МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

BIOLOGICAL CHEMISTRY
Part 1
THE GENERAL PRINCIPLES OF METABOLISM

Study guide
for students of general medicine faculty

БІОЛОГІЧНА ХІМІЯ
Частина 1
ЗАГАЛЬНІ ЗАКОНОМІРНОСТІ ОБМІНУ РЕЧОВИН

Методичні вказівки
для підготовки студентів медичних факультетів
до практичних занять

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CLASS 1 (4 hours)

Topic 1 (2 hours): Introduction to biochemistry. Development of biochemistry as a science. Biochemical components of cells. Features of labor in biochemical laboratories.

Importance. Biochemistry is the science of molecular essence of life. It examines the chemical nature of the substances that make up living organisms, their transformation and the relationship between these changes and the activities of cells, organs, tissues and organism as a whole. Biochemistry examines the chemical basis of life under normal and pathological conditions and therefore it has important practical implications for medicine.

Objective. Consider the stages of the biochemistry development as fundamental biomedical science and the role of biochemical investigation of the functional state of the human organism under normal and pathological conditions. Study the biochemical functions of the major classes of biomolecules in cells. Master the safety rules during work in biochemical laboratories.

THEORETICAL QUESTIONS

1. Biological chemistry as a science: subject, objectives, major historical stages and modern trends of development.
2. Purposes and methods of biomedical research, its clinical and diagnostic value.
3.* Relations of biochemistry to other medical and biological sciences. Medical biochemistry. Clinical biochemistry. Laboratory diagnostics.
4.* International history of biochemistry and development of biomedical research in Ukraine.
5.* Chemical composition of living organisms, its features compared with inanimate objects. The chemical composition of the human body.
6.* Biochemical components of cells (biomolecules) and their functions.
7.* The structure of prokaryotic and eukaryotic cells.
8.* Autotrophic and heterotrophic organisms.

QUESTIONS TO CONTROL THE BASIC LEVEL OF KNOWLEDGE

1. General concepts of bioorganic chemistry: polarity, hydrophobicity and hydrophilicity of organic molecules, acidic, basic and amphoteric properties of organic molecules.
2. Characteristic features of the structure of alcohols, aldehydes, ketones, carboxylic acids and amines.
3. Structure of individual representatives of classes of organic compounds: ethanol, glycerol, acetic, succinic, fumaric, palmitic, oleic, pyruvic, oxaloacetic, ketoglutaric, lactic, malic acids, acetaldehyde, acetone, ethanolamine, choline.

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* – Questions to self-study

4. The mechanism of esters formation (for example, triacylglycerols) and their biological role.

5. The general idea of lipids and their classification. The biological role of different classes of lipids.

6. Features of the structure and biological role of sugars (glucose, fructose, galactose, ribose, deoxyribose), oligosaccharides (lactose, sucrose, maltose), polysaccharides (starch, glycogen, cellulose).

7. Classification and properties of α-amino acids. Structure of individual respesentatives of amino acids (glycine, alanine, cysteine, serine, glutamic acid, lysine, phenylalanine, tryptophan, methionine).

8. Proteins: mechanism of peptide bond formation, levels of structural organization, biological role.

9. Nucleic acids, nucleosides, nucleotides: specificity of structure and composition, biological role.

INSTRUCTION
Safety rules during work in the laboratories of the Biochemistry Department of KhNMU

General rules

1. All work must be carried out in the laboratory in laboratory uniform (medical coat and cap). If working with reagents, pay strong attention to the label on containers with chemicals.

2. All procedures (measuring reagents, their transfusion, heating, etc.) may be performed only on the chemical table in a fume hood.

3. Do not perform reactions, whose outcome is unknown.

4. Make all experiments with poisons and substances that have unpleasant odor in the fume hood.

5. Do not walk in the laboratory with concentrated acids, pour them only in designated places.

6. If you need to smell the odor of a chemical, fan some of vapor toward your nose with one hand.

7. Do not contaminate reagents during operation (do not confuse corks from different glasses containing various reagents, do not pour back taken reagent excess into glass, each reagent type is taken by separate pipette, in any case do not confuse them).

8. After work put reagents to their places, wash the dishes, clean your workplace.

9. After work wash your hands.

10. No eating in the workplace.

11. Cover the flames with cloth or sand in case of a fire in the laboratory.

Rules for working with acids, alkalis and other potent reagents

1. Never permit acids or alkalis to come in contact with your skin, face, eyes and clothes.

2. All work with acids, alkalis and other potent chemicals should be done very carefully and cautiously.

3. Strong acids and alkalis (10 % and above), as well as other potent reagents are not taken into pipettes with mouth. It can cause chemical burns of oral cavity. These reagents should be taken using rubber bulbs, cylinder or dropper.

4. Dip reagent pipette to the bottom of a glass during measurement. During filling pipettes, watch them.

5. Do not put the dropper on the table after measuring reagent, put it in bowl for washing.

6. Collect flammable liquids in special airtight containers for later regeneration or disposal. Do not pour the reagents in the sewer.

7. If the reagent comes in contact with your skin, wash the affected area with water, then neutralize it with 3 % Na_2CO_3 solution if it is acid or with 3 % acetic acid in case of alkali.

8. If the reagents are spilled on the table, neutralize acids with soda and alkalis with a weak solution of acetic acid, then clean the table with water.

Rules for working with an open flame

1. The test tube should be held far from other students during heating, do not touch the source of flame with the test tube, always be very careful during heating, avoid spitting of fluid (occasionally move the test tube from the flame, do not heat it in a vertical position); do not approach your face to the test tube where the liquid is heating.

2. When the liquid is boiling remove it from the flame.

3. Use special clips for tubes in case of prolonged boiling.

4. Put cotton with alcohol on the affected area when you burn your skin.

Rules for work with electrical appliances

1. Flammable substances (ether, gasoline, alcohol, etc.) must not be placed near electrical appliances.

2. Cones with boiling liquid must be removed from the tiles with special clamp.

3. Do not touch electrical appliances and knife switches with wet hands.

REFERENCES

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Topic 2 (2 hours): Principles of biocatalysis. The structure and physical and chemical properties of enzymes. Classification and nomenclature of enzymes. Influence of temperature and pH on enzyme activity.

Importance. Enzymes are biological catalysts that are present in all cells, tissues and biological fluids providing a course of chemical reactions in the organism. Unlike inorganic catalysts (metals, acids etc.) enzymes have high efficiency and high specificity of action and can accelerate the reaction under mild conditions. Enzymes are thermolabile, their activity depends on the pH values of the medium. Synthesis and catalytic activity of enzymes are controlled by different regulatory mechanisms. Modern methods of isolation and purification of enzymes make possible to study their structure, conditions of activity manifestation, mechanism of action.

Objective. Identify the basic principles of catalysis. Study the biochemical concepts of structure and functioning of the different classes of enzymes. Familiarize with classification and nomenclature of enzymes. Be able to illustrate the difference between the enzymes and inorganic catalysts (specificity of action, high efficiency of catalysis, ability to catalyze under mild conditions, ability to be regulated, etc). Study the effect of temperature and pH on the activity of salivary α-amylase.

THEORETICAL QUESTIONS

1. The general idea of catalysis. Basic principles of catalysis.
2. The theory of biological catalysis.
3. The chemical nature of enzymes. Factors that contribute to a variety of enzymes.
4. The difference between enzymes and inorganic catalysts.
5. Structure of simple and complex enzymes. The concept of apoenzyme, cofactor, coenzyme and prosthetic group. Features of the structure of the active site of simple and complex enzymes. Allosteric site.
6. General properties of enzymes: thermolability, dependence on pH, specificity of action.
7.* Oligomeric proteins-enzymes, multienzyme complexes and membrane-associated enzymes.
8.* Isoenzymes: structural peculiarities, localization of synthesis in the human body (for example, lactate dehydrogenase and creatine phosphokinase isoenzymes), role in the diagnosis of diseases.
9. Classification and nomenclature of enzymes. Characteristics of types of chemical reactions that underlie the classification of enzymes.

Indicative list of theoretical questions for self-study

Content	*The main theses*
1. Oligomeric proteins-enzymes consist of several polypeptide chains (subunits, protomers), linked by non-covalent bonds.	*1.1. Oligomeric enzymes, consisting of identical or different chemical nature subunits, capable of performing catalysis after association into complex. Examples: hexokinase, LDH.* *1.2. Oligomeric enzymes consisting of different subunits on structure and biochemical functions. Examples: aspartate carbamoyltransferase (6 catalytic and 6 regulatory subunits).*
2. Polyenzyme complexes catalyze the reactions of the serial conversion of the substrate.	*2.1. The biological sence of associating several enzymes in the complex: a distance at which a molecule intermediates should move from the enzyme to the enzyme is sharply reduced; and therefore a sufficiently high total rate of metabolic pathways is provided.* *2.2. Examples: pyruvate dehydrogenase multienzyme complex – catalyzes reactions of serial conversion of pyruvate to acetyl-CoA; α-ketoglutarate dehydrogenase multienzyme complex – catalyzes reactions of serial conversion of α-ketoglutarate into succinyl-CoA in the citric acid cycle.* *2.3. Types in the cell:* *– soluble multi enzyme complexes in which the permanent association between enzymes is absent, and the substrates and reaction products diffuse between individual enzymes;* *– multienzyme complexes, in which the enzymes are linked by non covalent bonds, which facilitates the transfer between them of substrates and products;* *– multienzyme complexes in which individual enzymes are associated with the lipid bilayer of subcellular organelles*
3. The membrane-associated enzymes are enzymes associated with the lipid bi-layer of the plasma membrane and membranes of cellular organelles (mitochondria, endoplasmic reticulum etc.)	*Association enzymes with membranes provides: their localization in a certain part of the cell and/or in the region of the membrane, which concentrates the substrate (e.g., acetylcholinesterase in the presynaptic membrane, where acetylcholine is concentrated); opportunity for coupling of processes of catalysis and transmembrane transport (Na^+, K^+ -ATPase); availability of water-insoluble substrates (e.g., protein kinase C, phospholipase); formation of the optimal microenvironment that creates native conformation and catalytic activity of the membrane-associated enzymes*

Content	*The main theses*
4. Isoenzymes are multiple molecular forms of the same enzyme, which differ in primary structure, physico-chemical properties, the activation conditions, but catalyze the same biochemical reaction; *Isoezymes are the result of the expression of different genetic loci.*	*4.1. The presence of isoenzymes in different organs and tissues, subcellular structures* *4.2. Oligomeric structure:* *– lactate dehydrogenase (LDH) – tetramer – has protomers of two types: H (heart) and M (muscle) LDH_1 (H_4), LDH_2 (H_3M_1) LDH_3 (H_2M_2) LDH_4 (H_1M_3) LDH_5 (M_4) → determination of the activity of isoforms in the blood has a diagnostic significance ($LDH_{1,2}$ – myocardial infarction, $LDH_{4,5}$ – infectious or toxic hepatitis, liver cirrhosis);* *– creatine phosphokinase (CPK, CK) – dimer – has protomers of two types: M (muscle) and B (brain): BB isoform of CK (brain), MB-isoform of CK (heart), the MM isoform of CK (muscle) → definition of the activity of isoforms in the blood has diagnotic significance (MB-CK – myocardial infarction, MM-CK – traumatic injury of muscles and muscular dystrophy; BB-CK isoform has no diagnostic significance, due to the failure to pass the blood-brain barrier)*

TESTS FOR SELF-CONTROL

1. The man adressed to the doctor after the onset of chest pain. A significant increase of enzyme activities (creatine kinase and its MB-isoform, aspartate aminotransferase) was revealed in the blood serum. Where is the localization of pathological process?

A. Lungs. *B. Heart.* *C. Muscle.* *D. Liver.* *E. Smooth muscle.*

2. The biological oxidation is the main molecular mechanism for providing energy of living organisms. Which class of enzymes catalyzes this process?

A. Hydrolases *C. Oxidoreductases* *E. Transferases*
B.Lyases *D. Ligases*

3. Biogenic amines are formed by decarbolylases. What is the class of these enzymes?

A. Lyases. *C. Oxidoreductases.* *E. Transferases.*
B. Isomerases. *D. Hydrolases.*

4. Five isoenzyme forms of LDH were identified from human serum and their properties were studied. What property indicates that the isoenzyme forms of the same enzyme were isolated?

A. The same molecular weight.
B. Tissue localization.
C. The same physical and chemical properties.
D. They catalyze the same reaction.
E. The same electrophoretic mobility.

5. The structural feature of regulatory enzymes is the presence of an allosteric center. Specify its role.

A. Binding the substrate.
B. Changing the structure of the substrate.
C. Binding the regulatory effector.
D. Promotes dissociation of coenzyme.
E. Binding coenzyme.

6. Indicate an enzyme class of glucokinase catalyzing the reaction of phosphate group transfer from ATP to glucose.

A. Transferases. *C. Isomerases.* *E. Lyases.*
B. Oxidoreductases. *D. Hydrolases.*

7. Enzyme D-amino acid oxidase catalyzes deamination of D-amino acids only. Which property of the enzyme provides this?

A. Stereochemical specificity. *D. Dependence on pH.*
B. Thermolability. *E. Absolute specificity.*
C. Rekative specificity.

8. Conversions of proline into hydroxyproline and lysine into hydroxylysine in collagen molecule are catalyzed by:

A. Hydroxylases. *C. Dehydrogenases.* *E. Dehydrases.*
B. Hydrolases. *D. Oxidases.*

9. The increase of LDH_4, LDH_5, alanine aminotransferase, ornithine carbamoyl transferase activities is observed in in the blood of the patient. Where is the localization of pathological process?

A. Heart (myocardial infarction). *C. Liver (hepatitis).* *E. Connective tissue.*
B. Skeletal muscle. *D. Kidney.*

10. The activity of some enzymes and their isozyme forms is determined in the blood for biochemical diagnosis of myocardial infarction. Which enzymatic test is the best one to confirm or exclude the diagnosis of myocardial infarction in the early period after the onset of chest pain?

A. Isoenzyme MM of creatine kinase.
B. Isoenzyme LDH_1 of lactate dehydrogenase.
C. Isoenzyme MB of creatine kinase.
D. Isoenzyme LDH_5 of lactate dehydrogenase.
E. Cytosolic isoenzyme of aspartate aminotransferase.

11. Choose the substance, which is not able to be substrate for enzymes of human organism:

A. Glucose. *C. Nitric acid.* *E. Glycogen.*
B. Fatty acid. *D. Active form of acetic acid.*

12. Indicate a substrate degraded by hydrolases:

A. Fatty acids. *C. Glucose.* *E. Carbon dioxide.*
B. Proteins. *D. Pyruvate.*

13. Indicate a feature that constitutes the basis of enzyme classification:

A. Reversibility of reaction. *D. Type of reaction catalyzed.*
B. Chemical structure of enzyme. *E. Chemical structure of substrate.*
C. Type of enzyme specificity.

14. Enzyme urease is able to break down only the structure of urea. Indicate the type of its specificity:

A. Stereochemical. *C. Absolute group.*
B. Absolute. *D. Relative group.*

15. How are enzymes called that catalyze the same reaction, but differ one from another by their primary structure and physico-chemical properties?

A. Isoenzymes *C. Zymogens* *E. Apoenzymes*
B. Holoenzymes *D. Cofactors*

16. Which of the below mentioned properties is characteristic only for biologic catalysts?

A. Increase a velocity of reaction, but are not consumed and are not ir reversibly changed.
B. Increase a velocity of reaction, decreasing energy of activation.
C. Do not change the state of equilibrium of chemical reaction.
D. Ability to regulation.

