

THE MINISTRY OF HEALTH OF UKRAINE  
ZAPORIZHZHIA STATE MEDICAL UNIVERSITY  
BIOLOGICAL CHEMISTRY DEPARTMENT

## **BIOCHEMISTRY**

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

**LABORATORY MANUAL**  
for independent work at home and in class,  
preparation for licensing examination 'KROK-1'  
for students of II year study

Specialty: 222 "Medicine"

Zaporizhzhia, 2017

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**Biochemistry. Module 1.** Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids and amino acids and its regulation. Laboratory manual for independent work at home and in class, preparation for licensing examination “KROK-1” for students of 2-d year study Specialty: 222 “Medicine”/ K. V. Aleksandrova, N. V. Krisanova, N. P. Rudko, D. G. Ivanchenko – Zaporizhzhia : ZSMU, 2017. - 98 p.

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

**Біохімія. Модуль 1.** «Загальні закономірності метаболізму. Метаболізм вуглеводів, ліпідів, амінокислот та його регуляція». Практикум для самостійної аудиторної та позааудиторної підготовки до практичних занять та ліцензійного іспиту «КРОК-1» студентів 2 курсу спеціальності 222 «Медицина» / К. В. Александрова, Н. В. Крісанова, Н. П. Рудько, Д. Г. Іванченко - Запоріжжя : [ЗДМУ], 2017. – 98 с.

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### **LABORATORY MANUAL**

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**gr. №** \_\_\_\_\_

**name, surname** \_\_\_\_\_

Zaporizhzhia, 2017



## INTRODUCTION

This methodological manual is adopted to new Curriculum on Biochemistry discipline for specialty 222 “Medicine” for medical universities in Ukraine that was proposed by Ministry of Public Health in 2016/2017 educational year.

It is recommended for students to use like additional material in preparation for practical class on Biochemistry. The plan to work with it is: 1) to prepare theoretical questions for topic using literature sources and summaries of lectures; 2) to make testing control yourself; 3) to answer tests recommended for licensing examination “Krok-1” preparation; 4) to be ready to answer teacher about principles of methods used for the determination of proposed biochemical indexes and about their clinical significance.

This manual contains: abstracts names to prepare them in a form of individual work and to deliver them orally for colleagues during the class. There are all the theoretical questions for test lesson of module 1 preparation in this manual, too.

## A Plan for Lectures of Module 1

<i>Module 1</i> <i>Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids and amino acids and its regulation</i>		<b>Amount of hours</b>
<b>1</b>	Biochemistry as a science. Enzymes: Structure, Common Properties, Mechanism of action and Regulation of their activity	<b>2</b>
<b>2</b>	Common regularities of Metabolism and Energy exchange. Citric Acid Cycle (Krebs Cycle).	<b>2</b>
<b>3</b>	Molecular bases of bioenergetics. Biological oxidation. Tissue respiration and oxidative phosphorylation.	<b>2</b>
<b>4</b>	Metabolism of carbohydrates N1: Glycolysis and Gluconeogenesis. Aerobic oxidation of monosaccharides up to carbon dioxide and water.	<b>2</b>
<b>5</b>	Metabolism of carbohydrates N2: Alternative metabolic pathways for monosaccharides Glycogen metabolism. The regulation of carbohydrate metabolism and its disorders.	<b>2</b>
<b>6</b>	Metabolism of lipids N1: A transport of Lipids in the blood. Metabolism of triacylglycerols. Metabolism of High Fatty Acids.	<b>2</b>
<b>7</b>	Metabolism of lipids N2: Metabolism of Cholesterol and of ketone bodies. The regulation and disorders of lipid metabolism.	<b>2</b>
<b>8</b>	Amino acids metabolism. Common pathways for amino acids. Ammonia metabolism. Specific pathways for some amino acids and their genetic disorders.	<b>2</b>
<b>9</b>	The role of Vitamins in metabolism of substances in human organism: Water-soluble vitamins, Fat-soluble vitamins.	<b>2</b>

## Plan for Practical Classes of Module 1

Module 1	<b><i>Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids, amino acids and its regulation</i></b>	Amount Of hours
1	Introduction into Biochemistry. Biochemical components of a cell.	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.	2.5
4	The mechanism of action and kinetic properties of enzymes. Enzyme activity regulation.	2.5
5	The principles of enzyme activity determination. Genetic deficiency of enzymes. Medical enzymology	2.5
6	Introduction into metabolism. Anabolic and catabolic processes. Krebs Cycle.	2.5
7	General bases of bioenergetics. Tissue respiration and oxidative phosphorylation in human tissues	2.5
8	<b><i>Sudmodule 1 testing: a control of knowledge for classes NN 1-7. Control work 1</i></b>	2.5
9	Anaerobic oxidation of glucose - Glycolysis and Gluconeogenesis..	2.5
10	Aerobic oxidation of carbohydrates. Hexose monophosphate shunt. Metabolism of Galactose and Fructose in humans.	2.5
11	.Metabolism of polysaccharides and its regulation. Carbohydrate metabolic pathways regulation. Pathologies of carbohydrate metabolism	2.5
12	Lipoproteins of blood plasma. Metabolism of Triacylglycerols and of Phospholipids	2.5
13	High Fatty Acids and Ketone Bodies metabolism	2.5
14	Cholesterol metabolism. The regulation and disorders of lipid metabolism: Obesity, Atherosclerosis	2.5
15	Digestion of proteins in gastro-intestinal tract. Common	

	metabolic pathways for amino acids: Gluco-genous and ketogenous amino acids.	2.5
16	Ways for ammonia utilization. Specific pathways for aromatic and sulfur-containing amino acids	2.5
17	Biochemical functions of vitamins in metabolism. Vitamin-similar substances. Antivitamins.	2.5
18	<b>Submodule 2 testing: the control for classes NN 9-17. Control work 2</b>	2.5
19	<b>TEST LESSON FOR MODULE 1</b>	2

## LIST OF PRACTICAL SKILLS, WHICH STUDENTS SHOULD GET IN MODULE 1:

### To be able:

1. To work with saliva, urine and blood serum in biochemical researches.
2. To draw the graphs for the influences of substrate, enzyme concentrations on the velocity of enzymatic reaction; for the influences of pH and temperature on enzymatic activity.
3. To write the structures of cofactors for enzymes as derivatives of vitamins B<sub>1</sub>, B<sub>2</sub>, PP, B<sub>6</sub>.
4. To determine the class of enzyme according the type of chemical reaction.
5. To determine the type of enzyme's inhibition according the curve of the graph using the Lineweaver-Burk method.
6. To discuss the results for amylase activity in urine and for choline esterase activity in the blood serum according the normal values of these indexes.
7. To show the order of stages, using the name of intermediate metabolites, for catabolic pathways of proteins, carbohydrates and lipids.
8. To write all the reactions of Krebs cycle and to name the factors for the regulation of each reaction.
9. To draw all the components of electron transport chain (long) and ATP synthetase in the inner membrane of mitochondria and to discuss their function and regulation.
10. To describe the coupling of oxidation and phosphorylation in mitochondria using Chemiosmotic Theory (P.Mitchell, 1961) and modern investigations.
11. To write the reactions of Glycolysis, Hexose Monophosphate Shunt (Pentose Phosphate Cycle, oxidative stage), breakdown and synthesis of glycogen.
12. To create the scheme of metabolic pathways for carbohydrates and lipids.



13. To explain the mechanisms of carbohydrate and lipid metabolisms regulation.
14. To discuss the results of biochemical indexes in the blood plasma and urine of patients to estimate the states associated with disorders of carbohydrate and lipid metabolism.
15. To create the schemes for some amino acid (phenylalanine, tyrosine, tryptophan, glutamic acid, cysteine, methionine, glycine) metabolism.
16. To analyze the regulation of some metabolic pathways for amino acids.
17. To discuss some genetic disorders in amino acid metabolism using blood and urine indexes.

**The individual tasks for Module 1 (as the essays):**

1. The history of Biochemistry development as a science.
2. The use of gel, ion-exchange and affinity chromatography methods in separation and clearance of proteins mixtures.
3. The role of vitamins in the formation of non-protein part of conjugated enzymes (vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, PP, B<sub>6</sub>, B<sub>12</sub>, H, K).
4. Multienzyme systems (Pyruvate dehydrogenase complex, Palmitate synthase complex): location, structure and function.
5. Modern notions about mechanisms of enzymatic catalysis.
6. Oxido-reductases: the peculiarities of structure, function and location in a cell.
7. Covalent modification of enzymes as the way for the regulation of their activity.
8. The importance of isozymes determination for differential diagnostic of pathologies.
9. The use of enzymes and their inhibitors as drugs.
11. The use of tissue respiration inhibitors and uncouplers of oxidative phosphorylation as drugs.
12. Features of digestion processes for proteins and lipids under the development of liver and pancreas gland disorders.
13. Sphingolipidoses: reasons for the development and diagnostics.
14. To create the scheme for the regulation of glucose metabolism in humans.
15. Lipid metabolism disorders at patients with obesity.
16. Biochemistry of diabetes mellitus.
17. Aminoaciduria states: reasons of the development, clinical symptoms, and diagnostics.
17. To create the scheme for specific pathways of Phenylalanine and Tyrosine metabolism.

## Lesson 1

### *THEME: INTRODUCTION INTO BIOCHEMISTRY. BIOCHEMICAL COMPONENTS OF A CELL*

#### *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 relations of biochemistry with other biomedical subjects. Medical biochemistry: clinical biochemistry and 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, hydrophilicity, lipophilicity of organic compounds.
  - 1.2. Acidic, alkaline 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,  $\alpha$ -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. A Creation of acetylcholine structure. A 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.  $\alpha$ -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.97)**

## **Lesson 2**

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

*QUESTIONS FOR PREPARATION:*

1. The chemical composition of peptides and proteins. 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 for the influence on proteins
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 to obtain fractions (chromatography methods, electrophoresis). Qualitative tests to prove proteins and amino-acid residues presence.
6. Methods to determine content of proteins in biological fluids due to spectrophotometry and photocolourimetry.

*LABORATORY WORKS:*

1. Qualitative reactions for proteins: biuretic reaction, Fole's test, the reaction with sulfosalicylic acid.
2. Sedimentation reactions for proteins by mineral acids

**Check up your home preparation using the tests:**

- 1. The reason of the damage of  $\alpha$ -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-methionine
  - C. Cysteine
  - D.  $\beta$ -alanine
  - E. Ornithine
- 6. The  $\beta$ -pleated sheet structure is very seldom in nature. Name the protein whose structure is based on it:**
  - A. Albumin of eggs
  - B.  $\alpha$ -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 out 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.  $\text{HNO}_3$
  - C.  $\text{Fe}^{2+}$
  - 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  $\text{Cu}^{2+}$ :**
- 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 glyco-  
proteins:**

- A.  $\text{Cu}^{2+}$
- B.  $\text{Fe}^{2+}$
- 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

## **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. 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 reagent (30% NaOH :  $\text{Pb}(\text{CH}_3\text{COO})_2$  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 ( $\text{PbS}$ ) should be formed.

RESULTS:

CONCLUSIONS:

### **1.3. 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:

### **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  $\text{SO}_4^{2-}$  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 correspondingly in two test tubes. Hold test tube with acid under the corner  $45^\circ$  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:

### ***A PREPARATION TO LICENSING EXAMINATION “KROK 1”***

1. Toxic affection of liver results in dysfunction of protein synthesis. It is usually accompanied by the following kind of dysproteinemia:

- A Absolute hypoproteinemia
- B Relative hypoproteinemia
- C Absolute hyperproteinemia
- D Relative hyperproteinemia
- E Paraproteinemia

#### **Literature (p.97)**

### **Lesson 3**

*THEME: ENZYMES: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES. CLASSIFICATION AND NOMENCLATURE OF ENZYMES*

#### **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<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, H).
3. Common properties of enzymes (factors of an influence: pH and temperature of environment, specificity of action).
4. Isozymes: structure and location of their synthesis in tissues (e.g.: Lactate dehydrogenase isozymes).
5. Classification and nomenclature of enzymes: features of reactions catalyzed by each class of enzymes
6. 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<sub>2</sub>. 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<sup>0</sup> C to 38<sup>0</sup> 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° C
- C. The presence of heavy metal ion as Hg<sup>2+</sup>
- 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<sup>o</sup> C. Then divide the content of each test tube into two parts and carry out qualitative reactions for starch and glucose (Trommer's test).

a) 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:

6) 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 in 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 phosphatic 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 the 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:

### ***A PREPARATION TO LICENSING EXAMINATION “KROK 1”***

**1. In case of enterobiasis acrihine - the structural analogue of vitamin B2 - 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. Researchers isolated 5 isoenzymic forms of lactate dehydrogenase from the human blood serum and studied their properties. What property**

**indicates that the isozyme forms were isolated from the same enzyme?**

- A Catalyzation of the same reaction
- B The same molecular weight
- C The same physicochemical properties
- D Tissue localization
- E The same electrophoretic mobility

**3. 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

**4. 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

## **Literature (p.97)**

### **Lesson 4**

*THEME: THE MECHANISM OF ACTION AND KINETIC PROPERTIES OF ENZYMES. THE REGULATION OF ENZYMATIC ACTIVITY*

#### *QUESTIONS FOR PREPARATION*

1. 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.

