reactive protein*

**49**. Paraproteins are proteins of the γ-globulin fraction that appear in the blood plasma of people with leukemia, myeloma, lymphosarcoma. Which of the following proteins is a paraprotein capable of forming a gelatinous precipitate when the temperature decreases?

*A. C-Reactive protein* *B. α1-Glycoprotein* *C. Fibronectin*
*D. Cryoglobulin* *E. Ceruloplasmin*

**50.** A patient with diabetes mellitus has a diabetic coma due to an acid-base balance disorder. Which kind of acid-base balance disorders occurs in this case?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**51.** A patient with diabetes mellitus was hospitalized in a severe precomatous state. Metabolic acidosis was found. What is the primary mechanism for the identified acid-base balance disorder?

*A. Impaired use of $O_2$ in cells* *B. Formation of underoxidized products*
*C. Abnormal blood buffer systems*
*D. Excretion of alkaline components in the urine* *E. A decrease in $CO_2$ excretion*

**52.** The toxic damage to the liver leads to the impairment of its protein-synthesizing function. Which kind of dysproteinemia is observed in this case?

*A. Absolute hyperproteinemia* *B. Relative hypoproteinemia*
*C. Absolute hypoproteinemia* *D. Relative hyperproteinemia*
*E. Paraproteinemia*

**53.** A patient underwent an examination and was diagnosed with hyperglycemia, ketonuria, polyuria, and glucosuria. Which form of acid-base balance disorders is observed?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**54.** A male patient with type 1 diabetes mellitus is hospitalized due to coma. Laboratory tests revealed hyperglycemia, ketonemia. Which of the metabolic disorders mentioned below can be found in this patient?

*A. Respiratory alkalosis* *B. Metabolic acidosis*
*C. Respiratory acidosis* *D. Metabolic alkalosis*
*E. No changes in acid-base balance*

**55**. A 2-year-old boy began to suffer from respiratory diseases, stomatitis, pustular skin lesions. Even small damages of gums and mucous membranes were complicated by long-lasting inflammation. It was found out that immunoglobulins of all classes were practically absent in his blood. The decrease in the functional activity of a cell population that underlies the described syndrome is observed. Which cell population is affected?

*A. Neutrophils* *B. NK-lymphocytes* *C. T-lymphocytes*
*D. B-lymphocytes* *E. Macrophages*

**56.** Inflammatory processes in the body are associated with the synthesis of acute phase proteins. Their synthesis is stimulated by:

*A. Interleukin-1* *B. Immunoglobulins* *C. Interferons*
*D. Biogenic amines* *E. Angiotensins*

## *PRACTICAL WORK*

### Quantitative determination of total protein in blood serum by biuret method

**Task.** Determine the content of total protein in blood serum.

***Principle***. The protein reacts with a solution of copper sulphate, which contains potassium-sodium tartrate, NaI, and KI (biuret reagent) in an alkaline medium to form a purple-blue complex. The optical density of this complex is proportional to the concentration of protein in the sample.

***Procedure***. Pour 25 µl of serum (without hemolysis) into the test sample, 1 ml of biuret reagent containing 15 mmol/L of potassium sodium tartrate, 100 mmol/L of sodium iodide, 15 mmol/L of potassium iodide and 5 mmol/L of copper sulfate. Add 25 µl of standard of total protein (70 g/l) and 1 ml of biuret reagent to the standard sample. Pour 1 ml of biuret reagent into the third test tube. All the test tubes should be well mixed and incubated for 15 min at 30-37 °C. Stand for 5 minutes at room temperature. Measure the absorbance of the test and standard samples against biuret re-

agent at 540 nm. The total protein concentration (X) in g/L is calculated using the formula: $X = (C_{st} \times E_{test})/E_{st}$, where $C_{st}$ is the concentration of total protein in the standard sample (g/L); $E_{test}$ is the optical density of the test sample; $E_{st}$ is the optical density of the standard sample.

***Clinical and diagnostic significance.*** *The content of total protein in the blood plasma of adults is 65-85 g/L. The content of proteins in plasma is higher than in serum because of fibrinogen whose concentration is 2-4 g/L. In newborns the content of blood plasma proteins is 50-60 g/L and during the first month is slightly reduced, but till three years it reaches the level of adults. An increase or decrease in the total protein content of blood plasma and individual fractions may be due to many reasons. These changes are not specific. However, they reflect a common pathological process (inflammation, necrosis, and tumor), dynamics, and severity of illness. They can be used to evaluate the treatment effectiveness. Changes in protein content can manifest in the form of hyper- and hypoproteinemia. Hypoproteinemia is observed in the insufficient intake of proteins in the body; altered digestion and absorption of dietary protein; disorders of protein synthesis in the liver; kidney diseases with nephrotic syndrome. Hyperproteinemia is observed in disorders of hemodynamics, fluid loss during dehydration (diarrhea, vomiting, diabetes insipidus), on the first days of severe burns, in the postoperative period. It is interesting to note that such changes as a dysproteinemia ma (the albumin-to-globulin ratio changes, whereas there ia a constant content of total protein) and paraproteinemia (the appearance of abnormal proteins, such as C-reactive protein, cryoglobulins) may be observed in acute infectious diseases, inflammatory processes, etc.*

## ***CLASS 2 (4 hours)***

**TOPIC 2 (2 hours): Biochemical composition of blood in normal and pathological conditions. Blood plasma enzymes. Non-protein organic substances of blood plasma (nitrogen-containing and nitrogen-free). Inorganic components of blood plasma. Kallikrein-kinin system. Determination of residual nitrogen of blood plasma.**

**IMPORTANCE.** Plasma is the blood without formed elements. Serum is formed after removal of fibrinogen from plasma. Blood plasma is a complex system. It contains more than 200 proteins that differ in their physico-chemical and functional properties. There are proenzymes, enzymes, enzyme inhibitors, hormones, transport proteins, coagulation and anticoagulation factors, antibodies, antitoxins, and others. In addition, blood plasma contains non-protein organic substances and inorganic components. The majority of pathological conditions, the influence of external and internal environmental factors, the use of pharmacological agents are accompanied mainly by changes in the content of individual components of blood plasma. The state of human health, the course of adaptation processes may be characterized according to the results of blood analysis.

**AIM.** Familiarize yourself with the biochemical composition of blood in normal and pathological conditions. Characterize the blood enzymes: the origin and

significance of determining the activity for diagnosing pathological states. Determine substances making up the total and residual nitrogen levels. Study nitrogen-free blood components, their contents, clinical significance of their quantitive determination. Consider the blood kallikrein-kinin system, its components and their role in the body. Familiarize yourself with the methods of quantitative determination of blood residual (rest) nitrogen and its clinical and diagnostic significance.

***THEORETICAL QUESTIONS***

1. Blood enzymes, their origin, clinical and diagnostic significance of their determination.
2. Non-protein nitrogen-containing compoudns: structure, levels, clinical significance of determination.
3. Total and residual (rest) nitrogen levels. Clinical significance of determination.
4. Azotemia: types, causes, methods of determination.
5. Non-protein nitrogen-free blood components: content, clinical significance of determination.

**6*.** Inorganic components of blood

**7*.** Kallikrein-kinin system and its role in the body. The use of kallikrein and inhibitors of kinin formation as drugs.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
|---|---|
| *1. Inorganic blood components.* | *Major blood inorganic components are:<br>- cations: calcium (2.25-2.75 mmol/L); sodium (135-155 mmol/L); potassium (3.5-5.3 mmol/L); magnesium (0.66-1.07 mmol/L); phosphorus (0.87-1.45 mmol/L); iron (11.6-31.3 μmol/L);<br>- anions: bicarbonates, chlorides, phosphates, sulphates, iodides.* |
| *2. Kallikrein-kinin system, its role in the organism. Drugs as inhibitors kinin formation.* | *2.1. Kallikrein-kinin system (KKS) is a key system involved in the regulation of a wide range of physiological functions of the body and the development of many pathological conditions.<br>2.2. Under the action of KKS, kallikreins are formed from prekallikreins, and biologically active peptides called kinins (bradykinin, callidin) are produced from kininogens under the action of kallikreins.<br>2.3. Role - participation in the regulation of various functions in the body: activity of hemocoagulation, fibrinolysis, renin-angiotensin system, vascular tone (dilation of microcirculatory vessels), microcirculatory processes, inflammation, allergic reactions, etc.<br>Bradykinin has a powerful vasodilating effect.<br>2.4. Medicines - inhibitors of kininoformation: contrical.* |

## *TESTS FOR SELF-CONTROL*

**1.** The content of residual (rest) nitrogen in patient's blood is 48 mmol/L; urea level reaches 15.3 mmol/L. Which organ disease may be the cause of such changes:

*A. Spleen* *B. Liver* *C. Stomach*
*D. Kidney* *E. Pancreas*

**2.** Which level of residual (rest) nitrogen is normal for adults?

*A. 14.3-25 mmol/L* *B. 25-38 mmol/L*
*C. 42.8-71.4 mmol/L* *D. 70-90 mmol/L*

**3.** Point out the component of blood, which belongs to nitrogen-free compounds:

*A. ATP* *B. Thiamin* *C. Ascorbic acid*
*D. Creatine* *E. Glutamine*

**4.** Which type of asotemia is observed as a result of dehydration?

*A. Absolute dehydration* *B. Relative dehydration* *C. Retention dehydration*
*D. Extrarenal dehydration* *E. Productive dehydration*

**5.** What is the action of bradykinin on vessels?

*A. Vasodilation* *B. Vasoconstriction* *C. An increase in blood pressure*
*D. An increase in blood clotting* *E. A decrease in vascular permeability*

**6.** A decrease in blood residual (rest) nitrogen level was revealed in a patient with liver insufficiency. The diminished blood non-protein nitrogen was due to:

*A. Urea* *B. Ammonium* *C. Amino acids*
*D. Bilirubin* *E. Uric acid*

**7.** A patient complains of vomiting, general weakness. Residual (rest) nitrogen in blood is 35 mmol/L, renal function is not affected. Which type of azotemia is developed?

*A. Relative azotemia* *B. Renal azotemia*
*C. Retention azotemia* *D. Productive azotemia*

**8.** Which components of blood residual (rest) nitrogen fraction prevail in productive azotemia?

*A. Ketone bodies* *B. Lipids, carbohydrates*
*C. Amino acids, urea* *D. Porphyrins, bilirubin*

**9.** A patient had pathological changes in the liver and brain. A sharp increase of copper in the urine and its decrease in the blood are observed. Wilson disease was diagnosed. Which enzyme activity in the blood serum should be investigated to confirm the diagnosis?

*A. Carbonic anhydrase* *B. Xanthine oxidases*
*C. Leucine aminopeptidases* *D.Ceruloplasmin* *E. Alcohol dehydrogenase*

**10.** A patient has hemorrhagic stroke. An increased concentration of kinins was found in the blood. The doctor prescribed contrical, which is an inhibitor of one of the foloowing proteinases:

*A. Pepsin* *B. Trypsin* *C. Chymotrypsin*
*D. Collagenase* *E. Kallikrein*

**11**. A significant increase in the activity of creatine phosphokinase MB and LDH1 was revealed in the patient's blood. What is the possible pathology?

*A. Myocardial infarction* *B. Hepatitis* *C. Rheumatism*
*D. Pancreatitis* *E. Cholecystitis*

**12**. According to clinical data, a patient was diagnosed with acute pancreatitis. Which biochemical test can confirm this diagnosis?

*A. Acidic phosphatase activity in the blood*
*B. Activity of alkaline phosphatase in the blood* *C. Blood amylase activity*
*D. Aminotransferase activity in the blood* *E. Blood creatinine level*

**13**. Rest (residual) nitrogen in the patient's blood was 48 mmol/L, urea - 15.3 mmol/L. Which organ is affected?

*A. Stomach* *B. Liver* *C. Kidney* *D. Intestine* *E. Spleen*

**14.** Hemolysis occurred while obtaining blood plasma for studying the LDH isoenzyme spectrum. Which LDH isoenzyme will be increased in this case?

*A. LDH4 and LDH5* *B. LDH3 and LDH5* *C. LDH2 and LDH5*
*D. LDH1 and LDH4* *E. LDH1 and LDH2*

**15**. Rest (residual) nitrogen and urea were determined in the patient's blood. The proportion of urea in the residual nitrogen is significantly reduced. Which organ is affected?

*A. Stomach* *B. Liver* *C. Kidney* *D. Intestine* *E. Heart*

**16.** Blood analysis revealed azotemia. The percentage of urea nitrogen in the (rest) residual blood nitrogen is significantly reduced. Which organ is affected?

*A. Stomach* *B. Liver* *C. Kidney* *D. Intestine* *E. Heart*

**17.** A patient suffering from chronic renal failure has an increase in the level of residual (rest) nitrogen to 35 mmol/L. More than half of its is urea. This type of azotemia is called:

*A. Hepatic* *B. Productive* *C. Retentional*
*D. Residual* *E. Mixed*

## PRACTICAL WORK

### Quantitative determination of residual (rest) nitrogen in the blood

**Task.** Determine the content of residual (rest) nitrogen in the blood.

***Principle.*** The method is based on the ability of ammonia formed as a result of mineralization of blood non-protein nitrogen-containing compounds with the Nessler's reagent to give a compound of yellow color whose intensity is proportional to the concentration of residual nitrogen.

**Procedure.** Pour 0.5 ml of water into a centrifuge tube and add 0.2 ml of blood, 1.3 ml of 10% trichloroacetic acid solution. Mix the content of the test tube with a glass rod, set the test tube aside for 20 min to provide precipitation of blood proteins. Then centrifuge for 10 min at 3,000 rpm. Transfer the supernatant liquid into a clean test tube. Pour 1 ml of protein-free centrifugate into another test tube, add 3 drops of concentrated sulphuric acid to it and 2 drops of 30% hydrogen peroxide solution. Put the test tube for about 30 minutes on a sand bath for mineralization (up to full decoloration of solution). After cooling the test tube to room temperature pour 10 ml of distilled water into it; scratch the inner wall of the test tube thoroughly with a glass rod to transfer the deposited mineralizate into solution; mix the content. Add 0.5 ml of 0.4 mol/L sodium hydroxide solution and Nessler's reagent to the test tube. After the appearance of yellow color measure the absorbance for the test sample against a control (20 ml water and 1 ml of Nessler's reagent) on a photocolorimeter at 610-650 nm

(red light filter) in a 2 cm thick cells. The calculation in g/L is carried out using the formula:

$(m \times 2 \times 5000) / (1 \times 1000) = m \times 10$, where m is the nitrogen amount in the sample, obtained from the calibration graph (mg); 5000 is the scaling factor for conversion per liter of blood; 2 is volume of trichloroacetic extract (ml); 1 is the amount of protein-free supernatant taken for analysis (ml).

**Clinical and diagnostic significance.** The composition of the residual nitrogen is nitrogen of urea, peptides, amino acids, ammonia and other nitrogen-containing low-molecular-weight compounds. In a healthy person, the residual nitrogen varies in the ranges of 14-25 mmol/L. Azotemia (increased levels of residual nitrogen in the blood) may be absolute and relative. Absolute azotemia is caused by a retention of nitrogenous wastes, or an increase in their formation in the body; relative azotemia is observed in dehydration (increased sweating, prolonged vomiting, diarrhea, etc.). Productive and retention azotemias are distinguished. Productive azotemia is observed in an excessive release of nitrogenous products to the blood as a result of accelerated degradation of tissues proteins in different states: inflammation, wounds, extensive burns, cachexia, and other states. Retention azotemia is caused by incomplete urinary excretion of nitrogen-containing products when their release into the bloodstream is normal. Retention azotemia may be renal and extrarenal. The first one is observed in the disorders of the renal excretory ability (acute and chronic nephritis, glomerulonephritis), and the level of azotemia depends on a severity of the pathological process. Extrarenal retention azotemia may arise due to acute circulatory insufficiency, low arterial pressure, or reduced renal blood flow. Also, the frequent cause of extrarenal retention azotemia is a urinary tract obstruction.

**TOPIC 3 (2 hours): Biochemistry of erythrocytes. Biochemistry of hemoglobin. Structure, properties and role of hemoglobin. Quantitative determination of hemoglobin in the blood.**

**IMPORTANCE.** Hemoglobin is a red respiratory pigment of red blood cells, transporting oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs; it helps maintain the blood pH. It consists of a protein (globin) and complex of iron with protoporphyrin (heme). Most of the blood diseases occur due to disturbances of the structure and synthesis of hemoglobin, including hereditary, leading to a change in its concentration.

**AIM.** View the structure, properties, the role and heterogeneity of hemoglobin.

***THEORETICAL QUESTIONS***

1. Biochemistry of red blood cells.
2. Structure, role and properties of hemoglobin.
3. Types of hemoglobin.
4. Heme, its structure and role in the functioning of hemoglobin.
5. Mechanism of hemoglobin participation in the transport of $O_2$ and $CO_2$.
6. Abnormal forms of hemoglobin.

## *TESTS FOR SELF-CONTROL*

**1**. RBCs require energy in the form of ATP. Which process provides these cells with the necessary amount of ATP?

*A. Pentose phosphate pathway* *B. Beta-oxidation of fatty acids*
*C. Anaerobic glycolysis* *D. Aerobic glucose oxidation*
*E. Tricarboxylic acid cycle*

**2**. Under the action of oxidizing agents (hydrogen peroxide, nitric oxide, etc.), hemoglobin that contains $Fe^{2+}$ is converted to a compound containing $Fe^{3+}$ that is unable to carry oxygen. What is the name of this compound?

*A. Methemoglobin* *B. Carboxyhemoglobin* *C. Carbhemoglobin*
*D. Oxyhemoglobin* *E. Glycosylated hemoglobin*

**3.** Laboratory investigation of the blood respiratory function showed the worsened $CO_2$ transport. Which enzyme is deficient in the red blood cells?

*A. 2,3-Diphosphoglycerate* *B. Adenylate cyclases*
*C. Carbonic anhydrase* *D. Protein kinases* *E. Phosphorylases*

**4**. Approximately 20% of the world population have a decrease in the activity of glucose-6-phosphate dehydrogenase in erythrocytes. Such people have a higher risk of hemolysis due to the impairment of:

*A. Hemoglobin synthesis* *B. Glycolysis in erythrocytes*
*C. Activities of calcium-magnesium-ATPase*
*D. Activity of sodium-potassium-ATPase*
*E. Antioxidant system of erythrocytes*

**5**. Along with the normal types of hemoglobin in adults, there are also pathological ones. Select one of them.

*A. $HbA_1$* *B. HbS* *C. $HbA_2$* *D. HbF* *E. $HbO_2$*

**6**. An increase in the concentration of carbon monoxide in the air can lead to poisoning. It affects the oxygen transport by hemoglobin from lungs to tissues. Which hemoglobin derivative is formed in this case?

*A. Oxyhemoglobin* *B. Methemoglobin* *C. Carboxyhemoglobin*
*D. Carbhemoglobin* *E. Hemochromogen*

**7.** Biochemical analysis of the baby's erythrocytes reaveled a marked glutathione peroxidase deficiency and low levels of reduced glutathione. Which pathological condition can develop?

*A. Pernicious anemia* *B. Megaloblastic anemia*
*C. Sickle cell anemia* *D. Hemolytic anemia* *E. Iron deficiency anemia*

**8**. It has been known that the pentose phosphate pathway actively functions in erythrocytes. What is the main function of this metabolic pathway in erythrocytes?

*A. Prevention of lipid peroxidation* *B. Detoxication of xenobiotics*
*C. Oxidation of glucose into lactate*
*D. Activation of microsomal oxidation* *E. Enhancement of lipid peroxidation*

**9**. A male was bitten by a snake. He started gasping, hemoglobin appeared in the urine. Hemolysis was revealed. The toxic action of led to:

*A. Acidosis* *B. Formation of lysolecithin* *C. Polyuria*
*D. Development of alkalosis* *E. Formation of triglycerides*

**10**. Hemoglobin of adultsis a protein-tetramer consisting of two α- and two β-peptide chains. What is the structure of this protein?

*A. Tertiary* *B. Secondary* *C. Quartenary*
*D. Primary* *E. -*

**11.** After the accident in the chemical plant, the environment was polluted with nitro compounds. People living in that area experienced weakness, headache, shortness of breath, dizziness. What was the cause of hypoxia?

*A. Inhibition of dehydrogenases* *B. Formation of carboxyhemoglobin*
*C. Reduced function of flavin-dependent enzymes*
*D. Formation of methemoglobin* *E. Inactivation of cytochrome oxidase*

**12**. Blood analysis revealed a decrease in hemoglobin. Which blood function was affected?

*A. Provision of immunity* *B. Transport of hormones*
*C. Transport of nutrients* *D. Transport of medicines* *E. Transport of gases*

**13**. A person suffers from diabetes mellitus, which is accompanied by fasting hyperglycemia (more than 7.2 mmol/L). Which plasma protein level allows assessing the level of glycemia retrospectively (for 4-8 weeks before the examination)?

*A. Glycosylated hemoglobin* *B. C-Reactive protein*
*C. Fibrinogen* *D. Ceruloplasmin* *E. Albumin*

**14.** A group of children ate watermelon. One of the children had weakness, dizziness, vomiting, shortness of breath, tachycardia, acrocyanosis. Laboratory analysis of watermelon showed the high content of nitrates. What is the leading mechanism in the pathogenesis of poisoning in this child?

*A. Superoxide dismutase insufficiency* *B. Cytochrome oxidase inhibition*
*C. Methaemoglobin reductase insufficiency*
*D. Glutathione peroxidase deficiency* *E. Catalase insufficiency*

**15**. After repairing the car in the garage, the driver was hospitalized with symptoms of poisoning with exhaust fumes. Which blood hemoglobin type will be increased in the blood?

