THE MINISTRY OF HEALTH OF UKRAINE
ZAPORIZHZHIA STATE MEDICAL AND PHARMACEUTICAL UNIVERSITY
Biological Chemistry Department

BIOCHEMISTRY LABORATORY MANUAL

Section 1
Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids and amino acids and its regulation

FOR INDEPENDENT WORK AT HOME AND IN CLASS
PREPARATION FOR LICENSING EXAMINATION 'KROK-1'

For students of II international faculty

Speciality: 221 "Dentistry"

ZAPORIZHZHIA
2023

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This manual is recommended to use for students of II International faculty (the second year of study) for independent work on Biochemistry discipline at home and in class.

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PREPARATION FOR LICENSING EXAMINATION 'KROK-1'

II International faculty second year student`s

_____**gr. №_____**
name, surname

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INTRODUCTION

An important place in the training of a dentist is given to the study of biological chemistry. Biochemistry is a science that studies the chemical composition and metabolism of substances in the body. Knowledge of chemical processes occurring in the body of a healthy person forms the prerequisites for the development of ideas about the formation of the material basis for the occurrence of pathological processes and ways of their possible correction with the help of pharmacological agents. The proposed practicum is a necessary additional methodical guide for the study of biological chemistry by students of the 2nd year of the medical faculty of the specialty "Dentistry".

The manual contains a thematic plan of lectures and practical classes of section 1. For each practical session, the relevance of the topic being studied, the goal, a list of theoretical questions that must be known when studying the corresponding section of biological chemistry in accordance with the approved program for students of the specialty "Dentistry" are indicated in ECTS conditions. Each practical lesson contains the principle of the method, the methodology of performing laboratory work. The student's independent work in the biochemical laboratory allows one to get an idea of research methods used in biochemistry, develop an algorithm for interpreting laboratory research data, and judge the clinical and diagnostic value of biochemical indicators. In addition, each practical session contains a test control of the initial level of knowledge. Also, the workshop contains the necessary information about the individual independent work of students, questions for preparation for passing the basic topics and differentiated assessment. All of the above will help students in preparing for practical classes and differentiated assessment.

A Plan for Lectures of Section 1

	Section 1 ***Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids and amino acids and its regulation***	**Amount of hours**
1	Biochemistry as a science. Enzymes: structure, properties, mechanism of action, and regulation of enzymatic processes.	**2**
2	Bioenergetics. Citric acid cycle. Biological oxidation, tissue respiration, and oxidative phosphorylation.	**2**
3	Carbohydrate Metabolism (part1). Glycolysis and Gluconeogenesis. Aerobic Oxidation of Glucose.	**2**
4	Carbohydrate Metabolism (part 2). Alternative Pathways of Monosaccharide Metabolism. Glycogen Metabolism. Regulation and Pathology of Carbohydrate Metabolism.	**2**
5	Lipid Metabolism (part 1). Lipid Transport in the Blood. Triglyceride Metabolism. Metabolism of Higher Fatty Acids.	**2**
6	Lipid Metabolism (part 2). Steroid and Ketone Body Metabolism. Regulation and Pathology of Lipid Metabolism.	**2**
7	Amino Acid Metabolism. General and Specialized Pathways of Amino Acid Transformation. Inherited Enzymopathies of Amino Acid Metabolism. Pathways of Ammonia Metabolism. Biosynthesis of Urea.	**2**
8	Purine and Pyrimidine Nucleotide Metabolism. Pathologies of Metabolism.	**2**
Sum total, hours		**16**

Plan for Practical Classes of Section 1

	Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids, amino acids and its regulation	**Amount Of hours**
1	Introduction to biochemistry. Biochemical components of the cell. Features of work in the biochemical laboratory. Briefing on safety precautions. Control of the initial level of knowledge.	2.5
2	Structure, physicochemical properties and functions of proteins in humans. Classification of proteins, Simple and conjugated proteins.	2.5
3	Structure and physicochemical properties of proteins-enzymes. Classification and nomenclature of enzymes. The mechanism of enzyme action.	2.5
4	Kinetic properties of enzymes. Regulation and determination of enzyme activity. Units of enzyme activity. Enzymopathies. Medical enzymology.	2.5
5	The general patterns of metabolism and energy. Krebs Cycle.	2.5
6	General bases of bioenergetics.	2.5
7	**Intermediate control on basic themes 1, 2. Control work № 1.**	2.5
8	Anaerobic oxidation of glucose – Glycolysis. Glucose biosynthesis – Gluconeogenesis.	2.5
9	Aerobic oxidation of carbohydrates. Hexose monophosphate shunt. Metabolism of Galactose and Fructose in humans.	2.5
10	Metabolism of polysaccharides and its regulation. Carbohydrate metabolic pathways regulation. Pathologies of carbohydrate metabolism.	2.5
11	Lipoproteins of blood plasma. Metabolism of Triacylglycerols and Phospholipids.	2.5
12	High Fatty Acids and Ketone Bodies metabolism.	2.5
13	Cholesterol metabolism. The regulation and disorders of lipid metabolism: Obesity, Atherosclerosis.	2.5
14	Common metabolic pathways for amino acids. Glucogenous and ketogenous amino acids.	2.5
15	Ways for ammonia utilization. Metabolism of individual amino acids. Molecular pathologies of amino acid metabolism.	2.5
16	**Graded test for section 1 «Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids and amino acids and its regulation».**	2.5

Independent work of students in Section 1

№	Theme	Hours
1	Rules for the work with biological fluids (blood serum, saliva, urine) in biochemical investigations	**1**
2	Principles of determining the activity of enzyme proteins in biological fluids. Classification and determination of enzyme class by type of chemical reaction.	**1**
3	Construction and interpretation of graphs showing the relationship between the rate of enzymatic reaction and substrate concentration, enzyme concentration, pH variation, and temperature change in the environment. Determination of the type of enzyme inhibition using the Lineweaver-Burk plot.	**1**
4	Enzyme diagnostics and enzyme therapy. Diagnostic significance of determining changes in quantitative content and activity of isoenzymes in pathologies. Utilization of enzymes and their inhibitors as pharmaceutical agents.	**1,2**
5	Reconstruction of the sequence of steps in the general pathways of protein, carbohydrate, and lipid catabolism. Writing the sequence of reactions for the conversion of intermediates in the tricarboxylic acid cycle.	**1,2**
6	Drawing up a diagram and explaining the structure and mechanism of action of the electron transport chain in the mitochondrion. Explanation of the mechanism of coupling of oxidation and phosphorylation (ATP synthesis) in the mitochondrion based on the provisions of Mitchell's chemioosmotic theory. The use of tissue respiration inhibitors and uncouplers of oxidative phosphorylation as pharmaceuticals.	**1,2**
7	**Preparation for intermediate control of basic topics 1, 2**	**1**
8	Writing enzymatic reactions of the transformation of intermediates in glycolysis. Clinical-diagnostic significance of detecting metabolites of anaerobic glucose oxidation under physiological and pathological conditions.	**1,2**
9	Alternative pathways of glucose metabolism. Write enzymatic reactions for the conversion of intermediates in the pentose phosphate pathway.	**1,2**
10	Hereditary disorders of the biosynthesis of glycogen metabolism enzymes. Assessment of the state of carbohydrate metabolism by biochemical parameters in normal and pathological conditions. Biochemistry of diabetes mellitus.	**1,2**

11	Plasma lipoproteins: characteristics, metabolism, functional significance. Principles of methods for determining phospholipids and total lipids in blood serum.	**1,2**
12	Metabolism of ketone bodies in pathology; mechanisms of excessive increase in the content of ketone bodies in diabetes mellitus and starvation.	**1,2**
13	The main pathways of cholesterol biotransformation and excretion. Assessment of lipid metabolism in normal and pathological conditions (atherosclerosis, obesity, diabetes mellitus). Changes in the plasma lipoprotein system (lipoproteinemia). Genetic disorders of phospholipid metabolism (sphingolipidosis).	**1,2**
14	Investigation of amino acid metabolism disorders in congenital and acquired metabolic disorders by biochemical parameters.	**1,2**
15	Drawing up diagrams of circulatory ammonia transport in the body. Analysis of changes in the ammonia scavenging systems in case of genetic abnormalities of its metabolic enzymes.	**1**
16	**Preparation for differentiated credit of section 1.**	**2**
Sum total, hours		19

Lesson 1

THEME: INTRODUCTION TO BIOCHEMISTRY. BIOCHEMICAL COMPONENTS OF THE CELL. FEATURES OF WORK IN THE BIOCHEMICAL LABORATORY. BRIEFING ON SAFETY PRECAUTIONS. CONTROL OF THE INITIAL LEVEL OF KNOWLEDGE.

RELEVANCE OF THE TOPIC: biochemistry is a science that studies the chemical composition of organisms, as well as the peculiarities of the structure of their components, their function and chemical transformation. The study of biochemistry is necessary for the formation of a general idea about the molecular mechanisms of the occurrence of pathological processes, the effect of drugs and the nature of their interaction with the body. This knowledge creates a solid foundation for further study of pathological physiology, general clinical pharmacology, clinical biochemistry, etc.

THE PURPOSE OF THE LESSON: to study the stages of development of biochemistry as a fundamental medical and biological science and to determine the role of biochemical studies of the functional state of the human body in normal and pathological conditions.

QUESTIONS FOR PREPARATION

1. Biochemistry as a science. A subject, tasks, general stages and trends in the development of biochemistry.
2. The purpose and methods of biochemical research, their clinical and diagnostic value.
3. The relations of biochemistry with other biomedical subjuects. Clinical biochemistry. Biochemical laboratory diagnostics.
4. The history of biochemistry as the science.

Questions for the control of initial level of knowledge in organic chemistry:

1. Common notions in organic chemistry:
1.1. Polarity, hydrophility, lipophility of organic compounds.
1.2. Acidic, basic and amphoteric properties of organic compounds.
2. General peculiarities of structure for alcohols, aldehydes, ketones, carboxylic acids, amines.
3. A structure of some organic compounds: ethanol, glycerol; carboxylic acids: acetic, palmitic, oleic, succinic, fumaric, pyruvic, oxaloacetic, □-ketoglutaric, lactic and malic; acetic aldehyde, acetone, ethanol amine and choline.
4. The mechanism of ester bond formation using structure of glycerol and any fatty acid to form triacylglycerol. Creation of acetylcholine structure. Biological role of esters in human body.
5. Common notions about lipids, their classification according their structure; the biological role of each class of lipids.

6. General peculiarities of structure and functions in humans for monosaccharides (alpha-D-glucose, alpha-D-fructose, alpha-D-galactose and beta-D-ribose) and polysaccharides (starch, glycogen and cellulose).
7. α-Amino acids: classification according structure and physicochemical properties. The structure of some amino acids: glycine. alanine, glutamic acid, aspartic acid, phenylalanine, tyrosine, tryptophan, cysteine and methionine.
8. Proteins: levels of organization; a mechanism of peptide bond formation; types of bond in the protein molecule; physicochemical properties and functions of proteins.
9. Types of nucleic acids, nucleotides and nucleosides: structure, composition and function for them.

Protocol N 1 **Date**_____

Safety technique of the work in biochemical laboratory

Literature (p. 78)

Lesson 2

THEME: STRUCTURE, PHYSICOCHEMICAL PROPERTIES AND FUNCTIONS OF PROTEINS IN HUMANS. CLASSIFICATION OF PROTEINS. SIMPLE AND CONJUGATED PROTEINS

RELEVANCE OF THE TOPIC: biological fluids of the body - blood, cerebrospinal fluid, bile, gastric and intestinal juices, intercellular fluid contain mixtures of proteins. Proteins are polymers consisting of amino acids linked by peptide bonds. Peculiarities of the physical and chemical properties of proteins are the basis of many methods used in clinical and biochemical laboratories, pharmaceutical practice and experimental biochemistry for their isolation, purification, separation.

THE PURPOSE OF THE LESSON: to study materials about the amino acid composition and structural levels of organization of simple and complex proteins, their classification and functions, physical and chemical properties, to learn the methods of qualitative study of proteins and individual amino acids.

QUESTIONS FOR PREPARATION:

1. Amino acid composition of proteins and peptides. The mechanism of peptide bond formation. Levels of protein molecule organization.
2. Globular proteins: their structure, physicochemical properties, distribution in tissues and functions in humans (examples). The factors for stability of globular proteins in colloid solution. Common notions about Denaturation and Renaturation factors influence on proteins in solution.
3. Fibrous proteins: their structure, physicochemical properties, distribution in tissues and functions in humans (examples).
4. A Classification of simple proteins and conjugated proteins. The use of conjugated proteins (chromoproteins, nucleoproteins, metalloproteins, glycoproteins, phosphoproteins, lipoproteins) in human tissues.
5. Common notions about methods to release proteins preparations from biological fluids (salting-out, ultracentrifugation, dialysis), to separate them in mixture for obtaining of fractions (chromatography methods, electrophoresis). Qualitative tests to prove proteins and amino-acid residues presence in solutions.
6. Spectrophotometry and photocolorimetry methods to determine content of proteins in biological fluids.

LABORATORY WORKS:

1. Qualitative reactions for proteins: biuretic reaction, the reaction with sulfosalicylic acid, xanthoproteic test, Fole's test.

2. The separation of egg albumins and globulins by salting-out.

2. Sedimentation reaction for proteins: reactions with heavy metal salts, mineral acids.

Check up your home preparation using the tests:

1. **The reason of the damage of α-helical structure of polypeptide chain may be the large concentration (> 30 %) of one amino acid residue. Name it:**
A. Asp
B. Pro
C. Tyr
D. Ser
E. Gly

2. **The tertiary structure of protein is formed mainly due to disulfide bonds between side radicals of one amino acid, only. Point out it:**
A. Cys
B. Met
C. Asp
D. Lys
E. His

3. **Primary structure of proteins is formed due to one type of bonds. Point out it:**
A. Peptide bond
B. Disulfide bond
C. Ester bond
D. Hydrogen bond
E. Metal bond

4. **Point out the minimal quantity of amino acid residues in the polypeptide chain allowing the formation of the tertiary structure:**
A. 10
B. 12
C. 5
D. 40
E. 3

5. **Polypeptide chains of collagen include specific amino acids. Name one of them:**
A. Hydroxyproline
B. Formyl-methyonine
C. Cysteine
D. α-alanine
E. Ornithine

6. **The β-pleated sheet structure is very seldom in nature. Name the protein whose structure is based on it:**
A. Albumin of eggs
B. α-Keratin of hair
C. Fibroin of a silk
D. Elastin of cartilages
E. Protamine of plants

7. **There are many important protein functions in the human organism. Point out that of them, which isn't peculiar for proteins:**
A. Catalyst
B. Transfer of substances
C. Antibody
D. Structural component of a cell
E. Solvent

8. **The solubility of proteins in saline solutions is determined by their native structure. Point out the protein, which will swell only in saline solution:**
A. Elastin
B. Albumin
C. Myoglobin
D. Immunoglobulin
E. Pepsin

9. **The proteins are able to carry out the regulatory function. Find out those protein**:
A. Aminopeptidase
B. Insulin
C. Collagen
D. Hemoglobin
E. Immunoglobulin G

10. **All proteins are divided into simple and conjugated ones. Find out the simple protein among these ones:**
A. Albumin of egg
B. Histone

C. Globulin of egg
D. Protamine
E. All the proteins above

11. **Choose the proteins which are included into the deoxyribonucleoprotein composition in eukaryotic cells:**
A. Albumins
B. Globulins
C. Glutelins
D. Histones
E. Collagens

12. **Find the conjugated protein among following ones:**
A. Albumin
B. Protamine
C. Prolamine
D. Hemoglobin
E. Histone

13. **The conjugated protein necessarily contains special component as a non-protein part. Choose the substance that can't carry out this function:**
A. Glucose
B. HNO_3
C. Fe^{2+}
D. Haem
E. Phosphate

14. **Which method is better suited to separate a mixture of compounds into its individual components and detects small amounts (microgram or even picogram) of material:**
A. Dialysis
B. Paper chromatography
C. Ultracentrifugation
D. Salting out
E. Spectrophotometry

15. **Name protein of blood plasma containing copper ion Cu^{2+}:**
A. Albumin
B. Gamma-globulin
C. Ceruloplasmin
D. Alpha-2-macroglobulin
E. Collagen

16. **Name the non-protein part for conjugated protein that is derived from vitamin:**
A. Thiamine pyrophosphate
B. Acetyl-galactose
C. Copper sulfate
D. Phosphoric acid
E. Ribose-5-phosphate

17. **Which method is appropriate for the determination of total protein content in the blood serum:**
A. Salting out
B. Fole's test
C. Dialysis
D. Electrophoresis
E. Biuretic method

18. **Choose the conjugated protein in possession of following characteristics: quaternary structure - 4 polypeptide chains; non-protein part – 4 haem; function – oxygen transport in the blood:**
A. Low Density Lipoprotein
B. Albumin
C. Immunoglobulin
D. Hemoglobin
E. Ceruloplasmin

19. **What compound serves as non-protein part of glycoproteins:**
A. Cu^{2+}
B. Fe^{2+}
C. Galactose
D. Haem
E. Phospholipid

20. **Which group of proteins being phosphoproteins posses an activity but being**

dephosphorylated have lost the activity:
A. Hormones
B. Transfer of lipids
C. Transfer of vitamins
D. Enzymes
E. Carriers through membrane

21. **Collagen is a water-insoluble fibrillar protein that is essential for the formation of tooth tissues. Which amino acids are in significant quantities in its composition?**
A. Hydroxylysine, hydroxyproline
B. Lysine, hydroxylysine, glycine
C. Proline, hydroxylysine
D. Proline, lysine
E. Lysine, glycine

22. **The presence of protein in the solution is detected by color reactions. Which of the following reactions will NOT give a positive result with complete hydrolysis of these molecules?**
A. Biuretic
B. Xanthoprotein
C. Ningidrinov
D. Sakaguchi
E. Foil

Protocol N 2 **Date**_____

Qualitative reactions for proteins

1.1. Biuretic reaction (Piotrovsky's test)

This reaction proves the peptide bond in proteins and peptides (starting from tripeptides). The protein solution during the interaction with copper ions gets blue-violet color complex in the alkaline environment. And incomplete hydrolysis products of it (peptones) give pink coloring.