17. Indicate the substrate of salivary amylase:

A. Protein. *C. Sucrose.* *E. Amino acid.*
B. Starch. *D. Glucose.*

18. Give the full name of conjugated enzyme, polypeptide chains of which are combined with nonprotein part:

A. Prosthetic group *C. Coenzyme.* *E. Holoenzyme.*
B. Cofactor. *D. Apoenzyme.*

19. Choose non-protein part of enzymes used for formation of fatty acid active forms:

A. CoQ. *B. HSCoA.* *C. TPP.* *D. NADP.* *E. FMN.*

20. Enzymes increase the velocity of reaction, because they:

A. Change free energy of reaction.
B. Decrease the velocity of reverse reaction.
C. Decrease the energy of activation.
D. Change the state of equilibrium of chemical reaction.

21. Indicate the optimal pH for pepsin:

A. 1.5–2.5. *B. 4–5.* *C. 6–7.* *D. 8–9.* *E. 10–11.*

22. Indicate the optimal pH for amylase:

A. 1.5–2. *B. 7–7.5.* *C. 8–9.* *D. 3.5–4.* *E. 4.5–5.*

23. Indicate the optimal temperature for action of the most enzymes:

A. 50–60 °C. *B. 15–20 °C.* *C. 80–100 °C.* *D. 35–40 °C.*

PRACTICAL WORK

Detection of enzymes in biological objects.
The influence of temperature and pH on enzyme activity

Task 1. Identify the salivary enzyme α-amylase that hydrolyzes starch to disaccharide maltose and dextrins.

α-amylase of saliva (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1), which has a relative group specificity, cleaves α-1,4-glycosidic bonds in polysaccharides and does not act on disaccharides.

Principle. The enzyme activity is estimated by its effect on the substrate: the disappearance of substrate or the appearance of its cleavage products. Breakdown of starch is detected by negative reaction with Lugol's iodine reagent.

Procedure. Add 0.5–1 ml of saliva to the test tube, add 3–5 ml of 1 % starch solution, mix and put in a thermostat at 37 °C for 15–20 min. Then add to the test tube 3–4 drops of Lugol's iodine reagent (solution of J_2 in KJ).

Task 2. Verify the thermolability of amylase.

***Principle*:** the same.

Procedure. Add to the test tube 0.5–1 ml of saliva, 1 ml of distilled water, heat it in a flame and cool, then pour 3–5 ml of 1 % starch solution, mix and put in a thermostat at 37 °C for 15–20 min. Then pour into the test tube 3–4 drops of iodine reagent.

Task 3. Verify the influence of pH on the activity of amylase.

***Principle*:** the same.

Procedure. Add 0.5–1 ml of saliva to two test tubes, pour into the first one 1 ml of 0.4 % solution of NaOH, into the second test tube pour 1 ml of 0.4 % HCl, then add 3.5 ml of 1 % starch solution and place test tubes in a thermostat at 37 °C for 15–20 min. Taking into consideration that NaOH reacts with J_2 ($NaOH + J_2 = NaJ + H_2O$), before adding iodine reagent to the test tube with NaOH pour 1 ml of 0.4 % HCI to neutralize the alkali.

Fill in the table:

№ of test tube	Enzyme	Substrate	Conditions of experiment	Results of reaction	Conclusions

Practical significance. Knowledge of the general properties is necessary for choosing the optimal conditions of enzymes during determination of their activity in research and clinical trials. Incorrectly chosen conditions lead to errors in the diagnosis of disease and monitoring the quality of enzymatic drugs.

1.** Prepare the abstract on the theme: "The occurrence of hyperamylasemia and hyperamylasuria in disturbance of the functioning pancreas."

2.** Prepare the abstract on the topic: "The ribozymes – biological catalysts of non-protein nature."

** – tasks for individual self-study

CLASS 2 (4 hours)

Topic 3 (2 hours): The mechanism of enzyme action and kinetics of enzymatic reactions. Determination of enzyme activity.

Importance. Methods for determination of the activity of enzymes in biological fluids and tissues are widely used in modern biochemistry. It helps to understand the pathogenesis of many diseases and to suggest methods of treatment using modern medicines.

Objectives. Be able to analyze the mechanisms of enzyme action and kinetics of enzymatic reactions. Familiarize yourself with the methods of qualitative and quantitative determination of enzyme activity in biological objects, which allow studying the properties of enzymes and especially their action and regulation, as well as the basis of the diagnosis and prognosis of many diseases. Determine the specificity of the enzymes salivary α-amylase and yeast sucrase.

THEORETICAL QUESTIONS

1. Modern theories of the mechanism of enzyme action: the concept of activation energy, formation of enzyme-substrate complex and the mechanisms of enzymatic reaction products formation (covalent and acid-base catalysis). The significance of E. Fisher's and D. Koshland's researches.

2. Activity of enzymes. Units of activity and quantity of enzymes: international unit, katal, specific activity.

3. Factors affecting enzyme activity: the concentration of substrate, enzymes and products of reaction, temperature, pH.

4. Methods of enzymes allocation from biological objects and their fractionation (ultracentrifugation, gel and ion exchange chromatography, affinity chromatography, electrophoresis) and analysis of enzyme activity.

5. Methods for determination of enzyme activity: by the quantity of the product formed by the enzyme per unit of time; by the quantity of substrate consumed per unit of time. Spectrophotometric methods for determination of enzyme activity and visualization of results of enzymatic reactions.

6.* Kinetics of enzymatic reactions: the effect of substrate and enzyme concentration on the rate of enzymatic reactions (graphic dependence). Michaelis-Menten equation. Michaelis constant, its determination and significance.

Indicative list of theoretical questions for self-study

Content	*The main theses*
1. The kinetics of enzymatic reactions is the section of chemical kinetics which studies the influence of different chemical and physical factors on the reaction rate	*1.1. The dependence of the rate of enzymatic reaction on the enzyme concentration, substrate concentration, temperature and pH of the medium, the action of inhibitors and activators.* *1.2. The general equation of the enzymatic reaction.* *1.3. Michaelis-Menten theory: dissociation constant and Michaelis constant; their definition and meaning.*

Content	*The main theses*
	1.4. Michaelis-Menten equation – the dependence of the reaction rate on the substrate concentration. *1.5. Lineweaver-Burk equation.*

TESTS FOR SELF-CONTROL

1. The Michaelis constant determines:

A. The affinity of enzyme to product of reaction.
B. The affinity of enzyme to substrate of reaction.
C. The affinity of enzyme to inhibitor.
D. Average velocity of enzymatic reaction.
E. Maximal velocity of enzymatic reaction.

2. Continue the sentence «Minimal change of pH of medium influences the enzyme molecule, changing…»:

A. Level of structure of enzyme molecule.
B. Ionization degree of acidic and basic groups of active site of enzyme.
C. Thickness of hydrate sheet of enzyme.
D. Optical properties of enzyme.

3. Which parameter is used for calculation of specific activity if the general activity is known?

A. Concentration of the enzyme in investigated sample.
B. Concentration of the protein in investigated sample.
C. Concentration of the substrate in investigated sample.
D. The Michaelis constant of this enzyme.
E. Maximal velocity of investigated enzymatic reaction.

4. Give the name of scientist, who proposed the hypothesis of «induced fit»:

A. H. Krebs. *C. M. Menten.* *E. K. Funk.*
B. D. Koshland. *D. F. Crick.*

5. Indicate the factor, which does not influence the constant of dissociation of enzyme-substrate complex:

A. Concentration of the substrate.
B. Chemical nature of enzyme.
C. Concentration of the enzyme.
D. Concentration of the enzyme-substrate complex.
E. The affinity of enzyme to substrate.

6. What happens during the formation of enzyme-substrate complex?

A. The conformation of substrate is changed.
B. The conformation of enzyme is changed.
C. The induced complementarity between the enzyme and the substrate is established.
D.The functional groups participating in catalysis are approached.

7. Which of below mentioned properties of enzymes lies in the base of their qualitative and quantitative determination in biological material?

A. Ability to express maximal activity in defined value of pH of medium.

B. Dependence on the presence in medium of the different activators and inhibitors.

C. Specificity.

D. Thermolability.

E. Inhibition of reaction by its products.

8. Yeast extract had been added to the test tube with unknown substrate. Mixture in the test tube gave the positive reaction with Fehling's reagent after 15 minutes of incubation. Which substrate was in the test tube?

A. Starch. B. Sucrose. C. Lactose. D. Glycogen. E. Cellulose.

9. Reaction mixture gives yellow color with iodine and positive Fellinge's reaction after 10 minutes of incubation of starch with saliva. The mixture contains:

A. Maltose and dextrins. *C. Sucrose D. Lactose.*

B. Fructose and glucose.

10. Which event lies in the base of mechanism of enzyme action?

A. The approchement of groups included into active site of enzyme.

B. The formation of enzyme-substrate complex.

C. The change of electrostatic charge of enzyme.

D. The change of spatial conformation.

E. The hydrolysis of enzyme.

11. Indicate the method used for separation of subcellular fractions from tissue homogenates:

A. Dialysis. *D. Differential centrifugation.*

B. Isoelectric focus. *E. X-ray analysis.*

C. Qualitative analysis.

12. Choose the unit of enzyme activity expressed by amount of enzyme that converts 1 mole of substrate per second under optimal conditions:

A. Katal. *D. Turnover number.*

B. Standart unit of activity of enzyme. *E. Molar activity.*

C. Specific activity.

PRACTICAL WORK

Determination of specificity of the enzymes salivary α-amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) and yeast sucrase (β-D-fructofuranoside fructohydrolase, EC 3.1.1.26)

Task 1. Prepare a yeast extract containing enzyme sucrase.

Procedure. Grind a piece of yeast with 10–15 ml of distilled water in a mortar, and filter to flask.

Task 2. Verify the action of amylase on starch and sucrose.

Principle. The enzyme activity is estimated by its effect on the substrate: the disappearance of substrate or the appearance of products. Breakdown of starch is detected by negative reaction with Lugol's iodine reagent. Cleavage

of sucrose is found by positive reaction with Fehling's reagent, which is positive for products of sucrose hydrolysis (glucose and fructose), but negative for sucrose.

Procedure. Add to two test tubes 0.5–1 ml of saliva: add to the first one 3.5 ml of 1 % starch solution and to the second one 3–5 ml of 1 % sucrose solution, mix well and put in a thermostat at 37 °C for 15–20 minutes. After this add to the first test tube 3–4 drops of Lugol's iodine reagent and make sure that starch is hydrolyzed; Fehling's reaction is carried out with the content of the second test tube and make sure that sucrose is not hydrolyzed.

Technique of Fehling's reaction: the same volume of Fehling's reagent ($CuSO_4$ + NaOH) is added to 1–2 ml of test solution and is heated on a flame. In the presence of monosaccharides in solution the red precipitate (Cu_2O) is formed as the result of reduction of copper due to oxidation of carbonyl groups of monosaccharides.

Task 3. Verify the action of sucrase on starch and sucrose.

***Principle*:** the same.

Procedure. Add to two test tubes 0.5–1 ml of yeast extract: add to the first one 3.5 ml of 1 % starch solution and to the second one 3–5 ml of 1 % sucrose solution, mix well and put in a thermostat at 37 °C for 15–20 minutes. After this add to the first test tube 3–4 drops of Lugol's iodine reagent and make sure that starch is not hydrolyzed; Fehling's reaction is carried out with the content of the second test tube and make sure that sucrose is hydrolyzed.

Fill in the table:

№ of test tube	Enzyme	Substrate	Conditions of experiment	Results of reaction	Conclusions

Practical significance. Enzymes with absolute substrate specificity are used in the clinic as analytical reagents for the determination of substances that are their substrates. For example, urease is used for determination of urea in biological material and medicines; glucose oxidase is used to determine the amount of glucose in the blood and urine. Enzymes with absolute and relative group substrate specificities have less selective action on substrates and are usually involved in the hydrolysis of nutrients or conversion of foreign compounds. For example, α-amylase exhibits specificity towards a definite type of glycosidic bonds in corresponding carbohydrates rather than to the structure of the substrate as entity.

Topic 4 (2 hours): Regulation of enzymatic processes. Inhibitors and activators of enzymes. Medical enzymology. Quantitative determination of the activity of α-amylase and lactate dehydrogenase in serum.

Importance. Achievements of enzymology (the science of enzymes) are widely used in medicine. Development of medical enzymology occurs in three main directions (enzymopathology, enzyme diagnostics and enzyme therapy), which solve the problems of enzymopathy pathogenesis, apply enzyme tests for the diagnosis of organic and functional disorders of organs and tissues, use enzymes and modulators of their action as medicines. Thus, increased activity of

alkaline phosphatase in serum is observed in rickets, tumors of bones, hyperparathyroidism, mechanical jaundice, viral hepatitis; and hypothyroidism, C-hypovitaminosis manifests by its decreased activity. Lactate dehydrogenase activity is increased in patients with damage to the myocardium, skeletal muscle, kidney, anemia, tumors, and acute hepatitis. Enzymes (pepsin, trypsin, hyaluronidase and others) are used as medicines, as well as analytical reagents (glucose oxidase, urease and others) are used in clinical and biochemical laboratories.

Objective. Learn and be able to analyze the mechanisms of regulation of enzymatic processes as the basis of metabolism under normal and pathological conditions; changes in the course of enzymatic processes and accumulation of metabolic intermediates in congenital (hereditary) and acquired defects of metabolism – enzymopathies; changes in activity of indicatory enzymes in the blood plasma in pathologies of certain organs and tissues, the use of enzymes and their modulators as pharmacological agents in certain pathological conditions. Familiarize with the methods of quantitative determination of activity of α-amylase and lactate dehydrogenase in serum and their clinical and diagnostic values.

THEORETICAL QUESTIONS

1. Regulation of enzymatic processes, its ways and mechanisms: allosteric interactions, covalent modification of enzymes, the effect of regulatory proteins called effectors (calmodulin, proteinases, and proteinase inhibitors). Cyclic nucleotides as regulators of enzymatic reactions and biological functions of cells.

2. Inhibitors and activators of enzymes. Types of enzyme inhibition: reversible (competitive, non-competitive) and irreversible. Examples.

3. Key aspects of modern enzyme diagnostics. Indicatory, secretory and excretory enzymes. Isoenzymes in enzyme diagnosctics, tissue specificity of isoenzyme distribution. Changes of enzyme activity in plasma and serum as diagnostic indices of pathological processes in organs and tissues.

4.* Application of enzyme diagnostics in cardiology, urology, oncology, etc.

5.* Disorders of enzymatic processes: congenital (hereditary) and acquired enzymopathies, congenital disorders of metabolism, their clinical and laboratory diagnosis.

6.* Enzyme therapy is the use of enzymes as medicines. Pharmacological application of enzymes of the gastrointestinal tract, coagulation and fibrinolytic systems, kallikrein-kinin and renin-angiotensin systems. Enzyme inhibitors as medicines.