2. 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.

3. The factors for enzyme activity regulation: concentration of substrate; concentration of product; concentration of enzyme; pH and temperature of environment.

4. 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).

5. Allosteric centre 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.

### LABORATORY WORKS

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

#### Check up your home preparation using the tests:

1. **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

2. **There are some factors influencing enzyme activity. Point out one of them resulting in complete loss of enzymatic activity:**

- A. Vitamin H
- B. Oxygen
- C.  $t^{\circ} C = 100^{\circ} C$
- D.  $P = 101325 Pa$
- E. Sodium chloride solution

3. **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

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  $25^{\circ} C$
- D. Allosteric inhibitor
- E. Water

5. **Point out 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 out 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 out the irreversible type of enzyme inhibition:**

- A. Competitive
- B. Noncompetitive
- C. Uncompetitive
- D. Allosteric
- E. Suicide

**9. Find out 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  $V_{max}$
- C. It is a substrate concentration for obtaining of 1/2  $V_{max}$
- 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

#### Protocol N 4

Date\_\_\_\_\_

**1. The influence of activators and inhibitors on the 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:

### Literature (p.97)

### Lesson 5

*THEME: PRINCIPLES OF ENZYME ACTIVITY DETERMINATION. GENETIC DEFICIENCY OF ENZYMES. MEDICAL ENZYMOLOGY*

#### QUESTIONS FOR PREPARATION

1. The principles of enzyme activity determination. Total and specific enzyme activity. The Units of enzyme activity. Turnover number of enzyme.
2. Common notions about enzymatic pathologies; the reasons of their development (examples).
3. 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. Determination of amylase activity in the urine by Volgemut's method.
2. Determination of cholinesterase activity in the blood serum.

#### Check up your home preparation using the tests:

1. **Lactate dehydrogenase (LDH) isozymes catalyze the transformation of pyruvate to lactic acid in different**

**types of tissues. Point out the structural distinctive peculiarity of each LDH isozyme:**

- A. Different active centre structure
  - B. Different level of structural organization in native molecule
  - C. Different by the type of coenzyme in native molecule
  - D. Different by the quantity of subunits
  - E. Different by the combination of subunits, forming a native molecule
2. **Point out the activator, used for the determination of urine amylase activity under Volgemut's method:**
- A.  $\text{CuSO}_4$
  - B. NaCl
  - C.  $\text{H}_3\text{PO}_4$
  - D. ATP
  - E.  $\text{Ca}^{2+}$
3. **Patient's amylase activity in the urine exceeds the normal values in ten times as much. Point out the possible diagnosis:**
- A. Viral hepatitis
  - B. Diabetes mellitus
  - C. Sharp pancreatitis
  - D. Influenza
  - E. Angina
4. **Point out the signs of multiple enzyme systems (MS):**
- A. The MS enzymes are united by their intracellular localization

- B. The MS enzymes form a single structural-functional complex
- C. The MS enzymes form several different metabolic products at once
- D. The MS enzymes use one and the same cofactor
- E. Positions A,B,C are right

5. **Find out the term for unit of enzyme activity that is estimated as the number of molecules of a substrate catalyzed upon in a period 1 second by a single enzyme molecule:**

- A. Total activity
- B. Specific activity
- C. Turnover number
- D. Katal
- E. The Unit of an enzyme

activity

6. **Find out the substrate used for amylase activity determination in the urine of patient:**

- A. Glucose
- B. Pyruvate
- C. Maltose
- D. Glycogen
- E. Starch

7. **Find out the method for separation of isozymes to determine their content in the blood serum of patient:**

- A. Dialysis
- B. Electrophoresis
- C. Spectrophotometry
- D. Gel chromatography
- E. Salting-out

8. Pyruvate dehydrogenase complex is multiple enzyme system because it contains:

- A. Two enzymes and one coenzyme
- B. Two enzymes and five coenzymes
- C. Three enzymes and three coenzymes
- D. Three enzymes and five coenzymes
- E. Five enzymes and five coenzymes

9. **There is the treatment of patients with achlorhydria (the absence of free hydrochloric**

**acid in the gastric juice of patient) by enzyme as a drug.**

**Name it:**

- A. Rennin
- B. Pyruvate
- C. Pepsin
- D. Trypsin
- E. Chymotrypsin

10. **Choose the enzyme used as diagnostic reagent for glucose content determination in the blood:**

- A. Glucose-6-phosphatase**
- B. Pyruvate kinase
- C. Maltase
- D. Glucose oxidase
- E. Amylase

#### **Protocol N 5**

Date \_\_\_\_\_

#### **1. 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<sup>0</sup>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:

$$\mathbf{X \text{ (units)} = 1 \cdot 2 \cdot \text{dilution};}$$

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.

**2. 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
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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:

$$E = E(\text{empty}) + E(\text{control}) - E(\text{test})$$

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  $\mu\text{mol}/\text{sec}\cdot\text{lit}$

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.

### ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

**1. Profuse foam appeared when dentist put hydrogen peroxide on the mucous of the oral cavity.**

**What enzyme caused such activity?**

- A Glucose-6-phosphatdehydrogenase
- B Cholinesterase
- C Acetyltransferase
- D Catalase
- E Methemoglobinreductase

**2. Twelve hours after an accute attack of retrosternal pain a patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?**

- A Viral hepatitis
- B Myocardium infarction
- C Collagenosis
- D Diabetes mellitus
- E Diabetes insipidus

**3. Marked increase of activity of MB-forms of CPK (creatine phosphokinase) and LDH-1 were revealed on the examination of the patient's blood. What is the most likely pathology?**

- A Cholecystitis
- B Hepatitis
- C Rheumatism
- D Pancreatitis
- E Miocardial infarction

**4. A patient presents high activity of LDH1, 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 kidneys and adrenals
- D In connective tissue

E In liver and kidneys

5. **During metabolic process active forms of the oxygen including superoxide anion radical are formed in the human body. With help of what enzyme is this anion**

**inactivated?**

- A Glutathione peroxidase
- B Catalase
- C Peroxidase
- D Superoxide dismutase
- E Glutathione reductase

## Literature (p.97)

### Lesson 6

*THEME: COMMON REGULARITIES OF METABOLISM. ANABOLIC AND CATABOLIC PROCESSES IN HUMANS. KREBS CYCLE*

*QUESTIONS FOR PREPARATION:*

1. Common notions about Metabolism and Energy exchange in organism. Anabolic, Catabolic and Amphibolic 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. **Point out the substrate of Krebs Cycle that can be the product of the last reaction of this process:**

- A. Oxaloacetate
- B. Citrate
- C.  $\alpha$ -Ketoglutarate
- D. Malate
- E. Succinate

2. **Nucleoside triphosphate is formed in Krebs Cycle. Point out its abbreviation:**

- A. ATP
- B. CTP
- C. GTP
- D. UTP
- E. TTP

3. **Only one dehydrogenase of Krebs Cycle has the non-protein part FAD. Name it:**

- A. Isocitrate dehydrogenase
- B.  $\alpha$ -Ketoglutarate dehydrogenase
- C. Malate dehydrogenase

- D. Succinate dehydrogenase  
E. Pyruvate dehydrogenase
4. **There is a multiple enzyme complex among enzymes of Krebs Cycle. Point out it:**  
A. Isocitrate dehydrogenase  
B.  $\alpha$ -Ketoglutarate dehydrogenase  
C. Malate dehydrogenase  
D. Succinate dehydrogenase  
E. Pyruvate dehydrogenase
5. **Vitamin B<sub>1</sub> (coenzyme TPP) is necessary for only one dehydrogenase function in Krebs Cycle. Point out it:**  
A. Malate dehydrogenase  
B.  $\alpha$ -Ketoglutarate dehydrogenase  
C. Isocitrate dehydrogenase  
D. Succinate dehydrogenase  
E. Lactate dehydrogenase
6. **HADH is formed as a product in Krebs Cycle. Point out the mole quantity of HADH per 1 mole of acetyl~SCoA incorporated into the process:**  
A. 1  
B. 2  
C. 3  
D. 4  
E. 1.5
7. **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
8. **Krebs Cycle is an amphibolic way. Choose the explanation of this sentence:**  
A. It forms CO<sub>2</sub> and H<sub>2</sub>O  
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
9. **Two reactions of Krebs Cycle are named as oxidative decarboxylation. Point out the enzyme for this type of reaction:**  
A. Citrate synthase  
B. cis-Aconitate hydratase  
C. Isocitrate dehydrogenase  
D. Succinate dehydrogenase  
E. Succinyl~SCoA synthase
10. **Point out the stage with maximum ATP energy formation for glycolose aerobic destruction up to CO<sub>2</sub> and H<sub>2</sub>O:**  
A. Glycolysis up to pyruvate  
B. Oxidative decarboxylation of pyruvate  
C. Krebs Cycle  
D. Glycolysis to lactate  
E. None of these processes



## **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-dichlorophenolindophenolate (as acceptor of hydrogen ions). It is transformed into a restored colourless 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-dichlorophenolindophenolate and mix the content of three test tubes. Put all test tubes into a thermostat at 37°C 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-dichlorophenolindophenolate characterizes the SDHase activity at the presence of the amber acid.

RESULTS:

CONCLUSIONS

### ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

1. Examination of a patient revealed II grade obesity. It is known that he consumes a lot of sweets and rich food, has sedentary way of life. That's why anabolic metabolism has the priority in his organism. Which of the following pathways is amphibolic?

- A. Glyconeogenesis
- B. Cycle of tricarboxylic acids
- C. Lipolysis
- D. Glycolysis
- E. Fatty acids oxidation

**Literature (p.97)**

## Lesson 7

### THEME: GENERAL BASES OF BIOENERGETICS (A SEMINAR)

#### QUESTIONS FOR PREPARATION

1. Types of reactions in biological oxidation (the function of dehydrogenases, oxidases and oxygenases), their biological role.

2. Tissue respiration. Stages of tissue respiration: location in a cell. Mitochondria: structure and functions.

3. Enzymes of mitochondria: the structure, composition and function in metabolic pathways (NAD-derivatives, flavoproteins, cytochromes).

4. The Electron Transport Chain (ETC, respiratory chain): structural organization in the inner membrane of mitochondria; complexes of the respiratory chain. Vitamins and mineral components promotion for ETC function. The mechanism of endogenous water formation in the mitochondria. The uncomplete restoration of oxygen derivatives and their destruction.

5. Oxidative phosphorylation. The location of three coupling sites in ETC. P/O ratio for oxidation of some substrates at normal condition of life span for living cell.

6. Chemiosmotic Theory (P.Mitchell, 1961) and modern representations in description of mechanism of oxidative phosphorylation; ATP synthase: structure and function. P/O ratio.change under the influence of Inhibitors of tissue respiration and Uncouplers of oxidative phosphorylation:

7. The regulation of tissue respiration. An estimation of respiratory control in a cell.

#### Check up your home preparation using the tests:

1. **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.  $\alpha$ -Ketoglutarate dehydrogenase

2. **The antibiotic oligomycin has been recently used in tuberculosis treatment.**

**Point out 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

3. **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 adsorbtion 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
4. **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
5. **The important catabolic processes are located in the mitochondrial matrix. Find out the catabolic process that isn't located in the mitochondria:**
- A. Krebs Cycle
  - B. Oxidation of fatty acids to acetyl-SCoA
  - C. Oxidative decarbo-xylation of pyruvate
  - D. Glycolysis
  - E. The formation of oxaloacetate from pyruvate
6. **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  $aa_3$
7. **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
8. **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.  $\alpha$ -Ketoglutarate dehydrogenase
9. **The increase of one substrate concentration occurs in the mitochondrial matrix during the inhibition of Citrate synthase in Krebs Cycle. Find out this substrate:**
- A. Pyruvate
  - B. Acetyl ~ SCoA
  - C.  $\alpha$ -Ketoglutarate
  - D. Malate

- E. Succinate
10. **The electrochemical potential ( $\Delta\mu\text{H}^+$ ) formation occurs on the inner membrane of mitochondria during the active work of the respiratory chain. Point**

**out the substance that can reduce the ( $\Delta\mu\text{H}^+$ ) value:**

- A. Succinate
- B. Malonic acid
- C. 2,4-dinitrophenol
- D. Citric acid
- E. Glucose

***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

1. **A patient is followed up in an endocrinological dispensary on account of hyperthyroidism. Weight loss, tachycardia, finger tremor are accompanied with hypoxia similar symptoms – headache, fatigue, eye flicker. Find out the result for the influence of high level of thyroid hormones on tissue respiration causing the development of hypoxia similar symptoms:**

- A. Specific binding of active centers of respiratory enzymes
- B. Intensification of respiratory enzymes synthesis
- C. Competitive inhibition of respiratory enzymes
- D. Inhibition of respiratory enzymes synthesis
- E. Uncoupling of oxidation and phosphorylation

2. **Increased production of thyroidal hormones T3 and T4, weight loss, tachycardia, psychic excitement and so on present on thyrotoxicosis. How do thyroidal hormones effect energy metabolism in the mitochondrion of cells?**

- A. Disconnect oxidation and oxidated phosphorylation
- B. Activates phosphorylation of substance
- C. Stops phosphorylation of substance
- D. Stops respiratory chain
- E. Activates oxidative phosphorylation

**Literature (p.97)**

**Lesson 8**

*THEME: TESTING CONTROL FOR CLASSES NN 1-7 (SUBMODULE 1).*

*CONTROL WORK 1*

***Common regularities of metabolism and energy exchange in humans***

*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 centres for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B<sub>1</sub>, B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub>).
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 (K<sub>m</sub>) 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 occurrence 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 Amphibolic 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. Chemiosmotic 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 submodules:**

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.97)

### Lesson 9

*THEME: ANAEROBIC OXIDATION OF GLUCOSE - GLYCOLYSIS.  
SYNTHESIS OF GLUCOSE – GLUCONEOGENESIS*

#### *QUESTIONS FOR PREPARATION*

1. Chemical composition of nutritive carbohydrates and their importance for human body. Daily requirement of carbohydrates for humans
2. Digestion of carbohydrates in the gastro-intestinal tract. Enzymes for digestion of carbohydrates: the location of their synthesis, their pH medium and specificity of action. Terminal products for carbohydrates digestion and mechanism of their absorption in the small intestine. Lactase deficiency in some patients.
3. Common notions about metabolic processes for monosaccharides in a cell.
4. Anaerobic glycolysis: the sequence of reactions, location in a cell and tissues, its biological role.
5. Glycolytic oxido-reduction reactions. Substrate phosphorylation in glycolysis. Energy balance for anaerobic glycolysis.
6. The key enzymes of glycolysis and their regulation.
7. Gluconeogenesis: initial substrates, location in tissues and in a cell, key reactions regulation. Common equation for glucose formation from pyruvate; energy balance for glucose synthesis from pyruvate, lactate and glycerol.
8. Metabolic and hormonal regulation of Gluconeogenesis. Reciprocal regulation of Glycolysis and Gluconeogenesis.
9. The importance of Cori cycle and Glucose-alanine cycle.

