Ministry of Education and Science of Ukraine
Sumy State University

BIOLOGICAL CHEMISTRY
Practical Lessons

Study guide

In two parts

Part 1

Submodule I “Basic Aspects of Metabolism”. Submodule II “Metabolism of Carbohydrates, Lipids and Its Regulation”

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The study guide in Biological Chemistry contains materials for preparing students for practical classes in biochemistry, a laboratory workshop that combines modern biochemical research methods, an algorithm for performing each work, reference information, and a list of recommended literature. The study guide is complited in accordance with the current biochemistry curriculum, meets the requirements of state standart of higher medical education.

For students of medical faculties of higher educational institutions of Ukraine.

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CONTENT

Introduction

Biological chemistry is a fundamental science that is essential in the training of future doctors. The development of a basic knowledge of the subject forms the foundation for the formation of biochemical thinking in students, as well as the progress of their basic skills and the ability to assess metabolic processes in healthy individuals and in the case of pathological processes.

A higher education reform carried out in Ukraine requires a modern presentation of materials that meet the goals and objectives of the current state standards of medical education and the principles of the Bologna Process (ECTS). The study guide is based on the current typical program of biological chemistry.

Part I of "Biological Chemistry: Practical Lessons" contains the themes of submodule I, "Basic Aspects of Metabolism," and submodule II, "Metabolism of Carbohydrates, Lipids, and Its Regulation." The study guide contains all necessary information for the preparation of students for practical classes, test questions of the module, and references. Each lesson indicates the importance of the theme, its goals and objectives, offers theoretical questions, and recommends literature. Each lesson also has a practical part that includes laboratory work with the methodology of its implementation and the clinical and diagnostic value of various biochemical parameters.

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

- clearly understand the principles of the laboratory work methods and the main stages of the experiment;
- know the basic safety rules;
- understand the clinical diagnostic value of detecting certain substances in biological materials;
- have protocols for laboratory work in their notebooks.

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

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

The authors hope that the study guide will help students quickly find a large amount of scientific information during their studies and gain knowledge about modern methods of biochemical research. To develop practical skills in the definition of certain substances in biological materials, students should have the ability to analyze and evaluate the results of laboratory tests that are important in their further studies of pharmacology, pathophysiology, and clinical sciences, as well as in their future careers.

Plans of practical lessons on biological chemistry by themes 3rd semester

Lesson No.	Theme	Hours
	Submodule 1. Basic aspects of metabolism	
1	Control of the knowledge initial level. Adoption of principles of biochemical laboratory research perfomance. Justification and clinical diagnostic value of biochemical indices changes	2
2	Methods of studying amino acid composition of biological liquids	2
3	Physical and chemical properties of proteins. Methods of extraction and separation of proteins. Classification of proteins. Characteristics of simple proteins and natural peptides	2
4	Classification, structural features and research methods of complex proteins. Study of the structure, functions and physical and chemical properties of nucleic acids	2
5	Structure, physical and chemical properties and classification of enzymes. Methods of enzyme activity definition	2
6	The definition of enzyme activity and mechanisms of its action. Kinetics of enzyme catalysis. Cofactors and coenzymatic vitamins', functions in the catalytic activity of enzymes	2
7	Rregulation of enzymatic processes and the analysis of the enzyme pathology origin. Medical enzymology	2
8	Metabolism: general characteristics. Stages of aerobic catabolism. Tissue respiration	2
9	TCA cycle: general characteristics, reactions, regulation and energetic balance	2
10	Mechanisms of biological oxidation, oxidative phosphorylation and ATP synthesis. Electron transport chain (ETC)	2
11	Basic principles of chemiosmotic theory. The analysis of the action of inhibitors and uncouplers of the oxidative phosphorylation	2
12–13	*Examination submodule 1 "Basic aspects of metabolism"*	2

	Submodule 2 Carbohydrate and lipid metabolism and its regulatio	
14	Digestion of carbohydrates. Glycolysis as an anaerobic oxidation of carbohydrates	2
15	Aerobic glucose oxidation	2
16	Catabolism and biosynthesis of glycogen. Regulation of glycogen metabolism. Metabolism of glycoconjugates	2
17	Gluconeogenesis and alternative pathways of carbohydrate metabolism. Definition methods of glucose concentration in blood	2
18	Mechanisms of metabolic and hormonal regulation of glucose metabolism and its concentration in blood. Biochemistry of diabetes mellitus	2
19	Test on situational tasks from "Step-1": " Basic aspects of metabolism"	2
20	General characteristics of lipids. Lipids of biomembranes. Lipolysisand its regulation	2
21	Beta–oxidation of fatty acids. Ketone body metabolism research	2
22	Biosynthesis of fatty acids, triacylglycerols and complex lipids. Determination of total phospholipid concentration in blood serum	2
23	Cholesterol biosynthesisand biotransformation. Blood lipoproteins	2
24	Metabolism in adipocytes. Metabolism of glycerol.Biochemistry of unsaturated fatty acids	2
25	Regulation and disorders of lipid metabolism. Interrrelationsbetween lipid and carbohydrate metabolisms	2
26–27	*Examination submodule 2 "Basic aspects of metabolism. Carbohydrate and lipid metabolism and its regulation"*	2

Lesson 1
Theme: CONTROL OF THE KNOWLEDGE INITIAL LEVEL. ADOPTION OF PRINCIPLES OF BIOCHEMICAL LABORATORY RESEARCH PERFOMANCE. JUSTIFICATION AND CLINICAL DIAGNOSTIC VALUE OF BIOCHEMICAL INDICES' CHANGES

Actuality of the theme*. Knowledge about the basic modern methods of laboratory diagnostics and principles of biochemical laboratory research allow a doctor to choose the methods of sick people examination correctly and take into account their pecularities, sensitiveness, analyse and interpret the results of analyses, after additional researches for setting adequate effective therapy and treatment monitoring.*

Objectives. A student should be able*: to characterize basic classes of organic substances; to explain principles of biochemical laboratory researches.*

Main tasks. *A student should be able:*

1. *To explain the basic rules of work in a biochemical laboratory.*
2. *To explain principles of biochemical researches, estimation criteria of laboratory methods, rules of standard preparation of experimental sample.*
3. *To characterize physical and chemical properties and to represent structures of basic classes of organic substances; to distinguish functional groups and types of chemical compounds.*

Referances

1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 15–21.
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Weil. 30th edition. – Lange Medical Books / McGraw–Hill, 2017. – P. 1–5.

Theoretical questions

1. The definition of biochemistry as a science. A place of biochemistry among other medical and biological subjects.
2. Achievements and prospects of the development of biochemistry, theoretical and molecular biology, biotechnology, gene engineering.
3. A purpose of biochemical laboratory researches; estimation criteria of laboratory methods: principles of collection of researche samples, errors of laboratory diagnostics.
4. Chemical composition of living organisms (biotic), their features as compared with the objects of abiotic environment. Chemical composition of human organism.
5. Biochemical components of cells, their biochemical functions. The major classes of biomolecules.
6. The general characteristic of cyclic compounds, proteins, lipids, carbohydrates, nucleic acids; features of chemical composition and structure, biological functions.

RULES FOR WORK IN A BIOCHEMISTRY LABORATORY

In biochemical laboratory it is necessary to know the following key rules:

1. Do not work alone in the laboratory.
2. Keep the benches and shelves clean and well–organized.
3. Prevent contamination of the chemicals; use only clean glassware and spatulas; label glassware in use.
4. Plan your experiments before starting their carry out.
5. Eating, drinking and smoking in the laboratory are strictly forbidden.
6. While heating a solution one should make sure not to overheat it, therefore, vigorous mixing of the solution by shaking or stirring is required. The mouth of the glassware containing the solution to be heated should never be pointed toward anyone.

7. Handling of strong acids and bases requires special attention. When diluting concentrated acids, the acid should be poured into the water and never on the contrary.
8. The pipets should never be filled with solutions of toxic substances, biological fluids, strong acids and bases by mouth suction. Use either automatic pipets or pipet pumps.
9. Volatile liquids and solids that are toxic or irritating should be handled under fume hoods.
10. While handling flammable liquids such as ether, alcohols, benzene, naked flame (burners, matches) must not be in use. The above liquids must not be stored near radiating heat sources, such as the laboratory oven.
11. Before using electrical appliances, make sure they are grounded.
12. Flasks with flat bottoms or thin walls should not be dessicated.
13. Before leaving the laboratory, electrical equiment should be turned off, and gas burners – extinguished. No tap water should be left running.

Rules to follow in case of accidents and injuries

Chemical splatters into the eye. First the eyelid should be opened by using the thumb and the pointing finger. Then, by using the eye wash kit, the eye should be rinsed with large amounts of water. When an acid or alkaline solution gets into the eye, the eye should be rinsed with 1 % $NaHCO_3$ or 1 % boric acid solutions, respectively. The victim should be taken to the doctor as soon as possible.

Burning. The burned spot on the skin should not be treated with water, rather a special bandage should be used. See a doctor if necessary.

Poisoning. Prompt medical treatement should be obtained.

All injuries and accidents must be reported to the teacher.

Lesson 2

Theme: METHODS OF STUDYING AMINO ACID COMPOSITION OF BIOLOGICAL LIQUIDS

Actuality of the theme. *Amino acids are organic low–molecular compounds, which are included into the structure of proteins and peptides, and also are in the cells of organism in the free state and take part in metabolism. Physical and chemical properties of amino acids are predetermined by features of structure and functions of proteins, possibility of low chemical transformations, for example, such as TCA cycle, detoxification of ammonia, synthesis of creatine and others.*

Theoretical knowledge about the structure, physical and chemical properties, definition of amino acid composition of proteins and peptides; their level in biological liquids (blood, urine) is important both for forming fundamental conceptions of molecular basis of life and for clinical and laboratory diagnostics.

Objectives*. A student should be able to characterize physical and chemical properties of amino acids, methods of definition of amino acid composition of proteins and peptides, amino acid spectrum of biological liquids.*

Main tasks. A student should be able:

1. *To use knowledge about physical and chemical properties of amino acids in the analyses of results of chromatography and electrophoresis.*
2. *To write chemical structure of amino acids and peptides.*

References

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Theoretical questions

1. The general characteristics and biological functions of proteins and peptides.
2. Amino acid composition of proteins and peptides: structure, classification and physical and chemical properties of amino acids.
3. The formation of peptide bonds. Levels of protein structure. Chemical bonds in protein molecules.
4. Methods of study of amino acids and proteins in the biological liquids. The colour reactions of amino acids and proteins. Methods of chromatography for separation of amino acids.

Laboratory work

1. Color reactions of proteins and amino acids

Results of many color reactions depend on the reactive groups in side chains of specific amino acid residues and also can be produced by the corresponding free amino acids.

1.1. Biuret reaction

Principle of the method. It is a qualitative reaction of a peptide bond (–CO–NH–). This reaction is produced by any compound having at least 2 peptide linkages in its molecule, e.g. proteins, peptides (except dipeptides) and biuret (formed by heating urea). If the protein solution is treated with solutions of diluted $CuSO_4$ and sodium hydroxide, a violet or pink color is produced due to the formation of violet – or pink – colored compounds, involving the nitrogen of more than one peptide bond and the oxygen of water. This is the basis of the biuret formation test for urea.

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

Reagent	Quantity (drops)
Egg protein	3–5
NaOH, 10 %	3–5
$CuSO_4$, 1 %	1–2

1.2. Xanthoproteic reaction

Principle of a method. It is the qualitative reaction of cyclic amino acids. Proteins with phenolic or indolic amino acids (phenylalanine, tyrosine, and tryptophan) give a yellow precipitate after boiling with HNO_3 (strong). The precipitate changes color to orange after the addition of alkali. The yellow color is due to the nitro derivatives of the aromatic amino acids present in the protein. The sodium salts of nitro derivatives are orange in color. Gelatins which are produced from collagens have the deficiency in these amino acides and cannot give the positive result of the test.

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

Reagent	Quantity (drops)
Egg protein, 1 %	5
HNO_3 (strong)	5
$CuSO_4$, 1 %	1–2
The solution should be boiled for 2 min, t =100°C. You can see yellow precipitation in the test tube	

1.3. Sulphur reaction (Fall reaction)

Principle of the method. It is qualitative reaction of sulfur–containing amino acid (cysteine). The sulphur in sulphur containing amino acids of the proteins in presence of NaOH, is changed into Na_2S which forms black lead sulphide when reacted with lead acetate.

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

Reagent	Quantity (drops)
Egg protein, 1 %	5
NaOH, 30 %	5
CH_3COOPb, 5 %	2
The solution should be boiled for 2 min, t =100°C. You can observe the black coloring	

1.4. Ninhydrin reaction

Principle of the method. The ninhydrin test is a test for amino acids and proteins. Heating a protein with ninhydrin produces a violet color due to the reaction between ninhydrin and the free α–amino group of the N–terminal amino acid residue of the protein. The formation of a complex called Ruhemann's purple due to condensation of two molecules of ninhydrin with one molecule of ammonia from amino acid is responsible for the violet color.

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

Reagent	Quantity (drops)
Egg protein, 1 %	5
Ninhydrin, 0,5 %	5
Warm up. You can observe the violet coloring	

1.5. Adamkiewicz reaction

Principle of the method. This reaction detects the amino acid tryptophan with the indole ring in the structure. The addition of the strong acetic and sulfuric acids to the solution of tryptophan leads to the formation of a red–violet ring appearing on the boundary of different liquids. Gelatins and collagens have deficiency in tryptophan and therefore, do not give positive Adamkiewicz reaction.

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

Reagent	Quantity (drops)
Egg protein, 1 %	5
CH_3COOH (strong)	5
H_2SO_4 (strong)	5
Warm up the test tube in a boiling water bath. The red–violet ring is formed on the boundary of different liquids	

1.6. Nitroprusside reaction

Principle of the method. If the solution of a protein with sulfur–containing amino acid (cysteine) is treated by sodium nitroprusside, in diluted ammonium hydroxide deep purple red color is obtained in solution. Heating denatures proteins and liberates free –SH radicals, if the protein contains such in the combined form.

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

Reagent	Quantity (drops)
Egg protein, 1%	10
NaOH, 10%.	10
Warm up the test tube in a boiling water bath for 5 min.. Cool it	
Sodium nitroprusside	3–5

The protocols of the laboratory work follow the experimental part. For this purpose, add the results to the table, then draw the conclusions.

