Ministry of Education and Science of Ukraine
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Methodical instructions for practical lessons

on discipline **“Biological chemistry”**

for students of speciality 222 “Medicine”
full-time form of education

In two parts
Part 2

Sumy
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Department of Biophysics, Biochemistry, Pharmacology and Biomolecular Engineering

CONTENT **P.**

Introduction

Biological chemistry is a fundamental science which is essential in the system of training of future doctors. The development of basic knowledge of the subject forms the foundation for biochemical thinking formation in students, the progress of their basic skills and skill of assessment of metabolic processes in healthy people and in the case of the pathological process.

A reform of higher education, which is carried out in Ukraine, requires a modern presentation of materials that meet goals and objectives of the current state standards of medical education and the principles of the Bologna Process (ECTS). Methodical recommendations are based on the current typical programme of biological chemistry.

Part 2 of the methodical recommendations contains the themes of module 2 «Metabolism of proteins. Molecular biology. Biochemistry of tissues, physiological functions and intercellular communications». Methodical recommendations contain all necessary information for preparation of students to practical classes, test questions of the module, reference. Each lesson indicates the importance of the theme, its goals and objectives, offers theoretical questions and recommends literature. Each lesson has a practical part which includes laboratory work with the methodology of its implementation and the clinical and diagnostic value of various biochemical parameters.

During the process of preparation to the laboratory part of the practical lesson students should know that the laboratory work can be done only by students who:

– clearly understand the principles of methods of the laboratory work and main stages of the experiment;

– know the basic safety rules;

– understand the clinical diagnostic value of detecting certain substances in biological materials;

– have protocols of laboratory works in their copybooks.

Based on their research, the students should establish a protocol according to next plan:

– a date of preparation and a number of the lesson;
– theme of the lesson;
– name of the laboratory work;
– the principle of the method;
– clinical and diagnostic value;
– results of the experiment;
– conclusions.

The authors hope that the methodical recommendations will help students to find quickly a large amount of scientific information during this study and to gain knowledge about modern methods of biochemical research. For getting practical skills in definition of certain substances in biological material the students should have the ability to analyze and evaluate the results of laboratory tests that matter in their further study of pharmacology, pathophysiology and clinical disciplines, and in their future career.

Plans of practical lessonson biological chemistry by themes
4th semester

Lesson №	Theme	Hours
	Submodule 3. Metabolism of proteins. Molecular biology. Biochemistry of intercellular communications	
28	Metabolism of simple proteins and amino acids. Common pathways of amino acids transformation	2
29	Metabolism of ammonia in human body. Ammonia detoxification and synthesis of urea	2
30	Specialized pathways of cyclic and acyclic amino acids metabolism. Disorders of nitrogen metabolism. Biosynthesis of porphyrins	2
31	Biosynthesis and catabolism of purine and pyrimidine nucleotides. Determination of the final products of their metabolism	2
32	DNA replication and RNA transcription	2
33	Protein biosynthesis on the ribosomes. Antibiotics as inhibitors of transcription and translation. Regulation of gene expression. Molecular mechanisms of mutation. DNA repair. Recombinant DNA	2
34	Molecular-cellular mechanisms of protein-peptide, catecholamine, steroid hormones action	2
35	Biochemical effects of protein-peptide and gastrointestinal tract hormones	2
36	Hormonal regulation of metabolism and cellular functions by thyroid hormones and catecholamines. Biochemical effects of eicosanoids	2
37	Biochemical effects of steroid hormones. Hormonal regulation of calcium and phosphate homeostasis	
38–39	*Examination submodule 3 "Metabolism of proteins. Molecular biology. Biochemistry of intercellular communications"*	2
	Submodule 4. Biochemistry of tissues and physiological functions	

40	Water-soluble (coenzyme) vitamins: B_1, B_2, B_5, B_6, B_{12}. Functional role in metabolism	2
41	Mechanisms of action and biochemical effects of vitamin C, PP, H, Bc, P. Methods for determination of vitamin C	2
42	Biochemical effects and methods for determining the fat-soluble vitamins. Determination of macro- and trace elements in biological material	2
43	Physiological and biochemical functions of blood: buffer system, acid-base status. Respiratory function of erythrocytes	2
44	Plasma proteins, acute-phase of inflammation proteins, indicator enzymes	2
45	Blood composition: non-protein organic components. Plasma lipoproteins. Coagulation and fibrinolytic systems of blood. Pathology of hemostasis. Biochemistry of immune processes and biochemical mechanisms of immunodeficiency	2
46	Biochemical functions of liver. Determination of activity of sorbitol dehydrogenase and γ-glutamyl peptidase in blood serum	2
47	The role of liver in the metabolism of bile pigments. Pathobiochemistry of jaundice. Biotransformation of xenobiotics and endogenous toxic substances.	2
48	Test on situational tasks from “Step-1”: IV semester	2
49	Functional activity in the kidneys. Chemical composition of urine	2
50	Biochemical transformations in the muscles. Determination of serum creatinine	2
51	Features of chemical composition and metabolism in the connective tissue. Determination of sialic acids in blood serum	2
52	Features of chemical composition and metabolism in the nervous tissue	2

53	Test on situational tasks from “Step-1”: III – IV semesters	2
54–55	*Examination submodule 4 "Biochemistry of tissues and physiological functions "*	2
56	Interrelation of metabolism in organs and body systems	2

Lesson 28
Theme: METABOLISM OF SIMPLE PROTEINS AND AMINO ACIDS. COMMON PATHWAYS OF AMINO ACIDS TRANSFORMATION

Actuality of the theme. *Modern ideas about the metabolic processes in the body impossible without the knowledge about metabolism of the various groups of major macromolecules. Amino acids are structural components of proteins, which have a lot of biological functions. Free amino acids are precursors of a number of biologically important compounds (carbohydrates, lipids, hormones, melanin, creatine, etc.). Knowledge of metabolic transformations of amino acids is an important component of the basic knowledge of future doctors in biochemistry.*

Objectives. A student should be able to *characterize biochemical mechanisms of protein digestion in the gastrointestinal tract; explain the general pathways of metabolism of free amino acids, describe the possibility of using the definition of activity of enzymes of amino acid metabolism for the diagnosis of diseases of the internal organs.*

Main tasks. A student should be able:

1. *To explain the protein digestion mechanism and absorption of their hydrolysis products in gastrointestinal tract.*
2. *To determine the activity of aminotransferases in serum, to interpret the results of the definition of AST and ALT in the blood serum.*
3. *To write down the chemical reaction of basic transformations of free amino acids: transamination, deamination, decarboxylation.*
4. *To define the acidity, concentration of hydrochloric acid, and pathological compounds of gastric juice.*

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Theoretical questions

1. The protein digestion in the gastrointestinal tract. Rotting of proteins in the intestine. Daily requirement of proteins for human body.
2. Pathways of formation and using of a pool of free amino acids in the human body.
3. Pathways of catabolism of free amino acids to end products.
4. Deamination of amino acids: types of deamination, sequence of reactions. Glutamate dehydrogenase reaction, its value and regulation.
5. Transamination of amino acids: reactions, biochemical value, the mechanism of action of aminotransferases.
6. The mechanism of indirect deamination of amino acids.
7. Decarboxylation of amino acids: enzymes, physiological value. Oxidation of biogenic amines.
8. Diagnostic significance of definition of aminotransferases activity.

Laboratory work

1. The definition of transaminase activity in blood serum

Principle of the method: As a result of transamination under the action of AST, aspartic acid is converted into oxalic acid, and alanine under the action of ALT - in pyruvic acid. Oxalic acid is capable in the process of enzymatic reaction to be transformed in pyruvic acid.

With the addition of acid 2.4-dinitrophenylhydrazine (2.4-DPH), the enzymatic process is stopped and dinitrophenylhydrazone of pyruvic acid is formed, which in a alkaline environment gives a brownish-red color. The intensity of color is proportional to the amount of formed pyruvic acid, which allows us to conclude about the activity of the enzyme. The activity of aminotransferases is measured in mcM pyruvic acid, formed by 1 ml of serum for 1 h incubation at a temperature of 37^0 C.

1.1.The definition of aspartate aminotransferase activity

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	**Experimental sample, ml**	**Control sample, ml**
Substrates (aspartic acid + α- ketoglutaric acid)	0.5	0.5
Test tubes are put in a thermostat for 5 min at 37 ° C		
Blood serum	0.1	–
H_2O	–	0.1
Test tubes are put in a thermostat for 30 min at 37° C		
2.4-Bisphosphoglyceric acid	0.5	0.5
Test tubes are put in a thermostat for 20 min at 25° C		
0.4N NaOH	5	5
Reagent	3.0	3.0
Mix all reagents ; test tubes hold for 10 min at 25 ℃ (for color formation). The optical density is measured at photometer at λ = 500–560 nm		

The activity of the enzyme is calculated by the formula:

$$X = E \cdot 133 \text{ unit/ml},$$

where

X – activity of the enzyme;

E – extinction;

133 – conversion coefficient.

1 μg of pyruvic acid – 0.015 U; 1 unit – 133 μg pyruvic acid, or according to the calibration graph.

Activity is measured in units per 1 ml of serum. 1 unit AsAT corresponds to such activity of an enzyme that is capable of forming 1 μg of pyruvic acid in these conditions.

When calculating the activity of the enzyme, consideration should be given to serum dilution

$$X = a \cdot 10,$$

where

X – unit of the enzyme;

10 – recalculation on 1 ml;

a – quantity of pyruvic acid, determined according to the calibration graph, μg.

Recalculation of the activity of the enzyme in the micromole of pyruvic acid formed during the incubation of 1 ml of serum for 1 h at 37^0 C is carried out according to the formula

$$\text{AST} = \frac{a \cdot 10 \cdot 2}{88},$$

where

a is the quantity of pyruvic acid, determined according to the calibration graph, μg;

88 is mass of 1 micromole of pyruvic acid, μg;

10 is coefficient of conversion to 1 ml of serum;

1 is conversion coefficient for 1 h incubation (only for AlT).

1.2. The definition of alanine aminotransferase activity

Course of work. The definition of ALT activity is carried out analogously to the AST, except that it take another substrates (alanine and α-ketoglutaric acid).

The calculation of the activity of the enzyme is performed on the calibration curve. To calculate the activity of ALT and its conversion into a pyruvic acid micromole, the same formulas as for AST are used. One ALT unit corresponds to the activity of an enzyme that is capable of producing 1 μg of pyruvic acid under these experimental conditions. In the blood serum of healthy people, the activity of ALT, determined by this method,

Diagnostic value of clinical tests. Normal valye of the AST activity in blood serum is ranges from 5 to 40 U / l (0.1 – 0.45 μmol / h·ml), ALT – from 5 to 30 U / l (0.1 – 0.68 μmol / h·ml).

AST and ALT are the most sensitive indicators of damage to liver parenchyma (especially ALT). The activity of ALT, which is 10 times or more than the upper limit of normal, is observed in acute hepatitis (viral and toxic); an increase in the activity of the enzyme in 5–10 times is characteristic for acute (viral, alcoholic, drug-induced) hepatitis, exacerbation of chronic active hepatitis and liver tumors. Indicators that are above the norm 1.5–5 times, are observed in all of diseases listed above, as well as in the first week of acute obturation of the bile duct.

The activity of AST varies similarly to ALT, but with less potential. Determining the activity of aminotransferases in serum is important for the diagnosis of heart disease. In myocardial infarction, the activity of AST rises in 10–100 times compared with normal. In the initial period of myocardial infarction, after 24–36 h, it is clearly expressed and only on the 3–7th day the enzyme activity is normalized.

2. The definition of acidity, concentration of total, free and bound hydrochloric acid in gastric juice

Course of work. 5 ml of filtered gastric juice, 1 drop of n-dimethylaminoazobensol and 2 drops of phenolphthalein are added to the titration flask. In the presence of free hydrochloric acid in gastric juice red color appears, in the absence of it – yellow or orange color appears. The free hydrochloric acid is titrated with 0.1N NaOH solution till orange color appears and result is written down (I mark). The titration is continued till yellow color appears, result is written down (II mark). The titration is continued till pink color appears (III mark).

The calculation of HCl concentration should be made in accordance with the formula:

$$X = \frac{\text{Mark} \cdot 1000 \cdot C}{V},$$

where

X is concentration of HCl, mmol / l;

Mark is volume of 0,1 N NaOH solution, ml;

C is concentration of NaOH solution (0.1 mmol / l);

V is volume of gastic juice (5 ml).

I mark corresponds to the concentration of free hydrochloric acid; III mark corresponds to the total acidity of gastric juice. The arithmetic average of II and III marks corresponds to the concentration of total hydrochloric acid.

For example, such amount of 0.1 N NaOH is spent for titration:

I mark – 1.6 ml;

II mark – 1.8 ml;
III mark – 2.4 ml,

Concentration of free HCl is $1.6 \cdot 1000 \cdot 0.1/5 = 32$ mmol/l.

Total acidity is $2.4 \cdot 1000 \cdot 0.1/5 = 48$ mmol/l.

Concentration of total HCl is

$$\frac{1.8 + 2.4}{2} \cdot \frac{1000 \cdot 0.1}{5} = 42 \text{ mmol/l},$$

The calculation of bound HCl concentration should be made in accordance with the formula:

bound HCl = total HCl – free HCl;

Concentration of bound HCl is 42 – 32 = 10 mmol/l.

Diagnostic value of clinical tests. The normal values:

– total HCl – 40–60 mmol/l,
– bound HCl – 2–15 mmol/l,
– free HCl – 20–40 mmol/l,
– pH = 1,5–2,5.

The definition of gastric juice acidity is important for diagnosis and treatment of diseases of the stomach and duodenum. Patients with hyperacidic gastritis or duodenal ulcer have increasing of concentration of free and total HCl in gastric juice. The decreasing of free HCl concentration and total acidity is observed at chronic atrophic gastritis. Hypochlorhydria, achlorhydria, achilia (absence of HCl and pepsin) are observed at stomach cancer.

3. The definition of pathological compounds of gastric juice

3.1. A qualitative Uffelmann's reaction to lactic acid

Principle of the method. lactic acid reacts with violet ferric phenolate, forming a yellow-green ferric lactate.

Course of work. Add 5 drops of gastric juice and 5 drops of Uffelmann's reagent (20 drops of 1 % phenol solution + 2 drops of 1 % ferric chloride solution) to the test tube. The appearance of yellow-green ferric lactate is observed.

Diagnostic value of clinical tests. Lactic acid is formed by bacteria in the case of decrease in the content or absence of free HCl in gastric juice. Lactic acid is also formed as a product of cancer cell

metabolism. The portions of gastric juice got fasting are tested for lactic acid.

3.2 The definition of blood in gastric juice

Principle of the method. In the presence of blood in gastric juice a peroxidase oxidazes bensedine, forming a blue color substance. Peroxidase uses H_2O_2 as electron acceptor.

Course of work. Add 5 drops of 3 % H_2O_2 solution, 5 drops of gastric juice and 5 drops of 1 % bensedine solution to the test tube. Blue colouration appears in the presence of blood in gastric juice.

Diagnostic value of clinical tests. Blood can be found in the gastric juice in case of bleeding from walls of stomach, esophagus, at pulmonary bleeding.

3.3 The definition of bile pigments

Principle of the method. Bile pigments are capable of producing colour circles at the boundary of two fluids by presence of concentrated nitric acid in such a sequence: green, blue, violet, red, red–yellow. The reaction can detect bilirubin at a dilution of 1 : 80000.

Course of work.

1st method. Add 1 ml of HNO_3 (conc.) to the test tube, gastric juice is layered carefully down the test tube walls. The appearance of color circles on the boundary of fluids is observed.

2nd method. A drop of gastric fluid is applied on filter paper, is given time to dry up and then a drop of HNO_3 containing some HNO_2 is applied at the center of a spot. The appearance of color circles is observed.

Diagnostic value of clinical tests. Bile pigments (bilirubin, biliverdin) can be found in gastric juice in result of intestinal antiperistalsic. The presence of bile pigments determines green or yellow color of gastric juice.

The results of experiments 1–2 should be written in a table:

Test tube number	Pathologic compounds of gastric juice		
	Lactic acid	Blood	Bile pigments

4. The definition of pepsin activity

Principle of the method. Pepsin convertes soluble caseinogen to insoluble casein. The conversation of lactic acetate mixture by pepsin at pH = 4.9 and 25°C is proportional to its activity. The unit of pepsin activity is the amount of enzyme that is capable of converting 5 ml of lactic acetate mixture in 60 seconds (this unit is equivalent to 0.01 mg of crystal pepsin).

Course of work. Add 0.1 ml of gastric juice to the test tube and 5 ml of lactic acetate mixture to another one. Both test tubes are set on water bath for 5 min at 25°C. Lactic acetate mixture is quickly transferred to the tube with tested gastric juice and shaken down; turn on the stopwatch. The test tube with mixture is hold in water bath and observed the appearance of first casein flakes on test tube walls. When they appear, the stopwatch is switched off and the time in seconds is written down.

To calculate pepsin activity in 1 ml of gastric juice, the number 60 is divided by number of found seconds.

By this way a number of pepsin units in 0,1 ml of gastric juice is calculated, and having multiplied by 10 – in 1 ml.

For example, found time is 15 seconds. Thus, 0,1 ml of gastric juice contains 4 pepsin units (60/15 = 4.0) and 1 ml – 40 units ($4 \cdot 10 = 4$) or 0.4 mg of crystal pepsin ($40 \cdot 0.01 = 0.4$).

Diagnostic value of clinical tests. The normal value of pepsin activity is 40 – 60 units in 1 mg of gastric juice. The pepsin activity decreases in case of atrophic gastritis and increases in case of stomach ulcer. The patients with stomach cancer have very low activity or absence of pepsin in gastric juice.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 29
Theme: METABOLISM OF AMMONIA IN HUMAN BODY. AMMONIA DETOXIFICATION AND SYNTHESIS OF UREA

Actuality of theme. *The result of the processes of deamination of amino acids, nucleotides, biogenic amines is the formation of ammonia. Ammonia is a toxic substance, therefore, in the body there are special ways of its detoxification. For future physicians, knowledge about the metabolism of ammonia and the formation of urea as the main product of its elimination are important for understanding the causes and consequences of liver, kidney, hormonal disturbances.*

Objectives. A student should be able to *characterize the main ways of formation and utilization of ammonia; explain the principles of the method and the diagnostic value of the definition of urea.*

Main tasks. A student should be able:

1. *To explain the mechanisms of formation and utilization of ammonia.*
2. *To write chemical reactions of the ornithine cycle.*
3. *To define the concentration of urea in blood serum.*
4. *To interpret the results of the definition of urea concentration in blood serum.*

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Theoretical questions

1. The sources of ammonia in an organism.
2. Toxicity of ammonia and pathways of its neutralization.

3. Transport of ammonia.
4. Biosynthesis of urea: a biological role, regulation, localization, sequence of reactions.
5. Interrelation of the ornithine cycle with transformation of fumarate and aspartic acids in the citric acid cycle.
6. Genetic disorders of the urea cycle enzymes. Hyperammonemia.

Laboratory work

1. The definition of urea concentration in blood serum

Principle of the method. Urea forms with diacetylmonoxime, Fe^{3+} and thiosemidcarbazide, a complex of red color, whose intensity of color determines its concentration.

Course of work: Add all reagents in accordance with the table to the 3 test tubes:

Reagent	Sample, ml		
	test	standard	control
Blood serum	0.02	–	–
Reference solution of urea	–	0.02	–
H_2O	–	–	0.02
Diacetylmiooxime solution	2.00	2.00	2.00
Thiosemid carbazide solution	2.00	2.00	2.00

To reduce the analysis error, it is recommended to follow the mixing procedure below.

Notes:

1) With a urea content of more than 25 mmol/l, the sample should be diluted with distilled water and repeat the analysis. The result is multiplied by dilution.

2) Hemolytic and lipemic serums are deproteinized. For this 0.1 ml of serum is mixed with 0.9 ml of trichloroacetic acid solution and centrifuged for 5 min. The same process for standard. For analysis, studies are selected as for serum without deproteinization. The same method can be used to examine blood.

The test tubes are closed with aluminum foil, the contents of the samples are mixed and boiled in a water bath for 10 min. At the same time, experimental, standard and control samples are treated. Then the test tubes are cooled rapidly under a stream of cold water. Colorimetric test sample and standard against control at a wavelength

of 530–560 nm (green filter). Measurement of optical density should be carried out within no more than 15 min after cooling.

If, after heating, the solution in the first tube is cloudy, then it is centrifuged for 5 min or deproteinized with a solution of trichloroacetic acid.