*A. Carboxyhemoglobin* *B. Methemoglobin* *C. Carbhemoglobin*
*D. Oxyhemoglobin* *E. Glycosylated hemoglobin*

**16**. To prevent the long-term consequences of four-day malaria, a patient was prescribed with primaquin. Abdominal and heart pain, dyspepsia, general cyanosis, hemoglobinuria appeared on the third day after the beginning of treatment with therapeutic doses of the drug. What was the reason for the development of the side effects?

*A. Potentiation of action by other drugs*
*B. A decrease in activity of microsomal liver enzymes*
*C. Genetic insufficiency of glucose-6-phosphate dehydrogenase*
*D. Low urinary excretion of the drug* *E. Cumulation of the drug*

**17**. RBCs don't contain mitochondria. What is the major pathway of ATP synthesis in them?

*A. Creatine kinase reaction* *B. Adenylate kinase reaction*
*C. Oxidative phosphorylation* *D. Aerobic glycolysis*
*E. Anaerobic glycolysis*

**18.** The ability of hemoglobin to bind oxygen decreases in:

*A. An increase in the content of 2,3-diphosphoglycerate in erythrocytes*

*B. A decrease in temperature* *C. An increase in pH*

*D. Reduced partial pressure of $CO_2$* *E. High $O_2$ partial pressure*

**19**. In erythrocytes, an additional intermediate metabolite of glycolysis is formed in a significant amount, which plays the role in allosteric regulation of hemoglobin function. Choose this metabolite.

*A. 3-Phosphoglycerate* *B. 1,3-Bisphosphoglycerate*

*C.2.3-Bisphosphoglycerate* *D. 2-Phosphoglycerate*

*E. Phosphoenolpyruvate*

**20.** The increased concentration of methemoglobin has therapeutic significance for the treatment of HCN and cyanide poisoning because methemoglobin forms non-toxic cyanmethemoglobin, which is slowly converted to Hb. Which of the following substances facilitates methemoglobin formation and is used for the treatment of cyanide poisoning?

*A. Methylene blue* *B. Glucose* *C. Nitrates*

*D. Ascorbic acid* *E. Adrenaline*

## PRACTICAL WORK

### Quantitative determination of hemoglobin in the blood by hemoglobin cyanide method

**Task.** Determine the content of hemoglobin in the blood by hemoglobin cyanide method.

**Principle.** Interaction of hemoglobin with potassium ferricyanide results in its oxidation to methemoglobin.

**Procedure.** Add 0.02 ml of blood (diluted 25-fold) to 5 ml of transforming solution, mix well. Determination is carried out in 10 minutes against the reagent blank (transforming solution). Determine the absorbance on a spectrophotometer at 540 nm in 1 cm thick cells. Standard solution of hemoglobin is prepared in the same way as a sample of whole blood. Hemoglobin content (B) in g/L is calculated in accordance with the formula: $B = (D_{test} \times 120)/D_{st}$, where $D_{test}$ is the optical density of the test sample, $D_{st}$ is the optical density of the standard sample, 120 is the hemoglobin content in the standard solution (g/L).

***Clinical and diagnostic significance.*** *The normal hemoglobin content in the blood of males is 130-160 g/L and it is 120-140 g/L in the blood of females. Determination of hemoglobin is the necessary stage of the diagnosis of various diseases. The increase in blood hemoglobin content may be associated with the following conditions: 1) diseases accompanied by an increase in the level of red blood cells; 2)an increase in the oxygen capacity of blood caused by hypoxia in cardiovascular diseases, living in the highlands, obesity, smoking, and the formation of inactive hemoglobin; 3) dehydration; 4) stress and high physical activity, 5) tumors in the kidney, liver, CNS, ovaries; 6) dysfunction of blood vessels; 7) changes in hormonal levels (the increase in corticosteroids or androgens); 8) chemical poisoning by carbon monoxide, nitrates, or drugs; 9) burns. The decrease in blood hemoglobin level may be due*

*to: 1) bleeding; 2) reduced level of red blood cells in different types of anemia (deficiency of vitamins $B_9$ and $B_{12}$, vitamin C, iron deficiency, intoxication or a disturbance of the kidney functioning, impaired maturation of red blood cells). Lower hemoglobin levels may be also caused by a variety of factors: malnutrition, disorders of the gastrointestinal tract; consumption of large quantities of tea, coffee and other products that inhibit iron absorption.*

## ***CLASS 3 (4 hours)***

**TOPIC 4 (2 hours): Metabolism of hemoglobin; its synthesis. Iron metabolism and its role in the formation of heme. Disorders of hemoglobin synthesis: porphyria and hemoglobinoses (hemoglobinopathies, thalassemia).**

**IMPORTANCE.** Hemoglobin is a red respiratory pigment of red blood cells, transporting oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs; it helps maintain the blood pH. It consists of a protein (globin) and complex of iron with protoporphyrin (heme). Most of the diseases of the blood occur due to disturbances of the structure and synthesis of hemoglobin, including hereditary, leading to a change in its concentration.

**AIM.** Learn the synthesis of heme, the role of iron in hemoglobin metabolism, iron metabolism in the body. Be able to describe porphyrias, hemoglobinopathies, and thalassemia.

### *THEORETICAL QUESTIONS*

1. The general scheme of the hemoglobin synthesis. Regulation of process.
**2*.** Iron, its role and metabolism in the body; daily requirement.
3. Porphyrias: causes, types.
**4*.** Hemoglobinoses: thalassemia and hemoglobinopathies. Their causes.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
|---|---|
| *1. Iron. Its role and metabolism in the body. Daily requirements.* | *1.1. Daily requirements reach 15-20 mg (about 10% is absorbed).*<br>*1.2. The role of iron:*<br>*- structural (it forms a part of iron-containing proteins: hemoglobin, myoglobin, cytochromes, etc.);*<br>*- participation in redox processes as a component of cytochromes and iron-sulfur proteins (transport of electrons in the respiratory chain);*<br>*- transport (as a component of hemoglobin it is involved in transfer of oxygen and carbon dioxide).*<br>*1.3. The major iron pools in the body:*<br>*- heme (cellular) – it forms a part of hemoglobin, myoglobin, enzymes (cytochromes, catalase, peroxidases), metalloproteins (aconitase, etc.);* |

| | |
|---|---|
| | *- non-heme;*<br>*- extracellular: free plasma iron and iron-binding serum proteins (transferrin, lactoferrin). involved in iron transport;*<br>*- Iron is stored in the body in the form of two protein compounds - ferritin and hemosiderin*<br><br>*1.4. Iron metabolism: absorption in the gastrointestinal tract (the main process in maintaining iron homeostasis in cells of the small intestine is the convertion of $Fe^{2+}$ to $Fe^{3+}$) → $Fe^{3+}$ transport by transferrin → intracellular metabolism (the iron pool plays an important role in regulating cell proliferation, synthesis of heme-containing proteins, expression of transferrin receptors, etc.) → storage (ferritin, hemosiderin) → utilization and reutilization → excretion.* |
| *2. Hemoglobinoses: hemoglobinopathies and thalassemias. Causes of development.* | *2.1. Hemoglobinoses are pathological conditions that develop due to the presence pathological forms of hemoglobin in blood, leading to the changes in the oxygen transport.*<br>*2.2. According to the mechanism of molecular defect, hemoglobinoses are divided into hemoglobinopathies and thalassemias.*<br>*- Hemoglobinopathies are hereditary changes in the primary structure of polypeptide chains. Examples: HbS (sickle-cell anemia) is caused by the substitution of glutamate by valine; in the β-chain in the 6 position; HbM is a replacement of histidine by tyrosine; Riverdale-Bronx Hb is a replacement of glycine by arginine; Kempsey Hb is a replacement of aspartate by asparagine.*<br>*- Thalassemias are hereditary disorders of polypeptide chain synthesis (α- and β-thalassemias).*<br>*2.3. Causes: gene mutations affecting the regulatory genes responsible for the expression of certain structural hemoglobin genes.* |

***TESTS FOR SELF-CONTROL***

**1**. Erythema and vesicular rash on the skin appeared in a child under the action of sunlight. The child complains of itching. Blood tests revealed a decrease in blood serum iron content, as well as an increase in urinary excretion of uroporphyrinogen I. The most likely hereditary pathology is:

*A. Methemoglobinemia B. Hepatic porphyria*
*C. Erythropoietic porphyria D. Coproporphyria E. Intermittent porphyria*

**2**. Erythrocytes are sickle-shaped in a patient with severe forms of hemolytic anemia. What is the molecular cause of this disease?

*A. Replacement of glutamate with valine* *B. Abnormal porphyrin synthesis*
*C. Disorders of hemoglobin alpha chain synthesis*
*D. Abnoral synthesis of hemoglobin beta-chain* *E. Impaired heme synthesis*

**3.** A patient had a hemoglobin gene mutation. This led to the development of sickle cell anemia. How is the pathological hemoglobin, formed in this disease, called?

*A. Bart-Hb* *B. HbF* *C. HbS* *D. HbA* *E. HbA1*

**4**. The content of iron in blood plasma increased in a male due to increased hemolysis. Which protein provides its deposition in tissues?

*A. Ferritin* *B. Transscortin* *C. Albumin*
*D. Haptoglobin* *E. Transferrin*

**5**. A patient had visually seen blisters and enhanced pigmentation after exposure to UV rays. Urine turns red after exposing to the air. Which parameter of the urine makes it possible to verify Gunther's disease?

*A. Hemoglobin* *B. Uroporphyrinogen I* *C. Bilirubin*
*D. Creatinine* *E. Acetone*

**6**. In patients with erythropoietic porphyria (Gunther's disease), teeth are fluoresced in the ultraviolet with a bright red color, the skin is sensitive to light, urine is red. Which enzyme insufficiency is observed?

*A. Delta-aminolevulinate synthase* *B. Uroporphyrinogen decarboxylase*
*C. Uroporphyrinogen I synthase* *D. Ferrochelatase*
*E. Uroporphyrinogen III cosyntase*

**7**. A patient was examined in a hospital. Since childhood, his hemoglobin has been varying from 90 to 95 g/L. Treatment with iron supplements was ineffective. There are the following blood indices: RBCs-3.2, Hb-85 g/L, color index – 0.78, anisocytosis, poikilocytosis, target cells, reticulocytes - 16%. The diagnosis is thalassemia. To which kind of hemolytic anemia belongs this disease?

*A. Hereditary membranopathy* *B. Acquired enzymopathy*
*C. Hereditary hemoglobinopathy* *D. Hereditary enzymopathy*
*E. Acquired membranopathy*

**8.** A patient was diagnosed with iron-deficiency sideroachristic anemia, which was accompanied by skin hyperpigmentation, development of pigment liver cirrhosis, damage to the pancreas and heart. The content of iron in the blood serum is increased. What is the reason for the abnormal iron metabolism?

*A. Excessive intake of iron from food*
*B. Abnormal iron absorption in the intestine*
*C. Iron is not used and is deposited in tissues*
*D. Increased consumption of iron by the body* *E. -*

**9**. Sickle cell anemia is common in some areas of South Africa. In this case, erythrocytes have the shape of a sickle due to the replacement of the amino acid glutamate with valine in the molecule of hemoglobin. What causes this disease?

*A. Genomic mutation* *B. Crossingover* *C. Gene mutation*
*D. Impaired mechanisms for the implementation of genetic information*
*E. Transduction*

**10**. During the catabolism of hemoglobin, iron is released. Then it enters the bone marrow as part of a special transport protein, and is again used for hemoglobin synthesis. This transport protein is called:

*A. Transferrin* *B. Transcobalamin* *C. Haptoglobin*
*D. Ceruloplasmin* *E. Albumin*

**11**. A patient is diagnosed with α-thalassemia. Which disorders of hemoglobin synthesis are observed in this disease?

*A. Inhibition of β-chain synthesis* *B. Inhibition of α-chain synthesis*
*C. Inhibition of γ- chain synthesis* *D. Activation of α-chain synthesis*
*E. Inhibition of δ- and β- chain synthesis*

**12**. A female patient, a worker of a paint and varnish factory, complains of general weakness, weight loss, apathy, drowsiness. Chronic lead intoxication was confirmed by laboratory methods: hypochromic anemia was revealed. Blood protoporphyrin level is increased and δ-aminolevulinic acid level is lowered, which indicates the abnormal synthesis of:

*A. DNA* *B. RNA* *C. Protein* *D. Mevalonic acid* *E. Heme*

**13**. A patient has an increased skin sensitivity to sunlight. When exposing to the air, urine turns dark red. What is the most likely cause of this condition?

*A. Hemolitic jaundice* *B. Porphyria* *C. Albinism*
*D. Pellagra* *E. Alkaponuria*

**14**. A patient complains of general weakness, dizziness, and rapid fatigue. The content of hemoglobin is 80 g/L. Microscopically, erythrocytes have abnormal shape. The cause of this condition is:

*A. Parenchymal jaundice* *B. Addison's disease*
*C. Acute intermittent porphyria* *D. Obturative jaundice* *E. Sickle-cell anemia*

**15**. The synthesis of iron-containing protein, which is the source of iron for heme synthesis, is impaired in the liver of a patient suffering from iron deficiency anemia. How is this protein called?

*A. Hemosiderin* *B. Transferrin* *C. Ceruloplasmin*
*D. Ferritin* *E. Hemoglobin*

**16**. A patient has been ill for 10 years. Periodically he complaints of acute pain in the abdomen, convulsions, impaired vision. His relatives have similar symptoms. Urine is red. The patient was hospitalized with acute intermittent porphyria. The cause of the disease may be the abnormal synthesis of:

*A. Insulin* *B. Bile acids* *C. Heme*
*D. Prostaglandin* *E. Collagen*

**17**. A patient has sickle-cell anemia. Which amino acid is replaced in the polypeptide chain of hemoglobin for valine?

*A. Glutamic acid* *B. Aspartic acid*
*C. Leucine* *D. Arginine* *E. Threonine*

**18**. Molecular analysis of the hemoglobin in a patient with anemia revealed a 6Glu substitution for 6Val in β-chain. What is the molecular mechanism of the pathology?

*A. Chromosomal mutation* *B. Genomic mutation*
*C. Gene mutation* *D. Gene amplification* *E. Gene transduction*

**19**. Severe form of hypoxia (shortness of breath, cyanosis) developed in a 3-month-old child. Which process of hemoglobin formation is affected?

*A. Replacement of hemoglobin F to hemoglobin M*
*B. Replacement of hemoglobin F to hemoglobin S*
*C. Replacement of hemoglobin F to glycosylated hemoglobin*
*D. Replacement of hemoglobin F to methemoglobin*
*E. Replacement of hemoglobin F to hemoglobin A*

**20.** In a number of hemoglobinopathies, amino acid substitutions occur in the α- and β-chains of hemoglobin. Which of them is typical for HbS (sickle cell anemia)?

*A. Aspartate-lysine* *B. Alanine-serine* *C. Methionine-histidine*
*D. Glycine-serine* *E. Glutamate valine*

**21**. Hereditary defects in heme synthesis enzymes are associated with the increased sensitivity of patients' skin to sunlight. Urine is red. Which hemoglobin metabolites are accumulated causing such symptoms?

*A. Mesobilinogens* *B. Stercobilinogens* *C. Urobilinogens*
*D. Porphyrinogens* *E. Bilirubin*

**22**. A patient was diagnosed with erythropoietic porphyria (Gunther's disease): urine is red, a noticeable red coloration of teeth is observed under the ultraviolet radiation. Which substance metabolism is affected?

*A. Heme* *B. Globin* *C. Adenine* *D. Creatine* *E. Cholesterol*

**23.** One of the reasons for the development of anemia is a heme synthesis disorder, which may be due to a decrease in the activity of the delta-aminolevulinate synthase whose coenzyme is:

*A. NAD* *B. TPP* *C. FAD* *D. PLP* *E. THF*

**24.** Substrates for the synthesis of pyrrol rings of porphyrin are:

*A. Acetyl-CoA and glycine* *B. Acetoacetyl-CoA and serine*
*C. Succinyl-CoA and serine* *D. Succinyl-CoA and glycine*
*E. Malonyl-CoA and serine*

**25.** Heme synthesis is regulated by feedback mechanism at the stage of:

*A. Incorporation of iron ion into protoporphyrin*
*B. Formation of δ-aminolevulinic acid*
*C. Condensation of porphobilinogen molecules*
*D. Formation of protoporphyrin III*
*E. Synthesis of porphobilinogen*

**26.** Which of the following statements about porphyrias is not correct?

*A. Genetic disturbance of heme synthesis*
*B. They are divided into erythropoietic and hepatic*
*C. They are accompanied by the increased excretion of bile pigments in urine and feces*
*D. They manifest by dermatitis and neuropsychiatric disorders*
*E. Some symptoms are similar to those caused by lead poisoning*

**27.** There is an abnomal formation of a metalloprotein, which is the source of iron for heme synthesis, in the liver of a patient with iron deficiency anemia. How is this protein called?

*A. Ceruloplasmin* *B. Ferritin* *C. Hemosiderin*
*D. Myoglobin* *E. Cytochrome c*

**28.** The excessive accumulation of iron in tissues is observed in a 42-year-old woman. The accumulation occurs due to transferrin deficiency. Each of the following statements about transferrin is correct except:

*A. Transferrin is a protein that binds iron and is secreted by neutrophiles*
*B. Transferrin is a glycoprotein secreted by parenchymatous cells of liver*
*C. Iron binding by transferrin is the mechanism of protection from iron toxicity*
*D. Iron and transferrin amounts are proportional in blood*
*E. Transferrin directs a flow of iron to cells which actively synthesize hemoglobin*

**29.** Indicate an inhibitor of the enzyme that catalyzes the first reaction of heme synthesis:

*A. Heme* *B. Glycine* *C. Porphobilinogen*
*D. Uroporphyrinogen* *E. Bilirubin*

**TOPIC 5 (2 hours): Hemoglobin metabolism; its degradation. Biochemistry of bile pigments. Types of jaundices. Quantitative determination of bilirubin and its fractions in blood serum.**

**IMPORTANCE.** The life span of red blood cells is about 120 days. After this period of time the erythrocytes are broken down, hemoglobin is released and is split into globin and heme in the spleen, bone marrow and Kupffer cells of the liver. The protein part undergoes transformations that are typical for simple proteins. Heme molecule undergoes a multi-step transformation, the end product of which is stercobilin, excreted in the feces and urine. Bile pigments (biliverdin, bilirubin, etc.) are formed as breakdown products of hemoglobin. Bilirubin is transported by blood albumin because of its weak solubility and toxicity, enters the sinusoidal surface of hepatocytes by facilitated transport. The conjugation of bilirubin with glucuronic acid (UDPGA) occurs with formation of mono- and dibilirubin glucuronides in the liver by means of the enzyme UDP-glucuronyl transferase. Conjugated bilirubin is secreted into the bile by active transport and enters the gastrointestinal tract with the bile, where it undergoes further transformations. Thus, there are two forms of bilirubin: 1) unconjugated, free (bilirubin-albumin) and 2) conjugated, bound (bilirubin glucuronides). The first one was called by van den Berg "indirect" (it gave positive Ehrlich's diazoreaction after pretreatment with methanol, caffeine reagent) and the the second one was called "direct" (it gave positive diazoreaction without pretreatment). Knowledge of pathways of hemoglobin breakdown is important for the differential diagnosis of jaundices, which may be associated with liver diseases and enzymopathies, as well as a possible correction of these disorders.

**AIM.** View the process of enzymatic breakdown of hemoglobin in the body; bile pigments formed in it, their transformation and significance of their determination in the blood, urine and feces. Pay particular attention to the fractions of bilirubin and

clinical and diagnostic significance of their determination in serum. Learn disorders of bile pigment metabolism and the factors that cause them. Be able to link theoretical knowledge of the material with the concrete results of laboratory studies and to use them as criteria for establishing some form of jaundice. Familiarize yourself with the method of quantitative determination of total bilirubin and its fractions in blood serum and its clinical and diagnostic significance.

### *THEORETICAL QUESTIONS*

1. The general scheme of the hemoglobin breakdown.
2. Bile pigments, their conversion, the significance of their determination in blood, urine, and feces.
3. Direct and indirect bilirubin levels. The significance of their determination.
4. Pathobiochemistry of jaundices: hemolytic, parenchymal, and obstructive.
5. Enzymatic, hereditary jaundice: Crigler-Najjar syndrome ("conjugated jaundice"), Gilbert's disease ("absorption jaundice"), Dubin-Johnson syndrome ("excretional jaundice").

**6*.** Enzymatic jaundices of newborns, methods of prevention and treatment.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
|---|---|
| *1. Physiological neonatal jaundice.* | *1.1. A temporary condition caused by the late activation of genes encoding UDP-glucuronyl transferase (found in about 80% of newborns):*<br>*- the most intensive jaundice is observed on the 2nd-3rd day of life, on the 7-10th day it disappears;*<br>*- prolonged jaundice: premature infants, imunological conflict, maternal and fetal incompatibility in Rh- or ABO systems, hypothyroidism, congenital hepatitis, sepsis, biliary atresia;*<br>*- development of physiological jaundice: increased breakdown of erythrocytes; immaturity of enzymatic systems in the liver, in particular, glucuronyl transferase.*<br>*1.2. Treatment of long-term and hereditary jaundices: phenobarbital is a universal inducer of enzymes for hepatic detoxification.* |

### *TESTS FOR SELF-CONTROL*

**1.** Select a reaction of hemoglobin catabolism occurring in the intestine:

*A. Glucuronyl transferase* *B. Biliverdin reduction*
*C. Stercobilinogen formation* *D. Heme oxygenase*
*E. Mesobilinogen breakdown to dipyrroles*

**2.** Which of the following substances is not excreted in the urine?

*A. Conjugated bilirubin* *B. Unconjugated bilirubin*

*C. Urobilinogen* *D. Stercobilinogen*

**3.** A male with yellow skin has anemia, splenomegaly, hyperbilirubinemia (indirect bilirubin), urobilinuria, dark-yellow feces. These changes are the most typical for:

*A. Hemolytic jaundice* *B. Obstructive jaundice*

*C. Hepatocellular jaundice* *D. Gilbert's syndrome* *E. Liver insufficiency*

**4.** Bilirubin content (indirect bilirubin) in a newborn is increased; feces are intensively colored (the enhanced level of stercobilin). Bilirubin is not found in urine. Which type of jaundice may be diagnosed?

