THE MINISTRY OF HEALTH OF UKRAINE
ZAPORIZHZHIA STATE MEDICAL AND PHARMACEUTICAL UNIVERSITY

Biological Chemistry Department

BIOLOGICAL CHEMISTRY

SECTION 1: COMMON REGULARITIES OF METABOLISM AND ENERGY EXCHANGE. METABOLISM OF CARBOHYDRATES, LIPIDS AND AMINO ACIDS AND ITS REGULATION

HANDBOOK FOR INDEPENDENT WORK
PREPARATION FOR COMPONENT OF USQE 'KROK-1, DENTISTRY'
for students of Speciality "Dentistry"

ZAPORIZHZHIA
2025

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

Editors:Dr. Sc., professor Shwets V.M.
Dr. Sc., professor Ivanchenko D.H.
PhD., senior teacher Mykhalchenko Ye.K.
Reviewers:

- Prykhodko O. B. – a Head of Medical Biology department, Dr. Sc., ass. professor, ZSMPhU
- Antipenko O. M. - lecturer of Organic Chemistry department, ass. professor, ZSMPhU

BIOLOGICAL CHEMISTRY. Section 1: Common regularities of metabolism and energy exchange. metabolism of carbohydrates, lipids and amino acids and its regulation handbook for independent work at home and in class preparation for component of USQE ‘KROK-1, Dentistry’ for students Speciality “Dentistry” / V. M. Shwets, D. H. Ivanchenko, Ye.K. Mykhalchenko – Zaporizhzhia : ZSMPhU, 2025. - 230 p.

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

INTRODUCTION

The concept of development of health care of Ukraine envisages the holding of license integrated exams in higher educational medical institutions for unified quality control of training of specialists in the field of health care. The proposed study guide contains the necessary material for successful preparation for passing the licensing integrated test exam «Krok 1. Dentistry» for students of the Faculty of Medicine, specialty «Dentistry». The notebook covers the entire curriculum of module 1. At the beginning of each topic, students are offered a short theoretical material on the topic, then they get a block of tests to which they must independently find the answer and justify it. This work is published in English.

1. **TOPIC:** STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF PROTEINS. FUNCTIONS OF PROTEINS IN THE HUMAN BODY. SIMPLE AND COMPLEX PROTEINS. CLASSIFICATION OF PROTEINS.

2. INFORMATIVE MATERIAL

Proteins are high-molecular natural polymers consisting of amino acid residues (AA) connected by a peptide bond; is the main component of living organisms and the molecular basis of life processes.

CLASSIFICATION OF PROTEINS

I. Functional (according to the function performed by the protein in the body)

1. Catalytic (more than 3000 proteins are enzymes).
2. Contractile (actin, myosin, etc.).
3. Structural (plasma membrane proteins, collagen, elastin, etc.).
4. Transport (transport of substances in blood and cells: hemoglobin, cytochrome C, lipoproteins, etc.).
5. Protective (fibrinogen, lysozyme are factors of the body's natural resistance, interferon is synthesized by the body against viral infection).
6. Regulatory (histones stabilize the structure of DNA and regulate the functioning of the genome; heat shock proteins, stress proteins; factors of cell growth and differentiation, etc.).
7. Hormonal (calcitonin, insulin, somatotropic hormone, etc.).
8. Buffers (hemoglobin protein buffer, maintenance of blood pH).
9. Reserve (casein, ovalbumin, etc.).
10. Receptor (rhodopsin, chemoreceptors, etc.).
11. Proteins supporting oncotic pressure in cells and blood (albumin).
12. Energetic (in very small quantity, i.e., protein hydrolysis products serve as a source of energy only under special conditions, for example, during starvation).

II. By the shape of the molecule:

1. Globular or spherical (they are spherical molecules, soluble in water, perform dynamic functions: hemoglobin and albumin are enzymes, immunoglobulins,

transport proteins). When globular proteins are formed, the hydrophobic part of the polypeptide chain is located inside the structure, and the hydrophilic part is outside. The presence of polar residues of amino acids on the surface of globular proteins determines their solubility in aqueous solutions.

2. Fibrillar or filamentous (have a rod-like elongated shape, insoluble in water, since they mainly include hydrophobic amino acids; physically strong, perform structural and protective functions: collagen, elastin, β-keratin).

III. According to the degree of complexity of the molecule:

1. Simple (consist only of amino acids).
2. Complex (the composition of the protein, in addition to AA, includes a non-protein substance what is a prosthetic group).

FUNCTIONS OF PEPTIDES

Peptides are compounds consisting of several dozen amino acids connected by peptide bonds. The number of amino acids in the composition of peptides can vary greatly. Peptides containing up to 10 amino acids are called “oligopeptides”. Peptides containing more than 10 amino acids are called “polypeptides”, and polypeptides consisting of more than 50 amino acid residues are usually called proteins.

Peptides which are discovered and studied to this day can be divided into groups according to their main physiological action:

1. Peptides with hormonal activity (oxytocin, vasopressin, releasing hormones are the hormones of the hypothalamus, glucagon, etc.).
2. Peptides that regulate the digestion process (gastrin, cholecystokinin, secretin,etc.).
3. Peptides that regulate vascular tone and blood pressure (bradykinin, kalidin, angiotensin II).
4. Peptides that regulate appetite (leptin, neuropeptide Y, melanocyte-stimulating hormone, β-endorphins).
5. Peptides that have an analgetic effect (enkephalin, endorphins and other opioid peptides). The analgetic effect of these peptides is hundreds of times superior to the analgetic effect of morphine.

6. Peptides involved in the regulation of higher nervous activity, in biochemical processes related to the mechanisms of sleep, learning, memory, the emergence of feelings of fear, joy, and so on (β-endorphin).

Monomers of proteins and peptides are proteinogenic amino acids: 19α-AK (the amino group is in the α-position) and proline, which is an imino acid. Non-proteinogenic AKs are not found in the composition of proteins, but perform other important functions in the body. For example, ornithine, citrulline are intermediate metabolites in the biosynthesis of urea.