The calculation should be made in accordance with the formula:

$$C = \frac{E_{test} \cdot \times 16{,}64 \text{ mmol /l}}{E_{st.}},$$

where

C is concentration of urea;

E_{test}- is the optical density of the experimental sample;

E_{ref}- is the optical density of the standart sample.

Diagnostic value of clinical tests. The normal urea contenttation:

- in blood serum 3.3–8.3 mmol/l ;
- in urine 333–583 mmol/day or 20–35 g/day.

The increase in the value is observed with heart failure, increased diuresis or perspiration, shock conditions, increased catabolism of proteins, high protein diet, acute and chronic kidney diseases.

The decrease is observed in a low protein diet and a high-carbohydrate diet, pregnancy, acromegaly, parenteral nutrition, liver diseases, and malabsorbtion.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 30
Theme: SPECIALIZED PATHWAYS OF CYCLIC AND ACYCLIC AMINO ACIDS METABOLISM. DISORDERS OF NITROGEN METABOLISM. BIOSYNTHESIS OF PORHYRINS

Actuality of theme. *For future physicians, knowledge of the metabolic transformations of individual amino acids will allow the interpretation of clinical manifestations in the diagnosis of inherited diseases, such as*

phenylketonuria, alpathonuria, albinism, maple syrup and Hartnup's disease and others. Amino acids are precursors of the synthesis of hormones (thyroid and catecholamines), biogenic amines, coenzymes, creatine, melanin, heme and others. Disruption of heme biosynthesis causes porhyria. The effective treatment of porphyria is based on the knowledge of defective stage of heme biosynthesis.

Objectives. A student should be able to *explain the basic metabolic transformation of acyclic, cyclic, sulfur-containing and branched chain amino acids; explain metabolic changes in inherited diseases of amino acids metabolism; explain the sequence of reactions of the heme biosynthesis; characterize the most common porphyrias.*

Main tasks. A student should be able:

1. To explain the metabolic pathways of transformations of amino acids.
2. To characterize chemistry of synthesis and catabolism of creatine.
3. To describe the main metabolic changes in inherited diseases of amino acids metabolism (homocysteinuria, cystinosis and Fanconi syndrome, maple syrup and Hartnup's disease, phenylketonuria, alpathonuria, albinism, histidinemia, disturbances of urea cycle).
4. To characterize the biological role of hemoproteins.
5. To explain the chemical reactions of the heme synthesis.
6. To define the concentration of creatinine in blood serum, to detect of porphobilinogen and coproporphyrin in urine; to interpret the results.

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Theoretical questions

1. Metabolism of the carbon chain of amino acids. Glucogenic and ketogenic amino acids.
2. Metabolism of aromatic and heterocyclic amino acids.
3. Metabolism of sulfur containing amino acids. Biological role of SAM.
4. Synthesis of creatine and creatinine. Diagnostic significance of definition of creatinine in blood serum.
5. Biological role of glutathione.
6. Metabolism of arginine. Formation and biological role of NO.
7. Metabolism of branched-chain amino acids. Biological role of vitamins B_{12} and H in metabolism of amino acids.
8. Metabolism of glycine and serine. Biological role of tetrahydrofolate in metabolism of amino acids.
9. Disorders of amino acids metabolism (phenilketonuria, alcaptonuria, albinism, maple syrup urine disease, Hartnup′s disease, histidinemia, cystinuria and homocystinuria).
10. Biosynthesis of heme: reactions, enzymes, regulation.
11. Porhyria: classification, clinical manifestations, treatment.

Laboratory work

1. The definition of the creatinine concentration in blood serum by Popper's method

The principle of the method. Creatinine reacts with picric acid in an alkaline medium to form a creatine picrate, which causes the appearance of an orange-red color, the intensity of which is proportional to the concentration of creatinine.

Course of work. Add all reagents in accordance with the table to the 3 test tubes:

Reagent	Sample, ml		
	test	standard	control
Blood serum, ml	1.0	–	–
Standard creatinine solution, ml	–	1.0	–
Picric acid (saturated)	3.0	3.0	3.0
All test tubes are put in a thermostat for 5 min at 25 ℃, then place in a water bath at 100 °C for 15–20 s			

	Centrifuge for 10′ at 3000 r/min		
Centrifugate, ml	2.0	–	–
NaOH 10%, ml	0.1	0.1	0.1

Total volume of solutions is adjusted to 10 ml with water. After 10 min (no later than 20 min), colorimetre at a wavelength of 500 – 560 nm (green filter) against the control sample.

The calculation should be made in accordance with the calibration graph or the formula:

$$X = \frac{E_{test} \cdot 0{,}1 \text{ mmol/l}}{E_{st}},$$

where

E_{test} is extinction of test sample;

E_{st} is extinction of a standard sample;

0.1 is concentration of creatinine in a standard test, mM/l.

WARNING! *Picnic acid is explosive. Do not allow it to contact with metals and their oxides, heating at a temperature above 100°C.*

Diagnostic value of clinical tests. The normal concentration of creatinine in blood serum of adults is: men 53–106 μmol/l; women 44–97 μmol/l.

An increase in the concentration of creatinine is observed in acute and chronic diseases of kidneys, acromegalia, gigantism, hyperthyroidism, hyperfunction of the adrenal glands. Lowering the level of creatinine in the blood is observed in muscle weakness, pregnancy (in the 1–2 trimesters).

2. The definition of porphyrin precursors in urine by Maiser and Granin method

The principle of the method. Porphobilinogen (PBG) reacts with n-dimethylaminobenzaldehyde (DAB) to form a reddish-colored compound. Compounds that give a similar reaction to DAB (urobilinogen, indole, scatol) are determined by extraction with chloroform in which PBG does not dissolve.

Course of work. Add 2 ml of urine and 2 ml of Erelih's reagent (2 % solution of n-dimethylaminobenzaldehyde in 4 N hydrochloric acid) into the test tube. Appears cherry-red color, which indicates the presence of porphobilinogen.

To exclude the possible presence of urobilinogen in the urine, add the same amount of chloroform to the mixture of urine and Erelih's reagent. The appearance of color in the lower chloroform layer indicates the presence of urobilin. The appearance of color in the higher layer indicates the presence of porphyrins in the urine.

3. The definition of coproporphyrin (CP) by Reznik and Fedorov semi-quantitative method

Principle of the method. The method is based on the extraction of coproporphyrins in an acid environment with ether followed by UV light exposure. The fluorescence intensity of the upper layer of the liquid is proportional to the amount of coproporphyrins, which is determined according to the scale.

Course of work. Add 1 ml of urine, 1 drop of acetic acid (to an acid reaction with litmus paper) and 1 ml of ether into the test tube with a stopper. The contents of the test tube vigorously shake during 3 – 5 min. After settling and separating the mixture, observe the fluorescence by placing the test tube underUV light.

Scale for definition of CP:

– 1 point (blue fluorescence) - normal CP content;

– 2 points (barely noticeable pink fluorescence) - a small increase in concentration of CP;

– 3 points (weak pink fluorescence) – a moderate increase in the concentration of CP;

– 4 points (clear pink fluorescence) - a large increase in the concentration of CP;

– 5 points (clear red fluorescence) - a significant increase in the concentration of CP.

The results of experiments 1–2 should be written in a table:

Test tube number	Coproporphyrin	Porphobilinogen
1		
2		
3		

Diagnostic value of clinical tests. Disturbances, in which precursors of porphyrins are found, are divided into 2 groups: porphyria and porphyrinuria. Porphyria is a group of diseases caused by deficiency of heme biosynthesis enzymes.

The appearance in urine of coproporphyrin, porphobilinogen and other precursors of porphyrins is observed in various forms of porphyrias, depending on the stage at which heme synthesis is disrupted. For example, in the case of hepatic porphyria the content of porphobilinogen and 5-aminolevulinic acid is significantly increased; in the case of erythropoietic porphyria (Gunther's disease) uroporphyrinogen I and coproporyrinogen I are accumulated in the urine.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 31
Theme: BIOSYNTHESIS AND CATABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES. DETERMINATION OF THE FINAL PRODUCTS OF THEIR METABOLISM

Actuality of theme. *Nucleotides have a lot of biological functions: structural components of nucleic acids (DNA and RNA), macroergic substances (ATP, ADP, GTP, GDP), second messengers (cAMP, cGMP), coenzymes (NAD, FAD), activators activation of molecules in the process of synthesis (UDP-glucose, CDP-cholin). Knowledge of the metabolism of purine and pyrimidine nucleotides provides an understanding of biochemical mechanisms, diagnosis and treatment of diseases such as gout, Lesch-Nyhan syndrome, orotic aciduria.*

Objectives. A student should be able to *characterize the catabolic and anabolic pathways of purine and pyrimidine nucleotides; explain the methodological approaches to the biochemical diagnosis of nucleotide metabolism disorders.*

Main tasks. A student should be able:

1. *To explain the origin of individual atoms in purine nucleotides.*
2. *To characterize the chemistry of the synthesis of pyrimidine nucleotides indicating the name of enzymes.*
3. *To explain the chemistry of the catabolism of purine nucleotides with the indication the name of enzymes.*

4. *To characterize the end products of pyrimidine nucleotides catabolism.*
5. *To explain the chemistry of the synthesis of deoxyribonucleotides.*
6. *To define the concentration of uric acid in blood serum and interpret the results.*

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Theoretical questions

1. Sources of separate atoms in the purine ring.
2. Synthesis of purine nucleotides de novo: localization, sequence of reactions, regulation. Biosynthesis of AMP, GMP, ATP, GTP.
3. The pathways of purine bases reutilization in the tissues.
4. Catabolism of purine nucleotides. Formation of uric acid.
5. Catabolism of pirimidine nucleotides.
6. Synthesis of pirimidine nucleotides: sequence of reactions, regulation.
7. Biosynthesis of deoxyribonucleotides. Formation of thymidine nucleotides, dTMP biosynthesis inhibitors as antitumor agents.
8. Disorders of nucleotide metabolism: gout, Lesch-Nyhan syndrome, orotic aciduria.

Laboratory work

1. The definition of uric acid concentration in blood serum

The principle of the method. The uric acid reduces a phosphorus-tungsten reagent to form a blue color complex. The intensity of the color is proportional to the concentration of uric acid.

Course of work. Add all reagents in accordance with the table to the 3 test tubes:

Reagent	**Sample, ml**		
	test	**standard**	**control**
Distilled water	4.0	4.0	4.5
Blood serum	0.5	–	–
Standard solution of uric acid	–	0.5	–
Sulfuric acid (0,35M)	0.25	0.25	0.25
Sodium tungstate	0.25	0.25	0.25
All test tubes are put in a thermostat for 10 min at 20–25 ° C; than centrifuged for 10 min at 3000 r/min			
Centrifugate (only for test sample)	3.0	3.0	3.0
Sodium carbonate solution	1.5	1.5	1.5
Phosphoric-tungsten reagent	1	1	1
***Note**. Mix, maintain 30 min at 20–25℃, measure extinction against a control sample at a wavelength of 500–700 nm (red filter)*			

The calculation should be made in accordance with the formula:

$$X = \frac{E_{test} \cdot C_{st}}{E_{st}},$$

where

X is concentration of uric acid, mmol/l;

E_{test} is extinction of test sample;

E_{st} is extinction of standard sample;

C_{st} is the concentration of uric acid in standard solution (0.3 mmol/l).

Diagnostic value of clinical tests. The concentration of uric acid in the blood are labile, due to factors such as age, a sex, the use of alcohol, smoking, etc.Normal values: men – 0.12–0.38 mmol/l, women – 0.12–0.46 mmol/l. Hyperuricemia is observed under: gout, Lesch-Nyhan syndrome, kidney diseases, after leukemia treatment by cytotoxic drugs. Hypouricemia is observed at Wilson disease, some malignant diseases.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 32
Theme: DNA REPLICATION AND RNA TRANSCRIPTION

Actuality of theme. *Mechanisms of replication and transcription are the basis of the processes of storing and realizing of genetic information. Knowledge of these mechanisms is fundamental not only for future doctors, but also for any educated person. In addition, understanding the processes of gene expression allows expanding approaches to the diagnosis, treatment and correction of metabolic changes of genetic diseases.*

Objectives. A student should be able to *explain the molecular mechanisms of replication and transcription; characterize the functioning of the enzymatic systems of these processes.*

Main tasks. A student should be able:

1. To characterize mechanisms of DNA replication: basic principles, enzymatic systems.

2. To explain mechanisms of transcription of RNA: stages, functioning of enzymes.

3. To explain the mechanisms of action of some antibiotics - inhibitors of transcription.

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Theoretical questions

1. Biological role of DNA replication. Semi-conservative mechanism of DNA replication; scheme of the Meselson – Stahl experiment.

2. General scheme of replication fork. Enzymes of DNA replication in prokaryotes and eukaryotes.
3. Molecular mechanisms of DNA replication: topological problems (topoisomerases, helicases); anti parallel strands of DNA; Okazaki fragments.
4. Formation of new strands of DNA. Biological role of primer.
5. Transcription: stages and mechanism. RNA polymerases of prokaryotes and eukaryotes.
6. Processing of RNA. Role of snRNA in RNA splising.
7. Replication of the genome of viruses. Biological role of reverse transcriptase.
8. Inhibitors of transcription: actinomycin D, rifampicin, streptolydigin, α-amanitin.

Lesson 33
Theme: PROTEIN BIOSYNTHESIS ON THE RIBOSOMES. ANTIBIOTICS AS INHIBITORS OF TRANSCRIPTION AND TRANSLATION. REGULATION OF GENE EXPRESSION. MOLECULAR MECHANISMS OF MUTATIONS. DNA REPAIR. RECOMBINANT DNA

Actuality of theme. *Knowledge of the mechanisms of translation and its regulation is fundamental for understanding of the causes and clinical correlation of molecular diseases. Mutations cause changes in the processes of the synthesis of enzymes and transport proteins. DNA repair is a protective mechanism by which a cell identifies and corrects damage of DNA structure. Knowledge of the functioning of DNA repair can be used to create the newest of treatment for genetic diseases that do not currently have effective therapies. The study of modern methods of DNA analysis and polymerase chain reaction is important for future physicians, because these methods are used in the system of medical diagnosis and treatment.*

Objectives. A student should be able to *explain the molecular mechanism of translation and its regulation; explain the mechanism of DNA repair; characterize the basic methods of DNA analysis: the creation of recombinant DNA, polymerase chain reaction.*

Main tasks. A student should be able:

1. *To characterize the genetic code and its properties.*

2. *To explain the main stages of translation.*
3. *To characterize the mechanisms of posttranslational modification of proteins.*
4. *To explain the mechanisms of action of antibiotics and toxins - inhibitors of translation.*
5. *To characterize the mechanisms of antivirus action of interferons.*
6. *To explain the mechanisms of regulation of gene expression in procatiotes by the lac-operon example.*
7. *To characterize the mechanism of gene amplification by the example of the genes of metallothionein and dihydrofolate reductase.*
8. *To explain the molecular mechanisms of mutations and DNA repair.*
9. *To describe the basic methodical techniques of genetic engineering and their application in medical practice.*

References

1. Gubsky Yu. Biological chemistry : textbook / Yu. Gubsky. – Vinnytsia : Nova Knyha, 2017. – P. 284–287, 291–299.
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Theoretical questions

1. The genetic code: properties, table of genetic code.
2. Translation: basic components of protein synthesis system, stages and mechanism. The biological role of protein translation factors.
3. Posttranslational modification of proteins.
4. Antibiotics and toxins as inhibitors of protein synthesis. Biochemical mechanisms of antiviral action of interferons.
5. Regulation of gene expression in prokaryotes: induction and repression. Structure of Lac-operon E.Coli.

6. Regulation of gene expression in eukaryotes. The system of transcriptional signals - promoter sequences, enhancers, attenuators, silencers. Regulation of globin synthesis.
7. Genetic recombinations; transposons. Amplification of genes (metallothioneins, dihydrofolate reductase).
8. Mutations: genomic, chromosomal, genetic. Biochemical mechanisms of action of mutagens.
9. Molecular mechanisms of DNA repair. Repair of UV-induced gene mutations; xeroderma pigmentosum. Clinical correlation of molecular disease.
10. Polymerase chain reaction (PCR); its biomedical application.
11. Biotechnology involving recombinant DNA.

Lesson 34
Theme: MOLECULAR-CELLULAR MECHANISMS OF PROTEIN-PEPTIDE, CATECHOLAMINES, STEROID HORMONES ACTION.

Actuality of theme. *The theoretical knowledge of these topic will help in the study of pharmacology, pathophysiology, endocrinology and other disciplines. Understanding the biochemical and physiological effects of hormones is possible in the presence of clear ideas about the molecular mechanisms of hormones action on the cells of the body.*

Objectives. A student should be able to *explain the classification and mechanisms of action of hormones .*

Main tasks. A student should be able:

1. To characterize the classification of hormones.

2. To explain mechanisms of regulation of synthesis and secretion of hormones.

3. To explain the membrane-cytosolic and cytosolic mechanisms of hormones action.

4. To characterize the principle of the method and application of the radioimmunological analysis (RIA) of hormones.

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Theoretical questions

1. Hormones and bioregulators in the system of intercellular integration of functions in an organism and their chemical nature.
2. Classification of hormones.
3. Mechanism of regulation of hormones synthesis and secretion.
4. Targets organs, receptors and second messengers of hormones.
5. Mechanism of action of polypeptide hormones and epinephrine.
6. Mechanism of action of steroid and thyroid hormones.
7. Radioimmunologycal method of definition of hormones concentration in blood.

Laboratory work

1. Radioimmunological method of definition of hormones concentration in blood

The principle of the method. The RIA method is based on the interaction of antigen (AG) – antibody (AB). Antigen is a hormone. Antibodies are excreted from blood of laboratory animals, which have been injected with the hormone for some time. The RIA method RIA uses a radioactive label, which enters in one of components of reaction (AG or AB). The use of a radioactive label makes it possible to determinate a very low concentration of substances (10^{-12} g).

To determine the concentration of the hormone (AG) in the blood of the patient are used: blood serum containing AG, to which add the complex AG*–AB, where AG* – a radioactive hormone.

After entering of a certain quantity of the AG*–AB complex into the blood serum, there will be a replacement of AG* in the AG*–AB complex on AG (depending on the concentration of AG in the blood serum) and a dynamic balance between the components of the reaction mixture is established. The amount of free AG* is directly proportional to the concentration of the hormone (AG) in the blood serum. The adsorption methods, fractional deposition and separation methods are used to divide the two labels in AG*–AB and AG*.

At the final stage, the radioactivity of the solution or precipitate is measured. The calculation is carried out according to the calibration curve, which is built on the results of the definition of known concentrations of AG in the reaction mixtures.

The RIA method is used in endocrinologic diagnosis to determine the concentration of total thyroxine, free thyroxine, triiodothyronine, thyroid stimulating hormone, thyroxine-binding globulin, thyroglobulin, insulin, ACTH, glucocorticoids, parathyroid hormone, calcitonin.

In clinical practice, the RIA method is used to determine the concentration of pituitary hormones: somatotropin, follicle stimulating and luteinizing hormones, prolactin, vasopressin.

Lesson 35
Theme: BIOCHEMICAL EFFECTS OF PROTEIN-PEPTIDE AND GASTROINTESTINAL TRACT HORMONES

Actuality of theme. *The protein-peptide hormones of hypothalamus and pituitary glandIn are played the main role in regulation of synthesis and secretion of hormones of peripheral endocrine glands. In addition, these hormones can directly affect various biochemical and physiological functions of the body. Therefore, study of the biochemistry of protein-peptide hormones is important because of the high frequency of endocrine diseases and the need to find new methods of diagnosis and treatment.*

Objectives. A student should be able to *explain the mechanisms of synthesis, secretion, biochemical and some physiological effects of the hormones of hypothalamus, pituitary gland, pancreas and the hormones of digestive system.*

Main tasks. A student should be able:

1. To analyze mechanisms of secretion, molecular action, biochemical and some physiological effects of protein-peptide hormones of hypothalamus and pituitary gland.

2. To characterize the clinical manifestations of hypo- and hypersecretion of protein-peptide hormones.

3. To explain mechanisms of action and the effects of insulin and glucagon on the metabolism of carbohydrates, lipids and proteins .

4. To explain the biochemical and physiological effects of the digestive system hormones.

5. To interpret the results of the qualitative definition of insulin and sulfur-containing amino acids in its composition.

References

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Theoretical questions

1. Hormones of hypothalamus: structure, the mechanism of action, biological role.
2. Growth hormone (somatotrophin) is hormone of anterior pituitary gland: structure, metabolic role, regulation and disorder of hormone secretion.
3. Tropic hormones of pituitary gland are prolactin (mammotrophin), gonadotrophins – follicle stimulating hormone (FSH), luteinizing hormone (LH): metabolic role, mechanism of action, regulation and disorder of hormones secretion.