*A. Hepatocellular* *B. Hemolytic* *C. Obstructive*

*D. Inherited* *E. Neonatal physiologic jaundice*

**5.** Skin, scleras and mucosa are yellow colored in a patient. Urine has the color of dark beer, feces are acholic. The increased levels of both direct and indirect bilirubin, enhanced AlAT, $LDH_4$ and $LDH_5$ activities are found in blood. Bilirubin is present in the urine. Which type of jaundice may be diagnosed?

*A. Inherited* *B. Hemolytic* *C. Obstructive*

*D. Hepatic* *E. Neonatal physiologic jaundice*

**6.** Neurologic abnormalities, yellow skin, an increase in blood serum unconjugated bilirubin levels were found in an ill 10-year-old child. Which enzyme abnormal synthesis leads to the development of Gilbert's syndrome?

*A. UDP dehydrogenase* *B. UDP-glucuronyl transferase*

*C. Glycerol kinase* *D. Galactose-1-phosphate uridyltransferase*

**7.** A patient was hospitalized with complaints of general weakness, abdominal pain, and bad appetite. Symptoms of jaundice were observed. Blood serum total bilirubin content was 77.3 μmol/L; conjugated bilirubin level was 70.76 μmol/L. Which diagnosis is the most possible?

*A. Obstructive jaundice* *B. Acute hepatitis* *C. Cirrhosis of liver*

*D. Hepatic jaundice* *E. Hemolytic jaundice*

**8.** Select a product of hemoglobin breakdown in the liver:

*A. Biliverdin* *B. Bilirubin* *C. Verdoglobin*

*D. Porphobilinogen* *E. Stercobilin*

**9**. Barbiturates activate UDP-glucuronyl transferase synthesis in the liver, which causes the formation of:

*A. Direct bilirubin* *B. Indirect bilirubin* *C. Biliverdin*

*D. Protoprophyrine* *E. Heme*

**10**. As a result of the transfusion of Rh antigen incompatible blood, hemolytic jaundice developed in a patient. Which laboratory blood parameter can confirm this type of jaundice?

*A. Accumulation of urobilinogen*

*B. A decrease in the content of unconjugated bilirubin*

*C. Accumulation of unconjugated bilirubin*

*D. Reduction of the content of sterbilin*

*E. Reduction of the content of conjugated bilirubin*

**11.** It was found in 1970s that the cause of severe neonatal jaundice was abnormal bilirubin conjugation in hepatocytes. Which substance is used to form the conjugate?

*A. Uric acid* *B. Sulfuric acid* *C. Lactic acid*
*D. Glucuronic acid* *E. Pyruvic acid*

**12**. A patient complains of jaundice, skin itching, general weakness. There is no urobilin in the urine. Which pathology can be diagnosed?

*A. Mechanical jaundice* *B. Parenchymal jaundice* *C. Acute liver failure*
*D. Haemolytic jaundice* *E. Chronic liver failure*

**13.** Jaundice of the skin and mucous membranes developed in a patient after the blood transfusion. Blood levels of total and indirect bilirubin are high. Urobilin is found in the urine. Stercobilin is found in the urine. Which kind of jaundice can be suspected?

*A. Hereditary* *B. Obturative* *C. Parenchymal*
*D. Jaundice of newborns* *E. Hemolytic*

**14**. A male complains of nausea, vomiting, pain in the right hypochondrium. The patient has skin and sclera jaundice, increased body temperature, enlarged liver, dark urine, hypocholic feces, hyperbilirubinemia (due to direct and indirect bilirubin), bilirubinuria, urobilinuria, hypoproteinemia, decreased blood clotting. Which of the conditions mentioned below are characterized by such changes?

*A. Hemolytic jaundice* *B. Cellular parenchymal jaundice*
*C. Acute pancreatitis* *D. Pedicular jaundice* *E. Acute cholecystitis*

**15**. A premature newborn on the second day of life has yellow coloration of the skin and mucous membranes. Which enzyme temporary deficiency is the cause of this condition?

*A. UDP-glucuronyltransferase* *B. Aminolevulinate synthase*
*C. Hemoxygenases* *D. Heme synthase* *E. Biliverdin reductase*

**16**. A young male has a hereditary UDP-glucuronyl transferase deficiency. Laboratory tests allowed determining hyperbilirubinemia, mainly due to the increase in blood concentrations of:

*A. Direct bilirubin* *B. Urolilinogen* *C. Indirect bilirubin*
*D. Sterkobilinogen* *E. Biliverdin*

**17**. The patient has an acute attack of cholelithiasis. What will be changed in laboratory tests?

*A. Positive reaction for stercobilin in feces*
*B. The presence of connective tissue in feces* *C. Fibers in feces*
*D. Negative reaction for stercobilin in feces*
*E. The presence of starch granules in feces*

**18.** A female patient with jaundice has an increase in the blood plasma content of total bilirubin due to indirect (free) bilirubin. The high content of stercobilin is found in feces and urine. The level of direct (conjugated) bilirubin in the blood plasma is normal. What typed of jaundice is observed?

*A. Parenchymal* *B. Hemolytic* *C. Mechanical*
*D. Neonatal jaundice* *E. Gilbert disease*

**19**. A female was hospitalized with complaints of weakness, irritability, sleep disturbance. The skin and sclera are yellow. An elevated level of direct bilirubin is

found. Feces are acholic. Dark color (bile pigments) of urine is observed. Which type of jaundice should be diagnosed?

*A. Hemolytic B. Mechanical C. Parenchymal*
*D. Gilbert syndrome E. Crigler-Najjar syndrome*

**20.** A male patient with jaundice has increased total bilirubin levels due to its indirect fraction. Urine and feces are intensely colored. What is the most likely mechanism of such disorders?

*A. Impaired outflow of bile from the liver B. Damage to the liver parenchyma*
*C. Increased hemolysis of erythrocytes D. Impaired formation of direct bilirubin*
*E. Abnormal urobilinogen metabolism in the liver*

**21.** In a patient the development of acute pancreatitis is accompanied by the obstruction of common bile duct. What can develop as a result?

*A. Hepatic coma B. Portal hypertension C. Mechanical jaundice*
*D. Haemolytic jaundice E. Parenchymal jaundice*

**22**. A woman suffering from cholelithiasis had jaundice. At the same time, urine turned dark yellow, and feces became colorless. Which substance concentration in the blood serum will increase at most?

*A. Conjugated bilirubin B. Free bilirubin C. Biliverdin*
*D. Mesobilirubin E. Urolilinogen*

**23**. A newborn has physiological jaundice. The level of free bilirubin in the blood significantly exceeds the normal values. Which enzyme deficiency is observed?

*A. Transaminases B. Xanthine oxidases C. Adenosine deaminases*
*D. Hemoxygenase E. UDP-glucuronyl transferase*

**24.** A patient with signs of jaundice due to viral hepatitis was hospitalized to the infectious department. Which of the following parameters is strictly specific, distinguishing parenchymal jaundice from the other types?

*A. Cholechemia B. Hyperbilirubinemia C. Bilirubinuria*
*D. Increased activity of ALT, ASAT E. Urobilinuria*

**25**. A patient with subacute septic endocarditis showed signs of the general pallor and jaundice of skin, sclera and visible mucous membranes. The increased content of indirect bilirubin was found in blood. What promotes yellowness of the skin and mucous membranes?

*A. Posthepatic jaundice B. Hemosiderosis C. Fatty liver*
*D. Hepatic jaundice E. Hypoplastic jaundice*

**26**. In patients with a genetic enzymatic disease (Gilbert's disease), conjugation of bilirubin in the liver is impaired. Which enzyme is blocked in this case?

*A. UDP-glucosopyrophosphorylase B. UDP-glycogentransferase*
*C. Ornithine carbamoyltransferase D. UDP-glucuronyl transferase*
*E. Phosphoribosyl pyrophosphate amidotransferase*

**27**. A patient has an increase in the blood plasma content of conjugated (direct) bilirubin against the background of a simultaneous increase in the level of unconjugated (indirect) bilirubin and a sharp decrease in the content of stericobilinogen in the feces and urine. Which kind of jaundice is described?

*A. Parenchymal B. Hemolytic C. Neonatal jaundice*
*D. Obstructive E. Gilbert disease*

**28**. A patient has immune hemolytic anemia. Which parameter is increased in the serum at most?

*A. Indirect bilirubin* *B. Direct bilirubin* *C. Protoporphyrin*
*D. Mesobilinogen* *E. Stercobilinogen*

**29.** A patient has been suffering from pain in the right hypochondrium for several days after eating fatty food. The jaundice of the sclera and skin is visually noted. Acholic feces are observed. Urine has a "color of beer." Which substance is present in the urine and causes a dark color of urine?

*A. Ketone bodies* *B. Indirect bilirubin* *C. Stercobilin*
*D. Bilirubin glucuronides* *E. Direct bilirubin*

**30**. A newborn has signs of jaundice. The administration of small doses of phenobarbital, which induces the synthesis of UDP-glucuronyl transferase, has contributed to the improvement of the child's health. Which of the following processes is activated in this case?

*A. Conjugation* *B. Microsomal oxidation* *C. Tissue respiration*
*D. Gluconeogenesis* *E. Glycogen synthesis*

**31**. A young man has a UDP-glucuronyltransferase hereditary deficiency. Which parameter in blood can confirm the diagnosis?

*A. Direct (conjugated) bilirubin* *B. Urobilin*
*C. Indirect (unconjugated) bilirubin* *D. Stercobilinogen* *E. Animal indican*

**32**. Select a type of jaundice, in which there is no direct bilirubin in the blood, but a lot of urobilinogen is present in the urine:

*A. Posthepatic* *B. Mechanical* *C. Prehepatic*
*D. Hepatic* *E. –*

**33.** A patient had jaundice of the skin, sclera and mucous membranes. In the blood plasma the level of total bilirubin was high. The level of sterocilin increased in feces. Urobilin level was elevated in the urine. Which type of jaundice can be diagnosed in a patient?

*A. Hemolytic* *B. Parenchymal* *C. Obstructive*
*D. Cholestatic* *E. Gilbert disease*

**34**. A patient with jaundice has the increased content of direct bilirubin and bile acids in blood. There is no sterocilinogen in the urine. Which type of jaundice can be diagnosed?

*A. Parenchymal* *B. Hepatic* *C. Hemolytic*
*D. Posthepatic* *E. Mechanical*

**35.** A worker of the chemical enterprise who suffered from the poisoning has an increase in the blood plasma content of total bilirubin due to its indirect fraction. The high content of stercobilin is found in feces and urine. The level of direct bilirubin in the blood plasma is normal. Which kind of jaundice can be diagnosed?

*A. Parenchymal* *B. Hepatic* *C. Hemolytic*
*D. Obstructive* *E. Mechanical*

**36**. A female patient with an acute attack of hepatic colic was hospitalized to the gastroenterological department. Body temperature is 38ºC, sclera, mucous membranes and skin are icteric, urine is dark, feces are lightly colored. The patient complains of itching. What is the cause of jaundice in this patient?

*A. Hepatocyte destruction* *B. Enhanced destruction of erythrocytes*

*C. Obstruction of the bile duct* *D. Impaired lipid metabolism*
*E. Prolonged use of carotene - containing products*

**37.** In a patient with a pronounced yellowness of the skin, sclera, mucous membranes, urine became of color of dark beer, feces were lightly colored. The content of direct bilirubin is elevated, bilirubin is found in urine. Which type of jaundice is observed?

*A. Obstructive* *B. Parenchymal* *C. Hemolytic*
*D. Conjugative* *E. Excretory*

**38**. A patient was hospitalized to the infectious hospital because of yellowness of the skin, sclera, mucous membranes. Laboratory tests revealed increased level of direct bilirubin in the blood. In urine, urobilinogen and bilirubin were found. Which disease is characterized by such changes?

*A. Kidney infarction* *B. Kidney tuberculosis* *C. Mechanical jaundice*
*D. Hemolytic jaundice* *E. Parenchymal jaundice*

**39**. A male complained of acute pain in the right hypochondrium. Upon examination, the doctor paid attention to the yellowed sclera. Laboratory tests showed increased ALT activity and negative reaction for stercobilin in the feces. Which disease is characterized by such symptoms?

*A. Hemolytic jaundice* *B. Hepatitis* *C. Chronic colitis*
*D. Chronic gastritis* *E. Cholelithiasis*

**40.** Enzymatic jaundices are associated with the abnormal activity of UDP-glucuronyl transferase. Which compound is accumulated in the serum in such cases?

*A. Indirect bilirubin* *B. Direct bilirubin* *C. Biliverdin*
*D. Mesobilirubin* *E. Verdoglobin*

## PRACTICAL WORK

### Quantitative determination of bilirubin and its fractions in the blood serum by the method of Jendrashik, Cleghorn and Groff

**Task.** Determine the content of total, bound and free serum bilirubin by the method of Jendrashik, Cleghorn and Groff.

***Principle.*** The method is based on the ability of the bound (conjugated) bilirubin and a dissociated form of free bilirubin to give, when reacting with diazophenylsulphonic acid, a compound azobilirubin colored pink-violet. Unconjugated bilirubin is converted to a soluble dissociated state by caffeine reagent and due to it total bilirubin is determined in the analysed sample. The free bilirubin concentration is determined by a difference between the total and conjugated bilirubins.

***Procedure***. Transfer the components listed in the table to three tubes and mix thoroughly the contents.

| Reagent | Total bilirubin, ml | Bound bilirubin, ml | Control, ml |
|---|---|---|---|
| Serum | 0.5 | 0.5 | 0.5 |
| Caffeine reagent | 1.75 | - | 1.75 |
| Sodium chloride solution | - | 1.75 | 0.25 |
| Diazoreagent | 0.25 | 0.25 | - |

While performing the determination of total bilirubin, the solutions should stand for 20 min for the color development; in the determination of bound bilirubin, this time is shortened to 5-10 min (if it is left to stand for a longer time, the free bilirubin begins to be involved in the color reaction). At the end of the indicated time, measure the absorbance for each of three solutions against distilled water on a photocolorimeter at wavelength of 520-560 nm (green light filter) in a 5 mm cuvette. The absorbance measured for the control solution (control for blood serum clouding) is subtracted from the absorbance values for total and bound bilirubin solutions and the respective concentrations of total and bound (conjugated) bilirubins (in μmol/L) are determined by means of plotted analytical curve. Unconjugated (free) bilirubin is calculated by the difference between the total and bound bilirubin concentrations. Calculate the total bilirubin and its fractions in the blood serum and make conclusions about the causes of possible variations of these parameters.

***Clinical and diagnostic significance.*** *Normally, the amount of total serum bilirubin is 8.0-21.0 μmol/L; the unconjugated (free) bilirubin accounts for 75% (6.0-15.0 μmol/L) and conjugated (bound) bilirubin, for 25% (2.0-5.0 μmol/L). The main factors causing the disorders of bile pigment metabolism include: increased hemolysis of erythrocytes, impaired synthesis of bilirubin glucuronides, impaired release of conjugated bilirubin from hepatocytes into the bile capillaries (destruction of hepatocytes), impaired bile flow as a result of extrahepatic biliary obstruction. Hepatic (parenchymal) jaundice occurs in hepatitis, liver cirrhosis and is characterized by a sharp increase in the content of bound bilirubin in the blood. As a result of it, the formation of bilirubin glucuronides is decreased in the liver, the content of free bilirubin in the blood increases. Hemolytic jaundice occurs due to the intensive hemolysis leading to increased formation of free bilirubin, as the liver is incapable of produceing bilirubin glucuronides in adequate amounts. Elevation of the bound bilirubin concentration in the case of obstructive jaundice is caused by the biliary tract overfilling due to blockage, rupture and further transition of bile components in the blood. Bound bilirubin is accumulated in blood (hyperbilirubinemia), urobilinogen disappears from the urine, but the urine gets dark in color due to bilirubinuria. Stercobilin disappears from feces. In the clinical and biochemical laboratories the determination of bilirubin and its fractions is carried out for the differential diagnosis of jaundices.*

**1****. Compare erythropoietic and hepatic porphyrias.
**2****. Make a table "Differential diagnosis of jaundice."

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**** - tasks for self-study**

## *CLASS 4 (4 hours)*

**TOPIC 6 (2 hours): Water-salt and mineral metabolism. Regulation. Disorders. Quantitative determination of calcium and inorganic phosphorus in blood serum.**

**IMPORTANCE.** The concepts of water-salt and mineral metabolism are not the same. Speaking about the water-salt metabolism, the metabolism of major mineral electrolytes (above all, the metabolism of water and NaCl) is kept in mind. Water and dissolved mineral salts constitute the internal environment of the human body, creating an environment for biochemical reactions. Kidneys and hormones that regulate their functions (vasopressin, aldosterone, atrial natruretic factor, and the renin-angiotensin system) play an important role in maintainance of water-salt homeostasis. The main parameters of the liquid medium of the body are the osmotic pressure, pH and volume. Osmotic pressure and pH of the extracellular fluid and blood plasma are practically the same, and the pH value of cells of different tissues may be different. Maintainance of homeostasis is provided by the constancy of the osmotic pressure, pH and volume of extracellular fluid and blood plasma. Knowledge of the water-salt metabolism and methods of correction of the basic parameters of the liquid medium in the body is necessary for the diagnosis, treatment and prognosis of such disorders as dehydration of tissues or edema, increased or decreased blood pressure, shock, acidosis, and alkalosis.

Mineral metabolism is a metabolism of any mineral components of the body, including those that do not affect the basic parameters of the liquid medium, but perform different functions related to catalysis, regulation, transport and storage of substances, structuring of macromolecules, etc. Knowledge of mineral metabolism and methods for its study are essential for diagnosis, treatment and prognosis of exogenous (primary) and endogenous (secondary) disorders.

**AIM.** Familiarize yourself with the water functions in vital processes, which are due to the peculiarities of its physico-chemical properties and chemical structure; learn the content and distribution of water in the body, tissues, cells, state of water, and metabolism of water. Have an idea of the water pool (ways of intake and excretion from the body), endogenous and exogenous water, water content in the body, its daily needs, age features. View the regulation of total body water volume and its movement between the liquid spaces, possible disorders. Learn and be able to characterize the macro-, oligo-, micro- and ultramicrobiogenic elements, their general and specific functions; electrolyte composition of the body; the biological role of major cations and anions; the role of sodium and potassium. Familiarize yourself with phosphate-calcium metabolism and its regulation and the disturbances. Define the role and the metabolism of iron, copper, cobalt, zinc, iodine, fluorine, selenium and other biogenic elements. Learn the daily requirements for minerals, their absorption and excretion from the body, the ability to be deposited and forms of deposition, disorders. Familiarize yourself with the methods of quantitative determination of calcium and phosphorus in the blood serum and their clinical and biochemical significance.