CLASSIFICATION OF AMINO ACIDS

I. According to the structure of the radical:

1. Acyclic, aliphatic (depending on the number of amino and carboxyl groups):
   - Monoaminocarbons (glycine-gly, alanine-ala, leucine-leu, isoleucine-ile, valine-val, containing a hydroxyl group – threonine-tre and serine-ser, methionine-met and cysteine-cis are containing sulfur);
   - Monoaminodicarbonic (aspartic acid-asp, glutamic acid-glu);
   - Diamino monocarbon (lysine-lyse, arginine-arg);
2. Cyclic:
   - Carbocyclic (aromatic): phenylalanine-phen, tyrosine-tyr.
   - Heterocyclic:
   - With a primary amino group in the side chain (tryptophan-three, histidine-his);
   - Imino acid (proline-pro).

II. According to acid-base properties:

1. Sour (asp, glu).
2. Basic (liz, arg, his).
3. Neutral (all others).

III. By polarity:

1. Non-polar (hydrophobic) (ala, val, leu, met, pro, tri, phen).
2. Polar:

a) Uncharged (hydrophilic) (ser, tre, cis, gly, tyr, asn, gln);

b) Charged:

- Negatively charged (glu, asp);
- Positively charged (lyz, arg, his).

IV. **By biological value:**

1. Indispensable (essential) amino acids that cannot be synthesized in the body and must come with food. They are needed to ensure and support growth: val, lei, liz, met, tre, tri, fen.
2. Partially substituted amino acids (arg and his) are synthesized in a complex way in small quantities. Most of them should come with food.
3. Conditionally interchangeable (tir and cis). Their synthesis requires essential amino acids, phenylalanine and methionine, respectively.
4. Substitute amino acids - synthesized in the body to meet biological needs: ala, asn, asp, glu, gln, gli, pro, ser.

V. **Based on metabolic transformations:**

1. Glycogenic, the carbon skeleton of which is the basis for the synthesis of glucose or glycogen (ala, arg, asp, glu, gly, his, met, pro, ser, tre, val, cis).
2. Ketogenic amino acid is leu, which is the basis for the synthesis of lipids.
3. Glycogenic and ketogenic amino acids, the carbon skeleton of which can be the basis for the synthesis of glucose and lipids (lys, phen, tri, tyr).

QUALITATIVE ANALYSIS OF PROTEINS AND AMINO ACIDS

Color reactions are used to establish the protein nature of substances, identify proteins and determine their amino acid composition in various biological fluids. In clinical laboratory practice, these methods are used to determine the amount of protein in blood plasma, amino acids in urine and blood, to detect hereditary and acquired pathologies of protein and amino acid metabolism in newborns.

Biuret reaction to a peptide bond (Piotrovsky reaction).

It is based on the ability of peptide bonds (-CO-NH-) to form colored complex compounds with copper sulfate in an alkaline medium, the intensity of its color depends on the length of the polypeptide chain. The protein solution gives a blue-violet color, and the products of its incomplete hydrolysis gives a pink color. The biuret reaction is used to detect proteins and peptides in solution, as well as for their quantitative determination (substances containing at least two peptide bonds give a positive result).

Ninhydrin reaction.

The essence of the reaction is the formation of a compound that has a blue-violet color, consists of ninhydrin and amino acid hydrolysis products. This reaction is characteristic of amino groups in the α-position present in natural amino acids and proteins.

Xanthoproteic reaction.

When concentrated nitric acid is added to the protein solution and heated, a yellow color appears, which turns orange in the presence of alkali. The essence of the reaction is the nitration of the benzene ring of cyclic amino acids with nitric acid with the formation of nitro compounds that precipitate.The reaction reveals the presence of aromatic amino acids in the protein like fen, tyr.

Millon's reagent.

Specific reaction to the presence of a hydroxyl group in the aromatic ring of tyrosine. Under conditions of heating of phenols and their derivatives with Millon's reagent (mixture of mercury nitrates (I) and (II)), mercury derivatives of brick-red color are formed.

Adamkiewicz reaction.

Amino acid tryptophan in an acidic environment, interacting with acid aldehydes, forms red-violet condensation products.

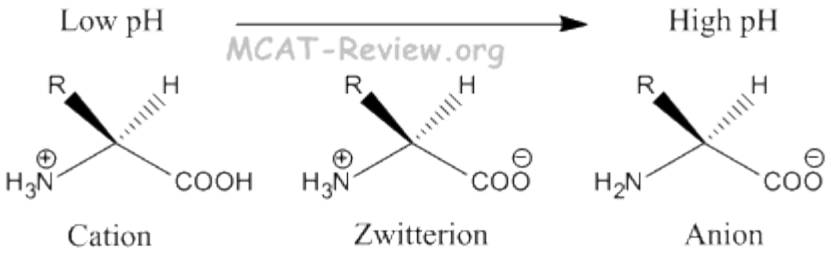
Foley's reaction.

Amino acids containing sulfhydryl groups, - SH, undergo alkaline hydrolysis with the formation of sodium sulfide Na_2S. The latter, interacting with sodium plumbite (formed during the reaction between lead acetate and NaOH), forms a black or brown precipitate of lead sulfide PbS.

PROPERTIES OF AMINO ACIDS

I. Acid-base properties. Amphotericity

Amino acids have 2 functional groups with opposite properties: an acidic carboxyl group and a basic amino group. Therefore, in an aqueous solution, amino acids exist in the form of a bipolar ion (pic. 1).



Pic. 1. Anionic, bipolar and cationic shape of amino acids in aqueous solvents.

When an additional amount of protons (acid) is added to the amino acid solution, the dissociation of carboxyl groups is inhibited and the number of NH^{3+} groups increases. At the same time, amino acids change into a cationic form (receive a positive charge). Adding alkali, on the contrary, increases the dissociation of carboxyl groups. Amino acids go into an anionic form (receive a negative charge). By changing the pH of the solution in this way, it is possible to change the charge of amino acid molecules.