Indicative list of theoretical questions for self-study

Content	*The main theses*
1. Enzymodiagnostics – diagnosis based on the determination of enzyme activity in body fluids	*1.1. Principles of enzymodiagnostics: organ specificity; increase in the activity of organospecific enzymes in blood as a result of damage to cell membrane; a sufficient amount of enzyme for determination in blood.*

Content	*The main theses*
	1.2. Enzymodiagnostics in cardiology: determination in blood of the activity of MB-isoform of creatine kinase; $LDH_{1,2}$; AsAT, etc. *1.3. Enzymodiagnostics in urology: determination in the blood of the activity of glycine aminidinotransferase, urokinase, arylsulfatase, AlAT, AsAT, LDH, aldolase, malate dehydrogenase (MDH), glutamine synthetase etc.* *1.4. Enzymodiagnostics in oncology: determining activity of enzymes of carbohydrate catabolism, anabolism of proteins and nucleic acids (in particular, hexokinase, pyruvate kinase, LDH, MDH, etc.) in the blood*
2. Enzymopathies – pathological conditions associated with defect enzymes	*2.1. The primary (hereditary) and secondary (acquired) enzymopaties. Causes.* *2.2. Affiliation to enzymopathies of inborn errors of metabolism: of simple and complex carbohydrates (galactosemia, fructosemia, glycogenoses, mucopolysaccharidoses, etc.); lipids (sphingolipidoses: Gaucher disease, Tay-Sachs, Niemann-Pick etc.); amino acids (phenylketonuria, alkaptonuria, tyrosinosis, albinism, hyperammonemia, cystinosis, maple syrup urine disease etc.); porphyrins (porphyria); purines and pyrimidines (Lesch-Nyhan syndrome, xanthinuria, orotic aciduria).*
3. Enzyme therapy – the use of enzymes as drugs	*3.1. Replacement therapy – normalization of digestion process:* *– pepsin, festal, trypsin, mezim, etc. (in deficiency of gastrointestinal tract enzymes, in particular, in inflammatory processes of stomach and intestine);* *– Creon, pancreatin, panzinorm (in disorders of pancreas function, in particular, pancreatitis, cystic fibrosis).* *3.2. Antitumor effect: asparaginase has antileukemic action, it hydrolyzes asparagine, which is necessary for development of leukemic cells.* *3.3. Improving the tissue respiration processes:* *– cytochrome c – in newborn asphyxia, asthma, chronic pneumonia, coronary heart disease, hepatitis.*

Content	*The main theses*
	3.4. Fibrinolytic agents: plasmin, urokinase, streptokinase – in the treatment of acute myocardial infarction. *3.5. Primary treatment of wounds, burns: trypsin, chymotrypsin.* *3.6. The treatment of viral diseases: nucleases (DNA-ase, RNA-ase) – in viral conjunctivitis.* *3.7. Using coenzymes: TPP – cocarboxylase – in the treatment of cardiovascular diseases.* *3.8. The use of enzyme inhibitors: in overdoses of thrombolytics (plasmin, streptokinase) – trasilol, contrycal, aminocapronic acid; in mechanical, thermal and chemical injuries, thrombosis; in oncology – inhibition of enzyme activity of tumor cells; in hypertension – angiotensin-converting enzyme inhibitor (enalapril, captopril, lysinopril)*

TESTS FOR SELF-CONTROL

1. An anti-inflammatory agent, that blocks the action of cyclooxygenase, was used for the patient treatment. This anti-inflammatory agent is referred to as:

A. Pepsin. *C. Allopurinol* *E. Analgin.*
B. Thiamine. *D. Aspirin.*

2. Proteolytic enzymes of the stomach and the pancreas are synthesized in an inactive form – as zymogens, and then activated in the digestive tract. Indicate the proteolytic enzyme of the stomach secreted in the inactive state.

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

3. To prevent attacks of acute pancreatitis a doctor prescribed trasylol (contrycal, gordox), which is an inhibitor of:

A. Trypsin. *C. Gastrixin.* *E. Carboxypeptidase.*
B. Chymotrypsin. *D. Elastase.*

4. Activity of which of the enzymes mentioned bellow is sharply increased in the blood and urine of patients with acute pancreatitis?

A. Pepsin. *C. Dipeptidase.* *E. Sucrase.*
B. α-Amylase. *D. Lactase.*

5. Name the enzyme, determination of which in blood is the most informative in the early hours after a myocardial infarction.

A. Aspartate aminotransferase B. Alanine aminotransferase.
C. Lactate dehydrogenase D. Glutamate dehydrogenase.
E. Creatine phosphokinase.

6. There are several groups of the molecular mechanisms that play an important role in the pathogenesis of cellular damage that contributes pathology. What processes provide the protein mechanisms of injury?

A. Inhibition of enzymes B. Lipid peroxidation
C. Activation of phospholipase D. The osmotic membrane tension
E. Acidosis

7. Organophosphates (highly toxic poisons of neuro-paralitic action) inhibit acetylcholinesterase by the formation of covalent bonds with the OH groups of serine in the active site of the enzyme. What type of inhibition is typical for this class of compounds?

A. Reversible. *D. Irreversible.*
B. Competitive. *E. Retroinhibition (feedback inhibition)*
C. Noncompetitive.

8. Indicate the type of inhibition in which the inhibitor is attached no to the active site of the enzyme, but to other specific region of the molecule.

A. Allosteric. *C. Uncompetitive.* *E. Competitive.*
B. Noncompetitive. *D. Substrate.*

9. Enzyme therapy is the direction of medical enzymology associated with the use of enzymes for the treatment of various diseases. Name the enzyme that is used in the complex therapy to eliminate edema, hematoma, keloid scars.

A. Carboxypeptidase. *C. Pepsin.* *E. Lipase.*
B. Collagenase. *D. Amylase.*

10. A woman with diagnosis of myocardial infarction was tbrought to the intensive care unit. The activity of which enzyme will be increased in the first two days?

A. Alanine aminotransferase. *D. LDH5.*
B. γ-Glutamyltranspeptidase. *E. LDH4.*
C. Aspartate aminotransferase.

11. The patient has acute pancreatitis. Which preparations must be prescribed by a doctor to prevent autolysis of the pancreas?

A. Protease activators. *C. Chymotrypsin.* *E. Protease inhibitors.*
B. Trypsin. *D. Amylase.*

12. One of the ways of regulating the activity of acetyl-CoA carboxylase (limiting enzyme in fatty acid synthesis) is retroinhibition by final product – palmitoyl-CoA. Feedback inhibition is an variant of:

A. Covalent modification of enzyme. *D. Allosteric inhibition.*
B. Competitive inhibition. *E. Noncompetitive inhibition.*
C. Irreversible inhibition.

13. The patient has progressive muscular dystrophy. Which of the following biochemical parameters have diagnostic value in this case?

A. Creatine phosphokinase. *D. Glutamate dehydrogenase.*
B. Pyruvate dehydrogenase. *E. Adenylate cyclase.*
C. Lactate dehydrogenase.

14. The activity of which enzymes should be determined with diagnostic and prognostic purpose in the patient with pathology of the cardiac muscle?

A. Lysozyme, citrate synthase, aldolase.
B. Neuraminidase, hexokinase, pyruvate kinase.
C. Malate dehydrogenase, pyruvate dehydrogenase, succinate dehydrogenase.
D. Creatine kinase, alanine and aspartate aminotransferase.
E. Arginase, peptidase, phosphatase.

15. Name type of inhibition in which the inhibitor chemical structure resembles the structure of the substrate.

A. Noncompetitive. *C. Uncompetitive.* *E. Irreversible.*
B. Competitive. *D. Substrate.*

16. Tabun, zarin, diisopropyl fluorophosphate (organophosphorus compounds) are poisons of neuro paralitic action. Which of the mentioned enzyme is inhibited by organophosphorus compoundsf?

A. Phospholipase A2. *D. Angiotensin converting enzyme.*
B. Acetylcholinesterase. *E. Tyrosine aminotransferase.*
C. Cytochrome P-450.

17. Enzymes are widely used as drugs. Which of the mentioned enzymes is used for the treatment of leukemia?

A. Asparaginase. *C. Enolase.* *E. Catalase.*
B. Dihydroorotase. *D. Fumarase.*

18. Enzyme therapy is the direction of medical enzymology associated with the use of enzymes for the treatment of various diseases. Name the enzyme that is used in myocardial infarction treatment.

A. Phosphofructokinase. *D. Hexokinase.*
B. Pyruvate kinase. *E. Glycerol kinase.*
C. Streptokinase.

19. In the analysis of the gastric juice of a patient with diagnosed hypoacidic gastritis the significant decrease of pepsin activity was shown. Select a possible biochemical mechanism for this phenomenon.

A. Enzyme denaturation.
B. Competitive inhibition of the enzyme.
C. Reducing the activation energy of the enzymatic reaction.
D. Lack of intrinsic factor (Castle factor) in gastric juice.
E. Disturbance of an enzyme formation from proenzyme.

20. Choose the activator of salivary amylase:

A. Sodium chloride. *C. Copper sulfate.* *E. Calcium gluconate.*
B. Ammonium sulfate. *D. Magnesium chloride.*

21. Indicate the type of inhibition when the product of reaction is the inhibitor of enzyme:

A. Competitive. *C. Stereochemical.*
B. Non-competitive. *D. Retroinhibition (feedback inhibition).*

22. Indicate the enzyme whose activity is determined in the blood plasma of patients with bone tissue pathology.

A. Pepsin. *C. Amylase.* *E. Alkaline phosphatase.*
B. Trypsin. *D. Acid phosphatase.*

23. What is the mechanism of inhibition of folic acid synthesis by sulfanylmides?

A. Competitive. *D. Irreversible.*
B. Non-competitive. *E. Binding with allosteric site of enzymes.*
C. Denaturation of enzyme.

PRACTICAL WORK

Quantitative determination of α-amylase activity in serum by Karavey's method

Task. Determine α-amylase activity in serum by Karavey's method

The principle of the method. α-Amylase (*α-1,4-glucan-4-glucano-hydrolase, EC 3.2.1.1*) catalyzes the hydrolytic cleavage of starch to form the final products, which do not give color with iodine. The enzyme activity is estimated by excessive starch, which is determined by the spectrophotometric method by changing of iodine-starch solution color.

Procedure. Determination of α-amylase activity in serum according to the following scheme:

Laboratory glassware	Volume of pipette, ml	Reagents	Experimental sample, ml	Control sample, ml	Stages
Laboratory test tubes	1	substrate-buffer solution	1	1	preincubation
	Incubate 5 minutes at 37 °C				
	0,1	blood serum	0,02	–	
	mix, incubate 7,5 minutes at 37 °C (enzymatic reaction)				
	1	iodine solution	1	1	color reaction
	0,1	blood serum	–	0,02	
		distilled water	to 10	to 10	
	mix, measure E_{ex} and E_c at a wavelength of 630–690 nm in a cuvette (10 mm) against distilled water for 5 min (photometry)				

Activity of α-amylase(X) in g/(L·h) in serum is calculated by the following formula:

$$X = (E_c - E_e) : E_c \times 160 \times K,$$

where E_c is an extinction of control sample, E_e is an extinction of experimental sample, 160 is a coefficient of calculation, which takes into account the amount of starch put into the experimental and control test tubes per 1 liter of biological fluid for 1 h incubation at 37 °C; K is a dilution coefficient of investigated blood serum.

Clinical and diagnostic significance. Normally, α-amylase activity in serum is 12–32 g/(h × L). The elevation in blood and urine is observed in pancreatic diseases. The activity of α-amylase in acute pancreatitis increases 10–30 times and it reaches its maximum on the first day of illness and within the second-sixth days it is quickly normalized. Hyperamylasemia also occurs in acute appendicitis, perforated gastric ulcer and duodenal ulcer, cholecystitis, gallbladder rupture,

burns, traumatic shock, pneumonia, prostatitis, uremia. Increased activity of α-amylase is promoted by several medicines (corticosteroids, catecholamines, furosemide, and anticoagulants), narcotics and alcohol. Reduction of α-amylase activity is observed in hepatitis, cirrhosis, malignant tumors of liver, diabetes mellitus, hypothyroidism and cachexia.

Quantitative determination of the activity of lactate dehydrogenase (EC 1.1.1.27) in serum by Sevel's and Tovarek's method

Task. Determine the activity of lactate dehydrogenase (LDH) in serum by Sevel's and Tovarek's method.

Principle of the method. Under the influence of LDH L-lactate is oxidized to pyruvate in the presence of nicotinamide adenine dinucleotide (NAD). Amount of formed pyruvate is determined photometrically by a color reaction with 2,4-dinitrophenylhydrazine leading to the formation of 2,4-dinitrophenylhydrazone which has a red-brown color in alkaline medium. Its intensity is directly proportional to the content of keto acid.

$$\underset{\text{lactate}}{CH_3\text{-}CH(OH)\text{-}COOH} + NAD^+ \xrightarrow{LDH} \underset{\text{pyruvate}}{CH_3\text{-}CO\text{-}COOH} + NADH(H^+)$$

Procedure. Add to the first test tube 0.1 ml of 3 fold diluted serum, 0.3 ml of freshly prepared solution of 0.02 mol/L NAD^+ and leave for 5 min in a water bath at 37 °C for heating the mixture. Transfer 0.8 ml of 0.03 mol/L sodium pyrophosphate solution to the second test tube, 0.2 ml of 0.45 mol/L sodium lactate solution and heat in a water bath at 37 °C. Pour the contents of the second tube in the first one, quickly mix with a glass rod without removing the tube from the bath and mark the start of incubation. After 25 min the reaction is stopped by adding 0.5 ml of 0.2 % solution of 2,4-dinitrophenylhydrazine in solution of 1 mol/L hydrochloric acid and leave the test tube for 20 minutes at room temperature to form hydrazones. Add 5 ml of 0.4 mol/L sodium hydroxide to the mixture, mix with a glass rod and after 10 min measure the extinction of test sample against a control one on PEC at a wavelength of 520–560 nm in 10 mm cuvettes. The control sample is prepared in the same wayl as experimental one, but diluted serum is added after incubation. The enzyme activity is calculated from the calibration graph that can be built according to data available in the table.

№ of test tube	Standard solution of sodium pyruvate, ml	Solution of 0.03 mol/L sodium pyro-phosphate, ml	Distilled water, ml	Amount of pyruvate in a sample, μmol	LDH activity mmol / L × h	Extinction
1	0.1	0.8	0.5	0.01	1.2	
2	0.2	0.8	0.4	0.02	2.4	
3	0.4	0.8	0.2	0.04	4.8	
4	0.6	0.8	-	0.06	7.2	
5	0.8	0.8	-	0.08	9.6	

The vertical axis shows extinction values and the horizontal axis displays corresponding units of LDH activity expressed in mmol/(L × h).

Clinical and diagnostic significance. Determination of LDH is used in clinical and biochemical laboratories for the diagnosis of diseases, as well as a test for recovery. Normally, the enzyme activity in serum is 0.8–4.0 mmol/L × h. It increases in myocardial damage, leukemia, kidney disease, sickle-cell anemia, hemolytic anemia, thrombocytopenia, infectious mononucleosis and progressive muscular dystrophy. When diseases are accompanied by tissue necrosis (myocardial infarction, necrotic kidney damage, hepatitis, pancreatitis, tumors) a sharp increase in LDH activity is observed in serum. In acute hepatitis, it is elevated during the first week of jaundice period; in mild and moderate forms of the disease the activity of LDH is quickly normalized. In patients with acute myocardial infarction the increase in total LDH and LDH_1 in serum is observed 8–18 h after the begining of the attack, which reaches its maximum after 24–72 h. Enzyme activity remains high during the first week and normalizes in 6–10 days. The determination LDG1 / LDG2 ratio is also important. Normally it constitutes 0.4–0.74, and in acute myocardial infarction it increases 5–10 times.

LDH activity in serum is not increased in angina pectoris.

1.** Prepare the abstract on the theme: "Peculiarities of the structure, kinetics and regulation of the activity of allosteric enzymes."

2.** Prepare the review of the scientific literature on the topic: "Serine proteinase. The use of proteolysis inhibitors in medicine. "

CLASS 3 (4 hours)

Topic 5 (4 hours): General characteristics of vitamins. Fat-soluble vitamins. Qualitative reactions on fat-soluble vitamins.

Importance. Vitamins are a group of organic compounds which have different structure and physical and chemical properties. They are essential for normal functioning of the body performing catalytic, regulatory or antioxidant functions directly or forming a part of more complex compounds. Typically, in the organism, they are not synthesized or stored, so always have to come from food. Deficiency of vitamins leads to the development of hypo- and avitaminosis.

Objective. Read the history of vitamins and the role of Ukrainian scientists in development of vitaminology. Make an overview of vitamins: biochemical concepts of their functioning as components of human nutrition, regulators of enzymatic reactions and metabolic processes; classifications based on physical and chemical properties, clinical and physiological action; molecular and biochemical mechanisms of hypo- and hypervitaminosis development; theoretical bases of polyvitamin drugs manufacturing. Consider and be able to describe the fat-soluble vitamins according to following plan: 1) name (chemical, biological); 2) chemical structure and its possible transformation; 3) biological role; 4) specific symptoms of hypo- and avitaminosis; 5) source and prophylactic dose.