Test No	Result of reaction (+/–)						Conclusion
	Biuret	Xanthoproteic	Fall	Ninhydrin	Adamkiewicz	Nitroprusside	

Lesson 3
Theme: PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS. METHODS OF EXTRACTION AND SEPARATION OF PROTEINS. CLASSIFICATION OF PROTEINS. CHARACTERISTICS OF SIMPLE PROTEINS AND NATURAL PEPTIDES

Actuality of the theme. *Proteins are the basis of structure and functions of living organisms. Properties, which distinguish living nature from non–living, are based on the variety of structure and functions of proteins, their efficiency and physical and chemical features. Changes of the composition, structure and properties of proteins result in the destabilization of biochemical processes in a cell that often is regarded as pathology. The modern methods of excretion and fractionation of proteins allow to separate proteins from other cellular structures and study the features of their composition and properties. They open the wide prospects of using these researches in medicine.*

Objectives. A student should be able *to explain physical and chemical properties of proteins; to characterize the basic methods of separation and fractionation of proteins; to classify proteins in accordance to their structure and composition.*

Main tasks. A student should be able:

1. *To use physical and chemical properties of proteins for characteristics of basic methods of their separation, fractionation, for studing amino acid composition.*
2. *To interpret the results of the research of proteins by the methods of precipitation, dialysis, ultracentrifugation, chromatography, electrophoresis.*
3. *To characterize the classes of simple proteins and peptides in accordance to modern classifications.*

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Theoretical questions

1. Physical and chemical properties of proteins. The amphoteric nature. The isoelectric point (pI).
2. Solubility of proteins. Thermodynamic stability of proteins and denaturation.
3. Methods of protein separation, fractionation, and the analysis of a structure; reactions of sedimentation of proteins and practical

application of these reactions; methods of centrifugation and chromatography; electrophoretic methods; methods of studying amino acid composition and structure of proteins and peptides.

4. Classifications of proteins. The general characteristics of simple proteins, their functions.
5. Natural peptides. The general characteristics of these molecules: the structure and functions.

Laboratory work

1. Protein precipitation reactions (salting out)

S*alting out* is the reversible reaction of the protein precipitation by high concentration of neutral salts. For *salting out* more often are used such salts: $(NH4)_2SO_4$, NaCI, Na_2SO_4, $MgSO_4$.

In these reactions the structure of proteins does not change essentially. The high concentration of salt results in the dehydration of proteins, thus reducing their solubility and precipitation. The precipitate can be dissolved again in an initial solvent. The protein molecule retains the previous properties.

The method of the *salting out* is widely applied for the fractionation of the protein mixture when it is necessary to separate one protein from another (for example, albumins and globulins). The *salting out* of the globulins is easier than of the albumins by the half–saturated solution of $(NH_4)_2SO_4$, whereas the albumins are salted out by the saturated solution of this salt.

Principle of the method. The *salting out* causes dehydration reaction of macromolecules of protein with simultaneous neutralization of their electric charge.

2. Irreversible denaturation (precipitation) of proteins by salts of heavy metals, concentrated acids and heating

2.1. Denaturation of proteins by salts of heavy metals.

Principle of the method. Salts of heavy metals (copper, lead, silver, mercury) precipitate proteins out from solutions and form complex compounds which are dissolved by excess of these salts, but can not be soluble in water.

The property of proteins to connect ions of heavy metals in the form of an insoluble precipitate in water is used in cases of poisoning by salts of mercury, copper, lead, etc. It is advised to take proteins of milk or eggs at once after poisoning while these salts are still in a stomach and have not been absorbed. Then the patient should be made to vomit to remove the poison.

Course of work. Add all reactants shown in the table to the three test tubes:

Reactant	**Test tube**		
	I	II	III
Egg white solution, 1 %	5 drops	5 drops	5 drops
$CuSO_4$, 5 %	2–3 drops	–	–
CH_3COOPb, 5 %	–	2 drops	–
$AgNO_3$, 5 %	–	–	2 drops
The formation of the insoluble precipitate			
$CuSO_4$, 1 %	5–10 drops	–	–
CH_3COOPb, 5 %	–	5–10 drops	–
$AgNO_3$, 5 %	–	–	5–10 drops
	the precipitate will be solved		the precipitate will not be solved

3.2. Denaturation of proteins by strong organic and mineral acids

Principle of the method. Under the action of strong mineral and organic acids on proteins the denaturation of protein molecules and the formation of complex salts of proteins with acids take place.

The precipitate dissolves in the excess of the acid, except nitric acid. Therefore, the reaction of sedimentation by the strong nitric acid underlies the quantitative definition of proteins by Roberts–Stalnikov–Brandberg method.

3.2.1. Denaturation of proteins by nitric acid.

Course of work. Add 1 ml of the strong nitric acid to the layer of 1 ml of the protein solution (1 %) accurately. On the border of these solutions the precipitate in the form of a white ring is formed.

3.2.2. Denaturation of proteins by sulfuric acid.

Course of work. On walls of a test tube, add some drops of the strong sulfuric acid to 1 ml of the egg white solution carefully. The precipitate is formed.

3.2.3. Denaturation of proteins by trychloracetate (TCA) and sulphosalicilic acids

Course of work. Add 1 ml of the protein solution to two test tubes. Add 1 ml of 20 % solution of sulphosalicilic acid to the first one and to another – 1 ml of TCA solution (10 %). Then protein precipitates out. Sulphosalicilic acid is used for the qualitative determination of proteins in urine.

4. The definition of gelatin pI

Principle of the method. The definition of gelatin isoelectric points is based on research of its solubility in various pH. The pI corresponds to the pH of a solution at which the solubility of gelatin decreases and it precipitates.

Course of work. Add all reactants, shown in the table, to three test tubes.

Reagent	**Test tube No (ml)**					
	1	2	3	4	5	6
H_2O	3.8	3.5	3.0	2.0	–	3.2
Acetic acid (0.1 M)	0.8	0.5	1.0	2.0	4.0	–
Acetic acid (1 M)	–	–	–	–	–	0.8
CH_3COONa (0.1 M)	2	2	2	2	2	2
Solution of gelatine (1 %)	2	2	2	2	2	2
Acetone	2	2	2	2	2	2
pH of a solution	5.6	5.3	5.0	4.7	4.4	4.1
Degree of the solution turbidity						

Define a degree of turbidity by a sign (+ or –) through 30 mines. The pH of the solution, where the greatest turbidity is observed, will be equal to the pI of gelatin.

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

Lesson 4
Theme: CLASSIFICATION, STRUCTURAL FEATURES AND RESEARCH METHODS OF COMPLEX PROTEINS. STUDY OF THE STRUCTURE, FUNCTIONS AND PHYSICAL AND CHEMICAL PROPERTIES OF NUCLEIC ACIDS

Actuality of the theme. *The detailed study of structural and functional features of complex proteins proves that their representatives take part in key metabolic processes. Breaking of functioning of such protein molecules results in pathological changes of metabolism which can have dangerous clinical manifestations. The haemoglobin synthesize disturbance results both in anaemia and inherited diseases like thalassemias and hemoglobinopathies. The reason of other inherited illnesses, for example Wilson–Konovalov disease, is the inherited defect of ceruloplasmine which provides the transportation of copper in the blood. Atherosclerotic changes of vessels can develop at the inherited breaking synthesis of lipoproteins. The result of this state is a heart attack of myocardium, stroke and other pathologies. Theoretical knowledge about complex proteins and methods of their research can be used for the study of subjects such as pathophysiology, pharmacology, clinical biochemistry and is an important in the future medical practice of doctors.*

Objectives. A student should be able to *interpret the results of structural and functional changes of complex proteins and to know methods of their research.*

Main tasks. A student should be able:

1. *To use knowledge about classifications, structure and functions of complex proteins for explanation of consequences of the inherited and accuired pathological changes which are related to functioning of separate representatives of complex proteins.*
2. *To determine glycoproteins (on an example of salivary mucin), hemoproteins (on the example of hemoglobin), nucleoproteins.*
3. *To interpret the results of determination of complex proteins.*

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Theoretical questions

1. Complex proteins: the classification, representatives of each class, the content in the organism and functions.
2. General characteristics of chromoproteins, structural features, biological functions.
3. Hemoproteins: myoglobin, haemoglobin, cytochromes. Their biological functions and structural features. Normal content of haemoglobin in the blood. General characteristics of hemoglobinopathies and thalassemias.
4. Flavoproteins: structural features and their functions in an organism.
5. Glycoproteins: the classification, structural features, distribution, biological functions.
6. Lipoproteins, phosphoproteins, metalloproteins: the structure, biological functions.
7. Nucleotides: the structure, nomenclature, biological functions. Minor nitrogenous bases and nucleotides. Free nucleotides, participating in metabolic reactions. Cyclic nucleotides.

8. Nucleic acids: features of structural organization, biological functions of DNA and RNA. Experimental proving the genetic role of DNA (phenomenon of transformation).
9. Study methods for the composition and structural features of complex proteins.

Laboratory work

1. Extraction of mucin from saliva and the qualitative test on the carbohydrate component

Principle of the method. Mucin is very soluble in the alkaline solutions and precipitate in the acid solutions.

1.1. Extraction of mucin from saliva

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

Reagent	Quantity
Saliva	2–3 ml
CH_3COOH (strong)	10–12 drops

You can see a clot of mucin in the test tube.

1.2. Qualitative test on the carbohydrate component

Course of work. T**o** the test tube with a clot of mucin, add all reagents in accordance with the table:

Reagent	Quantity (drops)
α–Naftol, 1 %	1–2
H_2SO_4 (strong)	1–2

A red–violet ring between two liquid layers can be observed. It is the evidence of the presence of carbohydrate component.

2. Hydrolysis of yeast nucleoproteins and opening of a carbohydrate component

For yeast hydrolysis one should add nucleoproteins and 20 ml of sulfuric acid (10 %) to 2.5 g of yeast. Hydrolysis is carried out in a round–bottom flask with air condenser during 1 hour.

2.1. Reaction on a carbohydrate component with α–naftol

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

Reagent	Quantity (drops)
Solution of hydrolyzed yeast	5
0.2 % α–Naftol	3
H_2SO_4 (strong)	20

The pink–violet solution coloring can be observed in the test tube.

2.2. Reaction on deoxyribose and ribose with difenylamine

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

Reagent	Quantity (drops)
Solution of hydrolyzed yeast	5
Difenilamin 1 %	20
Boil in water bath for 15 ′	

The dark–blue solution coloring can be observed in the test tube.

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

Lesson 5
Theme: STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES AND CLASSIFICATION OF ENZYMES. METHODS OF ENZYME ACTIVITY DEFINITION

Actuality of the theme. *Enzymes, as high–specific biological catalysts, provide thousands of interrelated chemical reactions with high rate. Enzymes supply the most important processes of life: the expression of genetic information, the production of energy, synthesis and breaking down of molecules. The study of the structure, functions and molecular mechanisms of enzyme action allows revealing of the essence of the basic processes of vital functions. Knowledge about the biocatalysts is also required in chemical and pharmaceutical industries, food industry, medicine.*

Objectives. ***A student should be able*** *to characterize enzymes as biological catalysts; to explain basic principles of the enzyme definition in biological samples.*

Main tasks. A student should be able:

1. *To characterize the basic features of enzymes as biological catalysts; to explain their physical and chemical properties.*
2. *To characterize the structure of simple and omplex enzymes.*
3. *To interpret biochemical conformities of structure and functioning of different classes of enzymes.*
4. *To classify coenzymes in accordance to their chemical nature, the type of reactions which are catalysed by them.*
5. *To represent the structural formulas of basic coenzymes those are vitamine derivatives.*

References

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Theoretical questions

1. Enzymes as biological catalysts of metabolism. General properties of enzymes.
2. The nomenclature of enzymes and the classification of them in accordance with the type of reaction.

3. The structure of enzymes; oligomeric enzymes; multienzyme complexes (functional enzyme systems), membrane–associated enzymes.
4. Cofactors and coenzymes. The structure and properties of coenzymes; vitamins as precursors in the biosynthesis of coenzymes. Coenzymes are derivatives of vitamin B_1, B_2, B_3, B_5, B_6, Bc, B_{12}, H and lipoic acid.
5. Classification of coenzymes in accordance with the chemical nature and the type of reaction which they catalyse.
6. Methods of separation of enzymes, their fractionation (ultracentrifugation, chromatography, electrophoresis) and analysis of the enzyme activity.

Laboratory work

1. Amylase thermolability

The principle of the method. The amylase activity of saliva depends on temperature. The degree of starch splitting is detected by Trommer′s reaction and reaction with I_2 (Lugol′s test).

Course of work. 2–3 ml of saliva should be collected in a clean test tube. Add 4 drops of saliva and 16 drops of H_2O to the empty test tube for the dilution of saliva (saliva is diluted 5 times). The remain of saliva is to be boiled.

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

Test tube No	Reagent	Quantity (drops)
1	Starch 10 %; Diluted saliva	10 10
2	Starch 10 %; Boiled saliva	10 10
3	Starch 10 %; H_2O	10 10
All test tubes are put in a thermostat for 10′, t°=38°C		

The solution in all test tubes should be divided into two parts (6 test tubes). Carry out the qualitative starch reactions (in 3 test tubes) and such products of hydrolysis as glucose and maltose (in other 3 test tubes).

1.1. Qualitative starch iodine reaction (Iodine (I_2) test)

Course of work. Add 1 drop of lugol′s solution (I_2 in KI) to three test tubes with starch and saliva (see above). The dark–blue color can be observed in the test tube with starch.

1.2. Trommer′s reaction

Course of work. Add all reagents in accordance with the table into the three remaining test tubes:

Reagent	Quantity (drops)
NaOH, 10 %	5
CuSO4, 1.0 %	3
Boil in water bath for 1 min	

The Trommer′s reaction is positive for glucose and maltose solutions. In this case the red precipitate can be observed in the test tube.

The results should be formed in the table:

Test tube No	Enz	S	t°	I_2 test (+ or –)	Trommer′s reaction (+ or –)
1					
2					
3					

2. The specificity of amylase

The principle of the method. Amylase hydrolyses starch and can not hydrolyze sucrose. Hydrolysis of starch is confirmed by positive Trommer′s reaction.