## LABORATORY WORKS

**Qualitative reaction for lactate (Uffelmann's reaction) made to prove its presence in skeletal muscular tissue and in gastric juice**

**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<sub>2</sub>, H<sub>2</sub>O
  - 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<sup>+</sup> 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
- 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 out 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. Alpha-Amylase

## Protocol N9

Date

### 1. The determination of lactate in muscular homogenate (Uffelmann's reaction)

#### *THE PRINCIPLE OF THE METHOD:*

It is based on Uffelmann'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 Uffelmann'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:*

## 2. The determination of lactic acid in gastric juice

### THE COURSE OF THE WORK:

Bring 20 drops of 1% phenol solution into a test tube. Add 2 drops of 1% iron (III) chloride solution. The violet coloring complex appears. Then pour some drops of gastric juice. If lactic acid is present in the gastric juice the violet coloring turns into yellow-green due to the formation of iron lactate.

RESULTS:

CONCLUSIONS:

### A PREPARATION TO LICENSING EXAMINATION "KROK 1"

- 1. A 45 y.o woman suffers from Cushing`s syndrome (steroid diabetes). Biochemical examination revealed hyperglycemia, hyperchloremia in this patient. Which of the processes below is the first to be activated?**

  - Gluconeogenesis
  - Glucose reabsorption
  - Glycogenolysis
  - Glycolysis
  - Glucose transport to the cell
  - Oxidative phosphorylation
  - Peroxide decomposition
  - Amino acids deamination
  - Anaerobic glycolysis
- 2. A 7-year-old girl has signs of anemia. Laboratory examination revealed pyruvate kinase deficiency in the erythrocytes. What process disturbance plays the main role in anemia development?**

  - Tissue respiration
- 3. The gluconeogenesis is activated in the liver after intensive physical trainings .What substance is utilized in gluconeogenesis first of all in this case:**

  - Glucose
  - Glutamate
  - Alanine
  - Lactate
  - Pyruvate
- 4. During starvation muscular proteins are broken into free amino acids. These compounds will be the most probably involved into the following process:**

  - Gluconeogenesis in the liver
  - Gluconeogenesis in muscles

C Synthesis of higher fatty acids

D Glycogenolysis

E Decarboxylation

**5. 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

**6. Myocyte cytoplasm contains a big number of dissolved metabolites of glucose oxidation.**

**Name one of them that turns directly into a lactate:**

A Pyruvate

B Oxaloacetate

C Glycerophosphate

D Glucose 6-phosphate

E Fructose 6-phosphate

**7. There is development of muscular hypoxia in untrained person after a sprint. This leads to the accumulation of the following metabolite in muscles:**

A Lactate

B Ketone bodies

C Acetyl CoA

D Glucose 6-phosphate

E Oxaloacetate

**8. As a result of exhausting muscular work a worker has largely reduced buffer capacity of blood. What acidic substance that came to blood caused this phenomenon?**

A Lactate

B Pyruvate

C 1,3-bisphosphoglycerate

D 3-phosphoglycerate

E. Alanine

**9. Untrained people often have muscle pain after sprints as a result of lactate accumulation. This might be caused by intensification of the following biochemical process:**

A Glycolysis

B Gluconeogenesis

C Pentose phosphate pathway

D Lipogenesis

E Glycogenesis

**10. Diseases of the respiratory system and circulatory disorders impair the transport of oxygen, thus leading to hypoxia. Under these conditions the energy metabolism is carried out by anaerobic glycolysis. As a result, the following substance is generated and accumulated in blood:**

A. Fumaric acid

B. Pyruvic acid

C. Glutamic acid

D. Citric acid

E. Lactic acid

**11. The genetic defect of pyruvate carboxylase deficiency is the cause of delayed physical and mental development and early death in children. This defect is characterized by lacticemia, lactaciduria, disorders of a number of metabolic pathways. In particular, the following processes are inhibited:**

A. Citric acid cycle and gluconeogenesis

- B. Glycolysis and glycogenolysis
- C. Pentose phosphate pathway and glycolysis
- D. Lipolysis and lipogenesis
- E. Glycogenesis and glycogenolysis

**12. A 30-year-old woman was diagnosed with insufficiency of exocrine function of pancreas.**

**Hydrolysis of what nutrients will be disturbed?**

- A. Proteins, fats, carbohydrates
- B. Fats, carbohydrates
- C. Proteins, carbohydrates
- D. Proteins, fats
- E. Proteins

**13. Examination of a man who hadn't been consuming fats but had been getting enough carbohydrates and proteins for a long time revealed**

**dermatitis, poor wound healing, vision impairment. What is the probable cause of metabolic disorder?**

- A. Lack of vitamins PP, H
- B. Lack of oleic acid
- C. Lack of linoleic acid, vitamins A, D, E, K.
- D. Lack of palmitic acid
- E. Low caloric value of diet

**14. A newborn develops dyspepsia after the milk feeding. When the milk is substituted by the glucose solution the dyspepsia symptoms disappear. The newborn has the subnormal activity of the following enzyme:**

- A Lactase
- B Invertase
- C Maltase
- D Amylase
- E Isomaltase

## Literature (p.97)

### Lesson 10

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

#### QUESTIONS FOR PREPARATION

1. Aerobic oxidation of glucose up to carbon dioxide and water: stages of oxidation, vitamins promotion and biological role; location in a cell of each stage.
2. Oxidative decarboxylation of pyruvate: the composition of pyruvate dehydrogenase complex, mechanism of its action and regulation.
3. Interrelations between aerobic and anaerobic oxidation of glucose in a cell. Pasteur effect.

4. Hydrogen ion and electron transport from cytosolic NADH into mitochondria matrix. Shuttle systems (malate-aspartate and glycerol phosphate) function.

5. Comparative characteristics of energy balance of aerobic glucose oxidation (up to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) and anaerobic glycolysis.

6. Pentose Phosphate Cycle: reactions, regulation and biological role. Interrelations between Pentose phosphate cycle and aerobic oxidation of Glucose. Genetic deficiency of Glucose-6-Phosphate dehydrogenase in erythrocytes.

7. Galactose and fructose incorporation into Glycolysis: key enzymes, their function and inherited disorders associated with their deficiency.

### LABORATORY WORKS

1. The determination of pyruvate content in the urine
2. Selivanoff's test for fructose.

### 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 out 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 out 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.  $\text{CO}_2$
- C. HADH
- D. Glycerol-3-phosphate
- E.  $\text{FADH}_2$

5. **Point out 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.  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and ATP
- C. Acetyl-SCoA and ATP
- D. Pyruvate and ATP
- E. Citric acid and ATP

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

- A. Insulin
- B. Excess pyruvate in a cell
- C. ATP/ADP ratio lesser than 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 pyruvate
- B. Krebs Cycle
- C. Pyruvate formation
- D. a-Ketoglutarate formation
- E. Acetyl-SCoA formation

9. **Point out 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. a-Ketoglutarate
- C. Glutamate
- D. Glycerate-3-phosphate
- E. Malate

## **Protocol N10**

**Date**\_\_\_\_\_

### **The determination of pyruvate content in the urine**

#### *THE PRINCIPLE OF THE METHOD:*

Pyruvic acid (PA), reacting with 2,4-dinitrophenylhydrazine 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 3 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-dinitrophenylhydrazine 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:

$[PA] \text{ mg/day} = A \cdot 1,5$  (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  $\mu\text{mol/day}$ ).

**RESULTS:**

**CONCLUSIONS:**

**Clinical significance:**

A large quantity of PA is accumulated in blood plasma and is excreted with urine during B<sub>1</sub> 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.

***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

**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-1,6-glucosidase
- C. Galactokinase
- D. Galactose-1-phosphate uridyltransferase
- E. Hexokinase

**3. Galactosemia is revealed in the child. Concentration of glucose in the blood is not considerably changed. Deficiency of what enzyme caused this illness?**

- A Galactose-1-phosphate uridyltransferase
- B Amylo-1,6-glucosidase
- C Phosphoglucomutase
- D Galactokinase
- E Hexokinase

**Literature (p.97)**

## Lesson 11

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

### QUESTIONS FOR PREPARATION

1. Polysaccharides (glycogen and glucose aminoglycans): a structure and biological role in humans.
2. Glycogen synthesis (glycogenesis): location in tissues, key reactions, significance of this process for organism.
3. Phosphorolytic way for glycogen breakdown (glycogenolysis) in the liver and muscles: key enzymes and terminal products according type of tissue.
4. Epinephrine, glucagon, insulin functions in the regulation of glycogen metabolism in the liver and muscles. Reciprocal cAMP-dependent regulation of Glycogen synthetase and Glycogen phosphorylase in tissues.
5. Inherited disorders in glycogen metabolism (glycogen storage diseases (I-V), aglycogenesis).
6. Common notions about glucose aminoglycans metabolism. Inherited disorders in glucose aminoglycans metabolism. Mucopolysaccharidosis.
7. Glucagon, insulin, epinephrine, glucocorticoids, somatotropin: their effects on the level of glucose in the blood, and the mechanisms which describe these effects.
8. Hyperglycemia and glucosuria states: all the reasons of their occurrence in humans. Hypoglycemia state.
9. Biochemical characteristics for diabetes mellitus (insulin-dependent and insulin-independent). Methods for diabetes mellitus diagnostic (glucose loading test; Shtaub-Traugotte test) in clinical biochemistry.
10. Inherited disorders for galactose and fructose metabolism in humans (fructosemia, galactosemia states).

### LABORATORY WORK

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

Check up your home preparation using the tests:

1. **What hormone decreases the glucose concentration in the blood, if its value is more than 6.8 mM/L:**

- A. Thyroxin
- B. Testosterone
- C. Glucagon
- D. Adrenalin

E. Insulin

2. **Choose the hormone whose secretion may be damaged at diabetes mellitus in person:**

- A. Cortisol
- B. Progesterone
- C. Growth hormone



- D. Insulin
  - E. Epinephrine
3. **Continue, please, the phrase: "Glycogenolysis in the liver is used for...":**
- A. The decrease of glucose level in the blood
  - B. The increase of glucose level in the blood
  - C. Glucose-6-phosphate formation, only
  - D. The change of blood pH
  - E. The lactate formation
4. **Choose the substance that can be the terminal product of glycogenolysis in muscles:**
- A. Glycogen
  - B. Glucose
  - C. Pyruvate
  - D. Fructose
  - E. Glucose-6-phosphate
5. **Point out 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 out 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 out the key enzyme of glycogen degradation in the liver:**
- A. Fructose-1,6-diphosphatase
  - B. Glycogen Phosphorylase
  - C. Glyceraldehyde-3-phosphatase
  - 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

### **Protocol N 11**

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 reabsorption;
- 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  $\beta$ -cells of pancreas.

### ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

**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 child is languid, apathetic. Liver is enlarged and liver biopsy revealed a significant amount of glycogen. Glucose concentration in the blood stream is below normal. What is the cause of low glucose levels:**

- A. Low {absent} activity of hexokinase
- B. High activity of glycogen synthetase
- C. Deficit of gene that is responsible for the synthesis

of glucose 1-phosphate uridine transferase

- D. High activity of glycogen phosphorylase in liver
- E. Low {absent} activity of glucose 6-phosphatase

**3. A nurse accidentally injected a nearly double dose of insulin to a patient with diabetes mellitus. The patient lapsed into a hypoglycemic coma. What drug should be injected in order to help him out of coma?**

- A Glucose
- B Lidase
- C Insulin
- D Somatotropin
- E Noradrenaline

**4. Characteristic sign of this glycogenosis is muscle pain during physical work. Blood examination reveals usually hypoglycemia. This pathology is caused by congenital deficiency of the following enzyme:**

- A Glycogen phosphorylase

B Glucose 6-phosphate dehydrogenase

C Alpha - amylase

D Gamma amylase

E Lysosomal glycosidase

5. **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

6. **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

7. **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 r-glutamyltransferase

8. **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

9. **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

10. **A child is languid, apathetic. Liver is enlarged and liver biopsy revealed a significant excess of glycogen. Glucose concentration in the blood stream is below normal. What is the cause of low glucose**

### concentration?

- A Low (absent) activity of glycogen phosphorylase in liver
- B Low (absent) activity of hexokinase
- C High activity of glycogen synthetase
- D Low (absent) activity of phosphofructokinase
- E Deficit of a gene that is responsible for synthesis of glucosyl- 1-phosphate uridine transferase

**11. Glycogen polysaccharide is synthesized from the active form of glucose. The immediate donor of glucose residues during the glycogenesis is:**

- A. Glucose-3-phosphate
- B. Glucose-6-phosphate
- C. UDP-glucose
- D. Glucose-1-phosphate
- E. ADP-glucose

**12. Inherited disease, such as mucopolysaccharidosis, is manifested in metabolic**

**disorders of connective tissue, bone and joint pathologies. The sign of this disease is the excessive urinary excretion of the following substance:**

- A. Lipids
- B. Amino acids
- C. Urea
- D. Glycosaminoglycans
- E. Glucose

**13. A 50-year old patient with food poisoning is on a drip of 10 % glucose solution. It does not provide the body with necessary energy only, but also performs the function of detoxification by the production of a metabolite that participates in the following conjugation reaction:**

- A. Glucuronidation
- B. Hydroxylation
- C. Methylation
- D. Sulfation
- E. Glycosylation

## Literature (p.97)

### Lesson 12

*THEME: LIPOPROTEINS OF THE BLOOD PLASMA. METABOLISM OF TRIACYLGLYCEROLS AND OF GLYCERO PHOSPHO-LIPIDS*

#### QUESTIONS FOR PREPARATION

1. Main classes of lipids place in human organism^ their structure and functions.
2. Daily requirement for lipids in human diet. A Digestion of lipids in the gastro-intestinal tract: enzymes , location of their synthesis and the factors for their activation, pH medium, specificity of function.

3. Bile acids function in the gastro-intestinal tract. Steatorrhea: the reasons of the development and its diagnostic. Plasma lipoproteins – the transport form of lipids in the blood: a classification, chemical composition, metabolism, and functions.
4. Triacylglycerols catabolism in adipose tissue (lipolysis): reactions, hormonal regulation (by epinephrine, glucagon, and insulin). The way for glycerol oxidation: reactions and energy balance.
5. Synthesis of Triacylglycerols in the intestine wall, liver, and adipose tissue: reactions, biological role, and regulation.
6. Glycerophospholipid lipolysis in a cell: location and functions of phospholipases A1, A2, C and D. The hormonal regulation of tissue phospholipases A2, C.
7. Glycerophospholipid synthesis in a cell (using the way for Phosphatidyl choline); the role of methionine derivative and CTP in this synthesis. Lipotropic factors to promote this type of synthesis.

### LABORATORY WORK

#### Determination of $\beta$ -lipoproteins (LDL) content in the blood serum

#### Check up your home preparation using the tests:

1. **Lipids are natural organic compounds that are:**
  - A. Good soluble in water
  - B. Insoluble in organic solvents
  - C. Insoluble in benzene
  - D. Soluble in organic solvents
  - E. Soluble in buffer solutions
2. **Point out the terminal product of triacylglycerol lipolysis in adipose tissue:**
  - A. Bile acids
  - B. Mineral acids
  - C. Glycerol
  - D. 2-Monoacylglycerol
  - E. Diacylglycerol
3. **Find out the enzyme of tissue lipolysis that is regulated by hormones:**
  - A. Triacylglycerol lipase
  - B. Diacylglycerol lipase
  - C. Monoacylglycerol lipase
  - D. Lecithin cholesterol acyl transferase
  - E. Lipoprotein lipase
4. **Point out the lipoproteins of the blood plasma containing the highest mass of triacylglycerols:**
  - A. HDL
  - B. LDL
  - C. IDL
  - D. Chylomicrons
  - E. VLDL
5. **Point out the biological role that is suitable for phospholipid:**
  - A. Membrane structural component
  - B. Component of multiple enzyme system

C. Substrate for steroidal hormones synthesis

D. Precursor for 1, 25-dioxycholecalciferol synthesis

E. Substrate for the bile acid formation

6. **Choose the separation method for lipoproteins of the blood plasm:**

A. Radioimmunal Assay

B. Extraction

C. Electrophoresis

D. Salting-out

E. Photocolorimetry method

7. **Name the signal in the liver cell causing the triacylglycerol synthesis:**

A. The accumulation of high fatty acids

B. The decrease of ATP/ADP ratio

C. The accumulation of carbon dioxide

D. The ketone bodies accumulation

E. The stimulation of protein degradation

8. **Point out the lipoproteins of blood plasma that keep the biggest amount of cholesterol ester:**

A. HDL

B. LDL

C. IDL

D. Chylomicrons

E. VLDL

9. **Choose the enzyme participating in  $H_3PO_4$  removal from glycerol residue in phosphatidic acid:**

A. Phospholipase A<sub>1</sub>

B. Phospholipase D

C. Phospholipase C

D. Phospholipase A<sub>2</sub>

E. Phosphatidate Phosphatase

10. **Point out the enzyme used for lysophospholipid formation during lipolysis of glycerophospholipid:**

A. Diglycerol lipase

B. Monoglycerol lipase

C. Triglycerol lipase

D. Phospholipase A<sub>2</sub>

E. Phospholipase D

11. **Point out the place of lipid products absorption in gastrointestinal tract:**

A. Duodenum

B. Small intestine

C. Stomach

D. Esophagus

E. Bottom parts of intestine

12. **Point out the terminal product of lipids digestion that is absorbed most intensively than others:**

A. Bile acids

B. Mineral acids

C. Glycine

D. 2-Monoacylglycerol

E. Triacylglycerol

13. **The bile acids participate in:**

A. The activation of trypsin

B. The lipids emulsification

C. The cholesterol synthesis

D. The ketone bodies synthesis

E. The protein transport activation

## Protocol N12

Date \_\_\_\_\_

### Determination of $\beta$ -lipoproteins (LDL) content in the blood serum

#### *THE PRINCIPLE OF THE METHOD:*

$\beta$ -Lipoproteins (LDL) precipitate in the presence of calcium chloride and heparin: the turbidity is appeared. It is explained that heparin can form with  $\beta$ -lipoproteins a complex, which is precipitated in the presence of calcium chloride. The concentration of  $\beta$ -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 1000 empirical coefficient Calculate LDL content according to the formula:

$$X = (E_2 - E_1) \cdot 1000, \text{ where}$$

X - concentration of LDL in the blood serum, mg %;

$E_1$  - optical density of experimental sample before heparin adding;

$E_2$  - optical density of experimental sample after heparin adding;

1000 – recalculation coefficient.

RESULTS:

CONCLUSIONS:

#### **Clinical significance:**

**The content of beta-lipoproteins in the blood serum is normal when it equals 300-450 mg% or 3,0-4,5 g/l.**

The increased  $\beta$ -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.



## **A PREPARATION TO LICENSING EXAMINATION "KROK 1"**

1. Synthesis of phospholipids is disordered under the liver fat infiltration. Indicate which of the following substances can enhance the process of methylation during phospholipids synthesis?
  - A. Glucose
  - B. Citrate
  - C. Methionine
  - D. Glycerin
  - E. Ascorbic acid
2. An experimental animal that was kept on protein-free diet developed fatty liver infiltration, in particular as a result of deficiency of methylating agents. This is caused by disturbed generation of the following metabolite:
  - A Choline
  - B DOPA
  - C Cholesterol
  - D Acetoacetate
  - E Linoleic acid
3. Increased amount of free fatty acids is observed in the blood of the patients with diabetes mellitus. It may be caused by:
  - A Increased activity of triglyceride lipase of adipocytes
  - B Storage of palmitoyl-CoA
  - C Activation of ketone bodies utilization
  - D Activation of apolipoproteins synthesis
  - E Decreased activity of phosphatidylcholine-cholesterol-acyltransferase blood plasma

### **Literature (p.97)**

### **Lesson 13**

#### *THEME: HIGH FATTY ACIDS AND KETONE BODIES METABOLISM*

#### *QUESTIONS FOR PREPARATION*

1. Essential and non-essential high fatty acids: their structure and ways to be used in human organism.
2.  $\beta$ -Oxidation of high fatty acids (HFA): intracellular location, reactions for one round, the balance equation, factors for regulation, biological role. Unsaturated HFAs  $\beta$ -Oxidation features. Vitamins promotion for oxidation of HFA.
3. Power effect of  $\beta$ -Oxidation of Stearic and Oleic acids.
4. High fatty acids synthesis: location in tissues, biological role. Composition and functions of acetyl-CoA carboxylase and Palmitate

synthase multiple enzyme system. Factors for regulation of these enzymes.

5. Monounsaturated HFA formation (a desaturation): location in a cell, composition of enzyme systems used in this process.
6. Ketone bodies metabolism: location in tissues, its regulation and importance for human organism. Ketonemia and ketonuria states: reasons for the development at starvation and diabetes mellitus.

## LABORATORY WORK

### The qualitative tests for ketone bodies

Check up your home preparation using the tests:

1. **Point out the terminal product of  $\beta$ -oxidation of Higher Fatty Acids (HFA) with odd number of carbon atoms:**

- A. Butyryl ~ SCoA
- B. Malonyl ~ SCoA
- C. Succinyl ~ SCoA
- D. Acetoacetyl ~ SCoA
- E. Propionyl ~ SCoA

2. **Point out the cellular location of saturated HFA synthesis:**

- A. Nucleus
- B. Plasmolemma
- C. Cytoplasm
- D. Mitochondrions
- E. Endoplasmic reticulum

3. **Choose the allosteric activator of acetyl-CoA-carboxylase (the key enzyme of HFA synthesis):**

- A. Malate
- B. Oxaloacetate
- C. Citrate
- D. Succinate
- E. Fumarate

4. **Point out the biological role of carnitine in cells:**

- A. Antioxidant
- B. Allosteric activator of enzymes

C. Transporter of acyl ~ SCoA across the mitochondria membranes

D. The component of respiratory chain

E. The enzyme inhibitor

5. **Point out the substrate for acyl-CoA-dehydrogenase ( $\beta$ -oxidation of HFA):**

A. Acetyl ~ SCoA

B. Enoyl ~ SCoA

C. Butyryl ~ SCoA

D.  $\beta$ -hydroxyacyl ~ SCoA

E.  $\beta$ -ketoacyl ~ SCoA

6. **Choose the bodies and tissues where lipogenesis occurs most intensively:**

A. Muscle

B. Brain

C. Liver

D. Kidneys

E. Myocardium

7. **Point out, how fatty acids are activated in catabolic process:**

A. Are phosphorylated by ATP

B. Don't change the structure

C. Are converted to acyl~ SCoA due to ATP energy

D. Are linked to HS-CoA without any energy use

E. Interact with carnitine

8. Point out the HFA that is the most presumable energy source for a cell:

- A. Palmitic acid
- B. Stearic acid
- C. Oleic acid
- D. Acetic acid
- E. Propionic acid

9. Point out the metabolite, concerning to ketone bodies:

- A. Acetyl ~ SCoA
- B.  $\beta$ -hydroxybutyric acid
- C. Butyric acid
- D. Palmitoleic acid

E. Malonyl ~ SCoA

10. Point out the process or reaction where acetone is formed as end-product:

- A.  $\beta$ -Oxidation of HFA
- B. Decarboxylation of acetoacetic acid
- C. Condensation of two acetyl-CoA molecules
- D. Synthesis of HFA
- E. Decarboxylation of  $\beta$ -hydroxybutyric acid

## Protocol N13

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.