THEORETICAL QUESTIONS

1.* History of vitamins discovery, the role of scientists in the development of vitaminology.

2. General characteristics of vitamins, their role in the human body. Classification of vitamins based on the physical and chemical properties, clinical and physiological action. Provitamins, formulas of known provitamins. Vitamers.

3.* Exogenous and endogenous hypo- and avitaminoses. Clinical and biochemical aspects of avitaminoses.

4. Vitamin A and β-carotenes: structure, participation in metabolism, sources, daily requirement for retinol and β-carotenes, hypo- and hypervitaminoses.

5. Vitamin E: structure, participation in metabolism, sources, daily requirement, deficiency symptoms.

6. Vitamin K: structure, participation in blood coagulation, sources, daily requirement. Vitamin K analogues and antagonists as drugs.

7. Vitamin D: structure, mechanism of action in the metabolism of calcium and phosphate, sources, daily requirement. Hypovitaminosis in children and adults. Symptoms of hypervitaminosis.

8.* Vitamin F (polyunsaturated fatty acids): the structure of the components of the complex, participation in metabolism, sources, daily requirement, deficiency symptoms.

Indicative list of theoretical questions for self-study

Content	*Basic theses*
1. History of the vitamins discovery; the role of scientists in the vitaminology development.	*1.1. Experimental studies of N. I. Lunin (1880) – the determination of the role of essential nutritional factors.* *1.2. Experimental studies of K. Sosin (1891) – confirmation of NI Lunin's works.* *1.3. Experimental studies of F. Hopkins (1906–1912) – "additional nutritional factors."* *1.4. Experimental studies of T. Takaki (1887) and C. Eykman (1897) – the study of beri-beri.* *1.5. Experimental studies VV Pashutin (1895–1901) – It was proved that scurvy was one of forms of starvation, developed due to the lack of some organic substance in food ; and a relationship of vitamins with enzymes was presumed.* *1.6. Experimental investigations of V.Stepp (1909) – Fat-soluble component was identified in milk and rye bread, it was called "vitamin A".* *1.7. K. Funk (1912) – proposed the name "vitamin" and received vitamin B_1.* *1.8. M. Zielinski (1921) established a link between vitamins and enzymes.*

Content	*Basic theses*
	1.9. AV Paladin (1919–1944) investigated the process of conversion of vitamins in the tissues of the animal organism, metabolic disorders in avitaminoses and hypovitaminosis. He created a synthetic vitamin preparation – vikasol.
2. Exogenous and endogenous hypo- and avitaminoses. Clinical and biochemical aspects of avitaminoses. Hypovitaminosis is a pathological condition characterized by a relative insufficiency of vitamin in the body. Avitaminosis is a pathological condition characterized by a full deficiency of vitamin in the body	*2.1. Causes:* *– Exogenous: reduction/lack of any vitamin in the food;* *– Endogenous: malabsorption of vitamins (diseases of the gastrointestinal tract, liver, gall bladder); antivitamin presence; increased needs (pregnancy, lactation, infectious diseases) etc.* *2.2. As a result of hypo- and avitaminosis the severe disorders of metabolic processes occur: the appearance of specific complexes of symptoms; a disturbance of functioning enzymes; impairment of regulatory functions of the body*
3. Vitamin F is the complex of polyunsaturated fatty acids (linoleic, linolenic, arachidonic).	*3.1. The chemical nature and properties.* *3.2. The biological role:* *– Precursors in the synthesis of eicosanoids (prostaglandins, thromboxanes, leukotrienes, prostacyclins);* *– anti-atherosclerotic effect (a decrease in blood cholesterol);* *– growth and regeneration of epidermis;* *– influence on spermatogenesis;* *– stimulation of immune system;* *– synthesis of phospholipids and glycolipids;* *– antiallergic action (inhibitor of histamine).* *3.3. Clinical symptoms of vitamin deficiency:* *– follicular hyperkeratosis;* *– disorder of lipid metabolism;* *– liver diseases;* *– atherosclerosis;* *– growth retardation;* *– reduction of the reproductive function;* *– development of cardiovascular diseases;* *– skin diseases (eczema, dermatitis).* *3.4. Daily requirement: 2–6 g to 10 g.* *3.5. Sources: vegetable oils, butter, eggs, red fish, nuts.*

TESTS FOR SELF-CONTROL

1. The signs of rickets are observed in children with hereditary renal disease. Vitamin D concentration in the blood is within the normal range. What of the below mentioned is the most likely cause of rickets?

A. Increased calcium excretion from the body.
B. Hyperparathyroidism.
C. Hypofunction of parathyroid glands.
D. Impairment of calcitriol synthesis.
E. Insufficient intake of calcium from food.

2. Linoleic and linolenic acids are necessary for the synthesis of eicosanoids. The main source of these acids is:

A. Alimentary factor.
B. Biosynthesis of fatty acids.
C. Cholesterol degradation.
D. Microsomal oxidation.
E. Oxidation of fatty acids.

3. Which vitamin has antixerophthalmic action?

A. Vitamin D_3.
B. Vitamin K.
C. Vitamin A.
D. Vitamin P.
E. Vitamin C.

4. A few days before the operation the vitamin K or its synthetic analog vikasol is prescribed to the patient. What type of posttranslational modification reactions of II, VII, IX, X blood coagulation factors is performed with participating vitamin K?

A. Carboxylation.
B. Decarboxylation.
C. Deamination.
D. Transamination.
E. Glycosylation.

5. Under the influence of ionizing radiation or avitaminosis E the increased lysosomal membrane permeability is observed in cell. What consequences may result from such pathology?

A. Intensive generation of energy.
B. Intensive synthesis of proteins.
C. Partial or complete destruction of cells.
D. Renovation of the cytoplasmic membrane.
E. Division spindle formation.

6. Which vitamin, because of the hydrophobic side chains, is integrated in the phospholipid matrix of biological membranes, stabilizes them and serves as a powerful bioantioxidants?

A. Tocopherol
B. Vitamin B_1.
C. Vitamin B_6.
D. Nicotinamide
E. Cyanocobalamin

7. To the patient with periodontosis the doctor prescribed the vitamin A applications. Which process activation under the influence of vitamin A provides the healing process?

A. Carboxylation of glutamic acid.
B. Dark vision.
C. Color vision.
D. Proline hydroxylation.
E. Cell growth and differentiation.

8. Which vitamin hormonal form induces the synthesis of Ca-binding proteins in enterocytes at the genome level and thus regulates the intestinal absorption of calcium ions necessary for the formation of dental tissue?

A. D_3. *B. B_1.* *C. E.* *D. A.* *E. K.*

9. Nyctalopia (night blindness) is observed in a patient. Which of the below mentioned substances will have a therapeutic effect?

A. Keratin. *C. Carotene.* *E. Carnosine.*
B. Creatine. *D. Carnitine.*

10. A patient complains of loss of appetite, headache, and insomnia. Hyperkeratosis, inflammation of eyes, loss of hair, general physical exhaustion are observed. It is known from anamnesis that the patient yas been consuming cod-liver oil during a long time. What is the cause of this state?

A. Hypervitaminosis of vitamin D. *D. Hypovitaminosis of vitamin A.*
B. Hypovitaminosis of vitamin D. *E. Hypervitaminosis of vitamin F.*
C. Hypervitaminosis of vitamin A.

11. Symptoms of K-hypovitaminosis:

A. Thromboses. *D. Increased blood clotting.*
B. Subcutaneous hemorrhages. *E. Dermatitis.*
C. Dedentition (shedding of teeth).

12. The mother of breast-fed baby complains of baby's insomnia, frequent weeping, irritability, disposition to sweat, bald back of the head. Which disease may be diagnosed?

A. Scurvy. *C. Beriberi.* *E. Anemia of Addison-Birmer.*
B. Rickets. *D. Pellagra.*

13. A newborn has symptoms of hemorrhagic disease due to hypovitaminosis K. Development of disease is explained by to the biological role of vitamin K, namely:

A. It is a cofactor of prothrombin.
B. It is a specific inhibitor antithrombin.
C. It influences the proteolytic activity of thrombin.
D. It is a cofactor γ-glutamate carboxylase.
E. It inhibits the heparin synthesis.

14. The possible anticancer activity of two fat-soluble vitamins is associated with their antioxidant properties. Point out these vitamins.

A. A and K. *C. D and K.* *E. A and E.*
B. E and K. *D. D and E.*

15. During an examination of the child the doctor found signs of rickets. Which substance deficiency in the child's body contributes to the development of this disease?

A. Naphthoquinone. *C. Tocopherol.* *E. Biotin.*
B. 1,25-Dihydroxycholecalciferol. *D. Retinol.*

16. The therapy with vitamin preparations was prescribed to pregnant woman with numerous spontaneous abortions in the anamnesis. Which vitamin facilitates the normal proceeding of pregnancy?

A. α-Tocopherol. *C. Cyanocobalamin.* *E. Ruthin.*
B. Folic acid. *D. Pyridoxal phosphate.*

17. Osteoporosis developed in the woman with chronic kidney disease. Deficiency of which of the below mentioned substances is a major cause of this complication?

A. D_3. *C. Cholesterol.* *E. 25-hydroxy-D_3.*
B. 1,25-dihydroxy-D_3. *D. D_2.*

18. Delayed teething, their wrong location is marked in the child. There are also complaints on dry mouth, cracks in the corner of the mouth with further suppuration. Which vitamin deficiency can lead to this state?

A. A. *B. D.* *C. C.* *D. E.* *E. K.*

19. The child has the pronounced signs of rickets. Digestion was not disturbed. The child is in the sun a prolonged time. Within two months, the child receives vitamin D_3, but manifestation of rickets has not decreased. What can explain the development of rickets in the child?

A. Disorders of calcitonin synthesis.
B. Disorders of parathyroid hormone synthesis.
C. Disorders of calcitriol synthesis.
D. Disorders of thyroxine synthesis.
E. Disorders of insulin synthesis.

20. Patients with bile duct obstruction have hemorrhages due to bad assimilability of vitamin:

A. Vitamin F. *C. Vitamin E.* *E. Vitamin K.*
B. Vitamin A. *D. Vitamin D.*

21. A complex of vitamins with vitamin E is recommended to old persons. What is the main function of this vitamin?

A. Antiscorbutic. *C. Antioxidant.* *E. Antidermatic.*
B. Antihemorrhagic. *D. Antineuritic.*

22. It is known that dicumarol administration to organism causes a sharp reduction of blood levels of prothrombin and other blood clotting factors. Dicumarol is antivitamin of:

A. Vitamin H. *C. Vitamin E.* *E. Vitamin K.*
B. Vitamin C. *D. Vitamin P.*

23. The physician found the increase in time of the patient's eye adaptation to darkness. Which vitamin deficiency may be the cause of that symptom?

A. Vitamin E. *C. Vitamin C.* *E. Vitamin D.*
B. Vitamin A. *D. Vitamin K.*

24. Treating the child with rickets using vitamin D_3 did not give a positive result. What is the most likely cause of ineffective treatment?

A. Lack of lipids in diet.
B. Impairment of hydroxylation of vitamin D_3.
C. Impairment of the incorporation of vitamin D_3 into the enzyme.
D. Improved use of vitamin D_3 by intestinal microflora.
E. Impairment of vitamin D_3 transport by blood proteins.

25. The patient has night blindness, dryness of the conjunctiva and cornea. Such changes may be the result of the deficiency:

A. Vitamin A. *C. Vitamin C.* *E. Vitamin B_{12}.*
B. Vitamin B_1. *D. Vitamin D.*

26. Vitamin D deficiency in children causes a disturbance of calcium-phosphorus metabolism, osteomalacia and rickets. Calcitriol regulates the absorption of Ca^{2+} ions in the intestine through the induction of the synthesis:

A. All mentioned.
B. Ca^{2+}-dependent ATPase in myocytes.
C. Na/K-ATPase in the enterocytes.
D. Ca^{2+}-binding protein in enterocytes.
E. Ca^{2+}-calmodulin in enterocytes.

27. Which hypovitaminosis manifests by the disturbance of reproductive function of organism and muscular dystrophy?

A. Vitamin B_1. *C. Vitamin K.* *E. Vitamin E.*
B. Vitamin A. *D. Vitamin D.*

28. The child has the intestinal dysbiosis, which led to development a hemorrhagic syndrome. What is the most likely cause of hemorrhages in this child?

A. Deficiency of vitamin A. *D. Deficiency of fibrinogen.*
B. Activation of tissue thromboplastin. *E. Hypocalcemia.*
C. Hypovitaminosis PP.

29. One of the mechanisms of vitamin K action is its participation in the enzyme system of gamma-carboxylation of protein factors of blood clotting, bone and the tooth mineralization that increases the affinity of their molecules to calcium ions. Which amino acid is carboxylated in proteins?

A. Serine. *C. Phenylalanine.* *E. Arginine.*
B. Valine. *D. Glutamic.*

PRACTICAL WORK

Qualitative reactions on fat-soluble vitamins

Task 1. Carry out reaction on retinol with concentrated sulfuric acid.

Principle. Concentrated sulfuric acid takes water from retinol with the formation of colored products.

Procedure. Add 2 drops of 0.05 % oil solution of retinol in chloroform (1:5) to a dry test tube and 1 drop of concentrated sulfuric acid. A red-violet color appears which gradually becomes reddish-brown.

Task 2. Carry out reaction on retinol with ferric sulfate.

Procedure. Add 2 drops of 0.05 % oil solution of retinol in chloroform (1:5) and 10 drops of glacial acetic acid saturated with ferric sulfate and 2 drops of concentrated sulfuric acid. A blue color appears which gradually turns into a pinkish-red. Carotene gives greenish color in this reaction.

Task 3. Carry out reaction on calciferol.

Principle. Interaction of vitamin D with aniline reagent causes the appearance of red color under heating.

Procedure. Add to a dry test tube 2 drops of cod liver oil and 10 drops of chloroform, then add 2 drops of aniline reagent continuously mixing with stirring rod. Gently heat and boil for 30 seconds with continuous stirring. In the presence of vitamin D yellow emulsion becomes green, then red.

Task 4. Carry out the reaction on naphthoquinone (vitamin K_1).

***Principle*.** Vicasol has lemon-yellow color in the presence of cysteine in alkaline medium.

***Procedure*.** Put 5 drops of 0.05 % solution of vicasol on microscope slide, add 5 drops of 0.025 % solution of cysteine and 1 drop of 10 % sodium hydroxide. Lemon-yellow color appears.

Fill in the table:

Vitamin	Chemical structure	Qualitative reaction	Mechanism of reaction	Observation
Retinol				
Calciferol				
Naphthoquinone				

Practical significance. Qualitative reactions on vitamins are based on color reactions characteristic for certain chemical group that serves as a part of their structure. These reactions help to find vitamins in drugs, foods and medicinal plants. The principles underlying the qualitative reactions on vitamins are also used for developing methods for their quantitative determination.

1.** Prepare an abstract on the theme: "Toxic effects of vitamins A and D hypervitaminosis."

2.** Prepare a presentation on the topic: "The absorption of fat-soluble vitamins in the gastrointestinal tract."

CLASS 4 (4hours)

Topic 6 (4 hours)*: Water-soluble vitamins. Vitamin-like substances. Antivitamins. Qualitative reactions on water-soluble vitamins. Quantitative determination of vitamin C in the urine and rose hip extract.

Importance. Knowledge of the role of vitamins in metabolism is necessary to explain the occurrence of specific symptoms, characteristic for the development of hypo- or avitaminosis and for understanding of the opportunities

and ways for their prevention and treatment. Conclusions about the body's vitamin supply can be based on the qualitative and quantitative determination of vitamins and some constants of biological fluids that depend on vitamins.