Neutral amino acids in water have no charge. Dicarboxylic amino acids have two carboxyl groups that dissociate, giving 2 protons, but since they have only one amino group that accepts one proton, such amino acids have the properties of acids and their solution has an acidic reaction. The amino acid ion itself is negatively charged.

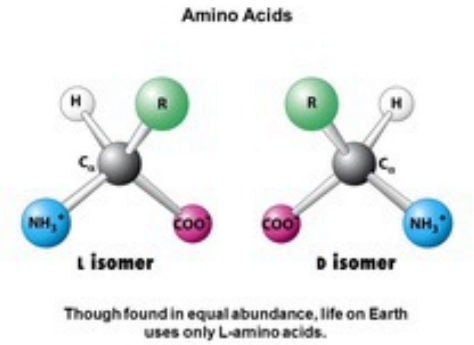
Diamino monocarboxylic amino acids react in aqueous solution as weak bases, since one proton, which is released during the dissociation of the carboxyl group of such amino acids, binds to one of the amino groups, and the second amino group binds a proton from the aqueous environment, as a result, the number of OH^- groups increases and pH increases. The ion charge of such amino acids will be positive.

By adding a certain amount of acid or alkali to the amino acid solution, you can change their charge. At a certain pH value, a state occurs in which the charge of the amino acid becomes neutral. This pH value is called the isoelectric point (IEP), while the concentration of AA does not affect the value of IEP. At a pH equal to the isoelectric point, amino acids do not move in an electric field. If the pH is below the isoelectric point, the amino acid cation moves to the cathode, and at pH above the IEP, the amino acid anion moves to the anode. The ability to separate them in an electric field (electrophoresis) is based on these properties of amino acids. Acidic amino acids have IEP in a weakly acidic environment, basic

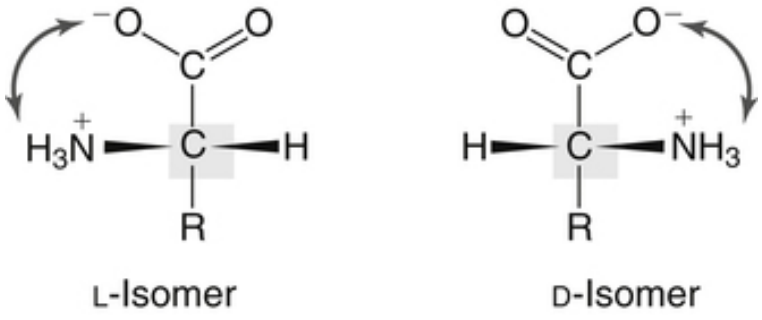
ones in a weakly basic one, and neutral ones in a neutral one.

II.Stereoisomerism

It is caused by the presence of an asymmetric carbon atom in an amino acid (called a chiral center) (pic. 2). According to the absolute configuration (standard is glycerol aldehyde), AA can be L - or D - stereoisomers (pic. 3). Only L-stereoisomers of amino acids are part of body proteins.



Pic. 2. Asymmetric carbon atom in amino acids.



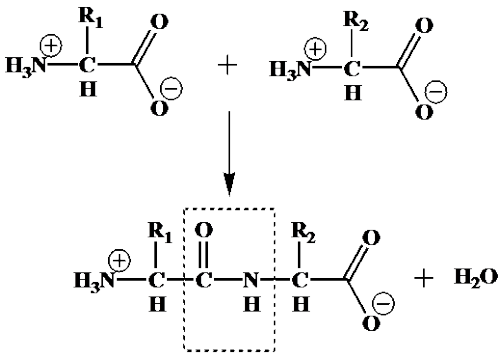
Pic. 3. L – and D – stereoisomers of AA.

Spectral properties

All amino acids absorb light in the infrared region of the spectrum. Three cyclic amino acids (phenylalanine, tyrosine and tryptophan) absorb light in the ultraviolet region at 280 nm.

LEVELS OF STRUCTURAL ORGANIZATION OF PROTEIN MOLECULES

Primary structure - this is the configuration of the polypeptide chain, which is formed as a result of the formation of a peptide bond between AA residues (Pic. 4).



Pic. 4. Formation of a peptide bond.

Postulates (principles of peptide bond formation) formulated by L.Pauling and R.Corey:

1) atoms forming a peptide bond are coplanar (located in the same plane); rotation of atoms or groups of atoms around a peptide bond is impossible;

2) the principle of equivalence of the contribution of AA residues in the formation of a peptide bond and, thereby, in the formation of a polypeptide chain (exclusion of proline);

3) the principle of the maximum of hydrogen bonds.

The primary protein structure is stabilized (maintained) by covalent:

- peptide bonds (between AK residues);
- disulfide bonds (between free SH groups of cysteine).

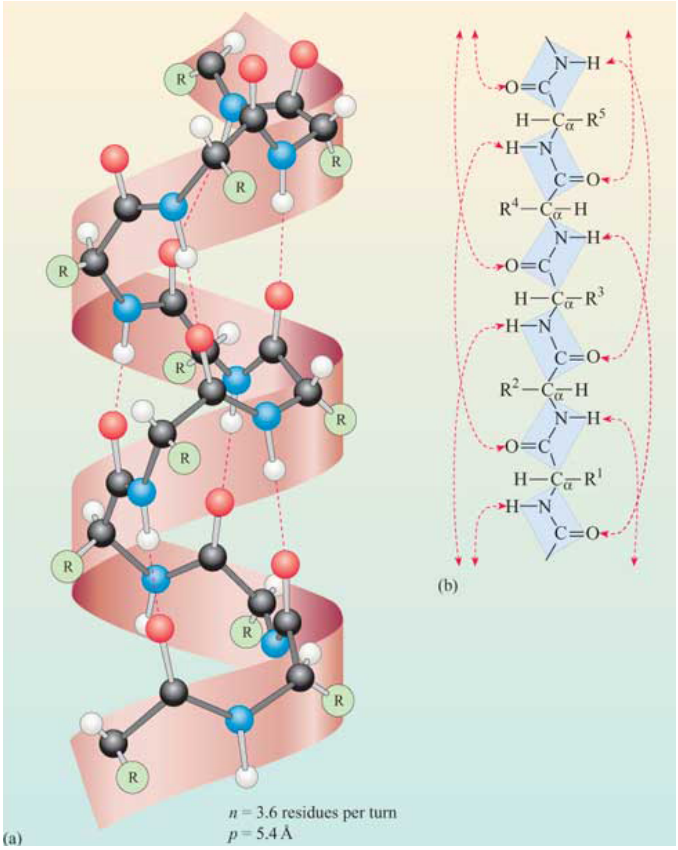
The primary structure of a protein carries information about its spatial structure.