THE COURSE OF THE WORK:

Add to 5 drops of 1 % egg protein solution, 5 drops of 10 % NaOH solution, 2 drops of 1 % copper sulfate solution, and all of them mix. The test tube contents will get violet colour. A copper sulfate shouldn't be added surplusly, as the dark blue residue of the copper hydroxide masks the characteristic violet colouring of the biuretic protein complex.

RESULTS:

CONCLUSIONS:

1.2. The reaction with sulfosalicylic acid

THE COURSE OF THE WORK:

Pour 2-3 ml solution of protein (or researched fluid) into a test tube and add 5-6 drops of 20% sulfosalicylic acid solution. You can see the appearance of white colour precipitate at the presence of protein. This test is the most sensitive reaction for proteins.

Clinical significance

This test is used to prove the presence of proteins in the urine of patients at nephritis, some cardiac diseases, during some forms of idiopathic hypertension and during pregnancy pathology.
RESULTS:
CONCLUSIONS:

1.3. Xanthoproteic test

The test is used to detect amino acids containing an aromatic nucleus (tyrosine, tryptophan and phenylalanine) in a protein solution which gives yellow color nitro derivatives on heating with conc. HNO_3. The aromatic benzene ring undergoes nitration to give yellow colored product.
THE COURSE OF THE WORK:
Pour 5 drops of 1 % egg protein solution into first test tube and 5 drops of 10 % gelatin solution into second test tube. Add 3 drops of conc. HNO_3 into both test tubes and gently heat. In the first test tube a precipitate is formed, which is coloured yellow. In the second test tube, **NO** yellow precipitate is formed (gelatin does not contain aromatic amino acids in its structure).

1.4. Fole's test

This test is used to prove the presence one amino acid residue, only, in the composition of proteins - Cysteine. The sodium hydroxide under the boiling will cause the denaturation of egg proteins to get free cysteine residues in polypeptide chains which are involved in the reaction with lead acetate to geve the product – lead sulfide (black sediment)
THE COURSE OF THE WORK:
Add 10 drops of Fole's reactive (30% NaOH : $Pb(CH_3COO)_2$ in correlation 1:1) to the 10 drops of 1 % egg protein solution, then boil intensively for 1 min and wait for 1-2 minutes. The black or brown sediment of lead sulfide (PbS) should be formed.
RESULTS:

CONCLUSIONS:

2. The separation of egg albumins and globulins by salting-out

Salting-out is a reversible reaction of the protein sedimentation from solutions by means of big neutral salts concentration: sodium, ammonium sulfates, magnesium sulfate. Albumins are besieged in saturated solutions of sulfate ammonium, globulins are besieged in the half-saturated one; because the molecular weight of globulins is greater than the molecular weight of albumin. And albumins have the higher solubility in solution.
THE COURSE OF THE WORK:
Add 20 drops of the saturated ammonium sulfate solution to 20 drops of egg protein solution and mix. The half saturated solution is got, where the egg

globulin sediment falls out. In 5 minutes it must be separated by filtration. There is another protein in solution. It is an egg albumin. For albumins salting-out the crushed powder of ammonium sulfate is added till full saturation, i.e. while a new portion of powder remains not dissolved. The dropped out albumin's sediment is filtered out. The biuretic reaction is made with the filtrate. The negative reaction proves the absence of a protein in the filtrate.
RESULTS:

CONCLUSIONS:

3. Sedimentation reaction for proteins

3.1. Sedimentation reaction for proteins under the influence of heavy metal salts

Proteins, when interacting with heavy metal salts (Cu, Pb, Ag, etc.), form insoluble water complexes (chelates). This is because metal ions bind to the functional groups of amino acids in the protein molecule, disrupting its native structure, leading to the precipitation of denatured protein. The dissolution of the formed precipitate upon the addition of an excess of heavy metal salts (except AgNO3 and HgCl2) is explained by the adsorption of metal ions on the surface of denatured protein and the development of a positive charge on the protein particles (adsorption peptization). In this case, the protein remains denatured in the solution.
THE COURSE OF THE WORK:

In two test tubes, add 5 drops of an egg white solution each, and then add the following:

1. To the first test tube, add 2 drops of a 5% solution of $CuSO_4$ (copper sulfate).
2. To the second test tube, add 2 drops of a 5% solution of $(CH_3COO)_2Pb$ (lead acetate).

In both test tubes, a water-insoluble precipitate forms. When excess of the corresponding precipitating agent is added (5-10 drops), you will observe the dissolution of the precipitates.
RESULTS:

CONCLUSIONS:

3.2. Sedimentation reaction for proteins under the influence of mineral acids

Mineral acids are found as denaturation agents for proteins in solution.
Sulfuric acid and nitric acids cause complete denaturation of protein molecules to form free polypeptide chains. Excess content of sulfuric acid can dissolve the sediment of protein, but it is not renaturation!. Sulfate ions SO_4^{2-} make soluble complexes with polypeptide chains to be dissolved under this condition. Nitric acid anion has no ability to form soluble complex with polypeptide chain, and sediment of protein is not dissolved in excess content of nitric acid.
THE COURSE OF THE WORK:
Pour 1 ml of strong sulfuric acid and 1 ml of strong nitric acid respectively in two test tubes. Hold test tube with acid under the corner 45º and add drop by drop on the wall of test tube 1% egg protein solution to obtain ring of protein sediment, then shake (it is like addition of excess content of acid).

RESULTS:

CONCLUSIONS:

PREPARATION FOR THE KROK-1 LICENSURE EXAM

Cationic glycoproteins are the main components of the parotid saliva. Which amino acids determine their positive charge?

A. Lysine, arginine, histidine
B. Aspartate, glutamate, glycine
C. Glutamate, valine, leucine
D. Aspartate, arginine, glutamate
E. Cysteine, glycine, proline

Literature (p. 78)

Lesson 3

THEME: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF PROTEINS-ENZYMES. CLASSIFICATION AND NOMENCLATURE OF ENZYMES. THE MECHANISM OF ENZYME ACTION

RELEVANCE OF THE TOPIC: enzymes are catalysts of protein nature. Among the enzymes, there are representatives that are simple (pepsin, chymotrypsin) or complex (transferase, dehydrogenase) proteins. Like non-protein catalysts, they accelerate the achievement of a state of chemical equilibrium in chemical reactions. At the same time, they are not consumed during the reaction. Unlike non-protein catalysts, enzymes have: high specificity of catalytic action, significantly higher catalytic power, ability to

show effect in mild conditions (pH close to neutral, temperature within 28-37 C, normal pressure). Diagnostic enzymology can help not only in making a correct and timely diagnosis, but also in checking the effectiveness of the used treatment method.

THE PURPOSE OF THE LESSON: to study the biochemical regularities of the structure and functioning of various classes of enzymes. Be able to show with examples the difference between enzymes and non-protein catalysts (specificity of action, high efficiency of catalysis, ability to act in mild conditions, etc.). To be able to analyze the mechanisms of action of enzymes and ways of regulation of enzymatic processes as the basis of metabolism in the body in normal and pathological conditions.

QUESTIONS FOR PREPARATION

1. The function of enzymes in the organism. Enzyme characteristics in the comparison to non-protein catalysts.
2. Simple and conjugated enzymes structure. A definition of apoenzyme, cofactor, coenzyme and prosthetic group. A structure of active centres for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B_1, B_2, B_3, B_5, B_6, H).
3. Modern notions about the mechanism of enzymatic catalysis: the definition of energy activation for enzymatic reaction; the stages of the formation of an enzyme-substrate complex; the mechanisms for products formation (covalent and acidic catalysis). A significance of scientific works written by D. Keilin, B. Chance, D. Koshland, L. Michaelis and M. Menten.
4. Common properties of enzymes (factors of an influence: pH and temperature of environment, specificity of action).
5. Isozymes: structure and location of their synthesis in tissues (e.g.: Lactate dehydrogenase isozymes).
6. Classification and nomenclature of enzymes: features of reactions catalyzed by each class of enzymes.
7. Multienzyme systems of a cell: types of composition and function.

LABORATORY WORKS

1. Specificity of salivary amylase.
2. Thermolability of salivary amylase.
3. The pH medium influence on amylase activity in saliva.

Check up your home preparation using the tests:

1. **Enzymes are the catalysts of protein nature. Name the property of enzymes which is not presented at the inorganic catalysts:**

A. Ability to be denaturated
B. Wide specificity
C. To be Inert to chemical substrates
D. Big half-life

E. Ability to lower the energy activation for the reaction

2. **One of the important properties of enzymes is their specificity of action. Check up a type of specificity for salivary amylase:**

A. Absolute
B. Absolute group
C. Absolute relative
D. Relative group
E. Stereochemical

3. **Some terms are used for the description of non-protein part of an enzyme. Point out the term of non-protein part that easily dissociates from polypeptide chain:**

A. Apoenzyme
B. Coenzyme
C. Prosthetic group
D. Cofactor
E. Metall ions

4. **Oxidoreductase can contain prosthetic group with vitamin B_2. Name it:**

A. Retinal
B. Flavin adenine dinucleotide (FAD)
C. Nicotinamide adenine dinucleotide (NAD)
D. Pyridoxal phosphate
E. Ascorbic acid

5. **The change of the temperature of invironment from 0^0 C to 38^0 C can cause this effect:**

A. The probability of ES complex formation is increased
B. A denaturation of enzymes occurs
C. The enzyme molecular charge changes
D. The substrate molecular charge changes
E. Enzyme action specificity varies

6. **The optimum pH for cytoplasmic enzymes activity varies from 7.2 to 7.6. Point out all possible changes in active centre structure of such enzyme at pH=7.1:**

A. Changes are not presented
B. Radicals of amino acids get negative charge
C. Neutralization of negatively charged radicals
D. Formation of ester bonds between radicals
E. Destruction of the active centre

7. **A substrate molecule is destructed upon enzyme action, and the water is used for the products structure formation. Name the enzyme class:**

A. Oxidoreductase
B. Hydrolase
C. Lyase
D. Ligase
E. Isomerase

8. **A qualitative composition of product molecule is completely identical to substrate one, but the structure is different. Name the enzyme class:**

A. Oxidoreductase
B. Hydrolase
C. Lyase
D. Ligase
E. Isomerase

9. **ATP molecules may be used for Transferases and Ligases function. Point out the signs of ATP use for Ligases class:**

A. ATP is used for a substrate dephosphorylation
B. ATP is used for a substrate phosphorylation

C. ATP is used for hydrolysis of a substrate bond
D. ATP is used for the new bond formation during the interaction of two substrates
E. ATP is used for a substrate decarboxylation.

10**. Choose the factor which can cause the block of enzyme activity in human tissue:**

A. The pH value about 2
B. The temperature about 60^0 C
C. The presence of heavy metal ion as Hg^{2+}
D. The presence of the substrate
E. Positions A, B,C are right, only

Protocol N 3 **Date**_____

1. Specificity of salivary amylase

THE PRINCIPLE OF THE METHOD:

Amylase splits starch, glycogen and does not react on sucrose. The specificity of the amylase action is proved by Trommer's test result.

THE COURSE OF THE WORK:

Pour 5 drops of the saliva dissolved in correlation (1:4) into 2 test tubes. Add 10 drops of 1 % starch solution into the 1-st test tube, and 10 drops 1 % of the sucrose solution into the 2-nd one. Put the both test tubes into the thermostat at 38°C for 10 minutes. Carry out the Trommer's test.

Trommer's test:

Pour 3 drops of 5 % copper sulfate (II) solution and a few drops of 10 % sodium hydroxide solution into each test tube until the blue transparent solution appears. Shake up the content of the test tubes. Then cautiously heat up the test tubes and boil for 1 minute. The appearance of red colouring proves the glucose presence.

RESULTS:

CONCLUSIONS:

2. The thermolability of salivary amylase

THE PRINCIPLE OF THE METHOD:

The influence of temperature on salivary amylase activity is judged at splitting of starch by this enzyme at various temperature conditions. The degree of starch splitting is determined by iodic test, the product formation might be proved by the Trommer's test.

THE COURSE OF THE WORK:

Collect 3 ml of saliva into a test tube. Take away 2 ml of saliva into another tube for to boil 5 minutes, and then cool. Into the third test tube add 1 ml of saliva and dissolve the volume in correlation (1:4). Take the new three test tubes, and pour into each test tube 10 drops of 1% starch solution, after that add 10 drops of the dissolved saliva into the 1-st test tube. Add 10 drops of boiled saliva into the 2-nd test tube. Add 10 drops of water into the 3-rd test tube (control tube). All three test tubes put into the thermostat for 10 minutes at 38^0C. Then divide the content of each test tube into two parts and carry out qualitative reactions for starch and glucose (Trommer's test).

а) Reaction to starch (iodic test):
Pour 1 drop of the solution of iodine in potassium iodide into all three test tubes. At the starch presence the blue coloured complex appears.

RESULTS: Test tube N1 –
Test tube N2 –
Test tube N3 -

CONCLUSIONS:

б) Trommer's test:
RESULTS: Test tube N1 –
Test tube N2 –
Test tube N3 -
CONCLUSIONS:

3. The influence of the pH environment on the salivary amylase activity

THE PRINCIPLE OF THE METHOD:

The influence of the pH-environment on amylase activity is judged by the starch splitting at various pH values. The degree of starch splitting is determined by iodic test, the optimum of pH corresponds to a negative iodic test.

THE COURSE OF THE WORK:

The saliva volume is dissolved in correlation (1:100). Take 6 test tubes and pour 2 ml of the phosphate buffer with various value of pH: 6,0; 6,4; 6,8; 7,2; 7,6; 8,0 into each test tube. Then add 1 ml of 0,5 % starch solution and 1 ml of the dissolved saliva into each one. Mix the content of test tubes and place them into thermostat at 38°C for 10 minutes. Then pour 1 drop of iodine solution into each tube, and mix. You can observe the colouring in each tube and mark the pH optimum.

RESULTS:

CONCLUSIONS:

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. **In case of enterobiasis acrihine - the structural analogue of vitamin B_2 – is administered. The synthesis disorder of which enzymes does this medicine cause in microorganisms?**

A. FAD-dependent dehydrogenases
B. Cytochromeoxidases
C. Peptidases
D. NAD-dependet dehydrogenases
E. Aminotransferases

2. **In clinical practice tuberculosis is treated with izoniazid preparation – that is an anti-vitamin able to penetrate into the tuberculosis bacillus. Tuberculostatic effect is induced by the interference with replication processes and oxidation-reduction reactions due to the buildup of pseudo-coenzyme:**

A. FMN
B. NAD
C. CoQ
D. FAD
E. TDP

Literature (p. 78)

Lesson 4

THEME: KINETIC PROPERTIES OF ENZYMES. REGULATION AND DETERMINATION OF ENZYME ACTIVITY. UNITS OF ENZYME ACTIVITY. ENZYMOPATHIES. MEDICAL ENZYMOLOGY.

RELEVANCE OF THE TOPIC: there are some general principles for quantifying enzyme activity based on the rate of product accumulation in the reaction mixture or the rate of substrate disappearance in the reaction mixture. Enzyme activity is determined under optimal conditions for the enzyme. Under these conditions, its magnitude is proportional to the enzyme content in the sample and can be used for the indirect estimation of its concentration. Determining enzyme activity and studying medical enzymology will allow future doctors to diagnose enzyme disorders in a timely manner and propose treatment options.

THE PURPOSE OF THE LESSON: to study changes in the course of enzymatic processes, the accumulation of intermediate products of

metabolism in congenital (genetic) and acquired metabolic disorders - enzyme deficiencies.

QUESTIONS FOR PREPARATION

1. Enzymes kinetics: the determination of kinetic indexes (K_m and V_{max}) using the Michaelis-Menten equation curve and Lineweaver-Burk equation curve. A significance of Michaelis constant determination for enzymes with relative group specificity.
2. The factors for enzyme activity regulation: concentration of substrate; concentration of product; concentration of enzyme; pH and temperature of environment.
3. Common notions about inhibitors. Inhibition Types: reversible - competitive, uncompetitive, noncompetitive; irreversible - suicide inhibition, affinity labels (examples). The change of kinetic indexes for enzyme under the influence of competitive, non-competitive inhibitors (the determination of inhibitor type using Lineweaver-Burk equation curves).
4. Allosteric center of enzyme: its location, structure and function in enzymatic catalysis. The common notion about Allosteric type of enzyme activity regulation. Feed-back type of inhibition.
5. The principles of enzyme activity determination. Total and specific enzyme activity. The Units of enzyme activity. Turnover number of enzyme.
6. Common notions about enzymatic pathologies; the reasons of their development (examples).
7. General trends in the development of medical enzymology: 1) the elaboration of diagnostic methods using enzymes as reagents; 2) enzymatic tests for diagnosis of diseases (examples); 3) the use of enzymes and their inhibitors as drugs (examples).