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

Test tube No	Reagent	Quantity (drops)
1	Diluted saliva Starch, 1.0 %	5 10
2	Diluted saliva Sucrose, 1.0 %	5 10
All test tubes are placed in a thermostat for 10′, t°=38°C		

After the placement, Trommer′s reaction should be done.

The results should be formed in the table:

Test tube No	Enz	Substrate	Trommer′s reaction (+ or –)
1			
2			

3. The pH influence on the amylase activity

The principle of the method. The influence of the pH on the amylase activity is detected by Lugol′s reaction for starch.

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

Test tube No	Reagent	Quantity
1	Saliva NaOH, 0.4 % Starch, 1.0 %	1.0 ml 20 dops 10 drops
2	Saliva HCl, 0.4 % Starch, 1.0 %	1.0 ml 20 drops 10 drops
All test tubes are placed in a thermostat for 15′, t°=38°C		

Add 20 drops of 0.4 % HCl solution to the 1st test tube (with NaOH) after the incubation. After it 1 drop of Lugol′s solution should be added to both test tubes.

The results should be formed in the table.

Test tube No	Enz	Substrate	pH	Lugol′s test (+ or –)
1				
2				

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

Lesson 6
Theme: THE DEFINITION OF ENZYME ACTIVITY AND MECHANISM OF ENZYME ACTION. KINETICS OF ENZYME CATALYSIS. COFACTORS AND COENZYMATIC VITAMINS', FUNCTIONS IN THE CATALYTIC ACTIVITY OF ENZYMES.

Actuality of the theme. *The definition of enzyme activity in blood and urine is used for diagnostics of many diseases with the prognostic purpose and during treatment. Knowledge of this definition basic principles and approaches gives the possibility to be directed not only in methods and to interpret the results, but also to create modern methods. The definition of activity of aspartat aminotransferase (AST), creatinphosphokinase (CPK), lactate dehydrogenase (LDH) and others are traditional methods which are successfully used in the medical practice.*

Besides, knowledge of mechanisms of the enzyme action is basic for understanding of the course of pathological processes at molecular level.

One of the characteristic properties of life is ability of living organisms to regulate chemical reactions kinetically, restraining an attempt to achieve thermodynamic equilibrium. Enzymatic kinetics studies the law of the influence of different factors on the reaction rate. The studies of kinetics of enzyme reactions give information for finding out molecular mechanisms of the enzyme action for provision of probability, direction and rate of reactions.

Objectives. A student should be able *to define the activity of enzymes with the use of photoelectric calorimeter; to interpret the results of the definition; to explain the basic kinetic aspects of enzyme reactions, functions of cofactors that are different by nature.*

Main tasks. A student should be able:

1. To explain thermodynamic aspects of enzyme catalysis.
2. To interpret the molecular effects of enzyme catalysis.
3. To define the activity of amylase in the serum of the blood with photoelectric calorimeter.
4. To analyse the results and to make the conclusions.
5. To represent and explain the chart of the enzyme activity dependency (rate of reaction) on the concentration of enzyme and substrate, pH and temperature.

6. To interpret the function of vitamins and their biologically active derivates in the mechanisms of enzyme catalysis.
7. To explain the mechanism of the formation of multiple forms of enzymes and advantages of isoenzymes' definition in blood and urine.

References

1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 33–51, 63–75.
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Theoretical questions

1. Mechanisms of enzyme action: thermodynamics of enzymatic catalysis, active centres of enzymes. Hypotheses of E. Fisher and D. Koshlend.
2. The sequence of stages of catalysis.
3. Methods of the enzyme activity definition. The basic principles of the definition.
4. Spectrophotometric methods of the enzyme activity definition and the visualization of the results of the enzyme reaction.
5. Units of the enzyme activity measurement and the amount of enzymes: international units, katal, specific activity of enzyme.
6. Kinetics of enzyme reactions: the reaction rate dependence on the concentration of enzyme and substrate, pH and temperature.

7. Michaelis–Menten constant (Km), its semantic value. The Michaelis–Menten equation processing by the method of double reciprocal coordinates (Lineweaver–Burk equation).
8. Use of Km for the characteristic of enzyme activity and the provision of probability of metabolic processes in a cell.
9. Isoenzymes are multiple molecular forms of enzymes and the result of the expression of different genetic loci. The diagnostic significance of isoenzyme definition in blood.

Laboratory work

1. The definition of the amylase activity in blood serum

The principle of the method. This method is based on the colorimetric definition of the nonhydrolyzed starch by the color reaction with iodine.

Course of work. Add to the two 50 ml volumetric flasks all reactants in accordance with the table:

Reagent	Check sample (ml)	Test sample (ml)
Starch 0.1 %	5	5
Incubation in the thermostat for 5', 37 °C		
Blood serum	–	0.1
Incubation in the thermostat for 7.5', 37 °C		
Blood serum	0.1	–
Iodine Solution	5	5

Then expend the volume of these solutions to 50 ml by distilled water. After that the optical density of these solutions should be defined (λ = 630–690 nm). Water is a reference solution.

The calculation should be made in accordance with the formula:

$$D = \frac{E_w - E_{ex}}{E_w} \times 2 \times 80,'$$

where

D is an amylase activity (mg/ml·h);

E_w is the optical density of the reference sample;

E_{ex} is the optical density of the test sample;
2 is an amount of starch (mg);
80 is a coefficient of the calculation.

Diagnostic value of clinical tests. The normal amylase activity is 12–30 mg/ml·h. The definition of the amylase activity is very important for the diagnosis of the pancreas disease (in the acute stage, there is 10–30–fold increase; chronic stage and cancer can not give sharp increase).

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

2. The definition of amylase activity in urine by Vol'gemut method

Principle of the method. The method is based on the definition of a minimal amount of amylase which is capable to split 1 ml of 0.1 % solution of starch completely during a certain time period in standard conditions. Unit of amylase is the amount of 0.1 % solution of starch in ml, which can be splited by amylase in 1 ml of urine at 38^0C during 30 minutes.

Course of work. Add 1 ml of 0.85 % NaCl solution to 8 test tubes. Add 1 ml to urine in the first test tube. Mix all liquids in the first test tube. Select 1 ml of deluted utine from the 1st test tube and pour into the next one. Mix the content of the 2nd test tube, select 1 ml of this compound and pour into the third test tube, etc. 1 ml of the solution from the last test tube is necessary to pour out for equalizing volumes in all test tubes. After this dilution you will have the line of test tubes with diluted urine where the enzyme concentration in each next test tube is twice less than in a previous one (diluted 2, 4, 8, 16, etc. times).

After that, you should add 2 ml of a starch solution into each test tube, mix and place them in thermostat for 30 mines at 38^0C. After the placement, the test tubes should be cooled, and in each of them, the reaction with iodine should be carried out. Mark the last test tube in this line of diluted urine with yellow color after iodine reaction (starch was splited completely).

The results should be formed in the table.

Parameter	Test tube No							
	1	2	3	4	5	6	7	8
The urine dilution								
Amount of 0.1 % starch solution								
Color after iodine reaction (+ or –)								

The calculation should be performed in accordance with the formula:

X = 1 x 2 x the urine dilution,

where 1 – quantity of urine, ml;

2 – quantity of 0.1 solution of starch, ml.

Diagnostic value of clinical tests. Normal value of amylase activity in urine is 16–64 units. In the case of a sharp pancreatitis the activity of amylase in urine increases 10–30 times.

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

Lesson 7
Theme: REGULATION OF ENZYMATIC PROCESSES AND THE ANALYSIS OF THE ENZYME PATHOLOGY

Actuality of the theme. *The passage of metabolic processes, their coordination, the direction and the efficiency of them are provided by the existence of regulatory mechanisms at molecular level where enzymes are necessary for transformations of molecules. There are certain ways and mechanisms of the regulation of enzymatic processes in an organism. Information about it and ability to use it in the analysis of mechanisms and the origin of pathological processes is the important element in forming of fundamental knowledge of future doctors. Studing of biochemical aspects of medical enzymology allows to create the complete conception about possible enzymopathies, diagnostics and treatment of them.*

Objectives. A student should be able *to explain basic ways and mechanisms of the regulation of enzyme processes and molecular reasons, biochemical changes and approaches to treatment of enzymopathies.*

Main tasks. A student should be able:

1. *To use knowledge about ways and mechanisms of regulation of enzyme processes.*

2. *To determine the activity of cholinesterase in the blood serum and to be able to interpret the results of researches.*

References

1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 52–63.
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Theoretical questions

1. Regulation of enzyme processes. Activators and inhibitors. Types of enzyme activity inhibition. Reversible (competitive and noncompetitive) and nonreversible inhibition of enzymes. Physiologically active compounds and xenobiotics as reversible and nonreversible inhibitors of enzymes.
2. Ways and mechanisms of regulation of enzyme processes:

2.1. Control of enzyme activity: allosteric enzymes; regulation of enzyme activity by covalent modification; feedback regulation; activation of proenzymes; regulation by protein–protein interaction; cyclic nucleotides as regulators of enzymatic reactions and biological functions of a cell.

2.2. Control of enzyme synthesis by repression and induction.

3. Use of enzymes in medicine: enzymes in clinical diagnosis; enzymes as laboratory reagents; therapeutic uses of enzymes. Inhibitors of enzymes as drugs.
4. Use of test definition of isoenzymes in the diagnostics.

5. The disturbance of enzyme processes: hereditary and acquired pathologies of enzymes; congenital disorders of metabolism and their clinical and laboratory diagnostics.

Laboratory work

1. The influence of activators and inhibitors on the activity of salivary amylase

The principle of the method. Sodium chloride is the activator and copper sulfate is the inhibitor of amylase. Their action on the enzyme activity is defined by the level of starch degradation on the basis of the qualitative reaction with I_2 (Lugol′s iodine test).

Course of work. Saliva is diluted 5 times (1 ml of saliva and 4 ml of water).

Add reactants to all test tubes according to the table.

Define the presence of the activator or inhibitor in accordance with the results. Write down the results in the table. On the basis of the results, the conclusions should be done.

Reactant	**Test tube**		
	1 (check)	2	3
Diluted saliva, ml	1.0	1.0	1.0
H_2O, drops	2	–	–
NaCl, 1.0 %, drops	–	2	–
$CuSO_4$,1.0 %, drops	–	–	2
Starch, 1.0 %, drops	5	5	5
All test tubes are incubated in the thermostat for 10′, t°=38°C. Add 1–2 drops of iodine solution into all test tubes after the incubation and mix reagents. Observe the colour change of solutions.			
The result of the reaction to iodine (+/–)			

2. The definition of serum cholinesterase (pseudocholinesterase) activity

The 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. Proserine is the inhibitor of cholinesterase.

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

Reactant	***Test sample (ml)***	***Check sample (ml)***
Buffer solution	5.0	5.0
H_2O	0.2	0.2
Blood serum	0.1	0.1
Acetylcholine chloride	0.2	0.2
Proserine	–	0.2
Incubation (in a thermostat) for 30′, t°=37°C.		
Proserine	0.2	–
After cooling the sample to room temperature, it is necessary to measure the optical density of the solution in the experimental test tube against the check sample at 500–560 nanometers (a green optical filter) in cuvette with a layer thickness of 5 mm.		

The activity of an enzyme is defined in accordance with the calibration graph. The enzyme activity 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 t°=37°C (μmol/h·l).

Diagnostic value of clinical tests. The normal activity of cholinesterase is *160–340 μmol/h·l.* Pseudocholinesterase of serum comes mainly from the liver. Reduction of serum pseudocholinesterase 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. Pseudocholinesterase activity in blood decreases in case of pesticide poisoning.

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

Lesson 8
Theme: METABOLISM: THE GENERAL CHARACTERISTICS. STAGES OF AEROBIC CATABOLISM. TISSUE RESPIRATION

Actuality of the theme. *The steady state of biological systems and vital functions of cells are supported thermodynamically due to transformation of organic compounds, that take place in living organisms, and substance and energy environmental exchange.*

The processes of catabolism and anabolism are interrelated through energy–rich compounds which appear in the tissue respiration. That provides the transmission of chemical energy from exergonic to endergonic processes and, also, its conversion into other types of energy (mechanical, thermal and others).

Obgectives. A student should be able *to explain basic aspects of metabolism, characterize the stages of aerobic catabolism, basic metabolic pathways and mechanisms of their regulation.*

Main tasks. A student should be able:

1. *To interpret general aspects of metabolism: catabolic, anabolic, amphibolic pathways of metabolis.*
2. *To characterize exergonic and endergonic biochemical reactions.*
3. *To characterize the types of biological oxidation reactions, represent the schemes of reactions.*
4. *To explain the functions of ATP and other energy–rich phosphates in the coupling of anabolic and catabolic processes.*
5. *To characterize the stages of aerobic catabolism of biomolecules.*
6. *To explain the biochemical mechanisms of regulation of catabolism and anabolism.*
7. *To explain the methods of metabolism studies.*

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Theoretical questions

1. General aspects of metabolism: catabolic, anabolic and amphibolic pathways of metabolism.
2. Interrelation of the energy production and use processes in the living systems.
3. Exergonic and endergonic biochemical reactions; the functions of ATP and other energy–rich phosphates.
4. The stages of aerobic catabolism of biomolecules in an organism.
5. Endocellular localization of enzymes and metabolic pathways; compartmentation of metabolic processes in a cell.
6. Biological oxidation and tissue respiration. Reactions of biological oxidation: types of reactions (dehydrogenase, oxydase, oxygenase) and their biological value.
7. Enzymes and coenzymes of biological oxidation: pyridine–linked and flavine–linked dehydrogenases, cytochromes.