The secondary structure of a protein is a local conformation caused by the rotation of individual sections of the polypeptide chain around single covalent bonds.

The main bonds that stabilize the secondary structure are hydrogen bonds.

Types of secondary structure:

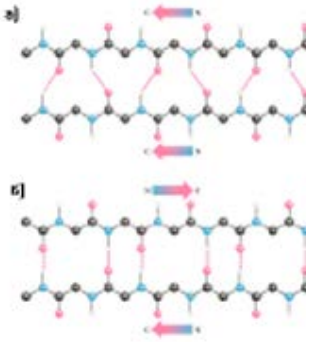
- ***α-helix(right-handed)***



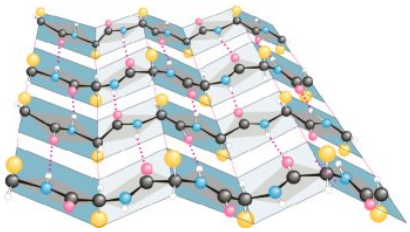
Pic. 5. Secondary protein structure: α-helix (the main type of bond is hydrogen). (https://files.mtstatic.com/site_4463/48531/0?Expires=1698681013&Signature=KtdBzmRvbLjNSmdrvq1I95848Xu4dC8OF4DzXlvtIsZqP6ZEbJFnYI-32LZwbWDRCCN2RIfz-cHQOLNxr8c~kNmiB5HU1wMwtnG9g7g9KGTAr-EWhamNDMKp-S19OED-p384JCVw~usAy8WfW7dKepl-xnEdi2ya17qxin1Ut~g_&Key-Pair-Id=APKAJ5Y6AV4GI7A555NA)

• ***β-structure***

β-layer

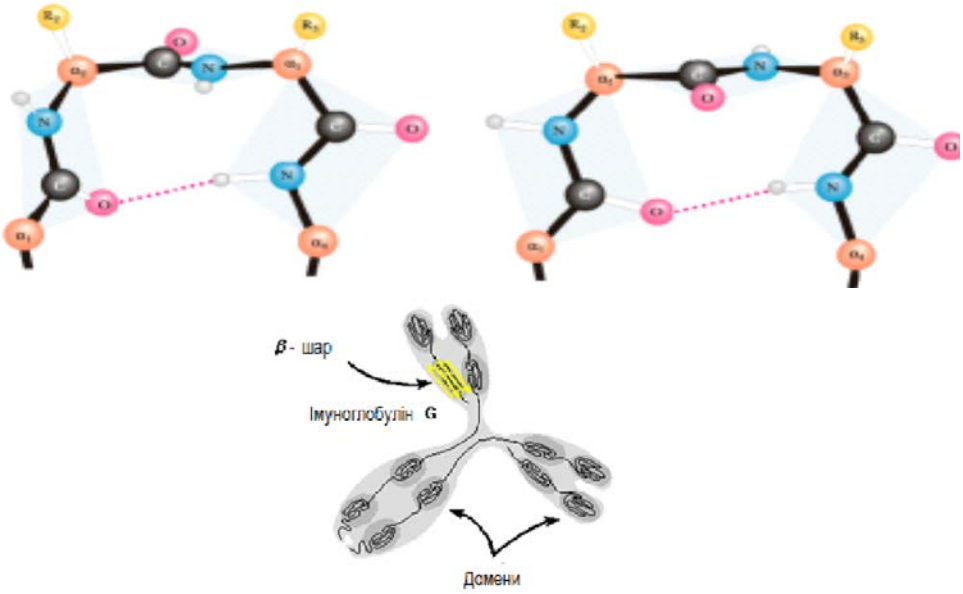


Parallel



Antiparallel

• ***β-turn***

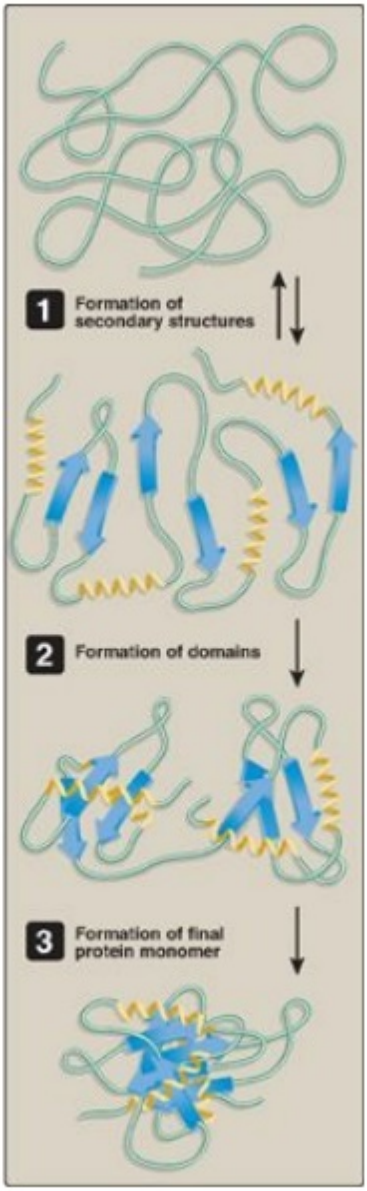


Мал.7 Доменна організація імуноглобуліну G

Pic. 6. Secondary protein structure: β-folding (the main type of bond is hydrogen). (https://www.researchgate.net/publication/372349375/figure/fig3/AS:1143128117 4783398@1689338565362.png)

Several areas of the polypeptide chain, organized in space in the form of an α-α-helix (Pic.5) or β-structures (Pic. 6), can combine to form a supersecondary structure. As a result, domains (functional or structural) are formed in the protein molecule (Pic. 7).