LABORATORY WORKS

1. The influence of activators and inhibitors on the salivary amylase activity.
2. Determination of amylase activity in the urine by Volgemut's method.
3. Determination of cholinesterase activity in the blood serum.

Check up your home preparation using the tests:

1. Specify an inhibitor of salivary amylase:
A. Sodium chloride
B. Ammonium sulfate
C. Copper sulfate
D. Magnesium chloride
E. Calcium gluconate

2. There are some characteristic sites in the enzyme structure. Choose the most important site for enzyme function:
A. Allosteric centre
B. Active centre
C. Cofactor
D. Apoenzyme
E. Catalytic site, only

3. Specify the class of enzymes that performs the process of substrate phosphorylation:
A. Transferases
B. Oxidoreductases
C. Isomerases
D. Lyases
E. Ligases

4. **Choose the factor that changes the cytoplasmic enzyme conformation mainly:**
A. Suicide inhibitor
B. Environmental pH value about 7.4
C. Environmental temperature value about 25^0 C
D. Allosteric inhibitor
E. Water

5. Point the way of proenzyme transformation to the active enzyme:
A. Limited proteolysis
B. Dehydration
C. Decarboxylation
D. Inhibitor action
E. Vitamin non-protein part dissociation from enzyme

6. Competitive inhibitor always interacts with enzyme active centre. Find the explanation of this phenomenon:
A. Inhibitor causes the denaturation of active centre
B. Inhibitor is similar to a substrate structure
C. Inhibitor is an exact copy of a substrate structure
D. Inhibitor is similar to the product's structure
E. Inhibitor forms a covalent type of bonds with amino acid residues of active centre

7. Covalent modification of inactive form of enzyme may be catalyzed by special enzyme in a cell. Name it:
A. Esterase
B. Ligase
C. Protein kinase
D. Hydroxylase
E. Oxygenase

8. Find the irreversible type of enzyme inhibition:
A. Competitive
B. Noncompetitive
C. Uncompetitive
D. Allosteric
E. Suicide

9. Find the mathematic sense of Michaelis constant (Km):
A. It is a time for complete degradation of a substrate
B. It is a 1/2 of a substrate concentration for obtaining of Vmax
C. It is a substrate concentration for obtaining of 1/2 Vmax
D. It is a constant for ES-complex dissociation
E. It is a product concentration formed after enzymatic reaction

10. **The active centre of the enzyme contains amino acid residues of Aspartic acid. The substrate for this enzyme is cyclic organic alcohol. Point out the type of bond that may be formed between this substrate molecule and active centre of this enzyme:**
A. Glycosidic bond only
B. Hydrogen bond mainly
C. Peptide bond
D. Ester bond mainly
E. Disulfide bond

11. E. Fisher`s theory explains the mechanism of enzyme action with the fixed type of specificity, only. Name it:

A. Absolute
B. Absolute group
C. Absolute relative
D. Relative group
E. Stereochemical

12. **Choose the factor that does not affect the value of the dissociation constant of the enzyme-substrate complex:**
A. Substrate concentration
B. The chemical nature of the enzyme
C. Enzyme concentration
D. Concentration of the enzyme-substrate complex
E. The degree of affinity of the enzyme to the substrate

13. **Choose a substance that is not able to perform the function of a substrate for enzymes of the human body:**
A. Glucose
B. Higher fatty acid
C. Nitric acid
D. Acetic acid in active form
E. Glycogen

Protocol N 4 **Date**_____

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

THE PRINCIPLE OF THE METHOD:

The activator of salivary amylase is sodium chloride, and the inhibitor of one is copper sulfate. The influence of these substances on the amylase activity is judged by the degree of starch hydrolysis under the enzyme influence at the presence of sodium chloride and copper sulfate.

THE COURSE OF THE WORK:

The saliva is dissolved in correlation (1:200). Take 3 test tubes. Pour on 2 drops of 1 % sodium chloride solution into the 1-st one, and 2 drops of 1 % copper sulfate solution into the 2-nd one, and 2 drops of water into the 3-rd one. Add 1 ml of the dissolved saliva and 5 drops of 1% starch solution into each test tube. Mix the content and keep it at a room temperature for 2 minutes. Pour 1 drop of iodine solution into each tube, mix and observe the colouring.

RESULTS:

CONCLUSIONS:

2.Determination of amylase activity in the urine (Volgemut`s method)

THE PRINCIPLE OF THE METHOD:

The Volgemut's method is based on the minimal quantity of the enzyme determination, which is capable to split completely 2ml of 1% starch solution. This quantity of enzyme is accepted for a unit of the amylase activity.

THE COURSE OF THE WORK:

Pour 1 ml of 0, 85 % sodium chloride solution into each test tube (8 test tubes). Add 1 ml of patient’s urine into the 1-st test tube and mix thoroughly.

Then transfer 1 ml of the mixture into the 2-nd test tube and repeat all the operations with the test tubes: from the 2-nd one into the 3rd one, etc. Pour 1 ml of liquid out of the 8-th test tube. Add 2 ml of 0,1 % starch solution into each test tube, mix and put them into the thermostat at 38°C for 30 minutes. At the end of the incubation take the test tubes out, cool them and add 2 drops of the iodine solution into each one. Mix the content of the tubes and mark the latest test tube with no coloured solution (where there was full starch splitting).
The calculation is made according to the formula:
X (units)= 1 • 2 • dilution;
1 - urine volume (1ml); 2 - volume of 0,1 % starch solution in ml; X-salivary amylase activity in standard units.
Dilution is in each test tube (respectively): N1 - 2; N2 - 4; N3 -8; N4 - 16; N5 - 32; N6 - 64; N7 - 128; N8 - 256.
RESULTS:

CONCLUSIONS:

The clinical significance of the test:
Normal values of the amylase activity in the urine (by Volgemut) are 16 - 64 units. At sharp pancreatitis the activity of amylase in the urine and the blood serum arises 10 - 30 times.

3. Cholinesterase activity determination in the blood serum

THE PRINCIPLE OF THE METHOD:
Cholinesterase (CE) hydrolyzes acetylcholine to obtain an acetic acid and choline. The acetic acid decreases the pH value of solution that is because an indicator changes its colour: from crimson colour to yellow one.

THE COURSE OF THE WORK:
Keep all the reagents for 10 minutes at 37°C in the thermostat. Take three test tubes and make all the operations according to scheme:

Add, ml	N1 (test sample)	N2 (control sample)	N3 (empty sample)
Indicator solution	2,5	-	2,5
Blood serum	0, 05	0, 05	-
0, 9% NaCl sol-n	-	2, 7	0,05
Acetylcholine sol-n	0, 1	-	0,1
Take all test tubes into the thermostat (37°C) and keep there for 30 minutes. Then add:			
Stop-reagent	0,1	-	0,1

You have to determine an optical density of each sample against dist. water at 540 nm (green colour filter) in cuvettes (5 mm). Calculate the E according the formula:
E = E(empty) +E(control) – E(test)
Use this E value to find out on a graph the CE activity.
RESULTS:

CONCLUSIONS:

The clinical significance of the cholinesterase (CE) determination in the blood serum:

The normal value of CE activity is 45-95 μmol/sec•lit
The distinct decrease of the CE activity in blood serum takes place at the diseases of the liver, hypothyroidism, the bronchial asthma, articulate rheumatism, heart attacks of the myocardium, burns, traumatic shocks, in postoperative conditions. In severe forms of Botkin's disease the CE activity is decreased. In a case of the aggravation of the disease the decrease of the cholinesterase activity outstrips the bilirubin peak, playing a role of a harbinger of the aggravation. The dynamics of CE activity changes plays a valuable prognostic role at the patient's treatment.

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. Those organisms which in the process of evolution failed the protection from H_2O_2 can exist only in anaerobic conditions. Which of the following enzymes can break hydrogen peroxide down?

A. Oxygenase and hydroxylase
B. Oxygenase and catalase
C. Cytochrome oxidase, cytochrome b5
D. Flavin-dependent oxidase
E. Peroxidase and catalase

2. 6 Hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isozyme should be expected in this case

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

3. A patient presents high activity of LDH1 and LDH2, aspartate aminotransferase, creatine Phosphokinase. In what organ (organs) is the development of a pathological process the most probable?

A. In the heart muscle (initial stage of myocardium infarction)
B. In skeletal muscles (dystrophy, atrophy)
C. In connective tissue
D. In liver and kidneys
E. In kidneys and adrenals

Literature (p. 78)

Lesson 5
THEME: THE GENERAL PATTERNS OF METABOLISM AND ENERGY. KREBS CYCLE.

RELEVANCE OF THE TOPIC: metabolism in a living cell is closely linked to energy exchange. Most biosynthesis reactions, the functioning of ion transport systems in specialized intracellular structures, are energy-dependent processes. The central role in providing energy to the cell is played by the tricarboxylic acid cycle, which is a common pathway for the catabolism of proteins, lipids and carbohydrates. Disruptions in energy metabolism are a crucial aspect of the pathogenesis of various diseases, and their correction forms the basis of treatment and prevention for these conditions.

THE PURPOSE OF THE LESSON: to study the biochemical principles governing the metabolism of substances and energy. To interpret the biochemical principles of functioning, regulatory mechanisms, and the key role of the tricarboxylic acid cycle (TCA) in the metabolism of substances and energy.

QUESTIONS FOR PREPARATION:
1. Common notions about Metabolism and Energy exchange in organism. Anabolic, Catabolic and Amphiobolic processes: definition and their interrelations.
2. Exergonic and endergonic reactions in metabolism.
3. Stages of catabolism. Common and specific ways of catabolism for exogenous and endogenous substrates. Terminal products of catabolic pathways for humans
4. Krebs Cycle: the location in a cell, all the reactions, the regulation and the biological role of this process. Energy balance for Krebs cycle.Vitamins promotion of Krebs cycle.

LABORATORY WORKS
The investigation of succinate dehydrogenase (SDHase) activity in muscles

Check up your home preparation using the tests:

1. Specify the cellular localization of Krebs cycle enzymes:
A. Mitochondria
B. Cytoplasm
C. Endoplasmic reticulum
D. Core
E. Lysosomes

2. Point the substrate of Krebs Cycle that can be the product of the last reaction of this process:
A. Oxaloacetate
B. Citrate
C. α-Ketoglutarate
D. Malate
E. Succinate

3. Nucleoside triphosphate is formed in Krebs Cycle. Point its abbreviation:
A. ATP
B. CTP
C. GTP
D. UTP
E. TTP

4. Only one dehydrogenase of Krebs Cycle has the non-protein part FAD. Name it:
A. Isocitrate dehydrogenase
B. α-Ketoglutarate dehydrogenase
C. Malate dehydrogenase
D. Succinate dehydrogenase
E. Pyruvate dehydrogenase

5. There is a multiple enzyme complex among enzymes of Krebs Cycle. Point it:
A. Isocitrate dehydrogenase
B. α-Ketoglutarate dehydrogenase
C. Malate dehydrogenase
D. Succinate dehydrogenase
E. Pyruvate dehydrogenase

6. Vitamin B_1 (coenzyme TPP) is necessary for only one dehydrogenase function in Krebs Cycle. Point it:
A. Malate dehydrogenase
B. α-Ketoglutarate dehydrogenase
C. Isocitrate dehydrogenase
D. Succinate dehydrogenase
E. Lactate dehydrogenase

7. NADH is formed as a product in Krebs Cycle. Point the mole quantity of NADH per 1 mole of acetyl~SCoA incorporated into the process:
A. 1
B. 2
C. 3
D. 4
E. 1.5

8. Citric Acid Cycle is one of stages in catabolic pathways. Point out the number of stage, which is related to it:
A. 1
B. 2
C. 3
D. 4
E. 5

9. Krebs Cycle is an amphiobolic way. Choose the explanation of this sentence:
A. It forms CO_2 and H_2O
B. It forms HADH
C. Intermediate metabolites may be used in anabolic ways
D. 1 mole of ATP will be formed in one cycle
E. The process is in mitochondrion

10. Two reactions of Krebs Cycle are named as oxidative decarboxylation. Point the enzyme for this type of reaction:
A. Citrate synthase
B. cis-Aconitate hydratase
C. Isocitrate dehydrogenase

D. Succinate dehydrogenase
E. Succinyl~SCoA synthase

11. Point the stage with maximum ATP energy formation for glycose aerobic destruction up to CO_2 and H_2O:

A. Glycolysis up to pyruvate
B. Oxidative decarboxylation of pyruvate
C. Krebs Cycle
D. Glycolysis to lactate
E. None of these processes

Protocol N 5 **Date**_____

The investigation of succinate dehydrogenase (SDHase) activity in muscles

THE PRINCIPLE OF THE METHOD:

SDHase oxidizes succinate into fumarate. The coenzyme of SDHase is flavin adenine dinucleotide (FAD). This enzymatic action can be observed in aerobic conditions at addition of sodium 2,6-dichlorphenolindophenolate (as acceptor of hydrogen ions). It is transformed into a restored colorless form from blue one.

THE COURSE OF THE WORK:

The muscular tissue (about 1 g) is crushed with scissors and pounded in a mortar with a small quantity of water (2-3 ml) for 1 minute. Then the muscular mass is transferred on a double layer of gauze placed on a funnel, is washed thoroughly with water, is placed on filtering paper and is dried up. Pour 3 ml of the phosphate buffer (pH =7,4) into three test tubes and place 1/3 of the muscular mass into each of them. Then add 5 drops of 3 % amber acid solution and 5 drops 0,1N NaOH solution (for neutralization) into an experimental test tube, and into a control test tube pour 10 drops of distilled water. Into the 3-rd test tube pour 5 drops of 3 % malonate solution, 5 drops of 3 % amber acid solution and 5 drops of 0,1N NaOH solution. Into each tube add 1 ml 0,001N solution of sodium 2,6-dichlorphenolindophenolate and mix the content of three test tubes. Put all test tubes into a thermostat at 37^0C for 40 minutes. After the incubation compare the colouring of the experimental tube with the content of the control one and the test tube, where the competitive inhibitor of SDHase - malonate was. The intensity of decoloration of sodium 2,6-dichlorphenolindophenolate characterizes the SDHase activity at the presence of the amber acid.

RESULTS:

CONCLUSIONS:

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. The central intermediate product of all exchanges (proteins, lipids, carbohydrates) are active form of acetic acid. Name her.

A. Acetyl-CoA
B. Lactate

C. Succinyl-CoA
D. Citrate
E. Glutamate

Literature (p. 78)

Lesson 6
THEME: GENERAL BASES OF BIOENERGETICS

RELEVANCE OF THE TOPIC: the existence of living organisms is linked to the absorption of energy released during biological oxidation and is stored in the high-energy bonds of various compounds. The respiratory chain within mitochondria, which is a sequence of carriers responsible for transporting electrons from reduced substrates to oxygen, primarily serves as a source of energy for macroergs.

THE PURPOSE OF THE LESSON: study the biochemical principles of biological oxidation processes and oxidative phosphorylation. Be able to analyze disruptions in ATP synthesis under the influence of various factors on the human body.

QUESTIONS FOR PREPARATION
1. Modern concepts of tissue respiration. Stages of tissue respiration.
2. Complexes of the respiratory chain in mitochondria and their interrelation. Endogenous water formation. Formation of products of incomplete oxygen reduction and their detoxification in the cell.
3. Inhibitors of tissue respiration: classification and representatives.
4. Oxidative phosphorylation. Chemiosmotic theory by P. Mitchell.
5. Proton gradient energy and its utilization pathways. Mechanism of proton gradient formation on the inner mitochondrial membrane.
6. Structure of the coupling factor and its role in ATP synthesis (mitochondrial ATP synthase).
7. Assessment of oxidative phosphorylation efficiency. The respiratory control ratio (P/O).
8. Uncouplers of oxidative phosphorylation and biological oxidation.
9. Regulation of tissue respiration. Respiratory control.