4. Thyrotrophin (TSH) is tropic hormone of pituitary gland: metabolic role, mechanism of action, regulation and disorder of hormones secretion.
5. Adrenocorticotrophic hormone (ACTH) is tropic hormone of pituitary gland: metabolic role, the mechanism of action, regulation and disorder of hormones secretion.
6. Biological role of the products of processing of Pro-opiomelanocortin (POMC).
7. Hormones of posterior pituitary gland are vasopressin and oxytocin: mechanism of action, metabolic role, clinical importance.
8. Insulin is hormone of pancreas: structure, biosynthesis and catabolism, mechanism of action, metabolic role, clinical aspects.
9. Glucagon is hormone of pancreas: structure, biosynthesis and catabolism, mechanism of action, metabolic role.
10. Hormones that regulate the utilization of nutrients are gastrin, secretin, cholecystokinin.

Laboratory work

1. Color reactions of insulin

1.1. Biuret reaction.

Principle of the method. It is a qualitative reaction of a peptide bond (–CO–NH–). If the protein solution is treated with solutions of diluted $CuSO_4$ and NaOH, a violet – or pink – colored compounds are produced.

Course of work. Add 5 drops of insulin solution, 5 drops of 10 % NaOH solution and 2 drops of 1 % $CuSO_4$ solution to the test tube.

1.2. A qualitative reaction of sulfur-containing amino acids

Principle of the method. As a result of the interaction of sulfur and Pb^{2+} a black-colored precipitate of PbS is formed.

Course of work. Add 10 drops of insulin solution and 10 drops of 10 % NaOH to the test tube. The mixture of reagents is heated to boiling. After cooling, add a few drops of lead acetate solution CH_3COOPb to the test tube, a black-colored precipitate of PbS appears.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 36
Theme: HORMONAL REGULATION OF METABOLISM AND CELLULAR FUNCTIONS BY THYROID HORMONES AND CATECHOLAMINES. BIOCHEMICAL EFFECTS OF EICOSANOIDS

Actuality of theme. *Pathological conditions associated with a disfunction of the synthesis and secretion of hormones of the thyroid gland and catecholamines are widespread. In order to understand the causes and consequences of such molecular-cellular endocrine diseases, clear insight about the chemical nature, mechanisms of action and regulation of secretion, and metabolic effects of hormones are required. Some eicosanoids are used as medicines, that is why study of the biochemical effects of these hormones is necessary component of the professional training of future physicians.*

Objectives. A student should be able to *characterize the structure,, mechanisms of action, biological role, disturbances of the secretion of thyroid hormones and catecholamines; explain the biochemical and some physiological effects of prostaglandins, prostacyclins, thromboxanes and leukotrienes.*

Main tasks. A student should be able:

1. To explain the chemistry of synthesis of T_3, T_4, epinephrine and norepinephrine.

2. To analyze the mechanisms of action and regulation of secretion of thyroid hormones.

3. To characterize molecular-cellular mechanisms of action and biological role of catecholamines.

4. To explain the metabolic changes and clinical manifestations of hypo- and hypersecretion of hormones of the thyroid gland and the adrenal glands.

5. To explain the mechanisms of synthesis and the biological role of eicosanoids.

6. To interpret the results of the qualitative definition of thyroxine and epinephrine.

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Theoretical questions

1. Hormones of the thyroid gland: structure, biosynthesis, the mechanism of action, biochemical effects, disorders of T_3 and T_4 secretion.
2. Adrenal medullar hormones: structure, biosynthesis, the mechanisms of action, metabolic role, disorders of hormones secretion.
3. Biogenic amines – dopamine, serotonin, melatonin, histamine: structure, biosynthesis, biochemical and physiological effects.
4. Eicosanoids: classification, chemistry, biosynthesis and catabolism.
5. Functions of prostaglandins, prostacyclins, thromboxanes, leukotriens and lipoxine. Clinical aspects.
6. Inhibitors and activators of prostaglandin synthesis.

laboratory work

1. A qualitative reaction to thyroxine

Principle of the method. As result of thyroidin destruction the potassium iodide is formed, which releases free iodine under action of potassium iodate. The free iodine gives a blue color to the starch:

$$5\,KI + KIO_3 + 3\,H_2SO_4 \rightarrow 3\,I_2 + 3\,K_2SO_4 + 3\,H_2O;$$

$$I_2 + starch \rightarrow blue\ color$$

Course of work. Add 1 ml of thyroxine containing solution, 3 drops of 1 % starch solution, 1 drop of phenolphthalein and 5 drops of 2 % KIO_3 solution to the test tube. Than add 10 - 15 drops of 10 % H_2SO_4 solution until the pink color disappears. The free iodine released is blue colored with starch.

2. A qualitative reactions to epinephrine

2.1. Reaction with ferric chloride

Principle of the method. Epinephrine (adrenaline) is oxidized in the air with the formation of red-colored adrenochrome. As result of the interaction of adrenochrome with ferric chloride a green-colored compound is formed.

Course of work. Add 10 drops of epinephrine containing solution and 1 drop of ferric chloride solution ($FeCl_3$) to the test tube. A green color appears. Add 3 drops of 10 % NaOH solution and observe a red color appearance.

2.2. Diazoreaction

Principle of the method. The interaction of epinephrine with diazo reagent results in the formation of a red-colored complex compound.

Course of work. Add 10 drops of epinephrine containing solution, 10 drops of diazo reagent solution (a mixture of 0.5 % $NaNO_3$ and 0.5 % sulfanilic acid solutions) to the test tube. Then add 3 drops of 1 % NaOH solution and observe the appearance of a red color.

The results of experiments 1–2 should be written in a table:

Test tube number	Reaction result			Conclusions
	1	2.1	2.2	

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 37
Theme: THE BIOCHEMICAL EFFECTS OF STEROID HORMONES. HORMONAL REGULATION OF CALCIUM AND PHOSPHATE HOMESOSTASIS

Actuality of theme. *Steroid hormones have a wide range of biochemical and physiological effects, the knowledge of which is necessary for the diagnosis and treatment of endocrine diseases, such as Itsenko – Cushing disease / syndrome, Addison disease and others. A disfunctions of the synthesis and secretion of parathormone, calcitonin and calcitriol are common. Therefore, the study of the hormones biochemistry that regulate the metabolism of calcium and phosphates is important for medical students.*

Objectives. A student should be able to *explain the molecular-cellular mechanisms of action, biochemical and some physiological effects of steroid hormones and hormones that are involved in the regulation of the metabolism of calcium and phosphates.*

Main tasks. A student should be able:

1. *To characterize the biochemical and some physiological effects of parathormonone and calcitonin.*
2. *To explain the biosynthesis of calcitriol, its effect on the metabolism of calcium and phosphates.*
3. *To explain the sequence of reactions to the synthesis of steroid hormones.*
4. *To characterize the biochemical and some physiological effects of steroid hormones.*
5. *To analyze the metabolic changes and clinical manifestations of disorders of steroid hormones secretion: Itsenko-Cushing disease, Addison disease.*
6. *To interpret the results of determining the concentration of calcium in the blood serum.*

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Theoretical questions

1. Steroid hormones: classification, biosynthesis, the mechanism of action.
2. Glucocorticoids: the biochemical effects, disorders of secretion.
3. Mineralocorticoids: the biochemical effects, disorders of secretion. Renin – angiotensin – aldosteron system.
4. Androgens, estrogens and progesteron: the biochemical and physiological effects, regulation of synthesis and secretion, disorders of secretion. Clinical application of analogs and antagonists of hormones of the gonads.
5. Biological role of Ca^{2+} in the body; molecular forms of calcium in human plasma. The role of bone tissue, small intestine and kidneys in calcium homeostasis.
6. Hormones that regulates calcium and phosphate homeostasis – calcitonin, parathyroid hormone, 1.25-dihydroxycholecalciferol (calcitriol): metabolism, the mechanism of action, the biochemical and physiological effects, disorders of secretion.
7. Clinical and biochemical characteristics of disturbances of calcium homeostasis (rickets, osteoporosis).

laboratory work

1. The definition of Ca^{2+} concentration in blood serum

Principle of the method. In alkaline medium Ca^{2+} react with o-crezolftaleincomplekson, forming a complex of violet color. The color intensity is proportional to the concentration of Ca^{2+} in the sample.

Course of work. Add all reagents in accordance with the table to the 3 test tubes:

Reagent	Sample, ml		
	test	standart	control
Chromogen	1.00	1.00	1.00
Blood serum	0.02	–	–
Standard Ca^{2+} solution	–	0.02	–
Buffer solution	1.00	1.00	1.00
H_2O	–	–	0.02

Wait for 10 min at 20–25°C, measure the optical density of the test and standard samples against the control sample at a wavelength of 500–600 nm (green filter).

The calculation should be made in accordance with the formula:

$$X = \frac{E_{test} \cdot C_{st}}{E_{st}},$$

where

X is concentration of Ca^{2+}, mmol/l;

E_{test} is extinction of test sample;

E_{st} is extinction of a standard sample;

Cst is the concentration of Ca^{2+} in the standard solution, (2.5 mmol/l).

Diagnostic value of clinical tests. The normal value of Ca^{2+} concentration in the blood serum is 2.15–2.5 mmol/l.

Hypercalciemia occurs in case of bone destruction, hypervitaminosis D. Hypocalcemia is observed in hypofunction of the parathyroid glands, hypovitaminosis D, impaired renal function, malabsorption.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lessons 38–39
Theme: EXAMINATION SUBMODULE 3 "METABOLISM OF PROTEINS. MOLECULAR BIOLOGY. BIOCHEMISTRY OF INTERCELLULAR COMMUNICATIONS"

Actuality of the theme. *Studying of the module topic questions is important for getting of fundamental knowledge in biochemistry of molecular-cellular processes. That's why a lesson for control of information and practical skills mastering is introduced, which allows estimating students' level of competence on issues relating to protein metabolism, molecular biology, biochemistry of hormones.*

Objectives. A student should be able to *systemize and formulate distinctly one's knowledge, use it in solving situational tasks and interpreting of clinical laboratory research data of some blood and urine values.*

Main tasks. A student should be able:

1. *To answer the questions of content module topics correctly and sequentially.*
2. *To characterize chemical transformations of amino acids denoting the names of enzymes.*
3. *To explain chemical transformations of nucleotides.*
4. *To describe the stages in replication, transcription and translation.*
5. *To explain molecular-cellular mechanisms of action of hormones.*
6. *To explain the biochemical changes in case of disorders of amino acids and nucleotides metabolism.*
7. *To explain biochemical consequences of hormone synthesis disorders.*

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Theoretical questions

1. Pathways of formation and using of a pool of free amino acids in the human body.
2. Pathways of catabolism of free amino acids to end products.
3. Deamination of amino acids: types of deamination, sequence of reactions. Glutamate dehydrogenation reaction, its value and regulation.
4. Transamination of amino acids: reactions, biochemical value, the mechanism of action aminotransferases.
5. The mechanism of indirect deamination of amino acids.
6. Decarboxilation of amino acids: enzymes, physiological value. Oxidation of biogenic amines.
7. Diagnostic significance of definition of aminotransferases activity.
8. The sources of ammonia in an organism.
9. Toxicity of ammonia and pathways of its neutralization. Transportation of ammonia.
10. Biosynthesis of urea: a biological role, regulation, localization, sequence of reactions.
11. Interrelation of the ornithine cycle with transformation of fumarate and aspartic acids.
12. Metabolism of the carbon skeletons of amino acids. Glycogenic and ketogenic amino acids.
13. Metabolism of aromatic and heterocyclic amino acids.
14. Metabolism of sulfur containing amino acids. Biological role of SAM.
15. Synthesis of creatine and creatinine. Diagnostic significance of definition of creatinine in blood serum.
16. Biological role of glutathione.
17. Metabolism of arginine. Formation and biological role of NO.

18. Metabolism of branched-chain amino acids. Biological role of vitamins B_{12} and H in metabolism of amino acids.
19. Metabolism of glycine and serine. Biological role of tetrahydrofolate in metabolism of amino acids.
20. Disorders of amino acids metabolism (phenilketonuria, alcaptonuria, albinism, maple syrup urine disease).
21. Disorders of amino acids metabolism (Hartnup′s disease, histidinemia, cystinuria and homocystinuria).
22. Sources of separate atoms in the purine ring.
23. Synthesis of purine nucleotides de novo: localization, sequence of reactions, regulation. Biosynthesis of AMP, GMP, ATP, GTP.
24. Pathways of purine bases reutilization in the tissues.
25. Synthesis of pyrimidine nucleotides: sequence of reactions, regulation, biosynthesis of deoxyribonucleotides.
26. Degradation of purine and pyrimidine nucleotides.
27. Disorders of nucleotide metabolism: gout, Lesch-Nyhan syndrome, orotic aciduria.
28. Replication of DNA: mechanism, enzymes.
29. Transcription: stages and mechanism.
30. Processing of RNA. Role of snRNA in RNA splising.
31. Inhibitors of RNA synthesis: actinomycin D, rifampicin, streptolydigin, α-Amanitin.
32. Genetic code: properties, table of genetic code.
33. Translation: basic components of the protein synthesis system, stages and mechanism.
34. Posttranslational modification of proteins.
35. Antibiotics as inhibitors of protein synthesis.
36. Regulation of protein synthesis in prokaryotes: induction and repression.
37. Regulation of protein synthesis in eukaryotes: repression of initiation synthesis of globin.
38. Biotechnology involving recombinant DNA.
39. Molecular mechanisms of DNA repair. Repair of UV-induced gene mutations; xeroderma pigmentosum. Clinical correlation of molecular disease.

40. Hormones and bioregulators in the system of intercellular integration of functions in an organism and their chemical nature.
41. Classification of hormones.
42. Mechanism of regulation of hormones synthesis and secretion.
43. Targets organs, receptors and second messengers of hormones.
44. Mechanism of action of polypeptide hormones and epinephrine.
45. Mechanism of action of steroid and thyroid hormones.
46. Radioimmunologycal method of definition of hormone concentration in blood.
47. Hormones of hypothalamus: structure, mechanism of action, biological role.
48. Growth hormone (somatotrophin) is hormone of anterior pituitary gland: structure, metabolic role, regulation and disorder of hormone secretion.
49. Tropic hormones of pituitary gland are prolactin (mammotrophin), gonadotrophins – follicle stimulating hormone (FSH), luteinizing hormone (LH): metabolic role, mechanism of action, regulation and disorder of hormones secretion.
50. Thyrotrophin (TSH) is tropic hormone of pituitary gland: metabolic role, mechanism of action, regulation and disorder of hormones secretion.
51. Adrenocorticotrophic hormone (ACTH) is tropic hormone of pituitary gland: metabolic role, the mechanism of action, regulation and disorder of hormones secretion.
52. Biological role of the products of processing of Pro-opiomelanocortin (POMC).
53. Hormones of posterior pituitary gland are vasopressin and oxytocin: mechanism of action, metabolic role, clinical importance.
54. Insulin is hormone of pancreas: structure, biosynthesis and catabolism, mechanism of action, metabolic role, clinical aspects.
55. Glucagon is hormone of pancreas: structure, biosynthesis and catabolism, mechanism of action, metabolic role.
56. Hormones that regulate the utilization of nutrients are gastrin, secretin, cholecystokinin.

57. Thyroid gland and its hormones: structure, biosynthesis, mechanism of action, biological effects of T_3, T_4, and clinical importance.
58. Adrenal medullar hormones: structure, biosynthesis, mechanism of action, metabolic role of catecholamines.
59. Eicosanoids: classification, chemistry, biosynthesis and catabolism.
60. Functions of prostaglandins, prostacyclins, thromboxanes, leukotriens and lipoxine. Clinical aspects.
61. Inhibitors and stimulators of prostaglandin synthesis.
62. Hormones of adrenal cortex. Glucocoricoids: mechanism of action, biological role, disorders of secretion.
63. Hormones of adrenal cortex. Mineralocorticoids: mechanism of action, biological role, disorders of secretion. Renin-angiotensin-aldosterons system.
64. Androgens, estrogens and progesterone: mechanism of action, metabolism, biological role, disorders of secretion.
65. Hormones that regulate Ca^{2+} and PO_4^{3-} metabolism: parathyroid hormone, 1,25-Dihydroxycholecalciferol, calcitonin.
    Metabolism, mechanism of action, biological role, disorders of secretion.

Lesson 40
Theme: WATER SOLUBLE VITAMINES: B_1, B_2, B_5, B_6, B_{12}. FUNCTIONAL ROLE IN METABOLISM

Actuality of the theme. *Water-soluble vitamins, which transform in coenzymes, play an important role in functioning of enzyme system. The knowledge of water-soluble vitamins biochemistry is essential for future doctors for diagnosis, correction and therapy of the avitaminosis, hypo- and hypervitaminosis.*

Objectives. A student should be able to *explain the role of coenzyme vitamins B_1, B_2, B_3, B_6, B_{12} in metabolic processes in a human organism.*

Main tasks. A student should be able:

1. *To explain the bochemical role of vitamins B_1, B_2, B_5, B_6, B_{12} in metabolism of proteins, lipids and carbohydrates.*

2. *To characterize biochemical changes of metabolism in case of avitaminosis and hypovitaminosis of B_1, B_2, B_5, B_6, B_{12}*
3. *To define the vitamins B_1 and B_2.*
4. *To write chemical structure of vitamins B_1, B_2, B_6 and their coenzymes.*

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Theoretical questions

1. The general characteristic of vitamins as components of a diet.
2. Classification and the nomenclature of vitamins.
3. Biological role of vitamins in a human organism. Coenzyme function of vitamins.
4. Avitaminosis, hypovitaminosis, hypervitaminosis: causes, general principles of prevention and treatment.
5. Biochemical characteristics of water-soluble vitamins (B_1, B_2, B_5, B_6, B_{12}): chemical structure, metabolism, biochemical role, coenzymes, daily requirement, the basic dietary sources, deficiency manifestations, clinical application.

Laboratory work

1 The definition of vitamin B_1 (thiamine)

1.1. The oxidation reaction

Principle of the method. Thiamine is oxidized to thiochrome by potassium ferrocyanide in an alkaline medium. Thiochrome has blue fluorescence under ultraviolet irradiation of the solution.

Course of work. Add 1 drop of 5 % vitamin B_1 solution, 5–10 drops of 10 % NaOH solution and 1–2 drops of 5 % potassium ferrocyanide to the test tube. All reagents are mixed. The fluoroscope is heat up for 10 min and blue fluorescence under ultraviolet irradiation is observed.

1.2. Diazoreaction

Principle of the method. Thiamine react with a diazoreagent to form a coordination complex of orange color in an alkaline medium.

Course of work. Add 1–2 drops of 5 % thiamine solution to diazoreagent, which consists of 15 drops of 1 % sulphanilic acid solution and 5 drops of 5 % sodium nitrate solition. Then 5–7 drops of 10 % sodium bicarbonate solution are carefully taken to the test tube walls. An orange circle appears on the boundary of two fluids.

2. The reaction to vitamin B_2 (riboflavin)

Principle of the method. Vitamin B_2 has redox properties. As result of conversion of reduced riboflavin to the oxidized color of solution changes from yellow (riboflavin) to red (rodoflavin) and colorless (leukoflavin). The conversion of reduced vitamin B_2 form to the oxidized occurs under action of concentrated hydrochloric acid in the presence of zinc.

Course of work. Add 1 ml of riboflavin solution, 0.5 ml of concentrated HCl and a small piece of metal zinc to the test tube. The change of solution color is observed.

3. The reaction to vitamin B_6 (pyridoxine)

Principle of the method. Vitamin B_6 reacts with ferric chloride to form a red-colorated coordination complex of ferric phenolate type.

Course of work. Add 5 drops of 1 % $FeCl_3$ solution and 5 drops of 1 % solution of vitamin B_6 to the test tube. The fluid is coloured in red.