**A PREPARATION TO LICENSING EXAMINATION “KROK 1”**

1. **An experimental animal has been given excessive amount of carbon labeled glucose for a week. In what compound can the label be found?**
  - A. Phenylalanine
  - B. Methionine
  - C. Palmitic acid
  - D. Vitamin A
  - E. Arachidonic acid
2. **A 1 y.o. child with symptoms of muscle affection was admitted to the hospital. Examination revealed carnitine deficiency in muscles. Biochemical base of this pathology is disturbed process of:**
  - A. Regulation of Ca<sup>2+</sup> level in mitochondria
  - B. Transporting of fatty acids to the matrix of mitochondria
  - C. Actin and myosin synthesis
  - D. Lactic acid utilization
  - E. Substrate phosphorylation
3. **A sportsman was recommended to take a medication that contains carnitine in order to improve his results. What process is activated by carnitine the most?**
  - A. Synthesis of steroid hormones
  - B. Fatty acids transport to mitochondria
  - C. Tissue respiration
  - D. Synthesis of ketone bodies
  - E. Synthesis of proteins
4. **Patients who suffer from severe diabetes and don't receive insulin have metabolic acidosis. This is caused by the increased concentration of the following metabolites:**

- A Ketone bodies
  - B Fatty acids
  - C Unsaturated fatty acids
  - D Triacylglycerols
  - E Cholesterol
5. **A sportsman needs to improve his sporting results. He was recommended to take a preparation that contains carnitine. What process is activated the most by this compound?**
- A Fatty acids transporting
  - B Amino acids transporting
  - C Calcium ions transporting
  - D Glucose transporting
  - E Vitamin K transporting
6. **Examination revealed carnitine deficiency in patient's muscles. What process disturbance is the biochemical basis of this pathology?**
- A Transporting of fatty acids to

- mitochondria
  - B Regulation of Ca<sup>2+</sup> level in mitochondria
  - C Substrate phosphorylation
  - D Lactic acid utilization
  - E Actin and myosin synthesis
7. **A patient with high rate of obesity was advised to use carnitine as a food additive in order to enhance "fat burning". What is the role of carnitine in the process of fats oxidation?**
- A Transport of free fatty acids (FFA) from cytosol to the mitochondria
  - B Transport of FFA from fat depots to other tissues
  - C It takes part in one of reactions of FFA beta-oxidation
  - D FFA activation
  - E Activation of intracellular lipolysis

## Literature (p.97)

### Lesson 16

*THEME: CHOLESTEROL METABOLISM. THE REGULATION AND DISORDERS OF LIPIDS METABOLISM: OBESITY, ATHEROSCLEROSIS*

#### QUESTIONS FOR PREPARATION

1. Cholesterol biosynthesis: location in tissues, initial substrates, the sequence of reactions, and regulation.
2. Catabolic pathways for cholesterol in humans (formation of corticosteroids, sex hormones, bile acids, vitamin D<sub>3</sub> derivatives): location, sequence of reactions, and importance.
3. The regulation of lipids metabolism by insulin, catecholamines, glucagon, thyroid hormones, glucocorticoides, STH, ACTH.

4. Biochemical mechanisms for Atherosclerosis of blood vessels development in human organism. Atherogenic and anti-atherogenic lipoproteins. Indexes of blood plasma for diagnostics of atherosclerosis.

5. The infringements of lipid metabolism at obesity and diabetes mellitus.

6. Disorders of phospholipid metabolism; Goshe's disease, Tay-Sach's disease, sphingolipidosis, gangliosidosis (reasons for the development and diagnostics).

### 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 out the key enzyme of cholesterol synthesis:**

- A. Acetyl ~ SCoA carboxylase
- B.  $\beta$ - hydroxybutyryl dehydrogenase
- C.  $\beta$ - hydroxy- $\beta$ -methylglutaryl-CoA reductase
- D. Palmitate synthetase
- E. Malonyl ~ SCoA-ACP transferase

2. **Point out 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. Antimycin A

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

- A. Adrenalin
- B. Thyroxin
- C. Cholesterol
- D. Phosphate

E. Glucose

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

- A. FADH<sub>2</sub>
- B. NAD
- C. Pyridoxal phosphate
- D. NADPH
- E. Biotin

5. **Find out 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 out 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 out the enzyme system that is used for bile acids formation from cholesterol:**
- A. Acetyl ~ SCoA carboxylase
- B.  $\beta$ - hydroxybutyryl dehydrogenase
- C. Acetoacetyl-CoA reductase
- D. 7-Monooxygenase cytochrome P<sub>450</sub> -linked system
- E. Malonyl ~ SCoA-ACP transferase
8. **Find out the vitamin derivative that is synthesized from cholesterol in humans:**
- A. Progesterone
- B. 1,25-dihydroxy cholecalciferol
- 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

**Protocol N 14**

**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. 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:

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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°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 of the development myocardial ischemia (MI) considerably grows at the 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.

### ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

1. After intake of food rich in fats a patient feels nausea and sluggishness; with time there appeared signs of steatorrhea. Blood

cholesterol concentration is 9,2 micromole/L. This condition was caused in the intestine by lack of:



- A. Phospholipids
  - B. Chylomicrons
  - C. Fatty acids
  - D. Bile acids
  - E. Amino acids
2. **A patient has disturbed absorption of fat digestion products. It might have been caused by a deficit of those components in the small intestine cavity:**
- A. Bile acids
  - B. Bile pigments
  - C. Lipolytic enzymes
  - D. Sodium ions
  - E. Lipid soluble vitamins
3. **Examination of cell culture got from a patient with lysosomal pathology revealed accumulation of great quantity of lipids in the lysosomes. What of the following diseases is this disturbance typical for?**
- A. Gout
  - B. Phenylketonuria
  - C. Wilson disease
  - D. Tay-Sachs disease
  - E. Galactosemia
4. **The patient with diabetes mellitus has been delivered in hospital in the state of unconsciousness. Arterial pressure free fatty acids accumulation in the blood results in these manifestations:**
- A. Ketone bodies
  - B. Monosaccharides
  - C. Cholesterol esters
  - D. High fatty acids
  - E. Amines
5. **A 6 year old child was delivered to a hospital. Examination revealed that the child couldn't fix his eyes, didn't keep his eyes on toys, eye ground had the cherry-red spot sign. Laboratory analyses showed that brain, liver and spleen had high rate of ganglioside glycometide. What congenital disease is the child ill with?**
- A Tay-Sachs disease
  - B Wilson's syndrome
  - C Turner's syndrome
  - D Niemann-Pick disease
  - E MacArdle disease
6. **A 12-year old patient was found to have blood serum cholesterol at the rate of 25 mmol/l. The boy has a history of hereditary familial hypercholesterolemia, which is caused by the impaired synthesis of the following protein receptors:**
- A. Low density lipoproteins
  - B. High density lipoproteins
  - C. Intermediate density lipoproteins
  - D. Very low density lipoproteins
  - E. Chylomicrons
7. **A 67-year old male patient consumes eggs, pork fat, butter, milk and meal. Blood test results: cholesterol – 12,3 mmol/l, total lipids – 8,2 g/l, increased low-density**

**lipoprotein fraction (LDL). What type of hyperlipoproteinemia is observed in the patient?**

- A. Cholesterolemia
- B. Hyperlipoproteinemia type IIa
- C. Hyperlipoproteinemia type I
- D. Hyperlipoproteinemia type IIb
- E. Hyperlipoproteinemia type IV

**8. One of the factors that cause obesity is the inhibition of fatty acids oxidation due to:**

- A. Choline deficiency
- B. Impaired phospholipids synthesis
- C. Low level of carnitine

- D. Lack of carbohydrates in the diet
- E. Excessive consumption of fatty foods

**9. A patient underwent a course of treatment for atherosclerosis. Laboratory tests revealed an increase in the anti-atherogenic lipoprotein fraction in the blood plasma. The treatment efficacy is confirmed by the increase in:**

- A. HDL
- B. LDL
- C. VLDL
- D. Chylomicrons
- E. IDL

## Literature (p.97)

### Lesson 15

*THEME: A DIGESTION OF PROTEINS IN GASTRO-INTESTINAL TRACT. COMMON METABOLIC PATHWAYS FOR AMINO ACIDS. GLUCOGENIC AND KETOGENIC AMINO ACIDS*

### QUESTIONS FOR PREPARATION

1. An importance of proteins use in human diet, daily requirement of proteins for humans. Digestion of proteins: enzymes for: digestion ; their the location of synthesis and factors for their activation, pH medium, specificity of function.
2. Transport systems for of amino acids absorption in the small intestine and placed in cellular membrane of tissues. Nitrogen balance (positive and negative);.
3. Total pool of amino acids in organism: the ways for the formation and utilization of amino acids in tissues.
4. Transamination of amino acids: the mechanism of aminotransferases action, and its biological role. Alanine- and aspartate aminotransferases, their clinical significance for diagnosis of some diseases.
5. Direct and indirect Deamination of L-amino acids. The role of Oxidases (L- and D-) and Glutamate dehydrogenase in the metabolism of amino acids; the regulation of latter reaction.
6.  $\alpha$ -Decarboxylation of L-amino acids: enzymes and coenzyme for this type of reaction. Biogenic amines: their formation and biological role (histamine,

tryptamine, serotonin,  $\gamma$ -aminobutyric acid). The utilization of biogenic amines in tissues.

7. Ways for the transformation of nitrogen-free residues of amino acids. 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:

- Point out the way of Amino Acids transformation that is not the common catabolic pathway:**
  - Transamination
  - Transdeamination
  - $\alpha$ -Decarboxylation
  - Oxidative deamination
  - Hydroxylation
- Point out the liver enzyme, which takes part in second step of transdeamination of Amino Acid:**
  - $\alpha$ -Ketoglutarate dehydrogenase
  - Glutamate dehydrogenase
  - Glutamate decarboxylase
  - L-Alanine oxidase
  - Tryptophan hydroxylase
- Point out the enzyme, whose activity is determined in the blood plasma during the unicteric period of viral hepatitis:**
  - Phenylalanine hydroxylase
  - Creatine phosphokinase
  - Glutamate dehydrogenase
  - Alanine transaminase
  - Ornithine carbomoyl phosphatase transferase
- Choose the enzyme of the blood plasma, whose activity increases in ten or more times for 3-4 hours after myocardium infarction:**
  - Alanine transaminase
  - Aspartate transaminase
  - Alcaline phosphatase
  - Arginase
  - Leucine aminopeptidase
- Point out the cofactor, which is used by D-Amino acid oxidase in oxidative deamination:**
  - NADP<sup>+</sup>
  - NAD<sup>+</sup>
  - FAD
  - FMN
  - TPP
- Name biogenic amine that is formed from 5-hydroxy-Tryptophan:**
  - Thiamine
  - Serotonin
  - Adrenalin
  - Dopamine
  - Histamine
- Choose the enzyme, whose genetic defect results in the**

**GABA levels decrease in the brain (GABA -  $\gamma$ -Amino Butyric Acid):**

- A. Tryptophan decarboxylase
- B. Phenylalanine hydroxylase
- C. Histidine decarboxylase
- D. Alanine hydroxylase
- E. Glutamate decarboxylase

**8. Point out the vitamin, whose hypovitaminosis causes the violations in the transamination and decarboxylation of Amino Acids:**

- A. Vitamin C
- B. Vitamin B<sub>1</sub>
- C. Vitamin B<sub>2</sub>
- D. Vitamin B<sub>9</sub>
- E. Vitamin B<sub>6</sub>

**9. Point out the glucogenic amino acids:**

- A. Glutamate
- B. Alanine
- C. Serine
- D. Aspartate
- E. All the positions above

are right

**10. Point out the liver enzyme catalyzing the reversible oxidative deamination:**

- A. Glutamate dehydrogenase
- B. Alanine transaminase
- C. Monoamino oxidase
- D. Aspartate transaminase
- E. Arginase

**11. Point out 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 out the group of peptidases which trypsin is belong to:**

- A. Amino peptidase
- B. Exopeptidase
- C. Endopeptidase
- D. Dipeptidase
- E. Carboxypeptidase

**13. Point out 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 out 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 out 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 out 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 N15**

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 AIAT 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:

<b>Add, in ml</b>	<b>Test sample</b>	<b>Control sample</b>
Substrate-buffer solution	0,5	0,5
Incubation in a dry-air thermostat at 37 <sup>0</sup> C for 3 min		
Stop reagent	-	0,5
Blood serum	0,1	0,1
Incubation in a dry-air thermostat at 37 <sup>0</sup> 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 ( $\alpha$ -ketoglutarate). The most important is the estimation of aspartate aminotransferase (AsAT) and alanine aminotransferase (AIAT) activities.

Determination of the activity of AIAT and AsAT is widely used for diagnostics of heart and liver diseases. AIAT activity increases at Botkin's disease (before the preicteric period). As a rule, the activity change reflects up severity of hepatic parenchyma lesion. AIAT 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/AIAT (de Ritis's factor) equals  $1.33 \pm 0.42$ . This factor grows considerably at severe infarction of myocardium, and it is decreased at patients with viral hepatitis up to value 0, 8.

### ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

- In course of histidine catabolism a biogenic amine is formed that has powerful vasodilatation effect. Name it:**
  - Noradrenalin
  - Dioxyphenylalanine
  - Serotonin
  - Dopamine
  - Histamine
- Glutamate decarboxylation results in the formation of inhibitory transmitter in CNS. Name it:**
  - Glutathione
  - Gamma amino butyric acid
  - Serotonin
  - Histamine
  - Asparagine
- According to clinical indications a patient was administered pyridoxal phosphate. What process is this medication intended to correct?**
  - Deamination of purine nucleotide
  - Synthesis of purine and pyrimidine bases.
  - Transamination and decarboxylation of amino acids
  - Protein synthesis
  - Oxidative decarboxylation of ketoacids
- 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 B6. This most probable cause of this effect is that vitamin B6 is a component of the following enzyme:**

- A Glutamate decarboxylase
- B Pyruvate dehydrogenase
- C NADPH-dependent glutamate dehydrogenase
- D Aminolevulinic acid synthase
- E Glycogen phosphorylase

**6. Glutamate decarboxylation results in formation of inhibitory transmitter in CNS. Name it:**

- A GABA
- B Glutathione
- C Histamine
- D Serotonin
- E Asparagine

**7. 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

**8. 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

**9. 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

**10. Pharmacological effects of antidepressants are connected with inhibition of an enzyme catalyzing degradation of biogenic amines nor-adrenaline 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

**11. A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B6**

**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

**12. 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

### **Literature (p.97)**

## **Lesson 16**

Theme:

*WAYS FOR AMMONIA UTILIZATION. SPECIFIC PATHWAYS FOR AROMATIC AND SULFUR-CONTAINING AMINO ACIDS*

### **QUESTIONS FOR PREPARATION**

1. Common notions about the ways for ammonia utilization in humans.
2. Urea synthesis: the sequence of reactions, regulation and biological role. Genetic disorders of some enzymes in Urea cycle.
3. Common notions about the metabolism of some amino acids (phenylalanine, tyrosine, tryptophan, methionine and cysteine). A significance of these ways for humans.
4. Glycine metabolism? Its association with metabolism of tetrahydrofolates. Glutathione: structure, synthesis and functions in humans.

- C. Addition of an amino group
- D. Removal of a methyl group
- E. Hydrolysis

**13. A 30-year-old woman was diagnosed with insufficiency of exocrine function of pancreas. Hydrolysis of what nutrients will be disturbed?**

- A. Proteins, fats, carbohydrates
- B. Fats, carbohydrates
- C. Proteins, carbohydrates
- D. Proteins, fats
- E. Proteins

**14. To prevent attacks of acute pancreatitis a doctor prescribed the patient trasyolol (contrycal, gordox), which is an inhibitor of:**

- A. Trypsin
- B. Carboxypeptidase
- C. Elastase
- D. Chymotrypsin
- E. Gastricsin



5. Inherited disorders of amino acids metabolism (phenylketonuria, alkaptonuria, albinism, tyrosinosis, tyrosinemia, aminoaciduria states: reasons for their development and biochemical diagnostics.

**LABORATORY WORK**

The determination of urea content in the blood serum

**Check up your home preparation using the tests:**

1. **Point out the index of the blood plasma that is used for the estimation of liver parenchyma damage:**
  - A. Glucose
  - B. Urea
  - C. Free Amino Acids
  - D. Cholesterol ester
  - E.  $Ca^{2+}$
2. **Choose the enzyme used for tyrosine formation from phenylalanine:**
  - A. Phenylalanine-4-hydroxylase
  - B. Phenylalanine transaminase
  - C. Dioxypyhenylalanine decarboxylase
  - D. Aspartate transaminase
  - E. Tyrosinase
3. **Point out the enzyme in Urea Cycle used for Arginine formation:**
  - A. Ornithine transcarbamoylase
  - B. Arginine transaminase
  - C. Argininosuccinate lyase
  - D. Aspartate transaminase
  - E. Arginase
4. **Point out the pathologic state that may be estimated in patients with genetic defect of Phenylalanine -4-hydroxylase:**
  - A. Glucosemia
  - B. Hyperuricemia
  - C. Aminoaciduria
  - D. Hypercholesterinemia
  - E. Dislipoproteinemia
5. **Point out the coenzyme used for hydroxylases structure in Phenylalanine and Tyrosine conversions:**
  - A. Tetrahydrobiopterin
  - B. Dihydrobiopterin
  - C. NADP
  - D. FAD
  - E. Biotin
6. **Point out the pathology that is estimated in patients with genetic deficiency of Dihydrobiopterin reductase:**
  - A. Classic phenylketonuria
  - B. Gout
  - C. Phenylketonuria type II
  - D. Diabetes mellitus
  - E. Ishemic heart disease
7. **Find out the main important pathway for ammonia utilization in the brain:**
  - A. Conversion into Glucose
  - B. Urea formation
  - C. Amino Acid decarboxylation
  - D. Synthesis of Glutamine from  $\alpha$ -ketoglutarate
  - E. Ammonia salts formation

8. **Choose the type of infringement in pathology named Alkaptonuria:**

- A. Genetic deficiency of enzyme
- B. The inhibition of amino acid formation
- C. The renal deiciency
- D. The damage of receptor synthesis
- E. The viral damage of hepatocyte

9. **Choose the hormone that is formed from tryptophan:**

- A. Epinephrine

- B. Thyroxin
- C. Nor-epinephrine
- D. Melatonin
- E. Histamine

10. **Point out the enzyme whose deficiency causes the hyperammonemia state in patient:**

- A. Ornithine transcarbamoylase
- B. Arginine transaminase
- C. Argininosuccinate lyase
- D. Aspartate transaminase
- E. Arginase

**Protocol N16**

**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:

$X = 16,65 \cdot (E_{\text{exper.}}/E_{\text{stand.}})$  (mmol/l), where

X – the concentration of urea in the blood serum.

$E_{\text{exper.}}$  - the optical density of the experimental solution;

$E_{\text{stand.}}$  – 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 can be increased at greenstones, feverish conditions, sepsis, tuberculosis of the kidneys and other kidney diseases. The increasing of residual nitrogen of the blood (first of all due to urea) is called azotemia.

## ***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

**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 decarboxylation
- 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 consciousness, convulsions. Examination revealed hyperammonemia. 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 hydroxyanthranilic 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 patient has pellagra. Interrogation revealed that he had lived mostly on maize for a long time and eaten little meat. This disease had been caused**

**by the deficit of the following substance in the maize:**

- A Tryptophan
- B Tyrosine
- C Proline
- D Alanine
- E Histidine

**11. 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

**12. A child has an acute renal failure. What biochemical factor found in saliva can confirm this diagnosis?**

- A Decrease in glucose concentration
- B Increase in glucose concentration
- C Increase in urea concentration
- D Increase in concentration of higher fatty acids
- E Decrease in nucleic acid concentration

**13. A 1,5-year-old child presents with both mental and physical lag, decolorizing of skin and hair, decrease in catechol-amine concentration in blood. When a few drops of 5% solution of trichloroacetic iron had been added to the child's urine it turned olive green. Such**

**alteration are typical for the following pathology of the amino acid metabolism:**

- A Phenylketonuria
- B Alkaptonuria
- C Tyrosinosis
- D Albinism
- E Xanthinuria

**14. 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

**15. 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 Aminoacids

**16. 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 keto-derivatives 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

20. **Depressions and emotional insanities result from the deficit of noradrenalin, serotonin and other biogenic amines in the brain. Their concentration in the synapses may be increased by means of the antidepressants that inhibit the following enzyme:**

- A. Phenylalanine-4-monooxygenase
- B. Monoamine oxidase
- C. D-amino-acid oxidase
- D. L-amino-acid oxidase
- E. Diamine oxidase

21. **A 1,5-year-old child presents with both mental and physical lag, decolorizing of skin and hair, decrease in catecholamine concentration in blood. When a few drops of 5% solution of chloride iron ( $Fe^{3+}$ ) had been added to the child's urine it becomes olive green in color. Such alterations are typical for the following pathology of the amino acid metabolism:**

- A. Xanthinuria
- B. Tyrosinosis
- C. Albinism
- D. Phenylketonuria
- E. Alkaptonuria

**Literature (p.97)**

## Lesson 17

*THEME: BIOCHEMICAL FUNCTIONS OF VITAMINS IN METABOLISM. VITAMIN-SIMILAR SUBSTANCES. ANTIVITAMINS*

### *THEORETICAL QUESTIONS FOR PREPARATION*

1. Common notions about Vitamins and their role in metabolism. Provitamins. Classification of vitamins. The features of absorption for water-soluble and fat-soluble vitamins in the gastro-intestinal tract.
2. Hypovitaminosis (vitamins deficiency): exogenous and endogenous reasons of their development. Avitaminosis (examples).
3. Water-soluble vitamins (H, B<sub>1</sub>, B<sub>2</sub>, PP (B<sub>3</sub>), B<sub>5</sub>, B<sub>6</sub>, B<sub>9</sub>, B<sub>12</sub>): structure, sources of reception, daily requirement, biological role.
4. Vitamins C and P: structure, mechanisms of function in humans, daily requirement, and clinical symptoms of their deficiency.
5. Vitamin-similar substances (CoQ, carnitine, lipoic acid): structure and function in humans.
6. Hypervitaminosis state for fat-soluble vitamins.
7. A group of vitamin A (retinol, retinal, retinoic acid) and  $\beta$ -carotenes: structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency
8. A group of vitamin E (tocopherols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
9. A group of vitamin D (D<sub>2</sub>, D<sub>3</sub>, calcitriols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency in children (1-3 years of age) and in adults.
10. A group of vitamin K (naphthoquinones): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
11. Vitamin F (a complex of unsaturated high fatty acids): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
12. Antivitamins: the examples of their mechanism of action.
13. Interaction of vitamins. Combined vitamin drugs (multi-vitamins) in the prophylactic and treatment of some diseases.

### *LABORATORY WORKS*

**The determination of ascorbic acid content in food products and in the urine of patient.**

**Check up your home preparation using the tests:**

1. **Choose the correct definition of vitamin:**
  - A. Essential food proteins
  - B. Food factors that cannot be synthesized in human organism
  - C. Essential biologic amines
  - D. Organic compounds, containing amino group
  - E. Essential energy sources
2. **Choose the vitamin, whose oxidation results in blue fluorescing product under UV-light:**
  - A. Pyridoxine
  - B. Rutin
  - C. Thiamine
  - D. Folic acid
  - E. Ascorbic acid
3. **Choose the vitamin that contains the isoalloxazine fragment in its structure:**
  - A. Thiamine
  - B. Riboflavin
  - C. Pyridoxine
  - D. Ubiquinone
  - E. Naphthoquinone
4. **Point out the vitamin, whose deficiency leads to pellagra:**
  - A. Vitamin P
  - B. Vitamin A
  - C. Vitamin C
  - D. Vitamin B<sub>3</sub>
  - E. Vitamin B<sub>2</sub>
5. **Name the metabolic pathway which is in need for vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub> supplement at the same time:**
  - A. Pentose Phosphate Cycle
  - B. Glycolysis
  - C. Urea Cycle
  - D. Krebs Cycle
  - E. Glycogenesis
6. **Find out the enzyme name whose activity is depended on the presence of vitamin B<sub>2</sub>:**
  - A. Pyruvate carboxylase
  - B. Succinate dehydrogenase
  - C. Malate dehydrogenase
  - D. Isocitrate dehydrogenase
  - E. Citrate synthase
7. **The avitaminosis of ascorbic acid is named as:**
  - A. Cushing`s syndrome
  - B. Addison`s disease
  - C. Kwashiorkor
  - D. Hemolytic anemia
  - E. Scurvy
8. **Propose the main food product to promote the intake of vitamin C:**
  - A. Parsley
  - B. Black currant
  - C. Beef
  - D. Milk
  - E. Butter
9. **Find out the vitamin whose deficiency is associated with damaged transamination of amino acids:**
  - A. Pyridoxine
  - B. Rutin
  - C. Thiamine
  - D. Folic acid
  - E. Ascorbic acid
10. **The glycolysis duration is in need for one vitamin, only. Name it:**
  - A. Pyridoxal phosphate
  - B. Riboflavin
  - C. Thiamine
  - D. Nicotinic acid
  - E. Ascorbic acid
11. **Point out the vitamin, which is soluble in lipids:**
  - A. Vitamin C



- B. Vitamin B<sub>1</sub>  
 C. Vitamin PP  
 D. Vitamin K  
 E. Vitamin H
12. **Choose the vitamin, whose precursor is named as b-carotene:**  
 A. Vitamin C  
 B. Vitamin D  
 C. Vitamin A  
 D. Vitamin B<sub>12</sub>  
 E. Vitamin P
13. **Choose the vitamin, whose molecule structure is unsaturated cyclic alcohol (one hydroxide-group only):**  
 A. Vitamin K  
 B. Vitamin F  
 C. Vitamin B<sub>5</sub>  
 D. Vitamin D<sub>2</sub>  
 E. Vitamin H
14. **Choose the vitamin, whose antivitamin is named as Dicoumarol:**  
 A. Vitamin A  
 B. Vitamin B<sub>6</sub>  
 C. Vitamin C  
 D. Vitamin D  
 E. Vitamin K
15. **Choose the vitamin, whose deficiency leads to osteomalacia at adults:**  
 A. Vitamin C  
 B. Vitamin E  
 C. Vitamin D  
 D. Vitamin K  
 E. Vitamin PP
16. **Choose the vitamin, which is a powerful natural anti-oxidant:**  
 A. Retinal  
 B. Tocopherol  
 C. Ergocalciferol  
 D. Riboflavin  
 E. Pyridoxine
17. **Name the blood plasma index whose low value will prove the deficiency of vitamin K in patient:**  
 A. Urea  
 B. Albumins  
 C. Immunoglobulin G  
 D. Prothrombin  
 E. C-reactive protein
18. **Name the vitamin whose level in the blood is depended on the secretion rate of parathyroid hormone:**  
 A. Ascorbic acid  
 B. Calcitriol  
 C. Thiamine  
 D. Tocopherol  
 E. Naphtoquinone
19. **Find out the fat-soluble vitamin whose function is hormone-similar one:**  
 A. Vitamin C  
 B. Vitamin E  
 C. Vitamin D  
 D. Vitamin K  
 E. Vitamin PP
20. **Vitamin A group contains substance whose function is associated mainly with stimulation of proliferation and differentiation processes in tissues. Name it:**  
 A. Retinal  
 B. Pantothenic acid  
 C. Retinoic acid  
 D. Nicotinic acid  
 E. Nicotine amide

## 1. The determination of ascorbic acid concentration in vegetables (a potato, cabbage).

### THE COURSE OF THE WORK:

Crush 5 g of potato (cabbage or other product) with a scalpel and pound it in a mortar, add 3 drops of 10 % hydrochloric acid solution and gradually 15 ml of distilled water. The mass received pour into a flask for titration. Titrate by 0.001 N DCIP solution up to the appearance of pink coloring, which will not disappear within 30 sec.