Check up your home preparation using the tests:

1. Specify the number of macroergic substrates that are synthesized due to substrate phosphorylation in one Krebs cycle:	A. 1 B. 3 C. 11 D. 12 E. 9

2. The patient suffering from sleeplessness was prescribed barbiturate sleeping pills. Name the enzyme of mitochondria that is inhibited by this drug:
A. Cytochrome oxidase
B. NADH-dehydrogenase
C. Succinate dehydrogenase
D. Isocitrate dehydrogenase
E. α-Ketoglutarate dehydrogenase

3. The antibiotic oligomycin has been recently used in tuberculosis treatment. Point the process in tuberculous bacillus that is inhibited by this drug:
A.Oxidative phospho-rylation
B. Translation
C. Anaerobic glycolysis
D. The active transport of substances across membranes
E. Phagocytosis

4. High concentrations of thyroid gland hormone (T_4) in the patient suffering from Basedow`s disease is followed by the infringement in the tissue energy supply. Point the right reason of this state:
A. T_4 intensifies the absorbtion of Ca^{2+} in the small intestine
B. T_4 plays the role of uncoupler of oxidation and phosphorylation
C. T_4 inhibits the dehydrogenases of Krebs Cycle
D. T_4 activates the lipolysis
E. T_4 increases the ATP/ADP ratio up to 1

5. The increasing of NH_3 in the blood plasma leads to the tissue respiration blockade. How will the ATP/ADP ratio change in the blood cells in this case?
A. ATP/ADP will rise
B. ATP/ADP will reduce
C. ATP/ADP will not change
D. ATP/ADP = 0
E. ATP/ADP becomes negative

6. The important catabolic processes are located in the mitochondrial matrix. Find the catabolic process that isn't located in the mitochondria:
A. Krebs Cycle
B. Oxidation of fatty acids to acetyl~SCoA
C. Oxidative decarboxylation of pyruvate
D. Glycolysis
E. The formation of oxaloacetate from pyruvate

7. The tissue respiration is inhibited after coal gas poisoning. Point the respiratory chain enzyme whose activity abruptly reduces in this condition:
A. Succinate dehydrogenase
B. NADH-dehydrogenase
C. Cytochrome b_1
D. Cytochrome c
E. Cytochrome aa_3

8. Rotenone (the inhibitor of the first complex of the respiratory chain) changes the P/O ratio for substrates that are oxidized in Krebs Cycle. Choose the value of P/O at the presence of this inhibitor per 1 mole of the malate that is oxidized:
A. <1
B. <2
C. <3
D. <4
E. 0

9. Name the Krebs Cycle enzyme whose activity is increasing

while the value of the respiratory control (ATP/ADP) is reducing:
A.Isocitrate dehydrogenase
B. Malate dehydrogenase
C. Pyruvate dehydrogenase
D. Succinate dehydrogenase
E. α-Ketoglutarate dehydrogenase

10. The increase of one substrate concentration occurs in the mitochondrial matrix during the inhibition of Citrate synthase in Krebs Cycle. Find this substrate:
A. Pyruvate
B. Acetyl~SCoA
C. α-Ketoglutarate
D. Malate
E. Succinate

11. The electrochemical potential ($\Delta\mu H^+$) formation occurs on the inner membrane of mitochondria during the active work of the respiratory chain. Point out the substance that can reduce the ($\Delta\mu H^+$) value:
A. Succinate
B. Malonic acid
C. 2,4-Dinitrophenol
D. Citric acid
E. Glucose

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. What is the process of ATP synthesis occurs in mitochondria conjugated with oxidation reactions involving the system respiratory enzymes?
A. Oxidative phosphorylation
B. Free oxidation
C. Peroxidation
D. Substrate phosphorylation
E. Photosynthetic phosphorylation

Literature (p. 78)

Lesson 7

THEME: INTERMEIATE CONTROL FOR CLASSES NN 1-6. CONTROL WORK

Common regularities of metabolism and energy exchange in humans

THE PURPOSE OF THE LESSON: assess the level of students' comprehension of the fundamental principles and general patterns of metabolism.

THEORETICAL QUESTIONS FOR PREPARATION
1. Biochemistry as a science. A subject, tasks, general stages and trends in the development of biochemistry.
2. The aims and methods for biochemical researches, their medical significance.

3. The connection of biochemistry with other biomedical sciences. Medical biochemistry. Clinical biochemistry.
4. The history of biochemistry as the science.
5. Biochemical components of a cell. All the classes of biological molecules; the ways for their formation in a cell.
6. The function of enzymes in the organism. Enzymes characteristic in the comparison to non-protein catalysts.
7. Simple and conjugated enzymes structure. Definitions of apoenzyme, cofactor, coenzymes and prosthetic group (examples).
8. Structure of active centers for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B_1, B_2, B_5, B_6).
9. The common properties of enzymes (factors of an influence: pH and temperature of environment, specificity of action).
10. Isozymes: structure, location in tissues and clinical significance of their determination in the blood plasma. (e.g.: lactate dehydrogenase isozymes).
11. The principles of enzymes` classification and nomenclature.
12. The mechanism of enzymatic catalysis and enzymes` kinetics. A significance of scientific works written by D. Keilin, B. Chance, D. Koshland, L. Michaelis and M. Menten.
13. The influence of substrate concentration, pH and temperature on the velocity of enzymatic reaction. Michaelis-Menten constant (Km) determination and its significance.
14. The regulation of enzymes activity. Activators and inhibitors for enzymes: the mechanisms of action, according examples.
15. Inhibition Types: reversible - competitive, noncompetitive; irreversible - suicide inhibition, affinity labels (examples).
16. Allosteric regulation of enzymes activity. Covalent modification of enzymes.
17. Common notions about of enzymatic pathologies and the reasons of their occurance in patients.
18. The use of enzymes for diagnosis of diseases (examples).
19. The use of enzymes, their activators and inhibitors as drugs (examples).
20. The principles and methods of enzyme activity determination. Types and units of enzyme activity.
21. Common notions about Metabolism and Energy exchange in organism. Anabolic, Catabolic and Amphiobolic processes: definition and their interrelations.
22. General stages of catabolism for proteins, carbohydrates and lipids. Terminal products of catabolic pathways in humans.
23. Krebs Cycle: the location in a cell, all the reactions, the regulation and the biological role of this process.
24. Energy balance for Krebs cycle.
25. Amphibolic role of Krebs cycle in a cell.

26. Types of reactions in biological oxidation (the function of dehydrogenases, oxidases and oxygenases), their biological role.
27. Tissue respiration. Stages of tissue respiration: location in a cell.
28. Enzymes of mitochondria: the structure, composition and function in metabolic pathways (NAD-derivatives, flavoproteins, cytochromes).
29. The respiratory chain: structural organization in the inner membrane; complexes of respiratory chain.
30. Oxidative phosphorylation. The location of three coupling sites in the respiratory chain. P/O ratio for oxidation of some substrates.
31. Chemioosmotic Theory (P.Mitchell, 1961) in the explanation of Oxidative phosphorylation mechanism. ATP synthase: location, structure and function.
32. Inhibitors of tissue respiration and Uncouplers of oxidative phosphorylation: mechanism of their action. The estimation of respiratory control in a cell.

The questions for laboratory works *of classes NN1-6:*

1. Principles of qualitative reactions on proteins and amino acids: Biuretic reaction, Fole's test, test with sulfur salicylic acid.
2. Explain, please, the general principles for the use of the use of iodic test and Trommer's test in study of enzymatic activity using as example the enzyme - salivary amylase.
3. Qualitative tests to prove protein nature of enzymes
4. An explanation enzymes thermolability using the method for salivary amylase investigation; a drawing of the graph for temperature influence on the enzyme activity.
5. A graph curve for the influence of pH medium on the enzyme activity using the results of laboratory work for salivary amylase.
6. The way to prove the relative group specificity for salivary amylase. Types of specificity for enzymes.
7. Explain, please, the functions of sodium chloride and copper sulfate in the laboratory work with salivary amylase.
8. Explain, please, the principle of the method in the research of the enzyme concentration influence (for salivary amylase) on the velocity of enzymatic reaction.
9. The principle of the method, normal values and clinical significance for determination of amylase activity in the urine.
10. The principle of the method, normal values and clinical significance for determination of choline esterase activity in the blood serum.
11. The principle of the method to investigate the activity of succinate dehydrogenase of muscles. Name, please, the location of this enzyme in the cell.
12. Describe, please, the inhibition of succinate dehydrogenase activity by malonic acid. Name the type of this inhibition. How can you protect this enzyme from the action of malonic acid? Draw, please, the curves for the

influence of substrate concentration on the velocity of enzymatic reaction without inhibitor (1) and in the presence of it (2), using one graph.

Literature (p. 78)

Lesson 8

THEME: ANAEROBIC OXIDATION OF GLUCOSE – GLYCOLYSIS. GLUCOSE BIOSYNTHESIS – GLUCONEOGENESIS.

RELEVANCE OF THE TOPIC: understanding the peculiarities of carbohydrate metabolism in human tissues is extremely important for future physicians. It allows for a comprehensive comprehension of the carbohydrate exchange process, both under normal conditions (physiological state) and in pathology, characterized by alterations in carbohydrate metabolism (such as diabetes, liver diseases, and others).

THE PURPOSE OF THE LESSON: to study the patterns of gluconeogenesis and glycolysis - the processes of energy production during physical exertion and maintenance of a relatively constant glucose level in biological fluids.

QUESTIONS FOR PREPARATION

1. Structure, classification, and biological roles of carbohydrates.
2. Daily requirements and digestion of carbohydrates. Carbohydrate-digesting enzymes: localization, pH optimum, and specificity of action. Inherited lactase deficiency.
3. End products of carbohydrate digestion and the mechanism of their absorption in the small intestine.
4. Intracellular catabolism pathways of monosaccharides; aerobic and anaerobic glucose oxidation, general characteristics of processes.
5. Anaerobic glucose oxidation – glycolysis: sequence of enzymatic reactions, biological roles, localization in the body and within cells.
6. Glycolytic oxidoreduction, substrate-level phosphorylation in glycolysis. Energetic balance of anaerobic glucose oxidation.
7. Regulation of glycolysis. Key enzymes of the process.
8. Alcoholic and other types of fermentation.
9. Gluconeogenesis: substrates, key enzymes, reactions, intramolecular localization, physiological significance of the process. Balanced equation for glucose formation from pyruvate. Energetic support of gluconeogenesis.
10. Metabolic and hormonal regulation of gluconeogenesis.
11. Interconnection and reciprocal regulation of glycolysis and gluconeogenesis in the body. Glucose-lactate (Cori cycle) and glucose-alanine cycles.

LABORATORY WORKS

Determination of lactic acid in muscles. Uffelmann's reaction.

Check up your home preparation using the tests:

1. Choose the terminal product of anaerobic glycolysis:
A. Pyruvate
B. Acetyl-SCoA
C. Lactate
D. CO_2, H_2O
E. Oxaloacetate

2. What enzyme catalyzes the glucose-6-phosphate formation from glucose in the liver and is not inhibited by excess level of glucose-6-phosphate:
A. Hexokinase
B. Glucokinase
C. Pyruvate kinase
D. Glucose-6-phosphatase
E. Phosphoglucomutase

3. Choose the condition in human organism which can cause the beginning of gluconeogenesis in the liver:
A. Hyperglycemia
B. Hypoglycemia
C. The decrease of diuresis
D. The hypoxia of liver tissue
E. The bile ducts obstruction

4. Choose the key enzyme for glycolysis:
A. Phosphofructokinase
B. Aconitase
C. Pyruvate carboxylase
D. Glucose-6-phosphatase
E. Phosphoglucomutase

5. Name the factors which are important to regulate the aerobic glycolysis duration:
A. ATP/ADP ratio in a cell
B. NADH/NAD+ ratio in a cell
C. Fructose-2.6-biphosphate level
D. Oxygen level in tissue
E. All the factors mentioned above

6. Choose the enzyme for the reaction of glucose formation due to dephosphorylation:
A. Glucokinase
B. Phosphofructokinase
C. Glucose-6-phosphatase
D. Aldolase
E. Aconitase

7. Choose the substance that can be the substrate for gluconeogenesis:
A. Glycogen
B. Glucose
C. Pyruvate
D. Fructose
E. Galactose

8. How glucocorticoids influence on the carbohydrate metabolism?
A. Stimulate the glycolysis from glucose
B. Stimulate the gluconeogenesis
C. Stimulate the starch hydrolysis in the small intestine
D. Inhibit the glycogen phosphorolysis
E. Stimulate the glycogenesis

9. Find the location of glucose-6-phosphatase in human tissues:
A. Gonads, only
B. Liver, kidney
C. Liver, only
D. Skeletal muscular tissue
E. Myocardium

10. Name the energy effect of anaerobic glycolysis per 1 mole of glucose incorporated into the process:
A. 2 ATP
B. 5 ATP

C. 8 ATP
D. 10 ATP
E. 3 ATP

11. Name the enzyme whose function is associated with digestion of polysaccharides in the small intestine:

A. Elastase
B. Renin
C. Pepsinogen
D. Maltase
E. α-Amylase

Protocol N8 **Date**

The determination of lactate in muscular homogenate (Uffelmann`s reaction)

THE PRINCIPLE OF THE METHOD:

It is based on Uffelman's reaction: lactic acid can react with phenol solution in the presence of iron (III) chloride. The yellow-green colouring will appear.

THE COURSE OF THE WORK:

Crush and pound in a mortar 1 g muscles with a small amount of quartz sand for 3 minutes, add 5 drops of water for reception of homogeneous mass. Then flow 3 ml of water, mix and filter through the cotton wool moistened with water.

Prepare Uffelman`**s** reagent: bring 20 drops of 1 % phenol solution in a test tube, add 2 drops of 1 % chloride of iron (III) solution. The solution is coloured in violet color of phenolate of iron. Then add 15 drops of the filtrate to Uffelmann`**s** reagent drop by drop. At the presence of the lactic acid violet colouring of the liquid passes in yellow-green one due to formation of iron lactate. For comparison carry out Uffelmann`s reaction, using a solution of lactic acid instead of the filtrate.

RESULTS:

CONCLUSIONS:

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. When blood circulation in the damaged tissue is restored, then lactate accumulation comes to a stop and glucose consumption decelerates. These metabolic changes are caused by activation of the following process:

A. Aerobic glycolysis
B. Anaerobic glycolysis
C. Lipolysis
D. Gluconeogenesis
E. Glycogen biosynthesis

Literature (p. 78)

Lesson 9

THEME: AEROBIC OXIDATION OF CARBOHYDRATES. HEXOSE MONOPHOSPHATE SHUNT. METABOLISM OF GALACTOSE AND FRUCTOSE IN HUMANS

RELEVANCE OF THE TOPIC: the majority of animal and plant cells exist under aerobic conditions in normal circumstances, and therefore carbohydrates are completely oxidized to CO_2 and H_2O. During this process, all biologically available free energy is released from glucose. Additionally, there is another pathway for carbohydrate oxidation in the body, the pentose phosphate pathway. Understanding both the aerobic and pentose phosphate pathways of glucose oxidation is crucial for future physicians to comprehend their roles in energy metabolism and plastic processes within cells, as it relates to the potential correction of these processes in pathological conditions.

THE PURPOSE OF THE LESSON: to study the theoretical material on intermediary carbohydrate metabolism. Be able to determine glucose and its derivatives in biological fluids.

QUESTIONS FOR PREPARATION

1. Stages of aerobic glucose oxidation.
2. Oxidative decarboxylation of pyruvic acid (enzymes, coenzymes, reaction sequence, regulation of pyruvate dehydrogenase complex functioning).
3. Interplay between anaerobic and aerobic carbohydrate oxidation pathways in the cell, Pasteur effect.
4. Oxidation of cytosolic NADH in mitochondria. Shuttle mechanisms for glycolytic NADH oxidation (glycerophosphate, malate-aspartate).
5. Comparative characteristics of the bioenergetics of aerobic and anaerobic glucose oxidation.
6. Pentose phosphate pathway of glucose oxidation: scheme and biological role of the oxidative phase. Non-oxidative phase of the process and its interaction with glycolysis. Inherited deficiency of glucose-6-phosphate dehydrogenase in erythrocytes.
7. Metabolism of fructose and galactose in the human body and its disorders.

LABORATORY WORKS

The determination of pyruvate content in the urine

Check up your home preparation using the tests:

1. Name the energy effect of aerobic glycolysis per 1 mole of glucose incorporated into the process:

A. 2 ATP
B. 5 ATP
C. 8 ATP
D. 10 ATP

E. 3 ATP

2. Point the vitamin that does not take part in aerobic oxidation of carbohydrates.

A. Thiamine
B. Nicotinamide
C. Lipoic acid
D. Folic acid
E. Pantothenic acid

3. Point the biological role of Pentose Phosphate Cycle:

A. HADPH and ribose-5-phosphate production
B. Acetyl-SCoA formation
C. ATP synthesis
D. Deoxyribose formation
E. Fructose formation

4. Choose a compound that isn't formed during oxidative decarboxylation of pyruvate:

A. Acetyl-SCoA
B. CO_2
C. HADH
D. Glycerol-3-phosphate
E. $FADH_2$

5. Point the location of oxidative decarboxylation of pyruvate in a cell:

A. Cytoplasm
B. Mitochondria
C. Lysosome
D. Endoplasmic reticulum
E. Nucleus

6. Choose the terminal products of aerobic glucose oxidation in a cell:

A. Lactate and ATP
B. CO_2, H_2O and ATP
C. Acetyl-SCoA and ATP
D. Pyruvate and ATP
E. Citric acid and ATP

7. Point the factor stimulating the pyruvate dehydrogenase complex activity:

A. Insulin
B. Excess pyruvate in a cell
C. ATP/ADP ratio lesser then 1
D. Excess glucose in a cell
E. Positions A, B, C above are right

8. Name the last stage of aerobic glucose oxidation:

A. Oxidative decarboxylation of piruvate
B. Krebs Cycle
C. Pyruvate formation
D. α-Ketoglutarate formation
E. Acetyl-SCoA formation

9. Point the energy effect of complete glucose oxidation in aerobic condition (glycerol phosphate shuttle mechanism is used):

A. 36 ATP
B. 38 ATP
C. 2 ATP
D. 3 ATP
E. 12 ATP

10. Name the substance that is used for electrons transport from cytoplasmic HADH to the matrix of mitochondria:

A. Aspartate
B. α-Ketoglutarate
C. Glutamate
D. Glycerate-3-phosphate
E. Malate

11. The child has vomiting and diarrhea after eating, general dystrophy, hepato- and splenomegaly. After stopping breastfeeding, the symptoms subside. Specify a possible metabolic disorder:

A. Hypersecretion of endocrine glands
B. Disorders of phenylalanine metabolism

C. Disorders of galactose metabolism
D. Disorders of tyrosine metabolism
E. Glucose-6-phosphate deficiency dehydrogenasesspartate

12. The patient was diagnosed with hypovitaminosis B1. Name the enzyme of the pentose phosphate cycle, the activity of which is reduced:
A. Transketolase
B. Glucose-6-phosphate dehydro-genase
C. Ketoisomerase
D. Transaldolase
E. Gluconolactone hydrolase

13. Specify the localization in tissue cells of the reactions and enzymes of the pentose phosphate pathway of glucose metabolism:
A. Core
B. Mitochondrial matrix
C. Cytoplasmic membrane
D. Cytosol
E. Ribosomes

Protocol N9 **Date**_____

The determination of pyruvate content in the urine

THE PRINCIPLE OF THE METHOD:

Pyruvic acid (PA), reacting with 2,4-dinitrophenilhydrazine in alkaline environment, forms hydrazone derivatives coloured yellow. The intensity of colouring is proportional to PA concentration.