Objective. Learn and be able to describe the water-soluble vitamins in accordance with the following plan: 1) name (chemical, biological); 2) chemical structure and its possible transformation; 3) biological role; 4) specific symptoms of hypo- and avitaminosis; 5) sources and prophylactic dose. Familiarize yourself with vitamin-like substances and antivitamins. Read the qualitative reactions for water-soluble vitamins and the method of quantitative determination of vitamin C.

THEORETICAL QUESTIONS

1. Vitamin B_1 (thiamine): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of TPP.

2. Vitamin B_2 (riboflavin): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of FAD, FMN.

3. Pantothenic acid: structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of HS-CoA.

4. Vitamin PP (nicotinic acid, nicotinamide, niacin): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of NAD and NADP.

5. Vitamin B_6 (pyridoxine): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of pyridoxal phosphate.

6. Vitamin B_7 (biotin): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms.

7. Vitamin B_9 (folic acid): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms.

8. Vitamin B_{12} (cobalamine): structure, biological properties, mechanism of action, role in metabolism, sources, daily requirement, deficiency symptoms.

9. Vitamin C (ascorbic acid): structure, biological properties, mechanism of action, role in metabolism, sources, deficiency symptoms. Prophylactic, protective and therapeutic doses.

10. Vitamin P (flavonoids): structure, biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.

11.* General characteristics of the vitamin-like substances. Role of carnitine, ubiquinone and lipoic acid in metabolism.

12.* Antivitamins: specificity of structure and action, application in medicine.

Indicative list of theoretical questions for self-study

Content	*Main theses*
1. Vitamin-like substances are compounds that do not correspond to the classical definition of vitamins according to some criteria: *– they are not strictly essential food factors;* *– they can perform different functions (not just cofactor, regulatory, antioxidant);* *– their deficiency is not accompanied by the development of a specific symptomocomplex*	*– Vitamin B_4 (choline) is a component of the phospholipids (components of cell membranes), the donor of methyl groups, lipotropic factor;* *– vitamin B_8 (Inositol) is a part of inositol phosphates, phosphatides, lipotropic factor;* *– vitamin B_{13} (orotic acid) stimulates protein synthesis, cell division, growth and development of organisms (anabolic effect);* *– vitamin B_{15} (pangamic acid) is a donor of methyl groups, lipotropic factor activator of oxidative processes in the body;* *– lipoic acid is the coenzyme in composition of oxidoreductases, prosthetic group of complex multienzyme complexes – pyruvate dehydrogenase and α-ketoglutarate dehydrogenase;* *– carnitine transports active forms of fatty acids into the mitochondria promoting the process of β-oxidation;* *– ubiquinone is proton and electron transporter in the respiratory chain;* *– paraaminobenzoic acid (PABA) is vitamin necessary for the microorganisms.*
2. Antivitamins are substances that inhibit the action of vitamins by means of their destruction; inhibition of absorption and conversion into active forms; replacement of them by compounds similar in structure but without vitamin action.	*2.1. Classification:* *– specific: structural analogues of native vitamins, blockers enzymatic active sites, antimetabolites;* *– nonspecific: substances preventing the absorption of vitamins, their conversion into active form; providing their rapid destruction (examples: avidin – to B_7 (H), ascorbase – to vitamin C, thiaminase – to B_1).* *2.2. Application in medicine:* *– creating the experimental models of hypo- and avitaminosis;* *– the treatment of diseases (acute leukemia, thrombosis, thrombophlebitis etc.);* *– using as antiinfectious drugs.* *2.3. Examples of antivitamins:* *– Warfarin, dicoumarol – K antivitamins;* *– Sulfonamides – B_{10} antivitamins;* *– Pteridines, methotrexate – B_9 antivitamines;* *– Isoniazid – B_5 and B_6 antivitamin.*

TESTS FOR SELF-CONTROL

1. A medicine from the group of sulfonamides was prescribed to the patient with catarrhal tonsillitis. Determine the mechanism of antibacterial action of sulfonamides:

A. Disturbance of protein synthesis of the cell wall.
B. Decreasing membrane permeability.
C. Inhibition of sulfhydryl groups of thiol enzymes.
D. A competitive antagonism with para-aminobenzoic acid.
E. Protein coagulation.

2. It is known that the carbon dioxide is used in the body in the biosynthesis of fatty acids, urea, gluconeogenesis etc. Which vitamin participates in carboxylation reactions?

A.Thiamin. *C. Biotin.* *E. Retinol.*
B. Riboflavin. *D. Nicotinamide.*

3. Seizures were observed in the newborn. They disappeared after the administration of vitamin B_6. This effect is most likely caused by involving the vitamin B_6 into the structure of the following enzyme:

A. Pyruvate dehydrogenase. *D. Aminolevulinate synthase.*
B. Glutamate decarboxylase. *E. Glycogen phosphorylase.*
C. Glycogen synthase.

4. Erythropoiesis is disturbed in the patient. Which vitamin deficiency may be observed in this case?

A. Folic acid. *C. Ascorbic acid.* *E. Arachidonic acid.*
B. Pantothenic acid. *D. Niacin.*

5. Coenzyme, which is included in the composition of subclass "dehydrogenases" enzymes, was prescribed to the child with a medical purpose. Which of these vitamins may be involved in its formation?

A. P u B_1. *B.* B_2 *u* B_6. *C.* B_1 *u* B_2. *D.* B_1 *u* B_5. *E. PP u* B_2.

6. Which vitamin is part of glutamate decarboxylase and participates in GABA formation, but its insufficiency is manifested by convulsions?

A. Cobalamin. *C. Folic acid.* *E. Pyridoxine.*
B. Tocopherol. *D. Ascorbic acid.*

7. The child is being treated at the hematology department with the diagnosis – Addison-Biermer's disease. What is the main drug for the treatment of this disease?

A. Folic acid. *C. Nicotinamide.* *E. Iron lactate.*
B. Vitamin B_{12}. *D. Ruthin.*

8. Hydroxyproline is the important amino acid included in collagen structure. Which vitamin participates in the formation of this amino acid by hydroxylation of proline?

A. C. *B. D.* *C.* B_1. *D.* B_2. *E.* B_6.

9. Which vitamin is the component of coenzyme A?

A. Paraaminobenzoic acid. *C. Carnitine.* *E. Pantothenic acid.*
B. Pyridoxin. *D. Orotic acid.*

10. Which vitamin influences the capillary permeability?

A. Nicotinamide. *C. Pyridoxin.* *E. Pangamic acid.*
B. Riboflavin. *D. Rutin.*

11. Which vitamin deficiency leads to disease accompanied by the increased permeability and fragility of blood vessels?

A. Vitamin B_1. *B. Vitamin PP.* *C. Vitamin B_6.* *D. Vitamin C.* *E. Vitamin E.*

12. The structural analogue of vitamin B_2 acrichin is prescribed to patients with enterobiosis. What enzyme synthesis is disturbed by this preparation?

A. Cytochrome oxydases. *D. NAD-dependent dehydrogenases.*
B. FAD-dependent dehydrogenases. *E. Aminotransferases.*
C. Peptidases.

13. The girl often experiences acute respiratory infections with multiple spotty hemorrhages in the places of clothes friction. What vitamin hypovitaminosis is present in the girl?

A. B_6. *B. A.* *C. C.* *D. B_2.* *E. B_1.*

14. In the patient's urine the level of pyruvic acid is 5 times higher than normal. The parenteral administration of cocarboxylase was prescribed to the patient. Which of the following coenzymes is appointed?

A. FAD. *B. NAD.* *C. TPP.* *D. FH_4.* *E. NADP.*

15. Epileptic convulsions due to a deficiency of vitamin B_6 are observed in the infant organism. This is caused by decrease of content of the inhibition mediator (γ-aminobutyric acid) in nervous tissue. What enzyme activity is decreased?

A. Pyridoxalkinase. *D. Glutamate decarboxylase.*
B. Alanine aminotransferase. *E. Glutamate synthetase.*
C. Glutamate dehydrogenase.

16. The bactericidal action of sulfonamides is based on the competitive relationship with para-aminobenzoic acid. Which vitamin synthesis is disturbed in bacteria under the influence of sulfonamides?

A. Cobalamin. *C. Folic Acid.* *E. Biotin.*
B. Pyridoxine. *D. Nicotinamide.*

17. A patient has dermatitis, diarrhea and dementia. Which vitamin deficiency is the cause of that state?

A. Folic acid. *C. Nicotinamide.* *E. Rutin.*
B. Ascorbic acid. *D. Biotin.*

18. A patient is diagnosed with seborrheic dermatitis caused by vitamin H (biotin) deficiency. Which enzyme activity is disturbed in the patient?

A. Acetyl-CoA carboxylase. *D. Pyruvate decarboxylase.*
B. Alcohol dehydrogenase. *E. Carbamoyl phosphate synthetase.*
C. Aminotranspherases.

19. Reactions of intermolecular transfer of one-carbon radicals are essential for the synthesis of proteins and nucleic acids. Which vitamin is necessary for the formation of coenzyme for the above mentioned reactions?

A. Folic acid. *C. Pantothenic acid.* *E. Ascorbic acid.*
B. Thiamine. *D. Riboflavin.*

20. Pernicious anemia was revealed in a patient with atrophic gastritis. Which vitamin malabsorption is observed?

A. Thiamine. B. Niacin. C. Cobalamin. D. Biotin. E. Retinol.

21. Thiamine deficiency caused polyneuritis in the patient, who lived exclusively on polished rice. The excretion of which of the below mentioned substances in the urine can be an indicator of this vitamin deficiency?

A. Methyl malonate. *C. Phenylpyruvate.* *E. Uric acid.*
B. Pyruvic acid. *D. Malate.*

22. Isoniazid was prescribed to the patient with pulmonary tuberculosis. Which vitamin deficiency can be caused by prolonged use of this drug?

A. Biotin. *C. Cobalamin.* *E. Pyridoxine.*
B. Thiamine. *D. Folic acid.*

23. A patient was diagnosed with megaloblastic anemia. What substance deficiency in the organism can result in development of this disease?

A. Copper. *C. Cyanocobalamin.* *E. Magnesium.*
B. Glycine. *D. Cholecalciferol.*

24. A patient has pellagra. Interrogation revealed that he had eaten mostly on maize and a little meat for a long time. This disease had been caused by the deficit of the following substance in the maize:

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

25. Pyridoxal phosphate was prescribed to a patient. Which processes are corrected by this preparation?

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

26. Vitamin B_6 deficiency can potentiate vitamin PP insufficiency, as the coenzyme form of vitamin B_6 is involved in the synthesis of NAD from tryptophan. Specify the coenzyme form of vitamin B_6.

A. Calcitriol. *D. Methylcobalamin.*
B. Thiamine pyrophosphate. *E. Carboxybiotin.*
C. Pyridoxal phosphate.

27. Dermatitis appears on the patient's skin after eating raw eggs. Which avitaminosis is developed?

A. Folic acid. *C. Pantothenic acid.* *E. Inositol.*
B. Biotin. *D. Paraaminobenzoic acid.*

28. Hypovitaminosis C leads to a reduction of the organic matrix formation, to disturbance of collagen synthesis, as this vitamin is involved in the processes of:

A. Proline hydroxylation. *D. Proline carboxylation.*
B. Lysine carboxylation. *E. Tryptophan hydroxylation.*
C. Arginine hydroxylation.

29. The mechanism of action of widely spread anticancer drug methotrexate is based on the fact that it is a structural analog of:

A. Nicotinic acid. *C. Cyanocobalamin.* *E. Retinoic acid.*
B. Para-aminobenzoic acid. *D. Folic acid.*

30. A woman has hypovitaminosis B2. The cause of specific symptoms (damage of epithelium, mucous membranes, skin, cornea) is probably the deficit of:

A. Cytochrome a_1. *C. Cytochrome b.* *E. Cytochrome c.*
B. Flavin coenzyme. *D. Cytochrome oxidase.*

31. Antivitamins of folic acid are often used as anticancer drugs. Which one of the below mentioned belongs to them?

A. Avidin. *B. Sulfonamide.* *C. Aminopterin.* *D. Isoniazid.* *E. Dicoumarin.*

32. A man with memory disorders, painful sensations along the nerves, reduced intellectual function, disorders of the cardiovascular system and dyspepsia adressed to the hospital. In history he has chronic alcoholism. Which vitamin deficiency can cause these symptoms?

A. Thiamine. *B. Riboflavin.* *C. Retinol.* *D. Niacin.* *E. Calciferol.*

33. Folic acid performs a cofactor function in the reactions:

A. Phosphorylation. *C. Deamination.* *E. Hydrolysis.*
B. Transfer of one-carbon groups. *D. Transamination.*

34. Concentration of pyruvate is increased in patient's blood. Its large amount is excreted with urine. What avitaminosis is observed in the patient?

A. Avitaminosis B_2. *C. Avitaminosis B_3.* *E. Avitaminosis B_1.*
B. Avitaminosis E. *D. Avitaminosis B_6.*

35. During regular prophylactic and medical attendance a physician found in the child organism symmetric roughness of skin, diarrhea, disturbances of nervous activity. What nutrient factor deficiency is the cause of this state?

A. Nicotinic acid, tryptophan. *D. Methionine, lipoic acid.*
B. Lysine, ascorbic acid. *E. Phenylalanine, pangamic acid.*
C. Threonine, pantothenic acid.

36. After course of medical treatment a physician recommends a patient with ulcer of duodenum usage of cabbage and potato juices. What substance present in these vegetables facilitates a prophylaxis and treatment of the ulcer?

A. Vitamin U. *C. Vitamin C.* *E. Vitamin K.*
B. Pantothenic acid. *D. Vitamin B_1.*

37. The child of 9 months feeds exclusively on mixtures which are not balanced on content of vitamin B_6. The child has pellagra – like dermatitis, convulsions, anemia. The development of seizures may be due to the disturbance of formation of:

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

38. After prolonged use of isoniazid a doctor observes in the patient polyneuritis, paresthesia, memory disorders, convulsions. What provides side effects of the drug?

A. Antagonism with PABA.
B. Inhibition of RNA synthesis.
C. Inhibition of pyridoxal phosphate formation.
D. Violations of the cell wall synthesis.
E. Protein synthesis inhibition.

39. After treatment of the patient with antibiotics due to inhibition of intestinal microflora it is possible the following vitamin deficiency:

A. B_{12}. *B. C. C. A. D. P. E. D.*

40. Vitamin B_1 deficiency results in disturbance of oxidative decarboxylation of pyruvate. The synthesis of which of the following coenzymes is disturbed?

A. Nicotinamide adenine dinucleotide (NAD).
B. Lipoic acid.
C. Flavine adenine dinucleotide (FAD).
D. Thiamine pyrophosphate.
E. Coenzyme A.

41. After resection of 2/3 stomach, a quantity of erythrocytes in the patient's blood is decreased, their volume is increased, hemoglobin level is decreased. What vitamin deficiency results in such changes in blood?

A. B_6. *B. C. C. P. D.* B_{12}. *E. PP.*

42. Symptoms of pellagra are especially pronounced in patients with a deficient protein nutrition, because a precursor of nicotinamide in the human body is one of the essential amino acids, namely:

A. Threonine. C. Histidine. E. Tryptophan.
B. Arginine. D. Lysine.

43. Which vitamin in combination with vitamin C enhances the therapeutic effect of the scurvy treatment?

A. K. B. D. C. E. D. A. E. P.

44. In the treatment of many diseases the pharmaceutical preparation cocarboxylase is used to provide cells with energy. Which metabolic process is activated in this case?

A. Glutamate deamination.
B. Decarboxylation of amino acids.
C. Decarboxylation of biogenic amines.
D. Oxidative decarboxylation of pyruvate.
E. Detoxification of harmful substances in the liver.