The results of experiments 1–2 should be written in a table:

Test tube number	Reaction result			Conclusion
	1.1.	1.2.	2	

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 41
Theme: MECHANISMS OF ACTION AND BIOCHEMICAL EFFECTS OF VITAMIN C, PP, H, B_C, P. METHODS FOR DETERMINATION OF VITAMIN C

Actuality of the theme. *The study of biochemical effects of such vitamins as C, PP, H, B_C, P is necessary for understanding of mechanisms and consequences of pathologic and physiological states, in which these vitamins are involved. Besides, the knowledge in biochemistry of vitamins is essential for diagnosis, correction and therapy of diseases such as scurvy, pellagra .*

Objectives. A student should be able to *characterize biochemical functions of vitamins C, PP, H, B_C ,P and explain metabolic changes in case of hypo- and avitaminosis of these vitamins.*

Main tasks. A student should be able:

1. *To explain biological functions and metabolic role of vitamins C, PP, H, B_C, P.*
2. *To characterize metabolic changes and clinical manifestations of hypo- and avitaminosis of C, PP, H, B_C, P.*
3. *To write chemical structure of vitamins PP, H, B_C and their coenzymes.*
4. *To define vitamin C concentration and interpret results.*

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Theoretical questions

1. Coenzyme vitamins B_5, H, Bc: chemical structure, biochemical functions, the basic dietary sources, daily requirement, deficiency manifestations, clinical significance.
2. Biochemical characteristic of vitamins C and P: chemical structure, biological role, the basic dietary sources and daily requirement, manifestations of hypovitaminosis.
3. Practical use of vitamins.
4. The relationship between certain vitamins in an organism.
5. Methods for the definition of vitamin C.

Laboratory work

1. Qualitative reactions to vitamin C

Principle of the method. The definition of vitamin C is based on its redox properties.

1.1 Reaction with 2,6-dichlorophenolindophenol

Course of work. Add 10 drops of 0.01 % 2.6-dichlorphenol-indophenol solution, 1 drop of 2 % HCl to the test tube; the fluid is coloured in red. Add 5 drops of ascorbic acid solution to the test tube; the fluid is discolored.

1.2 Reaction with iodine

Course of work. Add 10 drops of distilled water and 1–2 drops of 0.1 % Lugol's iodine (solution of I_2 in KI) to the two test tubes. In the first test tube add 10 drops of water, in the second – 10 drops of ascorbic acid solution. The iodine solution in the second test tube is discolored.

2. The reaction to vitamin PP (nicotinic acid)

Principle of the method. Vitamin B_3 reacts with copper acetate to form a blue precipitate (copper salt of nicotinic acid).

Course of work. 5–10 mg of vitamin B_3 are dissolved in 3 ml of 10 % acetic acid solution when heated. The solution is heated till boiling and 3 ml of copper acetate solution are added. The fluid is coloured in blue, copper salt of nicotinic acid precipitates.

3. The reaction to vitamin P

Principle of the method. Vitamin P reacts with ferric chloride to form a green-colorated coordination complex.

Course of work. Add 0.5 ml of 1 % $FeCl_3$ solution and 1 ml of vitamin P solution to the test tube. Green colouration appearance is observed.

The results of experiments 1–2 should be written in a table:

Test tube number	Reaction result			Conclusion
	1.1.	1.2.	2	

4. Definition of vitamin C concentration in the urine

Principle of the method. The definition of vitamin C is based on its ability to reduce 2, 6-dichlorphenolindophenol. This pigment has red color in acidic medium, blue – in alkaline medium and colorless – by reduction.

Course of work. Add 10 ml of urine, 10 ml of distilled water and 20 drops of 10 % HCl into the titration flask. The solution is titrated with 0.001 M 2.6-dichlorphenolindophenol till pink color appears.

The calculation of vitamin C concentration in urine should be made in accordance with the formula:

$$X = \frac{0{,}088 \cdot A \cdot B}{V},$$

where

X is ascorbic acid concentration in the urine, mg/day;

0,088 is recalculating index (1 ml of 0.001 M 2.6-dichlor-phenolindophenol solution is equavalent to 0.088 mg of ascorbic acid);

V is urine volume, taken for titration (10 ml);

B is daily urine volume (1500 ml for men, 1200 ml for women);

A is volume of 0.001 M 2.6-dichlorophenolindophenol, ml.

Diagnostic value of clinical tests. Normally, 20–30 mg or 113.5–170.3 μmol of ascorbic acid is excreted daily. There is a correlation between the concentration of vitamin C in the blood and

urine. The quantifying of vitamin C in the urine informs about supply of organism with it.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 42
Theme: BIOCHEMICAL EFFECT AND METHODS FOR DETERMINING THE FAT-SOLUBLE VITAMINS. DETERMINATION OF MACRO – AND TRACE ELEMENTS IN BIOLOGICAL MATERIAL

Actuality of the theme. *Fat-soluble vitamins have regulatory, antioxidant and hormonal properties. They can accumulate in the body. Excess amount of vitamins in the diet can lead to hypervitaminosis.*

Vitamin-similar substances can produce in various metabolic processes or coming from food. Their failure also leads to development of hypovitaminosis.

Inorganic substances are integral components of living organisms. Water is the largest part of all inorganic substances in cells. It forms the basic medium for vital physicochemical and biochemical processes. Electrolytes maintain homeostasis of the internal environment of the organism. They form complexes with macromolecules and low molecular weight substances and their composition are involved in the implementation of various functions of cells and tissues.

Disorder the normal functioning of homeostatic mechanisms leads to manifestation of clinical symptoms of changes in osmotic pressure and volume of fluid in the body, as well as development of deficiency diseases and diseases of accumulation of certain minerals.

Objectives. A student should be able to *characterize the biological functions of some fat-soluble vitamins, vitamin-similar substances and explain the biochemical causes of hypo- and hypervitaminosis; characterize the biological functions of water and some macro- and microelements; explain the mechanisms of regulating homeostasis of inorganic substances; explain the causes of deficiency diseases and diseases of accumulation of trace elements; qualitatively determine the fat-soluble vitamins, macro-and trace elements in biological objects.*

Main tasks. A student should be able:

1. *To characterizethe biological properties, sources, daily requirement and role of fat soluble vitamins and vitamin-similar substance in metabolism.*
2. *To interpret the concept of pro-vitamins and explain the mechanism of their conversion to the active forms.*
3. *To write down the chemical formulas of vitamins (A, E, K, D, F).*
4. *To explain the causes and analyze the molecular and biochemical mechanisms of hypo- and hypervitaminosis.*
5. *To characterize the antioxidant properties of fat-soluble vitamins.*
6. *To explain the mechanisms of action of antivitamins and their use in medicine.*
7. *To characterize the biological role of water and some macro- and microelements in the metabolic processes in the body.*
8. *To explain the mechanisms of regulation and causes of disturbance of water-salt metabolism.*
9. *To characterize the manifestations of diseases of macro- and microelements deficiency.*
10. *To interpret the results of the qualitative and quantitative determination of fat-soluble vitamin, Ca^{2+}, Mg^{2+} and phosphate in serum, make conclusions according to the norm.*

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Theoretical questions

1. Biochemical characteristic of the fat-soluble vitamins (A, D, E, K, F): chemical structure, biological properties, the daily requirement, sources, role in metabolism, metabolism and mechanism of action.
2. Deficiency diseases of fat-soluble vitamins, hypervitaminosis.
3. The antioxidant properties of fat-soluble vitamins.
4. Vitamin-similar substances: structure and role in metabolism.
5. Antivitamins: mechanisms of action, use in medicine.
6. The biological role of water and its redistribution in the body.
7. Regulation and disturbance of water-salt balance.
8. Biological functions of macroelements (Na, K, Ca, Mg, P) and manifestations of deficiency.
9. The role of trace elements in human nutrition, manifestations of trace elements deficiency.
10. Biological role and metabolism of iron in the body.

Laboratory work

1. Detection of vitamin A

1.1. Reaction with sulfuric acid

Principle of the method. Sulfuric acid as a dehydrating factor helps to transform of vitamin A in the purple-red compound. Reaction is nonspecific.

Course of work. Two drops of fish oil is applied to the dry glass slide and add one drop of sulfuric acid (concentrated). Red-violet color appears, which changes to red-brown.

1.2. Reaction with ferrous sulfate

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity (drop)
Fish oil or 0,05 % vit.A (in chloroform)	1–2
Glacial acetic acid saturated with iron sulfate	5–10
H_2SO_4 (concentrated)	1–2
You can observe the occurrence of blue color, which gradually changes to pink	

2. Detection of vitamin E

2.1. Reaction with ferric chloride

Principle of the method: alcohol solution α-tocopherol oxidized by ferric chloride (Fe^{+3}) in tocopheryl quinone, which is red.

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
α-Tocopherol, 0.1 %	4–5 drops
$FeCl_3$, 1 %	0.5ml
You can observe the occurrence of red color	

2.2. Reaction with ferric chloride

Principle of the method. Tocopherol is oxidized by concentrated nitric acid to form a product with quinoid structure. The reaction mixture is colored in red. This reaction is used for the quantitative determination of vitamin E

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
α-Tocopherol, 0.1 %	5 drops
Sucrose	few crystals
HNO_3 (concentrated)	10 drops
You can observe the occurrence of red color	

3. Determination of vicasol

Principle of the method. Vicasol solution in alkaline medium in the presence of cysteine is colored in lemon-yellow.

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
Vicasol, 0.05 %	5 drops
Cysteine, 0.025 %	5 drops
NaOH, 10 %	5.0 ml
You can observe the occurrence of lemon-yellow color	

4. Bromchloroform test for vitamin D

Principle of the method. The interaction of vitamin D with a solution of bromine in chloroform (1:60) appears greenish-blue color.

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
Fish oil, ml	1
Bromine in chloroform (1:60), ml	2
You can observe the occurrence of green color	

Note. Practical works 1.1–4 you should perform as research. The results should be formed in the table.

Number of test tube	Results of reaction						Conclusion about the presence of vitamins
	1.1	1.2	2.1	2.2	3	4	

5. Qualitative reactions on Ca, Mg, P in blood serum

Principle of the method. Qualitative reactions on mineral elements based on the formation of precipitates of insoluble salts and colored compounds:

а) $Ca^{2+} + C_2O_4^{2-} \rightarrow CaC_2O_4$;

precipitate of calcium oxalate

б) $Mg^{2+} + NH_4OH + HPO_4^{2-} \rightarrow MgNH_4PO_4 + H_2O$;

precipitate of magnesium ammonium phosphate

в) $PO_4^{3-} + MoO_4^{2-} + NH_4^+ + nH^+ \rightarrow (NH_4)_3PO_4\ 12\ MoO_3\ 6\ H_2O$.

yellow crystals of phosphomolybdic acid

Phosphomolybdic acid is reduced by ascorbic acid to "molybdenum blue" (a mixture of various oxides of molybdenum).

5.1. Identification of Ca^{2+} ions

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
Blood serum, ml	0.5–1
Ammonium oxalate (saturated solution), drop	3–4
You can observe the occurrence of calcium oxalate precipitate CaC_2O_4	

5.2. Identification of Mg^{2+} ions

Course of work. The precipitate of calcium oxalate, which is obtained in the previous experiment, filtered. To the filtrate add 3 – 4 drops of concentrated ammonia solution. We observe sedimentation of double salts of magnesium (magnesium ammonium phosphate).

5.3. Identification of PO_4^{3-}

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
Blood serum	0.5–1 ml
Solution of ammonium molybdenum	5–6 drops
You can observe the formation of a yellow precipitate of phospho-molybdic acid. Add a few drops of 1 % solution of ascorbic acid. Solution in a test tube is painted in intensive blue color. Molybdenum blue formed	

6. Determination of inorganic phosphates in blood serum

Principle of the method. Determination of phosphorus is based on measuring the intensity of color of "molybdenum blue", which appears in the recovery of phospho-molybdic acid in an acidic medium. Eykonogen, ascorbic acid and other reagents are used as reducing agents.

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Sample, ml	
	Test	Standard
Blood serum	1	
Standard solution of phosphate		1
H_2O	4	4
Trichloroacetic acid, 10 %	5	5
After 10 min centrifuge at 3000 r/min for 10 min. Five mililiters of protein-free filtrate taken from both test tubes and add:		
Molybdic acid ammonium, 5 %	1	1
Eykonogen or ascorbic acid, 1 %	0.2	0.2
H_2O	1.8	1.8
Optical density of both samples should be measured after 20 min against the water at 630–690 nm (a red optical filter) in ditches with thickness of a layer of 10 mm		

The calculation is carried out according to the formula:

$$X = (E_{test} \cdot C_{st}) : E_{st},$$

where

X is phosphate concentration in blood serum, mmol/l;

E_{test} is the optical density of experimental test;

E_{st} is the optical density of standard test;

C_{st} is phosphate concentration is the standard test, mmol/l (with a known concentration).

Diagnostic value of clinical tests. The normal amount of inorganic phosphate in serum is 0.646–1.292 mmol/l.

Index increased under renal failure, hypoparathyroidism, an overdose of vitamin D; reducing phosphate observed at malabsorption, rickets, renal diseases.

7. Determination of magnesium in serum

Principle of the method. Magnesium reacts in alkaline medium with an indicator (kalmagit) and forms a colored complex which is determined photometrically at a wavelength of 520 nm.

Course of work. Add all reagents in accordance with the table to the test tube:

Reactants	Test sample, ml	Standard sample, ml	Cheek sample, ml
Working solution	3	3	3
Blood serum	0.05	–	–
Standard solution of Mg^{2+}	–	0.05	–
H_2O	–	–	0.05
Note. *Optical density of test and standard samples should be measured after 5 min against the cheek samplel at 520 nm in ditches with thickness of a layer of 10 mm*			

Magnesium content is calculated by the formula:

$$[Mg^{2+}] = (E_{test} \cdot\cdot\ 0{,}823) / E_{st},$$

where

E_{test} and E_{st} are the optical density of experimental and standard sample with the concentration of magnesium 0.823 mmol/l (20 mg/l), respectively.

Diagnostic value of clinical tests. The normal concentration of magnesium in blood serum is 0.78–0.98 mmol/l.

Reducing the concentration of magnesium in serum observed in toxemia of pregnancy, cancer, chronic heart failure, acute and chronic pancreatitis.

Increasing magnesium content observed in anuria, chronic renal failure and in renal disease, which is accompanied by hyperkalemia.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 43
Theme: PHYSIOLOGICAL AND BIOCHEMICAL FUNCTIONS OF BLOOD: BUFFER SYSTEMS, ACID-BASE STATUS. RESPIRATORY FUNCTION OF ERYTHROCYTES

Actuality of the theme. *Blood is a liquid tissue, which is due to transportation of chemical substances makes the integration of biochemical processes in various cells and intercellular spaces in a single system.*

Pathological conditions accompanied by disorders of systemic hemodynamics and microcirculation, lead to profound disturbances of physiological and biochemical functions.

Objectives. A student should be able to *characterize basic physiological and biochemical function of blood, the mechanisms of maintaining its basic parameters.*

Main tasks. A student should be able:

1. *To characterize respiratory, trophic, excretory, protective and regulatory functions of blood.*
2. *chematically the structure of various types of human haemoglobin, to characterize their properties and mechanisms of participation in the transport of oxygen and* CO_2.
3. *To explain the molecular causes of haemoglobinopathies and thalassemies.*
4. *To characterize the features of metabolic processes in the erythrocyte.*
5. *To classify buffer systems of blood and explain the mechanisms of regulation and maintenance of acid-base status.*
6. *To evaluate acid-base status of the human body based on parameters that are investigated in the clinic.*
7. *To explain the causes and mechanisms of acidosis, alkalosis, hypoxia and techniques of laboratory diagnosis.*

8. *To interpret the results of determination of haemoglobin and its compounds in blood serum.*

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Theoretical questions

1. General characteristics of the physiological and biochemical functions of blood.
2. Respiratory function of erythrocytes. Haemoglobin: structure and properties. Participation of haemoglobin in transportation of oxygen and carbon dioxide.
3. Pathobiochemistry of haemoglobin: variants and pathological forms. Disorders of haemoglobin structure: thalassemies and haemoglobinopathies.
4. Acid-base balance (ABB) of the human body. Mechanisms of regulation and support of ABB: buffer systems of blood, lung and kidney function.
5. The forms of disorders of acid-base balance and indexes of ABB.
6. Metabolism in an erythrocyte.
7. Types of hypoxia, the mechanisms of its occurrence, laboratory diagnostics.

Laboratory work

1. Determination of haemoglobin in the blood by haemoglobincyanide method

Principle of the method. Haemoglobin is oxidized by red blood salt to methaemoglobin (Hb-OH), which reacts with acetonecyanohydrin and forms a colored cyanmethaemoglobin. Color intensity of solution is proportional to haemoglobin content.

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity
Blood	0.02 ml
Transforming solution (dilution in 251 times)	5 ml
The test tube contents mix. Optical density of solution should be measured after 10 min against the control at 540 nm (a green filter) in ditches with thickness of a layer of 10 mm. Haemoglobin cyanide standard solution used undiluted. Measured under the same conditions as the experimental sample	

Calculation of haemoglobin content carried by the formula:

$$Hb = \frac{E_{exp}}{E_{st}} \cdot C_{st} \cdot K \cdot 0{,}01,$$

where

Hb is content of haemoglobin, g %;

E_{exp} and E_{st} are optical density of experimental and standard sample;

C_{st} is concentration of haemoglobin cyanide in the standard solution, 150 g/l or 59,75 mg %;

K is dilution factor of blood;

0.01 is coefficient for conversion, mg % in g/l.

Diagnostic value of clinical tests. The normal concentration of haemoglobin:

– males – 135–180 g/l;

– females – 120–140 g/l.

Anemia develops due to reduced haemoglobin in the blood. Sharp decline of this index is incompatible with life through oxygen starvation and disturbance of tissue metabolism. Haemoglobin concentration is significantly reduced when acute hemorrhage, hypoplastic anemia under hemolytic crisis. Haemoglobin concentration decreased more than the increase. The increase in

haemoglobin in the blood can be seen in erythremia, cardiac decompensation, myeloproliferative diseases.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Tasks for practical work

Fill in the tables:

Table 1 – Buffer systems of blood

No	Name of the buffer system	Components	The mechanism of action	
			at pH < 7	at pH > 7

Table 2 – Parametrs of acid-base balance (ABB)

No	Acid-base status	Blood parametrs		Change in pH relative to norm	Diseases
		HCO_3^-	ρCO_2		

Lesson 44
Theme: PLASMA PROTEINS: ACUTE-PHASE OF INFLAMMATION PROTEINS, INDICATOR ENZYMES

Actuality of the theme. *Modern physico-chemical methods can identify and describe approximately 100 individual proteins in blood plasma that differ in their physical and chemical properties and functions. Blood proteins form a unified system with tissue proteins and reflect the state of protein metabolism in the whole body. Therefore, knowledge of biochemistry of blood plasma proteins is an important element of basic knowledge of future doctors.*

Objectives. A student should be able to *analyze the chemical composition of blood, explain the principles of the methods for determination of plasma proteins, characterize their properties and functions, interpret clinical diagnostic value of determination of individual plasma proteins.*

Main tasks. A student should be able:

1. *To explain the basic functions of blood proteins.*
2. *To characterize the biochemical properties of plasma proteins and their clinical significance.*

3. *To depict proteins electrophoregram and explain clinical diagnostic value of determination of individual protein fractions.*
4. *To interpret the concept of acute phase proteins of inflammation.*
5. *To characterize plasma enzymes and explain their significance in enzyme diagnostics.*
6. *To explain the biochemical basis of the system of regulation of blood pressure (kallikrein-kinin and renin-angiotensin system) and scientifically grounded use of antihypertensive medications - angiotensin-converting enzyme.*
7. *To interpret the results of determination of total protein and protein fractions of blood serum and explain the diagnostic value of these parameters.*

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Theoretical questions

1. Plasma proteins and their clinical-biochemical characteristics.
2. Separation of blood proteins on fractions. Proteinogram of blood proteins in normal and under pathological conditions.
3. Components of nonspecific resistance of the organism and proteins of "acute phase" of inflammatory processes.
4. Plasma enzymes and their importance in the enzyme diagnosis of diseases of internal organs.
5. Kallikrein-kinin system.

Laboratory work

1. Determination of serum total protein by biuret method

Principle of the method. Method is based on the formation of colored complex of peptide bonds of protein with copper sulfate in an alkaline medium. The complex has a violet color. The color intensity is directly proportional to the concentration of serum protein and determined photometrically.

Course of work. Add all reactants as shown in the table:

Reactants	Test sample, ml	Standard sample, ml	Cheek sample, ml
Blood serum	0.1	–	–
Standard solution of protein	–	0.1	–
Sodium chloride, 0.9 %	–	–	0.1
Biuret reagent	5.0	5.0	5.0
Note. *The test tube contents mix. Optical density of test sample and standard sample should be measured after 30 min against the cheek sample at 540 nm (a green optical filter) in ditches with thickness of a layer of 10 mm*			

Protein content is calculated by the formula:

$$C_{st} = \frac{E_{exp}}{E_{st}} \cdot C_{st},$$

where

C_{st} is content of protein in blood serum, g / l;

E_{exp} and E_{st} are optical density of experimental and standard sample;

C_{st} is concentration of protein in the standard solution.