THE COURSE OF THE WORK:

Use dry test tubes, pipettes and cuvettes. Take 2 test tubes, add 1 ml of distilled water into each of two test tubes, and add to the 3-rd one 1 ml of the urine. Then pour 1 ml of 2,5% KOH alcoholic solution into every test tube, mix the content of all test tubes for 1 minute, pour 0,5 ml of 0,1% 2,4-dinitophenilhydrazine solution into each of them. Mix and let them stay for 15 minutes on the table. After that the optical density of a test sample is measured against control test in cuvettes (5 mm) using a blue colour filter. The content of PA (mcg/ml) is determined using the graph (A).

Calculation by the formula:

[PA] mg/day= A • 1,5 (or 1,2), where

A - index of PA according to the graph;

1,5 (or 1,2) - the factor that correlates with diuresis for men or for women.

Normal PA content in the urine is 10-25 mg / day (113,7-283,9 μmol/day).

RESULTS:

CONCLUSIONS:

Clinical significance:

A large quantity of PA is accumulated in blood plasma and is excreted with urine during B_1 hypovitaminosis in human organism. The content of this acid increases in the urine during diabetes mellitus, cardiac insufficiency, pituitary-

adrenal system superstimulation. The quantity of pyruvic acid increases during the drugs treatment: camphor, strychnine, adrenalin.
The content of pyruvic acid reduces during anesthesia.

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. A child's blood presents high content of galactose, glucose concentration is low. There are such presentations as cataract, mental deficiency, adipose degeneration of liver. What disease is it?
A. Diabetes melluitis
B. Steroid diabetes
C. Fructosemia
D. Lactosemia
E. Galactosemia

2. A child has got galactosemia. Concentration of glucose in blood has not considerably changed. Deficiency of what enzyme caused this illness?
A. Phosphoglucomutase
B. Amylo-l,6-glucosidase
C. Galactokinase
D. Galactose-1-phosphate uridyltransferase
E. Hexokinase

3. Galactosemia has been revealed in a child. Concentration of glucose in the blood has not considerably changed. What enzyme deficiency caused this illness?
A. Galactose-1-phosphate uridyltransferase
B. Amylo-1,6-glucosidase
C. Phosphoglucomutase
D. Galactokinase
E. Hexokinase

Literature (p. 78)

Lesson 10

Theme: *METABOLISM OF POLYSACCHARIDES AND ITS REGULATION. CARBOHYDRATE METABOLIC PATHWAYS REGULATION. PATHOLOGIES OF CARBOHYDRATE METABOLISM*

RELEVANCE OF THE TOPIC: carbohydrates, along with proteins, nucleic acids, and other substances, are considered essential components of cells. In animal tissues, the proportion of carbohydrates is relatively small compared to proteins, for example, but their physiological importance is significant due to the various functions they serve (energetic, structural, protective, etc.). Changes in carbohydrate metabolism are objectively reflected in alterations in the concentration of carbohydrates (such as glucose and glycogen) and their

metabolites, as well as changes in the activity of carbohydrate metabolism enzymes in biosubstrates. These indicators are used in the diagnosis of various diseases.

THE PURPOSE OF THE LESSON: to study the specifics of carbohydrate metabolism under normal and pathological conditions. Be able to explain the results of laboratory practical work using theoretical knowledge on the topic of the session.

QUESTIONS FOR PREPARATION

1. Structure and biological role of polysaccharides (glycogen, glycosaminoglycans).
2. Glycogen biosynthesis (glycogenesis): chemistry, key enzymes, physiological significance.
3. Phosphorolytic breakdown of glycogen in the liver and muscles (glycogenolysis).
4. The role of adrenaline, glucagon, and insulin in regulating glycogen metabolism in muscles and the liver. Mechanisms of cAMP-dependent regulation of glycogen phosphorylase and glycogen synthase activities.
5. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis.
6. Genetic disorders of glycogen metabolism enzyme function (glycogen storage diseases, glycogenoses).
7. General concepts of glycosaminoglycan metabolism.
8. Effects and mechanisms of action of glucagon, adrenaline, glucocorticoids, growth hormone, and insulin on blood glucose levels.
9. Normal blood glucose levels and their disturbances (hyperglycemia, hypoglycemia). Glucosuria.
10. Clinical and biochemical characteristics of diabetes mellitus (insulin-dependent and non-insulin-dependent types).
11. Diagnosis of latent diabetes using glycated hemoglobin concentration measurement and the oral glucose tolerance test (Staub-Traugott test, double sugar load method).
12. Mucopolysaccharidoses: genetic disorders of glycosaminoglycan metabolism.

LABORATORY WORK

Determination of glucose content in the blood serum (Glucose oxidase method)

Check up your home preparation using the tests:

1. Name the metabolic process, the rate of which is reduced in insulin-dependent diabetes::

A. Absorption of glucose by tissues
B. Glycogenolysis
C. Gluconeogenesis
D. Proteolysis

E. Lipolysis

2. An elderly woman developed cataracts due to diabetes. Name the process, the stimulation of which causes clouding of the lens:

A. Glycosylation of proteins
B. Proteolysis of proteins
C. Ketogenesis
D. Lipolysis
E. Gluconeogenesis

3. In the patient's blood, the fasting glucose was 5.55 mmol/l, 1 hour after the sugar load – 8.55 mmol/l, and 2 hours later – 4.95 mmol/l. Such indicators are typical for:

A. A healthy person
B. A patient with thyrotoxicosis
C. A patient with a hidden form of diabetes
D. A patient with insulin-dependent diabetes mellitus
E. A patient with non-insulin-dependent diabetes mellitus

4. In a child of the first year of life, an increase in the liver and kidneys, growth retardation, convulsions (as a result of hypoglycemia) were detected. Further research showed the absence of the enzyme glucose-6-phosphatase. Select the type of glycogenosis associated with a hereditary defect in the synthesis of this enzyme:

A. Bitter's disease
B. Pompe's disease
C. Andersen's disease
D. McArdle's disease
E. Thomson's disease

5. Point the enzyme whose deficiency can cause Gierke's disease development:

A. Alpha - 1,4 -glycosidase
B. Amylo- 1,6 -glycosidase
C.Glycogen-branching enzyme
D. Glucose-6-phosphatase
E. Glycogen phosphorylase

6. Point the process that is activated in the liver first of all during essential hyperglycemia in patient:

A. Gluconeogenesis
B. Glycogenolysis
C. Glycogen synthesis
D.Pentose Phosphate Cycle
E.Glucoseaminoglycans synthesis

7. How glucocorticoids influence the carbohydrates metabolism?

A. Stimulate the glycolysis from glucose
B. Stimulate the gluconeogenesis
C. Stimulate the starch hydrolysis in the small intestine
D. Inhibit the glycogen phosphorolysis
E. Stimulate the glycogenesis

8**. Point the key enzyme of glycogen degradation in the liver:**

A.Fructose-1,6-diphos-phatase
B. Glycogen Phosphorylase
C.Glyceraldehyde-3-phos-phatase
D. Glucose -6-phosphatase
E. Glucose oxidase

9. **How does adrenalin influence the glucose level in the blood?**

A. Increases, stimulating the glycogen destruction
B. Decreases, stimulating the gluconeogenesis
C. Does not influence
D. Decreases, inhibiting the glycogen synthesis
E. Decreases, inhibiting the glycolysis

10. Name the most important state associated with the beginning of disorder – diabetes mellitus:
A. Hypoglycemia
B. Hyperglycemia
C. Cholecystitis
D. Steatorrhea
E. Azotemia

11. The patient complains of increased fatigue, constant thirst. The doctor's preliminary diagnosis is diabetes. Select the blood plasma glucose concentration value that confirms this diagnosis:
A. 8.5 mmol/l
B. 2 mmol/l
C. 4.5 mmol/l
D. 5 mmol/l
E. 3.3 mmol/l

12. With a chronic overdose of glucocorticoids, the patient develops hyperglycemia. Name the process of carbohydrate metabolism due to which the concentration of glucose increases:
A. Gluconeogenesis
B. Glycogenolysis
C. Glycogenesis
D. Aerobic glycolysis
E. Pentose phosphate cycle

13. Laboratory examination revealed excessive accumulation of glycogen in the patient's liver. Specify the name of the disease in which it is observed:
A. Bitter's disease
B. Addison's disease
C. Maple syrup disease
D. Down's disease
E. Botkin's disease

14. Specify the enzyme whose hereditary absence is the cause of fructosemia:
A. Fructokinase
B. Phosphofructokinase
C. Hexokinase
D. Glucokinase
E. Pyruvate kinase

15. The concentration of which substance should be determined in the blood plasma of a patient with type I glycogenosis:
A. Glucose
B. Fructose
C. Galactose
D. Alanine
E. Uric acid

Protocol N 10 **Date**_____

Determination of glucose content in the blood serum (Glucose oxidase method)

THE PRINCIPLE OF THE METHOD:

Glucose is oxidized at the presence of glucose oxidase up to gluconic acid and hydrogen peroxide. The hydrogen peroxide is formed during the reaction due to air oxygen. Under the peroxidase action the hydrogen peroxide transforms to a pink or red coloured complex. This complex is obtained with reagents: phenole and 4 - amino-phenazone. This colouring is proportional to the glucose content.

THE COURSE OF THE WORK:

Put 1ml of solution N1 into two test tubes. Add into the 1-st test tube 0,02 ml 0,9% sodium chloride solution and into the 2-d - 0,02 ml of blood serum. Mix and leave test tubes on the table for 20 minutes. The optical density is estimated for the 2-d test tube (experimental) against the 1-st one in cuvettes (3 mm thick layer) with yellow colour filter.
The glucose concentration is determined using the graph curve.
RESULTS:

CONCLUSIONS:

Clinical significance:
Normal concentration of glucose in serum (plasma) is 4, 22 - 6,11 mmole/L.(due to this method determination)
The increase of glucose concentration in blood (more then 6,11 mmole/L) is called hyperglycemia state. It is observed at the following conditions:
1) After plentiful reception with food, containing carbohydrates - alimentary hyperglycemia;
2) A diabetes, a sharp pancreatitis, pancreatic cirrhoses (it is connected to the lack of insulin in organism);
3) Hyperfunction of a thyroid gland, adrenal glands, hypophysis;
4) Strong emotional and mental excitation;
5) Toxic, traumatic, mechanical irritation CNS: trauma, tumor of a brain, epilepsy, meningitis, poisoning by carbon monoxide, a hydrocyanic acid, an ether, mercury is accompanied so-called central (nervous) hyperglycemia.
A decrease of glucose levels up to 2,5-2,8 mmole/L is named hypoglycemia state. It takes place at:
1) Starvation, an unbalanced diet - hypoglycemia;
2) The infringement of carbohydrates digestion and absorption due to diseases of the thin intestine;
3) The high dose of insulin at the treatment of diabetes;
4) The disease of kidneys with the pathology of the renal tubules reabsorbtion;
5) The intimate insufficiency (sometimes);
6) The decrease of hormonal secretion for glucocorticoids, glucagon.
7) Poisoning with phosphorus, benzene, chloroform;
8) The big loss of blood;
9) Hyperfunction of β-cells of pancreas.

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. Patient with diabetes mellitus experienced loss of consciousness and convulsions after an injection of insulin. What might be the result of biochemical blood analysis for concentration of sugar?
A. 3,3 mmole/L

B. 10 mmole/L
C. 8,0 mmole/L
D. 1,5 mmole/L
E. 5,5 mmole/L

2. A patient was delivered to the hospital by an emergency team. Objectively: grave condition, unconscious, adynamy. Cutaneous surfaces are dry, eyes are sunken, face is cyanotic. There is tachycardia and smell of acetone from the mouth. Analysis results: blood glucose – 20,1 mmole/l (standard is 3,3-5,5 mmole/l), urine glucose – 3,5% (standard is – 0). What is the most probable diagnosis?

A. Anaphylactic shock
B. Hypoglycemic coma
C. Acute heart failure
D. Acute alcoholic intoxication
E. Hyperglycemic coma

3. A 62-year-old female patient has developed a cataract (lenticular opacity) secondary to the diabetes mellitus. What type of protein modification is observed in case of diabetic cataract?

A. Glycosylation
B. Phosphorylation
C. ADP-ribosylation
D. Methylation
E. Limited proteolysis

4. A 3 year old child with fever was given aspirin. It resulted in intensified erythrocyte haemolysis. Hemolytic anemia might have been caused by congenital insufficiency of the following enzyme:

A. Glycogen phosphorylase
B. Glucose 6-phosphatase
C. Glucose 6-phosphate dehydrogenase
D. Glycerol phosphate dehydrogenase
E. γ-Glutamiltransferase

5. A patient is ill with diabetes mellitus that is accompanied by hyperglycemia of over 7,2 millimole/l on an empty stomach. The level of what blood plasma protein allows to estimate the glycemia rate retrospectively (4-8 weeks before examination)?

A. Glycated hemoglobin
B. Albumin
C. Fibrinogen
D. C-reactive protein
E. Ceruloplasmin

6. On the empty stomach in the patients blood glucose level was 5,65 mmol/L, in an hour after usage of sugar it was 8,55 mmol/L, in a 2 hours – 4,95 mmol/L. Such indicators are typical for:

A. Healthy person

B. Patient with hidden diabetes mellitus
C. Patient with insulin-dependent diabetes mellitus
D. Patient with non-insulin dependent diabetes mellitus
E. Patient with tireotoxicosis

Literature (p. 78)

Lesson 11
THEME: LIPOPROTEINS OF BLOOD PLASMA. METABOLISM OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS.

RELEVANCE OF THE TOPIC: the importance of lipids in animal organisms cannot be overstated. They are diverse compounds with various physical and chemical properties, and they play multiple biological roles, including energy storage, structural components, and acting as sources of fat-soluble vitamins, among others. Several human diseases, such as hypertension, ischemic heart disease, strokes, type 2 diabetes, obesity, are, in part, linked to imbalances in lipid content (cholesterol, triglycerides) and plasma lipoproteins (HDL, LDL). The high prevalence of these pathologies in developed countries underscores the importance of future doctors thoroughly studying the causes of dyslipoproteinemias (abnormal percentages of different lipoprotein fractions in the blood plasma) and especially their diagnosis based on blood plasma indicators.

THE PURPOSE OF THE LESSON: to study the general principles of transport and metabolism of triacylglycerols, glycerophospholipids in the human body. Be able to determine the level of HDL cholesterol in blood serum.

QUESTIONS FOR PREPARATION
1. Structure, classification, and biological functions of lipids.
2. Daily requirements and digestion of lipids. Enzymes involved in lipid digestion: localization of synthesis and activation of enzymes, pH optimum, and specificity of action of the active form of enzymes.
3. The role of bile acids in lipid digestion and absorption. Steatorrhea: types (pancreatic, hepatic, enterogenic), causes, diagnosis.
4. Plasma lipoproteins - transport forms of lipids: their classification, chemical composition, functions, and metabolism.
5. Catabolism of triacylglycerols in adipocytes of adipose tissue (lipolysis), sequence of reactions. Neurohumoral regulation of lipolysis by adrenaline, noradrenaline, glucagon, and insulin. Oxidation of glycerol (enzymatic reactions, energy effects of the process).

6. Mechanism and biological role of triacylglycerol synthesis in enterocytes of the intestine, in the liver, and in adipose tissue.
7. Lipolysis of glycerophospholipids in cells: localization and specific actions of phospholipases A_1, A_2, C, and D.
8. Biosynthesis of glycerophospholipids using phosphatidylcholine as an example. The role of the active form of methionine in phosphatidylcholine synthesis.

LABORATORY WORK
Determination of β-lipoproteins (LDL) content in the blood serum

Check up your home preparation using the tests:

1. Specify which enzyme is involved in the formation of lysophospholipids, which have a strong hemolytic effect:
A. Phospholipase C
B. Phospholipase A_1
C. Triglyceride lipase
D. Phospholipase A_2
E. Phospholipase D

2. Select enzymes that break down phospholipids:
A. Pancreatic lipase
B. Monoglyceridlipase
C. Lysophospholipase
D. Intestinal lipase
E. Phospholipases A_1, A_2, C, D

3. Specify the lipids, the transport of which is mainly provided by blood chylomicrons:
A. Endogenous triglycerides
B. Exogenous triglycerides
C. Cholesterol
D. Phospholipids
E. Cholesterol and its esters

4. Specify the hormone-sensitive regulatory enzyme of lipolysis in adipose tissue:
A. Triglyceride lipase
B. Diglyceridlipase
C. Monoglyceridlipase
D. Phospholipase
E. Cholesterolesterase

5. Select the secondary mediator involved in the activation of hormone-sensitive triglyceride lipase:
A. cGMP
B. cAMP
C. Diacylglycerol
D. Ca^{2+}
E. Inositol triphosphate

6. Choose a macroerg whose energy is used in the synthesis of triacylglycerides:
A. CTF
B. GTFS
C. ATP
D. UTF
E. ADF

7. Name the compound that is a precursor in the synthesis of phosphatidylcholine:
A. Phosphatidylethanolamine
B. Phosphatidylserine
C. Phosphatidylinositol
D. Plasmalogen
E. Cardiolipin

8. Specify the substrate from which glycerol-3-phosphate is formed in the process of triglyceride biosynthesis in adipose tissue:
A. Glyceraldehyde phosphate
B. Glycerin

C. Glyceric acid
D. Dioxyacetone phosphate
E. Pyruvic acid

9. Specify the class of lipoproteins that contain the most protein:

A. Lipoproteins of intermediate density
B. Very low density lipoproteins
C. High density lipoproteins
D. Low density lipoproteins
E. Chylomicrons

10. Name the lipoproteins that transport cholesterol to tissues:

A. Lipoproteins of intermediate density
B. Low density lipoproteins
C. Chylomicrons
D. Very low density lipoproteins
E. High density lipoprotein

Protocol N12 **Date**_____

Determination of β-lipoproteins (LDL) content in the blood serum

THE PRINCIPLE OF THE METHOD:

β-Lipoproteins (LDL) precipitate in the presence of calcium chloride and heparin: the turbidity is appeared. It is explained that heparin can form with β-lipoproteins a complex, which is precipitated in the presence of calcium chloride. The concentration of β-lipoproteins in the blood serum correlates with the rate of turbidity.