***THEORETICAL QUESTIONS***

1. The biological significance of water and its content, daily requirements. Exogenous and endogenous water.
2. Properties and biochemical functions of water. The distribution and state of water in the body.
3. Metabolism of water in the body, age-related features, regulation.
4. Water balance of the body and its types.
5. Functions of mineral salts in the body.
**6*.** Neurohumoral regulation of water salt metabolism.
7. Electrolite composition of liquids in the body, its regulation.
8. Minerals of the human body, their content, role.
9. Classification of biogenic elements, their role.
10. Functions and metabolism of sodium, potassium, chlorine.
11. Functions and metabolism of iron, copper, cobalt, iodine, zinc, fluorine, selenium.
**12*.** Phosphate-calcium metabolism, role of hormones and vitamins in its regulation. Inorganic and organic phosphates. Phosphates in the urine.
13. The role of hormones and vitamins in the regulation of mineral metabolism.
14. Pathological conditions associated with metabolic disorders of mineral substances.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
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| *1. Humoral regulation of water-salt metabolism.* | *1.1. The main hormones responsible for the humoral regulation of water-salt metabolism are antidiuretic hormone, aldosterone, atrial natriuretic factor, and renin-angiotensin system.*<br>*1.2. Antidiuretic hormone (vasopressin):*<br>*- It interacts with V2-receptors in renal tubules, increases the level of cAMP, phosphorylates proteins, increases the permeability of membranes for water and its reabsorption: an increase in the osmotic pressure of the blood plasma → excitation of hypothalamic osmoreceptors → ADH secretion from the secretory granules → distal renal tubules (activation of hyaluronidase) → depolimerization of hyaluronic acid → increased permeability of distal tubules for water → decreased diuresis (water is retained in the body);*<br>*- It interacts with V1-receptors of smooth muscle cells in blood vessels, increases the concentration of calcium in cells, causes muscular contraction, narrowing blood vessels and increasing arterial pressure.* |

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| | *1.3. Aldosterone:*<br>*- It is responsible for the constancy of electrolyte composition, promotes sodium retention and potassium excretion from the body: a decrease in NaCl in the blood → aldosterone secretion → an increase in sodium and chlorine reabsorption in the tubules → NaCl retention.*<br>*1.4. The renin-angiotensin system:*<br>*- increases blood volume (especially after bleeding, vomiting, diarrhea) by narrowing vessels, retaining water and sodium chloride;*<br>*- enhances the filtering capacity of the renal glomerular apparatus;*<br>*- increases urine production;*<br>*- influences natriuretic factor synthesis.*<br><br>*Low blood volume and arterial pressure*<br>↓<br>*Activation of renal juxtaglomerular volume receptors*<br>↓<br>*Low perfusion pressure in renal glomeruli*<br>↓<br>*Renin excretion*<br>↓<br>*Angiotensin I*<br>↓<br>*Angiotensin II (octopeptide) → vasoconstriction*<br>↓<br>*Aldosterone release (NaCl retention),*<br>*Vasopressin secretion (water reabsorption).*<br>*1.5. Atrial natriuretic factor:*<br>*- its effects are opposite to the renin-angiotensin system;*<br>*- It increases the filtering capacity of the renal glomerular apparatus, increases urine formation;*<br>*- powerful vasodilator;*<br>*- It reduces the water volume, tubular sodium reabsorption, sodium concentration in the blood, the volume of circulating blood and blood pressure;*<br>*- It increases the pressure in the glomerular capillaries and glomerular filtration rate;*<br>*- It inhibits renin secretion, reduces aldosterone secretion.* |
| *2. Phosphate-calcium metabolism. Role of hormones* | *2.1. Blood plasma phosphate levels are 0.8-1.4 mmol/L; daily requirements reach 1-2 g.* |

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| *and vitamins in its regulation.* | *The role of phosphorus:*<br>*- structural (it forms a part of bone tissue, teeth and other tissues of the body, serves as a structural element of phosphoproteins, phospholipids, which are the main components of cell membranes);*<br>*- participation in energy transfer in the form of macroergic bonds (ADP, ATP);*<br>*- participation in phosphorylation reactions;*<br>*- participation in the regulation of acid-base balance (phosphate buffer system);*<br>*- participation in growth, cell division, storage and use of genetic information (as a component of nucleic acids).*<br>*2.2. Regulation of phosphate metabolism:*<br>*- Parathyroid hormone: It stimulates the functional activity of osteoclasts, releases phosphates into the bloodstream; reduces tubular phosphate reabsorption and causes phosphaturia;*<br>*- Calcitonin: It suppresses osteoclasts, resulting in inhibition of resorption of both organic and inorganic components in the bone matrix, which leads to a decrease in the release of phosphates into the bloodstream;*<br>*- vitamin D and its metabolites: It increases intestinal phosphorus absorption.*<br>*2.3. Serum calcium concentration is 2.25-2.75 mmol/L; daily requirements reach 0.8-1.2 g.*<br>*The role of calcium:*<br>*- participation in neuromuscular excitation (in hypercalcemia excitation decreases, and in hypocalcemia excitation increases);*<br>*- participation in muscular contraction, myocardial contractility;*<br>*- participation in blood coagulation (it binds to plasma factors II, VII, IX, X);*<br>*- reduces the permeability of capillaries;*<br>*- acts as an activator of enzymes (trypsinogen, lipase, amylase, ATPase);*<br>*- second messenger in the transmission of the hormonal signal, promotes the secretion of mediators, the release and action of hormones;*<br>*- participation in the regulation of protein phosphorylation;*<br>*- structural (for example, calcium salts provide a rigid structure of bones and teeth, calcium binds to* |

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| | *phosphorus and forms mineral salts of bones and teeth).*<br>*2.4. Regulation of calcium metabolism.- Parathyroid hormone: It stimulates mobilization of calcium from bone tissue; stimulates calcium reabsorption in the distal renal tubules, activates calcitriol synthesis in the kidneys, which leads to an increase in intestinal calcium absorption (hypercalcemic action);*<br>*- calcitriol: It induces calcium-binding protein synthesis in the intestine, which provides calcium absorption; stimulates calcium reabsorption in the distal renal tubules; promotes calcium mobilization from bone tissue; maintains calcium and phosphate concentrations necessary for the formation of hydroxyapatite crystals deposited in collagen fibers of bone (in disorders of calcitriol synthesis the formation of new bones is suppressed and the regeneration of the bone tissue is impaired);*<br>*- Calcitonin: It inhibits the release of calcium from bone tissue, reduces the activity of osteoclasts; It reduces calcium reabsorption in the distal renal tubules (hypocalcemic action).* |

## *TESTS FOR SELF-CONTROL*

**1**. Potassium blood plasma level in a patient is 8 mmol/L (normal levels are 3.6-5.3 mmol/L). This state is accompanied by:

*A. Tachycardia B. Arrhythmia, cardiac arrest C. A decrease in arterial pressure D. A increase in arterial pressure E. Bradycardia*

**2**. A characteristic feature of cholera is the loss of large amounts of water and $Na^+$ by the body. The biochemical action of cholera toxin is based on:

*A. Activation of adenylate cyclase in the small intestine*
*B. Activation of atrial natriuretic factor synthesis*
*C. Inhibition of antidiuretic hormone synthesis in the hypothalamus*
*D. Enhancement of renin secretion by cells of the juxtaglomerular apparatus in renal arterioles E. Oxidation of aldosterone in adrenal cortex*

**3**. A 50-year-old patient complained of general weakness, loss of appetite, cardiac arrhythmia. Muscular hypotension, flaccid paralysis, and weakened intestinal peristalsis were observed. The reason for such conditions can be:

*A. Hyponatremia B. Hypophosphatemia C. Hypokalemia D. Hypoproteinemia E. Hyperkalemia*

**4.** Addison-Biermer's disease (pernicious hyperchromic anemia) develops due to vitamin $B_{12}$ deficiency. Choose a metal incorporated to this vitamin:

*A. Zink B. Cobalt C. Molybdenum D. Magnesium E. Iron*

**5**. During the thyroid surgery, parathyroid glands had been mistakenly removed from a patient with a diffuse toxic goiter. There were cramps, tetany. Which bioelement metabolism is affected?

*A. Magnesium B. Potasium C. Iron D. Sodium E. Calcium*

**6**. It has been known that in some biogeochemical areas endemic goiter is widespread. Which bioelement deficiency can cause this disease?

*A. Iron B. Zinc C. Iodine D. Copper E. Cobalt*

**7**. Which element excess in food and water is associated with tooth fluorosis?

*A. Fluorine B. Phosphorus C. Calcium D. Sodium E. Potassium*

**8**. Which microelement has the most pronounced cariogenic effect?

*A. Barium B. Strontium C. Selenium D. Iron E. Copper*

**9**. A child has abnormal ossification and points on the enamel. Which microelement metabolism is impaired?

*A. Zinc B. Chromium C. Copper D. Iron E. Fluorine*

**10**. Against the background of treatment with diuretics, a patient who abuses alcohol has a strong muscle and heart weakness, vomiting, diarrhea, blood pressure - 100/60 mm. Hg, depression. The cause of this condition is a higher urinary excretion of:

*A. Sodium B. Potassium C. Chlorine D. Calcium E. Phosphates*

**11**. A dog after 1-2 days after the surgical removal of the parathyroid glands showed signs of lethargy, thirst, a sharp increase in neuromuscular excitability with the development of tetany. Which disorder of electrolyte metabolism takes place in this case?

*A. Hypermagnesia B. Hypomagnesemia C. Hypocalcemia D. Hypercalcemia E. Hyponatremia*

**12**. While examining a group of people living on the same territory, a dentist drew attention to the same symptoms of the disease - dark yellow spots of the tooth enamel. Which microelement is excessive in food or drinking water?

*A. Iodine B. Nickel C. Fluorine D. Calcium E. Copper*

**13**. Microelement selenium deficiency manifests by cardiomyopathy. The likely cause of this condition is a decrease in the activity of the selenium-containing enzyme called:

*A. Catalases B. Cytochrome oxidase C. Succinate dehydrogenase D. Lactate dehydrogenase E. Glutathione peroxidase*

**14**. Biochemical blood serum tests of a patient with hepatolenticular degeneration (Wilson disease) revealed a decrease in the content of ceruploplasmin. Which ion concentration will be increased in the serum of this patient?

*A. Copper B. Calcium C. Phosphorus D. Potassium E. Sodium*

**15**. A male has a parathyroid adenoma. Select a substance whose metabolism is impaired in the patient first of all:

*A. Calcium B. Potassium C. Sodium D. Water E. Phosphate*

**16**. Excessive formation of angiotensin II is observed in a patient with a pathology of the cardiovascular system. It is synthesized with the participation of the enzyme called:

*A. Kallikrein B. Kininase C. Urokinase*
*D. Angiotensin-converting enzyme E. Cyclooxygenases*

**17**. Captopril was prescribed to a patient with hypertension. It is an inhibitor of angiotensin-converting enzyme. Which substance is formed in low amounts as a result?

*A. Bradykinin* *B. Serotonin* *C. Angiotensin II*
*D. Histamine* *E. Renin*

**18**. Insulin was prescribed to a patient diabetes mellitus type I. Which microelement forms a part of this drug?

*A. Zinc* *B. Copper* *C. Magnesium* *D. Sodium* *E. Phosphorus*

**19**. An increase in the volume of circulating blood under the influence of aldosterone and antidiuretic hormone will lead to the activation of:

*A. Angiotensin II* *B. Angiotensinogen* *C. Renin*
*D. Atrial natruretic factor* *E. Melatonin*

**20**. What is the normal blood calcium level?

*A. 1.50-1.75 mmol/L* *B. 1.75-2.00 mmol/L* *C. 2.25-2.75 mmol/L*
*D. 3.0-4.5 mmol/L* *E. 0.65-1.60 mmol/L*

**21.** Prolonged vomiting in a patient led to dehydration. Which hormone oversecretion contributes to water retention in the body?

*A. Vasopressin* *B. Calcitonin* *C. Somatostatin*
*D. Aldosterone* *E. Thyroxine*

**22**. Intestinal sodium absorption is reduced in a male patient. Which substances will be absorpted normally in this case?

*A. Proteins* *B. Water* *C. Fats* *D. Carbohydrates* *E. Chlorides*

**23**. Clinical and biochemical manifestations in a patient include: anemia, abnormal collagen synthesis, and skeletal deformation. Which element is deficient?

*A. Iron* *B. Copper* *C. Magnesium* *D. Manganese* *E. Cobalt*

**24**. A female patient complained of general weakness, drowsiness, apathy, and edema. After the examination, endemic goiter was diagnosed. Which element deficiency can lead to this pathology?

*A. Iron* *B. Fluorine* *C. Calcium* *D. Magnesium* *E. Iodine*

**25**. A hormone was injected to a dog, which led to an increase in the glomerular filtration rate by dilating the renal arterioles and reducing sodium and water reabsorption in the tubules. Which hormone was injected?

*A. Oxytocin* *B. Adrenaline* *C. Atrial natriuretic factor*
*D. Thyroxine* *E. Testosterone*

**26**. A female patient has limited renal blood flow, increased blood pressure. Which hormone hypersecretion can cause the increase in pressure?

*A. Renin* *B. Adrenaline* *C. Erythropoietin*
*D. Norepinephrine* *E. Vasopressin*

**27**. A patient has reduced vasopressin synthesis, which leads to polyuria and, as a result, to severe dehydration. What is the mechanism of polyuria development?

*A. A decrease in tubular reabsorption of sodium ions*
*B. Increased glomerular filtration rate*
*C. A decrease in protein tubular reabsorption*
*D. Decrease in tubular water reabsorption* *E. Decreased glucose reabsorption*

**28**. A patient complains of thirst and polyuria. Urinalysis revealed: daily diuresis

is 10 L; urine density is 1.001. Which disease causes such changes?

*A. Diabetes mellitus* *B.Steroid diabetes* *C. Thyrotoxicosis*
*D. Acromegaly* *E. Diabetes insipidus*

**29**. A patient complains of thirst, intake of large water amounts(up to 5 liters), exretion of significant amounts of urine (up to 6 liters per day). Blood glucose concentration is 4.4 mmol/L, the level of ketone bodies is not increased. Urine is colorless, its specific gravity reaches 1.002. Sugar in urine is not found. Which hormone deficiency can lead to such changes?

*A. Vasopressin B. Insulin C. Aldosterone D. Glucagon E. ACTH*

**30**. A patient with liver cirrhosis has edema. What is the cause of its appearance?

*A. A decrease in blood albumin content*
*B. Reduction in the content of haptoglobin in the blood*
*C. Increased blood transferrin*
*D. An increase in the blood content of gamma globulins*
*E. Decreased blood glucose*

**31**. Signs of kidney damage and high blood pressure were revealed in a hospitalized boy. Which biologically active peptide is increased under such circumstances?

*A. Glucagon* *B. Callidin* *C. Angiotensin II*
*D. Antidiuretic hormone* *E. Insulin*

**32.** A tourist exposed to the heat for a long period of time experienced a significant loss of water, which was accompanied by a sharp decrease in diuresis. Which hormone is secreted excessively in this case?

*A. Glucocorticoids and insulin* *B. Vasopressin and aldosterone*
*C. Thyroxine and triiodothyronine* *D. Serotonin and dopamine*
*E. Adrenaline and norepinephrine*

**33**. A patient was hospitalized to the cardiovascular department with complaints of constant headache in the occipital region, tinnitus, dizziness. His examination revealed: arterial pressure - 180/110 mm. Hg, heart rate - 95 per min. X-ray revealed a narrowing of one of the renal arteries. Which system was activated and caused the hypertension?

*A. Hemostatic* *B. Sympatoadrenal* *C. Immune*
*D. Renin-angiotensin* *E.Kinins*

**34.** After drinking 1.5 liters of water, the amount of urine increases significantly, and its relative density decreases to 1.001. These changes are caused by a decrease in water reabsorption in the distal tubules due to the decreased secretion of:

*A. Aldosterone* *B. Angiotensin II* *C. Vasopressin*
*D. Renin* *E. Prostaglandins*

**35.** Osteoporosis developed in a woman with chronic kidney disease. Which of the following substances may be deficient, causing this complication?

*A. 1,25-dihydroxy-D3* *B. 25- dihydroxy -D3* *C. D3*
*D. D2* *E. Cholesterol*

**36.** A patient was hospitalized to the infectious department with complaints of incessant vomiting. Which disorder of water-salt metabolism is developed?

*A. Isoosmolar dehydration* *B. Hyperosmolar dehydration*

*C. Hypoosmolar hyperhydration* *D. Hyperosmolar hyperhydration*
*E. Hypoosmolar dehydration*

**37.** A young man had an increased amount of potassium in the secondary urine. Which hormone oversecretion could cause such changes?

*A. Oxytocin* *B. Adrenaline* *C. Glucagon*
*D. Aldosterone* *E. Testosterone*

**38.** An animal underwent 2-week experimental narrowing of the renal artery. Which factor of humoral regulation acted on the vessels in this case?

*A. Cortisol* *B. Aldosterone* *C. Angiotensin II*
*D. Vasopressin* *E. Dopamine*

**39.** A man with a basilar skull fracture excreted a large volume of urine with a low relative density. The cause of changes in urine formation is a disorder of synthesis and secretion of the hormone called:

*A. Vasopressin B. TSH C. ACTH D. Oxytocin E. Somatotropic hormone*

**40.** An animal underwent an intravenous injection of a concentrated sodium chloride solution, which led to a decrease in tubular sodium reabsorption. Which changes in the secretion of hormones were observed?

*A. High aldosterone* *B. Reduced vasopressin* *C. Reduced aldosterone*
*D. Increased vasopressin* *E. Decreased natriuretic factor*

**41.** Those who are adapted to the action of high temperature, intense perspiration is not accompanied by a loss of high amounts of sodium chloride. Which hormone acts on the sweat glands and promotes this effect?

*A. Vasopressin* *B. Cortisol* *C. Thyroxine*
*D. Natriuretic factor* *E. Aldosterone*

**42.** A male with complaints of a decrease in the density of the dental tissue, increased fragility of it after intake of solid food visited a dentist. Which mineral deficiency is most likely observed in this patient?

*A. Calcium* *B. Potassium* *C. Sodium* *D. Magnesium* *E. Iron*

**43.** The main symptoms of primary hyperparathyroidism include osteoporosis and kidney damage with the development of urolithiasis. Which substances form stones in case of this disease?

*A. Uric acid* *B. Cystine* *C. Calcium phosphate*
*D. Bilirubin* *E. Cholesterol*

**44.** The decrease in the activity of antioxidant enzymes activates lipid peroxidation. Which microelement deficiency reduces the activity of glutathione peroxidase?

*A. Molybdenum* *B. Cobalt* *C. Manganese* *D. Copper* *E. Selenium*

**45.** In toxic damage to hepatocytes with their abnormal protein-synthesizing function, the albumin content in plasma and the oncotic plasma pressure decrease sharply. What will be observed?

*A. A decrease in diuresis* *B. Edema* *C. Low ESR*
*D. An increase in the volume of circulating blood* *E. High blood viscosity*

**46.** In acute experiment, vasopressin was administered to an anesthetized dog, which reduced the amount of urine. What is the effect of vasopressin on the kidneys?

*A. Increased water reabsorption* *B. Increased reabsorption of sodium ions*
*C. Reduction of water reabsorption* *D. Reduction of calcium reabsorption*
*E. Increased reabsorption of calcium ions*

**47**. When the isolated heart of a mammal was perfused with a solution with the high content of ions, cardiac arrest in the diastole occurred. Which ions are excessive in the solution and caused the cardiac arrest?

*A. Magnesium B. Calcium C. Chlorine D. Potassium E. Sodium*

**48**. Restriction of water consumption led to dehydration. Which mechanism is activated under such conditions to retain water in the body?

*A. Increased secretion of somatostatin*

*B. Increased secretion of vasopressin C. Reduced secretion of vasopressin*

*D. Increased secretion of aldosterone E. Decreased secretion of aldosterone*

**49.** Less water is excreted from the body than is consumed. Which disease can lead to such conditions?

*A. Kidney disease B. Hepatitis C. Pancreatitis*

*D. Infectious diseases E. Myocardial infarction*

**50.** Calcium ions are referred to as second messengers in cells. They activate glycogen catabolism by interacting with:

*A. Calmodulin B. Calcitonin C. Calcipherol*

*D. Glutamine E. Phospholipase C*

**51.** Which electrolyte determines the blood osmotic pressure?

*A. Potasium B. Calcium C. Magnesium D. Zinc E. Sodium*

**52.** Select a hormone that affect blood sodium and potassium levels?

*A. Calcitonin B. Histamine C. Aldosterone D. Thyroxine E. Parathyroid hormone*

**53.** Which elements are oligobiogenic?

*A. Gold, silver, tungsten B. Copper, zinc, cobalt*

*C. Potassium, sodium, magnesium D. Iodine, bromine, fluorine*

*E. Nickel, molybdenum*

**54.** A significant weakening of cardiac activity leads to edema. Select a type of water balance disorder, observed in this case.

*A. Positive B. Negative C. Dynamic equilibrium*

**55.** Endogenous water is formed in the body as a result of:

*A. Reduction B. Decarboxylation C. Carboxylation*

*D. Oxidation E. Hydroxylation*

**56.** Daily water requirement for adults is:

*A. 30-50 ml/kg B. 75-100 ml/kg*

*C. 75-80 ml/kg D. 100-120 ml/kg*

**57.** Pathological changes in the liver and brain were revealed in a 27-year-old-patient. Copper concentration is decreased in blood plasma and increased in the urine. Wilson's disease was diagnosed. Which enzyme activity in the blood serum should be examined to prove the diagnosis?

*A. Carbonic anhydrase B. Xanthine oxidase C. Alcohol dehydrogenase*

*D. Leucine aminopeptidase E. Ceruloplasmin*

58. How many ml of endogenous water are formed in human organism per day under rational nutrition?

*A. 50-75 ml B. 100-120 ml C. 150-250 ml*

*D. 300-400 ml E. 500-700 ml*

## *PRACTICAL WORK*

## Quantitative determination of calcium and inorganic phosphorus in blood serum

**Task 1. Determine the content of calcium in blood serum.**

***Principle.*** Serum calcium is precipitated by a saturated solution of ammonium oxalate [$(NH_4)_2C_2O_4$] in the form of calcium oxalate ($CaC_2O_4$). The latter is transformed with sulphuric acid into oxalic acid ($H_2C_2O_4$), which is titrated with $KMnO_4$.

***Procedure.*** The centrifuge tube is filled with 1 ml of blood serum and 1 ml of the solution [$(NH_4)_2C_2O_4$]. Keep 30 min and centrifuge. Crystalline precipitate of calcium oxalate is formed at the same time at the bottom of the tube. Remove clear supernatant. Pour 1-2 ml of distilled water into the sediment, stir with a glass rod, and then centrifuge. After centrifugation, remove the supernatant. Add 1 ml of 1N $H_2SO_4$ to a test tube with the precipitate, mix well the precipitate with a glass rod and put the tube in a water bath at 50-70 $^0$C. The precipitate is dissolved at the same time. The content of the tube is titrated with the hot solution of 0.01 N $KMnO_4$ until the appearance of pink color that persists for 30 seconds. Each ml of $KMnO_4$ corresponds to 0.2 mg of Ca. Calcium content (X) in mg% in serum is calculated in accordance with the formula: $X = 0.2 \times A \times 100$, where A is the amount of $KMnO_4$ used for titration. The content of calcium in the blood serum in mmol/L is equal to the content in mg% × 0.2495.