45. The young man adressed to a doctor with complaints of general weakness, fatigue, irritability, decreased ability to work, bleeding gums, petechiae on the skin. Which vitamin deficiency may be in this case?

A. Ascorbic acid. C. Riboflavin. E. Folate.
B. Thiamine. D. Retinol.

46. A dietitian recommends to the patient during treatment of pernicious anemia to consume the half raw liver in the diet. Which vitamin of this product stimulates erythropoiesis?

A. Vitamin B_1. *C. Vitamin* B_{12}. *E. Vitamin C.*
B. Vitamin H. D. Vitamin B_2.

47. Patients with alcoholism receive most calories with alcoholic drinks. Typical deficiency of thiamine (Wernicke-Korsakov's syndrome) can appear in their organism. Disturbances of central nervous system, psychoses, amnesia are observed in this syndrome. Which enzyme activity decrease is the disease linked with?

A. Alcohol dehydrogenase. *D. Aldolase.*
B. Pyruvate dehydrogenase. *E. Hexokinase.*
C. Transaminase.

48. The examination of the patient revealed an increase of blood pyruvate level and reduced transketolase activity in erythrocytes. Which vitamin deficiency is indicated by these biochemical results?

A. Biotin. *C. Tocopherol.* *E. Folate.*
B. Thiamine. *D. Retinol.*

49. The influence of hypovitaminosis C on the collagen fiber structure is due to decreased activity of the enzyme:

A. Procollagen peptidase. *D. Lysine hydroxylase.*
B. Collagenase. *E. Lysine oxidase.*
C. Glycosyltransferase.

50. What of the below mentioned indicates providing the body with vitamin B_1?

A. Determination of pyruvate content in the urine.
B. Performing Ratner's test.
C. Performing Robert-Stolnikov's test.
D. Determining the content of ketone bodies in urine.
E. Determining pH of the blood.

51. The year after subtotal gastrectomy the changes were detected in the blood analysis: anemia, leuko- and thrombocytopenia, color index – 1.3, the presence of megaloblasts and megalocytes. Which factor deficiency is the cause of anemia development?

A. Gastrin. *C. Mucin.* *E. Hydrochloric acid.*
B. Castle's factor. *D. Pepsin.*

52. Crimson "varnished" tongue was revealed in the patient during his visit to dentist. In the blood: decreased number of red blood cells and hemoglobin concentration, the blood color index – 1.3, there are signs of megaloblastic type of hematopoiesis, degenerative changes in white blood cells. Which disease of the blood was found in the patient?

A. Hemolytic anemia. *D. Aplastic anemia.*
B. Myeloid leukemia. *E. B_{12}-folate deficiency anemia*
C. Iron deficiency anemia.

53. High-protein diet is recommended to a patient. Which vitamin requirements will increase?

A. B_1. *B. B_{12}.* *C. B_6.* *D. B_2.* *E. B_9.*

54. A patient complains of frothy diarrhea. Macrocytic anemia is in the disease history. Which vitamin deficiency is observed?

A. Pantothenic acid. *C. Folic acid.* *E. Ascorbic acid.*
B. Niacin. *D. Pangamic acid.*

55. Long time unhealed cracks in the corners of the mouth, nasolabial fold dermatitis are clinical manifestations of the following vitamin deficiency:

A. B_6. *B. B_1.* *C. B_2.* *D. B_5.* *E. C.*

56. A child with symptoms of stomatitis, gingivitis and dermatitis of open skin areas was delivered to a hospital. Examination revealed inherited disturbance of neutral amino acid transporting in the bowels. These symptoms were caused by the deficiency of the following vitamin:

A. Niacin. *C. Cobalamin.* *E. Vitamin A.*
B. Pantothenic acid. *D. Biotin.*

PRACTICAL WORK

Qualitative reaction on water-soluble vitamins.
Quantitative determination of vitamin C in urine and rose hip extract.

Task 1. Carry out qualitative reactions on water-soluble vitamins.

A) Diazo reaction for thiamine.

***Principle*.** In an alkaline medium diazo reactive forms an orange complex with thiamine**.**

Procedure. Add to diazo reactive consisting of 5 drops of 1 % solution of sulfanilic acid and 5 drops of 5 % sodium nitrate, 1–2 drops of 5 % solution of thiamine and then add on the wall carefully 5–7 drops of 10 % sodium carbonate. Orange ring is formed at liquid-liquid interface.

B) The oxidation of thiamine in thiochrome.

Principle. In alkaline medium thiamine is oxidized to potassium thiochrome hexacyanoferrate (III). Thiochrome gives blue fluorescence under ultraviolet irradiation of solution by fluoroscope.

Procedure. Add 5–10 drops of 10 % sodium hydroxide, 1–2 drops of 5 % solution of potassium ferricyanide (III) to 1 drop of 5 % solution of thiamine and shake. Heat fluoroscope in advance for 10 minutes and observe the blue fluorescence during irradiation of solution with ultraviolet light.

C) Ferric chloride test for pyridoxine.

Principle. Addition of ferric chloride to a solution of pyridoxine leads to the formation of red color (complex compound of iron phenolate is formed).

Procedure. Up to 5 drops of 1 % solution of pyridoxine add an equal amount of 1% solution of ferric chloride and mix. Red color appears.

D) Reduction of potassium ferricyanide by ascorbic acid.

Principle. Ascorbic acid reduces potassium ferricyanide $K_3[Fe(CN)_6]$ to potassium ferrocyanide $K_4[Fe(CN)_6]$ which forms a blue color precipitate called Prussian blue with ferric chloride.

Procedure. Pour 1 drop of 5 % solution of potassium ferricyanide, 1 drop of 1 % solution of ferric chloride and 5 drops of 1 % solution of ascorbic acid into the test tube. Fluid into the test tube acquires a greenish-blue color and blue precipitate of Prussian blue appears on the bottom of the test tube.

Fill in the table*:*

Vitamin	Chemical structure	Quantitative reaction	Mechanism of reaction	Observation
Thiamine				
Pyridoxine				
Vitamin C				

Task 2. Determine the content of ascorbic acid in the urine.

***Principle*.** Ascorbic acid in acidic medium reduces molecular iodine, while its reduced form becomes oxidized. The appearance of a blue color indicates that all molecules of ascorbic acid from reduced forms are converted to oxidized ones and the first excessive drop of iodine solution gives a blue color in the presence of starch.

Procedure. Add 5 ml of urine and 5 ml of 1 N HCl into the flask, pour 5 drops of starch solution. Titrate with 0.001 N iodine solution till formation of blue color which disappears in 30 seconds.

***Calculation*.** According to the result of the titration daily excretion of ascorbic acid is calculated, taking into consideration the fact, that 1 ml of 0.001 N iodine solution corresponds to 0.5 μmol of ascorbic acid. The calculation is carried out in the following way:

$$\text{Quantity of ascorbic acid in μmol/day} = \frac{a \times 0.5 \times 1500}{5} = a \times 150,$$

where *a* is ml of 0.001 N iodine solution; 0.5 μmol ascorbic acid corresponding to 1 ml of 0.001 N J_2; 1 500 is daily diuresis (ml); 5 is a volume of urine in the sample (ml). 284–568 μmol ascorbic acid can be excreted normally per day in the urine.

Task 3. Determine the content of ascorbic acid in the rose hip extract.

Principle. Determination is based on the redox reaction between ascorbic acid and 2,6-dichlorophenolindophenol which is converted to leucoform during reduction. Acidified aqueous extract, which titrates 2,6-dichlorophenolindophenol, is prepared from investigated product for the determination of ascorbic acid. Acidification of vitamin aqueous extract increases the specificity of the method.

Procedure. Pour 5 000ml of water into 150 grams of rose hips, leave for a few hours, then filter through gauze and fill the microburette with the prepared extract. Add 0.05 ml of 2,6-dichlorophenolindophenol to the centrifuge tube and titrate by burette dropwise with extract of rose hips, shaking the tube. From the first drop the solution turns red (due to the indicator properties of 2,6-dichlorophenolindophenol). Titration continues until the solution turns colorless.

Calculation. Vitamin C concentration is calculated by the following formula:

Quantity of ascorbic acid = (0.88 × 0.05 × *B* × 100) / (*A* × *C*) = mg%,

where 0,88 is a titer of 2,6-dichlorophenolindophenol by ascorbic acid (mg); 0.05 is 2,6-dichlorophenolindophenol volume (ml) in the experiment; *A* is a volume of extract used for the titration (ml); *B* is a total volume of extract (5 000 ml); *C* is a weight of rose hips (150 g).

Practical significance. Qualitative reactions on vitamins are based on color reactions characteristic for certain chemical groups that are components of their structure. The perfomance of these reactions helps to find vitamins in drugs, food and medicinal plants. The principles underlying the qualitative reactions on vitamins are also used for developing methods for their quantitative determination.

1.** Prepare a presentation on the topic: "Vitamin B_{12} – history of discovery, participation in metabolism, malabsorption and formation of coenzyme forms."

2.** Review the scientific literature on the topic: "The role of ascorbic acid in metabolism of connective tissue."

3.** Prepare the abstract on the topic "Bioflavonoids (vitamin P) are plant antioxidants."

CLASS 5(4hours)

Topic: Bioenergy processes: biological oxidation, oxidative phosphorylation, ATP synthesis. Chemiosmotic theory of oxidative phosphorylation. Inhibitors and uncouplers of oxidative phosphorylation. Determination of blood catalase activity.

Importance. Biological oxidation is the major molecular mechanism that provides the energy needs of the body. It is realized by complicated multienzyme complexes of mitochondrial inner membrane. The result of these reactions is the generation of macroergic bonds in molecules of ATP. Biological oxidation and oxidative phosphorylation coupled to it are the basis of bioenergetic processes in the organism. Investigation of properties, characteristics and regulation of respiratory chain enzymes will provide correct understanding of pathologies caused by disorders of bioenergetic processes in hypoenergetic states (tissue hypoxia as a result of reduced concentration of oxygen in the air, diseases of cardiovascular and respiratory systems, anemia of various origins, vitamin deficiencies, starvation, effect of various poisons, etc.).

Objective. Study the biochemical basis of biological oxidation and oxidative phosphorylation; be able to explain the role of biological oxidation, tissue respiration and oxidative phosphorylation in the generation of ATP in aerobic conditions, be able to analyze impairment of ATP synthesis under the action of pathogenic factors of chemical, physical and biological nature on the human body; be able to explain biochemical basis of such processes as endogenous toxins detoxification by enzymes of microsomal oxidation (cytochrome P-450 and b_5); familiarize yourself with chemiosmotic theory, with inhibitors and uncouplers of oxidative phosphorylation. Read the method of blood catalase activity determination and its clinical and diagnostic significance.

THEORETICAL QUESTIONS

1. Relationship between formation and consumption of energy in living systems. The energy of chemical bonds as the main form of energy that cells use for their biological processes.

2. Biological oxidation reactions: types of reactions, enzymes (dehydrogenases, oxidases, oxygenases) and their biological significance. Modern concepts of tissue respiration, its stages.

3. Modern ideas about the structure and functions of mitochondria.

4. Enzymes of biological oxidation in mitochondria: pyridine- and flavin-dependent dehydrogenases, cytochromes.

5. Sequence of electrons transfer in the respiratory chain. Components of the respiratory chain as redox pairs: NAD, flavoproteins, coenzyme Q, cytochromes, their redox potentials.

6. Molecular complexes of mitochondrial inner membrane: NADH-coenzyme Q-oxidoreductase; succinate: coenzyme Q-oxidoreductase; coenzyme Q:cytochrome c oxidoreductase; cytochrome oxidase. Ways of reductive equivalents incorporation in the mitochondrial respiratory chain.

7. Oxidative phosphorylation. Coupling electron transport and phosphorylation. Coefficient of oxidative phosphorylation.

8.* Mitochondrial ATP synthase, the structure and principles of its functioning. F_0 and F_1 subunits of ATP synthase, their functional significance.

9.* Chemiosmotic theory of oxidative phosphorylation.

10. Inhibitors of electron transport (rotenone, amital, barbiturates, antimycin A, cyanides) and oxidative phosphorylation uncouplers (2,4-dinitrophenol, thyroid hormones, free fatty acids) and their biomedical significance.

11.* Pathways of ATP synthesis in cells: substrate and oxidative phosphorylation.

12.* Regulation of tissue respiration. Respiratory control.

13.* Disorders of ATP synthesis under action of pathogenic factors of chemical, biological and physical origins on the human body.

14. Microsomal oxidation and its role in the body.

15. Lipid peroxidation: biological significance and role in the occurrence of pathological conditions.

Indicative list of theoretical questions for self-study

Content	*Main theses*
1. Mitochondrial ATP synthase (complex V).	*1.1. Structure: protein with quaternary structure, composed of several subunits forming components Fo and F_1.* *1.2. Localization: integral protein of the inner mitochondrial membrane close to the respiratory chain.*

Content	*Main theses*
	1.3. Structure Fo and F_1: *– Fo (got its name due to inhibition by oligomycin) is hydrophobic complex in the membrane; base, on which ATP-synthetase is fixed in the membrane. It consists of several subunits that form the channel for the transfer of protons into matrix.* *– F_1 (first open and isolated "fraction" of all elements of the respiratory chain) is protruded into mitochondrial matrix. It is composed of 9 subunits (3α, 3β, γ, ε, δ); α, β subunits form a "head". Three active sites are located between α- and β-subunits → ATP synthesis. γ-, ε-, δ-subunits bind complexes Fo and F_1.*
2 Mitchell's chemiosmotic theory of oxidative phosphorylation.	*2.1. The main postulate: coupling the electron transport in the mitochondria with the biochemical system of ATP synthesis is provided by electrochemical potential of protons formed during the functioning electron transport chain.* *2.2. Key points:* *– functioning of the respiratory chain of mitochondrial inner membrane is accompanied by generation of proton electrochemical gradient;* *– chain components → proton pump → proton transport in the direction: "mitochondrial matrix» → outer surface of inner membrane;* *– view of the respiratory chain – three redox "loops" (I, III, IV enzyme complexes);* *– electrochemical proton potential is a driving force of ATP synthesis from ADP and Pi;* *– the existence of the enzymatic system → using the energy of electrochemical proton potential (reverse proton translocation) → ATP synthesis;* *– effects of various factors (physical, chemical, biological) – uncouplers → damage to the integrity of mitochondrial membranes, and dissipation of an electrochemical gradient energy, ATP synthesis disturbance.*

Content	*Main theses*
3. Ways of ATP synthesis in the cells.	*3.1. Oxidative phosphorylation is a process in which the chemical energy, released during the movement of the electrons along the respiratory chain, is used for ATP synthesis from ADP and Pi:* *– ATP synthesis is coupled to redox reactions;* *– release of chemical energy in the respiratory chain;* *– sites of ATP formation;* *– coefficient of oxidative phosphorylation is the ratio between formation of ATP and consumption of 1/2O_2.* *3.2. Substrate level phosphorylation is the formation of ATP from the energy that is released as a result of the splitting macroergic bonds; for example, in the TCA cycle in the reaction of succinyl-CoA conversion to succinate catalyzed by succinate thiokinase.*
4. Regulation of tissue respiration.	*– electron transport inhibitors – compounds that affect functioning the mitochondrial respiratory chain by binding to certain enzymatic proteins or coenzymes (e.g., rotenone, amobarbital, cyanides);* *– the presence of respiratory control.*
5. Respiratory control – the dependence of the intensity of mitochondrial respiration on the concentration of ADP.	*– substrate oxidation and ADP phosphorylation in mitochondria are coupled;* *– ATP utilization rate controls the speed of electron flow in the respiratory chain;* *– high accuracy of respiratory control mechanism;* *– value: the rate of ATP synthesis corresponds the needs of cell energy.*
6. Impairment of ATP synthesis by the action of pathogenic factors.	*– chemical factors (natural and synthetic toxins);* *– biological factors (microbial toxins);* *– physical factors (ionizing radiation, temperature, etc.).* *The mechanism ofimpairment – uncoupling of respiration and oxidative phosphorylation by disrupting the ability to create and maintain a proton potential on the membranes of mitochondria.*

TESTS FOR SELF-CONTROL

1. The cell was treated by substance that blocks phosphorylation of nucleotides in mitochondria. Which process of cell activity will be affected firstly?