Tasks for practical work

Fill in the tables:

Table 1 – Types of biological oxidation reactions

No	Type of reaction	Class (subclass) of enzymes	Coenzyme	Scheme or reaction

Table 2 – Enzymes of biological oxidation

No	Type of Enz	CoEnz	Common scheme of reaction	Example

Lesson 9
Theme: TCA CYCLE: GENERAL CHARACTERISTICS, REACTIONS, REGULATION, AND ENERGETIC BALANCE

Actuality of the theme. *The work of organism's cellsis provided by metabolic transformations which include anabolism and catabolism of compounds. Citric acid cycle (tricarboxylic acid cycle, TCA cycle or the Krebs cycle) is the general catabolic pathway of basic fuel molecules, such as carbohydrates, lipids, proteins. It is one of the fundamental processes of metabolism. The breakup of TCA cycle is incompatible with life. Therefore, the study of this process is obligatory for forming the bases of biochemical knowledge for future doctors.*

Objectives. A student should be able *to characterize biological functions, reactions, enzymes and regulation of TCA cycle.*

Main tasks. A student should be able:

1. *To write the chemical formulas of TCA cycle intermediates.*
2. *To know the calculation of energetic balance of TCA cycle.*
3. *To know regulatory mechanisms which provide the work of TCA cycle.*

References

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Theoretical questions

1. General characteristics of TCA cycle: a scheme of the sequence of reactions, characteristics of enzymes, biochemical value.
2. Enzymatic reactions of TCA cycle. Features of α–keto–glutarate dehydrogenase multienzyme complex.
3. The reaction of the substrate–level phosphorylation in TCA cycle. Total balance of ATP molecules (energetic balance) of TCA cycle.
4. Anaplerotic reactions and amphibolic nature of TCA cycle.
5. The regulation of TCA cycle: key enzymes, activators and inhibitors.

Task for practical work

Fill in the table"Reactions of TCA cycle"

No	Substrate (structure)	Enzyme	Product (structure)	Coenzyme	Regulation	
					Inhibitor	Activator

Lesson 10
Theme: MECHANISMS OF BIOLOGICAL OXIDATION, OXIDATIVE PHOSPHORYLATION AND ATP SYNTHESIS. ELECTRON TRANSPORT CHAIN (ETC)

Actuality of the theme. *Life of organisms depends on the intake of oxygen, which is used mainly in the process of energy storage in the form of ATP (oxidative phosphorylation process). The oxidative phosphorylation process allows aerobic organisms to catch the significant amount of free energy from the partial oxidation of substrates. Breakup of this process is not compatible with life. And that is why the amount of genetic abnormalities of this system is negligible.*

Objectives. A student should be able *to explain a concept of tissue respiration and biological oxidation, the general principle of oxidative phosphorylation; to explain the biochemical bases of energy generation processes in a cell.*

Main tasks. A student should be able:

1. *To interpret the functions of biological oxidation, tissue respiration and oxidative phosphorylation in ATP generation.*
2. *To characterize the enzymes of biological oxidation in mitochondrias: pyridine–, flavine–linked dehydrogenases, and cytochromes.*
3. *To represent the sequence of carriers of mitochondrial electron transport chain, and to explain the principles of its functioning.*
4. *To explain the mechanisms of oxidation coupling and the process of energy storage in the form of ATP.*
5. *To calculate the coefficient of the oxidative phosphorylation.*

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Theoretical questions

1. Pathways of ATP synthesis: substrate and oxidative phosphorylation.
2. Enzymes of biological oxidation: pyridine–, flavine–linked dehydrogenases, cytochromes.
3. Molecular organization of mitochondrial chain of biological oxidation: components of respiratory chain, their redox potential; molecular complexes of the electron transport chain (ETC).
4. Pathways that include reductive equivalents into mitochondrial ETC.

5. Oxidative phosphorylation: coefficient of oxidative phosphorylation, points of coupling of oxidation and phosphorylation.

Laboratory work

1. The definition of catalase number (CN) of blood

Principle of the method. The activity of blood catalase is defined by quantity of hydrogen peroxide that should be splited per time unit. The quantity of hydrogen peroxide is defined by titration according to reaction

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2.$$

The difference in $KMnO_4$ quantity used for titration before and after the action of catalase is a characteristic of the enzyme activity.

Course of work

Diluting blood 1000 times: Add 10 ml of the distilled water and 0,1 ml of blood into a measuring flask (volume 100 ml). Mix and add the distilled water up to the marking.

Add reactants into two flasks according to the table.

Reactant	Check sample, ml	Test sample, ml
Diluted blood	1	1
H_2O (dist.)	7	7
H_2O_2, 1 %	2	2
*H_2SO_4, 10 %	5	–
Incubation for 30', room temperature		
*H_2SO_4, 10 %	–	5

*H_2SO_4 is necessary for inhibition of catalase

After that, the solution in each flask is titrated by 0.1n solution of $KMnO_4$, and its colour turns pink.

The calculation of CN should be done in accordance with the formula

$$CN = (A - B) \cdot 1{,}7,$$

where A – quantity of 0.1n $KMnO_4$ which has been used for titration of the check sample;

B – quantity of 0,1N $KMnO_4$ which has been used for titration of the test sample.

Diagnostic value of clinical tests. Normal CN ranges from 10 to 15 units. Catalase (H_2O_2:H_2O_2–oxidoreductase) splits hydrogen peroxide with subsequent formation of molecular oxygen and water:

$$2H_2O_2 \rightarrow O_2 + 2H_2O_2.$$

The catalase number is the quantity (mg) of hydrogen peroxide which has been splited by catalase in 1 ml of blood for 30 minutes. The catalase activity decreases with anemia, tuberculosis, cancer diseases; it increases in conditions of toxic hepatitis by ionizing radiation exposure and salts of heavy metals.

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

Lesson 11
Theme: BASIC PRINCIPLES OF CHEMIOSMOTIC THEORY. THE ANALYSIS OF THE ACTION OF INHIBITORS AND UNCOUPLERS OF THE OXIDATIVE PHOSPHORYLATION

Actuality of the theme. *The basic process of ATP generation in a cell is a process of the oxidative phosphorylation (OP), which takes place with the participation of a respiratory chain in mitochondria. The importance of this process specifies the fact that breakup function of respiratory chain leads to the hypoenergetic states and sometimes, also, to a cell death. That is why the study of OP mechanisms, inhibition and uncoupling of this process is an important forforming the bases of biochemical knowledge of doctors.*

Objectives. A student should be able *to explain the mechanisms of the oxidative phosphorylation, the influence of inhibitors and uncouple agents on the cell energy production.*

Main tasks. A student should be able:

1. *To explain basic postulates of Mitchell's chemiosmotic theory.*
2. *To know inhibitors of OP and points of their action.*
3. *To know the mechanism of the action of uncoupling agents, examples of these compounds, the mechanism of uncoupling in brown adipose tissue.*

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Theoretical questions

1. Chemiosmotic theory of oxidative phosphorylation: the explanation of a molecular mechanism of ATP generation. Basic postulates of Mitchell's chemiosmotic theory.
2. ATP synthase of mitochondria: the structure and principles of work. $F_0$and F_1 subunits of ATP synthase: their functional value.
3. Conditions of the effective association of oxidation and phosphorylation in mitochondria. Electron transport inhibitors (rotenone, antimycin A, cyanides carbon monoxide) and ATP synthase (oligomycin).
4. Uncouplers of the oxidative phosphorylation (2,4–dinitrophenol, thyroid hormones, free fatty acids), their biomedical value.
5. Blocking of ATP synthesis in conditions of pathogenic factors of chemical, biological and physical origin action on the organism.
6. Microsomal oxydation: cytochrome P_{450}, the molecular organization of microsomal redox–chain.
7. Reactive oxygen species: production and inactivation. Antioxidants.

Lessons 12–13
Theme: EXAMINATION SUBMODULE 1
"BASIC ASPESTS OF METABOLISM"

Actuality of the theme. *The development of biochemical knowledge of students and teaching methods of study. This subject begins from the studies of general aspects of metabolism in an organism. Control of this knowledge is an important for information systematization and more detailed examination of subject matters.*

Besides, an important element of control is an estimation of practical skills which students have got during study of submodule themes.

Objectives. A student should be able *to systemize his knowledge and use it for the solution of clinical cases and the interpretation of indices of biochemical analyses of blood and urine.*

Main tasks. A student should be able:

1. *To answer the questions on the themes of submodule 1 clearly and succinctly.*
2. *To know chemical formulas of 20 proteinogenic amino acids, structures of peptides, oligonucleotides, nucleotides, nucleosides, nitrogen bases, NAD^+, FAD, CoQ.*
3. *To explain the mechanism of the enzyme action.*
4. *To know the chemical reactions of TCA cycle, to explain principles of regulation of key enzymes.*
5. *To know the structure of respiratory chain and to explain the mechanism of it's functioning.*
6. *To explain principles of methodology and the value of the experimental works of submodule for clinical diagnostics.*

References

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Theoretical questions

1. The general characteristics and biological functions of proteins and peptides.
2. Amino acid composition of proteins and peptides: structure, classification and physical and chemical properties of amino acids. The formation of peptide bonds.
3. Levels of protein structure. Chemical bonds in proteins.
4. Methods of study and research of amino acids and proteins in biological fluids. Colour reactions of amino acids and proteins. Chromatographic methods of amino acid separation.
5. Physical and chemical properties of proteins. Amphoteric nature. Isoelectric point (pI).
6. Solubility of proteins. Thermodynamic stability of proteins and denaturation.
7. Methods of protein separation, fractionation and analysis of their structure.
8. Reactions of protein sedimentation and practical application of these reactions.
9. Methods of centrifugation and chromatography.
10. Electrophoretic methods.
11. Methods of study of amino acids composition and structure of proteins and peptides.
12. Classifications of proteins. The general characteristics of simple proteins, their functions.
13. Natural peptides. The general characteristics of these molecules (structure and functions).
14. Complex proteins: classification, representation by groups, their content in the living organisms and functions.

15. General characteristics of chromoproteins, structural features, biological functions.
16. Haemoproteins: myoglobin, haemoglobin, cytochromes. Biological functions and the structural features of haemoproteins. The normal quantity of haemoglobin in the blood. General characteristics of haemoglobinopathies and thalassemias.
17. Flavoproteins: structurae features and functions in an organism.
18. Glycoproteins: classification, structural features, distribution, biological functions.
19. Lipoproteins, phosphoproteins, metalloproteins: structure, biological functions.
20. Nucleotides: structure, nomenclature, biological function. Minor nitrogenous bases and nucleotides. Free nucleotide: participating in metabolic reactions. Cyclic nucleotides.
21. Nucleic acids: features of structural organization, biological functions of DNA and RNA. Experimental proving of DNA genetic role (phenomenon of transformation).
22. Methods of the study of the composition and structure features of complex proteins.
23. Enzymes as biological catalysts of metabolic processes. General properties of enzymes.
24. Classification and nomenclature of enzymes in accordance with the reaction types.
25. The structure of enzymes; oligomeric enzymes; multienzyme complexes (functional enzyme systems), membrane–associated enzymes.
26. Cofactors and coenzymes. The structure and properties of coenzymes; vitamins as predecessors in the biosynthesis of coenzymes. Coenzymes are derivates of vitamins B_1, B_2, B_3, B_5, B_6, Bc, B_{12}, H and α–lipoic acid.
27. Classification of coenzymes in accordance with their chemical nature; the type of reaction which they catalyze.
28. Methods of separation of enzymes from bio–objects, their fractionation (ultracentrifugation, chromatography, electrophoresis) and enzyme activity analysis.

29. Mechanisms of enzyme action: thermodynamics lowers the enzymatic catalysis; active centres of enzymes. Hypotheses of E. Fisher and D. Koshland.
30. Basic steps of the catalytic process.
31. Methods of the definition of enzyme activity. The basic principles of the definition.
32. Spectrophotometric methods of the definition of enzyme activity and the visualization of the results of the enzymatic reaction.
33. Units of activity and amount of enzymes: the international units, the katal, specific enzyme activity.
34. Kinetics of enzyme reactions: the dependence of reaction velocity on the enzyme and substrate concentration, pH and temperature.
35. Michaelis–Menten constant (Km), its semantic value. Processing of Michaelis–Menten equation by the method of double reciprocal coordinates (Lineweaver–Burk equation).
36. Use of Km for characteristic of enzyme activity and the foresight probability of metabolic processes in a cell.
37. Isoenzymes are multiple molecular forms of enzymes and the result of the expression of different genetic locus. The diagnostic significance of isoenzyme definition in the blood.
38. Regulation of enzymatic processes. Activators and inhibitors. Types of enzymes activity inhibition. Reversible (competitive and noncompetitive) and nonreversible inhibition of enzymes. Physiologically active compounds and xenobiotics as reversible and nonreversible enzyme inhibitors.
39. Pathways and mechanisms of regulation of enzymatic processes:
    a) control of enzyme activity; allosteric enzymes; regulation of enzyme activity by covalent modification; feedback regulation; proenzyme activation; regulation by protein–protein interaction; cyclic nucleotides as regulators of enzymatic reactions and biological functions of a cell;
    b) control of enzyme synthesis by repression and induction.
40. Use of enzymes in medicine: enzymes in clinical diagnosis; enzymes as laboratory reagents; therapeutic use of enzymes. Enzyme inhibitors as drugs.

41. Use of isoenzymes in diagnostic.
42. Disturbance of enzymatic processes: hereditary and acquired pathologies of enzymes, congenital disorders of metabolism and their clinical–laboratory diagnostics.
43. General aspects of metabolism: catabolic, anabolic and amphibolic prthways of metabolism.
44. Interrelation of processes of energy production and use in the living systems.
45. Exergonic and endergonic biochemical reactions; functions of ATP and other energy–rich phosphates.
46. The stages of aerobic catabolism of biomolecules in an organism.
47. Endocellular localization of enzymes and metabolic pathways, compartmentation of metabolic processes in a cell.
48. Biological oxidation and tissue respiration. Reactions of biological oxidation: types of reactions (dehydrogenase, oxydase, oxygenase) and their biological value.
49. Enzymes and coenzymes of biological oxidation: pyridine–linked and flavine–linked dehydrogenases, cytochromes.
50. General characteristics of TCA cycle: a scheme of the sequence of reactions, characteristics of enzymes, biochemical value.
51. Enzymatic reactions of TCA cycle. Features of α–ketoglutarate dehydrogenase multienzyme complex.
52. A reaction of the substrate phosphorylation in TCA cycle. Total balance of ATP molecules (energetic balance) of TCA cycle.
53. Anaplerotic and amphibolic reactions of TCA cycle.
54. The regulation of TCA cycle: key enzymes, enzyme activators and inhibitors.
55. Pathways of ATP synthesis in cells: substrate and oxidative phosphorylation.
56. Enzymes of biological oxidation: pyridine–, flavine–linked dehydrogenases, cytochromes.
57. Molecular organization of the mitochondrial chain of biological oxidation: components of respiratory chain, their redox potential; molecular complexes of the electron transport chain (ETC).
58. Pathways of reductive equivalents' inclusion into mitochondrial ETC.