THE COURSE OF THE WORK:

Pour 2 ml of 0.27 % calcium chloride solution and 0.2 ml of blood serum in a test tube, mix. Determine the optical density of this solution (E_1) against 0.27 % of calcium chloride solution at red color filter in cuvettes (5 mm thick layer). A solution from experimental ditch pour in a test tube, add 0.04 ml of 1 % heparin solution, mix and exactly (in 4 minutes) determine the optical density of this one again (E_2) with the same conditions. Calculate the difference between the optical densities and multiply it on 10 (empirical coefficient). Calculate LDL content according to the formula:

$X= (E_2 - E_1) \cdot 10$, where

X - concentration of LDL in the blood serum, g/l;
E_1 - optical density of experimental sample before heparin adding;
E_2 - optical density of experimental sample after heparin adding;
10 – recalculation coefficient.

RESULTS:

CONCLUSIONS:

<u>Clinical significance:</u>

The content of beta-lipoproteins in the blood serum is normal when it equals 3,0-4,5 g/l.

The increased β-lipoproteins content is observed at states: hyperlipoproteinemia: such types as II a, II b, III (Fredrikson E., at all classification), which correlates with the increase of the total cholesterol content in the blood plasma. The specified conditions promote the

development of atherosclerotic damages of the vessels at patients with a hypertension, myocardial ischemia (MI) or at diseases, which are accompanied with development of secondary hyperlipoproteinemia: diabetes (obvious and the latent form): hypothyroid edema, nephritis syndrome, chronic kidney insufficiency.

Literature (p. 78)

Lesson 12

THEME: HIGH FATTY ACIDS AND KETONE BODIES METABOLISM

RELEVANCE OF THE TOPIC: high fatty acids serve as a substrate for the synthesis of triglycerides, phospholipids (including biological membranes), and also play the role of an energy reserve. Disorders in the metabolism of high fatty acids are observed in obesity and diabetes.

THE PURPOSE OF THE LESSON: to study the theoretical principles of high fatty acids metabolism and its regulation. To be able to determine ketone bodies in blood plasma.

QUESTIONS FOR PREPARATION

1. Beta-oxidation of high fatty acids (HFA) of saturated and unsaturated series. The role of carnitine in the transport of fatty acids from the cytoplasm to the mitochondria.
2. The energy value of beta-oxidation of HFA in cells (for stearic and oleic acids).
3. Biosynthesis of high fatty acids. Characteristics and functions of acetyl-CoA carboxylase and palmitate synthase complex. Regulation of the process.
4. Biosynthesis of monounsaturated high fatty acids in the human body.
5. Ketone bodies. Biosynthesis and utilization reactions of ketone bodies: localization in the body, biological significance. Ketonemia and ketonuria in diabetes, fasting.

LABORATORY WORK

The qualitative tests for ketone bodies

Check up your home preparation using the tests:

1. Name the blood proteins that transport fatty acids:

A. Globulins
B. Hemoglobin
C. Albumins
D. α-Lipoproteins
E. β-Lipoproteins

2. Specify the location of the process of β-oxidation of fatty acids in the cell:

A. Core
B. Cytosol
C. Mitochondria
D. Lysosomes

E. Golgi apparatus

3. Name the vitamin-like substance involved in the transport of fatty acids from the cytoplasm to the mitochondria:

A. Coenzyme A
B. Carnitine
C. C. Biotin
D. Pantothenic acid
E. Folic acid

4. Indicate by how many carbon atoms the carbon chain of higher fatty acids becomes shorter during one cycle of β-oxidation:

A. 3
B. 4
C. 2
D. 1
E. 05.

5. Select an additional enzyme necessary for the oxidation of unsaturated fatty acids:

A. $\Delta^{3,4}$-cis-$\Delta^{2,3}$-trans-enoyl~CoA–isomerase
B. Acyl~CoA dehydrogenase
C. Enoyl~CoA hydratase
D. Oxyacyl~CoA dehydrogenase
E. Thiolase

6. Specify the final product of β-oxidation of fatty acids with an odd number of carbon atoms:

A. Succinyl~CoA
B. Acetyl~CoA
C. Acetoacetyl~CoA
D. Propionyl~CoA
E. Oxymethylglutaryl~CoA

7. Name the representative of ketone bodies in the body:

A. Acetic acid
B. B. Butyric acid
C. Palmitic acid
D. Oleic acid
E. Acetoacetic acid

8. Specify the place of synthesis of ketone bodies in the body:

A. Liver
B. Kidneys
C. Muscles
D. Pancreas
E. Lungs

9. Name the product formed by the condensation of two acetyl-CoA molecules during the biosynthesis of ketone bodies:

A. Oxybutyrate
B. Acetoacetate
C. Acetone
D. Succinyl-CoA
E. Acetoacetyl-CoA

10. Select the pathology in which ketonemia is observed in the body:

A. Myocardial infarction
B. Atherosclerosis
C. Diabetes mellitus
D. Rheumatism
E. Acute viral infections

Protocol N12 **Date**_____

1. The qualitative tests for ketone bodies:

1.1. Liben`s test

THE PRINCIPLE OF THE METHOD:

Acetone reacts with iodine turning into iodoform in the presence of alkali. The formation of it is recognized by a specific odour.

THE COURSE OF THE WORK:

Add 5-6 drops of 10% NaOH solution and 3-4 drops of Lugol's reagent to 1 ml of acetone solution. Iodoform will be formed. In case of large quantity of acetone in the urine the crystalline precipitate of iodoform may be formed.
RESULTS:

CONCLUSIONS:

1.2. Legal's reaction
THE PRINCIPLE OF THE METHOD:
In alkaline environment acetone and acetoacetic acid form an orange-red colour complex with sodium nitroprusside. After adding of glacial acetic acid (100% solution) a cherry-colored compound will be formed.
THE COURSE OF THE WORK:
Pour 1 ml of acetone into a test-tube, add some drops of 10% NaOH solution and then pour some drops of fresh sodium nitroprusside solution. The red colouring will appear. The intensity of colouring grows due to the addition of acetic acid.
RESULTS:

CONCLUSIONS:

Clinical significance of these reactions : They are used usually for the determination of ketone bodies in the urine of patients at long time starvation, in severe form of diabetes mellitus, in patients with high rate of tissue lipolysis.

Literature (p. 78)

Lesson 13
THEME: CHOLESTEROL METABOLISM. THE REGULATION AND DISORDERS OF LIPID METABOLISM: OBESITY, ATHEROSCLEROSIS

RELEVANCE OF THE TOPIC: knowledge of the peculiarities of cholesterol metabolism and methods for its determination in the blood holds significant practical importance for future specialists, enabling the diagnosis of various conditions such as ischemic heart disease, hypertension, and others.

THE PURPOSE OF THE LESSON: to study the peculiarities of cholesterol and bile acid metabolism in the norm. Be able to apply theoretical knowledge to explain the results of lipid metabolism tests as diagnostic indicators.

QUESTIONS FOR PREPARATION

1. Biosynthesis of cholesterol: localization, initial substrates, reaction scheme, regulation of the process.
2. Biotransformation pathways of cholesterol, localization in the body: Esterification; formation of bile acids, steroid hormones, active forms of vitamin D_3.
3. Biochemical mechanisms of atherosclerosis development. Atherogenicity coefficient. Atherogenic and antiatherogenic lipoproteins.
4. Disruptions in lipid metabolism in obesity, diabetes.
5. Genetic disorders in phospholipid metabolism: sphingolipidoses.

LABORATORY WORKS

The determination of total cholesterol content in the blood serum (Ilk`s method)

Check up your home preparation using the tests:

1. Point the key enzyme of cholesterol synthesis:

A. Acetyl~SCoA carboxylase
B.β-Hydroxybutyryl dehydrogenase
C.β-Hydroxy-β-methylglutaryl-CoA reductase
D. Palmitate synthetase
E. Malonyl~SCoA-ACP transferase

2. Point the drug used for the decrease of cholesterol level in the blood of patients - allosteric inhibitor for key enzyme of cholesterol synthesis:

A. Aspirin
B. Lovastatin
C. Barbiturate
D. Indomethacin
E. Antimicin A

3. Point the substance that decreases the rate of cholesterol synthesis:

A. Adrenalin
B. Thyroxin
C. Cholesterol
D. Phosphate
E. Glucose

4. Find the coenzyme used in some reactions of cholesterol synthesis:

A. $FADH_2$
B. NAD
C. Pyridoxal phosphate
D. NADPH
E. Biotin

5. Find the substance synthesized from cholesterol in human organism:

A. Cortisol
B. Aldosterone
C. Calcitriol
D. Lipoic acid
E. The positions A, B, C above are right

6. Find the product of cholesterol transformation in the liver whose content is important for lipids digestion duration in the small intestine:

A. Glycocholic acid
B. Butyric acid
C. Acetone
D. Acetic acid
E. Taurine

7. Find the enzyme system that is used for bile acids formation from cholesterol:
A. Acetyl~SCoA carboxylase
B. β-Hydroxybytyryl dehydrogenase
C. Acetoacetyl-CoA reductase
D. 7-Monooxygenase cytochrome P_{450} -linked system
E. Malonyl~SCoA-ACP transferase

8. Find the vitamin derivative that is synthesized from chole-sterol in humans:
A. Progesterone
B.1,25-dihydroxy cholecalci-ferol
C. Estradiol
D. Cholesterol ester
E. Testosterone

9. Choose the substance, whose level is increased in the blood serum of patient with atherosclerosis of blood vessels:
A. Carnitine
B. Albumins
C. High fatty acids
D. Cholesterol
E. Hemoglobin

10. Name lipoproteins whose content is in need to determine in the blood plasma of patient with atherosclerosis of blood vessels:
A. VLDL
B. LDL
C. HDL
D. Chylomicrons
E. The positions A, B, C above are right

11. Name the compound from which cholesterol is synthesized in the body:
A. Crotonil-CoA
B. Palmytil-CoA
C. Oxybutyryl-CoA
D. Acetyl-CoA
E. Butyryl-CoA

12. Choose in which organ cholesterol synthesis is most actively carried out:
A. Kidneys
B. Liver
C. Intestine
D. Adrenal cortex
E. Reproductive organs

13. Specify the functions of cholesterol in the human body:
A. Mandatory component of biological membranes
B. Bile acids are synthesized from cholesterol
C. Precursor of corticosteroids, sex hormones
D. Precursor of vitamin D_3
E. All the mentioned functions

14. Specify the compound formed after the condensation of three acetyl-CoA molecules and subsequent reduction in the process of cholesterol synthesis:
A. Mevalonic acid
B. Butyric acid
C. Oxymethylglutaryl-CoA
D. Fumaric acid
E. Citric acid

15. Specify the final product into which mevalonic acid is converted in the second stage of cholesterol synthesis:
A. Lanosterol
B. Isoprene
C. Farnesyl pyrophosphate
D. Squalene
E. Geranyl pyrophosphate

16. Name the regulatory enzyme of the cholesterol synthesis process:
A. Acetyl-CoA-acetyltransferase
B. Oxymethylglutaryl-CoA reductase
C. Oxymethylglutaryl-CoA synthetase
D. Acetyl-CoA-carboxylase

E. Thiolase

17. The main end product of cholesterol metabolism in the liver is:

A. Vitamin D_3
B. Hippuric acid
C. Animal indicant
D. Bile acids
E. Skatol

18. Select the transport form of cholesterol from the tissues to the liver:

A. Very low density lipoproteins
B. Chylomicrons
C. Low density lipoproteins
D. High density lipoproteins
E. Lipoproteins of intermediate density

19. Name the products that are not formed during cholesterol catabolism:

A. CO_2 and H_2O
B. Bile acids
C. Vitamin D_3
D. Corticosteroids
E. Sex hormones

20. Choose the process that changes with a large intake of cholesterol with food:

A. The synthesis of endogenous cholesterol is accelerated
B. Cholesterol catabolism to CO_2 and H_2O is activated
C. Cholesterol synthesis in the liver decreases
D. The activity of oxymethylglutaryl-CoA reductase increases
E. The activity of oxymethylglutaryl-CoA synthetase decreases

Protocol N 13 **Date**_____

The determination of total cholesterol content in the blood serum (Ilk`s method)

THE PRINCIPLE OF THE METHOD:

Cholesterol (CHL) at the presence of reagent N 1 (a mixture of acetic anhydride, acetic and sulfuric acids) will have a green colouring (this is Lieberman-Burhardt reaction to prove the presence of cholesterol in the medium). The intensity of colouring is proportional to the cholesterol concentration.

THE COURSE OF THE WORK:

Use the reagent N1 very carefully in ventilation system only!

Prepare reactive solution according to scheme:

Add (in ml)	N1 (test sample)	N2 (control sample)
Blood serum	0, 05	—
0, 9% NaCl sol-n	--	0, 05
Reagent N1	1, 2	1, 2

Pour 0.05 ml of blood serum on the bottom of a dry test tube, then add reagent N1. Shake up the content of the test tube quickly and vigorously for 10-12 times (in ventilation system), close and leave them for 20 minutes in the thermostat (37^{o}C). The green colouring will appear. If there is the sediment in the test tube you have to centrifuge them. Measure the optical density at red colour filter (630-690 nm) in cuvettes (3 mm) against control solution (reagent N1). Use the obtained value to find out the content of cholesterol by calibration graph. Multiply the cholesterol index using the factor of conversion into SI units (mmol/l) - 0.0258. The total cholesterol content (free and esters) in the blood serum of healthy adults varies within 2.97-6, 46 mmol/l.

RESULTS:

CONCLUSIONS:

Clinical significance:

At newborns the total CHL concentration is very low (< 2,6 mmol/l), and till 10 years does not increase (usually 4,1 mmol/l). Then CHL concentration will grow in the early period of puberty. Risk for the development of myocardial ischemia (MI) considerably grows at adult person at CHL>5, 2 mmol/l, therefore it is more preferable to estimate the concentration ratio concerning the ideal for given one individually. In the domestic literature a range of norm is in wider limits (2,6 - 7,6 mmol/l), than in the foreign literature (2,6-6,5 mmol/l).

Hypercholesterinemia is observed at patients with a hypertension, MI, diabetes mellitus, obesity, hypothyroid edema, nephritis syndrome, kidney's insufficiency, cholestase, and also at some infringements of lipid exchange. Hypercholesterinemia accompanies hyperlipoproteinemia: such types as IIa, II b, III, IV and V.

Hypocholesterinemia is observed at parenchyma damages of the liver, starvation, tuberculosis, hyperthyreosis, cancer of some organs, the infringement of CNS function.

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. Examination of an ill child's blood revealed inherited hyperlipoproteinemia. Genetic defect of what enzyme synthesis causes this phenomenon?

A. Lipoprotein lipase
B. Glycosidase
C. Proteinase
D. Hemsynthetase
E. Phenylalanine hydroxylase

Literature (p. 78)

Lesson 14

THEME: COMMON METABOLIC PATHWAYS FOR AMINO ACIDS. GLUCOGENOUS AND KETOGENOUS AMINO ACIDS.

RELEVANCE OF THE TOPIC: proteins, the synthesis of which primarily depends on the availability of necessary amino acids, are the most crucial component of any animal and human cell. The level of amino acids in the cell is determined by the speed of their transport through the cellular membrane and the activity of enzymes involved in amino acid transformations. Understanding the fundamental transformations of amino acids in tissues will enable future doctors to correctly utilize methods of chemical analysis of protein and amino acid compounds, explaining vital processes in both healthy and diseased organisms.

THE PURPOSE OF THE LESSON: to study the pathways of amino acid transformations in the body. Be able to determine the activity of alanine transaminase in blood plasma (aminotransferase test), which is used in clinics for diagnosing diseases, as well as for prognosis and assessing the effectiveness of treatment methods.

QUESTIONS FOR PREPARATION

1. Daily protein needs. Understanding the concepts of positive and negative nitrogen balance in the human body. Enzymes involved in protein metabolism: localization of synthesis, activation of proenzymes, optimum pH, and specificity of active enzyme forms.
2. Amino acid absorption mechanisms.
3. Free amino acid pool in the body: processes of formation and utilization of free amino acids in tissues.
4. Amino acid transamination: mechanism of action of aminotransferases, biological significance of the reaction. Clinical and diagnostic importance of determining aminotransferase activity in blood plasma.
5. Direct and indirect deamination of L-amino acids: role of oxidases and glutamate dehydrogenase in deamination.
6. Alpha-decarboxylation of L-amino acids in the human body: role of biogenic amines and their neutralization in the body.
7. Metabolism pathways of non-nitrogenous residues of amino acids in the human body: glucogenic and ketogenic amino acids.