Diagnostic value of clinical tests. Concentration of total protein in blood serum is 65–85 g/l. Increase of total protein in blood serum (hyperproteinemia) can be relative (water loss during burns, diarrhea) and absolute (myeloma, infections and rheumatic diseases).

Hypoproteinemia is to reduce the protein content in the serum. It occurs mainly due to the decrease in albumin and observed in nephrotic syndrome, liver damage, increased permeability of the vessel walls, protein deficiency.

2. Electrophoretic separation of blood plasma proteins into fractions

Principle of the method. Method is based on different mobility of proteins in an electric field according to charge and molecular weight of the protein.

Paper electrophoresis of serum proteins can allocate 5 fractions: albumin, α_1-, α_2-, β-, γ-globulins.

Diagnostic value of clinical tests. Electrophoretic separation of proteins is widely used in medical practice for the diagnosis of various diseases and the identification of individual serum proteins fractions.

Disproteinemia is a change in the ratio between the individual protein fractions. Reduction of albumin observed under liver and kidneys diseases. Quantity of γ-globulin increases in infectious processes and reduce of this fraction may indicate the AIDS. A significant reduction of albumin and increase of all globulin fractions occurs in malignant tumors.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Tasks for practical work

Fill in the tables:

Table 1 – Indicator blood enzymes

No	Name of enzyme	Place of synthesis	Type of metabolism	The reaction catalyzed by the enzyme	Diagnostic significance

Table 2 – Blood enzymes

No	Group of enzymes	Name of enzyme	Place of synthesis	Reaction catalyzed by the enzyme	Diagnostic significance
1	Secretory				
2	Indicator				
2.1	Organspecific enzymes				
2.2	Nonspecific				
3	Excretory				

Lesson № 45

Theme: BLOOD COMPOSITION: NON-PROTEIN ORGANIC COMPONENTS. PLASMA LIPOPROTEINS. COAGULATION AND FIBRINOLYTIC SYSTEMS OF BLOOD. PATHOLOGY OF HEMOSTASIS. BIOCHEMISTRY OF IMMUNE PROCESSES AND BIOCHEMICAL MECHANISMS OF IMMUNODEFICIENCY

Actuality of the theme. *Non-protein nitrogen and nonnitrous blood components are final products and intermediates of nitrogen, carbohydrate and lipid metabolism. The contents of these substances in the blood relatively stable and depends on the flow of various nutrients from food, and changes significantly in certain pathological conditions, which may be used in diagnosis.*

Hemostatic system prevents leakage of blood from damaged vessels. It is closely linked and equilibrated with anticoagulation and fibrinolytic systems. Disturbance of these systems is accompanied by thrombosis or hemorrhages.

The immune system provides identification, binding and destroying antigens of infectious and non-infectious origin by means of cellular and humoral mechanisms.

Objectives. A student should be able to *analyze the biochemical composition of blood; explain the diagnostic role of determining non-protein nitrogen-containing compounds, nonnitrous organic constituents of blood in normal and pathological conditions; analyze the state of human health on the basis of biochemical parameters of intermediate and final products of metabolism in the blood; explain biochemical mechanisms of coagulation, anti-coagulation, fibrinolytic and immune systems..*

Main tasks. A student should be able:

1. *To analyze the biochemical composition of blood, to characterize nonnitrouse and inorganic components of plasma and explain their diagnostic value.*
2. *To interpret the term "rest nitrogen" and to explain causes of development of different types of azotemia.*
3. *To classify plasma lipoproteins and explain changes in their composition during the circulation in the blood, to describe primary and secondary lipoproteinemias and principles of laboratory diagnosis dislipoproteinemias.*

4. *To explain the basic principles of work of hemostatic system in humans.*
5. *To characterize the individual components of the coagulation system and the mechanisms of activation and operation of the cascade system of blood coagulation, to explain the role of vitamin K in coagulation reactions and the effect of drugs - agonists and antagonists of vitamin K.*
6. *To characterize components of anti-coagulation and fibrinolytic systems of blood.*
7. *To characterize components of the immune system and the mechanisms of regulation of immunoglobulin synthesis, to explain the biochemical mechanisms of immunodeficiency*
8. *To determine rest nitrogen level and interpret results of research.*

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Theoretical questions

1. Non-protein nitrogen components of blood. Azotemia. Clinical, biochemical and diagnostic significance of determination of urea, creatine, creatinine and bilirubin concentration in blood serum.
2. Nonnitrous organic compounds of blood. Classes of lipoproteins. Lipoproteinemias. Inorganic blood components.
3. Functional and biochemical characteristics of the hemostatic system in humans. Characteristics of the main components of the

blood coagulation system: the blood clotting cascade, internal and external pathways of coagulation.

4. Role of vitamin K in coagulation reactions. Drugs - agonists and antagonists of vitamin K. Hereditary disturbances of blood coagulation.
5. Functional characterization of the components of the anti-coagulation blood system - heparin, antithrombin III, citric acid, prostacyclin. Role of vascular endothelium.
6. Fibrinolytic system of blood: the stages and components of fibrinolysis. Drugs with fibrinolytic activity.
7. Blood coagulation, thrombosis and fibrinolysis in atherosclerosis and hypertension.
8. General characteristics of the immune system, cellular and biochemical components. Immunoglobulins: structure, functions and mechanisms of regulation of synthesis and properties of individual classes.
9. Neurotransmitters and hormones of the immune system, cytokines. Biochemical components of the complement system: the classical and alternative mechanisms of activation.
10. Biochemical mechanisms of immunodeficiency; primary and secondary immunodeficiencies; Acquired Immune Deficiency Syndrome.

Laboratory work

1. Determination of rest nitrogen in blood serum

Principle of the method. Rest nitrogen of blood defines after precipitation of blood proteins with subsequent mineralization of protein-free filtrate with concentrated sulfuric acid. Nitrogen from all investigated fractions released in the form of ammonia, which is reacts with sulfuric acid to form ammonium sulfate. Yellow-orange compound is formed by the interaction of ammonium sulfate with Nessler reagent. The color intensity is proportional to the concentration of ammonia and thus rest nitrogen in the blood.

Diagnostic value of clinical tests. Increased of blood of rest nitrogen is called azotemia. Azotemia can be ***absolute*** (accumulation of components of rest nitrogen in blood) and ***relative*** (dehydration

due to vomiting, diarrhea). Absolute azotemia is divided into ***retention*** and ***productive***. It depends on the cause of the disease.

Retention azotemia occurs as a result of insufficient urinary excretion of nitrous compounds under normal admission of their in blood. Retention azotemia is divided into ***renal*** and ***extrarenal***. Renal azotemia is accompanied by an increase in the concentration of rest nitrogen in the blood due to disturbance of excretory renal function. This type of azotemia is observed in glomerulonephritis, pyelonephritis, renal tuberculosis and amyloidosis. Extrarenal retention azotemia caused by the violation of hemodynamics and accordance decrease in glomerular filtration. They arise as a result of cardiovascular decompensation, violating local blood flow in the renal arteries.

Productive azotemia arises on excessive admission of waste products of tissue proteins degradation into the blood. Renal function is not impaired. This type of azotemia observed under cachexia, leukemia, malignant tumors, glucocorticoid therapy.

Content of rest nitrogen is reduced under malnutrition and sometimes during pregnancy.

Tasks for practical work

Fill in the table:

Table 1 – Nonprotein blood components

No	Nonprotein components	Place of synthe-sis	Type of metabolism	Diagnostic significance	
				Increase	Decrease
	Nitrous				
	Nonnitrous				

Table 2 – Clotting factors

Index of factor	Name of factor	Place of synthesis	Mechanism of activation	Product of activation	Biological role

Lesson № 46
Theme: BIOCHEMICAL FUNCTIONS OF LIVER. DETERMINATION OF ACTIVITY OF SORBITOL DEHYDROGENASE AND γ-GLUTAMYLPEPTIDASE IN BLOOD SERUM

Actuality of the theme. *Liver plays a central role in the regulation and integration of biochemical homeostasis of the whole organism. Liver diseases are the most common pathology.*

Objectives. A student should be able to *explain the homeostatic role of the liver in the metabolism of the whole organism and biochemical basis of liver diseases.*

Main tasks. A student should be able:

1. *To explain the biochemical functions of hepatocytes.*
2. *To analyze the changes of biochemical parameters in acute and chronic liver damage.*
3. *To explain the relationship of disorders of excretory liver function with the gastrointestinal tract diseases.*
4. *To interpret the results to determine the activity of liver enzymes and determine their clinical and diagnostic value.*

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Theoretical questions

1. Homeostatic role of the liver in the metabolism of the whole organism. Biochemical functions of hepatocytes.

2. Carbohydrate liver function and its disorders.
3. Liver function in the regulation of lipid composition of the blood.
4. Liver function in the metabolism of proteins. Role of liver in the synthesis of urea.
5. Role of liver in the metabolism of vitamins and minerals.
6. Biochemical composition of bile and its formation in the liver. Role of liver in the metabolism of bile pigments.
7. Disorders of biochemical processes in the liver under some diseases.

Laboratory work

1. Determination of sorbitoldehydrogenase (SDH) activity in blood serum by unified method with resorcinol

Sorbitol dehydrogenase catalyzes the reversible conversion of sorbitol to fructose. The highest enzyme activity was found in the liver, much less - in the prostate, kidney, spleen.

Principle of the method. Sorbitol is converted to fructose by the enzyme in the presence of NAD^+. Fructose forms with a pinkish-red color with resorcinol, the intensity of which is proportional to the amount of fructose.

Diagnostic value of clinical tests. Normal enzyme activity up to 5.6 nmol/(s · l), or 0.02 mmol/(h · ml). A significant increase is observed in acute infectious hepatitis (5–10 times higher than normal).

A moderate increase is observed in toxic hepatitis (poisoning by Amanita phalloides, alcohol, drugs), exacerbation of chronic hepatitis, cirrhosis.

Determination of sorbitol dehydrogenase activity is important for the differential diagnosis of parenchymal and obstructive jaundice. Enzyme activity is normal under uncomplicated inflammation jaundice and dramatically increased in acute hepatitis.

This parameter is highly reliable and informative compared to other enzymes that are used for this purpose (alkaline phosphatase, 5-nukleotidase).

2. The sedimentary tests

2.1. Thymol test

Principle of the method. Pathologically elevated β-globulins, γ-globulins and lipoproteins precipitate from blood serum at pH = 7.55 of buffer solution, which saturated by thymol. The intensity of the turbidity of the solution depends on the content of protein fractions and their quantitative relationships.

Course of work. Add all reactants as shown in the table:

Reactants	Test sample, ml
Thymol reagent	4.8
Blood serum	0.08
Note. *The test tube contents mix. Optical density of test sample should be measured after 30 min against the thymol reagent at 630–690 nm. The turbidity of the solution is determined by the calibration graph.*	

Diagnostic value of clinical tests. Normal values are 0 – 4 unit S-H (according to Shank i Hoagland). Index increases under inflammation of the liver parenchyma and acute hepatitis.

2.2 Veltman's test

Principle of the method. Colloidal stability of blood proteins is disturbed by heating and adding $CaCl_2$.

Course of work. Add all reactants as shown in the table:

Reactants	Test sample, ml
H_2O	4.9
Blood serum	0.1
$CaCl_2$, 0,5 %	0.1
Note. *The test tube is shaken. Test tube heat to boiling of mixture. The test tube is cooled. If turbidity is absent, add 0.1 ml of 0.5 % $CaCl_2$ and boil again. The procedure is repeated until the appearance of white flakes. Results are expressed in quantities $CaCl_2$ ml.*	

Clinical diagnostic value. Normal values are 0.4 – 0.5 ml $CaCl_2$. Parameter increases under rheumatoid arthritis, pulmonary tuberculosis, decreases under malaria and parenchymal liver damage.

3. Determination of activity of γ-glutamyltranspeptidase (GGTP) in blood serum

Principle of the method. Gamma-glutamyltranspeptidase catalyzes the transfer of residue of L-glutamine from chromogenic

substrate on glycile glycine. N-nitroaniline is liberated in the reaction, its optical density is measured photometrically.

Course of work. Add all reagents in accordance with the table to the test tube:

Reactants	Test sample, ml	Cheek sample, ml
Substrate-buffer solution	0.5	0.5
Placed in the thermostat for 5 min at 37ºC		
Blood serum	0.05	–
Contents of the test tubes mixed and incubated 15 min at 37ºC		
Acetic acid, 10.0 %	3.0	3.0
Blood serum	–	0.05
Note. *Optical density of test sample should be measured against the cheek sample at a wavelength of λ = 400–420 nm in ditches with thickness of a layer of 5 mm. Enzyme activity is determined by the calibration graph.*		

Diagnostic value of clinical tests. Normal values of enzyme activity are:

– male 250–1767 nmol/s · l (0.9–6.4 μmol/h · ml);

– female 167–1100 nmol/s · l (0.6–0.4 μmol/h · ml).

The enzyme has maximum activity in liver, kidneys and pancreas. Enzyme activity is significantly increased under liver diseases with symptoms of obstruction of biliary tract and hepatitis. A moderate increase of the enzyme activity observed under liver tumor, chronic alcoholism, chronic renal failure, poisoning by hepatotropic poisons.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 47
Theme: THE ROLE OF LIVER IN THE METABOLISM OF BILE PIGMENTS. PATHOBIOCHEMISTRY OF JAUNDICE. BIOTRANSFORMATION OF XENOBIOTICS AND ENDOGENOUS TOXIC SUBSTANCES

Actuality of the theme. *Liver has important role in the catabolism of haemoglobin and other haemoproteins. Bilirubin and biliverdin are products of heme catabolism. They are produced by hepatocytes and excreted in the composition of bile through the intestine. Violation of bile*

pigments synthesis in the liver and disturbance of their excretion lead to the development of jaundice.

The most important function of liver is the detoxification of xenobiotics which could cause toxic, mutagenic, carcinogenic effects. Enzymatic systems of liver can support chemical homeostasis of the internal medium of the human body by transforming toxic compounds into less toxic molecular forms that can be excreted from the human body through the various excretory systems.

***Objectives. A student should be able to** characterize the role of liver in the metabolism of bile pigments, determine the basic biochemical parameters which can be used for the diagnosis of jaundice and interpret their results; characterize antitoxic function of the liver and explain the role of cytochrome P-450-containing electron transport chains in the biotransformation of xenobiotics.*

Main tasks. A student should be able:

1. *To explain the role of the liver in the metabolism of bile pigments.*
2. *To depict schematically catabolism of haemoglobin in the body.*
3. *To explain the causes of various types of jaundice.*
4. *To analyze differential changes of biochemical parameters of blood and urine (free and conjugated bilirubin) to assess of patochemistry of jaundice.*
5. *To explain the principle of the method of determining the total, direct, indirect bilirubin in serum and interpret research results.*
6. *To explain the role of microsomal oxidation reactions and conjugation in biotransformation of xenobiotics and endogenous toxins.*
7. *To write down the types of reactions of microsomal oxidation, to explain the effect of inducers and inhibitors of microsomal monooxygenases.*
8. *To write down the structure of the electron transport chain of endoplasmic reticulum and explain the role of cytochrome P-450 system in the functioning of biotransformation.*

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Theoretical questions

1. Catabolism of haemoglobin. Metabolism of bile pigments.
2. Patochemistry of jaundice: haemolytic, parenchymal, obstructive jaundice.
3. Hereditary diseases of metabolism of bile pigments.
4. Biochemical tests in the diagnosis of jaundice.
5. Detoxification function of the liver: biotransformation of xenobiotics and endogenous toxins.
6. The phases and types of reactions of biotransformation of foreign compounds in the liver. Inducers and inhibitors of microsomal monooxygenases.
7. Theconjugation reactions in hepatocytes: biochemical mechanisms, functional significance.
8. Electron transport chains of microsomal oxidation in the endoplasmic reticulum.
9. Genetic polymorphism and regulation of synthesis of cytochrome P_{450}. Nature of the drugs tolerance.

Laboratory work

1. Quantitative determination of the total, direct and indirect bilirubin in serum by Indrashek method

Principle of the method. Direct bilirubin reacts with diazoreagent to form azobilirubin that has pink color. The intensity of color is proportional to the concentration of direct bilirubin and can be determined photometrically. Indirect (free) bilirubin can be converted into a soluble state after adding caffeine reagent to the serum. Caffeine reagent leads to increase the solubility of pigment. Concentration of free bilirubin can determine by diazoreaction. Total bilirubin is the sum of direct and indirect bilirubin. The difference

between the amount of total and direct bilirubin can determine the level of indirect bilirubin.

Course of work. Serum diluted 1:1 with a solution of NaCl (0,9 %). Add all reactants as shown in the table:

Reactants	Direct bilirubin	Cheek sample	Total bilirubin
	1	2	3
Blood serum, ml	0.5	0.5	0.5
NaCl (0,85 %), ml	1.75	0.25	–
Diazoreagent, ml	0.25	–	0.25
Caffeine reagent, ml	–	1.75	1.75
Note. *The test tube contents mix and wait for 10 min. Optical density of the first and third samples should be measured against the cheek sample at 530 nm (a green optical filter) in cuvette with thickness of a layer of 5 mm*			

The concentration of direct and total bilirubin is calculated by the formula. Indirect bilirubin is calculated from the difference between total and direct bilirubin

The calculation is carried out by the formula:

$$X = E \cdot 11.5 \cdot 17.1 \text{ μmo l/l}$$

Diagnostic value of clinical tests. Normal values of bilirubin in blood serum are:

- *total bilirubin* 8.5–20.5 μmol/l (0.1–1.2 мg/100 ml);
- *indirect bilirubin* 1.7–17.1 μmol/l (0.1–1.0 мg/100 ml);
- *direct bilirubin* 0.86–5.1 μmol/l (0.05–0.25 мg/100 ml);
- *newborns bilirubin* – 23.1 μmol/l.

Increase bilirubin level above 27.36–34.20 μmol/l leads to its accumulation in tissues and development of jaundice.

The concentration of indirect bilirubin will increase under **hemolytic jaundice**, because liver has not time to connect indirect (free) bilirubin that is formed as a result of intensive hemolysis of red blood cells.

Parenchymal jaundice arises under hepatitis (viral, toxic) and liver cirrhosis. Direct bilirubin returns back partially into the blood due to damage of membranes of hepatocytes. In addition, the liver's ability to neutralize indirect bilirubin decreases. Under parenchymal jaundice is observed bilirubinaemia due to increase fractions of direct and indirect bilirubin.

Under **obstructive jaundice** bile goes into the blood stream due to obstruction of bile duct by stones or tumors. Hyperbilirubinaemia (above the 170–700 μmol/l) occurs mainly due to the direct bilirubin fraction.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Tasks for practical work

Fill in the table:

Table 1 – Types of reactions of microsomal oxidation

Type of reaction	Chemistry of reaction	Enzymes	Examples

Lesson 48
Theme: TESTS ON SITUATIONAL TASKS FROM "STEP - 1": IV SEMESTER

Actuality of the theme. *Understanding the common pathways of metabolism on a background of organs and tissues is essential to the formation of clinical thinking of the future doctor.*

Objectives. A student should be able to *use the theoretical knowledge to solve situational problems.*

Main tasks. A student should be able:

1. *To estimate a clinical picture, that is represented in a situational task.*
2. *To interpret biochemical indices.*
3. *On the estimation and interpretation of basis biochemical indices, draw a conclusion with regard to a choice of the right answer among the standard answers.*

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   part 1 – P. 3–14 (All questions); P. 22–47 (All questions);
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part 3 – P. 83 (questions 11–13); P. 95 (questions 53–58); P. 96 (questions 59–110).

Theoretical questions

1. Biochemistry and metabolism of amino acids, proteins and nucleic acids.
2. Metabolism and function of lipids.
3. Biochemistry of hormones and neurotransmitters.
4. Metabolism of porphyrins. Biochemistry of blood and urine.
5. Biochemistry of vitamins and digestion.
6. Functional biochemistry.