59. Oxidative phosphorylation: coefficient of oxidative phosphorylation, points of oxidation and phosphorylation coupling.
60. Chemiosmotic theory of oxidative phosphorylation: the explanation of a molecular mechanism of ATP generation. Basic postulates of Mitchell's chemiosmotic theory.
61. ATP–synthase of mitochondrias: the structure and principles of work. $F_O$and F_1 subunits of ATP–synthase: their functional value.
62. Conditions of the effective association of oxidation and phosphorylation in mitochondria. Inhibitors of electrons transport (rotenone, antimycin A, cyanides, carbon monoxide) and ATP synthase (oligomycin).
63. Uncoupling of the oxidative phosphorylation (2,4–dinitrophenol, thyroid hormones, free fatty acids), their biomedical value; uncoupling in a brown adipose tissue.
64. ATP synthesis blocking in conditions of pathogenic factors of chemical, biological and physical origin action on the organism.
65. Microsomal oxydation: cytochrome P_{450}, molecular organization of the microsomal oxydation chain.
66. Reactive oxygen spesies: production and inactivation. Antioxidants.
67. The principle of the method and clinical diagnostic value for the definition of the amylase activity in the blood serum.
68. The principle of the method and clinical diagnostic value for the definition of amylase activity in urine by the Vol'gemut method.
69. The principle of the method and clinical diagnostic value for the definition of serum cholinesterase (pseudocholinesterase) activity.
70. The principle of the method and clinical diagnostic value for the definition of catalase number (CN) of the blood.

Lesson 14
Theme: DIGESTION OF CARBOHYDRATES. GLYCOLYSIS AS AN ANAEROBIC OXIDATION OF CARBOHYDRATES

Actuality of the theme. *Carbohydrates are the main source of energy for cells. This is not the only, but their main function. Glucose is the key substance of the metabolism of carbohydrates. Catabolism of glucose can occur in aerobic and anaerobic conditions. Small amount of energy produced by anaerobic glycolysis, and in case of lack of oxygen it is the way of ATP synthesis. That is why the study of glucose catabolism is very important in forming basic biochemical knowledge of future doctors.*

Objectives. A student should be able *to explain the biochemical transformations of carbohydrates and glucose catabolism under anaerobic conditions; should be able to quantitatively and qualitatively determine the concentration of glucose and some of its metabolic intermediates in blood and urine.*

Main tasks. A student should be able:

1. *To classify carbohydrate according to the features of their structure.*
2. *To represent the chemical reactions of glycolytic transformation of carbohydrates.*
3. *To perform qualitative tests on mono– and disaccharides.*
4. *To determine the concentration of lactic acid in blood serum by Boehner`s method.*

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Theoretical questions

1. Classification, structure and role of carbohydrates (mono– and oligosaccharides, homo– and heteropolysaccharides; glycosides, amino sugars).
2. Digestion and absorption of carbohydrates.
3. Anaerobic glycolysis: reactions, localization in the cell, biological role. Regulation of glycolysis, key reactions, energy balance.
4. Glycolitic redox cycle: pyruvate as the hydrogen acceptor during oxygen deficiency.
5. Diagnostic value of determination of LDH activity in blood serum.

Laboratory work

1. Qualitative determination of monosaccharides. Tromer's reaction

Principle of the method. Monosaccharides in alkaline solution can be oxidized. Copper salt is reduced to protoxide. This reaction is used for the qualitative definition of reducing (or reduced) monosaccharides (that contain a free anomeric carbon (the carbonyl group) that can be oxidized).

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

Reagent	Test tube
Glucose, ml	1–2
NaOH 5 %, ml	1–2
$CuSO_4$ 5 %, ml	2 (to add in drops)
Solution color turns into a dark blue color. A test tube is carefully (on a small fire) heated until boiling.	

Results. A yellow precipitate of copper (II) hydroxide $Cu(OH)_2$ will appear, at first. Then, it will turn to red sediment of copper (I) oxide Cu_2O.

2. Reaction of disaccharides. Barfoed's test.

Principle of the method. This reaction is based on a quick formation of monosaccharides from disaccharides of maltose type, which have reducing properties (lactose, maltose, celeobiose). In the acidic solution, disaccharides are hydrolyzed and react with a Barfoed's reagent. As a result, the red sediment of Cu_2O is formed within 15–20 minutes. Monosaccharides give the positive reaction with a Barfoed's reagent in 2–3 minutes (they keep reducing properties in the acidic solution. Unlike them, disaccharides reduce metals only in the alkaline medium).

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

Reagent	**Test tube**	
	1	2
Barfoed's reagent, ml	1	1
Glucose 1 %, ml	1	–
Lactose 1 %, ml	–	1

Shake tubes and heat them in a water bath. Red precipitate of copper (I) oxide (Cu_2O) appears in the test tube with glucose within 2–3 min. Reduction reaction is observed in the test tube with disaccharide only after 15–20 minutes of immersing in a water bath.

Note. The first and the second reactions are carried out as an experimental one.

Mono– and disaccharides, determined in biological fluids, are placed in several tubes. Enter results into the table and make appropriate conclusions:

Test tube No	**Result**	
	Tromer's reaction	Barfoed's test

3. Determination of lactic acid concentration in blood serum by Boehner's method.

Principle of the method. Lactic acid is converted to acetaldehyde by heating with concentrated sulfuric acid. Aldehyde reacts with hydroquinone and forms a red–brown compound.

Diagnostic value of clinical tests. The concentration of lactic acid in the blood of a healthy person is 1,2 mmol/l. Lactic acid content in blood increases during intensive muscular work, epilepsy, tetany, tetanus, hypoxia, malignant tumours and liver diseases.

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

Lesson 15
Theme: AEROBIC GLUCOSE OXIDATION

Actuality of the theme. *Glucose is the main substrate of energy metabolism. The metabolism of most tissues is supported by aerobic oxidation of glucose. Nervous tissue, cardiac muscles are adapted to work in aerobic conditions. They are very sensitive to hypoxia.*

Objectives. A student should be able *to characterize the biochemical regularities and stages of aerobic glucose metabolism.*

Main tasks. A student should be able:

1. *To characterize the stages of aerobic oxidation of glucose and calculate the energy balance of each stage.*
2. *To explain the basic principles of operation and the mechanisms of regulation of pyruvate dehydrogenase complex.*
3. *To compare the energy efficiency of aerobic and anaerobic oxidation of glucose.*
4. *To explain the molecular mechanisms of the Pasteur effect.*
5. *To characterize the structure and mechanisms of action of the shuttle systems for transporting of glycolitic* $NADH{\cdot}H^{+}$.

References

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Theoretical questions

1. Stages of aerobic glucose oxidization.
2. Oxidative decarboxylation of pyruvate. Structure, function, reactions and mechanisms of activity regulation of pyruvate dehydrogenase multi–enzyme complex.
3. Comparative characteristics of the energy effect of aerobic and anaerobic oxidization of glucose. Pasteur effect.
4. Shuttle mechanisms for the glycolytic $NADH{\cdot}H^+$ transporting from the cytosol to mitochondria under aerobic conditions.

Laboratory work

1. Quantitative determination of glucose in blood by glucose oxidase method.

Principle of the method. Glucose oxidase is commonly used for glucose oxidation by oxygen in the air to form gluconic acid and hydrogen peroxide.

$$\textbf{Glucose} + H_2O + O_2 \longrightarrow \textbf{Gluconic acid} + 2H_2O_2$$

Concentration of formed hydrogen peroxide is exactly equal to the concentration of glucose, which is determined. The hydrogen peroxide, under the action of peroxidase, oxidizes orthotolidin to a deep blue chromogen. The intensity of color is proportional to the concentration of glucose.

Course of work. Fill three test tubes with reagents according to the table below:**Reagent**	**Test sample, ml**	**Check sample, ml**	**Standard sample, ml**
NaCl, 0,85 %	1.1	1.1	1.1
$ZnSO_4$, 5,0 %	0.4	0.4	0.4
NaOH, 3N.	0.4	0.4	0.4
Serum	0.1	–	–
Standard glucose solution	–	–	0.1
H_2O	–	0.1	–
Let it stand for 10 min. Centrifuge at 3000 r/pm for 10 min			
The supernatant liquid from the respective tubes	0.1	0.1	0.1
Working reagent	3.0	3.0	3.0
Optical density of experimental and standard samples should be measured against the control at 630 nanometers (a red optical filter) in ditches with thickness of a layer of 10 mm			

Calculation. The calculation should be done according to the formula:

$$C = C_{stand} \cdot K \cdot E_{exp}/E_{stand},$$

where C – glucose concentration, mmol/l;

K – coefficient of dilution;

E_{exp} – optical density of the test sample;

E_{stand} – optical density of the standard sample;

C_{stand} – glucose concentration in the standard sample, mmol/l (with a known concentration).

Diagnostic value of clinical tests. The normal concentration of glucose in the blood is 3,33–5,55 mmol/l.

Physiological hyperglycemia is observed in patients under emotional stress, and if a diet is rich in carbohydrates.

Pathological hyperglycemia characterizes endocrine diseases, diabetes mellitus, tumors of the adrenal cortex and the hypothalamus, hyperthyroidism, liver diseases with dysfunction etc.

Hypoglycemia is observed in patients with insulome, hypothyroidism, hypofunction of the adrenal cortex and hypothalamus, starvation, heavy physical exercises, overdose of insulin, malabsorption of carbohydrates, kidney diseases.

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

LESSON 16
Theme: CATABOLISM AND BIOSYNTHESIS OF GLYCOGEN. REGULATION OF GLYCOGEN METABOLISM. METABOLISM OF GLYCOCONJUGATES

Actuality of the theme. *The biological role of glycogen (homopolysaccharide) is the accumulation of glucose. Glycogen accumulates, primarily in the liver and muscles, but the use of this polysaccharide in these tissues is differen. Liver glycogen is used for support of normal blood glucose level. Muscle glycogen is used for energy supply of muscle contraction. Glycogen storage diseases are heavy hereditary diseases in which enzyme deficiencies occur mainly in glycogen degradation or conversion to glucose. Knowledge of glycogen metabolism and disorders of its metabolism is an important element of fundamental knowledge of medical students. Understanding of the metabolism of glycoconjugates is also important due to the fact that most molecules in the cells of the body are complex and include components of different chemical nature.*

Objectives. A student should be able *to determine glycogen and starch in the test sample by qualitative reactions. Students should know and understand the metabolism of glycogen and glycoconjugates in an organism.*

Main tasks. A student should be able:

1. *To explain metabolism of glycogen in an organism, using the chemical transformations.*
2. *To explain the features of the regulation of glycogen metabolism.*
3. *To explain the features of metabolism of N– and O–linked glycoconjugates.*
4. *To explain the reasons and consequences of inherited disorders of glycogen metabolism.*
5. *To perform qualitative reactions on starch and glycogen.*

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1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 151–160.
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Theoretical questions

1. Glycogen degradation and biosynthesis: enzymatic reactions of glycogenesis and glycogenolysis.
2. Cascade mechanisms of cAMP–dependent regulation of glycogen phosphorylase and glycogensynthetase activity. Hormonal regulation of glycogen metabolism in muscles and liver.
3. Genetic disorders of enzymes of glycogen metabolism: glycogen storage diseases, aglycogenosis.
4. Metabolism of carbohydrate components of glycoconjugates. Biosynthesis of O– and N–linked glycoproteins. Enzymes of glycoconjugate catabolism.
5. Genetic disorders of glycoconjugate metabolism. Mucopolysaccharidoses, gangliosidoses (sphingolipidoses).

Laboratory work

1. The reaction of starch with iodine

Principle of the method. The reaction of iodine with starch, which gives a blue color, is the most specific reaction (Lugol′s test). The coloration is caused by amylose in starch. When starch is present you can see the dark blue color in the test tube. The coloration

disappears when starch is heated, but it is restored when starch solution is cooled.

Course of work. Fill three test tubes with reagent in accordance with the table.

Reagent	Quantity
0,5 % starch	1–2 ml
Lugol′s solution	1–2 drops
You can see a dark–blue colour in the test tube	
t=100℃, 1 min – blue color disappears when the solution is heated and is restored upon cooling	

This reaction is used to determine the test tube with starch solution.

2. The reducing properties of starch

Course of work. Fill two test tubes with reagents in accordance with the table:

Test tube No	Reagent	Quantity
1	1.0 % starch HCl (conc)	4–5 ml 3 drops
2	1.0 % starch H_2O	4–5 ml 3 drops
t =100℃, 10–15 min		

Trommer′s reaction is carried out after cooling the test tubes.

Reagent	Quantity
10 % NaOH	5 drops
1.0 % $CuSO_4$	3 drops
t =100℃, 1 min	

You can see a red precipitation of copper (I) oxide in the first test tube. This shows the hydrolytic starch degradation and releases substances that have reducing properties. In the second test tube, reaction is negative.

This demonstrates the hydrolytic cleavage of starch degradation and releases substances that have the reducing properties (glucose and maltose). In the second test tube, reaction is negative.