LABORATORY WORK

The determination of alanine aminotransferase activity in the blood serum

Check up your home preparation using the tests:

1. Point the way of Amino Acids transformation that is not the common catabolic pathway:
A. Transamination
B. Transdeamination
C. α-Decarboxylation
D. Oxidative deamination
E. Hydroxylation

2. Point the liver enzyme, which takes part in second step of transdeamination of Amino Acid:
A. α-Ketoglutarate dehydrogenase
B. Glutamate dehydrogenase
C. Glutamate decarboxylase
D. L-Alanine oxidase
E. Tryptophan hydroxylase

3. Point the enzyme, whose activity is determined in the blood plasma during the unicteric period of viral hepatitis:
A. Phenylalanine hydroxylase
B. Creatine phosphokinase
C. Glutamate dehydrogenase
D. Alanine transaminase
E. Ornithine carbomoyl phoshate transferase

4. Choose the enzyme of the blood plasma, whose activity increases in ten or more times for 3-4 hours after myocardium infarction:
A. Alanine transaminase
B. Aspartate transaminase
C. Alkaline phoshatase
D. Arginase
E. Leucine aminopeptidase

5. Point the cofactor, which is used by D-Amino acid oxidase in oxidative deamination:
A. $NADP^+$
B. NAD^+
C. FAD
D. FMN
E. TPP

6. Name biogenic amine that is formed from 5-hydroxy-Tryptophan:
A. Thiamine
B. Serotonin
C. Adrenalin
D. Dopamine
E. Histamine

7. Choose the enzyme, whose genetic defect results in the GABA levels decrease in the brain (GABA – γ-Amino Butyric Acid):
A. Tryptophan decarboxylase
B. Phenylalanine hydroxylase
C. Histidine decarboxylase
D. Alanine hydroxylase
E. Glutamate decarboxylase

8. Point the vitamin, whose hypovitaminosis causes the violations in the transamination and decarboxylation of Amino Acids:
A. Vitamin C
B. Vitamin B_1
C. Vitamin B_2
D. Vitamin B_9
E. Vitamin B_6

9. Point the glucogenic amino acids:
A. Glutamate
B. Alanine
C. Serine
D. Aspartate
E. All the positions above are right

10. Point the liver enzyme catalyzing the reversible oxidative deamination:
A. Glutamate dehydrogenase
B. Alanine transaminase
C. Monoamino oxidase
D. Aspartate transaminase
E. Arginase

11. Point the class of enzymes that catalyze the digestion of proteins in gastro-intestinal tract:
A. Transferases
B. Lyases
C. Hydrolases
D. Oxidoreductases
E. Ligases

12. Point the group of peptidases which trypsin is belong to:
A. Amino peptidase
B. Exopeptidase
C. Endopeptidase
D. Dipeptidase
E. Carboxypeptidase

13. Point the value of pH, which is optimal for the activity of pepsin:
A. 6.8-7.2
B. 3.5-4.8
C. 7.2-8.5
D. 1.5-2.5
E. 8.5-10.0

14. Point the couple of amino acids participating in the formation of peptide bond that is cleaved by trypsin:
A. Arginine, lysine
B. Leucine, valine
C. Glycine, Glutamine
D. Alanine, valine
E. Isoleucine, alanine

15. Point the endopeptidase that is produced by pancreas and is activated by trypsin:
A. Proelastase
B. Renin
C. Pepsinogen
D. Gastricsin
E. Alpha-Amylase

16. Find the values for total acidity associated with hypochlorhydria in patient:
A. 40 mmol/L
B. 50 mmol/L
C. 60 mmol/L
D. 20 mmol/L
E. 55 mmol/L

Protocol N14 **Date**_____

The determination of alanine aminotransferase activity in the blood serum

THE PRINCIPLE OF THE METHOD:

Glutamate and pyruvate are formed under the action of AlAT from a-ketoglutarate and alanine. Pyruvate can act with 2.4-dinitrophenylhydrazine to produce dinitrophenylhydrazone of a brown colour. The intensity of colouring is proportional to the quantity of pyruvic acid released during the reaction.

THE COURSE OF THE WORK:

Prepare reactive solutions according to scheme:

Add, in ml	**Test sample**	**Control sample**
Substrate-buffer solution	0,5	0,5
Incubation in a dry-air thermostat at 37°C for 3 min		
Stop reagent	-	0,5
Blood serum	0,1	0,1

Incubation in a dry-air thermostat at 37°C for 30 min		
Stop reagent	0,5	-
Let them stay at room temperature for 20 min		
0,4 N NaOH	5	5
Let them stay at room temperature for 10 min		

Measure the optical density of experimental test against control one in cuvettes (10mm) at green filter.
Calculation of enzyme activity in the blood serum is made by the graph. Alanine aminotransferase activity of the blood serum at healthy people equals 5-30 units/ml (0.1-0.7 μmol/ml) · 30 min.
RESULTS:

CONCLUSIONS:

Clinical significance:
Aminotranferases - enzymes, which are intermolecular transfers of amine-group from amino acid to ketoacid (α-ketoglutarate). The most important is the estimation of aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) activities.
Determination of the activity of AlAT and AsAT is widely used for diagnostics of heart and liver diseases. AlAT activity increases at Botkin's disease (before the preicteric period). As a rule, the activity change reflects up severity of hepatic parenchyma lesion. AlAT activity increases during exacerbation of chronic hepatitis, during toxic lesion of hepatic parenchyma. AsAT activity increases as early as in 4-6 hours after the attack of acute paroxys pains in myocardium and is high during 3-7 days. Therefore the indication of two serum aminotransferase activities is very important test. Normally a ratio of two activities AsAT/AlAT (de Ritis's factor) equals 1.33±0.42.This factor grows considerably at severe infarction of myocardium, and it is decreased at patients with viral hepatitis up to value 0,8.

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. In course of histidine catabolism a biogenic amine is formed that has powerful vasodilatation effect. Name it:
A. Noradrenalin
B. Dioxyphenylalanine
C. Serotonin
D. Dopamine
E. Histamine

2. Glutamate decarboxylation results in the formation of inhibitory transmitter in CNS. Name it:
A. Glutathione

B. Gamma amino butyric acid
C. Serotonin
D. Histamine
E. Asparagine

3. According to clinical indications a patient was administered pyridoxal phosphate. What process is this medication intended to correct?

A. Deamination of purine nucleotide
B. Synthesis of purine and pyrimidine bases.
C. Transamination and decarboxylation of amino acids
D. Protein synthesis
E. Oxidative decarboxylation of ketoacids

4. A patient diagnosed with carcinoma of bowels was admitted to the hospital. Analysis revealed high production of serotonin. It is known that this substance is formed of tryptophan amino acid. What biochemical mechanism underlies this process?

A. Formation of paired compounds
B. Decarboxylation
C. Transamination
D. Microsomal oxidation
E. Desamination

5. A newborn child has convulsions that have been observed after prescription of vitamin B_6. This most probable cause of this effect is that vitamin B_6 is a component of the following enzyme:

A. Glutamate decarboxylase
B. Pyruvate dehydrostase
C. Netoglubarate dehydromine
D. Aminolevulinate synthase
E. Glycogen phosphorylase

6. During hypersensitivity test a patient got subcutaneous injection of an antigen which caused reddening of skin, edema, pain as a result of histamine action. This biogenic amine is generated as a result of transformation of the following histidine amino acid:

A. Decarboxylation
B. Methylation
C. Phosphorylation
D. Isomerization
E. Deamination

7. A patient complained about dizziness, memory impairment, periodical convulsions. It was revealed that these changes were caused by a product of decarboxylation of glutamic acid. Name this product:

A. GABA
B. Pyridoxal phosphate
C. TDP
D. ATP

E. THFA

8. A patient presents with dysfunction of cerebral cortex accompanied by epileptic seizures. He has been administered a biogenic amine synthesized from glutamate and responsible for central inhibition. What substance is it?

A. Gamma-amino butyric acid
B. Serotonin
C. Dopamine
D. Acetylcholine
E. Histamine

9. Pharmacological effects of antidepressants are connected with inhibition of an enzyme catalyzing degradation of biogenic amines noradrenaline and serotonin in the mitochondria of cerebral neurons. What enzyme participates in this process?

A. Monoamine oxidase
B. Transaminase
C. Decarboxylase
D. Peptidase
E. Lyase

10. A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B_6 concentration. The infant presents with pellagra-like dermatitis, convulsions, anemia. Convulsions development might be caused by the disturbed formation of:

A. Dopamine
B. Histamine
C. Serotonin
D. DOPA
E. GABA

11. It is known that the monoamine oxidase (MAO) enzyme plays an important part in the metabolism of catecholamine neurotransmitters. In what way this enzyme inactivates these neurotransmitters (norepinephrine, epinephrine, dopamine)?

A. Oxidative deamination
B. Carboxylation
C. Addition of an amino group
D. Removal of a methyl group
E. Hydrolysis

Literature (p. 78)

Lesson 15
Theme*:*
WAYS FOR AMMONIA UTILIZATION. SPECIFIC PATHWAYS FOR AROMATIC AND SULFUR-CONTAINING AMINO ACIDS.

RELEVANCE OF THE TOPIC: future specialist needs to understand the pathways of ammonia detoxification and the transformation of specific amino acids. Disorders in ammonia utilization pathways and genetic enzymopathies in the metabolism of certain amino acids lead to the development of diseases associated with changes in the physical and mental state of the person.

THE PURPOSE OF THE LESSON: to study the main pathways of ammonia utilization in the body. Be able to interpret the biochemical peculiarities of the metabolism of individual amino acids under conditions of molecular pathologies. Be capable of determining the urea content in blood plasma and explaining the obtained result.

QUESTIONS FOR PREPARATION
1. Pathways of ammonia formation and detoxification in the body (mainly in the brain, liver, and kidneys).
2. Urea biosynthesis: sequence of enzymatic reactions, genetic anomalies of enzymes in the urea formation cycle.
3. Metabolism of phenylalanine and tyrosine: their metabolic pathways and genetic disorders.
4. Tryptophan metabolism and Its disorders.
5. Cysteine and methionine metabolism: structure, biosynthesis, and functions of glutathione in the human body.
6. Features of valine, leucine, isoleucine metabolism and their genetic disorders.
7. Glycine metabolism: synthesis of glutathione and its function in the human body.

LABORATORY WORK
The determination of urea content in the blood serum

Check up your home preparation using the tests:

1. After processing the baby's urine with $FeCl_3$ solution, a green color appears. Indicate which amino acid metabolism disorder it corresponds to:
A. Histidine
B. Cysteine
C. Phenylalanine
D. Glutamine
E. Lysine

2. Specify the enzyme whose hereditary defect is the cause of phenylketonuria:
A. Tyrosinase

B. Aspartate aminotransferase
C. Phenylalanine hydroxylase
D. Hexokinase
E. Pyruvate decarboxylase

3. Specify the regulatory enzyme of the ornithine cycle of urea formation:
A. Ornithine decarboxylase
B. Citrulline synthetase
C. Carbamoyl phosphate synthetase
D. Arginase
E. Argininsuccinatlyase

4. Indicate in which tissue the process of urea formation is mainly localized:
A. Kidney
B. Intestine
C. Livers
D. Muscles
E. Pancreas

5. Specify the cellular localization of the urea formation process:
A. Golgi apparatus
B. Mitochondria, cytosol
C. Lysosomes
D. Ribosomes
E. Core

6. Specify the process by which ammonia is neutralized in kidney tissue:
A. Synthesis of ammonium salts
B. Reductive amination
C. Indirect demining
D. Urea synthesis
E. Synthesis of biogenic amines

7. Specify the process due to which ammonia neutralization in nerve tissue mainly occurs:
A. Transamination
B. Urea synthesis
C. Formation of amides of dicarboxylic amino acids
D. Synthesis of ammonium salts
E. Synthesis of biogenic amines

8. Phenylpyruvic acid was detected in the patient's urine. Indicate the consequence of the violation of which exchange is this:
A. Phosphorous-calcium
B. Lipid
C. Metabolism of amino acids
D. Carbohydrate
E. Water-salt water

9. From the given list, choose the transport form of blood ammonia:
A. Alanine
B. Isoleucine
C. Ammonium salt
D. Glutamine
E. Urea

10. Albinos do not tolerate exposure to the sun, they quickly get burns. Specify the metabolic disorder that is the basis of this phenomenon:
A. Destruction of melanin
B. Disorders of cholesterol transport
C. Absence of tyrosinase
D. Violation of cholesterol hydroxylation
E. Destruction of vitamin D_3

Protocol N15 **Date**_____

The determination of urea content in the blood serum

THE PRINCIPLE OF THE METHOD:

Urea forms with dimethylmonooxime at the presence Fe^{3+} ions and thiosemicarbazide a red colouring complex, which content is proportional to urea concentration.

THE COURSE OF THE WORK:
Take two test tubes (Experimental and Control) and pour into them solutions according the table:

Solution, ml	Experimental test tube	Control test tube
Blood serum	0, 02	-
0,9% NaCl	-	0, 02
Dimethylmonooxime	2	2
Thiosemicarbazide	2	2

Close the test tubes with aluminum foil and place them into boiling water bath for 10 minutes.
Then cool the content of the test tubes quickly under cold water and determine an optical density of experimental solution (A) against control. The measurement should be carried out within no more than 15 minutes after cooling at a light green optical filter in cuvettes (10 mm).
Calculation:
$X = 16,65 \cdot (E_{exper.}/E_{stand.})$ (mmol/l), where
X – the concentration of urea in the blood serum.
$E_{exper.}$ - the optical density of the experimental solution;
$E_{stand.}$ – the optical density of the urea standard solution, obtained in the same condition; B = 0,16.
There is 3,3-6,6 mmol/l of urea in the blood serum of healthy adults.
RESULTS:

CONCLUSIONS:

Clinical significance:
The decrease of urea content is observed at parenchymatic hepatitis, cirrhosis of the liver and during pregnancy.
The content of urea may be be increased at greenstones, feverish conditions, sepsis, tuberculosis of kidneys and other kidney diseases. The increasing of residual nitrogen of the blood (fist of all due to urea) is named **azotemia.**

PREPARATION FOR THE KROK-1 LICENSURE EXAM

1. Ammonia is a very toxic substance, especially for the nervous system. What substance takes the most active part in ammonia detoxification in the brain tissue?

A. Lysine

B. Glutamic acid
C. Histidine
D. Proline
E. Alanine

2. Nappies of a newborn have dark spots that witness the presence of homogentisic acid oxidation product. Choose the substance whose metabolic disorder is associated with accumulation of homogentisic acid in the organism:

A. Cholesterol
B. Galactose
C. Tyrosine
D. Tryptophan
E. Methionine

3. A 4 y.o. boy has had recently serious viral hepatitis. Now there are such clinical symptoms as vomiting, unconsciousness, fits. There is hyperammoniemia in patient, too. Disturbance of which biochemical process caused such pathological condition of the patient?

A. Increased putrefaction of proteins in bowels
B. Inhibition of transamination enzymes
C. Disturbed neutralization of ammonia in the liver
D. Activation of amino acid decaboxylation
E. Disturbed neutralization of biogenic amines

4. Albinos can't stand sun impact – they don't require sun-tan but get sunburns. Disturbed metabolism of what amino acid underlies this phenomenon?

A. Histidine
B. Phenylalanine
C. Tryptophan
D. Glutamic acid
E. Methionine

5. Cerebral trauma caused the increase of ammonia formation. What amino acid takes part in removal of ammonia from cerebral tissue?

A. Tryptophan
B. Lysine
C. Glutamic acid
D. Valine
E. Tyrosine

6. After a serious viral infection a 3-year-old child has repeated vomiting, loss of conscious- ness, convulsions. Examination revealed

hyperammoniemia. What may have caused changes of biochemical blood indexes of this child?

A. Activated processes of amino acids decarboxylation
B. The increased putrefaction of proteins in intestines
C. The inhibited activity of enzymes for transamination
D. Disorder of ammonia neutralization in ornithine cycle
E. Disorder of biogenic amines neutralization

7. Examination of a patient suffering from cancer of urinary bladder revealed high rate of serotonin and hydroxylanthranilic acid. It is caused by excess of the following amino acid in the organism:

A. Tyrosine
B. Alanine
C. Histidine
D. Methionine
E. Tryptophan

8. A 13-year-old boy complains of general weakness, dizziness, tiredness. He is mentally retarded. Increased level of valine, isoleucine, leucine is in the blood and urine. Urine has specific smell. What is the diagnosis?

A. Graves' disease
B. Addison's disease
C. Tyrosinosis
D. Histidinemia
E. Maple syrup urine disease

9. Ammonia is a very toxic substance, especially for nervous system. What substance takes the most active part in ammonia detoxication in brain tissues?

A. Histidine
B. Lysine
C. Proline
D. Glutamic acid
E. Alanine

10. A 2-year-old child with mental and physical retardation has been delivered to a hospital. He presents with frequent vomiting after having meals. There is phenyl pyruvic acid in urine. Which metabolism abnormality is the reason for this pathology?

A. Amino acid metabolism
B. Lipidic metabolism
C. Carbohydrate metabolism

D. Water-salt metabolism
E. Phosphoric calcium metabolism

11. The greater amount of nitrogen is excreted from the organism in a form of urea. Inhibition of urea synthesis and accumulation of ammonia in the blood and tissues are induced by the decreased activity of the following liver enzyme:

A. Urease
B. Aspartate aminotransferase
C. Carbamoyl phosphate synthetase
D. Amylase
E. Pepsin

12. A patient suffers from hepatic cirrhosis. Examination of which of the following substances excreted by urine can characterize the state of antitoxic function of liver?