Lesson 49
Theme: FUNCTIONAL ACTIVITY IN THE KIDNEYS. CHEMICAL COMPOSITION OF URINE

Actuality of the theme. *The kidneys support the constancy of internal medium and remove the final products metabolism. Renal dysfunction causes changes in metabolism of the whole organism.*

Objectives. A student should be able to *characterize the basic biochemical renal functions and mechanisms of their regulation; analyze the biochemical composition of urine in norm and under pathology.*

Main tasks. A student should be able:

1. *To characterize the basic biochemical renal functions.*
2. *To explain the mechanisms of regulation of pH and electrolyte composition of body fluids.*
3. *To characterize the biochemical mechanisms of urine synthesis.*
4. *To analyze the biochemical composition of urine.*
5. *To estimate the functional significance of the final products of metabolism.*
6. *To analyze the health of a person on the basis of biochemical parameters of blood and urine.*

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Theoretical questions

1. Water-salt metabolism in the body. Features of the chemical composition of intracellular and extracellular fluids.
2. Features of metabolism in the kidney.
3. The role of the kidney in the regulation of electrolyte composition and pH of body fluids.
4. Mechanism of urine synthesis in the kidneys.
5. Hormonal regulation of water-salt balance and kidney function. Renin-angiotensin system.
6. Antihypertensive drugs as inhibitors of angiotensin-converting enzyme.
7. Biochemical composition of urine in norm and under pathology. Diagnostic significance of urine analysis
8. Urolithiasis: conditions of stone formation, their chemical composition and preventive measures.
9. Clinical and biochemical changes in various kidney diseases. Diagnostics of chronic renal failure.

Laboratory work

1. Quantitative reaction on protein in urine

1.1. Qualitative reaction on a protein with sulfosalicylic acid

Principle of the method. Healthy human urine contains a small amount of protein that cannot be detected by conventional reactions which used in clinical laboratories. Protein in urine determine by precipitation reactions with nitric acid or sulfosalicylic acid. The latter is the most sensitive.

Course of work. Three drops of 20 % sulfosalicylic acid solution add to 1 ml of urine. If the protein is present in the urine, the white precipitate (or turbidity) is formed, the degree of which depends on the concentration of protein in urine. The same reaction with the urine of a healthy person you must do for comparison.

1.2. Geller reaction

Course of work. Ten drops of concentrated nitric acid added to a test tube. A test tube tilt an angle of 45º C. Ten drops of urine poured gently along the wall of the test tube. The two fluids should not be mixed. A precipitate formed as white ring on the boundary between two layers if the protein is present in liquid. If you add an excess of concentrated nitric acid, the precipitate disappears.

Diagnostic value of clinical tests. Protein is found in the urine under nephritis (inflammation of the glomeruli in the kidney when increasing their permeability) in cardiac decompensation, with increasing pressure, sometimes during pregnancy, as well as inflammation of the urinary tract.

2. Qualitative test on blood pigments in urine (benzidine test)

Principle of the method. Benzidine is oxidized by atomic oxygen, which is formed under the decomposition of hydrogen peroxide with help haemoglobin that has peroxidase activity.

Course of work. 1 – 2 ml of fresh urine pour into a test tube, boiled and cooled. Add the same volume of benzidine and a few drops of H_2O_2. Products of benzidine oxidation have blue and green color. This coloration of urine is observed under positive benzidine test (blood in urine).

Diagnostic value of clinical tests. Hematuria (blood in urine) occurs under damage of the urinary tract, haemoglobinuria (blood pigments in the urine) observed at the action of hemolytic poisons, as diseases that are accompanied by hemolysis of red blood cells. In the case of hematuria and haemoglobinuria urine contains protein.

3. Qualitative test on glucose in urine (Trommer′s reaction)

Course of work. Add all reagents in accordance with the table to the test tube:

Reagent	Quantity, ml
Urine	1.0
NaOH, 10 %	1.0
$CuSO_4$, 1 %	0.5
The solution should be boiled for 1 min at 100° C. You can observe the red color - positive reaction for the presence of glucose	

4. Qualitative reaction on ketone bodies

4.1. Legal's test for acetone and acetoacetic acid

Principle of the method. In alkaline medium, acetone and acetoacetic acid together with sodium nitroprusside give red colour of the substance. Cherry-red color of the complex salt is formed when it is added to the solution of the concentrated acetic acid.

Course of work. Pour 0,5 ml of urine in two test tubes: the first – the urine of a healthy person (check sample), the second – the urine of the patient with diabetes mellitus (test sample). Add 0.5 ml of sodium hydroxide solution in both test tubes and 5–7 drops of sodium nitroprusside. Observe the appearance of red color in the second test tube. Acidify the solution by few drops of concentrated acetic acid. Red color becomes cherry.

4.2. The Gerhard's reaction on acetoacetic acid

Principle of the method. Phosphate **(Fe_3PO_4)** precipitate when ferric chloride solution is added to urine. In the presence of acetoacetic acid, after the addition of excess ferric chloride, cherry-red color appears. After some time color turns pale due to decarboxylation of acetoacetic acid that converts it into acetone. When heated, the rate of reaction is much faster.

Course of work. Pour 2 ml of urine (healthy human and diabetics) in two test tubes, and then, drop wise, add 10 % solution of ferric chloride to stop the sediment formation of phosphates. The precipitate is filtered. Add a few drops of $FeCl_3$ to the filtrate and observe the advent of cherry color in the test tube with the diabetic urine.

Diagnostic value of clinical tests. Number of ketone bodies, which are excreted in the urine of a healthy human, is 20–40 mg/day. Ketonemia and ketonuria is observed in diabetes, starvation, deficiency of carbohydrates in the diet, disorders of the

gastrointestinal tract, overproduction of insulin-antagonists (corticosteroids, thyroxine, hormones of the anterior pituitary gland secretion, and etc.).

Decrease of ketone bodies in the blood has no clinical significance. In early childhood, prolonged disorders of the gastrointestinal tract (toxemia, dysentery) lead to development of ketonemia due to chronic starvation and malnutrition.

5. Qualitative reaction on bile pigments

5.1. Gmelin's test

Principle of the method. Gmelin's test on bile pigment is based on ability of bilirubin and biliverdin to oxidize with formation of products, painted in a different color (biliverdin – green, bilitsian – blue).

Course of work. Pour 20 drops of nitric acid in test tube and add urine gently along the wall of the test tube. The two fluids should not be mixed. Rings, painted in different colors (green, blue, purple and red, yellow) appear on the boundary of two liquids. Yellow ring appears first.

6. Reaction with an alcoholic solution of iodine (unified Rosin's test)

Principle of the method. Urine bilirubin is oxidized to biliverdin under action of iodine.

Course of work. Pour 5 ml of urine in test tube and add iodine solution gently along the wall of the test tube. The appearance on the border of two liquids green ring indicates the presence of bilirubin.

Diagnostic value of clinical tests. Bile pigments formed from haemoglobin when red blood cells are destroyed. Bile pigments (bilirubin, biliverdin) appear in the urine in the form of alkaline salts under hepatitis.

Note. Practical works 1 – 5 you should perform as research for definition of pathological components of urine in all test tubes.

The results should be formed in the table.

Number of test tube	Name of reaction	The results of experiments in vitro			
		1	2	3	4

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 50

Theme: BIOCHEMICAL TRANSFORMATIONS IN THE MUSCLES. DETERMINATION OF SERUM CREATININE

Actuality of the theme. *Muscle is a system in which the chemical energy of ATP is transformed into mechanical energy of reduction and movement. Study of Muscle biochemistry opens possibilities to explain the molecular mechanisms of muscles diseases and helps to develop effective treatments and training of athletes.*

Objectives. A student should be able to *explain the biochemical basis of energy supply and molecular mechanisms of muscle contraction.*

Main tasks. A student should be able:

1. *To characterize the chemical composition of muscle and molecular mechanisms of muscle contraction.*
2. *To explain the bioenergetics of muscle tissue, the role of different energy sources in conditions of physical activity.*
3. *To characterize the changes of biochemical processes in muscles under myopathies.*
4. *To explain the features of cellular organization and metabolism in the heart muscle in normal and pathological conditions.*
5. *To determine the concentration of creatinine in blood serum and analyze the results.*

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Theoretical questions

1. Chemical composition of skeletal muscles. Proteins of myofibrils: myosin, actin, tropomyosin, troponin. Molecular organization of thick and thin filaments.
2. Molecular mechanisms of muscle contraction.
3. Bioenergy of muscle tissue: sources of ATP in the muscle; synthesis of creatine and creatine phosphate.
4. Cell organization, the features of metabolism and bioenergetic processes in the myocardium. Regulation of cardiomyocytes contraction.
5. Metabolic disorders in the coronary vessels and cardiac muscle in acute myocardial. Biochemical diagnosis of diseases of the myocardium.
6. Pathobiochemistry of muscle (myopathies).
7. Pathobiochemistry of hypertension. Damage of the heart in some diseases.

Laboratory work

1. Determination of creatinine in blood serum by Popper's method

Principle of the method. Creatinine reacts with picric acid in an alkaline medium with formation of red tautomer of creatinine picrate, which causes the appearance of orange-red color. The color intensity is proportional to the concentration of creatinine.

Course of work. Add all reagents in accordance with the table to the test tube:

Reactants	ample		
	Test sample	Standard sample	Cheek sample
Blood serum, ml	1.0	–	–
Standard solution of creatinine, ml	–	1.0	–
Picric acid, ml	3.0	3.0	3.0

Caffeine reagent, ml	–	1.75	1.75
Test tubes leave for 5 min at 25° C, then place in a water bath at 100° C on 15–20 s			
	Centrifuge at 3 000 r/pm for 10 min		
The centrifugate	2.0	–	–
NaOH (10 %), мл	0.1	0.1	0.1
The total volume of the solutions adjusted to 10 ml with water. The test tube contents mix and wait for 10 min. Optical density of the first and third samples should be measured against the cheek sample at 500–560 nm (a green optical filter) in cuvette with thickness of a layer of 1 cm			

The calculation is carried out by the formula:

$$X = \frac{E_{exp} \cdot 0.1 \text{ mmol/l} \cdot}{E_{st}},$$

where

X is concentration of creatinine, mmol/l;

E_{st} is optical density of standard sample;

E_{exp} is optical density of experimental sample;

0.1 is concentration of creatinine in the standard solution, mmol/l.

Note. *If the standard sample has another concentration of creatinine, ratio of the optical density of experimental and standard samples multiplies on its value.*

Straight-line relationship between the magnitude of optical density and creatinine concentration is stored only at concentrations within 0.026 – 0.352 mg/l. At higher concentration of creatinine serum should be diluted in 2 – 4 times and more by physiological solution. In the above formula must enter this value (multiply the results on the amount of dilution).

Diagnostic value of clinical tests. The normal concentration of serum creatinine is 53 – 106.1 μmol/l. The concentration of creatinine in blood serum reflects the glomerular filtration in the kidney.

Concentration of creatinine in the blood increases under acute and chronic disorders of the kidney, intestinal obstruction, urinary tract obturation, hyperfunction of the adrenal glands.

Creatinine is a non threshold substance. It is filtered in the glomerulus and not absorbed backward in the tubules. Reduction of serum creatinine observed under muscle weakness and pregnancy.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Tasks for practical work

Fill in the table:

Table 1 – Sources of energy in the muscles

Type of muscle tissue	Main energy substrates	Type of metabolism	Chemical reactions	Biological role

Table 2 – Enzymes of the muscles

Name	Type of metabolism	Chemical reaction	Diagnostic value of clinical tests

Lesson 51
Theme: FEATURES OF CHEMICAL COMPOSITION AND METABOLISM IN THE CONNECTIVE TISSUE. DETERMINATION OF SIALIC ACIDS IN BLOOD SERUM

Actuality of the theme. *Connective tissue is about 50 % of the body weight. It is present in all organs and serves as the basis for their formation and correction of damage. All types of connective tissue built on a single principle, despite the morphological differences. Disorders of synthesis or activity of hydrolytic enzymes of connective tissue is base of development of inherited and acquired diseases.*

Understanding of biochemical mechanisms of pathologies of connective tissue are the basis for the development of new methods of treatment and diagnosis of diseases.

Objectives. A student should be able to *describe the features of the chemical composition of connective tissue and mechanisms of development of collagenoses and mucopolysaccharidoses.*

Main tasks. A student should be able:

1. *To explain the features of the chemical structure of connective tissue.*
2. *To characterize the main stages of the synthesis of collagen and formation of fibrillar structures.*
3. *To explain the mechanisms of participation of glycosaminoglycans in connective tissue metabolism and their redistribution in organs and tissues.*
4. *To explain the biochemical mechanisms of connective tissue diseases.*
5. *To interpret the results of determination of sialic acids in serum.*

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Theoretical questions

1. Chemical composition of connective tissue. Proteins of fibers of connective tissue are collagen and elastin. Biosynthesis of collagen and formation of fibrillar structures.
2. Glycosaminoglycans as the complex carbohydrates of connective tissue. Role of glycosaminoglycans in the formation of the basic substance of loose connective tissue.
3. Metabolism of proteoglycans.
4. Pathobiochemistry of connective tissue. Biochemical mechanisms of development of mucopolysaccharidoses and collagenoses, their clinical and biochemical characteristics.

Laboratory work

1. Determination of sialic acids in blood serum

Principle of the method. The method is based on the color reaction that occurs during heating of sialic acid with Hess reagent. The intensity of brownish-pink color depends on the concentration of sialic acids.

Course of work. Pour 1 ml of blood serum in centrifuge test tube and then add 1 ml 10 % solution of trichloroacetic acid. Shake.
Test tube placed in a boiling water bath for 5 min and cool. This leads to release of molecules of N-acetylneuraminic acid from the protein portion of glycoproteins. Solution is centrifuged. Pour 0.4 ml of centrifugate in test tube and add 5 ml of Hess reagent. Test tube boiled in a boiling water bath for 30 min. Brownish-pink color appears in the test tube. Optical density of the sample should be measured against the water at 540 nm (a green optical filter) in cuvette with thickness of a layer of 1 cm. The resulting value of extinction multiplied by 1 000. Results are expressed in conventional units of extinction.

Diagnostic value of clinical tests. The normal value of extinction is 100 – 195 conventional units μmol/l. The concentration of creatinine, which corresponds to the concentration of sialic acids from 550 to 790 mg/L (0.7 g/l N-acetylneuraminic acid). Increasing the number of sialic acids in the blood observed under rheumatism, tuberculosis, myocardial infarction, malignant bone tumors, lung cancer.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Task for practical work

Fill in the table:

Table 1 – Chemical composition of connective tissue

No	Group of chemical components	Name of chemical compound	Features of structure	Pathological or physiological state	Change of structure and metabolism

Lesson 52
Theme: FEATURES OF CHEMICAL COMPOSITION AND METABOLISM IN THE NERVOUS TISSUE

Actuality of the theme. *The nervous tissue has common features with the cells of other tissues, as well as specific features that are caused by functions performed by the nervous system in whole organism. Understanding the molecular mechanisms of the nervous system, the study of the chemical composition and metabolism in normal and pathological conditions allows the development of modern methods of diagnosis and treatment of nervous diseases.*

Objectives. A student should be able to *explain the features of chemical composition and metabolism of nerve tissue.*

Main tasks. A student should be able:

1. *To characterize the biochemical composition of nervous tissue.*
2. *To explain the features of metabolism in the nervous system and molecular mechanisms of action of neurotransmitters.*
3. *To characterize the changes of energy metabolism in nervous tissue under different physiological conditions.*
4. *To explain the neurochemical mechanisms of action of psychotropic drugs.*
5. *To explain the biochemical basis of violations of metabolism of brain mediators and modulators under mental disorders.*

References

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Theoretical questions

1. Chemical composition of nervous tissue: the features of the changes in ontogenesis.
2. Myelin: chemical composition, role in the functioning of the nervous tissue.
3. Metabolism of carbohydrates, lipids, proteins and amino acids in the nervous tissue. Features of energy metabolism.
4. Formation and inactivation of neurotransmitters, their role in the functioning of the nervous system. Receptors for neurotransmitters and physiologically active compounds.
5. Biochemical basis of memory.
6. Neurochemical mechanisms of action of psychotropic drugs.

Laboratory work

1. Determination of cholinesterase activity (CE) in blood serum

Principle of the method. Cholinesterase catalyzes the hydrolysis of acetylcholine into choline and acetic acid. This acetic acid reduces pH of a solution and one can see the change of colour of the incubated solution. Color changes from crimson on yellowish Proserin is the inhibitor of cholinesterase.

Course of work. Carry out a laboratory test according to the table:

Reagent	Test sample, ml	Check sample, ml	Standard sample, ml
Buffer solution	2.5	–	2.5
H_2O	–	2.7	0.05
Blood serum	0.05	0.05	–
Acetylcholine chloride	0.1	–	0.1
Incubation (in a thermostat) for 30 min at 37 °C			
Proserin	0.1	–	0.1
After cooling the sample to room temperature, it is necessary to measure the optical density of the solution in the test tubes against water at 540 nm (a green optical filter) in cuvette with a layer thickness of 1 cm.			

The calculation is carried out by the formula:

$$E_{st} + E_{ch} - E_{test},$$

where

E_{st}, E_{ch}, E_{test} are optical density of the solutions of standard, check and test samples.

The activity of an enzyme is defined in accordance with the calibration graph. The activity of an enzyme should be expressed in μmol of the acetic acid which is formed at the incubation of 1 ml of blood serum during 1 hour at 37°C (μmol/h · l).

Diagnostic value of clinical tests. The normal activity of cholinesterase is 160–340 μmol/h · l or 45–95 μmol/s · l.

Cholinesterase is active mainly in the liver, pancreas, blood serum, brain. Cholinesterase activity in healthy persons can vary. Unlike most enzymes activity of cholinesterase is reduced under pathology.

Reduction of serum cholinesterase activity in blood may be caused in cases of most hepatic disorders. Nutritional deficiencies, cachexia, cancer, uremia, tuberculosis, and even the treatment by muscle relaxants like succinylcholine chloride can lower serum enzyme activity. Cholinesterase activity in blood decreases in case of pesticides poisoning.

After the experiment, you should fill in the protocol of the laboratory work with results and conclusions.

Lesson 53
Theme: TEST OF SITUATIONAL TASKS FROM "STEP-1": III – IV SEMESTERS

Actuality of the theme. *Understanding the basic regularities of macromolecules metabolism on the background of organs and systems is the bases for formation of future doctor's clinical thinking.*

Objectives. A student should be able to *use the theoretical knowledge to solve situational problems.*

Main tasks. A student should be able:

1. *To estimate a clinical picture, that is represented in a situational task.*
2. *To interpret biochemical indices.*
3. *On the estimation and interpretation of basis biochemical indices, draw a conclusion with regard to a choice of the right answer among the standard answers.*

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   part 1 – P. 3–14 (All questions); P. 22–47 (All questions);
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Theoretical questions

1. Amino acids and proteins: structure and functions.
2. Enzymes: structure, functions and general properties.
3. Bioenergetics and energy metabolism.
4. Metabolism of carbohydrates.
5. Biochemistry and metabolism of amino acids, proteins and nucleic acids.
6. Metabolism and function of lipids.
7. Biochemistry of hormones and neurotransmitters.
8. Metabolism of porphyrins. Biochemistry of blood and urine.
9. Biochemistry of vitamins and digestion.
10. Functional biochemistry.

Lessons 54–55
Theme: EXAMINATION SUBMODULE 4 "BIOCHEMISTRY OF TISSUES AND PHYSIOLIGICAL FUNCTIONS"

Actuality of the theme. *Understanding the basic laws of molecular-cellular processes and intercellular communication features of metabolic transformations of biomolecules on a background of organs and tissues is the basis of clinical thinking of medical students. Control of knowledge is important for ordering of information, its generalization and practical application.*

Objectives. A student should be able to *systematize and clearly formulate reasonable answers to the questions; make substantiated conclusion; interpret the results of laboratory research and parameters of blood and urine; use gained knowledge to solve situational problems.*

Main tasks. A student should be able:

1. *To reproduce chemical conversions of amino acids with an indication of name of the appropriate enzymes.*
2. *To explain the chemical conversions of nucleotides.*
3. *To depict the sequence of stages of replication, transcription and translation.*
4. *To explain the molecular-cellular mechanisms of hormone action.*
5. *To explain the fundamentals of biochemical changes in disturbance of amino acids and nucleotides metabolism*
6. *To explain the biochemical consequences of disturbance of the hormones synthesis.*
7. *To write down schematically the major metabolic pathways in individual organs and tissues.*
8. *To explain the mechanisms of regulation of metabolic processes and pathways of integration of metabolism in different organs and systems.*
9. *To explain the principles of the methods and clinical diagnostic value of main biochemical parameters of blood and urine.*
10. *To interpret the results of biomedical research*

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Theoretical questions

1. Digestion of proteins, amino acids absorption. Rotting of proteins in intestine. Clinical correlation.
2. Common characteristic of vitamins, as components of the diet. Classification and nomenclature of vitamins.
3. Biological role of vitamins, their interconnection with enzymes.
4. Vitamin deficiency diseases and the typical reasons of their occurrence.
5. Biochemical characteristic of water-soluble vitamins (B_1, B_2, B_3, B_6, B_{12}): structure, metabolism, basic dietary sources, daily requirement, metabolic role and functions, deficiency manifestation, clinical significance.
6. Vitamins in medicine.
7. Coenzyme vitamins are PP, H, a folic acid: structure, biological role, sources, daily requirement.
8. Biochemical characteristic of vitamin C and P: chemical structure, biological role, sources, daily requirement.
9. Practical use of vitamins.
10. Interrelation of vitamins in an organism.
11. Manifestation of vitamin insufficiency.