Put results of the research in the table:

Test tube No	Lugol′s test	Trommer′s reaction
1		
2		

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

Lesson 17
Theme: GLUCONEOGENESIS AND ALTERNATIVE PATHWAYS OF CARBOHYDRATE METABOLISM. DEFINITION METHODS OF GLUCOSE CONCENTRATION IN BLOOD

Actuality of the theme. *Exogenous polysaccharides, monosaccharides – fructose and galactose are the main sources of glucose as a metabolic fuel for the human body. In addition, there is a pathway of gluconeogenesis, which provides glucose to organism due to its synthesis from noncarbohydrate biomolecules. Pentose phosphate pathway is an alternative pathway of glucose oxidation. As a result, this process of glucose metabolism produces another kind of metabolic fuel ($NADPH•H^+$), that is used in the reducing synthesis in the cytosol of cells.*

Obgectives. A student should be able *to explain the basic biochemical regularities of gluconeogenesis and alternative metabolic pathways of monosaccharides; to characterize the express method for determination of glucose concentration in the blood.*

Main tasks. A student should be able:

1. *To depict the schemes of key reaction, explain the mechanisms of regulation of gluconeogenesis.*
2. *To calculate energy expenditure for the synthesis of glucose from various substrates.*
3. *To explain the mechanisms of glucose–alanine and Cori cycles.*
4. *To represent the sequence of enzymatic reactions of PPP (pentose phosphate pathway); to explain the biological role and the features of its operation in different tissues.*
5. *To represent the scheme of metabolic pathways of fructose and galactose transformation in the human body.*
6. *To characterize the hereditary enzymopathies of alternative pathways of carbohydrate metabolism.*

7. *To interpret the results of express determination of glucose concentration in the blood.*

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Theoretical questions

1. Gluconeogenesis (GNG) is biosynthesis of glucose: physiology significance, substrates, enzymatic reactions, regulatory enzymes, energy of the process.
2. Lactate and alanine as substrates of GNG: glucose–lactate (Cori cycle) and glucose–alanine cycles.
3. Pentose phosphate pathway of glucose oxidation (Hexose monophosphate (HMP) shunt): biological role, sequence of reactions, feature of functioning in different tissues.
4. Metabolic pathways and enzymatic reactions of fructose transformation in the human organism.
5. Metabolic pathways and enzymatic reactions of galactose metabolism in the human body.
6. Hereditary enzymopathies of pentose phosphate pathway, fructose and galactose metabolism.

Laboratory work

1. The use of photometer "Glucophot" for the quantitative determination of glucose in blood

Principle of the method. Photometer is an electronic device that converts the value of the refractive index (light reflectivity) from a reactive indicator strip (RIS) to elevate the glucose concentration in the blood, which is applied to the strip**.**

Before operation and every two hours, the device is calibrated to the correct setting of the initial signal.

Course of work. Determination of glucose concentration in the blood is carried out by measuring the refractive index of the strip, which is treated by the blood

To do this:

- apply 3 drops of blood on the indicator zone RIS, covering the entire surface area of the indicator;
- keep strip with blood in a horizontal position for 30 seconds;
- wash the blood from the RIS by running distilled water;
- blot strips between sheets of filter paper;
- put the RIS into the groove–locking plate of photosensor;
- click MEASUREMENT;
- read and record the result of the analysis;
- open the lid of photodetector, remove the RIS;
- after that, you are allowed to conduct this analysis.

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

LESSON 18
Theme: MECHANISMS OF METABOLIC AND HORMONAL REGULATOIN OF GLUCOSE METABOLISM AND ITS CONCENTRATION IN BLOOD. BIOCHEMISTRY OF DIABETES MELLITUS

Actuality of the theme. *Various regulatory mechanisms provide coordination of metabolic processes in the body: at the cellular level – regulation of enzymatic processes, at the level of tissues and organs – hormonal regulation. Insulin, glucagon, glucocorticoids, catecholamines, growth hormone, T3, T4, ACTH and other hormones involved in the regulation of carbohydrate metabolism. Hormonal imbalance leads to impaired metabolism of carbohydrates, which primarily affects the concentration of glucose in the blood. Changes in the amount of glucose in the blood are observed in physiological and pathological conditions such as long starvation, stress, prolonged exercises, diabetes mellitus, insuloma, hypo– and hyperthyroidism, pheochromocytoma and others. The study of carbohydrate metabolism regulation and its changes creates conditions for possible practical uses of biochemical knowledge in medical practice of doctors to be.*

Objectives. A student should be able *to determine the basic biochemical indices that are used to diagnose diabetes mellitus and to interpret the results.*

Main tasks. A student should be able:

1. *To explain the mechanisms of enzymatic regulation of basic processes of carbohydrate metabolism: glycolysis, glycogenesis and glycogenolysis, PPP and gluconeogenesis.*
5. *To know the biochemical effects of hormones which influence carbohydrate metabolism.*
6. *To explain metabolic changes in an organism – those caused by disturbance of carbohydrate metabolism under diabetes mellitus.*
7. *To know the principles of biochemical tests, which are used for diagnostics of diabetes mellitus, and to be able to interpret the research results.*

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Theoretical questions

1. Hormones are regulators of glucose metabolism: glucagon, epinephrine (adrenalin), corticosteroids, growth hormone, insulin. The hormonal effects and mechanisms of influence on the glucose level in blood.
2. Normal concentration of glucose in the blood. Factors that provide the normal blood glucose concentration.
3. Causes of hypo– and hyperglycemia. Glucosuria.
4. Diabetes mellitus: insulin dependent and insulin independent forms; clinical and biochemical characteristics.
5. Laboratory tests for the diagnosis of diabetes mellitus: glucose–tolerance test, double sugar loading test, determination of glycosylated hemoglobin (HbA1c) and fructosamine.

Laboratory work

1. Quantitative determination of glucose in urine by the Althauzen method (in modification of K. A. Kost).

Principle of the method. The method is based on the fact that urine, containing sugar, takes different shades of brown color (from yellow to dark brown) by boiling with alkali. Lactic acid and humic substances are the products of the reaction.

Course of work. Add 1,0 ml of 10 % solution of sodium hydroxide or potassium hydroxide to 4,0 ml of urine. Than put the

test tube in the boiling water bath for 5 min. After that, cool it till ambient temperature. Optical density of the solution is determined by a photoelectrocolorimeter at λ = 490 nm (green filter) in a cuvette with the 5 mm thickness of layer within 10 min. Water or (better) 4,0 ml of urine with addition of 1,0 ml of water are used for test. Results are expressed in percentage. If there is sugar in urine, the content of the tube becomes one of the shades by Althauzen's scale in colour. You can compare your results with a standard scale.

Clinical diagnostic value. Amount of glucose in daily urine, normally, should not exceed 0,5 mmol/l. Qualitative and quantitative determination of glucose in urine has a great diagnostic and prognostic value.

Glucosuria can occur in diabetes mellitus, with acute inflammation of pancreas, due to hyperthyroidism, acromegaly, Cushing's syndrome, liver diseases (due to disturbance of glycogen synthesis), inflammatory processes in the kidney, in pregnancy, excessive use of carbohydrates with meals.

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

2. Glucose tolerance test (sugar loading test)

Course of work. Take fasting blood sample from a patient's finger and determine the concentration of glucose in it. Then give water solution of glucose (the proportion is 1 g of glucose per 1 kg of body weight) to a patient to drink. Every 30 minutes, during 3 h., determine the amount of glucose in the patient's blood by glucose–oxidase method. Sugar curve is built on the basis of the obtained results. The vertical axis indicates the amount of glucose in mM/l and the horizontal axis – the time in minutes (Fig. 1).

In a healthy person, after 15 minutes of physical activity, the concentration of glucose in the blood begins to grow. The maximum concentration is observed after 30–60 min, then, it is followed by a decrease. After 120 minutes, the amount of glucose in the blood may be lower than the original. Normalization of the blood glucose level is observed, usually, after 180 min.

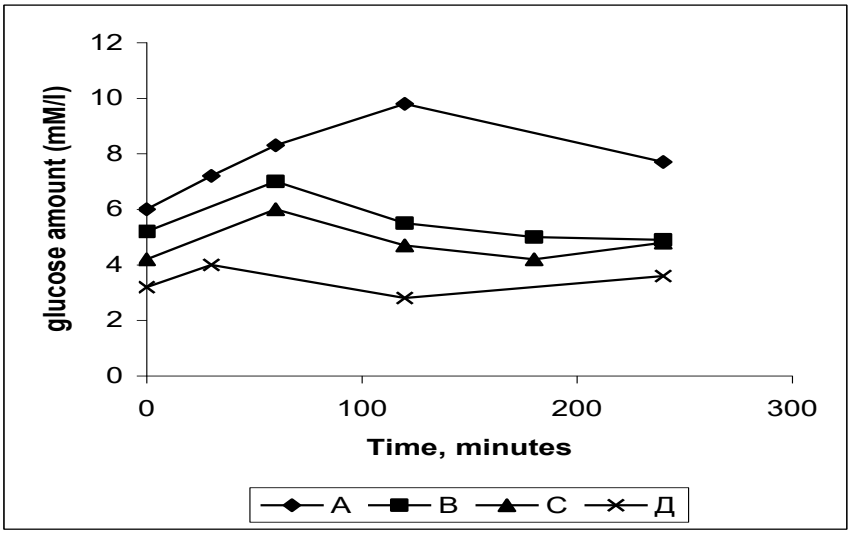

Figure 1 – Types of glycemic curves

Designations: A – diabetes mellitus; B – hyperthyroidism; C – norm; D – Addison's disease, hypothyroidism, hyperinsulinaemia

Diagnostic value of clinical tests. Glucose tolerance test is used to determine the body's tolerance to glucose, diagnosis of latent forms of diabetes mellitus and disorders of glycogen synthesis in the liver. Significant increase of blood glucose (up to 22 Mmol/l) and slow decrease are observed in diabetes mellitus, acromegaly, hyperthyroidism, hepatitis, and cirrhosis of the liver.

3. Definition of glucose–binding to haemoglobin (HbA_{1c}) and fructosamine

Nonenzymatic glycosylation of blood proteins occurs in their interaction with glucose. This process depends on the concentration of glucose in the blood and the rate of the protein metabolism. Quantity of $HbA1_C$ indicates the average blood glucose level during 4–8 weeks (if life duration of red blood cells does not decrease as, for example, at haemolytic anemia). Normal glucose–binding to haemoglobin in the blood is 4–7 % of the total haemoglobin level. This index is used for the diagnosis of latent forms of diabetes and to control hyperglycemia in diabetics. Increased content of $HbA1_c$ does

not occur in hyperglycemia of nervous origin and can be observed under renal failure, pregnancy and its complications, sickle cell anemia, and other diseases with disturbed carbohydrate metabolism. This parameter is determined during prophylactic examinations because it facilitates the identification of more patients with diabetes compared with a single definition of blood glucose and is safer than using the glycemic curves.

Fructosamine is the product of glycosylation of total serum proteins. More than 60 % of total blood protein is albumin, which can react with glucose. Fructosamine exists in the bloodstream 20 days.

The concentration of fructosamine indicates the level of blood glucose over the past 1–3 weeks.

Normal concentration of fructosamine in blood serum is 2.1 – 2.8 mmol/l. The method is used for treatment and monitoring of diabetes mellitus.

LESSON 19
Theme: TEST ON SITUATIONAL TASKS FROM "STEP–1": "BASIC ASPECTS OF METABOLISM"

Actuality of the theme. *Understanding general patterns of metabolism is the basis for clinical thinking of a 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 basis of biochemical indices, draw a conclusion with regard to a choice of the right answer among the standard answers.*

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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.

LESSON 20
Theme: GENERAL CHARACTERISTICS OF LIPIDS. LIPIDS OF BIOMEMBRANES. LIPOLYSIS AND ITS REGULATION

Actuality of the theme. *Triacylglycerols (TAG) are deposited in the adipocytes and act as energy stores. Hydrolysis of TAG is known as lipolysis. Glucagon, glucocorticoids, STH, ACTH activate lipolysis, insulin inhibits lipolysis. Free fatty acids (FFA) and glycerol are formed by lipolysis. FFA is the energy source for body tissues. Glycerol is used in the liver and kidneys for the synthesis of glucose, simple and complex lipids. Starvation, stress, endocrine diseases cause the changes in the hormonal states in the body, which leads to changes in lipid metabolism. Future doctor should clearly direct his efforts toward causes and consequences of changes in lipid metabolism and use their knowledge in his clinical practice.*

Objectives. A student should be able *to determine the total lipids concentration in the blood by photoelectro colorimetric method to interpret the results.*

Main tasks. A student should be able:

1. *To explain the features of structure, properties and biological role of basic classes of lipids.*
2. *To explain the molecular mechanism of hydrolysis of TAG in an organism and its regulation.*

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Theoretical questions

1. Lipids: structure, classification, biological functions of the basic classes.
2. Composition of lipids – saturated and unsaturated fatty acids: physical and chemical properties, content in human tissues.
3. Structure and functions of biological membranes:
   a) lipids of membranes, features of their structural organization, lipid composition of subcellular membranes;
   b) fluid–mosaic model of membrane structure;
   c) biophysical properties of membranes;
   d) pathology of membranes.
4. Digestion and absorption of lipids.
5. Catabolism of triacylglycerols: sequence of reactions, mechanisms of regulation of tryglycerol lipase (TAG–lipase) activity. Hormonal regulation of lipolysis.

Laboratory work

1. Definition of total lipids in blood serum.

Principle of the method. Products of disintegration of unsaturated lipids, after hydrolysis by a sulfuric acid, interact with phospho–vanillin reagent. The formed complex has a pink color and a maximum of absorption at the wavelength of 530 nanometers.

Course of work

Reagent, ml	Test sample	Check sample	Standard sample
Blood serum	0.01	–	–
Water	–	0.01	–
Stand. solution of lipids	–	–	0.01
Sulfuric acid (strong)	1.0	1.0	1.0
Water bath – 10 minutes at 100℃			
To cool 5 minutes in cold water			
Phospho–vanillin reactant	2.0	2.0	2.0
To mix, to cool 2 minutes in cold water			

Measure optical density at a wavelength of 500–560 nanometers in a ditch with the thickness of a liquid layer of 1 cm according to the test in 10 minutes, not later.

Calculation

$$C_c = \frac{E_{ex} \cdot C_{st.}}{Est.},$$

where C_c – concentration of the total lipids in blood serum (g/l);
E_{ex} and E_{st} – optical density of the test and standard samples;
C_{st} – concentration of lipids in the standard sample (800 mg/100 ml or 8 g/l).

Diagnostic value of clinical tests. The normal content of total lipids in the blood is 4–8 g/l.

Hyperlipemia is common in patients with obesity, stress, diabetes mellitus, lipid nephrosis, primary biliary cirrhosis, acute hepatitis, acute and chronic glomerulonephritis, atherosclerosis, pancreatitis, alcoholism.