***Clinical and diagnostic significance.*** *The normal concentration of calcium in blood serum is 2.25-2.75 mmol/L (9-11 mg%). Increased concentration of blood serum calcium (hypercalcemia) is observed in hypervitaminosis D, hyperparathyroidism, and osteoporosis. Reduced concentration of calcium (hypocalcemia) is observed in hypovitaminosis D (rickets), hypoparathyroidism, and chronic renal failure.*

**Task 2.** Determine the content of inorganic phosphorus in the blood serum.

***Principle.*** Inorganic phosphorus, interacting with molybdenum reagent in the presence of ascorbic acid, forms molybdenum blue. Its color intensity is proportional to the amount of inorganic phosphorus.

***Procedure.*** Pour 2 ml serum, 2 ml of 5% trichloroacetic acid solution into the test tube, mix and leave for 10 minutes to precipitate the proteins, and then filtrate. Then measure 2 ml of the filtrate (it corresponds to 1 ml of blood serum), pour into the test tube; add 1.2 ml of molybdenum reagent, 1 ml of 0.15% ascorbic acid solution and then fill with water to 10 ml (5.8 ml). Mix thoroughly and leave for 10 minutes for color development. Measure the absorbance on the photoelectrocolorimeter at a red light filter. The amount of inorganic phosphorus is found in calibration curve and the content of it (B) in the sample in mg/L is calculated according to the formula: $B = (A \times 1000) / 31$, where A is the content of inorganic phosphorus in 1 ml of serum (according to the calibration curve), 31 is its atomic mass, 1000 is the conversion factor per liter.

***Clinical and diagnostic significance.*** *Normally, the concentration of phosphorus in the blood serum is 0.8-1.48 mmol/L (2.5 mg%). Increased concentration of phosphorus in the blood serum (hyperphosphatemia) is observed in renal failure, hypoparathyroidism, overdoses of vitamin D. Decreased concentration of phosphorus (hypophosphatemia) is observed in disorders of the absorption in the intestine, galactosemia, rickets.*

**TOPIC 7 (2 hours): Biochemistry of kidneys and urine. Physical and chemical properties of urine under normal conditions. Biochemical urine analysis.**

**IMPORTANCE.** Kidneys are one of the most important organs whose main purpose is to maintain the constancy of the dynamic internal environment. Kidneys are involved in the regulation of fluid and electrolyte balance, blood pressure, in supporting acid base balance, osmotic pressure of body fluids, stimulation of erythropoiesis, etc. Kidneys produce urine from the components of blood plasma. About 150 different substances are excreted in the urine. Daily urine contains on average about 40 g of organic substances and approximately 20 g of inorganic substances. Biochemical examination of the urine plays an important role in the clinical practice for the diagnosis and prognosis, treatment effectiveness monitoring.

**AIM.** Familiarize yourself with basic physical and chemical properties of urine (diuresis, pH, density, color, transparency, smell) and chemical composition of normal urine. Familiarize yourself with the methods of clinical and biochemical analysis of urine. Memorize some physico-chemical constants and biochemical parameters in normal urine for correct estimation of deviations from the norm in order to use them for diagnosis of diseases.

***THEORETICAL QUESTIONS***

**1*.** Renal functions and features of metabolism in kidneys.

**2*.** Biochemical regulatory mechanisms of renal functions.

3. General properties and chemical composition of normal urine. Significance of urinalysis in clinical practice.

4. Organic and inorganic components of normal urine. Aging-related changes.

5. Physical and chemical properties of urine: urine output, specific gravity, pH, odor, color, and transparency. Significance of their investigation. Possible deviations from the normal ranges.

6. Clinical and diagnostic significance of quantitative and qualitative analysis of urine.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
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| *1. Functions of kidneys and features of their metabolism.* | *1.1. Functions of kidneys:*<br>*- formation of urine (in nephrons about 180 liters of liquid is filtered and reabsorbed per day);*<br>*- excretory, i.e. the release of end products of metabolism in urine (urea, uric acid, ammonium salts, indican, etc.);*<br>*- regulatory: volume regulation (maintenance of water volume), osmoregulation (maintenance of certain concentrations of osmotically active substances by means of water and electrolyte excretion or reabsorption of these substances), regulation of acid-base balance (via the exchange of sodium ions for hydrogen ions in the distal tubules* |

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| | *with the participation of carbonic anhydrase; sodium reabsorption is accompanied by a shift of urine pH to the acidic side; the level of alkaline reserve does not change in the body; the inhibition of carbonic anhydrase by diuretics suppresses hydrogen ion secretion, excretion of ammonia by the distal tubules, which is formed from glutamine under the action of glutaminase, largely contributes to the preservation of alkaline reserves; kidneys can be a source of $HCO_3$-, formed as a result of oxidation of metabolites); regulation of blood pressure (the renin-angiotensin system);*<br>*- Endocrine: erythropoietin and calcitriol synthesis;*<br>*- Metabolic.*<br>*1.2. Features of metabolism in the kidney:*<br>*- synthesis of ATP*<br>*- gluconeogenesis, glycolysis, glucose aerobic oxidation; in the cortex, the aerobic type of metabolism prevails; In the medulla, anaerobic metabolism prevails;*<br>*- synthesis of calcitriol, phospholipids, tri-acylglycerols, prostaglandins, ketone bodies (ketogenesis), and ketolysis occur intensively;*<br>*- synthesis of biologically active substances - renin, erythropoietin;*<br>*- processes of amino acid trans- and deamination with the release of ammonia and the formation of its transport forms - glutamine and asparagine;*<br>*- synthesis of creatine;*<br>*- the presence of specific enzymes: LDH1, 2 (cortex), LDH3, 4 (medulla), isoforms of alanine aminopeptidase - AAP3, transamidinases (the appearance of these enzymes in the blood and urine indicates damage to the kidney tissue).* |

***TESTS FOR SELF-CONTROL***

**1.** An athlete who had had high-protein diet showed the acidic reaction of urine. Which substances were present in the urine and caused such changes?

*A. Chlorides and phosphates* *B. Chlorides and sulfates*
*C. Phosphates and sulfates* *D. Urates and sulfates* *E. Urates and chlorides*

**2.** The transparency of urine is one of the parameters investigated in analysis. Which substances in the urine cause its clouding and provide alkaline reaction?

*A. Calcium phosphate* *B. Magnesium sulfate* *C. Urates*
*D. Chlorides* *E. Ammonia salts*

**3.** Kidneys perform all functions, except:

*A. Excretion of the final products of metabolism*

*B. Regulation of water-salt metabolism C. Supporting osmotic pressure*

*D. Regulation of blood pressure E. Breakdown of urea to $CO_2$ and $H_2O$*

**4.** The organ-specific enzyme for kidneys is:

*A. Lactate dehydrogenase B. Succinate dehydrogenase*

*C. Aspartate aminotransferase D. Transamidinase E. Creatine phosphokinase*

**5.** After blood plasma filtration all substances enter the primary urine, except:

*A. Monosacharides (glucose and fructose) B. Urea and uric acid*

*C. Low-molecular proteins D. Creatine and creatinine*

*E. Middle- and high-molecular-weight proteins*

**6.** Which physico-chemical index of urine depends on diuresis?

*A. Color B. pH C. Density*

*D. Odor E. Transparency*

**7.** Which pH and density urine indices are normal in adults?

*A. pH=4.8; density 1.001 B. pH=8.0; density 1.040*

*C. pH=8.5; density 1.029 D. pH=3.5; density 1.020 E. pH=6.0; density 1.021*

**8.** How can be called the pathological state of adults when the urine excretion is absent?

*A. Enuresis B. Oligouria C. Anuria*

*D. Nicturia E. Dysuria*

**9.** How can be called the pathological state of adults when the daily diuresis is below 500 ml?

*A. Anuria B. Polyuria C. Nicturia*

*D. Oligouria E. Pollakiuria*

**10.** Ammonia content in urine is an important parameter of acid-base balance. Ammonia amount increases both in respiratory and metabolic acidosis. It is connected with stimulation of one of the enzymes in the renal epithelial cells in acidosis. Indicate this enzyme:

*A.Glutaminase B. Krebs cycle enzymes C. Carbonic anhydrase*

*D. ATPase E. Hyaluronidase*

**11**. In the experimental model, a morphological damage to the epithelial cells of the distal tubules was caused in rats. Which functional processes are weakened in the kidneys?

*A. Glucose reabsorption B. Sodium and glucose reabsorption*

*C. Reabsorption of electrolytes and water D. Reabsorption of proteins*

*E. Filtration*

**12**. Which substances are excreted in high amounts, causing the development of urolithiasis?

*A. Urates and phosphates B. Sulfates and chlorides*

*C. Sulfates and urea D. Amino acids and indican*

*E. Carbonates, bicarbonates, and citrate*

**13**. After a hemorrhage into the brain with damage to the hypothalamic nucleus, diabetes insipidus developed in a female patient. What was the cause of polyuria in this case?

*A. Hypoglycemia* *B. Reduced reabsorption of potassium ions*
*C. Increased glomerular filtration* *D. Hyperglycemia*
*E. Reduction of water reabsorption*

**14**. A patient experienced polyuria at the early stage of diabetes mellitus. What is its cause?

*A. Ketonemia* *B. Hyperkalemia* *C. Hyperglycemia*
*D. Hypocholesterolemia* *E. Hypercholesterolemia*

**15**. As a result of acute renal failure, oliguria developed in a patient. Which daily amount of urine is excreted in this case?

*A. 500-1,000 ml* *B. 1,000-1,500 ml* *C. 50-100 ml*
*D. 100-500 ml* *E. 1,500-2,000 ml*

**16**. A patient has impaired renal function. To assess the state of the renal filtration capacity, a clearance of one of the following substances should be measured:

*A. Creatinine* *B. Glutamine* *C. Uric acid*
*D. Indole* *E. Hydrocarbonate*

## PRACTICAL WORK
## The study of physical and chemical properties and chemical composition of normal urine

**Task 1.** Determine the daily volume of urine (diuresis).

The normal daily urine volume is on average 1,200-1,800 ml. Volumes of more than 2,000 ml and less than 500 ml per day indicate pathology. Amount of excreted urine can be reduced (oliguria), increased (polyuria); the complete cessation of urine excretion is also possible (anuria).

***Procedure***. The daily amount of urine is measured using a graduated cylinder for 1 or 2 liters.

**Task 2.** Determine the relative density (specific gravity) of urine.

*Relative density depends on the amount of dissolved substances in the urine and is closely related to the amount of excreted urine. It normally ranges from 1.010 to 1.025, but typically is 1.017-1.020. Discrepancy between relative density and the amount of urine is observed in diabetes mellitus, when the relative density is high (hypersthenuria), despite the large amount of urine. Relative density changes in various pathological conditions. Its decrease (hyposthenuria) is observed in polyuria due to diabetes insipidus, etc.*

***Principle.*** Determination of the relative density of urine is carried out with the help of special small areometer (urometer).

***Procedure***. Place a glass cylinder vertically on an even surface and pour the investigated urine into it. If the urine is foamy, apply a piece of filter paper to remove the formed foam. Submerge cautiously a dry urometer in the urine; make sure that the urometer freely floats and doesn't touch the cylinder wall. Read on the urometer scale against the mark coincident with the lower meniscus of the liquid. Measure the urine temperature with a thermometer. The urine density (in kg/liter) is read directly on the urometer scale, but since the certified calibration of urometers is carried out at a definite temperature (most commonly at 15 °C), a correction for the actual urine temperature should be applied. Add 0.001 kg/L increment per 3 °C to the measured density

value if the urine temperature is above 15 °C. On the contrary, if the urine temperature is below 15 °C, subtract 0.001 kg/L per 3 °C from the measured value.

**Task 3. Characterize the color of urine.**

*The urine color is estimated visually. The normal urine color is straw-yellow due to the occurrence of pigments in the urine, such as urochrome (intentively yellow), urobilin (pale pink), and uroerythrin (pale red). The urine color is changed in pathology or in dietary intake of nutrients containing natural colorants. A pale yellow or nearly colorness urine is observed in polyuria (diabetes mellitus and diabetes insipidus). The red urine color occurs either in hemoglobin- and myoglobinuria, or due to consumption of food colorants present in candies, bilberries, currants, red beet, etc, and intake of certain drugs. The presence of bile pigments in the urine favours green or yellow-brown color formation. Urine opalesces in the presence of pus. A dark-brown color is observed in akaptonuria or melanuria. A green-blue color occurs in bacterial contamination of the urine or in an excess of urinary indican that can be converted to blue indigo.*

**Task 4. Estimate the transparency of urine.**

*The transparency of the urine is estimated visually. Normally, the urine is transparent; on longer standing, it deposits a loose mucous mass composed of mucous bodies and cast-off epithelium from the urinary tract walls. A cloudy urine can originate in the presence of excessive salts (urates, phosphates, oxalates, and carbonates), or mucus, pus, bacteria, and cast-off cells.*

**Task 5. Determine the odor of urine.**

*Freshly excreted urine has no unpleasant odor, but upon standing it gets strong smell of ammonia generated due to the splitting of urea by urease of microorganisms. Freshly released urine has such odor in cystitis, pyelitis, pyelonephritis, etc. Fragrant nutrients or drugs (garlic, asparagus, validol, etc.) can give urine odor proper to these nutrients. The urine odor has diagnostic vsignificance in diabetes mellitus (the smell of unripe apples), inherited disorders of amino acid metabolism (the smell of mould in phenylpyruvate oligophrenia, smell of "maple syrup" in maple syrup urine disease).*

**Task 6. Determine pH of urine.**

*Normally, urine pH ranges from 5.3 to 6.5. The pH shift to the acid side is observed in urinary excretion of ketone bodies (diabetes mellitus, starvation, renal failure, prolonged fever, consumption of meat products). The pH shifts toward alkaline side in the use of dietary bicarbonates, alkaline mineral water, dairy and vegetable products, cystitis, after prolonged vomiting.*

***Procedure.*** Dip a strip of universal indicator paper into the tested urine and determine the pH value by comparing the developed color to the pH reference color scale; or apply 1-2 drops of urine in the middle of the test paper "Rifon" and determine the pH value by comparing the developed color to the color of one of the colored strips coinciding with the test strip color.

**Task 7.** Identify the chlorides in the urine by reaction with silver nitrate. *Daily excretion of chloride under normal conditions is 8-15 g.*

***Procedure***. Add few drops of 2% solution of $AgNO_3$ to 3.2 ml of urine. Silver chloride is precipitated.

**Task 8.** Identify sulfates in urine by reaction with barium chloride. *Daily excretion of sulfates under normal conditions is 2-3 g.*

***Procedure***. Add few drops of $BaCl_2$ to 3.2 ml of urine. Insoluble precipitate of barium sulfate is formed.

**Task 9.** Identify phosphates in the urine.

***Procedure***. Add 1 ml of a 10% solution of NaOH or $NH_4OH$ to 3.2 ml of urine and heat. In alkaline medium a precipitate of alkaline earth metals (calcium and magnesium) phosphates is formed.

**Task 10.** Identify calcium cations in urine.

***Procedure***. Add 1 ml of a saturated solution of ammonium oxalate to 3.2 ml of urine. Crystalline precipitate of calcium oxalate is formed.

**Determination of urea, uric acid, creatinine in urine is described in the chapters of the metabolism of proteins and nucleotides.**

***Clinical and diagnostic significance.*** *General and special biochemical analysis of urine is carried out in the clinical and biochemical laboratories. General analysis includes the study of physical and chemical properties of urine, determination of the presence of pathological components in it, such as sugar, ketone bodies, protein, hemoglobin, pigments, indican, etc. Biochemical analysis includes the study of normal urine components (urea, uric acid, creatinine, ammonia). If it is necessary, the number of red blood cells and white blood cells, epithelial cells in the urine is counted. The general analysis is required for the initial examination of the patient and dispensary supervision. Special analysis (identification of other components of the urine, such as metabolites, enzymes, certain minerals, etc.) is carried out in case of a suspected lesion of certain organs or specific metabolic links.*

**1**.** Make a review of researches: "Renal insufficiency, biochemical changes in blood and urine."

**2 **.** Prepare a report: "Modern biochemical methods for the assessment of kidney function."

## ***CLASS 5 (4 hours)***

**TOPIC 8 (2 hours): Pathological components of urine. Biochemical analysis of urine.**

**IMPORTANCE.** Qualitative and quantitative composition of urine varies in many pathological states. The study of pathological components of the urine is important for disease diagnosis and prognosis, monitoring and treatment effectiveness.

**AIM.** Learn basic pathological components of urine and causes of their appearance. Familiarize yourself with methods of qualitative and quantitative determination of urine pathological components and their clinical and diagnostic significance.

## *THEORETICAL QUESTIONS*

1. Proteins as pathological components of urine. Possible causes of their occurrence. Methods of determination. Types of proteinuria.

2. Glucose as a pathological component of urine. Causes and types of glycosuria. Methods of determination.

3. Creatine as a pathological component of urine. Possible causes of its occurrence. Methods of determination. Physiological creatinuria.

4. Ketone bodies as pathological components of urine. Possible causes of ketonuria. Methods of determination.

5. Blood pigments (hemoglobin, methemoglobin) as pathological components of urine. Possible causes of their occurrence. Methods of determination.

6. Bile pigments as pathological components of urine. Possible causes of their occurence. Methods of determination.

7**.** Indican as a component of urine. Possible causes of its increased urinary excretion. Methods of determination.

## *TESTS FOR SELF-CONTROL*

**1.** It is possible to examine one of the following substances in urine to control the patient's diet with diabetes mellitus, namely proportional intake of fats and carbohydrates. Select this substance:

*A. Ketone bodies* *B. Lipids* *C. Carbohydrates*
*D. Proteins* *E. Phosphates*

**2.** A patient, who has been working for a long period of time at the chlorine-organic compounds factory, has hypotonus and muscle weakness. Myodistrophy is diagnosed. The decrease of which index in urine is informative to verify the diagnosis?

*A. Homogentisinic acid* *B. Creatine phosphate* *C. Creatinine*
*D. Creatine sulfate* *E. Glycocyamine*

**3.** The part of dietary proteins is not broken down in the gastrointestinal tract and is exposed to microorganisms in the large intestine. This process is called protein putrefaction. Which substance in urine indicates the intensity of putrefaction?

*A. Protein* *B. Urea* *C. Creatine and creatinine*
*D. Urates* *E. Indican*

**4.** A patient has glycosuria, glucose content in blood is normal. Which process disorder can lead to glycosuria?

*A. Gluconeogenesis* *B. Breakdown of kidney glycogen*
*C. Glycolysis* *D. Function of pancreas* *E. Function of renal tubules*

**5.** When the excretion of ketone bodies is observed?

*A. Diabetes insipidus and bronze disease* *B. Rickets and pellagra*
*C. Diabetes mellitus and starvation D. Hemolytic anemia* *E. Obstructive jaundice*

**6.** A patient complains of periodic pain in big toe joints and their swelling. Urinalysis showes sharply acidic urine and its pink coloration. Which substance can cause such changes?

*A. Uric acid salts* *B. Chlorides* *C. Ammonium salts*
*D. Calcium phosphate* *E. Magnesium sulfate*

**7.** Chronic glomerulonephritis is diagnosed in a patient. Edema has developed during last 6 months. What is the reason of its development?

*A. Hyperaldosteronism B. Overproduction of vasopressin*
*C. Proteinuria D. Hyperosmolarity of plasma*
*E. Disturbance of hepatic protein-synthesizing function*

**8.** Urinalysis was carried out for a patient with progressive muscular dystrophy. Which substance appearance in large quantities in the urine can confirm the diagnosis?

*A. Porphyrins B. Creatinine C. Creatine*
*D. Hippuric acid E. Urea*

**9**. In a man suffering from chronic intestinal obstruction, protein putrefaction in the large intestine is intensified. What can confirm this fact?

*A. Indicanuria B. Hyperuricuria C. Bilirubinuria*
*D. Glucosuria E. Creatinuria*

**10**. A patient complains of dry mouth, weight loss, despite the increased appetite, and increased urination. Which test should be done first to confirm the diagnosis?

*A. Zimnitsky's urinanalysis B. Urinalysis*
*C. Determination of sugar level in daily urine*
*D. Protein fractions in blood serum E. Coagulogram*

**11**. A patient has acute glomerulonephritis. Which substance appearance in the urine confirms the damage to the basal membrane of the glomerular capillaries?

*A. Fructose B. Protein C. Indican D. Creatine E. 17-Ketosteroids*

**12**. Alkaponuria leads to deviations from the normal composition of urine. Which acid concentration increases in the urine?

*A. Acetoacetic acid B. Phenylpyruvic acid C. Homogentisic acid*
*D. Oxalic acid E. Pyruvic acid*

**13.** Renal pathology is associated with the appearance of pathological components in the urine. Which pathological component of the urine confirms the increased permeability of the glomerular membrane?

*A. Proteinuria B. Glucosuria C. Aminoaciduria*
*D. Alkaponuria E. Pyuria*

**14**. The examination revealed sugar in the patient's urine. What is the possible mechanism of the changes found if the blood sugar content is normal?

*A. Impaired glucose filtration in glomeruli B. Insulin resistance*
*C. Hyperproduction of glucocorticoids by the adrenal glands*
*D. Insufficient production of insulin by the pancreas*
*E. Impaired glucose reabsorption in the tubules of nephrons*

**15**. A patient has reduced blood indican levels. Its daily urinary excretion is also low. Which organ functions improperly?

*A. Kidney B. Heart C. Lung D. Liver E. Pancreas*

**16.** According to the results of the glucose load test, tolerance to carbohydrates in a patient is normal. At the same time, glucose (5 mmol/L) is determined in the urine of the patient. Renal diabetes is diagnosed. Which changes in the kidneys cause glucosuria in this case?