A. Fragmentation of big mitochondria into small ones.
B. Mitochondrial protein synthesis.
C. Integration of functional protein molecules.
D. Oxidative phosphorylation.
E. All the mentioned.

2. The sharp increase in the formation of reactive oxygen species (superoxide anion radical, hydrogen peroxide, hydroxyl radical) is observed in neutrophils during phagocytosis. Apart from these, another substance with a high bactericidal action is formed in neutrophils by the enzyme myeloperoxidase. This substance is:

A. Hypochlorite anion.
B. Hydroperoxide radical.
C. Peroxynitrite.
D. Radicals of saturated fatty acids.
E. Radicals of unsaturated fatty acids.

3. Point out an anticoagulant that uncoupes respiration and phosphorylation:

A. Menaquinone. *C. Barbiturates.* *E. Naphthoquinone.*
B. Rotenone. *D. Dicoumarol.*

4. In the patient with scurvy the disturbance of proline and lysine hydroxylation in the composition of collagen was revealed. Which biochemical process inhibition leads to this impairment?

A. Tissue respiration. *D. Lipid peroxidation.*
B. Peroxidase oxidation. *E. Microsomal oxidation.*
C. Oxidative phosphorylation.

5. A patient addressed to the physician with complaints of dyspnea, dizziness. It turned out that he was working at a chemical plant for the production of hydrocyanic acid. Which enzyme activity impairment is associated with these symptoms?

A. Catalase. *D. Pyruvate dehydrogenase.*
B. Cytochrome oxidase. *E. Succinate dehydrogenase.*
C. Lactate dehydrogenase.

6. In thyrotoxicosis the increased production of thyroid hormones T_3 and T_4, weight loss, tachycardia, psychic excitability are observed. How thyroid hormones affect energy metabolism in mitochondria of cells?

A. They activate the substrate level phosphorylation.
B. They block the substrate level phosphorylation.
C. They block the respiratory chain.
D. They uncouple oxidation and oxidative phosphorylation.
E. They activate oxidative phosphorylation.

7. Organisms lacking systems of protection from H_2O_2 are able to exist only under anaerobic conditions. Which of the below-mentioned enzymes are able to destroy hydrogen peroxide?

A. Oxygenases and hydroxylases. *D. Peroxidase and catalase.*
B Cytochrome oxidase. *E. Flavin-dependent oxidases.*
C. Oxygenase and catalase.

8. Cyanide poisoning leads to instant death. What is the mechanism of cyanide action on the molecular level?

A. They inhibit cytochrome oxidase. *D. They inactivate oxygen.*
B. They bind substrates of TCA. *E. They inhibit cytochrome b_5.*
C. They block succinate dehydrogenase.

9. Biological oxidation and neutralization of xenobiotics is performed by the heme-containing enzymes. Which metal ions are obligatory component of these enzymes?

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

10. The process of ATP synthesis related to oxidative reactions with the participation of the mitochondrial respiratory enzymes is called:

A. Free oxidation.
B. Substrate level phosphorylation.
C. Photosynthetic phosphorylation.
D. Peroxide oxidation.
E. Oxidative phosphorylation.

11. Monooxygenases of endoplasmic reticulum of hepatocytes are known to oxidize foreign substances. Name this process.

A. Mitochondrial oxidation. *D. Microsomal oxidation.*
B. Induced synthesis. *E. Dehydrogenation.*
C. Repression.

12. During uncoupling of respiration and oxidative phosphorylation the energy is dissipated as heat, i.e., uncouplers increase a body temperature (pyrogenic effects). Which of the following substances have this effect?

A. All of these substances. *D. 2,4-Dinitrophenol.*
B. Dicoumarol. *E. None of these substances.*
C. Thyroxine.

13. Which of the following substances is not supposed to be a cofactor of mitochondrial oxidation:

A. FAD. *B. FMN.* *C. NADP.* *D. NAD.* *E. CoQ.*

14. A dead body was found. A large concentration of cyanides was found in the blood of the deceased. Inhibition of which complex of mitochondrial respiratory chain was a cause of death?

A. III. *B. II.* *C. IV.* *D. V.* *E. I.*

15. In the presence of 2,4-dinitrophenol substrate oxidation can continue, but the synthesis of ATP molecules is impossible. Which is the mechanism of its actions?

A. Activation of ATPase.
B. Uncoupling of oxidation and phosphorylation in mitochondria.
C. Transfer of substrates from mitochondria.
D. Stimulation of ATP hydrolysis.
E. Inhibition of cytochrome oxidase.

16. In the study of the food coloring conversion it has been found that detoxification of xenobiotic occurs only in one phase – microsomal oxidation. Indicate the component of this phase.

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

17. Under pathological processes, which are accompanied by hypoxia, an incomplete reduction of oxygen molecules in the respiratory chain and the accumulation of hydrogen peroxide occur. Which enzyme ensures its destruction?

A. Catalase. *D. α-Ketoglutarate dehydrogenase.*
B. Cytochrome oxidase. *E. Aconitase.*
C. Succinate dehydrogenase.

18. The medical examiner during the 20-year-old girl's autopsy found that death was caused by cyanide poisoning. Which process enzyme activity is inhibited by cyanides to the greatest extent?

A. Hemoglobin synthesis.
B. Urea synthesis.
C. Tissue respiration.
D. Transport of oxygen by hemoglobin.
E. Hydrogen transport via malate-aspartate mechanism.

19. Reactive oxygen species, including superoxide anion-radical, are formed in the body during metabolism. This anion is inactivated by the enzyme:

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

20. A patient with insecticide (rotenone) poisoning has been delivered to the hospital. Which portion of the mitochondrial electron transport chain is blocked with this substance?

A. Coenzyme Q-cytochrome c reductase. *D. Cytochrome c oxidase.*
B. NADH-coenzyme Q-reductase. *E. ATP synthase.*
C. Succinate-Coenzyme Q-reductase.

21. All the mentioned substances are the substrates in the process of tissue respiration, except for:

A. Succinate. *C. Cholesterol.* *E. α-Ketoglutarate.*
B. Isocitrate. *D. Malate.*

22. What unites cytochrome, catalase and hemoglobin?

A. Catalysis of reductive-oxidative reactions.

B. Transport of oxygen.

C. Availability of non-heme iron.

D. The presence of heme.

E. Hydrogen transfer.

23. Unconscious patient in serious conditions has been delivered to the intensive care unit. Overdose of barbiturates, which led to the phenomenon of tissue hypoxia, was diagnosed. At the which level of electron transport chain was blocked?

A. Cytochrome oxidase.

B. Cytochrome b-cytochrome c1.

C. Ubiquinone.

D. ATP-synthase.

E. NADH-coenzyme Q-reductase.

24. In biological systems, the general principle of energy transport from exergonic to endergonic reactions is its transport in the form of chemical bonds. Which substance is a universal carrier of energy in the body?

A. ATP.

B. Phosphoenolpyruvate.

C. Creatine phosphate.

D. Phospho succinate.

E.Acyl-CoA.

25. The difference between the tissue respiration and other types of biological oxidation is the presence of one of the below mentioned components as obligatory acceptor of hydrogen:

A. NAD. *B. FAD.* *C. O_2.* *D. NADP.* *E. Pyruvate.*

26. Cytochromes were found to be distributed in respiratory chain between CoQ and oxygen. What predetermines the sequence of their incorporation in respiratory chain?

A. Redox potential.

B. Molecular mass.

C. The presence in structure of different metal ions.

D. Amount of peptide chains.

E. Difference of heme structure.

27. Indicate the index to estimate the energy effect of reaction obtained by oxidative phosphorylation:

A. Respiratory control (ATP/ADP).

B. Coefficient of phosphorylation (P/O).

C. Ratio NADH/NAD^+.

D. Ratio $CoQH_2$/CoQ.

E. Ratio HSCoA/acetyl-CoA.

28. All the substances belong to tissue respiration except:

A. Thiamine pyrophosphate.

B. Riboflavin.

C. Pantothenic acid.

D. Niacin.

E. Pyridoxal phosphate.

29. The substrates of microsomal oxidation are:

A. Pyruvate and acetyl-CoA.

B. Succinate and malate.

C. Steroids hormones and cholesterol.

D. Isocitrate and α-ketoglutarate.

30. The purpose of respiratory chain in mitochondria is:

A. Transformation of substances and energy.

B. Oxidation of substances to CO_2 and H_2O.

C. Providing of cells with NAD^+ and FAD.

D. The transfer of hydrogen atoms from $NADH_2$ to oxygen with formation of ATP and water.

E. Transfer of electrons to cytochromes.

31. Show the point of coupling oxidation and phosphorylation in respiratory chain blocked by barbiturate:

A. $FMNH_2DH \rightarrow CoQ$.

B. $CoQH_2 \rightarrow 2b(Fe^{3+})$.

C. $2b(Fe^{2+}) \rightarrow 2c_1 (Fe^{3+})$.

D. Cytochrome oxidase $\rightarrow 1/2O_2$.

E. NADH → FMNDH.

32. Show the point of coupling oxidation and phosphorylation in respiratory chain blocked by antibiotic antimycin A:

A. $FMNH_2DH \rightarrow CoQ$.

B. $CoQH_2 \rightarrow 2b(Fe^{3+})$.

C. $2b(Fe^{2+}) \rightarrow 2c_1 (Fe^{3+})$

D. Cytochrome oxidase $\rightarrow 1/2O_2$.

E. NADH → FMNDH.

33. Show the point of coupling oxidation and phosphorylation in respiratory chain blocked by carbon monooxide:

A. $FMNH_2DH \rightarrow CoQ$.

B. $CoQH_2 \rightarrow 2b(Fe^{3+})$.

C. $2b(Fe^{2+}) \rightarrow 2c_1 (Fe^{3+})$.

D. Cytochrome oxidase $\rightarrow 1/2O_2$.

E. NADH → FMNDH

34. Experimental animals were treated by preparation, that removed the pH gradient on the inner mitochondrial membrane to uncouple the tissue respiration and oxidative phosphorylation. Which substance was injected?

A. Dinitrophenol. *B. Cholesterol.* *C. Ketone bodies.* *D. Urea.* *E. Somatotropin.*

35. After the treatment with phenobarbital, which is the inductor of cytochrome P_{450} synthesis, the following process was activated in a patient:

A. Microsomal oxidation.

B. Peroxide oxidation of lipids.

C. Biological oxidation.

D. Oxidative phosphorylation.

E. Substrate level phosphorylation.

36. Macroergic bonds are:

A. Chemical bonds whose formation requires a lot of energy.

B. Bonds present in carbohydrates, lipids, proteins.

C. Chemical bonds whose cleavage is accompanied by the release more than 21 kJ of energy.

D. Bonds whose hydrolysis is accompanied by therelease of 15 kJ of energy.

E. Bonds formed by carbonic acid

37. The function of brown fat tissue in newborns is:

A. Serving as the plastic material.

B. Serving as the thermoinsulator.

C. Serving as a source of heat by means of uncoupling oxidation and phosphorylation.

D. Performing the mechanic protection of tissues and organs.

E. Being the source of ketone bodies formation.

PRACTICAL WORK

Splitting of hydrogen peroxide by blood catalase
Determination of blood catalase number

Task 1. Identify the action of catalase.

Procedure. Pour 10–15 drops of 3 % H_2O_2 solution into the test tube and add 1 drop of blood. The rapid release of oxygen occurs: the liquid foams, the foam fills the entire test tube.

Task 2. Determine the blood catalase number**.**

Principle. The method is based on the determination of hydrogen peroxide, split by the enzyme during certain period of time. The number of split hydrogen peroxide can be estimated by the difference of $KMnO_4$ expended for titration before and after the action of catalase:

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

Procedure. Pour 1 ml of diluted blood (1:1 000) and pour 7 ml of H_2O (dyst.) into two flasks for titration. Then add 2 ml of 1 % H_2O_2 in the test sample and 5 ml of 10 % H_2SO_4 solution in control sample. Action of catalase in acidic medium (the control sample) stops, because it acts at pH = 7.4. Leave both samples at room temperature for 30 minutes, pour into the test flask 5 ml 10% H_2SO_4 and 2 ml of 1 % solution of H_2O_2 in control sample. Titrate the content of each flask with 0.1 N $KMnO_4$ solution to slightly pink color. Calculate catalase number (CN) according to the formula:

$$CN\ (U) = (A - B) \times 1.7,$$

where *A* is the amount of 0.1 N $KMnO_4$ for titration of control sample, ml; *B* is the amount of 0.1 N $KMnO_4$ for titration of test sample, ml; 1.7 is number of H_2O_2, which is equivalent to 1 ml of 0.1 N $KMnO_4$, mg (1 ml of 0.1 N $KMnO_4$ is equivalent to 1 ml of 0.1 N H_2O_2).

Clinical and dianogstic significance. Catalase (EC 1.11.1.6) is an enzyme that breaks down hydrogen peroxide into molecular oxygen and water. Catalase activity indicator is the catalase number. It is an amount of hydrogen peroxide in mg broken down by one microliter (10^{-6} liters) of blood during certain period of time. Normally catalase number ranges from 10 to 15 units. It is reduced in some diseases accompanied by cachexia (cancer, anemia, tuberculosis).

1.** Prepare the abstract on the topic: "Uncouplers of oxidative phosphorylation and tissue respiration, regulation of thermogenesis ."

2.** Prepare a presentation on the topic: "The development of conceptions about biological oxidation."

3.** Review the scientific literature on the topic: "The regulation of oxidative phosphorylation."

CLASS 6 (4 hours)

Topic 8 (2 hours): Basic principles of metabolism. Common pathways of catabolism: oxidative decarboxylation of pyruvate, tricarboxylic acid cycle (Krebs cycle). Determination of succinate dehydrogenase in muscles.

Importance. Oxidative decarboxylation of pyruvate and tricarboxylic acid cycle (Krebs cycle) are general metabolic processes of complete intracellular breakdown of proteins, fats and carbohydrates. They are located in mitochondria, ensuring uninterrupted transfer of electrons and protons to the respiratory chain. Krebs cycle performs integrative, hydrogengenerating, energy and amphybolic functions. The metabolism of a living cell is closely associated with the metabolism of energy. Disorders of energy metabolism in most cases are an important link in the pathogenesis of various diseases and the correction of energy metabolism is the basis of their prevention and treatment.

Objective. Study the biochemical principles of energy metabolism, oxidative decarboxylation of pyruvate, regulatory mechanisms and pivotal role of the tricarboxylic acid cycle in metabolism. Familiarize yourself with the determination of succinate dehydrogenase activity of muscles and its competitive inhibition by malonic acid.

THEORETICAL QUESTIONS

1. General understanding of substance and energy metabolism in the body. Catabolic and anabolic pathways, their relationship.

2.* Exergonic and endergonic biochemical reactions; the role of ATP and other high energy phosphates in coupling the processes which are accompanied by accumulation and release of energy.

3. Stages of exogenous and endogenous biomolecules catabolism in the body. Common and specific pathways of catabolism. The end products of catabolic pathways in humans.

4. Intracellular localization of enzymes and metabolic pathways, compartmentalization of metabolic processes in the cell.

5.* Methods of metabolism investigation. Methods of studing the metabolism.

6. Oxidative decarboxylation of pyruvate: a sequence of reactions characteristic of pyruvate dehydrogenase multienzyme complex.

7. Citric acid cycle (the tricarboxylic acid cycle, TCA cycle or Krebs cycle): intracellular localization and characteristic of enzymes, the sequence of reactions, regulation and biological roles. Energy balance of TCA cycle.