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

13. A patient has been diagnosed with alkaptonuria. Choose an enzyme whose deficiency can be the reason for this pathology:

A. Pyruvate dehydrogenase
B. Phenylalanine hydroxylase
C. Glutamate dehydrogenase
D. Homogentisic acid oxidase
E. Dioxyphenylalanine decarboxylase

17. Laboratory examination of a child revealed increased concentration of leucine, valine, isoleucine and their ketoderivatives in blood and urine. Urine smelt of maple syrup. This disease is characterized by the deficit of the following enzyme:

A. Dehydrogenase of branched amino acids
B. Aminotransferase
C. Glucose-6-phosphatase
D. Phosphofructokinase
E. Phosphofructomutase

18. A newborn child was found to have reduced intensity of sucking, frequent vomiting, hypotonia. Urine and blood exhibit increased concentration of citrulline. What metabolic process is disturbed?

A. Cori cycle

B. Tricarboxylic acid cycle
C. Glycolysis
D. Glyconeogenesis
E. Ornithinic cycle

19. A male patient has been diagnosed with acute radiation disease. Laboratory examination revealed a considerable reduction of platelet serotonin level. The likely cause of platelet serotonin reduction is the disturbed metabolism of the following substance:

A. Phenylalanine
B. Tyrosine
C. Histidine
D. 5-Oxytryptofan
E. Serine

Literature (p. 78)

Lesson 17

DIFFERENTIATED CREDIT FOR SECTION 1

"Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids, amino acids and its regulation"

THE PURPOSE OF THE LESSON: to determine the level of students' understanding of the basic principles and general patterns of carbohydrate, lipid, and amino acid metabolism and its regulation.

THEORETICAL QUESTIONS FOR PREPARATION

1. Biochemistry as a science. A subject, tasks, general stages and trends in the development of biochemistry.
2. The purpose and methods of biochemical research, their clinical and diagnostic value.
3. The relations of biochemistry with other biomedical subjuects. Clinical biochemistry. Biochemical laboratory diagnostics.
4. The history of biochemistry as the science.
5. Amino acid composition of proteins and peptides. The mechanism of peptide bond formation. Levels of protein molecule organization.
6. Globular proteins: their structure, physicochemical properties, distribution in tissues and functions in humans (examples). The factors for stability of globular proteins in colloid solution. Common notions about Denaturation and Renaturation factors influence on proteins in solution.
7. Fibrous proteins: their structure, physicochemical properties, distribution in tissues and functions in humans (examples).

8. A Classification of simple proteins and conjugated proteins. The use of conjugated proteins (chromoproteins, nucleoproteins, metalloproteins, glycoproteins, phosphoproteins, lipoproteins) in human tissues.
9. Common notions about methods to release proteins preparations from biological fluids (salting-out, ultracentrifugation, dialysis), to separate them in mixture for obtaining of fractions (chromatography methods, electrophoresis). Qualitative tests to prove proteins and amino-acid residues presence in solutions.
10. Spectrophotometry and photocolorimetry methods to determine content of proteins in biological fluids.
11. The function of enzymes in the organism. Enzyme characteristics in the comparison to non-protein catalysts.
12. Simple and conjugated enzymes structure. A definition of apoenzyme, cofactor, coenzyme and prosthetic group. A structure of active centres for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B_1, B_2, B_3, B_5, B_6, H).
13. Modern notions about the mechanism of enzymatic catalysis: the definition of energy activation for enzymatic reaction; the stages of the formation of an enzyme-substrate complex; the mechanisms for products formation (covalent and acidic catalysis). A significance of scientific works written by D. Keilin, B. Chance, D. Koshland, L. Michaelis and M. Menten.
14. Common properties of enzymes (factors of an influence: pH and temperature of environment, specificity of action).
15. Isozymes: structure and location of their synthesis in tissues (e.g.: Lactate dehydrogenase isozymes).
16. Classification and nomenclature of enzymes: features of reactions catalyzed by each class of enzymes.
17. Multienzyme systems of a cell: types of composition and function.
18. Enzymes kinetics: the determination of kinetic indexes (Km and Vmax) using the Michaelis-Menten equation curve and Lineweaver-Burk equation curve. A significance of Michaelis constant determination for enzymes with relative group specificity.
19. The factors for enzyme activity regulation: concentration of substrate; concentration of product; concentration of enzyme; pH and temperature of environment.
20. Common notions about inhibitors. Inhibition Types: reversible - competitive, uncompetitive, noncompetitive; irreversible - suicide inhibition, affinity labels (examples). The change of kinetic indexes for enzyme under the influence of competitive, non-competitive inhibitors (the determination of inhibitor type using Lineweaver-Burk equation curves).
21. Allosteric center of enzyme: its location, structure and function in enzymatic catalysis. The common notion about Allosteric type of enzyme activity regulation. Feed-back type of inhibition.

22. The principles of enzyme activity determination. Total and specific enzyme activity. The Units of enzyme activity. Turnover number of enzyme.
23. Common notions about enzymatic pathologies; the reasons of their development (examples).
24. General trends in the development of medical enzymology: 1) the elaboration of diagnostic methods using enzymes as reagents; 2) enzymatic tests for diagnosis of diseases (examples); 3) the use of enzymes and their inhibitors as drugs (examples).
25. Common notions about Metabolism and Energy exchange in organism. Anabolic, Catabolic and Amphiobolic processes: definition and their interrelations.
26. Exergonic and endergonic reactions in metabolism.
27. Stages of catabolism. Common and specific ways of catabolism for exogenous and endogenous substrates. Terminal products of catabolic pathways for humans
28. Krebs Cycle: the location in a cell, all the reactions, the regulation and the biological role of this process. Energy balance for Krebs cycle.Vitamins promotion of Krebs cycle.
29. Modern concepts of tissue respiration. Stages of tissue respiration.
30. Complexes of the respiratory chain in mitochondria and their interrelation. Endogenous water formation. Formation of products of incomplete oxygen reduction and their detoxification in the cell.
31. Inhibitors of tissue respiration: classification and representatives.
32. Oxidative phosphorylation. Chemiosmotic theory by P. Mitchell.
33. Proton gradient energy and its utilization pathways. Mechanism of proton gradient formation on the inner mitochondrial membrane.
34. Structure of the coupling factor and its role in ATP synthesis (mitochondrial ATP synthase).
35. Assessment of oxidative phosphorylation efficiency. The respiratory control ratio (P/O).
36. Uncouplers of oxidative phosphorylation and biological oxidation.
37. Regulation of tissue respiration. Respiratory control.
38. Structure, classification, and biological roles of carbohydrates.
39. Daily requirements and digestion of carbohydrates. Carbohydrate-digesting enzymes: localization, pH optimum, and specificity of action. Inherited lactase deficiency.
40. End products of carbohydrate digestion and the mechanism of their absorption in the small intestine.
41. Intracellular catabolism pathways of monosaccharides; aerobic and anaerobic glucose oxidation, general characteristics of processes.
42. Anaerobic glucose oxidation – glycolysis: sequence of enzymatic reactions, biological roles, localization in the body and within cells.
43. Glycolytic oxidoreduction, substrate-level phosphorylation in glycolysis. Energetic balance of anaerobic glucose oxidation.

44. Regulation of glycolysis. Key enzymes of the process.
45. Alcoholic and other types of fermentation.
46. Gluconeogenesis: substrates, key enzymes, reactions, intramolecular localization, physiological significance of the process. Balanced equation for glucose formation from pyruvate. Energetic support of gluconeogenesis.
47. Metabolic and hormonal regulation of gluconeogenesis.
48. Interconnection and reciprocal regulation of glycolysis and gluconeogenesis in the body. Glucose-lactate (Cori cycle) and glucose-alanine cycles.
49. Stages of aerobic glucose oxidation.
50. Oxidative decarboxylation of pyruvic acid (enzymes, coenzymes, reaction sequence, regulation of pyruvate dehydrogenase complex functioning).
51. Interplay between anaerobic and aerobic carbohydrate oxidation pathways in the cell, Pasteur effect.
52. Oxidation of cytosolic NADH in mitochondria. Shuttle mechanisms for glycolytic NADH oxidation (glycerophosphate, malate-aspartate).
53. Comparative characteristics of the bioenergetics of aerobic and anaerobic glucose oxidation.
54. Pentose phosphate pathway of glucose oxidation: scheme and biological role of the oxidative phase. Non-oxidative phase of the process and its interaction with glycolysis. Inherited deficiency of glucose-6-phosphate dehydrogenase in erythrocytes.
55. Metabolism of fructose and galactose in the human body and its disorders.
56. Structure and biological role of polysaccharides (glycogen, glycosaminoglycans).
57. Glycogen biosynthesis (glycogenesis): chemistry, key enzymes, physiological significance.
58. Phosphorolytic breakdown of glycogen in the liver and muscles (glycogenolysis).
59. The role of adrenaline, glucagon, and insulin in regulating glycogen metabolism in muscles and the liver. Mechanisms of cAMP-dependent regulation of glycogen phosphorylase and glycogen synthase activities.
60. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis.
61. Genetic disorders of glycogen metabolism enzyme function (glycogen storage diseases, glycogenoses).
62. General concepts of glycosaminoglycan metabolism.
63. Effects and mechanisms of action of glucagon, adrenaline, glucocorticoids, growth hormone, and insulin on blood glucose levels.
64. Normal blood glucose levels and their disturbances (hyperglycemia, hypoglycemia). Glucosuria.
65. Clinical and biochemical characteristics of diabetes mellitus (insulin-dependent and non-insulin-dependent types).

66. Diagnosis of latent diabetes using glycated hemoglobin concentration measurement and the oral glucose tolerance test (Staub-Traugott test, double sugar load method).
67. Mucopolysaccharidoses: genetic disorders of glycosaminoglycan metabolism.
68. Structure, classification, and biological functions of lipids.
69. Daily requirements and digestion of lipids. Enzymes involved in lipid digestion: localization of synthesis and activation of enzymes, pH optimum, and specificity of action of the active form of enzymes.
70. The role of bile acids in lipid digestion and absorption. Steatorrhea: types (pancreatic, hepatic, enterogenic), causes, diagnosis.
71. Plasma lipoproteins - transport forms of lipids: their classification, chemical composition, functions, and metabolism.
72. Catabolism of triacylglycerols in adipocytes of adipose tissue (lipolysis), sequence of reactions. Neurohumoral regulation of lipolysis by adrenaline, noradrenaline, glucagon, and insulin. Oxidation of glycerol (enzymatic reactions, energy effects of the process).
73. Mechanism and biological role of triacylglycerol synthesis in enterocytes of the intestine, in the liver, and in adipose tissue.
74. Lipolysis of glycerophospholipids in cells: localization and specific actions of phospholipases A_1, A_2, C, and D.
75. Biosynthesis of glycerophospholipids using phosphatidylcholine as an example. The role of the active form of methionine in phosphatidylcholine synthesis.
76. Beta-oxidation of high fatty acids (HFA) of saturated and unsaturated series. The role of carnitine in the transport of fatty acids from the cytoplasm to the mitochondria.
77. The energy value of beta-oxidation of HFA in cells (for stearic and oleic acids).
78. Biosynthesis of high fatty acids. Characteristics and functions of acetyl-CoA carboxylase and palmitate synthase complex. Regulation of the process.
79. Biosynthesis of monounsaturated high fatty acids in the human body.
80. Ketone bodies. Biosynthesis and utilization reactions of ketone bodies: localization in the body, biological significance. Ketonemia and ketonuria in diabetes, fasting.
81. Biosynthesis of cholesterol: localization, initial substrates, reaction scheme, regulation of the process.
82. Biotransformation pathways of cholesterol, localization in the body: Esterification; formation of bile acids, steroid hormones, active forms of vitamin D_3.
83. Biochemical mechanisms of atherosclerosis development. Atherogenicity coefficient. Atherogenic and antiatherogenic lipoproteins.
84. Disruptions in lipid metabolism in obesity, diabetes.
85. Genetic disorders in phospholipid metabolism: sphingolipidoses.

86. Daily protein needs. Understanding the concepts of positive and negative nitrogen balance in the human body. Enzymes involved in protein metabolism: localization of synthesis, activation of proenzymes, optimum pH, and specificity of active enzyme forms.
87. Amino acid absorption mechanisms.
88. Free amino acid pool in the body: processes of formation and utilization of free amino acids in tissues.
89. Amino acid transamination: mechanism of action of aminotransferases, biological significance of the reaction. Clinical and diagnostic importance of determining aminotransferase activity in blood plasma.
90. Direct and indirect deamination of L-amino acids: role of oxidases and glutamate dehydrogenase in deamination.
91. Alpha-decarboxylation of L-amino acids in the human body: role of biogenic amines and their neutralization in the body.
92. Metabolism pathways of non-nitrogenous residues of amino acids in the human body: glucogenic and ketogenic amino acids.
93. Pathways of ammonia formation and detoxification in the body (mainly in the brain, liver, and kidneys).
94. Urea biosynthesis: sequence of enzymatic reactions, genetic anomalies of enzymes in the urea formation cycle.
95. Metabolism of phenylalanine and tyrosine: their metabolic pathways and genetic disorders.
96. Tryptophan metabolism and Its disorders.
97. Cysteine and methionine metabolism: structure, biosynthesis, and functions of glutathione in the human body.
98. Features of valine, leucine, isoleucine metabolism and their genetic disorders.
99. Glycine metabolism: synthesis of glutathione and its function in the human body.

The questions for laboratory works of section 1:

1. The principle of the method, the normal value and clinical significance for determination of amylase activity in the urine.
2. The principle of the method, the normal value and clinical significance for determination of choline esterase activity in the blood serum.
3. The principle of glucose oxidase method for glucose content determination in the blood plasma or serum. The clinical significance of this test.
4. The identification of ketone bodies (acetone, etc.) in the blood plasma and in the urine by the reactions with sodium nitroprusside and chloric iron (III). Iodoformic test for acetone. The clinical significance of these tests.
5. Pyruvic acid content determination in the urine (the principle of the method, and its clinical significance).

6. Total cholesterol content determination in the blood plasm by Ilk`s method (the principle of the test, and its clinical significance).
7. The determination of transaminases (AlAT, AsAT) activity in the blood serum. De Rittis coefficient. The clinical significance of these tests.
8. The determination of urea content in the urine and the blood serum. Clinical significance of these tests.
9. Determination of β-lipoproteins (LDL) content in the blood serum (the principle of the test, and its clinical significance).
10. Principle of the method and the importance of ascorbic acid content determination in food sources and in the urine of patients

LITERATURE

Basic

1. Biological and bioorganic chemistry : national textbook : in 2 books. Book 2. Biological chemistry / Yu. I. Gubsky [et al.] ; ed. by: Yu. I. Gubsky, I. V. Nizhenkovska. - 2nd ed. - Kyiv : AUS Medicine Publishing, 2021. - 544 p.
2. Gubsky, Yu. I. Biological chemistry : textbook for students of medical and pharmaceutical faculties / Yu. I. Gubsky ; ed. by.: Yu. I. Gubsky. - 2nd ed. - Vinnytsya : Nova Knyha, 2018. - 488 p.
3. Skorobogatova, Z. M. Biochemistry. Short course : study guide. Pt. 1 / Z. M. Skorobogatova ; ed. of the English version: O. V. Matviyenko ; reviewed by.: A. L. Zagaiko, D. A. Novikov ; National Academy of Sciences of Ukraine, L. M. Litvinenko Institute of Physical-Organic and Coal Chemistry. - Kyiv : Biocomposite, 2018. - 108 p.
4. Skorobogatova, Z. M. Biochemistry. Short course : study guide. Pt. 2 / Z. M. Skorobogatova ; ed. by of the English version: O. V. Matviyenko ; reviewed by.: A. L. Zagaiko, D. A. Novikov ; National Academy of Sciences of Ukraine, L. M. Litvinenko Institute of Physical-Organic and Coal Chemistry. - Kyiv : Biocomposite, 2019. - 127 p.
5. Skorobogatova, Z. M. Metabolic pathways atlas : study guide / Z. M. Skorobogatova ; рец.: Anatoliy F. Popov, Karine S. Neporada ; translation from Ukrainian by.: Z. M. Skorobogatova, M. A. Stashkevych ; National Academy of Sciences of Ukraine, L. M. Litvinenko Institute of Physical-Organic and Coal Chemistry. - Kyiv : Biocomposite, 2018. - 76 p.

Additional

1. Biochemistry. Module 2. Molecular biology. The biochemistry of cell-to-cell interrelations : laboratory manual for students of second year study spesialty "Medicine" / The Ministry of Health of Ukraine, Zaporizhzhia State Medical University ; ed. by.: K. V. Aleksandrova [et al.] ; рец.: S. I. Kovalenko, O. V. Gancheva. - Zaporizhzhia : ZSMU, 2018. - 106 p.
2. Biological and bioorganic chemistry : national textbook : in 2 books. Book 1. Bioorganic chemistry / B. S. Zimenkovsky [et al.] ; ed. by.: B. S. Zimenkovsky, I. V. Nizhenkovska ; reviewers: V. P. Chernykh, V. O. Kalibabchuk, V. P. Novikov. - 3rd ed. - Kyiv : AUS Medicine Publishing, 2020. - 288 p.
3. USMLE. Step 1. 2018. Biochemistry and Medical Genetics : lecture notes / ed. by.: S. Turco ; contributor: R. Lane, R. M. Harden. - New York : Kaplan Medical USMLE, 2018. - 423 p.

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