The tertiary structure of the protein is the location in space of the entire polypeptide chain, individual sections of which have their own local conformation (Pic. 8).



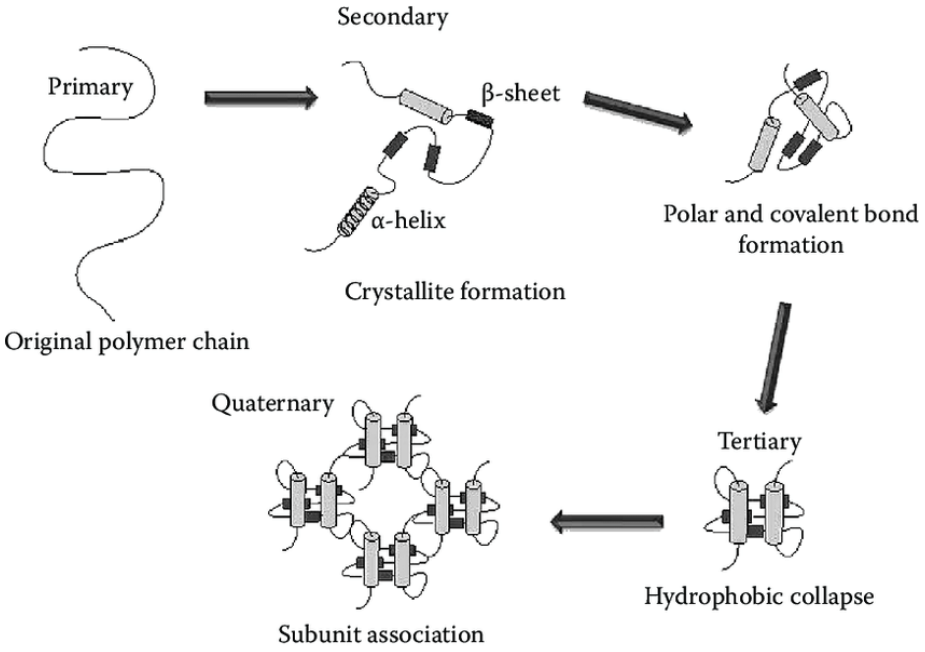
Pic.8. Stages of tertiary structure formation. (https://www.pharmacy180.com/media/imgph02/j3mjklE.jpg)

The maintenance of the tertiary structure of the protein is facilitated by hydrophobic bonds that are formed inside the molecule. Nonpolar radicals of amino acids participate in the formation of these bonds. Other non-covalent bonds can also be formed.

In a protein with a tertiary structure, an area is formed on the surface of the molecule that can attach other molecules to itself, called ligands. This area is called the active center and is formed from amino acid radicals that come close to each other during the formation of the tertiary structure. The high specificity of the

interaction between the protein and the ligand is ensured by the *complementarity* of the structure of the active center with the structure of the ligand.

A quaternary structure is formed by combining several polypeptide chains having a tertiary structure. The protein formed in this way has a new function (Pic. 9).



Pic. 9. Quaternary protein structure

Proteins with a quaternary structure (for example, hemoglobin) are called oligomeric, and the individual polypeptide chains that make them up are called protomers or monomers. Such connections are stabilized by hydrogen bonds and electrostatic interactions between AA residues located on the surface of protomers. The study of the spatial structure of the protein is carried out using X-ray structural analysis.

CLASSIFICATION OF SIMPLE PROTEINS

Protamines. These are relatively small proteins with a molecular weight of up to 10,000 Da. The molecular composition of these proteins contains up to 85% of amino acids with positively charged radicals (usually arginine) and a limited set (6-8) of other amino acids, which determines their main properties.

Protamines are soluble in weak acid solutions, do not precipitate upon boiling, have an isoelectric point at pH 10-12, are part of nucleoprotein proteins, do not contain tryptophan and sulfur.

Histones. They are the main proteins with a molecular weight of 12,000 to 20,000 Da, containing 20-30% of amino acids with positively charged radicals (usually arginine and lysine). Histones do not contain tryptophan, are soluble in dilute acids (0.2 M HCl), are precipitated by ammonia and ethanol, and have an isoelectric point at pH 8.5.

Histones are found mainly in the nuclei of animal and plant cells, where they play an important role in the structure of chromatin (a filamentous complex of DNA, histones and non-histone proteins).

Albumin refers to proteins that are widely distributed in animal and plant organisms.These proteins are found in blood serum, egg whites, muscles, and milk.

Albumins are dissolved in water, salted out from aqueous solutions with ammonium sulfate at full saturation, precipitated in the form of clots of denatured protein when boiled.

Globulins are proteins insoluble in water, but soluble in dilute solutions of neutral salts (4-10%); precipitate from the solution at half-saturation with ammonium sulfate. Representatives of this group of proteins are blood serum globulins, milk globulins, egg globulin, etc.

Prolamins is a group of proteins that are well soluble in 60-80% aqueous ethanol solution. They are vegetable proteins (the most studied is oryzenin from rice), characteristic exclusively for cereal seeds, they are not found in the living world. Prolamins are part of gluten what is a protein clot that provides elasticity and elasticity of the dough.

Glutelins dissolve well in weak alkali solutions (0.1-0.2%), but are insoluble in water, ethanol solutions, and neutral salts. This group of proteins is found in the seeds of cereals and other crops, as well as in the green parts of plants. Glutelins together with prolamins are part of gluten. Glutelins contain up to 45% glutamic acid.