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

Lesson 21
Theme: BETA–OXIDATION OF FATTY ACIDS. KETONE BODY METABOLISM RESEARCH

Actuality of the theme. *The process of energy production in the body occurs continuously. Fatty acids are the most powerful energy sources in the cells of the organism: their oxidation produces more energy than the oxidation of carbohydrates. Ketone bodies are products of incomplete oxidation of fatty acids. They are formed in the liver and used as additional energy fuel in extrahepatic cells. Synthesis of ketone bodies increases in certain pathological and physiological states. Ketone bodies are acids. Increase in their synthesis leads to changes in acid–base balance of the body and to development of ketoacidosis. Study of β–oxidation of fatty acids and ketone body metabolism is important for understanding of the mechanisms of energy production in the body, as well as awareness of metabolic changes in pathological processes (diabetes, obesity, starvation, etc.).*

Objectives. A student should be able *to determine ketone bodies in urine and interpret the results.*

Main tasks. A student should be able:

1. *To explain the mechanisms of β–oxidation of fatty acids.*
2. *To write the chemical reaction of the synthesis and utilization of ketone bodies.*
3. *To explain the biochemical mechanisms of development of ketonemia and ketonuria in diabetes and starvation.*

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Theoretical questions

1. Oxidation of fatty acids (β–oxidation): biological role, stages, mechanism of activation of fatty acids.
2. Role of carnitine in the transport of fatty acids into the mitochondria.
3. The sequence of enzymatic reactions of β–oxidation.
4. Energy effect of β–oxidization of fatty acids.
5. Biosynthesis and utilization of ketone bodies, their physiological significance.
6. Metabolism of ketone bodies in pathologic conditions. Mechanisms of ketoacidosis in diabetes mellitus and starvation.

Laboratory work

1. Qualitative determination of acetone and acetoacetate in urine

1.1. 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 color 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 drop 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.

1.2. The Gerhard's reaction on acetoacetic acid

Principle of the method. Ferum (III) phosphate (Fe_3PO_4) precipitate forms when ferric chloride solution is added to urine. In the presence of acetoacetic acid, after the addition of an excess of 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 (of healthy human and diabetics) in two test tubes, and then, dropwise, 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.

Carry out a research, enter the results in the table.

Test tube No	Qualitative reaction	
	Legal's test	Gerhard's reaction

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 are 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.

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

Lesson 22
Theme. BIOSYNTHESIS OF FATTY ACIDS, TRIACYLGLYCEROLS AND COMPLEX LIPIDS. DETERMINATION OF TOTAL PHOSPHOLIPID CONCENTRSTION IN BLOOD SERUM

Actuality of the theme. *Biosynthesis of lipids is an important part of their metabolism. It provides the body with metabolic fuel reserves in the form of triacylglycerols, which accumulate in the adipose tissue and in the cells of other organs, and is necessary to update the structural components of biomembranes.*

Objectives. A student should be able *to characterize the main pathways of synthesis of fatty acids, simple and complex lipids in humans.*

Main tasks. A student should be able:

1. *To depict the sequence reactions of synthesis of saturated fatty acids.*
2. *To explain synthesis of fatty acids by the fatty acid synthase multienzyme complex, which contains acyl carrier protein (ACP).*
3. *To characterize metabolic sources and mechanisms of regulatin of fatty acids synthesis.*
4. *To explain the mechanisms of fatty acid elongation and desaturation.*
5. *To represent schematically reactions of synthesis of triacylglycerols, phospholipids and sphingolipids.*
6. *To characterize genetic disorders of lipid metabolism – sphingolipidosis and gangliosidosis.*
7. *To interpret the results of investigation of the phospholipid concentration in blood serum.*

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Theoretical questions

1. Biosynthesis of saturated fatty acids: metabolic sources, regulation of the process, the sequence of enzymatic reactions of palmitate synthesis.
2. Elongation of saturated fatty acids, formation of mono– and polyunsaturated fatty acids in an organism.
3. Biosynthesis of triacylglycerols (TAG): substrates, the sequence of reactions, enzymes.
4. Biosynthesis of phospholipids in human tissues. The concept of lipotropic factors.
5. Metabolism of sphingolipids.
6. Genetic anomalies of sphingolipid metabolism – sphingolipidosis. Lysosomal storage disorders: Niemann–Pick disease, GM1 gangliosidosis, Tay–Sachs disease / GM2 gangliosidosis, Gaucher disease.

Laboratory work

1. Definition of total phospholipid in blood serum according to the concentration of phosphate

Principle of the method. The total concentration of phospholipids is determined by the content of lipid phosphorus. Phospholipids precipitated by trichlor acetic acid (TCAA) together with blood proteins. Quantity of phosphorus is determined in the resulting sediment by colorimetric method.

Course of work. Research is carried out in a sequence which is shown in the table.

Reagent	Test sample, ml	Check sample, ml	Standard sample, ml
Water	3.0	–	–
Blood serum	0.2	–	–
TCAA – 10 % (dropwise 1.5 ml, shaking the tube)	3.0	–	–

Leave for 1–2 min., centrifuge for 5 min 3000 r/min, the supernatant is drained and added to precipitate			
50–80 % perchloric acid	1.0	–	–
Heat in a boiling water bath during 20–30 min until the solution loses its color			
Working standard solution	–	–	2.0
50–80 % perchloric acid	–	0.8	0.8
Water	Bring to a 7 ml		
2.5 % ammonium molybdate	1.0	1.0	1.0
1 % solution of ascorbic acid	1.0	1.0	1.0
Note. Mix, bring the volume of solution in each tube with distilled water to 10 ml			

In 20 minutes measure the extinction coefficient to compare to the check sample at λ = 630–690 nm (red filter) in a cuvette with a fluid layer thickness of 1 cm.

Calculation. The total phospholipid content is determined by the formula

$$C_{ex} = \frac{E_{ex} \times 0{,}05}{E_{st} \times 0{,}2} 25,$$

where E_{ex} i E_{st} – value for extinction test and standard solutions;

0,05 – content of phosphorus in the standard solution, mg/ml;

0,2 – volume of serum sample;

25 – recalculation for total phospholipids;

C_{ex} – total phospholipid concentration, mg/ml.

In order to convert the result to mmol/l, multiply it by 0,3229.

Diagnostic value of clinical tests. The normal content of total phospholipids in the blood serum is 1,5 – 3,6 g/l.

Hyperphospholipidemia – increases the level of phospholipids in the blood and – is observed in severe forms of diabetes, nephrosis, chronic nephritis, and jaundice.

Hypophospholipidemia is observed in atherosclerosis, anemia, fever, malnutrition, and liver diseases.

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

Lesson 23
Theme: CHOLESTEROL BIOSYNTHESIS AND BIOTRANSFORMATION. BLOOD LIPOPROTEINS

Actuality of the theme. *Cholesterol is a main representative of the sterols of animal origin. It is necessary for the normal functioning of cells, maintaining the normal state of biomembranes, synthesis of steroid hormones and vitamins. Violation of cholesterol transport and TAG by blood lipoprotein occurs with many diseases.*

Objectives. A student should be able *to characterize the main stages of the biosynthesis of cholesterol, pathways of its biotransformation, transport forms of lipoproteins.*

Main tasks. A student should be able:

1. *To depict the main stages of cholesterol synthesis and explain the mechanisms of the process regulation.*
2. *To depict schematically the pathways of cholesterol biotransformation and characterize the biological role of the intermediate and final products of these pathways.*
3. *To explain the mechanism of TAG resynthesis in enterocytes and indicate a biological role of this process.*
4. *To characterize the classes of blood lipoproteins, methods of their separation.*
5. *To explain the biological role of different classes of blood lipoproteins.*
6. *To interpret the results of determination of total blood (or serum) cholesterol and explain the diagnostic value of this parameter.*

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Theoretical questions

1. Biosynthesis of cholesterol: metabolic precursors, the sequence of reactions of synthesis, mechanisms of regulation.
2. Pathways of cholesterol biotransformation: esterification, formation of bile acids, steroid hormones, vitamin D_3, body cholesterol excretion.
3. The role of cytochrome P–450 in the biotransformation of physiologically active steroids.
4. Transport of lipids. TAG resynthesis in the enterocytes.
5. Classes of blood lipoproteins: chemical composition, formation, biological role, methods of separation, apoproteins.

Laboratory work

1. Determination of total blood cholesterol by Ilk method.

Principle of the method. Cholesterol, in the presence of acetic anhydride, and a mixture of acetic and sulfuric acid forms a green compound. Quantity of cholesterol is determined by the intensity of solution coloration by colorimetric method.

Course of work. The research is carried out in the sequence which is shown in the table. ***Note.*** *All laboratory dishes should be dry.*

Reagent	Test sample, ml	Standard sample, ml
Working solution (reagent 1)	2.0	2.0
Standard solution	–	0.1
Blood serum	0.1	–
Mix by shaking 10–12 times and put the sample in a thermostat at t=37^{0}C for 20 min.		
Measure values of extinction against water on PEC at λ = 630–680 nm. with a red filter, cuvette thickness – 5 mm		

Calculation. Determine the cholesterol concentration by formula

$$X = \frac{E_{ex} \times E_{st}}{E_{st}},$$

where X – cholesterol concentration in the blood serum (mg/100 ml);

E_{st} – cholesterol concentration in a standard solution (180 mg/100 ml);

E_{ex} – extinction (optical density) of the experimental test;

E_{st} – extinction (optical density) of the standard solution;

Recalculation coefficient to IS units, mmol/l – 0,0258.

Diagnostic value of clinical tests. Normally, the concentration of cholesterol in serum is 3,3 – 5,2 mmol/l.

Hypercholesterolemia is observed in patients with anemia, tuberculosis, starvation, hyperthyroidism, cancer cachexia, parenchymatous jaundice, fever, and after insulin injection.

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

Lesson 24
Theme: METABOLISM IN ADIPOCYTES. OXIDATION OF GLYCEROL. BIOCHEMISTRY OF UNSATURATED FATTY ACIDS

Actuality of the theme. *Regulation of lipolysis and lipogenesis in adipocytes depends on the energy needs of the body. These processes are regulated by insulin, glucagon, growth hormone, ACTH, catecholamines, etc. They are also closely related to carbohydrate metabolism. First of all, lipogenesis in adipocytes does not occur without the participation of glucose*.

Understanding of biochemical transformations in adipocytes allows the future physician to know about not only the possible molecular effects of pathology, but also to use this knowledge for practical purposes in the treatment of various diseases.

Objectives. A student should be able to *characterize the molecular mechanisms of lipolisis and lipogenesis in adipose tissue, to explain hormonal regulation of these processes; to characterize the biological role of vitamin F and features of glycerole metabolism.*

Main tasks. A student should be able:

1. *To explain the chemistry of lipolysis in adipocytes and its hormonal regulation.*

2. *To interpret the role of glucose in lipogenesis in adipocytes and hormonal regulation of TAG synthesis.*
3. *To explain the chemical reactions of glycerole metabolism.*
4. *To know the biochemistry of vitamin F.*

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Theoretical questions

1. Biosynthesis of unsaturated fatty acids: formation of monounsaturated fatty acids; features of transformations of polyunsaturated fatty acids.
2. Vitamin F: daily requirement, sources, biological role, the consequences of deficiency.
3. Features of lipogenesis and lipolysis in adipose tissue and their interaction with the metabolism of carbohydrates. Hormonal regulation of processes.
4. Features of glycerole metabolism: sequence of catabolic reactions, energy effect, participation in anabolic reactions.

Laboratory work

1. Definition of free fatty acids in blood serum

Principle of the method. Copper salts of fatty acids are able to form colored complex compounds with sodium diethyldithiocarbamate. The color intensity is proportional to the concentration of free fatty acids and is determined by a colorimetric method.

Course of work. Take two test tubes with cork stoppers. In the first tube, pour 0,5 ml of plasma, in the second – 1 ml of palmitic acid into chloroform (standard test). In the experimental test tube should be 5 ml, and in the standard – 4,5 ml of chloroform. In both test tubes, add 2,5 ml of copper reagent. In the third tube (check), pour 5 ml of chloroform and 2,5 ml of copper reagent. Close test tubes by cork stoppers and shake them during 3 min. Then transfer their content to a centrifuge tube and spin it at 3000 r/min for 15 min. The mixture in the test tube will divide into three layers: chloroform, protein and water. The upper aqueous phase will contain an excess of copper reagent. The protein layer should by push aside against the tube wall by a glass rod. A chloroform layer with extracted fatty acids should be transfered to a clean tube. Then add 0,5 ml of 0,1 % solution of sodium diethyldithiocarbamate in butanol. The contents of the test tubes should be mixed. Optical densities of test and standard samples are determined by the photocolorimetric method in ditches with 5 mm thickness with a blue filter against a check sample.

The content of free fatty acids is calculated by the formula

$$X = \frac{E_{ex} \times C_{st} \times 1000}{E_{st} \times 0,5},$$

where X – concentration of free fatty acids in the blood serum (μmol/l);

C_{st} – concentration of standard solution of palmitic acid;

E_{ex} – extinction (optical density) of the experimental test;

E_{st} – extinction (optical density) of standard solution;

0,5 – amount of plasma, taken for an analysis;

1000 – recalculation into μmol/l.

Diagnostic value of clinical tests. The blood of a healthy human contains the 640–880 mmol/l of free fatty acids. The increase of fatty acid concentrations in the blood is observed in diabetes, starvation, overproduction or introduction of adrenaline. Reduction of the content of free fatty acids happens after the introduction of insulin and glucose.

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

Lesson 25
Theme: REGULATION AND DISORDERS OF LIPID METABOLISM. INTERRELATION OF LIPID AND CARBOHYDRATE METABOLISM

Actuality of the theme. *Disorders of lipid metabolism, such as atherosclerosis, obesity and diabetes, are the leading causes of morbidity and mortality of people.*

Objectives. A student should be able *to characterize the basic mechanisms of regulation and causes of lipid metabolism disorders.*

Main tasks. A student should be able:

1. *To explain the regulatory mechanisms of the main pathways of lipid metabolism.*
2. *To characterize disturbance of lipid metabolism in patients with diabetes mellitus, obesity, atherosclerosis, fatty liver and cholelithiasis.*
3. *To classify the types of hyperlipoproteinemia and to explain the role of atherogenic lipoproteins in the origin of atherosclerosis.*
4. *To explain the interrelation of the main pathways of lipid and carbohydrate metabolism.*
5. *To interpret the results of determination of serum lipoproteins and to explain the diagnostic value of the index.*

References

1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 187–192.