The calculation will be carried out according to the formula:

$$X = \frac{0.088 \cdot A \cdot 100}{5} \text{ mg \%}, \text{ where}$$

X - the content of vitamin C, mg %;

0.088 - the equivalent of ascorbic acid, which is titrated by 0,001N DCIP solution;

A - the quantity of DCIP (ml), used for titration;

100 - recalculation at 100 g of the product;

5 - quantity (g) of the product taken for the analysis.

Compare the received results with the content of vitamin C in foodstuff: in potatoes (6-20 mg %), cabbage (20-50 mg %), apples (20-40 mg%), lemons (40-55 mg%), needles (150-250 mg %), onions (30 mg %), parsley (150 mg %), cauliflower (70 mg %).

RESULTS:

CONCLUSIONS:

## 1.2. Determination of ascorbic acid content in the urine.

### THE COURSE OF THE WORK:

Pour 10 ml of the urine into a flask for titration and add 10 ml of distilled water, then add 20 drops of 10 % hydrochloric acid. Titrate from the tube 0.001N of DCIP solution up to permanent pink coloring. Calculate daily excretion of vitamin C according to the formula:

$$X = \frac{0.088 \cdot A \cdot B}{C}, \text{ where}$$

X - daily excretion of vitamin C, in mg;

0.088 - the equivalent of an ascorbic acid, which is titrated with 1 ml 0.001 N DCIP solution

- A – the volume of the indicator spent for titration;
- B - daily average volume of the urine: men - 1500ml, women -1200 ml;
- C - urine volume taken for titration.

RESULTS:

CONCLUSIONS:

**The significance of vitamin C determination in the blood plasma and urine:**

**In norm the content of vitamin C in the urine is 20-30 mg / daily.**

It is very important to define this index during the stage: the latent form of vitamin C Hypovitaminosis. The patient drinks the ascorbic acid - glucose solution, containing a daily norm of vitamin C (correlated with the patient's age). In 2-3 hours later the vitamin C concentration is determined in the patient's urine. If the result correlates with normal value, you can say about the latent form of vitamin C Hypovitaminosis.

***A PREPARATION TO LICENSING EXAMINATION "KROK 1"***

1. **A patient suffers from vision impairment – hemeralopia (night blindness). What vitamin preparation should be administered the patient in order to restore his vision?**
  - A. Pyridoxine
  - B. Retinol acetate
  - C. Vicasol
  - D. Thiamine chloride
  - E. Tocopherol acetate
2. **There is disturbed process of Ca<sup>2+</sup> absorption through intestinal wall after the removal of gall bladder in patient. What vitamin will stimulate this process?**
  - A. K
  - B. C
  - C. D<sub>3</sub>
  - D. PP
  - E. B<sub>12</sub>
3. **A 6 y.o child was administered vicasol to prevent postoperative bleeding. Vicasol is a synthetic analogue of vitamin K. Name post-translation changes of blood coagulation factors that will be activated by vicasol:**
  - A. Carboxylation of glutamic acid residues
  - B. Polymerization
  - C. Partial proteolysis
  - D. Glycosylation
  - E. Phosphorylation of serine radicals
4. **A patient who was previously ill with mastectomy as a result of breast cancer was prescribed radiation therapy. What vitamin preparation has marked radioprotective action**

**caused by antioxidant activity?**

- A. Tocopherol acetate
  - B. Riboflavin
  - C. Folic acid
  - D. Ergocalciferol
  - E. Thiamine chloride
5. **There is an inhibited coagulation in the patients with bile ducts obstruction, bleeding due to the low level of absorption of vitamin. What vitamin is in deficiency?**

- A. K
- B. E
- C. D
- D. A
- E. Carotene

6. **A 2-year-old child has got intestinal dysbacteriosis, which results in hemorrhagic syndrome. What is the most likely cause of hemorrhage of the child?**

- A. Activation of tissue thromboplastin
- B. PP hypovitaminosis
- C. Fibrinogen deficiency
- D. Vitamin K insufficiency
- E. Hypocalcemia

7. **During examination of an 11-month-old infant a pediatrician revealed osteoectasia of the lower extremities and delayed mineralization of cranial bones. Such pathology is usually provoked by the deficit of the following vitamin:**

- A. Thiamine
- B. Riboflavin

- C. Bioflavonoids
- D. Pantothenic acid
- E. Cholecalciferol

8. **A patient presents with twilight vision impairment. Which of the following vitamins should be administered?**

- A. Cyanocobalamin
- B. Ascorbic acid
- C. Nicotinic acid
- D. Retinol acetate
- E. Pyridoxine hydrochloride

9. **After the disease a 16-year-old boy is presenting with decreased function of protein synthesis in the liver as a result of vitamin K deficiency. This may cause disorder of:**

- A. Erythropoietin production
- B. Erythrocyte sedimentation rate
- C. Blood coagulation
- D. Osmotic blood pressure
- E. Anticoagulant production

10. **Examination of a patient with frequent hemorrhages from internals and mucous membranes revealed proline and lysine being a part of collagen fibers. What vitamin absence caused disturbance of their hydroxylation?**

- Vitamin A
- Thiamine
- Vitamin K
- Vitamin E
- Vitamin C

11. A woman who has been keeping to a clean-rice diet for a long time was diagnosed with polyneuritis (beriberi). What vitamin deficit results in development of this disease?

Folic acid

Thiamine

Ascorbic acid

Riboflavin

Pyridoxine

12. Most participants of Magellan expedition to America died from avitaminosis. This disease declared itself by general weakness, subcutaneous hemorrhages, falling of teeth, gingival hemorrhages. What is the name of this avitaminosis?

Biermer's anemia

Polyneuritis (beriberi)

Pellagra

Rachitis

Scurvy

13. The structural analogue of vitamin B2 is administered (acrichine) in a case of enterobiasis. The disorder of which enzyme synthesis is caused by this medicine in microorganisms?

- A. NAD-dependent dehydrogenases
- B. Cytochrome oxidases
- C. FAD-dependent dehydrogenases
- D. Peptidases

E. Aminotransferases

14. Pyruvate concentration in the patient's urine has increased 10 times from normal amount. What vitamin deficiency can be the reason of this change:

A. Vitamin B6

B. Vitamin A

C. Vitamin E

D. Vitamin C

E. Vitamin B1

15. Hydroxylation of endogenous substrates and xenobiotics requires a donor of protons. Which of the following vitamins can play this role?

A. Vitamin C

B. Vitamin E

C. Vitamin P

D. Vitamin A

E. Vitamin B6

16. A 10-year-old girl often experiences acute respiratory infections with multiple hemorrhages in the places of clothes friction. Hypovitaminosis of what vitamin is in this girl organism?

A. A

B. B2

C. B1

D. B6

E. C

17. A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B6 concentration. The infant

**presents with pellagral dermatitis, convulsions, anaemia. Convulsions development might be caused by the disturbed formation of:**

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

**18. In clinical practice tuberculosis is treated with isoniazid preparation – that is an antivitamin 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

**19. Some infections diseases caused by bacteria are treated with sulfanilamides, which block the synthesis of bacteria growth factor. What is the mechanism of their action?**

A. They inhibit the absorption of folic acid

B. They are allosteric enzyme inhibitors

C. They are allosteric enzymes

D. They are anti-vitamins of para-amino benzoic acid

E. They are involved in red-ox processes

**20. A 20-year old male patient complains of general weakness, rapid fatigability, irritability, decreased performance, bleeding gums, petechiae on the skin. What vitamin deficiency may be caused of these changes?**

- A. Riboflavin
- B. Ascorbic acid
- C. Retinol
- D. Thiamine
- E. Folic acid

**21. A number of disorders can be diagnosed by evaluation activity of blood transaminases. What vitamin is one of cofactors for these enzymes?**

- A. B6
- B. B1
- C. B5
- D. B2
- E. B8

**Literature (p.97)**

## Lesson 18

*THEME: CONTROL FOR CLASSES NN9-17  
(SUBMODULE 2 TESTING).  
CONTROL WORK 2*

### *THEORETICAL QUESTIONS FOR PREPARATION*

1. Chemical composition of nutritive substances, and their significance for human body. Biological value of carbohydrates, lipids, proteins, vitamins, and mineral components from foods for humans.
2. Daily requirement of carbohydrates for humans, and their digestion in the gastro-intestinal tract. Enzymes for digestion of carbohydrates: the location of the synthesis, their pH medium and specificity of function. Lactase deficiency in some patients.
3. Terminal products for digestion of carbohydrates, and their mechanism of absorption in the small intestine.
4. Daily requirement of lipids for humans, their digestion in the gastro-intestinal tract. Enzymes for digestion of lipids: the location of synthesis and the factors for their activation, pH medium and specificity of function.
5. Bile acids function in the gastro-intestinal tract. Steatorrhea: the reasons of the development and its diagnostic.
6. Nitrogen balance (positive and negative); daily requirement of proteins for humans. Enzymes for digestion of proteins: the location of synthesis and the factors for their activation, pH medium and specificity of function.
7. Mechanisms of amino acids absorption in the small intestine.
8. Aerobic oxidation of glucose: stages of oxidation, biological role, a location of each stage in a cell.
9. Aerobic and anaerobic glycolysis: reactions, location in a cell and biological role. The key enzymes of glycolysis and their regulation. Interrelation between anaerobic and aerobic ways in a cell. Hydrogen ions transport from cytosolic NADH into mitochondria matrix. Shuttle systems function. Power balance of process in both conditions.
10. Oxidative decarboxylation of pyruvate. The composition of pyruvate dehydrogenase complex. The mechanism and the regulation of this complex function.
11. Power balance of full aerobic glucose oxidation up to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .
12. Pentose Phosphate Cycle: the reactions and regulation of two stages, and biological role. Interrelation between Pentose Phosphate Cycle and glycolysis.
13. Gluconeogenesis: the condition of its stimulation in the liver and kidney, substrates, the key enzymes, the reactions of process from lactate, a biological role. The balance equation of glucose formation from pyruvate. Energy provision of gluconeogenesis from lactate.

14. Correlation between glycolysis and gluconeogenesis. The Reciprocal regulation of glycolysis and gluconeogenesis. A Cori Cycle.
15. Glycogen breakdown in the liver and muscles: the key enzyme and its mechanism of stimulation in special conditions. Debranching enzyme function in glycogenolysis.
16. Glycogen synthesis in the liver and muscles: the key enzymes, the factors for their stimulation at hyperglycemia state. The reciprocal regulation of glycogen phosphorylase and glycogen synthetase in the liver tissue.
17. Hormonal control of carbohydrates metabolism. The significance of epinephrine, glucagon, insulin, STH, glucocorticoids in the regulation of carbohydrates metabolism.
18. Metabolic pathways for fructose and galactose utilization in humans. Inherited disorders of these ways.
19. Pathologies of carbohydrates metabolism: Diabetes mellitus (two types); Glycogen storage diseases.
20. Carbohydrates and their derivatives as medicines.
21. Triacylglycerols metabolism (synthesis, lipolysis) in the liver and adipose tissue: enzymes, their regulation of activity, and biological role of processes. The aerobic oxidation of glycerol in tissues.
22. Glycerophospholipids: structure and biological role, their metabolism in tissues. Sphingolipidoses and Mucopolipidoses – the inherited disorders of phospholipids breakdown in tissues.
23. Cholesterol synthesis and its breakdown in the liver. Bile acids: their structure, and biological role.
24. Cholesterol catabolic pathways in adrenal cortex: the key metabolites, and terminal products function in humans. The calcitriols formation from cholesterol in humans.
25. Plasma lipoproteins: a classification; the structure and composition; metabolism; the biological role; researching methods.
26. Synthesis and  $\beta$ -oxidation of high fatty acids: reactions, the balance equation, power provision (or effect), intracellular location, regulation, biological role. The features of unsaturated high fatty acids metabolism.
27. Ketone bodies metabolism, its regulation and significance. The infringement of this metabolism at starvation and at diabetes mellitus.
28. Hormonal regulation of lipids metabolism (insulin, epinephrine, somatotropin, corticotropin, lipotropin, thyroid hormones).
29. Pathologies associated with the infringement of lipids` metabolism (atherosclerosis, obesity, diabetes mellitus): the molecular mechanism of clinical symptoms development, and diagnostic using the indexes of blood plasm.
30. The metabolic pathways providing the pool of free amino acids in the blood. Non-essential and essential amino acids.
31. The Transport mechanism for amino acids across the cellular membrane.