Indicative list of theoretical questions for self-study

Content	*The main theses*
1. Exergonic biochemical reactions are accompanied by energy release (ΔG is negative).	*1.1. The reaction occurs spontaneously and is accompanied by a decrease in the free energy.* *1.2. If the absolute value of ΔG is large, the reaction occurs almost to the end (irreversible).* *1.3. They serve as energy sources for other reactions or processes.* *1.4.Catabolic reactions.*
2. Endergonic biochemical reactions occur with consumption of energy (ΔG is positive).	*2.1. The reaction only occurs when there is a supply of free energy.* *2.2. If the absolute value of ΔG is high, the system is stable and the reaction doesn't occur.* *2.3. They are always energetically coupled reactions, since they need a delivery of energy from exergonic reactions.* *2.4. Anabolic reactions.*
3. The role of ATP and other macro energy phosphates in coupling the processes accompanied by delivery and consumption of energy.	*3.1. In biological systems, endergonic reactions can take place only at the expense of energy of exergonic reactions → energy coupling reactions (the role of coupling factor in the majority of cases is performed by ATP).* *3.2. In the body, there is a whole group of organic phosphates whose hydrolysis leads to the release of a large amount of free energy. Such compounds are called high-energy phosphates (1,3-bisphosphoglycerate, phosphoenolpyruvate, creatine phosphate, ATP, pyrophosphate, etc.).* *3.3. ATP contains two phosphoanhydride bonds → in the hydrolysis of terminal phosphoanhydride bond ATP is converted into ADP and orthophosphate, ΔG = -7,3 kcal/mol → ATP is the main directly used donor of free energy in biological systems to perform endergonic reactions, different types of work (muscle contraction, active transport, etc.).*
4. Methods of studying the metabolism.	*To study metabolic processes in the body a variety of instructional approaches at different levels of organization (the whole organism, isolated organs, tissue slices, homogenates,*

	extracts, subcellular structures, biological fluids, and others) is used. *Basic research methods:* *– dialysis;* *– centrifugation;* *– optical methods:* *1) refractometry;* *2) polarimetry;* *3) photometry:* *a) absorption (spectrophotometry, nephelometry, atomic absorption photometry);* *b) emission (fluorometry, flame photometry, atomic emission spectral analysis);* *– chromatography (ion exchange, absorption, gas, distribution, affinity chromatography and other types);* *– radioisotope, radioimmunoassay methods;* *– linked immunosorbent assay;* *– immunofluorescence assay;* *– an analysis based on the use of polymerase chain reaction;* *– saturation assay (radioimmunoassay, immunoradiometric assay - IRMA);* *– electrophoresis (frontal, zone, isoelectric focusing, immunoelectrophoresis, electrophoresis in agarose gel, electrophoresis in starch gel, electrophoresis on paper, etc.);* *– gel filtration;* *– sedimentation analysis method.*

TESTS FOR SELF-CONTROL

1. Choose the cellular localization of Krebs cycle enzymes:

A. Mitochondria. *C. Endoplasmic reticulum.* *E. Lysosomes.*
B. Cytosol. *D. Nucleus.*

2. A pesticide contains sodium arsenate that blocks lipoic acid. Which enzyme activity is affected?

A. Microsomal oxidation. *D. Glutathione reductase.*
B. Methemoglobin reductase. *E. Pyruvate dehydrogenase complex.*
C. Glutathione peroxidase.

3. Tricarboxylic acid cycle is the other name of Krebs cycle. Choose tricarboxylic acid of Krebs cycle:

A. α-Ketoglutarate. *C. Succinate.* *E. Malate.*
B. Isocitrate. *D. Fumarate.*

4. Indicate the first reaction product of Krebs cycle:

A. cis-Aconitate. *C. Citrate.* *E. Malate.*
B. Isocitrate. *D. α-Ketoglutarate.*

5. Choose the enzyme of Krebs cycle whose activity limits the rate of the entire pathway:

A. Citrate synthase.
B. Succinate dehydrogenase.
C. Isocitrate dehydrogenase.
D. Succinyl-CoA thiokinase (Succinyl-CoA synthetase).
E. Malate dehydrogenase.

6. Indicate the enzyme of Krebs cycle necessary for GTP synthesis:

A. Citrate synthase.
B. Succinate dehydrogenase.
C. Isocitrate dehydrogenase.
D. Succinyl-CoA thiokinase (Succinyl-CoA synthetase).
E. Malate dehydrogenase.

7. Choose the metabolite of Krebs cycle that is macroergic substance:

A. Citrate. *C. Isocitrate.* *E. Fumarate.*
B. Succinate. *D. Succinyl-CoA.*

8. What is the energy effect of Krebs cycle provided by oxidative phosphorylation?

A. 8 ATP. *B. 11 ATP.* *C. 12 ATP.* *D. 9 ATP.* *E. 3 ATP.*

9. All vitamins participate in reaction of oxidative decarboxylation of pyruvate, except for:

A. Vitamin B_5. *C. Vitamin B_2.* *E. Vitamin B_7.*
B. Vitamin B_3. *D. Vitamin B_1.*

10. In tissue respiration the universalization of energy occurs by ATP formation. How many molecules of ATP are formed in conversion of α-ketoglutarate into succinyl-CoA?

A. 5 molecules. *C. 3 molecules.* *E. 12 molecules.*
B. 6 molecules. *D. 2 molecules.*

11. A central intermediate of protein, lipid, and carbohydrate metabolism is:

A. Succinyl-CoA. *C. Oxaloacetate.* *E. Citrate.*
B. Acetyl-CoA. *D. Lactate.*

12. Pyruvic acid, as an intermediate metabolite of carbohydrate, lipid and amino acid metabolism, undergoes oxidative decarboxylation. Which nutrient absence in diet is the cause of this process disturbance?

A. Thiamine. *C. Pangamic acid.* *E. Pyridoxine.*
B. Citrine. *D. Ascorbic acid.*

13. There are three main stages of catabolism in the enzymatic degradation of complex bioorganic compounds in humans. In the first stage the reactions occur according to the mechanism:

A. Reduction. *C. Hydrolysis.* *E. Phosphorylation.*
B. Oxidation. *D. Carboxylation.*

14. A chemical plant worker was delivered to the hospital with symptoms of poisoning. The elevated arsenium concentrations were revealed in the woman's hair. Arsenium blocks lipoic acid. Which process impairment is a likely cause of the poisoning?

A. Microsomal oxidation.
B. Methemoglobin reduction.
C. Glutathione reduction.
D. Oxidative decarboxylation of pyruvate.
E. Elimination of superoxide ions.

15. Reactions, metabolites of which may be included both in catabolic and anabolic processes, belong to:

A. Catabolic. *C. Exergonic.* *E. Amphibolic.*
B. Anabolic. *D. Endergonic.*

16. Oxidation of acetyl-CoA in Krebs cycle plays an important role in the energy supply of each of these cells (tissues, organs), except for:

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

17. How many ATP molecules can be formed in the citric acid cycle without tissue respiration?

A. 12. *B. 11.* *C. 2.* *D. 1.* *E. 3.*

18. The oxidative decarboxylation of pyruvic acid is catalyzed by multi-enzyme complex with involving several functionally related coenzymes. Specify this complex.

A. FAD, FH4, PLP, TPP, choline.
B. NAD, PLP, TPP, methyl cobalamin, biotin.
C. TPP, FAD, HS-CoA, NAD, lipoic acid.
D. HS-CoA, FAD, PLP, FH4, carnitine.
E. Lipoic acid, FH4, carboxy biotin, methyl cobalamin.

19. What is acid – an intermediate of Krebs cycle – is involved in the binding of calcium ions?

A. Malate. *C. Succinate.* *E. Alpha-ketoglutarate.*
B. Acetate. *D. Citrate.*

20. It is known, that in the biological cell membranes, some enzymes are capable of forming poly enzymatic complexes which catalyze the sequences of subsequent biochemical reactions. Which of the below mentioned enzymes belongs to such complex?

A. Pyruvate dehydrogenase. *D. Phosphorylase.*
B. Hexokinase. *E. Phosphofructokinase.*
C. Lactate dehydrogenase.

21. In experimental animals lipoic acid was eliminated from the diet that led to inhibition of pyruvate dehydrogenase complex. For this enzyme lipoic acid is:

A. Inhibitor. *C. Allosteric regulator.* *E. Coenzyme.*
B. Substrate. *D. Product.*

PRACTICAL WORK

Activity of succinate dehydrogenase in muscles and its competitive inhibition by malonate

Objective. Identify the effect of succinate dehydrogenase of muscles and competitive inhibition of its activity by malonate.

Principle. The effect of succinate dehydrogenase (SDH) that catalyzes the oxidation (dehydrogenation) of succinic acid ($HOOC\text{-}CH_2\text{-}CH_2\text{-}COOH$) to fumaric acid ($HOOC\text{-}CH{=}CH\text{-}COOH$), can be estimated by discoloration of added to the mixture hydrogen acceptor 2,6-dichlorophenolindophenol, which is reduced and converted to leucoform. Discoloration of the reaction mixture does not occur in the presence of malonic acid ($HOOC\text{-}CH_2\text{-}COOH$), which is a competitive inhibitor of SDH.

Procedure. 1–2 g of fresh muscles are shredded by scissors and ground in a mortar with a little amount of water (2–3 ml) for 1 min. Then muscular pulp is transferred to a double layer of gauze in a funnel and washed with 25 ml of distilled water. Rinsed pulp is wrung out, transferred to a test tube and suspended by glass rod with 4 ml of water. The resulting suspension is poured evenly into three test tubes. Boil the first test tube for 1–2 min to inactivate the enzyme, than pour into the test tube reagents according to the scheme given in the table below:

№ of test tube	Succinate, ml	Water, ml	Malonate, ml	2,6-dichlorophenolindophenol
1	1	0.5	–	2 drops
2	1	0.5	–	2 drops
3	1	–	0.5	2 drops

The disappearance of blue color is observed in the second test tube after 15 minutes.

Practical significance. In clinical studies biochemical methods are used for determination of redox enzymes in bioptates in order to estimate energy metabolism in various pathological conditions, as well as in toxicology and pharmacology in the study of drugs and poisons that can be uncouplers or inhibitors.

1.** Prepare the abstract on the topic: "Mechanisms of regulation of common pathways of catabolism".

2.** Prepare a presentation on the topic: "Citric acid cycle is a common metabolic pathway of carbohydrate, lipid and amino acid metabolism."

CONCLUDING CONTROL WORK ON PART 1 "GENERAL FEATURES OF METABOLISM" (2 HOURS) LIST OF QUESTIONS

1. Biological chemistry as a science. Place of biochemistry among other biomedical disciplines.

2. The objects of study and biochemistry tasks. The leading role of biochemistry in determining the molecular mechanisms of human diseases pathogenesis.

3. Connection of biochemistry with other biomedical sciences. Medical biochemistry. Clinical biochemistry. Laboratory diagnostics.

4. History of biochemistry, development of biomedical research in Ukraine.

5. Biochemical components of cells and their functions. Classes of biomolecules. Hierarchy of biomolecules and their origin.

6. Enzymes: definition, properties as biological catalysts.

7. Classification and nomenclature of enzymes, characteristic of some classes of enzymes.

8. Structure and mechanisms of enzyme action. Active and allosteric (regulatory) sites.

9. Cofactors and coenzymes. Structure and properties of coenzymes, vitamins as precursors in the biosynthesis of coenzymes.

10. Coenzymes. Types of reactions that are catalyzed by separate classes of coenzymes.

11. Isoenzymes: structural features and functioning, the significance in the diagnosis of diseases.

12. The mechanism of action and kinetics of enzymatic reactions: the dependence of reaction velocity on substrate concentration, pH and temperature.

13. Mechanisms of enzyme activity regulation. Allosteric enzymes, covalent modification of enzymes. Activators and inhibitors of enzymes: examples and mechanisms of action.

14. Types of enzyme inhibition: reversible (competitive, non-competitive) and irreversible.

15. General understanding of enzymopathies and their causes.

16. Enzyme diagnostics of pathological processes and diseases.

17. Enzyme therapy. The use of enzymes, their activators and inhibitors in medicine.

18. Principles and methods of enzyme detection in biological objects. Units of enzyme activity.

19. History of discovery of vitamins, Lunin's and Funk's role in the development of vitaminology.

20. General characteristics of vitamins. The role of vitamins in the body. Classifications based on physical and chemical properties and clinical and physiological effect. Provitamins, their structures.

21. General characteristics of hypo- and avitaminosis, their classification, causes.

22. Vitamin A and β-carotene: structure, role in metabolism, sources, daily requirement for retinol and β-carotene, hypo- and hypervitaminosis.

23. Vitamin E: structure, role in metabolism, sources, daily requirement, deficiency symptoms

24 Vitamin K: structure, role in blood coagulation system; sources, daily requirement. Vitamin K analogues and antagonists as drugs.

25. Vitamin D: structure, mechanism of action, role in the metabolism of calcium and phosphate, sources, daily requirement. Hypovitaminosis in children and adults. Symptoms of hypervitaminosis.

26. Vitamin F (complex of polyunsaturated fatty acids): the structure of the components of the complex, role in metabolism, sources, daily requirement, deficiency symptoms.

27. Vitamin B_1 (thiamine): structure, biological properties, role in metabolism, sources, daily requirement, deficiency symptoms. Structure of TPP.

28. Vitamin B_2 (riboflavin): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms. Structure of FAD, FMN.

29. Pantothenic acid: structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms. Structure of HS-CoA.

30. Vitamin PP (nicotinic acid, nicotinamide): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms. Structure of NAD and NADP.

31. Vitamin B_6 (pyridoxine): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms. Structure of PALP.

32. Vitamin B_7 (biotin): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms.

33. Vitamin B_9 (folic acid): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms.

34. Vitamin B_{12} (cobalamin): structure, biological properties, mechanism of action in metabolism, sources, daily requirement, deficiency symptoms.

35. Vitamin C (ascorbic acid): structure, biological properties, mechanism of action in metabolism, sources, deficiency symptoms. Prophylactic, protective and therapeutic doses.

36. Vitamin P (flavonoids): structure, biological properties, mechanism of action, the manifestations of deficiency, sources, daily requirement.

37. General characteristics of the vitamin-like substances. Role of carnitine, ubiquinone and lipoic acid in metabolism.

38. Antivitamins: specificity of structure and action, use in medicine.

39. Metabolism: the general features of catabolic and anabolic processes.

40. General stages of intracellular catabolism of biomolecules: proteins, carbohydrates, lipids.

41. Citric acid cycle (TCA cycle): localization, sequence of enzymatic reactions, importance in metabolism.

42. Energy balance of TCA cycle.

43. Amphibolic function of TCA cycle.

44. Biological oxidation reactions; types of reactions (dehydrogenase, oxidase, oxygenase reactions) and their biological significance.

45. Tissue respiration: stages, localization in the cell.

46. Enzymes of biological oxidation in mitochondria: pyridine and flavin-dependent dehydrogenases, cytochromes.

47. Sequence of components of respiratory chain in mitochondria. Molecular complexes of mitochondrial innner membranes.

48. Oxidative phosphorylation: electron transport and oxidative phosphorylation coupling points, the coefficient of oxidative phosphorylation.

49. Chemiosmotic theory of oxidative phosphorylation, mitochondrial ATP-synthase.

50. Inhibitors and uncouplers of electron transport and oxidative phosphorylation.

51. Microsomal oxidation: cytochrome P-450 and b_5; molecular organization of electron transport chain.

52. Lipid peroxidation: biological significance and role in the occurrence of pathological conditions.

Навчальне видання

БІОЛОГІЧНА ХІМІЯ
Частина 1
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BIOLOGICAL CHEMISTRY
Part 1
THE GENERAL PRINCIPLES OF METABOLISM

Study guide
for students of general medicine faculty