12. Biochemical characteristic of fat-soluble vitamins (A, D, E, K, F): chemical structure, biological properties, daily requirement, sources, role in the metabolism, mechanism of action.
13. Vitamin deficiency diseases of fat-soluble vitamins.
14. Antioxidant properties of fat-soluble vitamins.
15. Antivitamins: mechanism of action, use in medicine.
16. Biological role of water and their redistribution in an organism.
17. Water and electrolytes balance and imbalance, mechanism of regulation.
18. Biological function and clinical importance of macroelements (Na, K, Ca, Mg, P).
19. Biological function and clinical importance of trace elements.
20. Physiological and biochemical functions of blood.
21. Respiratory function of erythrocytes. Haemoglobin: structure, properties, functions.
22. Variants and pathological forms of haemoglobin.
23. Acid-base balance. Buffer systems of blood.
24. Acid-base imbalance.
25. Biochemical composition of blood.
26. Plasma proteins: biochemical characteristic, methods of separation, electrophoregrams of serum proteins in norm and under pathology.
27. Total blood protein. Hypo-, hyper-, dis- and paraproteinaemies.
28. Blood enzymes, their clinical importance.
29. Nonprotein organic components of blood. Inorganic components of plasma. Azotemia.
30. Biochemistry of coagulation and fibrinolytic systems of blood.
31. Biochemistry of immune processes. Antibodies: structure, biological functions.
32. Specificity of a metabolism in kidneys.
33. Formation of urine in kidneys: glomerular filtration, tubular reabsorbtion, tubular secretion.
34. Normal urine composition.
35. Regulation of acid-base status in the kidneys.
36. Renin- angeiotensin and kallikrein-kinin systems.
37. Pathological components of urine.

38. Renal function test.
39. Functional organization and biochemical liver function.
40. Participation of liver in carbohydrates metabolism, its disorder.
41. Liver function in lipid metabolism.
42. Liver function in proteins metabolism.
43. Role of liver in metabolism of vitamins and mineral elements.
44. Biochemical composition and functions of bile.
45. Detoxification in the liver. Reactions of biotransformation. Microsomal oxidation and the role of cytochrome P-450.
46. Catabolism of haemoglobin. Metabolism of bile pigments.
47. Types of jaundice: hemolytic, hepatic and obstructive.
48. Hereditary diseases of metabolism of bile pigments.
49. Biochemical tests for diagnostics of jaundice.
50. Chemical composition of skeletal muscles.
51. Chemical changes under muscle contraction.
52. Metabolism of carbohydrate, lipid and amino acids in muscle cells.
53. Fuel utilization in skeletal and cardiac muscles.
54. Fuel utilization in the muscles in rest state and under physical exercises.
55. Proteins of fibers of connective tissue are collagen, elastin, glycoproteins and proteoglycans. Biosynthesis of collagen. Pathobiochemistry of connective tissue.
56. Chemical composition of nervous tissue.
57. Structure and synthesis of myelin.
58. Metabolism of energy, carbohydrates, lipids and amino acids in nervous tissue.
59. Neurotransmitters (catecholamines, serotonin, histamine, acetylcholine, glutamate, GABA and other neurotransmitters): general properties, synthesis, accumulation, release and inactivation.
60. Metabolic encephalopathies and neuropathies.

Lessons 56
Theme: INTERRELATION OF METABOLISM IN ORGANS AND BODY SYSTEMS

Actuality of the theme. *All conversions in the human body are closely interlinked, coordinated, regulated by neurohumoral mechanisms and integrated in whole process of metabolism. The intensity of exchange between human body and the environment and the rate of intracellular metabolic processes maintains constant internal environment and the integrity of the body. Violation of the dynamic status of the body accompanied of the development of pathological states. Their severity and duration determined by the degree of damage to the structure and functions of individual molecular and supramolecular components of cells.*

Objectives. A student should be able to *explain the relationship between metabolism of carbohydrate, lipid, protein in some organs and tissues and impaired coordination of metabolic processes.*

Main tasks. A student should be able:

1. *To characterize the main pathways of metabolism of carbohydrates, lipids, proteins and explain pathways of coordination of these processes in the body.*
2. *To explain the mechanisms of hormonal regulation of metabolism.*
3. *To depict schematically the stages of biomolecules catabolism.*
4. *To explain the various pathways of energy supply of metabolic processes.*
5. *To interpret the relationship of metabolic processes in separate organs and tissues.*
6. *To explain the causes of pathologies under disorders of coordination of metabolic processes.*

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Theoretical questions

1. Coordination of metabolic processes in the body. Systems of integration.
2. Hormonal regulation of metabolism, anabolic and catabolic hormones.
3. Stages of biomolecules cleavage. The total energy supply of various metabolic processes.
4. The relationship of carbohydrate, lipid and protein metabolisms. Common precursors and intermediates.
5. Relationship of metabolism in separate organs and tissues.
6. Disorders of coordination of metabolic processes. The development of pathologies.

Tasks for practical work

Show schematically the relationship of metabolic processes in:

a) liver and adipose tissue;
b) liver and muscles;
c) liver and kidneys;
d) liver and nervous tissue.

Appendix A
The list of questions for admission to the examination (if the total score for the current year academic progress is less than 72.0)

1. What compounds are called proteins? What kind of chemical bonds support the structure of protein molecules?
2. Draw the general structure of an amino acid.
3. What are the basic physical and chemical properties of amino acids?
4. Explain the amphoteric property of amino acids. What is pI?
5. Name the basic physical and chemical properties of proteins.
6. Explain what is the electrophoretic mobility of proteins.
7. What is the denaturation of proteins?
8. Which levels of structural organization of proteins do you know?
9. What are simple proteins? Name the classes of simple proteins.
10. What are complex proteins? Name the classes of complex proteins.
11. Give examples of hemoproteins.
12. What are oligomeric proteins?
13. Name structural components of nucleic acids.
14. Give examples of purine and pyrimidine nucleotides.
15. Give the definition of “enzymes”.
16. What are the functions of the active and allosteric sites of enzymes?
17. Give the definition of “multienzyme” complex.
18. Give the definition of “co-enzymes”.
19. Give examples of “co-enzymes”.
20. Give the definition of allosteric enzymes.
21. What is covalent modification of enzymes?
22. Give the definition of isoenzymes.
23. Name the isoenzymes of lactate dehydrogenase and creatine kinase.
24. Give the definition of “anabolism”, “catabolism”, amphibolim.
25. Define the term “oxidative phosphorylation”.

26. Draw the structure of the mitochondrial respiratory chain. Explain its biological role.
27. Explain the term “substrate phosphorylation”.
28. Explain the mechanism of oxidative phosphorylation according to Mitchell's theory.
29. Name the compounds that are uncouplers of oxidative phosphorylation. Explain the mechanism of their action.
30. Specify the functions of the TCA cycle.
31. Specify the cellular localization of the TCA cycle. Name the enzymes.
32. Specify the reaction of substrate phosphorylation in the TCA cycle.
33. Give the definition of “glycolysis”.
34. Specify the end product of glycolysis and amount of ATP, which is formed under anaerobic conditions.
35. Name the final products of aerobic glucose oxidation and ATP, which are generated under these conditions.
36. What reaction is catalyzed by pyruvate dehydrogenase complex. Specify its cellular localization.
37. Explain the role of the malate-aspartate and glycerol-phosphate shuttle mechanisms.
38. Name the key enzymes of glycolysis.
39. Which vitamins take part in work of pyruvate dehydrogenase complex?
40. Specify the biological role of the pentose phosphate pathway of glucose oxidation.
41. Hereditary deficiency of which enzyme of pentose phosphate pathway can cause a hemolytic anemia.
42. Name the enzyme deficiency which can lead to development of galactosemia.
43. Name the enzyme deficiency which can lead to a disease that called “fructose intolerance”.
44. What is “gluconeogenesis”? In which organs does it occur?
45. Name the substrates of gluconeogenesis.
46. What is the “Cory cycle”?
47. What is the glucose-alanine cycle?

48. Name the hormones that activate the gluconeogenesis.
49. Name the hormones that have hyperglycemic effect.
50. Specify the normal range of blood glucose level.
51. Specify the biological role of liver glycogen.
52. What are the key enzymes of glycogenesis and glycogenolysis.
53. Explain the effect of insulin on glycogen metabolism in the muscles?
54. What diseases are called "glycogen storage diseases"? How does the level of glucose in the blood change in patient with these diseases?
55. Specify the biological functions of TAG in the body.
56. What compounds are synthesized from cholesterol in the body?
57. What are lipolysis and lipogenesis?
58. What hormones activate and inhibit lipolysis?
59. Name the enzyme of lipid metabolism, which is activated in adipose tissue by epinephrine.
60. Specify the biological function of β-oxidation of fatty acids. What is the function of carnitine in this process?
61. What are ketone bodies? Specify the location of their synthesis and biological function of them.
62. Explain the terms "ketoacidosis", "ketonemia", "ketonuria".
63. What are the substrates and key enzymes of fatty acid synthesis?
64. Which fatty acids are called saturated fatty acids? Give examples.
65. Which fatty acids are called unsaturated fatty acids? Give examples.
66. Explain the term "vitamin F".
67. Explain the term "lipotropic factors". Name them.
68. Name the classes of blood lipoproteins. Which of them are atherogenous and antiatherogenous?
69. What is the normal concentration of serum cholesterol?
70. Explain the functions of chylomicrons, VLDL, LDL and HDL in the human body.
71. Name four main pathways of ammonia formation in a body.
72. Name four main pathways of ammonia detoxification in a body.
73. Name enzymes that catalyze the reaction of amino acids transamination; indicate coenzymes; give examples.

74. Name enzymes and substrates for the formation of biogenic amines. Give an example.
75. Specify three amino acids for the transport of ammonia in the blood.
76. Name the process of urea formation, its function and organ localization.
77. Specify the normal concentration of urea in the blood serum.
78. Specify the amino acid and its active form for the methylation reactions. Give an example of one process with this amino acid.
79. Explain which disease is called "metylmalonic aciduriya", specify the reason.
80. Which compounds are formed in a body from phenylalanine?
81. Specify stages and organ localization for creatine synthesis.
82. Explain the molecular cause of phenylketonuria.
83. Explain the molecular cause of alkaptonuria.
84. Explain the molecular cause of albinism.
85. Explain the reason of the high urea concentration revealed in the urine of patients with diabetes.
86. Explain the molecular cause of Hartnup's disease.
87. Name compounds that participate in the synthesis of purine nucleotides ring.
88. Name the end product of purine nucleotide catabolism. Specify the names of two diseases that are accompanied by increasing of this product accumulation in a body.
89. Specify the molecular cause of orotic aciduria.
90. Name enzymes which participate in DNA replication.
91. Explain which process is called "splicing".
92. What are the basic properties of the genetic code?
93. What are the main stages of translation? Name the enzyme that is involved in the activation of amino acids during translation.
94. Explain the term "gene amplification". Give an example.
95. Name major classes of hormones, according to the classification by chemical structure.
96. Name secondary messengers of hormonal action.
97. Indicate which classes of hormones have a membrane-cytosolic mechanism of action.

98. Specify which hormones are characterized by cytosolic mechanism of action.
99. Name processes of carbohydrate metabolisms, which are activated by insulin.
100. Explain the hypoglycemic effect of insulin.
101. Name hormones that have hyperglicemic effect. Name processes that are activated by one of these hormones for the realization such effect.
102. Name hormones which increase the free fatty acids concentration in the blood. Name the process that is activated in cells for it.
103. Name eicosanoids. Specify the substrate for synthesis of them.
104. Name hormones of the thyroid gland. Specify the hypothalamus and pituitary hormones, which are involved in the regulation of thyroid hormones secretion.
105. Name hormones that are involved in the regulation of calcium and phosphorus in the blood.
106. Specify how the concentration of calcium and phosphorus in the blood is changed by the action of these hormones.
107. Explain the effect of glucocorticoids on carbohydrate metabolism.
108. Explain the effect of glucocorticoids on proteins metabolism.
109. Name proteolytic enzymes and specify the localization of them in GIT.
110. Name GIT enzymes for the digestion of carbohydrates and lipids.
111. Specify the functions of bile acids.
112. Explain which bile acids are known as primary and secondary. Give examples.
113. Name coenzyme of water soluble vitamins: B_1, B_2, B_6, B_{12}, PP, folic acid, pantothenic acid and biotin.
114. Give examples of three enzymes with the coenzyme forms of vitamin PP.
115. Give examples of three enzymes with the coenzyme forms of vitamin B_2.

116. Give examples of two processes, in which vitamin B_6 is involved.
117. Give examples of three processes, in which vitamin C is involved.
118. Give one example of processes involving folic acid and vitamin B_{12}.
119. Explain the mechanism of vitamin D_3 synthesis and activation.
120. Specify the biological functions of vitamin A.
121. Explain the mechanism of the blood clotting with the participation of vitamin K.
122. Specify the biological functions of vitamin E.
123. Name the main fractions of blood serum proteins.
124. Explain the functions of albumin.
125. Explain the function of haptoglobin.
126. Explain the functions of ceruloplasmin and transferrin.
127. Explain the functions of antitrypsin.
128. Specify the normal concentration of blood serum proteins.
129. Explain the term "rest nitrogen of blood". Which compounds are in this fraction?
130. Explain which compounds are the bile pigments.
131. Name the enzyme for the conjugation of bilirubin which is localized in a liver. Specify the function of this reaction.
132. Explain the terms "direct bilirubin", "indirect bilirubin".
133. What is the normal concentration of total, direct and indirect bilirubin in blood serum?
134. Specify the function of cytochrome P_{450}.
135. Give examples of three compounds are needed for the conjugation of xenobiotics in the liver.
136. Explain the hippuric acid test.
137. Which proteins are the stromal and sarcoplasmic muscle proteins?
138. Which amino acids are specific for the collagen structure?
139. Which compounds are used as energy sources for muscles in prolonged physical exercises?
140. What components are normal and pathological for urine?

Appendix B
Questions to prepare for the exam in biochemistry

1. The general characteristic and biological functions of proteins and peptides. Levels of protein structure. Chemical bonds in protein molecules.
2. Amino acid composition of proteins and peptides: structure, classifications, biological functions of amino acids.
3. Physical and chemical properties of amino acids.
4. Methods of protein separation, fractionation and protein structure analysis (chromatography, electrophoresis).
5. Physical and chemical properties of proteins. Amphoteric nature. Isoelectric point (pI).
6. Solubility of proteins. Thermodynamic stability of proteins and denaturation.
7. Classification of proteins. General characteristics of simple proteins, their functions.
8. Natural peptides. The general characteristics of these molecules (structure and functions).
9. Complex proteins: classification, the content in the organism, and functions.
10. General characteristics of chromoproteins, structural features, biological functions.
11. Hemoproteins: myoglobin, haemoglobin, cytochromes. Their biological functions and structural features. The normal concentration of haemoglobin in the blood. General characteristics of haemoglobinopathies and thalassemias.
12. Flavoproteins: the structural features and their functions in an organism.
13. Glycoproteins: classification, the structural features, distribution, biological functions.
14. Lipoproteins, phosphoproteins, metalloproteins: the structure, biological functions.
15. Nucleotides: the structure, nomenclature, biological functions. Free nucleotides: participation in metabolic reactions. Cyclic nucleotides.

16. Nucleic acids: features of structural organization, biological functions of DNA and RNA.
17. Enzymes as biological catalysts of metabolism. General properties of enzymes. The nomenclature of enzymes and their classification. Classes of enzymes.
18. The structure of enzymes; active cites of enzymes; oligomeric enzymes; multienzyme complexes (functional enzyme systems), membrane-associated enzymes.
19. Cofactors and coenzymes. The structure and properties of coenzymes; coenzymes are derivatives of vitamins B_1, B_2, B_3, B_5, B_6, Bc, B_{12}, H and lipoic acid.
20. Mechanisms of enzyme action: E. Fisher's and D. Koshland's hypotheses of enzymatic catalysis. Molecular mechanisms of enzymatic catalysis.
21. Mechanisms of enzyme action: stages of catalytic process, formation of enzyme-substrate complex. Thermodynamic laws of enzymatic catalysis.
22. Kinetics of enzymatic reactions: the dependency of the reaction rate on the concentration of enzyme and substrate, pH and temperature. Kinetics of allosteric enzymes.Michaelis-Menten constant (Km), it semantic . Km using for measurement of enzyme activity.
24. The basic principles and methods of enzyme activity definition. Units of enzyme activity (international units, the katal).
25. The multiple forms of enzymes - isoenzymes. Using of isoenzymes for differential diagnosis.
26. Regulation of enzyme activity. Activators and inhibitors. Types of inhibition of enzyme activity.
27. Pathways and mechanisms of the regulation of enzymatic processes: regulation of catalytic activity of enzymes; allosteric enzymes; regulation by covalent modification of enzymes.
28. Pathways and regulatory mechanisms of enzymatic processes: feedback regulation; activation of proenzymes; regulation by protein-protein interactions, compartmentation of enzymatic processes.

29. Pathways and regulatory mechanisms of enzymatic processes: control of enzyme synthesis by repression and induction; cyclic nucleotides as regulators of enzymatic reactions.
30. Use of enzymes in medicine. Immobilized enzymes.
31. Enzymopathies - hereditary defects of carbohydrate and lipid metabolism.
32. Enzymodiagnostics of pathological processes and diseases. Enzymotherapy - the use of enzymes, their activators and inhibitors in medicine.
33. General aspects of metabolism: catabolic, anabolic and amphibolic pathways. Anaplerotic reactions. Stages of catabolism in the body.
34. General description of the citric acid cycle: intracellular localization, biological functions, the scheme of its.
35. Enzymatic reactions of TCA cycle. Amphibolic and anaplerotic reactions of TCA cycle.
36. Regulation and energy balance of TCA cycle.
37. Exergonic and endergonic biochemical reactions; the functions of ATP and other energy-rich phosphates.
38. Biological oxidation: types of reactions of biological oxidation (dehydrogenase, oxydase, oxygenase) and their biological . Tissue respiration.
39. Enzymes and coenzymes of biological oxidation: pyridine-linked and flavine-linked dehydrogenases, cytochromes.
40. Molecular organization of the mitochondrial chain of biological oxidation: components of respiratory chain, their redox potential; molecular complexes of the electron transport chain (ETC).
41. Chemiosmotic theory of oxidative phosphorylation. Basic postulates of Mitchell's chemiosmotic theory of energy transfer. Mitochondrial ATP-synthetase.Oxidative phosphorylation: coefficient of oxidative phosphorylation, points of coupling of oxidation and phosphorylation. The function of brown adipose tissue in thermogenesis.
43. Regulation of respiration and oxidative phosphorylation. Respiratory control. Inhibitors and uncouplers of electron

transport and oxidative phosphorylation, their biomedical importance.

44. Microsomal oxydation: cytochrome P_{450}, molecular organization of the chain of microsomal oxydation.
45. Reactive oxygen species and mechanisms of their inactivation.
46. Carbohydrate: classification, structure, properties and functions of the representatives of certain classes.
47. Anaerobic oxidation of glucose: the sequence of reactions, enzymes. Clinical aspects of glucose metabolism in anaerobic conditions.
48. Anaerobic oxidation of glucose: regulation, reactions of substrate-level phosphorylation, energy effect. Glycolytic oxidation-reduction (Redox) cycle. Features of glucose metabolism in erythrocytes.
49. Aerobic glucose oxidation: stages, energy balance. Shuttle mechanisms for the oxidation of NADH. The Pasteur's effect.
50. Oxidative decarboxylation of pyruvate: reactions, regulation, clinical aspects.
51. Glycogenolysis in the liver and muscles. Regulation of glycogen phosphorylase activity. Genetic disorders of glycogen metabolism.
52. Glycogen biosynthesis: enzymatic reactions, physiological value. Regulation of glycogen synthase activity.
53. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis. The functions of epinephrine, glucagon and insulin in the hormonal regulation of glycogen metabolism in muscles and liver.
54. Gluconeogenesis: substrates, enzymes and physiology value of the process. Cori cycle and glucose-alanine cycles.
55. Pentose phosphate pathway of glucose oxidation (hexose monophosphate (HMP) shunt): the scheme of the process, biological functions, regulation, process disturbance.
56. Metabolic pathways of fructose transformation in the human organism. Hereditary enzymopathies of fructose metabolism.
57. Metabolic pathways of galactose transformation in the human body and hereditary enzymopathies of its metabolism.