Proteinoids (scleroproteins). A characteristic feature of proteinoids is complete insolubility in water, solutions of neutral salts, dissolved acids and alkalis. Proteinoids belong to fibrillar proteins. These proteins are part of the skin, tendons, bones, cartilage (collagen), hair, horns, hooves, feathers (keratin), web and silk thread (fibroin). Keratins contain up to 3% sulfur.

PHYSICO-CHEMICAL PROPERTIES OF PROTEINS

The amino acid composition and structure of the protein molecule determine

its properties. Proteins combine basic and acidic properties determined by amino acid radicals: the more acidic amino acids in a protein, the more pronounced its acidic properties are. The ability to give and add H+ is determined by the buffer properties of proteins: one of the most powerful buffers is hemoglobin in erythrocytes, which maintains blood pH at a constant level. There are soluble proteins (fibrinogen), insoluble proteins, and those that perform mechanical functions (fibroin, keratin, collagen). There are chemically active proteins (enzymes), there are chemically inactive, resistant to various environmental conditions and extremely unstable.

External factors (heating, ultraviolet radiation, heavy metals and their salts, changes in pH, radiation, dehydration) can cause a violation of the structural organization of the protein molecule what's called denaturation.

DENATURATION is the destruction of the higher structures of the protein molecule with the preservation of the primary structure and the loss of the protein's native physico-chemical and biological properties. Destruction of the primary structure occurs only as a result of hydrolysis of the protein molecule during prolonged boiling in an acid or alkali solution.

If the denaturation is not accompanied by the destruction of the primary structure, then it can be **reversible**, in which case the protein's characteristic conformation is self-restored. For example, membrane receptor proteins are subject to such denaturation. The process of restoring the protein structure after denaturation is called **renaturation**. If the restoration of the spatial configuration of the protein is impossible, then the denaturation is called **irreversible**.

Factors that cause denaturation:

I. *Chemical factors*: strong acids or alkalis, organic solvents, concentrated salts, heavy metals.
II. *Physical factors*: temperature, pressure, mechanical action, ultrasonicandionizing radiation.

METHODS OF SEPARATION, ISOLATION AND PURIFICATION PROTEIN PREPARATIONS

I.Separation methods:

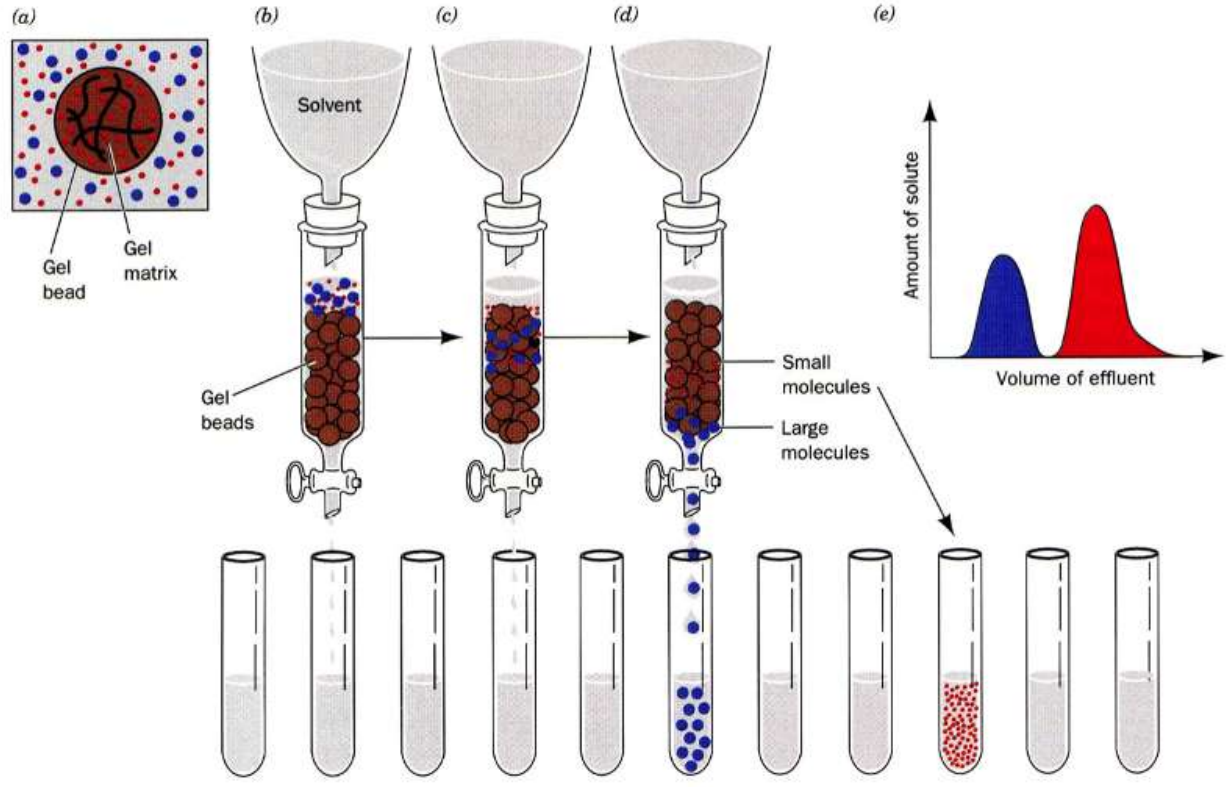
Salting out.

The method of protein separation is based on differences in their solubility at different salt concentrations in the solution. Salts of alkali and alkaline earth metals cause reversible precipitation of proteins, that is, after their removal, proteins regain the ability to dissolve, while retaining their native properties. Most often, different concentrations of ammonium sulfate salts $(NH4)_2SO_4$ are used to separate proteins by salting out. The higher the solubility of the protein, the higher the concentration of salt required for its salting out. For example, globulins precipitate at half saturation, and albumin at full saturation $(NH4))_2SO_4$.