*A. Increased activity of glucose reabsorption enzymes*
*B. Higher threshold for glucose reabsorption*

*C. Reduced activity of glucose reabsorption enzymes*
*D. Intensity of glucose secretion* *E. Increased glucose filtration*

**17**. Significant polyuria appeared in a patient due to a craniocerebral injury. It was not accompanied by glucosuria. What is damaged?

*A. Anterior pituitary* *B. Adrenal medulla* *C. Adrenal cortex*
*D. Posterior pituitary* *E. Pancreas*

**18**. A patient has been sufferring from pain in the right hypochondrium after eating fatty foods for several days. The jaundice of the sclera and skin can be visually noted. Acholic feces and urine that has "color of beer" are found. Which substance is present in the urine and provides the dark color of urine?

*A. Ketone bodies* *B. Direct bilirubin* *C. Sterilbilin*
*D. Indirect bilirubin* *E. Indican*

**19**. Glucosuria and hyperglycemia were found in a patient. There were complaints about dry mouth, skin itching, frequent urination, and thirst. Diabetes mellitus was diagnosed. What is the cause of polyuria in this patient?

*A. Reduced cardiac output* *B. Increased osmotic pressure of urine*
*C. Increased oncotic plasma pressure* *D. An increase in filtration pressure*
*E. Decreased oncotic plasma pressure*

**20**. A patient with diabetes mellitus had hyperglycemia (19 mmol/L), which clinically manifested by glucosuria, polyuria, and polydipsia. Which mechanism is associated with the development of glucosuria?

*A. Threshold glucose reabsorption* *B. Polyuria* *C. Tissue dehydration*
*D. Polydipsia* *E. Non-enzymatic glycosylation of proteins*

**21**. A patient has ketonuria. Which disease is associated with the appearance of ketone bodies in the urine?

*A. Urolithiasis* *B. Diabetes mellitus* *C. Renal tuberculosis*
*D. Acute glomerulonephritis* *E. Kidney infarction*

**22**. A hospitalized patient has glucosuria. His blood glucose level is 3.0 mmol/L. What is the most likely cause of glucosuria?

*A. Hypertensive disease* *B. Pellagra* *C. Kidney disease*
*D. Diabetes insipidus* *E. Myxedema*

**23**. У больного, который жалуется на полиурию и полидипсию, обнаружен сахар в моче. Содержание сахара в плазме крови нормальное. С чем связан механизм глюкозурии у больного?

Sugar is found in the urine of the patient who complains of polyuria and polydipsia. The sugar content in the blood plasma is normal. What is the mechanism of glucosuria in a patient?

*A. Insufficient production of insulin by the pancreas*
*B. Insulin resistance of cell receptors*
*C. Abnormal filtration of glucose in the glomerular part of the nephron*
*D. Impaired glucose reabsorption in the tubules of nephrons*
*E. Hyperproduction of glucocorticoids by the adrenal glands*

**24**. A newborn child was diagnosed with phenylketonuria in the maternity hospital. Which metabolite should be determined in the urine to confirm the diagnosis?

*A. Phenylpyruvate* *B. Fumaryl acetoacetate*
*C. Hydroxyphenylpyruvate* *D. Homogentisic acid* *E. Fumarate*

**25**. A patient was diagnosed with glucosuria. The blood glucose content is normal. Which process can be affected?

*A. Glycogen breakdown in kidneys* *B. Functions of the pancreas*
*C. Gluconeogenesis* *D. Glycolysis* *E. Functions of renal tubules*

**26**. Amylase activity is increased and trypsin is present in the patient's urine. Amylase activity is also increased in the blood. Which organ pathology can be suspected?

*A. Pancreas* *B. Liver* *C. Stomach* *D. Kidney* *E. Intestine*

**27**. A patient visited a doctor with complaints of shortness of breath, which occurs after exercise. Clinical examination revealed anemia and paraproteins in the gamma globulin zone. Which parameter in the urine should be determined to confirm myeloma?

*A. Ceruloplasmin* *B. Antitrypsin* *C. Bence-Jones protein*
*D. Hemoglobin* *E. Bilirubin*

## *PRACTICAL WORK*

### Determination of pathological components of urine

**Task 1.** Carry out qualitative reactions for protein in urine.

***Procedure.*** A) *Heat coagulation test:* Check pH of urine by litmus. If the urine is acidic, it (2-3 mL) is heated in a test tube at once; if the urine is alkaline, it is firstly acidified by adding dropwise a 1% solution of acetic acid and its pH is checked by litmus paper. A white turbility indicates the presence of proteins or phosphates. Add few drops of 10% acetic acid. If precipitate is not dissolved, it is due to proteins. B) *Nitric acid test (Heller's test):* Add 1 ml of concentrated nitric acid to a clean dry test tube. Make layers (urine above the acid). Appearance of white ring at the place of their interface indicates the presence of proteins. C) *Sulphosalicylic acid test*: Pour 1-2 ml of urine into a test tube. Add 2-3 drops of fresh 20% solution of sulphosalicylic acid to it. Appearance of white precipitate indicates the presence of proteins in urine.

**Task 2**. Carry out quantitative determination of urinary protein by Brandberg-Roberts-Stolnikov method.

***Principle***. The method is based on Heller's when protein-containing urine after contact with concentrated nitric acid forms a cloudy white ring of denaturated protein at the liquid-liquid interface. It has been experimentally established that solutions containing 0.033 g/L of protein give the characteristic ring within the time interval between the $2^{nd}$ and $3^{rd}$ minutes after the solution/nitric acid contact.

***Procedure***. Transfer 20 drops of concentrated nitric acid to a test tube and add cautiously, using a pipette, some urine to form an upper liquid layer to carry out Heller's test for normal and pathological urine. If the tested urine contains protein, the appearance of a cloudy ring at the liquid-liquid interface is noted within 2-4 minutes. Use the urine sample that gives a positive Heller's test for the determination of urinary protein. Pour 2 ml of distilled water into each of five test tubes to prepare dilute urine samples. Transfer 2 ml of urine to the first test tube, mix by shaking, take 2 ml of the

mixture and add it to the second test tube, and repeat the dilution operation once again; proceed until all the five test tubes are used. Discard 2 ml of diluted solution from the fifth test tube. Thus, equal volumes of diluted 2-, 4-, 8-, 16-, and 32-fold urine samples are obtained. Add 2 ml of concentrated nitric acid to five clean test tubes and cautiously, using a pipette, add 2 ml from the respective five test tubes of diluted urine to form an upper liquid layer. Note the maximally diluted urine sample in which a cloudy ring is developed within the time interval between the second and the third minutes. Multiply the urine dilution ratio value to a concentration factor 0.033 g/L to estimate the urinary protein concentration. For example, presume that denaturated protein ring is formed in the fourth test tube with the dilution ratio 16. It means that the protein concentration in the tested urine sample is 0.033x16=0.548 g/L.

***Clinical and diagnostic significance.*** *Proteinuria is observed in organic lesion of the nephron; in increased size of the pores of the glomerular filter; reduced blood flow rate in the glomeruli; in lesioned urinary tracts, in affected prostate gland. Bence Jones proteins are found in urine in multiple myeloma.*

***Task 3****. Determine glucose in urine (see module 3, class 4 "Self-study guide for students of general medicine faculty in biochemistry", part 1).*

***Clinical and diagnostic significance.*** *Very small amount of glucose is excreted in urine under normal conditions (not more than 0.4 g/day), which is not detected by the reagents used for its determination. Considerable excretion of glucose with urine is observed in disorders of hormonal regulation of carbohydrate metabolism, in pancreatic diseases and in disturbances of reabsorption capacity of kidneys.*

**Task 4**. Carry out qualitative reactions for ketone bodies (see module 3, class 9 "Self-study guide for students of general medicine faculty in biochemistry", part 1).

***Clinical and diagnostic significance.*** *Very small amounts of ketone bodies are excreted in urine normally (not more than 0.01 g/day), which are not detected by qualitative reactions. These reactions are positive in diabetes mellitus, starvation, and carbohydrate free diet. Ketonutia may be observed in thyrotoxicosis, subarachnoidal hemorrhage; craniocerebral injury; infectious diseases (scarlet fever, influenza, tuberculosis, meningitis). In infancy acute gastrointestinal diseases (dysentery, enterocolitis), prolonged hyperthermia are accompanied by ketonemia and ketonuria.*

**Task 5**. Carry out qualitative reactions for blood pigments in urine.

***Procedure****.* A) *Sample boiling with alkalis:* Pour 4-5 ml of unfiltrated urine into the test tube. Add 5-6 drops of alkalis solution and boil a content of the test tube. A precipitate of phosphates is formed. The precipitate is darker than urine in the presence of blood pigment. The precipitate of phosphates is lighter than urine in the absence of blood pigment. B) *Benzidine test:* It is based on the peroxidase activity of hemoglobin, which decomposes hydrogen peroxide and released oxygen oxidizes benzidine to give a blue solution. Transfer 5 drops of normal urine to the test tube and 5 ml of pathological urine to another test tube; add 3 drops of benzidine reagent and 3 drops of hydrogen peroxide solution to both test tubes. The occurrence of blood pigments in the sample tested manifests by the appearance of green-blue coloration.

***Clinical and diagnostic significance.*** *The appearance of blood in urine is called hematuria; the presence of blood pigments is called hemoglobinuria. Hematuria occurs in inflammation or traumatism of the urinary tract, renal lithiasis, on-*

*cological diseases of urinary bladder, cystitis, acute nephritis. Hemoglobinuria is mainly observed in hemolytic substance poisoning and in diseases associated with erythrocytes hemolysis.*

**Task 6.** Carry out qualitative reactions for bile pigments in urine.

***Procedure***. *A) Gmelin's test*: Place 2-3 ml of concentrated nitric acid to the test tube and cautiously, using a pipette, add 1-2 ml of urine. The appearance ofgreen ring at the liquid-liquid interface indicates the presence of bilirubin. B*) Rosin's test*: The metod is based on the property of iodine to convert bilirubin into green-colored biliverdin. Transfer 2-3 ml of urine to a test tube. Using pipette, add cautiously some iodine solution to form an upper liquid layer. The presence of bilirubin in the tested sample manifests by the appearance of a green ring at the liquid-liquid interface.

***Clinical and diagnostic significance.*** *Bile pigments are formed from hemoglobin in degradation of erythrocytes. Products of bilirubin reduction are mainly excreted with feces as stercobilinogen, which is oxidized to stercobilin. Part of stercobilinogen is absorbed to blood hemorrhoidal veins and excreted with urine. The appearance of the other product of bilirubin reduction called urobilinogen in urine is very important index of insufficiency of liver function. Urobilinogen content in urine can achieve 2 g/day in disorder of liver function and some infectious diseases. Bile pigments (biliverdin, bilirubin, etc) appear in urine as alkaline salts in jaundices.*

**Task 7.** Carry out qualitative reaction for indican in urine.

***Principle***. Concentrated chloric acid hydrolyzes indican to indoxyl, which is oxidized into indigo blue in the presence of $FeCl_3$.

***Procedure***. Transfer 3-5 ml of urine to the test tube. Add 1-2 ml 20% of plumbous acetate to precipitate substances, which prevent the determination of indigo. Filtrate. Add the equal volume of Obermayer's reagent (solution of $FeCl_3$ in chloric acid) to 2-3 ml of filtrate. The solutions are allowed standing to develop brown coloration. Add 1-2 ml of chloroform and shake strongly. Indigo is dissolved in chloform and gives blue-violet coloration.

***Clinical diagnostic significance.*** *Normal urine contains very small amount of indican (0.01-0.04 g/day). A content of indican increases in some pathological processes (constipation, intestinal obstruction, tuberculosis, peritonitis) and meat-rich diet.*

**Task 8**. Determine pathological components in urine by means of combined test-strips (pH, protein, glucose, ketone bodies, urobilinogen and blood).

***Procedure***. Mix thoroughly urine. Dip the whole test-strip in urine for 1 sec. Remove the excess of urine touching to wall of test tube. After 30-60 sec compare the color developed to standard scale. Change of color, which appears only on the border of indicator zone or only within 2 min, does not have diagnostic significance. Urine does not have to stand more than 4 h at room temperature before analysis.

*Protein*. The change in test-strip color from yellow to green is observed in the presence of protein (0.3; 1.0; 5.0 g/L); pathological proteinuria is observed if protein level is higher than 0.25 g/L. *Glucose*. Positive reaction from orange to brown color is noted after 60 sec (5.55; 16.65; 55.5 mmol/L). Coloration appears even in small amount of glucose (2.2 mmol/L). *Ketone bodies*. Positive reaction from pink to violet color (+, ++, +++). A sensitivity of a test-strip to acetoacetate is higher than to acetone. A test-strip does not react on β-hydroxybutyrate. Ranges: 100 mg/L for acetoace-

tate and more than 400 mg/L for acetone. *Urobilinogen*. Positive reaction from pink to red color (range of revealence is 4 mg/L). *Blood*. A separate scale is given to erythrocytes and hemoglobin. Intact erythrocytes are found as single or multiple green points against yellow background (5-10, 50, 250 erythrocytes/μL). Green coloration indicates the presence of free hemoglobin or hemolyzed erythrocytes, or myoglobin.

Wrong results are usually obtained after intake of large amount of vitamin C and some drugs.

***Presentation of results***. Write down the experimental results in the form of a table.

| Patological components of urine | Possible causes of appearance | Qualitative reaction | Observed effects |
|---|---|---|---|

**TOPIC 9 (2 houts): Biochemistry of the liver. Biotransformation of xenobiotics and endogenous toxins.**

**IMPORTANCE.** Liver plays the central role in regulation and integration of metabolism. Features of the enzymatic composition of the liver and the anatomic relationships with other organs enable it to participate in the regulation of virtually all metabolic pathways and provide the dynamic constancy of many vital component contents in the body. On the one hand, biochemical processes in the liver focus on the formation of different substances for other organs and, on the other hand, on the protection of these organs from the endo- and exogenous toxic substances. Liver damage leads to the disintegration of metabolism in the body and reducing of its adaptive properties.

**AIM.** Familiarize yourself with the peculiarities of the chemical composition of the liver, the major biochemical functions and their disorders in pathological conditions; role in the metabolism of carbohydrates, lipids, proteins, pigments, processes of xenobiotics biotransformation, microsomal oxidation. Familiarize yourself with the thymol turbidity test and its clinical and diagnostic significance.

***THEORETICAL QUESTIONS***

1. Biochemical functions of liver in the body.
**2*.** Role of liver in carbohydrate metabolism.
**3*.** Role of liver in lipid metabolism.
**4*.** Role of liver in protein metabolism.
**5*.** Role of liver in pigment metabolism.
6. Bile formation in the liver. Chemical composition of bile.
7. Detoxification functions of liver. Types of reactions of xenobiotics and endogenous toxins biotransformation.
8. Systems of conjugation for detoxification of toxic substances in the liver.
9. Disorders of liver functions in diseases. Liver tests.
10. Xenobiotics: concept, principle of classification, pathways of entering the body, transport through cellular membranes.
11. Microsomal oxidation. Characteristic of microsomal monooxygenase chains.
12. Cytochromes P-450 and $b_5$: features of structure, mechanism of action in hy-

droxylation reactions. Genetic polymorphism and regulation of cytochrome P-450 synthesis (inducers and inhibitors).

**13*.** Types of conjugation of xenobiotics in hepatocytes: biochemical mechanisms, functional significance.

**14*.** Pathways of removal of xenobiotic biotransformation products from organism.

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
|---|---|
| *1. Role of liver in carbohydrate metabolism.* | *1.1. The main role of the liver in carbohydrate metabolism is the maintenance of blood glucose concentration at a constant level of 3.3-6.1 mmol/L.*<br>*1.2. The glycogen-synthesizing function implies storage of glycogen (glucose polymer).*<br>*1.3. Gluconeogenesis (activated when glycogen storage is depleted).*<br>*1.4. Glucose breakdown (first of all, it provides metabolites for the biosynthesis of fatty acids, etc.).*<br>*1.5. Pentose phosphate pathway of glucose oxidation (formation of NADPH for the reductive synthesis of fatty acids, cholesterol and other steroids, as well as the formation of ribose-5-phosphate that is necessary for the synthesis of nucleic acids).*<br>*1.6. Metabolism of other monosaccharides (enzymatic systems that metabolize D-fructose, D-galactose and D-mannose into phosphorylated glucose and glycolysis intermediates).* |
| *2. Role of liver in lipid metabolism.* | *2.1. The liver is involved in virtually all stages of lipid metabolism, beginning with the digestion of lipids and ending with specific metabolic pathways of individual lipid fractions:*<br>*- synthesis of bile acids and the formation of bile;*<br>*- β-oxidation of fatty acids;*<br>*- biosynthesis of fatty acids;*<br>*- synthesis and secretion of triacylglycerols;*<br>*- formation of ketone bodies;*<br>*- synthesis and breakdown of phospholipids;*<br>*- cholesterol synthesis and formation of its esters;*<br>*- synthesis of transport forms of lipids - VLDL, HDL;*<br>*- hydroxylation of vitamin D on the 25th position;*<br>*- glycerol metabolism.* |
| *3. Role of liver in protein metabolism.* | *3.1. The protein-synthetizing function: synthesis of most blood plasma proteins (albumins, about 80% of globulins); synthesis of protein blood coagulation factors, components of anticoagulant and fibrinolytic blood systems.*<br>*3.2. Formation of urea.* |

| | |
|---|---|
| | *3.3. Synthesis of choline.*<br>*3.4. Synthesis of creatine.*<br>*3.5. Transamination, deamination, decarboxylation of amino acids.*<br>*3.6. Maintenance of constant blood amino acid composition.* |
| *4. Role of liver in pigment metabolism.* | *4.1. The pigment function: catabolism of hemoglobin and other heme-containing proteins (formation of pigments - bilirubin, biliverdin).* |
| *5. Types of xenobiotics conjugation in hepatocytes: biochemical mechanisms, functional significance.* | *5.1. Conjugation is an addition of other molecules or groups that increase hydrophilicity and reduce toxicity to the functional groups formed in the first phase or already existing in xenobiotics.*<br><br>*5.2. Conjugation can occur with glucuronate, glycine, sulfate, acetate, methyl group, and glutathione.*<br>*5.3. Transferases participate in this process. They attach different conjugates to hydrophilic groups of toxic substances. The resulting product, as a rule, is readily soluble and can be easily excreted from the body with bile and urine.* |
| *6. Ways of excretion of xenobiotics biotransformation products.* | *6.1. Renal excretion (exretion of volatile organic xenobiotics with urine is insignificant ).*<br>*6.2. Excretion through the lungs (volatile substances and volatile metabolites of non-volatile substances are mainly excreted; the main mechanism of excretion is the diffusion of the toxicant circulating in the blood through the alveolar-capillary barrier; the transfer of the volatile substance from the blood to the air of the alveoli is determined by the gradient concentration or partial pressure).*<br>*6.3. Excretion through the gastrointestinal tract (the substance or its metabolites are excreted in feces as a result of incomplete absorption in the gastrointestinal tract; by biliary excretion without subsequent reabsorption in the intestine or as a result of the discharge of the gastrointestinal mucosa; such processes can occur both separately and in a complex).*<br>*6.4. Excretion by the liver (as for xenobiotics that enter the bloodstream, the liver acts both as the main organ of their metabolism and as the organ of excretion; xenobiotics are released in two stages:* |

| | |
|---|---|
| | *hepatocytes take them up and excrete into the bile; both stages can occur in the form of simple diffusion or active transport).* |

### *TESTS FOR SELF-CONTROL*

**1.** Detoxification of natural metabolites and xenobiotics is affected in the patient's liver. Which chromoprotein deficiency can cause this?

*A. Cytochrome b* *B. Hemoglobin* *C. Cytochrome oxidase*
*D. Cytochrome P450* *E. Cytochrome $c_1$*

**2.** Fatty liver is found in a patient. Which substance synthesis is affected?

*A. Cholic acid* *B. Urea* *C. Phosphatidic acid*
*D. Tristearylglycerols* *E. Phosphatidylcholines*

**3.** One of the liver functions is the synthesis and secretion of bile, which contains bile acids, bile pigments, cholesterol, final products of metabolism. Select a substance that belongs to bile pigments:

*A. Stercobilin* *B. Melatonin* *C. Hemoglobin*
*D. Bilirubin* *E. Myoglobin*

**4.** A male is hospitalized to reanimation with hepatic coma. Arginine is involved in the complex of intensive therapy because it activates:

*A. Urea synthesis* *B. Glucose synthesis* *C. Protein synthesis*
*D. Skatole detoxification* *E. Cholesterol synthesis*

**5.** Microsomal oxidation is a reaction of:

*A. Oxygen using for energetic purpose* *B. Oxygen using for plastic purpose*
*C. Breakdown of fatty acids in the organism*
*D. Amino acid breakdown* *E. Aerobic breakdown of carbohydrates*

**6.** Liver cirrhosis is observed in a lean woman. Hypoalbuminemia, hyperglobulinemia are revealed in laboratory analysis; edema of hands, feet and eyelids is present. The most likely reason is a disorder of:

*A. Acid-base balance* *B. Detoxification function of liver*
*C. Glycogen synthesis in the liver* *D. Albumin synthesis in the liver*
*E. Lipoprotein synthesis in the liver*

**7.** Liver secretes synthesized fats into the bloodstream in the form of:

*A. VLDL* *B. LDL* *C. HDL*
*D. Chylomicrones* *E. Freely*

**8.** The damage of liver (hepatitis, cirrhosis, and tumor) leads to all disorders mentioned below, except:

*A. Hypoalbuminemia* *B. Hemorrhages* *C. Hyperaminoacidemia*
*D. Azotemia* *E. Hyper α-globulinemia*

**9.** Which compound takes part in the bilirubin conjugation in hepatocytes?

*A. Glucose* *B. Fructose* *C. Gluconic acid*
*D. Glucuronic acid* *E. Glycine*

**10.** The liver is the central regulator of blood glucose levelsdue to the presence of one of the following enzymes in hepatocytes:

*A. Glycogen synthetase* *B. Glycogen phosphorylase* *C. Hexokinase*

*D. Glucose-6-phosphatase* *E. Phosphofructokinase*

**11.** Reactions of biotransformation of xenobiotics and endogenous toxins occur in hepatocytes either by oxidoreductive and hydrolytic conversions or by conjugation. During the conjugation, a compound, which should be detoxified, cannot bind the moiety of:

*A. Gluconic acid* *B. Glucuronic acid* *C. Sulfuric acid*
*D. Glycine* *E. Glutathione*

**12**. A patient has liver cirrhosis. Which substance is excreted in the urine and can characterize the state of liver detoxification?