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3. Chatterjea M. N. Textbook of medical biochemistry / M. N. Chatterjea, Rana Shinde. 8th edition. – New Delhi: Jaypee, 2012. – P. 451–460.
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Theoretical questions

1. Regulatory mechanisms of the major pathways of lipid metabolism. Hormonal regulation of lipid metabolism.
2. Disturbance of lipid metabolism in diabetes mellitus.
3. Metabolic causes of fatty liver disease development.
4. Disorders of cholesterol metabolism: biochemical mechanisms in the development of gallstone disease.
5. Hyperlipoproteinemia: classification, general characteristics. Hyperalphalipoproteinemia. Role of atherogenic lipoproteins in the development of atherosclerosis.
6. Biochemistry of obesity.
7. Interrelation between lipids and carbohydrate metabolism.

Laboratory work

1. Determination of β–lipoproteins (LDL) in blood

Principle of the method. The method is based on the ability of β–lipoproteins to precipitate in the presence of calcium chloride and heparin that leads to turbidity of the solution. The turbidity of the solution is proportional to the content of lipoproteins.

Course of work. Pour 2 ml of 0,025M $CaCl_2$ solution in the test tube and add 0,2 ml of whey protein to mix and measure the optical density of the solution (E_1) on the photocolorimeter at a wavelength of 360–630 nanometers (red light filter) in the cuvette with a layer thickness of 0,5 cm against distilled water. From the cuvette, the solution should be poured in a test tube. Then top up 0,04 ml of 1 %

heparin solution with a micropipette and mix. Determine the optical density (extinction) of the solution (E_2) determine exactly after 4 min under the same conditions (versus distilled water).

Difference of optical density ($E_2 – E_1$) corresponds to the optical density caused by concentration of β–lipoproteins (LDL).

Calculation. The concentration of β–lipoproteins (LDL) is expressed in optical density units multiplied by 100.

$$X = (E_1 – E_2) \times 100$$

Diagnostic value of clinical tests. The normal concentration of LDL in the blood is 35 – 55 units. The increase of LDL is observed in atherosclerosis, obstructive jaundice, hepatitis, diabetes mellitus, obesity and in others diseases.

There is a correlation between the contents of triacylglycerols (TAG), cholesterol and β–lipoproteins. It was found, that the increase in plasma concentrations of cholesterol and TAG is accompanied by increased contents of β–lipoproteins.

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

Lessons 26–27
Theme: EXAMINATION SUBMODULE 2 "BASIC ASPECTS OF METABOLISM. CARBOHYDRATE AND LIPID METABOLISM AND IT'S REGULATION"

Actuality of the theme. *Mastering of biochemical knowledge by students during the study of biochemistry starts with learning the general principles of macromolecule metabolism in the human body. Control of this knowledge is important for organization and use of the information and for the completion of the topics that are not fully mastered.*

The metabolism of carbohydrates occupies a central place in the metabolism of biomolecules in the human organism and is closely related to the transformation of lipids.

Understanding of the basic principles of biomolecule transformation and regulatory processes of different metabolic pathways is based on subsequent mastering of biochemical knowledge.

Control of knowledge is important for the systematization of the mastered information, its generalization and practical application.
Additionally, the evaluation of practical skills, that students have for studying the themes of the module 1, is an important element of control.

Objectives. A student should be able *to systematize and clearly formulate the mastered material, to give reasonable answers to the questions, to draw conclusions, to use gained knowledge to solve situational problems and interpret the results of clinical laboratory tests.*

Main tasks. A student should be able:

1. *To answer clearly and concisely the questions of the module 2.*
2. *To depict structural formulas of representatives of certain classes of lipids and carbohydrates, the basic schemes of metabolic pathways.*
3. *To explain the mechanisms of regulation of carbohydrate and lipid metabolism.*
4. *To explain the principles of the methods and clinical diagnostic value of the main biochemical parameters.*
5. *To interpret the results of biomedical research.*

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1. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook / Yu. I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. – 2–nd. ed. – Vinnitsa : Nova Knyha, 2021. – P. 128–192.
2. Marks Dawn B. Biochemistry / Dawn B. Marks. 3th edition – Baltimore, Philadelphia: Williams & Wilkins, 2014. – P. 6, 231–237, 255–256.
3. Chatterjea M. N. Textbook of medical biochemistry / M. N. Chatterjea, Rana Shinde. 8th edition. – New Delhi: Jaypee, 2012. – P. 23–64, 319–460.
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Theoretical questions

1. Carbohydrate: classification, structure, properties and role of the representatives of certain classes.
2. Digestion and absorption of carbohydrates.

3. Anaerobic oxidation of glucose: the sequence of reactions, enzymes. Clinical aspects of glucose metabolism in anaerobic conditions.
4. Anaerobic oxidation of glucose: regulation, reactions of substrate phosphorylation, energy effect. Glycolytic oxidation–reduction (Redox) cycle. Features of glucose metabolism in erythrocytes.
5. Aerobic glucose oxidation: stages, energy balance. Shuttle mechanisms for the oxidation of NADH. The Pasteur effect.
6. Oxidative decarboxylation of pyruvate: reactions, regulation, clinical aspects.
7. Glycogenolysis in the liver and muscles. Regulation of glycogen phosphorylase activity. Genetic disorders of glycogen metabolism.
8. Glycogen biosynthesis: enzymatic reactions, physiological significance. Regulation of glycogen synthase activity.
9. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis. The role of epinephrine, glucagon and insulin in the hormonal regulation of glycogen metabolism in muscles and liver.
10. Gluconeogenesis: substrates, enzymes and physiology significance of the process. Lactic acid (the Cori cycle) and glucose–alanine cycles.
11. Pentose phosphate pathway of glucose oxidation (hexose monophosphate (*HMP shunt*): the scheme of the process, biological role, regulation, process disturbance.
12. Metabolic pathways of fructose transformation in the human organism. Hereditary enzymopathies of fructose metabolism.
13. Metabolic pathways of galactose transformation in the human body and hereditary enzymopathies of its metabolism.
14. Metabolism of carbohydrate components of glycoconjugates: synthesis of O– and N–linked glycoproteins. Genetic disorders of metabolism of glycoconjugates.
15. Mechanisms of regulation of blood glucose levels. Disorders of carbohydrate metabolism in diabetes mellitus.

16. General characteristics of lipids: structure, classification, func–tions of representatives of individual classes. Fatty acids: structure and role.
17. Digestion and absorption of lipids.
18. Catabolism of triacylglycerols in adipose tissue: sequence of reactions, regulatory mechanisms of TAG–lipase activity. Hormonal regulation of lipolysis.
19. Oxidation of fatty acids (β–oxidation), the role of carnitine in the transport of fatty acids into the mitochondria.
20. Energy balance of fatty acids β–oxidation in cells. Glycerol catabolism: enzymatic reactions and the energy of the process.
21. Biosynthesis and utilization of ketone bodies, their biological role.
22. Metabolic disturbances caused by ketone bodies in diabetes mellitus and starvation.
23. Biosynthesis of saturated fatty acids. Stages and reactions of palmitate biosynthesis.
24. Biosynthesis of fatty acids: sources of $NADPH{\cdot}H^{+}$, the total equation of palmitate synthesis, the regulation of the process.
25. Elongation of long–chain fatty acids. Biosynthesis of mono– and polyunsaturated fatty acids in an organism.
26. Biosynthesis of TAG. Features of lipogenesis in adipocytes.
27. Biosynthesis of phospholipids in an organism. Concept about lipotropic factors.
28. Metabolism of sphingolipids. Genetic anomalies of its biosynthesis – sphingolipidoses.
29. Biosynthesis of cholesterol: scheme of reactions, regulation of synthesis. Pathways of cholesterol biotransformation.
30. Transport of lipids. Plasma lipoproteins.
31. Classification of hyperlipoproteinemias.
32. Pathology of lipid metabolism: atherosclerosis, obesity, diabetes mellitus.

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 chemical bonds are used to stabilize the structure of protein molecules?
2. Draw the general structure of an amino acid.
3. What are the main physical and chemical properties of amino acids?
4. Describe the amphoteric property of amino acids
5. What are the main physical and chemical properties of proteins?
6. Describe the electrophoretic mobility of proteins.
7. What is the denaturation of proteins?
8. Describe the levels of structural organization of proteins.
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 with the explanation functions of them
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 for the term "multienzyme complex”.
18. Give the definition for the term "coenzymes".
19. Give examples of co–enzymes.
20. Give the definition for the term “allosteric enzymes”.
21. What is covalent modification of enzymes?
22. Give the definition for the term “isoenzymes”.
23. Name the isoenzymes of lactate dehydrogenase and creatine kinase.

24. Give the definition of "anabolism", "catabolism", "amfibolism.
25. Give the definition for the term "oxidative phosphorylation".
26. Draw the structure of the mitochondrial respiratory chain. Explain its biological role.
27. Give the definition for 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 and explain the functions of the TCA cycle.
31. Specify the cellular localization of the TCA cycle. Name the enzymes.
32. Specify and explain the reaction of substrate phosphorylation in the TCA cycle.
33. Give the definition for the term "glycolysis".
34. Specify the final product of glycolysis and amount of ATP, which is formed under anaerobic conditions.
35. Name the final products of aerobic glucose oxidation and amount of ATP, which are generated under these conditions.
36. What kind of reaction is catalyzed by the 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. Specify the enzyme of the pentose phosphate pathway of glucose oxidation hereditary deficiency of which can cause hemolytic anemia.
42. Specify the enzyme hereditary deficiency of which can cause galactosemia.
43. Specify the enzyme hereditary deficiency of which can cause"fructose intolerance".

44. What is "gluconeogenesis”? In which organs does it occur?
45. What are 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 and muscles 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 glycogen storage diseases?
55. Specify the biological role of TAG in the body.
56. What compounds are synthesized from cholesterol in the body?
57. What are lipolysis and lipogenesis?
58. What hormones can activate and inhibit lipolysis?
59. Name the enzyme of lipid metabolism, which is activated in adipose tissue under the action of epinephrine?
60. Specify the biological role of β–oxidation of fatty acids. What is the role of carnitine in the process?
61. What are ketone bodies? Specify the location of their synthesis and biological role.
62. Explain the term "ketoacidosis".
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 main classes of blood lipoproteins. Which of them are atherogenous and antiatherogenous?
69. What is the normal concentration of serum cholesterol (in mmol/l)?
70. Explain the biological role 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 removal of ammonia 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, which provide the transport of ammonia in the blood.
76. Name the process of urea formation, its role 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 these products production 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 increas 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 role of bile acids.
112. Explain which bile acids are known as primary and secondary. Give examples.
113. Name coenzyme for water soluble vitamins: B1, B2, B6, B12, 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 B2.
116. Give examples of two processes, in which vitamin B6 is involved.
117. Give examples of three processes, in which vitamin C isinvolved.
118. Give one example of processes involving Folic acid and vitamin B12.
119. Explain the mechanism of synthesis and activation of vitamin D_3.
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. What are the main fractions of blood serum proteines?
124. Explain the functions of albumin.
125. Explain the functions of haptoglobin.
126. Explain the functions of ceruloplasmin.
127. Explain the functions of antitrypsin.
128. Specify the normal concentration of blood serum proteins. Explain the function of transferrin.
129. Explain the term "rest nitrogen of blood". What 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, modern classifications, biological functions of amino acide.
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; multienzym 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 and D. Koshland 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 differentialdiagnosis.
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. 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. Active forms of oxygen 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 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 $NADPH{\cdot}H^{+}$, the total equation of palmitate synthesis, the regulation of the process.
68. Elongation of long–chain fatty acids. Biosynthesis of mono– and polyunsatu–rated 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 final 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. Transportation 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. Hereditary infringements of nucleotides 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 syntheses of hemoglobin.
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 Pro–opiomelanocortin (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 prosses. 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 (B1, B2, B3, B6, B12): 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. Hemoglobin: structure, properties, functions. Variants and pathological forms of hemoglobin.
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 paraproteinaemies.
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 hemoglobin. 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. Mucopolysacrides 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 neuropathies.

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

1. Electrophoresis as a method for protein fractionation. Electrophoregram of serum proteins in healthy person.
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 glycated hemoglobin 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.

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

1. 20 standard amino acids; 4–hydroxyproline, 5–hydrooxylysine.
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 phosphatidylserines (two 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 (1st 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 A2, thromboxane A2, leukotriene A4.
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 E

Table E.1 – Laboratory parameters

Complete blood count (CBC)	
1	2
Index	*Norm*
Erythrocytes	Male: 4.0–5.0 · 10^{12} /l
	Female: 3.9–4.7 · 10^{12} /l
Haemoglobin	Male: 135–180 g/l
	Female: 120–140 g/l
Color index	0.85–1.15
Biochemical blood test	
Total protein	65–85 g/l (6.0 to 8.3 g/dL)
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 (0.3 to 1.9 mg/dL)
free (indirect, unconjugated)	1.7–17.1 μmol/l (0.2 to 1.2 mg/dL)
binding (direct, conjugated)	0.86–5.1 μmol/l (less than 0.3 mg/dL)
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

1	2
Cholesterol (total)	< 5.2 mmol/L (< 200mg/dL)
Lipoproteins:	
VLDL	1.5–2.0 g/l (90.63–0.69 mmol/l)
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 (70 to 99 mg/dL)
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 (6–20 mg/dL)
Creatinine	53–106.1 μmol/l
Creatine	Male: 15.25–45.75 μmol/l Female: 45.75–76.25 μmol/l
Uric acid	Male: 0.24–0.51 mmol/L (4.0–8.5 mg/dL) Female: 0.16–0.43 mmol/L (2.7–7.3 mg/dL)
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)

1	2
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
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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Посібник містить матеріали для підготовки студентів до практичних занять з біологічної хімії: лабораторний практикум з алгоритмом виконання кожної роботи та клінічною оцінкою окремих показників, довідкову інформацію, список рекомендованої літератури. Посібник укладений відповідно до чинної програми з біохімії та відповідає вимогам державних стандартів вищої медичної освіти.

Для студентів вищих медичних навчальних закладів ІІІ–ІV рівнів акредитації.