Separation of proteins by molecular weight Gel chromatography

The chromatographic column is filled with gel granules (sephadex), which has pores of a certain size (pic. 10). A mixture of proteins is introduced into the column.

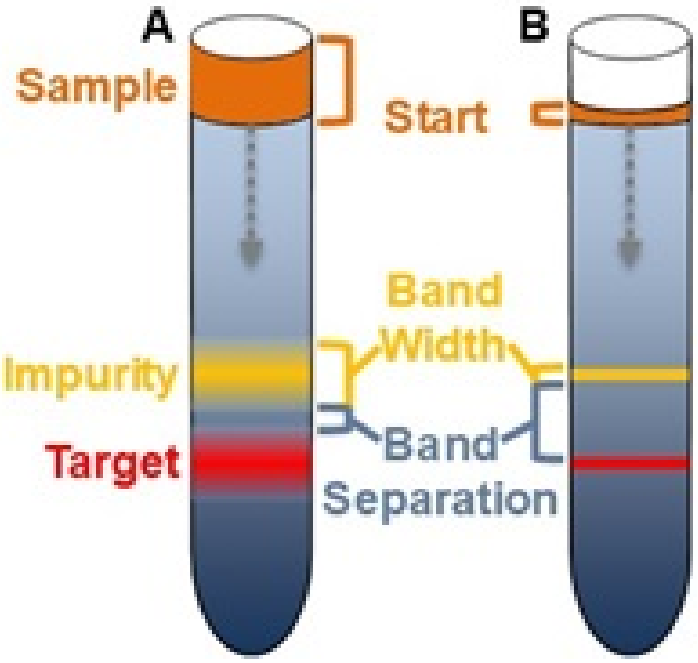
Proteins, the size of which is smaller than the size of the Sephadex pores, are retained in the column because they are "stuck" in the pores, while others freely leave the column. The size of the protein depends on its molecular weight.



Pic. 10. Gel chromatography method (https://comis.med.uvm.edu/vic/coursefiles/MD540/MD540Protein_Methods_Learning_Module_10400_593281210/Protein-methods/gel_filtration2.jpg)

Ultracentrifugation

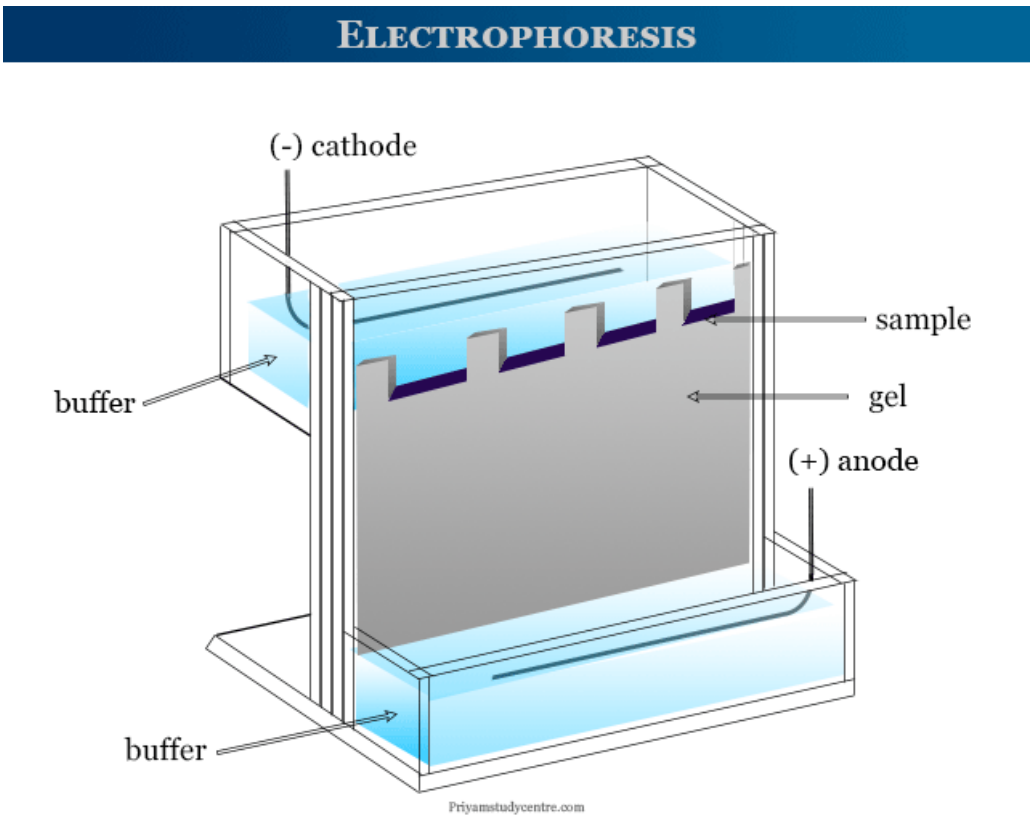
This method is based on the different rate of sedimentation (precipitation) of protein molecules in solutions with different density gradients (sucrose buffer or cesium chloride) (Pic. 11).



Pic. 11. Ultracentrifugation method (https://media.beckman.com/-/media/centrifugation/content/ultracentrifugation/centrifuges-density-gradient-ultracentrifugationfigure4202303.jpg?h=187&w=200&sc_lang=en&hash=5ADDB270B54E980DC15041F685E40B12)

Electrophoresis

This method is based on the different speed of migration of proteins and peptides in an electric field depending on their charge. Carriers for electrophoresis can be gels, cellulose acetate, agar, paper. Under the action of an electric current, the separated molecules move in the gel depending on their size: those of them that have large sizes are delayed when passing through the pores of the gel. Smaller molecules encounter less resistance and therefore move faster. As a result, after conducting electrophoresis, large molecules are closer to the start, than smaller ones (Pic. 12).