*A. Ammonium salts* *B. Creatinine* *C. Hippuric acid*
*D. Uric acid* *E. Amino acids*

**13**. Detoxification of xenobiotics (drugs, epoxides, aldehydes, nitroso derivatives) and endogenous metabolites occurs in the liver by their conjugation with:

*A. Glutathione* *B. Aspartic acid* *C. Glycine*
*D. S-Adenosylmethionine* *E. Phosphadenosine*

**14.** The detoxifying function of the liver was assessed in a patient with chronic hepatitis by the sodium benzoate load. Which acid is excreted in the urine and characterizes the detoxifying function of the liver?

*A. Phenylacetic acid* *B. Valeric acid* *C. Oxalic acid*
*D. Glutamic acid* *E. Hippuric acid*

**15**. Toxic damage to the liver cells with the impairment of their functions caused the development of edema in a patient. Which changes in the composition of blood plasma are the main cause of edema development?

*A. An increase in the content of globulins* *B. Reduction of albumins*
*C. Reduction of fibrinogen* *D. Reduction in the content of globulins*
*E. An increase in albumins*

**16**. A male with liver damage underwent the Quick's test, a low urinary level of one of the following substances was observed:

*A. Hydroxyproline* *B. Sodium benzoate* *C. Creatinine*
*D. Hippuric acid* *E. Amino acids*

**17**. After administration of adrenalin blood tests did not change significantly in a patient with persistent hypoglycemia. The doctor suspected a liver disorder. Which liver function is impaired?

*A. Ketogenic* *B. Glycolytic* *C. Cholesterol-forming*
*D. Glycogen-producing* *E. Excretory*

**18**. A male has impaired urea synthesis. Which organ is affected?

*A. Kidney* *B. Liver* *C. Brain* *D. Muscle* *E. Lung*

**19**. Detoxification of xenobiotics and active endogenous metabolites often occurs due to the incorporation of oxygen in the substrate molecule. Select the process involved in the detoxification:

*A. Hydroxylation* *B. Decarboxylation*
*C. Reamination* *D. Phosphorylation* *E. Deamination*

**20**. A patient with hepatitis received choline to prevent liver damage. Its therapeutic effect is associated with:

*A. Activation of glycogen phosphorylase B. Inhibition of cholesterol synthesis*
*C. Lipotropic action, prevention of fatty liver*
*D. Activation of glycogen synthase E. Inhibition of ketogenesis*

**21**. There is a phrase "to swell from hunger". Which function of the liver can lead to the appearance of edema in starvation?

*A. Cholesterol-producing B. Bile-producing*
*C. Glycogen storage D. Detoxification E. protein-synthesizing*

**22.** A patient with alcoholic liver damage has abnormalities in biotransformation of xenobiotics and endogenous toxic compounds. Which chromoprotein activity is low?

*A. Cytochrome P-450 B. Cytochrome oxidase C. Cytochrome c1*
*D. Hemoglobin E. Cytochrome b*

**23**. Hepatic insufficiency developed in a patient after hepatitis. Which abnormal liver function triggers the mechanism of edema formation in this case?

*A. Antitoxic B. Barrier C. Bile-forming*
*D. Protein-forming E. Glycogen-forming*

**24.** A patient was prescribed with methionine-containing drug to maintain liver functions. This promotes synthesis of:

*A. Phosphatidylcholine B. Phosphatidylserine C. Citrate*
*D. Lactate E. Pyruvate*

**25.** To determine the antitoxic liver function, a patient was prescribed with sodium benzoate, which is converted into hippuric acid in the liver. Which compound is used for this process?

*A. Cysteine B. Methionine C. PAPS*
*D. UDP-glucuronic acid E. Glycine*

**26.** A female patient with chronic hepatitis complains of an increased sensitivity to barbiturates, which she used to consume without symptoms of intoxication. Which liver function disorder may cause such changes?

*A. Bile formation B. Metabolic C. Hemodynamic*
*D. Hematopoietic E. Phagocytic*

**27**. A patient has low blood serum indican levels, as well as its reduced daily urinary excretion. Which organ failure is observed?

*A. Liver B. Kidney C. Heart D. Lung E. Pancreas*

**28.** While studying the biotransformation of food additive, it was found that the detoxification of this xenobiotic occured only in one phase - microsomal oxidation. Select a component of this phase.

*A. Cytochrome aa3 B. Cytochrome b C. Cytochrome c*
*B. Cytochrome P-450 E. Cytochrome oxidase*

**29.** The plasma albumin content and the oncotic plasma pressure decreased sharply in a patient with toxic damage to hepatocytes with impaired protein-synthesizing function. What will be the result of such changes?

*A. Edema B. A decrease in diuresis C. A decrease in ESR*
*D. An increase in the volume of circulating blood*
*E. Increase in blood viscosity*

**30**. A patient with food poisoning was prescribed with a drip of 10% glucose solution. Glucose not only provides the energy needs of the organism but also performs a

detoxifying function due to the formation of the metabolite that takes part in the conjugation reaction:

*A. Sulfation* *B. Methylation* *C. Glucuronation*
*D. Glycosylation* *E. Hydroxylation*

**31**. A boy was diagnosed with acute viral hepatitis. Which blood parameter should be measured to confirm the acute damage to hepatic cells?

*A. Conjugated and unconjugated bilirubin*
*B. Erythrocyte sedimentation rate(ESR)* *C. Cholesterol content*
*D. Aminotransferase activity (ALT and ASAT)* *E. Protein fractions*

**32.** A male patient with chronic hepatitis often has bleeding from the nose and gums, hemorrhagic rashes appear on the skin and mucous membranes. These symptoms are a consequence of:

*A. Increased blood levels of aminotransferases*
*B. Reduction of serum albumins*
*C. Increased blood levels of macroglobulins and cryoglobulins*
*D. Low activity of blood cholinesterase*
*E. Reduced synthesis of prothrombin and fibrinogen*

## *PRACTICAL WORK*

## Thymol turbidity test

**Task. Determine the index of thymol turbidity test in the serum.**

**Principle.** β-Globulins and lipoproteins are precipitated from serum at pH 7.55 by a buffer solution with a high content of thymol. Measure the intensity of turbidity depending on the content of protein fractions and their quantitative ratio.

***Procedure.*** Add 0.05 ml of non-hemolyzed blood serum to 3 ml of thymol reagent. Mix the content of the test tube and allow standing for 30 min at room temperature. Then measure the absorbance against a control sample (3 ml of thymol reagent) on a photocolorimeter at 660 nm (red light filter) in 10 mm cuvettes. The calculation is performed according to the calibration curve.

***Clinical and diagnostic significance.*** *Normally, the value of thymol tubidity test for healthy people is 0-4 units. Increased values are an important confirmation of inflammatory lesions of the liver. The sample is positive in 90-100% of cases of toxic, infectious (viral) hepatitis, as well as in patients who suffer from collagen diseases, malaria, and viral infections. In obstructive jaundice, this test is negative in approximately 75% of cases. This underlies the use of the test for differential diagnosis of jaundices.*

**1**.** Prepare a presentation: "Methods for determining the detoxification function of the liver."

**2 **.** Prepare a report: "Changes in parametes of the urine in diabetes mellitus."

## *CLASS 6 (4 hours)*

**TOPIC 10 (2 hours): Biochemistry of nervous, muscular and connective tissues.**

**IMPORTANCE.** In humans, the coordinated course of all the biological processes is defined by regulatory systems. The central chain of regulation in organism is the nervous system. The knowledge of features of chemical composition, metabolism, functioning of nervous tissue gives the possibility to understand the processes which underlie of its role in organism. The study of muscular tissue biochemistry is important to understand molecular mechanisms of its functioning in normal conditions, in different diseases, to choose the effective training methods of athletes whose profession needs a high physical preparation. The importance of the study of connective tissue biochemistry is defined by its distribution in organism and by important role in providing the functioning of other tissues.

**AIM.** Familiarize yourself with chemical composition of nervous tissue; features of metabolic processes; biochemical bases of neurons functioning; mechanisms of neurochemical transmission of nervous impulse; biochemical bases of nervous system pathology. Learn organelles of muscular cells; biochemical characteristic of muscular tissues; features of chemical composition; proteins of sarcoplasm, myofibrils, stroma; features of enzymatic composition; mechanisms of muscular contraction; features of metabolism of smooth and cardiac muscles; diagnosis of lesions of muscular tissues using biochemical parameters of blood and urine. Familiarize yourself with biochemical characteristic of connective tissue components; features of amino acid composition and physico-chemical properties of major structural proteins; collagen biosynthesis; proteo- and glycosaminoglycans of extracellular matrix; features of metabolism and its regulation; aging-relatedchanges, disorders in collagenoses, mucopolysaccharidoses.

### *THEORETICAL QUESTIONS*

1. Major classes of substances in the nervous tissue, their ratio in different regions of the nervous system.
2. Lipids of nervous tissue: classes, representatives, role. Diseases develop due to disorders of lipid metabolism in nervous tissue (Tay-Sach's, Gaushe's, Niemann-Pick's diseases).
3. Myelin: chemical composition, properties, role.
4. Proteins of nervous tissue: classification, composition, properties. Specific proteins of nervous tissue.
5. Macroergic compounds of nervous tissue. Features of energy metabolism.

**6*.** Amino acids and peptides of nervous tissue (neuropeptides): characteristic, role.

**7*.** Features of metabolism in the nervous system.

**8*.** Neurochemical impulse transmission. Synapses. Neurotransmitters. Synaptic receptors.

9. Features of structure in muscular fibers, thick and thin filaments.

10. Chemical composition of muscular tissue. Features of chemical composition and metabolism of substances in cardiac and smooth muscles.

11. Characteristics of muscle proteins, basic non-protein nitrogen-containing substances.

**12*.** Bioenergetics of muscular tissue; sources of ATP; role of creatine phosphate in providing muscular contraction with energy.

**13*.** Changes in muscles in muscular dystrophy, hypodynamia, avitaminosis E.

14. Features of connective tissue structure.

15. Fibronectin: features of structure and role.

16. Elastin: features of amino acid composition and physico-chemical properties.

17. Collagen: structure, role, biosynthesis.

**18*.** Major classes of proteo- and glycosaminoglycans, structure, role.

19. Features of metabolism regulation in connective tissue.

20. Changes in connective tissue in aging.

21. Connective tissue diseases. Molecular pathology of connective tissue (Marfan's syndrome, Menke's disease, Ehlers–Danlos syndrome).

***Recommendations for self-study of theoretical questions***

| *Question* | *Information* |
|---|---|
| *1. Amino acids, peptides and proteins of the nervous tissue: characteristics, role.* | *1.1 Up to 75% of the total number of amino acids in the nervous tissue are aspartate, glutamate, as well as the products of their transformations or substances synthesized with their participation (glutamine, acetyl derivatives, glutathione, GABA, etc.). The role of glutamate in the nervous tissue includes: energy (associated with reactions with intermediate metabolites of the tricarboxylic acid cycle); Participation (together with aspartate) in deamination reactions of other amino acids and the temporary ammonia detoxification; the precursor of the GABA neurotransmitter; participation in glutathione synthesis. Some amino acids (glycine, aspartate, glutamate) act as neurotransmitters in neurons (stored in synapses and released when a nervous impulse arrives).*<br>*1.2. Neuropeptides serve as mediators, hormones, form behavior (food, emotional, sexual, as well as learning, attention, memory, reaction to pain, sleep, etc.). The main representatives are: neurohypophyseal hormones (vasopressin, liberins, statins), opiate-like peptides (endorphins, enkephalins). They are formed by reactions of limited proteolysis of the precursor called proopiomelanocortin. They interact with the same receptors as opiates (for example, morphine),* |

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| | *thereby imitating their effect, causing anesthesia, developing euphoria with improved mood. They are easily destroyed by proteinases, so their pharmacological effect is insignificant.*<br>*1.3. Proteins of the nervous tissue:*<br>*- Simple: neuroalbumins (they make up 90% of all soluble proteins of the nervous tissue, form complexes with lipids, nucleic acids, carbohydrates, they are the main components of phosphoproteins, virtually they do not occur in the free state); Neuro-globulins (they make up about 5% of all soluble proteins); histones (main representatives of cationic proteins); neuroscleroproteins (neurocollagens, neuroelastins, neurostromins) are structural and supporting proteins, which account for 8-10% of the number of simple proteins in the nervous tissue;*<br>*- complex: nucleoproteins, lipoproteins, proteolipids, phosphoproteins, glycoproteins;*<br>*- specific: for example, protein S100 that belongs to a family of acidic low-molecular-weight proteins with a high content of glutamate and aspartate residues; they regulate phosphorylation of other proteins and their enzymatic activity, intracellular and axonal transport, division and transcription, proliferation and apoptosis, cellular metabolism, etc.; Proteins of synaptic vesicle membranes participate in binding to the surface of synaptic vesicles of cytoskeleton components, regulate the release of the neurotransmitters from the vesicles into the synaptic membrane.* |
| *2. Features of metabolism in the nervous tissue.* | *1. Consumption of a significant amount of oxygen, used in the aerobic glucose oxidation.*<br>*2. High intensity of cellular respiration (active aerobic processes).*<br>*3. The predominance of glucose use over other substrates.*<br>*!! Glucose is practically the only source of energy for brain cells (only in prolonged starvation additional sources of energy are used, namely ketone bodies).*<br>*4. Up to 70% of ATP is used to maintain ionic gradients.*<br>*5. High speed of gas exchange (20-25% of general needs).* |

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| | *6. High intensity of oxidative deamination, amino acid transamination.*<br>*7. Ammonia detoxification by its interaction with glutamate and glutamine formation.*<br>*8. Biosynthesis and breakdown of glutamate in the nervous tissue are considered as a by-product of the cytric acid cycle (GABA shuttle).* |
| *3. Neurochemical conductance. Synapses. Neurotransmitters. Synaptic receptors.* | *3.1. Synapses are morphofunctional formations in which a chemical signal is transmitted from the presynaptic to the postsynaptic cell by releasing neurotransmitters (acetylcholine, norepinephrine, etc.); Mechanism: excitation of the neuron with "quanta" by exocytosis of synaptic vesicles → an increase in calcium ion concentration in the nerve endings → penetration of calcium ions into cells via calcium channels → the release of neurotransmitters through the presynaptic membrane.*<br>*3.2. Neurotransmitters are biological molecules that facilitate the conduction of impulses in the nervous system from one neuron to the other or from the neuron to the effector organ (acetylcholine, biogenic amines - noradrenaline, dopamine, serotonin, amino acids and their derivatives - GABA, glycine, glutamate, aspartate, peptides - endorphins, enkephalins, etc.*<br><br>*3.3.Receptors of neurotransmitters are membrane-bound proteins (glycoproteins): they are localized in postsynaptic membranes of neurons, plasma membranes of effector cells; they are capable of binding effectors (neurotransmitters, various physiologically active compounds, psychotropic compounds); there are ionotropic and metabotropic receptors.* |
| *4. Bioenergetic processes in muscles; sources of ATP, role of creatine phosphate in energy supply of muscular contraction.* | *4.1. Skeletal muscles that work with maximal activity require ATP: conversion of ATP energy to mechanical. Sources of ATP in muscles: glycogenolysis, glucose oxidation, oxidative phosphorylation in sarcosomes (red muscles), adenylate kinase reaction of ATP formation, creatine phosphokinase reaction - generation of ATP from creatine phosphate.*<br>*4.2. The role of creatine phosphate is the fastest mechanism for ATP formation when it is necessary to urgently activate the process of muscular contraction (provision of intensive muscle work for 2-5 seconds).* |

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| *5. Changes in muscles in muscular dystrophy, hypodynamia, vitamin E deficiency.* | *5.1. Changes in muscles in muscular dystrophy, myopathies: a decrease in the content of myofibrillar proteins, an increase in the content of collagen and elastin, a decrease in myosin ATPase activity, low activity of glycolytic and other sarcoplasmic enzymes, an increase in the activity of lysosomal enzymes, a decrease in ATP concentration, changes in the phospholipid composition of membranes, reduction in the formation of creatine phosphate. Diagnosis: creatinuria, a decrease in urine creatinine, an increase in blood activity of CPK (MM-isoforms) and aminotransferases.*<br>*5.2. In hypovitaminosis E, hypodynamia - the loss of muscle mass, creatinuria, a decrease in the excretion of creatinine in the urine.* |
| *6. Basic classes of proteoglycans and glycosaminoglycans, structure and role.* | *6.1. Proteoglycans are high-molecular-weight compounds consisting of protein (5-10%) and glycosaminoglycans (GAG) (90-95%); proteins are represented by a polypeptide chain, GAG are linear negatively charged heteropolysaccharides, consisting of repeating "disaccharide units."*<br>*6.2. The role of proteoglycans:*<br>*- form the main substance of the intercellular matrix of connective tissue;*<br>*- provide intercellular interaction;*<br>*- ensure the formation and maintenance of the shape of cells and organs;*<br>*- provide the formation of a framework when forming tissues;*<br>*- specifically interact with collagen, elastin, fibronectin, laminin and other proteins of the intercellular matrix;*<br>*- as polyanions, they attach water, cations (sodium, potassium, calcium), form the turgor of various tissues;*<br>*- serve as a molecular sieve in the intercellular matrix;*<br>*- prevent the spread of pathogenic microorganisms;*<br>*- spring function in articular cartilage (hyaluronic acid;)*<br>*- acts as a filtration barrier in the kidneys (heparan-containing proteoglycans);*<br>*- Transparency of the cornea (keratan- and dermatan-sulfates);*<br>*- anticoagulants (heparin);* |

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| | *- components of cell plasma membrane, serve as receptors, participate in adhesion and intercellular interactions (heparan sulfate);*<br>*- components of synaptic vesicles.*<br>*6.2. Classes of GAG and the components of their disaccharide units: hyaluronic acid (D-glucuronate, N-acetyl-D-glucosamine), chondroitin-4-sulfate (D-glucuronate, N-acetyl-D-galactosamine-4-sulfate), chondroitin-6-sulfate (D-glucuronate, N-acetyl-D-galactosamine-6-sulfate), dermatan sulfate (L-iduronate, N-acetyl-D-galactosamine-4-sulfate), keratan sulfate (D-galactose, N-acetyl-D-galactosamine-6-sulfate), heparan sulfate (D-glucuronate-2-sulfate, N-acetyl-D-galactosamine-6-sulfate).* |

## *TESTS FOR SELF-CONTROL*

**1.** Liver glycogen is the source of glucose for different tissues, but muscular glycogen is used only by muscles. Which enzyme determines this fact?

*A. Glucose-6-phosphatase* *B. Phosphoglucomutase*
*C. Glycogen phosphorylase* *D. Amylo-1,6-glycosidase*
*E. Glycogen synthase*

**2.** Brain cells are characterized by the high ability to utilize glucose as a main source of energy. Which enzyme activity determines this feature?

*A. Phosphorylase* *B. Phosphofructokinase*
*C. Glucose-6-phosphate dehydrogenase*
*D. Hexokinase* *E. Glucose-6-phosphatase*

**3.** Some amino acids and their derivatives perform neurotransmitter functions in the CNS. Which of the aforementioned substances does not have neurotransmitter properties?

*A. Leucine* *B. Serotonin* *C. Noradrenaline*
*D. Dopamine* *E. Taurine*

**4.** Ammonia toxicity (especially for brain) occurs due to its capacity to affect Krebs cycle as a result of the removal of one of the following substances from the cycle:

*A. Malate* *B. Citrate* *C. α-Ketoglutarate*
*D. Succinate* *E. Fumarate*

**5.** GABA (γ-aminobutyric acid) that belongs to inhibitory neurotransmitters is a product of glutamate decarboxylation. Which vitamin prescription is used in convulsion states due to the decreased formation of GABA?

*A. Vitamin $B_9$* *B. Vitamin $B_6$* *C. Vitamin $B_1$*
*D. Vitamin $B_5$* *E. Vitamin $B_2$*

**6.** A female complains of Duchenne-type muscular dystrophy progression. Which enzyme activity changing is a diagnostic test in this case?

*A. Glutamate dehydrogenase* *B. Lactate dehydrogenase*
*C. Pyruvate dehydrogenase* *D. Creatine phosphokinase*
*E. Adenylyl kinase*

**7.** Psycho-pharmacologic drugs with antidepressive action inhibit oxidative deamination of noradrenaline and serotonin in mitochondria of brain by means of inhibition of:

*A. Monoamine oxidase* *B. Cytochrome oxidase*
*C. Pyruvate dehydrogenase* *D. Aldolase* *E. Succinate dehydrogenase*

**8.** Tabun, zarin, diisopropylfluorophosphate (phosphororganic substances) are poisons of neuro-paralytic action. Which of the enzymes mentioned below is inhibited by phosphororganic substances?