58. Metabolism of carbohydrate components of glycoconjugates: synthesis of O- and N-linked glycoproteins. Genetic disorders of metabolism of glycoconjugates.
59. Mechanisms of regulation of blood glucose levels. Disorders of carbohydrate metabolism in diabetes mellitus.
60. General characteristics of lipids: structure, classification, functions of representatives of individual classes. Fatty acids: structure and functions.
61. Catabolism of triacylglycerols in adipose tissue: sequence of reactions, regulatory mechanisms of TAG-lipase activity. Hormonal regulation of lipolysis.
62. Oxidation of fatty acids (β-oxidation), the function of carnitine.
63. Energy balance of β-oxidation of fatty acids in cells. Glycerol catabolism: enzymatic reactions and the energy of the process.
64. Biosynthesis and utilization of ketone bodies, their biological function.
65. Metabolic disturbances of ketone bodies metabolism in diabetes mellitus and starvation.
66. Biosynthesis of saturated fatty acids. Stages and reactions of palmitate biosynthesis.
67. Biosynthesis of fatty acids: sources of NADPH · H^+, the total equation of palmitate synthesis, the regulation of the process.
68. Elongation of long-chain fatty acids. Biosynthesis of mono- and polyunsaturated fatty acids in an organism.
69. Biosynthesis of TAG. Features of lipogenesis in adipocytes.
70. Biosynthesis of phospholipids in an organism. Concept about lipotropic factors.
71. Metabolism of sphingolipids. Genetic anomalies of its biosynthesis - sphingolipidoses.
72. Biosynthesis of cholesterol: scheme of reactions, regulation of synthesis. Pathways of cholesterol biotransformation.
73. Transport of lipids. Plasma lipoproteins. Hyperlipoproteinemia.
74. Biochemistry of lipid metabolism pathology: atherosclerosis, obesity, diabetes mellitus.

75. Pathways of formation and using of a free amino acids pool in the human body. Pathways of transformation of free amino acids to end products.
76. Deamination of amino acids: types of deamination, sequence of reactions. Glutamate dehydrogenation reaction, its value and regulation.
77. Transamination of amino acids: reactions, biochemical value, the mechanism of action aminotransferases.
78. The mechanism of transdeamination of amino acids, physiological value.
79. Decarboxilation of amino acids: enzymes, physiological value. Oxidation of biogenic amines.
80. Diagnostic value of definition of aminotransferases activity.
81. The sources of ammonia in an organism. Toxicity of ammonia and pathways of its detoxyfication. Transport of ammonia in blood.
82. Biosynthesis of urea: biological function, regulation, localization, sequence of reactions.
83. Interrelation of the ornithine cycle with transformation of fumarate and aspartic acids.
84. Metabolism of the carbon skeletons of amino acids. Glycogenic and ketogenic amino acids.
85. Metabolism of aromatic and heterocyclic amino acids.
86. Metabolism of sulfur containing amino acids. Biological role of SAM. Biological function of glutathione.
87. Synthesis of creatine and creatinine. Diagnostic value of definition of creatinine in blood serum.
88. Metabolism of arginine. Formation and biological role of NO.
89. Metabolism of branched-chain amino acids. Biological function of vitamins B_{12} and H in metabolism of amino acids.
90. Metabolism of glycine and serine. Biological functions of tetrahydrofolate in metabolism of amino acids.
91. Disorders of amino acids metabolism (phenilketonuria, alcaptonuria, albinism, maple syrup urine disease).
92. Disorders of amino acids metabolism (Hartnup′s disease, histidinemia, cystinuria and homocystinuria).

93. Sources of separate atoms in the purine ring. Synthesis of purine nucleotides de novo: localization, sequence of reactions, regulation. Biosynthesis of AMP, GMP, ATP, GTP.
94. Pathways of purine bases reutilization in the tissues.
95. Synthesis of pyrimidine nucleotides: sequence of reactions, regulation, biosynthesis of deoxyribonucleotides.
96. Degradation of purine and pyrimidine nucleotides.
97. Disorders of nucleotide metabolism: gout, Lesch-Nyhan syndrome, orotic aciduria.
98. Replication of DNA: mechanism, enzymes.
99. Transcription: stages and mechanism.
100. Processing of RNA. Role of snRNA in RNA splising.
101. Inhibitors of RNA synthesis: actinomycin D, rifampicin, streptolydigin, α-amanitin.
102. Genetic code: features, table of genetic code.
103. Translation: basic components of the protein synthesis system, stages and mechanism.
104. Posttranslational modification of proteins.
105. Antibiotics as inhibitors of protein synthesis.
106. Regulation of protein synthesis in prokaryotes: induction and repression.
107. Regulation of protein synthesis in eukaryotes: repression of initiation synthesis of haemoglobin.
108. Biotechnology involving recombinant DNA. Clinical correlation of molecular disease.
109. Hormones and bioregulators in the system of intercellular integration of functions in an organism and their chemical nature.
110. Classification of hormones.
111. Mechanism of regulation of hormones synthesis and secretion.
112. Targets organs, receptors and second messengers of hormones.
113. Mechanism of action of polypeptide hormones and epinephrine.
114. Mechanism of action of steroid and thyroid hormones.
115. Hormones of hypothalamus: structure, mechanism of action, biological functions.

116. Growth hormone: structure, metabolic functions, regulation and disorder of hormone secretion.
117. Tropic hormones of pituitary gland are prolactin (mammotrophin), gonadotrophins – follicle stimulating hormone (FSH), luteinizing hormone (LH): metabolic functions, mechanism of action, regulation and disorder of hormones secretion.
118. Thyrotrophin (TSH): metabolic function, mechanism of action, regulation and disorder of hormones secretion.
119. Adrenocorticotrophic hormone (ACTH): metabolic function, the mechanism of action, regulation and disorder of hormones secretion.
120. Biological function of the products of processing of Proopiomelanocortin (POMC).
121. Hormones of posterior pituitary gland are vasopressin and oxytocin: mechanism of action, metabolic functions, clinical importance.
122. Insulin: structure, biosynthesis and catabolism, mechanism of action, metabolic function, clinical aspects.
123. Glucagon: structure, biosynthesis and catabolism, mechanism of action, metabolic function.
124. Hormones of GIT: gastrin, secretin, cholecystokinin.
125. Thyroid gland and its hormones: structure, biosynthesis, mechanism of action, biological effects of T_3, T_4, and clinical importance.
126. Adrenal medullar hormones: structure, biosynthesis, mechanism of action, metabolic functions of catecholamines.
127. Eicosanoids: classification, chemistry, biosynthesis and catabolism. Functions of prostaglandins, prostacyclins, thromboxanes, leukotriens and lipoxine. Clinical aspects. Inhibitors and stimulators of prostaglandin synthesis.
128. Hormones of adrenal cortex. Glucocoricoids: mechanism of action, biological functions, disorders of secretion.
129. Hormones of adrenal cortex. Mineralocorticoids: mechanism of action, biological functions, disorders of secretion. Renin-angiotensin-aldosterons system.

130. Androgens, estrogens and progesterone: mechanism of action, metabolism, biological functions, disorders of secretion.
131. Hormones that regulate Ca^{2+} and PO_4^{3-} metabolism: parathyroid hormone, 1,25-dihydroxycholecalciferol, calcitonin. Metabolism, mechanism of action, biological functions, disorders of secretion.
132. Metabolic fuel and dietary components. Fed state, fasting and starvation: general metabolic changes. Clinical correlation.
133. Digestion of proteins, amino acids absorption. Rotting of proteins in intestine. Clinical correlation.
134. Digestion of carbohydrates. Clinical correlation.
135. Digestion of dietary lipids. Mechanisms of absorption. Functions of bile salts in these processes. Clinical correlation.
136. Common characteristic of vitamins as components of the diet. Classification and nomenclature of vitamins. Biological functions of vitamins, their interconnection with enzymes. Vitamin deficiency diseases and the typical reasons of their occurrence.
137. Biochemical characteristic of water-soluble vitamins (B_1, B_2, B_5, B_6, B_{12}): structure, metabolism, basic dietary sources, daily requirement, metabolic functions, deficiency manifestation, clinical value.
138. Coenzyme vitamins (PP, H, folic acid): structure, biological functions, sources, daily requirement.
139. Biochemical characteristic of vitamin C and P: chemical structure, biological functions, sources, daily requirement.
140. Vitamins in medicine. Practical using of vitamins. Interrelation of vitamins in an organism. Manifestation of vitamin insufficiency.
141. Biochemical characteristic of fat-soluble vitamins (A, D, E, K, F): chemical structure, biological properties, daily need, sources, functions in the metabolism, mechanism of action.
142. Vitamin deficiency diseases of fat-soluble vitamins. Antioxidant properties of fat-soluble vitamins. Antivitamins: mechanism of action, use in medicine.

143. Water and electrolytes balance and imbalance, mechanism of regulation.
144. Biological function and clinical importance of macroelements (Na, K, Ca, Mg, P).
145. Biological function and clinical importance of trace elements.
146. Physiological and biochemical functions of blood. Respiratory function of erythrocytes. Haemoglobin: structure, properties, functions. Variants and pathological forms of haemoglobin.
147. Acid-base balance. Buffer systems of blood. Acid-base imbalance.
148. Biochemical composition of blood. Plasma proteins: biochemical characteristic, methods of separation, electrophoregrams of serum proteins in norm and under pathology. Total blood protein. Hypo-, hyper-, dis- and paraproteinaemias.
149. Blood enzymes, their clinical importance.
150. Nonprotein organic components of blood. Inorganic components of plasma. Azotemias.
151. Specificity of a metabolism in kidneys. Formation of urine in kidneys: glomerular filtration, tubular reabsorbtion, tubular secretion.
152. Normal urine composition. Pathological components of urine.
153. Regulation of acid-base status in the kidneys. Renin-angeiotensin and kallikrein-kinin systems. Renal function test.
154. Participation of liver in carbohydrates metabolism, its disorder.
155. Liver functions in lipid metabolism.
156. Liver functions in proteins metabolism.
157. Functions of liver in metabolism of vitamins and mineral elements.
158. Biochemical composition and functions of bile.
159. Detoxification in the liver. Reactions of biotransformation. Microsomal oxidation.
160. Catabolism of haemoglobin. Metabolism of bile pigments.
161. Types of jaundice: hemolytic, hepatic and obstructive.
162. Hereditary diseases of metabolism of bile pigments. Biochemical tests for diagnostics of jaundice.

163. Chemical composition of skeletal muscles.
164. Metabolism of carbohydrate, lipid and amino acids in muscle cells.
165. Chemical changes under muscle contraction. Fuel utilization in skeletal and cardiac muscles.
166. Proteins of connective tissue: collagen, elastin, glycoproteins and proteoglycans. Biosynthesis of collagen. Pathobiochemistry of connective tissue.
167. Mucopolysaccharides of connective tissue: structure, functions, clinical aspects of metabolism.
168. Chemical composition of nervous tissue. Structure and synthesis of myelin.
169. Metabolism of energy, carbohydrates, lipids and amino acids in nervous tissue.
170. Neurotransmitters (catecholamines, serotonin, histamine, acetylcholine, glutamate, GABA and other neurotransmitters): general properties, synthesis, accumulation, release and inactivation. Metabolic encephalopathies and neuropathias.

Appendix C
Practical skills
(the list of laboratory works which are required to know)

1. Electrophoresis as a method of protein separation. Electrophoregram of serum proteins were normal.
2. Clinical diagnostic value of definition of amylase activity, LDH isoenzymes, CPK, AST, ALT in the blood.
3. The glucose tolerance test: the principle of the method and analysis of results.
4. Clinical diagnostic value of definition of glycosylated haemoglobin in blood.
5. Clinical diagnostic value of definition of ketone bodies in blood and urine. Analysis of the mechanisms of ketonemia in diabetes and starvation.
6. Clinical diagnostic value of definition of the urea concentration in blood.
7. Clinical diagnostic value of definition of the concentration of uric acid in the blood.
8. Clinical diagnostic value of definition of the total protein concentration in the blood.
9. Method and clinical diagnostic value of definition of bilirubin in the blood.
10. Biochemical composition of urine in normal and pathological states: clinical diagnostic value of definition of normal and pathological components.

The list of compounds and processes the structural formulas of which are required to know

1. 20 standard amino acids; 4-hydroxyproline, 5-oxylysine.
2. Di- and tripeptides, which are formed by standard amino acids.
3. Nitrogenous bases, nucleotides, nucleosides, a fragment of the primary structure of DNA and RNA.
4. Glucose, galactose and fructose.
5. Glucosamine, galactosamine.

6. Lactose, galactose, maltose.
7. N-acetylgalactosamine, N-acetylglucosamine.
8. Fatty acids: palmitic, stearic, palmitooleic, oleic, linoleic, linolenic, arachidonic.
9. Triacylglycerols, cholesterol, cholesterol ester, phosphatidylserine, -ethanolamine, phosphatidylcholine.
10. Coenzymes: NAD^+, FAD, FMN, TPP, ubiquinone, pyridoxal phosphate.
11. The secondary messengers of hormone action: cAMP, cGMP, DAG.
12. ATP, creatine phosphate.
13. Citric acid cycle.
14. Glycolysis.
15. The oxidative stage of pentose-phoshate pathway (PPP).
16. Metabolism of fructose and galactose.
17. Gluconeogenesis.
18. UDP-1-glucose, glucose-6-phosphate, glucose-1-phosphate.
19. Lipolysis, TAG synthesis, synthesis of phosphatidylserine (2 pathways).
20. β-oxidation of fatty acids.
21. Oxidation of glycerol.
22. Biosynthesis and catabolism of ketone bodies.
23. Biosynthesis of fatty acids.
24. The biosynthesis of cholesterol (1-st stage).
25. Bile acids: cholic acid, chenodeoxycholic acid, glycocholic acid, taurocholic acid.
26. Transamination: alanine aminotransferase and aspartate aminotransferase reaction.
27. Glutamate dehydrogenase reaction.
28. Synthesis of GABA and histamine.
29. Ornithine cycle.
30. Formation of S-adenosyl methionine.
31. Synthesis of creatine.
32. Glutathione, taurine (reactions of synthesis from Cis).
33. Catabolism of Val, Ile, Met to succinyl-CoA.
34. The formation of NO from Arg.

35. Catabolism of Phe to homogentisic acid.
36. Synthesis of serotonin.
37. Biosynthesis of purine nucleotides: 5 phosphorybosylamine formation, sources of separate atoms of purine ring.
38. The formation of AMP and GMP from IMP.
39. The biosynthesis of pyrimidine nucleotides: synthesis of UMP, CTP.
40. Synthesis of deoxyribonucleotides, ribonucleotides.
41. Formation of dTMP, dUMP.
42. Catabolism of purine nucleotides.
43. Catabolism of pyrimidine nucleotides: end products of catabolism.
44. Formation of aminoacyl-tRNA.
45. The structure and synthesis of T_3, T_4.
46. Synthesis of catecholamines.
47. Cortisol, corticosterone, aldosterone, progesterone.
48. Estradiol, testosterone.
49. The synthesis of calcitriol.
50. Prostaglandines A_2, thromboxane A_2, leukotriene A_4.
51. Resynthesis of TAG in the small intestine.
52. Vitamins: B_1, B_2, B_6, PP, C, A, β-carotene, D_3, choline, carnitine.
53. Formation of vitamin A from β-carotene.
54. UDP-glucuronic acid, PAPS.
55. Synthesis of animal indican (from Trp).
56. The formation of hippuric acid.
57. The formation of bilirubin from biliverdin.
58. Bilirubin diglucuronide.
59. Synthesis of acetylcholine.
60. The hydrolysis of acetylcholine.

Appendix D

Table D.1 – Laboratory parameters

Common blood test	
1	2
Index	*Norm*
Erythrocytes	Male: $4.0–5.0 \cdot 10^{12}$ /l
	Female: $3.9–4.7 \cdot 10^{12}$ /l
Haemoglobin	Male: 135–180 g/l
	Female: 120–140 g/l
Color index	0.85–1.15
Biochemical blood analysis	
Total protein	65–85 g/l
Albumin	35–50 g/l (52–65 %)
Globulins:	23–35 g/l (35–48 %)
α_1-globulins	2–4 g/l (4.2–7.2 %)
α_2-globulins	5–9 g/l (6.8–12 %)
β- globulins	6–11 g/l (9.3–15 %)
γ- globulins	11–15 g/l (15–19 %)
A/G coefficient	1.2–2.0
Immunoglobulins:	
IgD	0–0.15 g/l
IgG	50–112.5 μmol/l
IgM	0.6–2.5 μmol/l
IgA	5.6–28.1 μmol/l
IgE	0.3–30 nmol/l
Bilirubin:	
total	8.5–20.5 μmol/l
free (indirect, unconjugated)	1.7–17.1 μmol/l
binding (direct, conjugated)	0.86–5.1 μmol/l
Lipids (total content)	5–7 g/l
Triacylglycerols	0.59–1.77 mmol/l
Fatty acids (total)	9.0–15.0 mmol/l
Phospholipids (total)	1.98–4.71 mmol/l
Cholesterol (total)	2.97–8.79 mmol/l
Lipoproteins:	
VLDL	1.5–2.0 g/l (90.63–0.69 mmol/l)

1	2
LDL	3–4.5 g/l (3.06–3.14 mmol/l)
HDL	1.25–6.5 g/l (1.13–1.15 mmol/l)
Chylomicrons	0–0.5 g/l (0–0.1 м mmol/l)
Glucose	3.3–5.5 mmol/l
Glycated haemoglobin	4–7 %
Lactic acid (in venous blood)	0.56–1.67 mmol/l
Pyruvic acid	45.6–114.0 μmol/l
Iron in blood	8.53–28.06 μmol/l
Potassium in blood (plasma)	3.8–5.2 mmol/l
Sodium in blood (plasma)	138–217 mmol/l
Calcium in blood (plasma)	0.75–2.5 mmol/l
Magnesium (plasma)	0.78–0.91 mmol/l
Phosphorus (inorganic), serum	0.646–1.292 mmol/l
Chlorides	97–108 mmol/l
Rest nitrogen	14.28–25 mmol/l
Urea	3.33–8.32 mmol/l
Creatinine	53–106.1 μmol/l
Creatine	Male: 15.25–45.75 μmol/l Female: 45.75–76.25 μmol/l
Uric acid	Male: 0.12–0.38 μmol/l Female: 0.12–0.46 μmol/l
Lactate dehydrogenase (LDH)	< 7 mmol/(hour · l)
Aldolase	0,2–1,2 mmol/(hour · l)
Alpha-amylase (diastase)	12–32 г/(hour · l)
Aspartate aminotransferase (AST)	0.1–0.45 mmol/(hour · l)
Alanine aminotransferase (ALT)	0.1–0.68 mmol/(hour · l)
Cholinesterase	160–340 mmol/(hour · l)
Alkaline phosphatase	0.5–1.3 mmol/(hour · l)
Creatine kinase	0.152–0.305 mmol/(hour · l)
Lipase	0.4–30 mmol/(hour · l)
Thymol turbidity test	Up to 5 units
C-reactive protein	Negative
Serum osmolality	275–295 mOsm/kg
Cortisol, serum	230–750 nmol/l
Parathyroid hormone, serum	42.6–9.31 pmol/l
Growth hormone	0–118 pmol/l

1	2
Thyroxine (T_4), serum	65–155 nmol/l
Triiodothyronine (T3), serum	1.77–2.43 nmol/l
Thyroid stimulating hormone (TSH), serum or plasma	128±28 pmol/l
Biochemical parameters of urine	
Relative density	1.016–1.022
Total protein	45.0–75.0 mg/day
Potassium	38–77 mmol/day
Calcium	2.5–7.5 mmol/day
Creatinine clearance	Male: 97–137 ml/min Female: 88–128 ml/min
Uric acid	1.48–4.43 mmol/day
Sodium	Varies depending on the diet
Oxalates	90–445 μmol/l
Chlorides	4.1–13.7 μmol/day
17-ketosteroids	Male: 27.7–79.7 μmol/day Female: 17.4–55.4 μmol/day
17-hydroxy corticosteroids	0.11–0.77 μmol/day
Alpha-amylase (diastase)	28–160 g/(hour · l)
Creatinine in urine	Male: 6.8–17.6 mmol/day Female: 7.1–15.9 mmol/day
Urea	30 g/day

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