32. The Conversions of amino acids by the action of intestinal microflora. Neutralization of toxic products in the liver. Quick's test.
33.  $\alpha$ -Decarboxylation as the way for biogenic amines formation. Biological role of histamine, serotonin,  $\gamma$ -aminobutyric acid, epinephrine). The utilization of biogenic amines by Monoaminoxidase.
34. Transamination: the mechanism of the reaction, structure of enzymes, and biological role. Clinical significance of transaminases activity determination in the blood plasma.
35. Types of Deamination. Oxidases (L- and D-) function. Glutamate dehydrogenase reaction, its significance and regulation. Indirect deamination.
36. The Ways for the transformation of nitrogen-free residues of amino acids. Glucogenic and ketogenic amino acids.
37. Common notions about the ways for formation and neutralization of ammonia in an organism. Urea cycle: partial reactions, the regulation, and biological role. Inherited disorders associated with the infringements of Urea cycle function.
38. The common representations about the metabolism of some amino acids (phenylalanine, tyrosine, tryptophan, methionine, cysteine). Synthesis of NAD from tryptophan. Synthesis of creatine and creatine phosphate, its significance for humans.
39. Glycine metabolism. Glutathione: structure and function in humans.
40. Hereditary pathologies of amino acids metabolism (phenylketonuria, alkaptonuria, albinism, tyrosinosis and tyrosinemia states, aminoaciduria states).
41. Common notions about Vitamins and their role in metabolism. Provitamins. Classification of vitamins. The features of absorption for water-soluble and fat-soluble vitamins in the gastro-intestinal tract.
42. Hypovitaminosis (vitamins deficiency): exogenous and endogenous reasons of their development. Avitaminosis (examples).
43. Water-soluble vitamins (H, B<sub>1</sub>, B<sub>2</sub>, PP (B<sub>3</sub>), B<sub>5</sub>, B<sub>6</sub>, B<sub>9</sub>, B<sub>12</sub>): structure, sources of reception, daily requirement, biological role.
44. Vitamins C and P: structure, mechanisms of function in humans, daily requirement, and clinical symptoms of their deficiency.
45. Vitamin-similar substances (CoQ, carnitine, lipoic acid): structure and function in humans.
46. Hypervitaminosis state for fat-soluble vitamins.
47. A group of vitamin A (retinol, retinal, retinoic acid) and  $\beta$ -carotenes: structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency
48. A group of vitamin E (tocopherols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.

49. A group of vitamin D (D<sub>2</sub>, D<sub>3</sub>, calcitriols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency in children (1-3 years of age) and in adults.
50. A group of vitamin K (naphthoquinones): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
51. Vitamin F (a complex of unsaturated high fatty acids): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
52. Antivitamins: the examples of their mechanism of action.
53. Interaction of vitamins. Combined vitamin drugs (multi-vitamins) in the prophylactic and treatment of some diseases.

### **The questions for laboratory works:**

1. The principle of glucose oxidase method for glucose content determination in the blood plasma or serum. The value of glucose content in the blood plasma of healthy and diseased patients (diabetes mellitus: latent and severe forms).
2. The determination of the presence of lactic acid - the terminal product of anaerobic glycolysis using the Uffelmann's test (the principle of the method).
3. 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.
4. Pyruvic acid content determination in the urine (the principle of the method, and its clinical significance).
5. Total cholesterol content determination in the blood plasma by Ilk's method (the principle of the test, and its clinical significance).
6. The determination of transaminases (AlAT, AsAT) activity in the blood serum. De Rittis coefficient. The clinical significance of these tests.
7. The determination of urea content in the urine and the blood serum. Try to write the sequence of reactions to form urea in humans.
8. Determination of  $\beta$ -lipoproteins (LDL) content in the blood serum (the principle of the test, and its clinical significance).
9. Principle of the method and the importance of ascorbic acid content determination in food sources and in the urine of patients

### **Literature (p.97)**

## **Lesson 19**

### **TEST LESSON OF MODULE 1**

#### **“Common regularities of metabolism and energy exchange in humans. Metabolism of carbohydrates, lipids, amino acids 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 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 centres for simple and conjugated enzymes. The role of vitamins in the formation of active centre of enzymes (B<sub>1</sub>, B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub>).
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 plasm. (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 (K<sub>m</sub>) 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 occurrence 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 Amphibolic 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. Chemiosmotic Theory (P.Mitchell, 1961) in the explanation of Oxidative phosphorylation mechanism. ATP synthetase: location, structure and function.
32. Inhibitors of tissue respiration and Uncouplers of oxidative phosphorylation: the representators and mechanism of their action. The estimation of respiratory control.
33. Chemical composition of nutritive substances, and their significance for human body. Biological value of carbohydrates, lipids, proteins, vitamins, and mineral components from foods for humans.
34. Daily requirement of carbohydrates for humans, and their digestion in the gastro-intestinal tract. Enzymes for digestion of carbohydrates: the location of the synthesis, their pH medium and specificity of function. Lactase deficiency in some patients.
35. Terminal products for digestion of carbohydrates, and their mechanism of absorption in the small intestine.
36. Daily requirement of lipids for humans, their digestion in the gastro-intestinal tract. Enzymes for digestion of lipids: the location of synthesis and the factors for their activation, pH medium and specificity of function.
37. Bile acids function in the gastro-intestinal tract. Steatorrhea: the reasons of the development and its diagnostic.
38. Nitrogen balance (positive and negative); daily requirement of proteins for humans. Enzymes for digestion of proteins: the location of synthesis and the factors for their activation, pH medium and specificity of function.
39. Mechanisms of amino acids absorption in the small intestine.

40. Aerobic oxidation of glucose: stages of oxidation, biological role, a location of each stage in a cell.
41. Aerobic and anaerobic glycolysis: reactions, location in a cell and biological role. The key enzymes of glycolysis and their regulation. Interrelation between anaerobic and aerobic ways in a cell. Hydrogen ions transport from cytosolic NADH into mitochondria matrix. Shuttle systems function. Power balance of process in both conditions.
42. Oxidative decarboxylation of pyruvate. The composition of pyruvate dehydrogenase complex. The mechanism and the regulation of this complex function.
43. Power balance of full aerobic glucose oxidation up to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .
44. Pentose Phosphate Cycle: the reactions and regulation of two stages, and biological role. Interrelation between Pentose Phosphate Cycle and glycolysis.
45. Gluconeogenesis: the condition of its stimulation in the liver and kidney, substrates, the key enzymes, the reactions of process from lactate, a biological role. The balance equation of glucose formation from pyruvate. Energy provision of gluconeogenesis from lactate.
46. Correlation between glycolysis and gluconeogenesis. The Reciprocal regulation of glycolysis and gluconeogenesis. A Cori Cycle.
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51. Pathologies of carbohydrates metabolism: Diabetes mellitus (two types); Glycogen storage diseases.
52. Carbohydrates and their derivatives as medicines.
53. Triacylglycerols metabolism (synthesis, lipolysis) in the liver and adipose tissue: enzymes, their regulation of activity, and biological role of processes. The aerobic oxidation of glycerol in tissues.
54. Glycerophospholipids: structure and biological role, their metabolism in tissues. Sphingolipidoses and Mucopolipidoses – the inherited disorders of phospholipids breakdown in tissues.
55. Cholesterol synthesis and its breakdown in the liver. Bile acids: their structure, and biological role.

56. Cholesterol catabolic pathways in adrenal cortex: the key metabolites, and terminal products function in humans. The calcitriols formation from cholesterol in humans.
57. Plasma lipoproteins: a classification; the structure and composition; metabolism; the biological role; researching methods.
58. Synthesis and  $\beta$ -oxidation of high fatty acids: reactions, the balance equation, power provision (or effect), intracellular location, regulation, biological role. The features of unsaturated high fatty acids metabolism.
59. Ketone bodies metabolism, its regulation and significance. The infringement of this metabolism at starvation and at diabetes mellitus.
60. Hormonal regulation of lipids metabolism (insulin, epinephrine, somatotropin, corticotropin, lipotropin, thyroid hormones).
61. Pathologies associated with the infringement of lipid metabolism (atherosclerosis, obesity, diabetes mellitus): the molecular mechanism of clinical symptoms development, and diagnostic using the indexes of blood plasm.
62. The metabolic pathways providing the pool of free amino acids in the blood. Non-essential and essential amino acids.
63. The Transport mechanism for amino acids across the cellular membrane.
64. The Conversions of amino acids by the action of intestinal microflora. Neutralization of toxic products in the liver. Quick's test.
65.  $\alpha$ -Decarboxylation as the way for biogenic amines formation. Biological role of histamine, serotonin,  $\gamma$ -aminobutyric acid, epinephrine). The utilization of biogenic amines by Monoaminooxidase.
66. Transamination: the mechanism of the reaction, structure of enzymes, and biological role. Clinical significance of transaminases activity determination in the blood plasma.
67. Types of Deamination. Oxidases (L- and D-) function. Glutamate dehydrogenase reaction, its significance and regulation. Indirect deamination.
68. The Ways for the transformation of nitrogen-free residues of amino acids. Glucogenic and ketogenic amino acids.
69. Common notions about the ways for formation and neutralization of ammonia in an organism. Urea cycle: partial reactions, the regulation, and biological role. Inherited disorders associated with the infringements of Urea cycle function.
70. The common representations about the metabolism of some amino acids (phenylalanine, tyrosine, tryptophan, methionine, cysteine). Synthesis of NAD from tryptophan. Synthesis of creatine and creatine phosphate, its significance for humans.
71. Glycine metabolism. Glutathione: structure and function in humans.
72. Hereditary pathologies of amino acids metabolism (phenylketonuria, alkaptonuria, albinism, tyrosinosis and tyrosinemia states, aminoaciduria states).

73. Common notions about Vitamins and their role in metabolism. Provitamins. Classification of vitamins. The features of absorption for water-soluble and fat-soluble vitamins in the gastro-intestinal tract.
74. Hypovitaminosis (vitamins deficiency): exogenous and endogenous reasons of their development. Avitaminosis (examples).
75. Water-soluble vitamins (H, B<sub>1</sub>, B<sub>2</sub>, PP (B<sub>3</sub>), B<sub>5</sub>, B<sub>6</sub>, B<sub>9</sub>, B<sub>12</sub>): structure, sources of reception, daily requirement, biological role.
76. Vitamins C and P: structure, mechanisms of function in humans, daily requirement, and clinical symptoms of their deficiency.
77. Vitamin-similar substances (CoQ, carnitine, lipoic acid): structure and function in humans.
78. Hypervitaminosis state for fat-soluble vitamins.
79. A group of vitamin A (retinol, retinal, retinoic acid) and  $\beta$ -carotenes: structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency
80. A group of vitamin E (tocopherols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
81. A group of vitamin D (D<sub>2</sub>, D<sub>3</sub>, calcitriols): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency in children (1-3 years of age) and in adults.
82. A group of vitamin K (naphthoquinones): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
83. Vitamin F (a complex of unsaturated high fatty acids): structure, sources of reception, daily requirement, biological role; clinical symptoms of deficiency.
84. Antivitamins: the examples of their mechanism of action.
85. Interaction of vitamins. Combined vitamin drugs (multi-vitamins) in the prophylactic and treatment of some diseases.

### **The questions for laboratory works of module 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 plasma 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  $\beta$ -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 (p.97)**



## LITERATURE

### Basic:

1. Berezov T. T. Biochemistry : translated from the Russian / T. T. Berezov, B. E. Korovkin. - M. : Mir, 1992. - 514 p.
2. Ferrier D. R. Biochemistry : Lippincott illustrated reviews / D. R. Ferrier ; ed. by.: R. A. Harvey. - 6th ed. - India : Lippincott Williams & Wilkins, 2015. - 560 p.
3. Murray R. K. Harper's Illustrated Biochemistry / R. K. Murray, D. K. Granner, V. W. Rodwell. - 27th ed. - Boston [etc.] : McGraw Hill, 2006. - 692 p.
4. Satyanarayana U. Biochemistry : with clinical concepts & case studies / U. Satyanarayana, U. Chakra Pani. - 4th ed. - India : Elsevier, 2015. - 812 p.

### Additional:

1. Davidson V. L. Biochemistry / V. L. Davidson, D. B. Sittman. – USA: Harwal Publishing. – 1994. – 584 p.
2. Lehninger A. Principles of Biochemistry / A. Lehninger, D. L. Nelson, M. M. Cox. - 4th ed. - New York : W.H. Freeman and Company, 2005. - 1216 p.
3. Lieberman M. Marks' Essential Medical Biochemistry / M. Lieberman, A. Marks, C. Smith. - 2nd ed. - Lippincott Williams & Wilkins. – 2007. – 540 p.
4. Marshall W. J. Clinical Chemistry / W. J. Marshall, S. K. Bangert. – Fifth edition. – China :Mosby. – 2004. – 422 p.
5. Smith C. Marks' Basic Medical Biochemistry: A Clinical Approach / C. Smith, A. Marks, M. Lieberman. - 2nd ed. – Lippincott Williams & Wilkins. – 920 p.

FOR NOTES

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