*A. Cytochrome P450* *B. Phospholipase $A_2$*
*C. Angiotensin-converting enzyme* *D. Tyrosine aminotransferase*
*E. Acetylcholine esterase*

**9.** Which myofibril protein performs both structural and enzymatic functions?

*A. Actin* *B. Myosin* *C. Tropomyosin*
*D. Troponin T* *E. Troponin C*

**10.** Which of the following substances belong to so-called "inhibitory amino acids"?

*A. Histidine, tyrosine* *B. Glutamate, glutathione* *C. Aspartate, asparagine*
*D. Proline, lysine* *E. GABA, glycine*

**11.** Some vitamin deficiency in the organism causes neuropathy and other neurological symptoms. Which process disturbance provides the damage of nervous system in vitamin $B_1$ deficiency?

*A. Synthesis of amino acids and nucleotides*
*B. Antioxidant defense* *C. Oxidative decarboxylation of α-ketoacids*
*D. Transamination and decarboxylation of amino acids*
*E. Dehydrogenation of tissue respiration substrates*

**12.** Different substances are used by skeletal muscles and myocardium as oxidation substrates to produce ATP. Which of them is used in myocardium, but is not used in skeletal muscles?

*A. Glycogen* *B. Glucose* *C. Lactate*
*D. Fatty acids* *E. Ketone bodies*

**13.** Disorders of myelinization process of nerve fibers lead to severe neurological abnormalities and mental retardation. This clinical picture is characteristic for inherited metabolic disorders of:

*A. Neutral fats* *B. Cholesterol* *C. Sphingolipids*
*D. Glycerophospholipids* *E. Lipoproteins*

**14.** A specific diagnostic sign of muscular dystrophy is an increased excretion of one of the following substances in urine:

*A. Creatinine* *B. Creatine* *C. Proteins*
*D. Indican* *E. Bilirubin*

**15.** A patient suffers from collagenosis. Which metabolite amount is probably increased in the urine?

*A. Indican* *B. Hydroxyproline* *C. Creatinine*
*D. Urea* *E. Urobilinogen*

**16**. Exophthalmus is observed in hyperthyroidism and is caused by the accumulation of substances that have the high water binding capacity in the retrobulbar tissue. What are these substances?

*A. Cholesterol* *B. ATP* *C. Glycosaminoglycans*
*D. Creatine* *E. Phospholipids*

**17**. It has been known monoamine oxidase plays a special role in the metabolism of catecholamines. This enzyme inactivates neurotransmitters (noradrenaline, epinephrine, dopamine) by:

*A. Addition of amino group* *B. Removal of methyl group*
*C. Carboxylation* *D. Hydrolysis* *E. Oxidative deamination*

**18.** The patient's examination revealed clinical manifistations of collagenosis. Select a parameter whose concentration increases in urine in this case.

*A. Ammonium salts* *B. Hydroxyproline* *C. Glucose*
*D. Mineral salts* *E. Arginine*

**19.** Which of the factors mentioned below will cause the greatest vasodilation of the microvasculature and increase the vascular permeability?

*A. Vasopressin* *B. Serotonin* *C. Histamine*
*D. Endothelin* *E. Norepinephrine*

**20.** The long-term course of hydrocortisone treatment was administered to a patient with rheumatoid arthritis. Hyperglycemia, polyuria, glucosuria, thirst developed as a result. Such complications were a consequence of the activation of:

*A. Gluconeogenesis* *B. Glycogenolysis* *C. Glycogenesis*
*D. Glycolysis* *E. Lipolysis*

**21**. Monoamine oxidase inhibitors are widely used as psychopharmacological drugs. In synapses they change the content of the neurotransmitter:

*A. L-glutamate* *B. Substance P* *C. ATP*
*D. Noradrenaline* *E. Acetylcholine*

**22**. Due to a decrease in the activity of an enzyme, the number of transverse bonds in collagen fibers decreases and their strength is impaired. Select this enzyme:

*A. Peroxidases* *B. Hydroxylases* *C. Lysyl oxidases*
*D. Catalases* *E. Lactate dehydrogenase*

**23**. Brain cells are very sensitive to energy deficiency whose cause can be a high content of ammonia, which stimulates the outflow of alpha-ketoglutarate from:

*A. Glycolicysis* *B. Glycogenolysis* *C. Ornithine cycle*
*D. Citric acid cycle* *E. Pentose phosphate pathway*

**24**. Osteolaterism leads to the diminished strength of collagen due to a marked decrease in the formation of cross-links in collagen fibres. The reason for this phenomenon is a decrease in the activity of:

*A. Lysyl hydroxylase* *B. Collagenase* *C. Lysyl oxidase*
*D. Monoamine oxidase* *E. Prolyl hydroxylase*

**25**. A young male visited a doctor. His complaints allowed suspecting an active form of rheumatism, which is the damage to connective tissue, accompanied by the destruction of heteropolysaccharides in glycoproteins. Which biochemical parameter in blood and urine should be investigated to clarify the diagnosis?

*A. Glucose* *B. Protein fractions* *C. Indican*
*D. Ketone bodies* *E. Sialic acids*

**26.** A patient was hospitalized with concussion. Against the background of neurologic symptoms, blood ammonia concentrations were elevated. Which substance should be prescribed to intensify ammonia detoxification in the brain?

*A. Histamine* *B. Ascorbic acid* *C. Serotonin*
*D. Nicotinic acid* *E. Glutamic acid*

**27**. A patient requested a cosmetologist to help him to get rid of a tattoo on his shoulder. Which substance present in the connective tissue limits the spread of the dye and makes this kind of "painting" possible?

*A. Heparin* *B. Elastin* *C. Fibronectin*
*D. Hyaluronic acid* *E. Gamma globulin*

**28**. After intense exercise, the chronic alcoholic lost consciousness. Select a possible cause of fainting in this case.

*A. Hypoglycemia* *B. Hyperglycemia* *C. Ketonemia*
*D. Azotemia* *E. Hyperammonemia*

**29**. In the lungs, carbonic acid is split into water and carbon dioxide by an enzyme. $CO_2$ is excreted with exhaled air. Which enzyme catalyzes this reaction?

*A. Peroxidase* *B. Carbonic anhydrase* *C. Cytochrome*
*D. Catalase* *E. Cytochrome oxidase*

**30**. After healing a wound, a scar is formed. Which substance is the main component of this type of connective tissue?

*A. Collagen* *B. Heparin* *C. Chondroitin sulfate*
*D. Keratan sulfate* *E. Elastin*

**31.** A child has physical and mental retardation, severe damage to the connective tissue of internal organs, keratan sulfates are found in urine. Which kind of metabolism is impaired?

*A. Elastin* *B. Glycosaminoglycans* *C. Fibronectin*
*D. Collagen* *E. Hyaluronic acid*

**32**. After prolonged physical activity during physical training, delayed onset muscle soreness developed in students. Its cause was the accumulation of lactic acid in skeletal muscles. It was formed after the activation of:

*A. Lipolysis* *B. Glycogenesis* *C. Gluconeogenesis*
*D. Pentose phosphate pathway* *E. Glycolysis*

**33**. Fibrillar elements of connective tissue include collagen, elastin, and reticulin. Select an amino acid, which is incorporated in collagen and whose determination in biological fluids is used to confirm connective tissue diseases.

*A. Proline* *B. Phenylalanine* *C. Hydroxyproline*
*D. Lysine* *E. Glycine*

**34**. Muscle proteins break down into free amino acids during starvation. They will be most likely used in:

*A. Gluconeogenesis in muscles* *B. Decarboxylation*
*C. Gluconeogenesis in the liver* *D. Synthesis of fatty acids*
*E. Glycogenolysis*

**35.** Depressions and emotional disorders are a consequence of the deficiency of norepinephrine, serotonin, and other biogenic amines. An increase in their content in synapses can be achieved by antidepressants, which inhibit the enzyme:

*A. L-amino acid oxidase* *B. Monoamine oxidase*
*C. Phenylalanine-4-monooxygenase* *D. D-Amino acid oxidase*
*E. Diamino oxidase*

**36.** Mucopolysaccharidosis are hereditary diseases that manifest by disorders of metabolism in the connective tissue, pathology of bones and joints. Which parameter of urinalysis confirms this pathology?

*A. Excessive lipid excretion* *B. Excessive excretion of glucose*
*C. Excessive excretion of amino acids*
*D. Excessive excretion of glycosaminoglycans*
*E. Excessive albumin excretion*

**37.** What is the fastest mechanism for ATP formation that is necessary for the immediate activation of muscular contraction?

*A. Aerobic glycolysis* *B. Anaerobic glycolysis*
*C. Generation of ATP from creatine phosphate*
*D. Glycogenolysis in muscles* *E. Oxidation of triglycerides*

**38.** It has been known that synovial fluid reduces friction of joint surfaces. In rheumatism or arthritis, its viscosity decreases due to depolymerization (destruction) of:

*A. Glycogen* *B. Hyaluronic acid* *C. Collagen*
*D. Heparin* *E. Albumin*

**39.** In medical practice teturam is widely used for the prevention of alcoholism. It is an aldehyde dehydrogenase inhibitor. Which metabolite increases in the blood and predetermines an aversion to alcohol?

*A. Ethanol* *B. Malonic aldehyde* *C. Propionic aldehyde*
*D. Methanol* *E. Acetaldehyde*

**40.** A patient went to the hospital with complaints of fatigue and exacerbated muscle weakness. His examination revealed an autoimmune disease, which caused the abnormal functional state of the receptors in the neuromuscular synapses. Which neurotransmitter is blocked?

*A. Dopamine* *B. Serotonin* *C. Norepinephrine*
*D. Acetylcholine* *E. Glycine*

**41.** При беге на короткие дистанции у нетренированного человека возникает мышечная гипоксия. К накоплению какого метаболита в мышцах это приводит?

*A. Oxaloacetate* *B. Lactate* *C. Ketone bodies*
*D. Glucose-6-phosphate* *E. Acetyl-CoA*

**42.** A young male is diagnosed with muscular dystrophy. Which substance level is elevated in the blood serum in this pathology?

*A. Myoglobin* *B. Myosin* *C. Lactate* *D. Creatine* *E. Alanine*

**43**. A female has signs of rheumatoid arthritis. Which of the following blood parameters will be the most significant for confirmation of the diagnosis?

*A. Lipoproteins* *B. Acid phosphatase* *C. Total glycosaminoglycans*
*D. Total cholesterol* *E. R-Glycosidases*

**44**. A child with signs of muscle damage was hospitalized. After the examination, carnitine deficiency in the muscles was found. Which process pathology underlies this disease?

*A. Synthesis of actin and myosin* *B. Regulation of $Ca^{2+}$ level in mitochondria*
*C. Lactic acid utilization* *D. Substrate-level phosphorylation*
*E. Transport of fatty acids in mitochondria*

**45**. People have intense muscle pain after prolonged physical exercise. What changes in muscles are the most likely cause of this?

*A. Increased levels of ADP* *B. Increased excitability*
*C. Accumulation of lactic acid* *D. Enhanced protein breakdown*
*E. Accumulation of creatinine*

**46**. A female has been ill for about a year: pain in the joints, their swelling, redness of the skin above them. The previous diagnosis is rheumatoid arthritis. The consequence of this disease is a change in the structure of the connective tissue protein:

*A. Mucin* *B. Collagen* *C. Myosin* *D. Ovalbumin* *E. Troponin*

**47**. It has been known that the collagen molecule contains amino acids hydroxyproline and hydroxylysine. Which of the following substances is involved in proline and lysine hydroxylation?

*A. Folic acid* *B. Pantothenic acid*
*C. Glutamic acid* *D. Aspartic acid* *E. Ascorbic acid*

**48**. Periodontosis leads to the destruction of protein and polysaccharide components of connective tissue. Which of the following proteins is a component of connective tissue?

*A. Collagen* *B. Albumin* *C. Transferrin*
*D. Ceruloplasmin* *E. Antitrypsin*

**49**. An infant has epileptiform seizures caused by vitamin $B_6$ deficiency. This is caused by a decrease in the content of the inhibitory neurotransmitter γ-aminobutyric acid in the nervous tissue. Which enzyme activity is low?

*A. Alanine aminotransferase* *B. Glutamate dehydrogenase*
*C. Glutamate decarboxylase* *D. Pyridoxal kinases*
*E. Glutamate synthase*

**50**. Patients with collagenosis have the destruction of connective tissue. Which compounds are elevated in blood and are used to confirm this diagnosis?

*A. Creatine and creatinine* *B. Isozymes of LDH*
*C. Hydroxyproline and hydroxylysine* *D. Transaminase* *E. Urates*

**51**. In psychiatry, biogenic amines are used to treat certain CNS diseases. Select a drug of this group, which is an inhibitiory neurotransmitter.

*A. Histamine* *B. Serotonin* *C. Dopamine*
*D. Taurine* *E. γ-Aminobutyric Acid*

**52**. Ammonia is a very toxic substance, especially for the nervous system. Which compound is actively involved in detoxifying ammonia in the brain tissues?

*A. Lysine* *B. Proline* *C. Glutamic acid*
*D. Histidine* *E. Alanine*

**53**. High activity of hyaluronidase was found in patient's blood serum. Which biochemical index of blood serum will make it possible to confirm the pathology of connective tissue?

*A. Bilirubin* *B. Uric acid* *C. Glucose*
*D. Sialic acids* *E. Galactose*

**54**. A patient who had eaten exclusively polished rice had polyneuritis caused by thiamine deficiency. Which compound is excreted in the urine and can serve as an indicator of this vitamin deficiency?

*A. Methylmalonic acid* *B. Phenyl pyruvate* *C. Malate*
*D. Uric acid* *E. Pyruvic acid*

**55**. A patient with a significant damage to muscle was hospitalized to the traumatology department. Which biochemical parameter of the urine will be increased in this case?

*A. Creatine* *B. Mineral salts* *C. General lipids*
*D. Glucose* *E. Uric acid*

**56.** Wilson disease is associated with impaired copper transport, which leads to its accumulation in the cells of the brain and liver. Which protein is synthesized in smaller amounts in the liver?

*A. Haptoglobin* *B. Transcobalamin* *C. Ceruloplasmin*
*D. Siderophilin* *E. Metallothionein*

**57**. A patient of the dental department was diagnosed with Paget's disease, which was accompanied by degradation of collagen. The decisive factor that allows confirming the diagnosis is the detection of high levels of one of the following metabolites in the urine:

*A. Hydroxyprolin* *B. Alanine* *C. Tryptophan*
*D. Arginine* *E. Serine*

**1 **.** Prepare a presentation: "Non-collagen proteins of the intercellular matrix. Structure, biological role."

**2 **.** Prepare a review: "Biochemical basis of muscle fatigue. The problem of ammonia detoxification and lactate excretion from muscular tissues."

**3 **.** Prepare a review of researches: "Features of neurotransmitter balance of the brain under stress."

**4 **.** Present stages of collagen biosynthesis.

## FINAL MODULE CONTROL № 4 – FUNCTIONAL BIOCHEMISTRY (2 hours)

### *CONTROL QUESTIONS*

1. The biological significance of water and its content, the daily requirement. Exogenous and endogenous water.
2. Properties and biochemical functions of the water. The distribution and state of water in the body.
3. Metabolism of water in the body, age features, regulation.
4. Water balance of organism and its types.
5. Neurohumoral regulation of water-salt metabolism. Role of vasopressin, aldosterone and renin-angiotensin system.
6. Functions of mineral salts in the body.
7. Electrolite composition of body liquids, its regulation.
8. Minerals of the human body, their content, role.
9. Classification of biogenic elements, their role.

10. Phosphate-calcium metabolism. Inorganic and organic phosphates. Phosphates of urine. Regulation.
11. Role of sodium, potassium, chlorine in vital processes.
12. Iron: role and metabolism in organism, daily requirement.
13. Pathological conditions associated with disorders of metabolism of mineral substances.
14. Role of the blood in the vital functions.
15. Physico-chemical properties of blood, serum, lymph: pH, osmotic and oncotic pressure, relative density, viscosity.
16. Acid-base balance of the blood, its regulation, disorders. Modern methods for the determination of acid-base balance of the blood.
17. Buffer systems of the blood. Their role in maintainance of the acid-base balance. Disorders: acidosis, alkalosis. Their causes.
18. The main fractions of blood plasma proteins. Methods of investigation.
19. Blood proteins: content, functions, content changes in pathological states.
20. Albumins, physical and chemical properties, the role.
21. Globulins, physical and chemical properties, the role.
22. Immunoglobulins of blood, structure, and functions.
23. Hyper-, hypo-, dys- and paraproteinemias; the causes of their appearance.
24. Acute phase proteins. Clinical and diagnostic significance of their determination.
25. Enzymes of blood, clinical and diagnostic significance of their determination.
26. Non-protein nitrogen-containing components. Total and residual nitrogen of blood. Clinical significance of determination.
27. Azotemia: types, causes, methods of determination.
28. Nitrogen-free blood components. Clinical significance of determination.
29. Inorganic components of blood.
30. Kallikrein-kinin system and its role in the body.
31. Structure, role and properties of hemoglobin.
32. Types of hemoglobin.
33. Heme, its structure and role in the hemoglobin functioning.
34. Mechanism of hemoglobin participation in the transport of $O_2$ and $CO_2$.
35. Abnormal forms of hemoglobin.
36. The general scheme of the hemoglobin synthesis. Regulation of process.
37. Porphyria: causes, types.
38. Hemoglobinoses: thalassemia and hemoglobinopathies.
39. The general scheme of the hemoglobin breakdown. Bile pigments, their conversion, the value of research in the blood, urine, and feces.
40. Direct and indirect bilirubin of blood. Significance of determination.
41. Pathobiochemistry of jaundices. Enzymatic, hereditary jaundices. Jaundices of newborns.
42. Renal functions and features of metabolism in kidneys.
43. General properties and chemical composition of normal urine. Significance of urine analysis in clinical practice.

44. Physical and chemical indices of urine. The significance of their investigation. Possible deviations from the norm.
45. Clinical and diagnostic significance of quantitative and qualitative analysis of urine.
46. Protein as a pathological component of urine. Possible causes of its occurrence. Methods of determination.
47. Glucose as a pathological component of urine. The causes and types of glycosuria. Methods of determination.
48. Creatine as a pathological component of urine. Possible causes of its occurrence. Methods of determination.
49. Ketone bodies as pathological components of urine. Possible causes of ketonuria. Methods of determination.
50. Blood pigments (hemoglobin, methemoglobin) as pathological components of urine. Possible causes of their occurrence. Methods of determination.
51. Bile pigments (biliverdin, bilirubin, urobilinogen) as pathological components of urine. Possible causes of their occurence. Methods of determination.
52. Indican as a component of urine. Possible causes of the increased urinary excretion. Methods of determination.
53. Biochemical functions of liver in the body.
54. Role of liver in carbohydrate metabolism.
55. Role of liver in lipid metabolism.
56. Role of liver in protein metabolism.
57. Role of liver in pigment metabolism.
58. Bile formation function of liver. Chemical composition of bile.
59. Detoxification function of liver. Types of reactions of xenobiotics and endogenous toxins biotransformation.
60. Systems of conjugation for detoxification of toxic substances in liver.
61. Disorder of liver functions in diseases. Liver tests.
62. Xenobiotics: concept, principle of classification, pathways of entering to the body, transport through cellular membranes.
63. Microsomal oxidation. Characteristic of microsomal monooxygenase chains. Cytochromes P-450 and $b_5$.
64. Types of conjugation of xenobiotics in hepatocytes: biochemical mechanisms, functional value.
65. Ways of the removal of xenobiotics biotransformation products from organism.
66. The main classes of substances of nervous tissue, their ratio in different regions of nervous system.
67. Lipids of nervous tissue: classes, representatives, role. Diseases developed due to disorders of lipid metabolism in nervous tissue.
68. Myelin: chemical composition, properties, role.
69. Proteins, amino acid and peptides of nervous tissue: classification, composition, properties.
70. Macroergic compounds of nervous tissue. Features of energy metabolism.
71. Neurochemical transmission of impulse. Synapses. Neurotransmitters. Synaptic receptors.

72. Features of structure of muscle fiber, thick and thin filaments.
73. Chemical composition of muscular tissue. Features of chemical composition and metabolism of substances in cardiac and smooth muscles.
74. Characteristics of muscle proteins, main non-protein nitrogen-containing substances.
75. Bioenergetics of muscular tissue; sources of ATP; role of creatine phosphate in providing of muscle contraction with energy.
76. Changes in muscles in muscular dystrophy, hypodynamia, avitaminosis E.
77. Features of connective tissue structure.
78. Fibronectin: features of structure and role.
79. Elastin: features of amino acid composition and physico-chemical properties.
80. Collagen: structure, role, biosynthesis.
81. Main classes of proteo- and glycosaminoglycans, structure, role.
82. Changes in connective tissue in aging.
83. Diseases of connective tissue. Molecular pathology of connective tissue (Marfan's syndrome, Menke's disease, Ehlers–Danlos syndrome).

*Навчальне видання*

Part 4
Functional biochemistry.

*Self-Study Guide for Students of*
*General Medicine Faculty in Biochemistry*

Частина 4
Функціональна біохімія

*Методичні вказівки*
*для підготовки до практичних занять з біологічної хімії*
*(для студентів медичних факультетів)*

Формат А5. Ум. друк. арк. 5,0
Редакційно-видавничий відділ
ХНМУ, пр. Науки, 4, м. Харків, 61022

Свідоцтво про внесеннння суб'єкта видавничої справи до Державного реєстру видавців,
виготівників та розповсюджувачів видавничої продукції серії ДК № 3242 від 18.07.2008 р.