Pic. 12. Electrophoresis (https://www.priyamstudycentre.com/wp-content/uploads/2021/10/electrophoresis.png)

II. The method of isolation of individual proteins:

Affinity chromatography

The method is based on the ability of proteins to firmly bind to certain molecules (ligands) with non-covalent bonds. Molecules of substances (ligands) to which certain proteins must specifically bind are covalently attached to particles of an inert substance and fill the column with it. Then the mixture of proteins is introduced into the column and the desired protein is firmly attached to the ligand. Other proteins leave the column freely. The retained protein can then be washed from the column using a buffer solution containing the free ligand.

This highly sensitive method allows for the isolation of very small amounts of protein in pure form from a cell extract containing hundreds of other proteins.

It's used for isolation and purification of enzymes, immunoglobulins, receptor proteins.

III. Protein purification method:

Dialysis (method of membrane sieves)- a method of cleaning proteins from low molecular weight impurities.

They use a dialysis membrane, which is a polymer and has pores of a certain size. Small molecules (low-molecular impurities) pass through the pores In the membrane, and large ones (proteins) are retained. Thus proteins are washed from impurities.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test:	Explanation:
1.	1. To accelerate the healing of the wound of the mucous membrane in the oral cavity, the patient is prescribed a drug that is a heat-stable protein and is contained in human tears, saliva, breast milk, and can also be detected in a freshly laid chicken egg. It is known that it is a factor of natural resistance of the body and is called: A. Imanin B. Complement C. Interleukin D. Interferon E. Lysozyme	
2.	Fibrous connective tissue proteins include collagen, elastin, and reticulin. Name the amino acid that is only part of collagen, the determination of which in biological fluids is used to diagnose connective tissue diseases: A. Proline B. Phenylalanine C. Lysyn D. Glycine E. Hydroxyproline	

3.	The presence of protein in the solution can be detected by using color reactions. Which of the following reactions will give a negative result with complete hydrolysis of the protein: A. Biuret B. Ninhydrin C. Xanthoproteic D. Fohl`s E. Sakaguchi	
4.	When there is an activation of the inflammatory process, some autoimmune and infectious diseases, the level of acute-phase proteins in the blood plasma increases sharply. Which of the following proteins is able to form a gel when the serum is cooled? A. C-reactive protein B. Haptoglobin C. Alpha-2-macroglobulin D. Ceruloplasmin E. Cryoglobulin	
5.	Indicate which of the following amino acids that contain a hydroxyl group is most important in the formation of the structure of collagen and the organic matrix of the tooth. A. Homoserine. B. Oxyproline. C. Serine. D. Tyrosine. E. Threonine.	

6.	A huge role in the implementation of immunological protection against infection of the oral cavity and teeth belongs to immunoglobulins. Indicate which of these immunoglobulins is being secreted and can perform this important function. A. IgA. B. IgG. C. IgM. D. IgD. E. IgE.	
7.	Name which of these proteins is of primary importance in the formation of the organic matrix of the tooth: A. Albumin. B. Globulin. C. Elastin. D. Collagen. E. Fibronectin.	
8.	Collagen is a non-water-soluble protein that is essential in tooth formation. What amino acids are included in a significant amount in the composition of collagen? A. Lysine, glycine. B. Lysine, hydroxylysine, glycine. C. Proline, hydroxylysine. D. Proline, lysine. E. Hydroxylysine, hydroxyproline.	

9.	It is known that the destruction of protein and polysaccharide components of connective tissue causes the development of periodontal disease. Indicate which of these proteins is a part of the connective tissue: A. Antitrypsin. B. Albumin. C. Collagen. D. Transferrin. E. Ceruloplasmin.	
10.	The presence of protein in the solution is determined by using color reactions. Which of the following reactions will give a positive result with complete hydrolysis of the molecules? A. Biuret. B. Xanthoproteic. C. Ninhydrin. D. Sakaguchi. E. Fohl`s.	
11.	A patient with liver failure underwent a study of the electrophoretic spectrum of blood serum proteins. What are the physical and chemical properties of protein molecules that underlie this method? A. Hydrophilicity. B. Hydrophobicity. C. Presence of charge. D. Failure to undergo dialysis. E. Optical activity.	
12.	Cationic glycoproteins are the main components of saliva of	

	the parotid glands. What are the main amino acids that cause their positive charge? A. Aspartate, arginine, glutamate. B. Aspartate, glutamate, glycine. C. Glutamate, valine, leucine. D. Lysine, arginine, histidine. E. Cysteine, glycine, proline.	
13.	The surgeon used a 70% solution of ethyl alcohol to sterilize the hands before surgery. What is the main mechanism of antiseptic action of the drug on microorganisms? A. Blockade of sulfhydryl groups of enzyme systems. B. Interaction with amino groups of protoplasmic proteins. C. Interaction with hydroxyl groups of enzymes. D. Denaturation of protoplasmic proteins. E. Oxidation of organic components of protoplasm	
14.	Before prescribing protein parenteral nutrition to the patient, the doctor ordered a laboratory study of the electrophoretic spectrum of blood proteins. What are the physicochemical properties of proteins used in this method? A. Presence of charge B. Viscosity C. Inability to denaturate	

	D. Hydrophilicity and ability to swell E. Optical activity	
15.	In Wilson-Konovalov disease, the transport of copper is disturbed, which leads to the accumulation of this metal in the cells of the brain and liver. What protein synthesis disorder is this associated with? A. Metallothionein B. Haptoglobin C. Transcobalamin D. Siderophyllin E. Ceruloplasmin	
16.	One of the main components of saliva is the complex protein mucin. As which group of proteins can it be classified? A. Glycoproteins. B. Lipoproteins. C. Metalloproteins. D. Nucleoproteins. E. Phosphoproteins.	
17.	In an anemic patient, molecular analysis of hemoglobin revealed a substitution in the β-chain from the N-terminus at the 6th position of the amino acid residue glutamic acid to valine. What is the molecular mechanism of pathology in this patient? A. Gene amplification. B. Gene mutation. C. Genomic mutation. D. Genetic transduction. E. Chromosomal mutation.	

18.	What is the level of structural organization of adult hemoglobin (HbA) - a tetramer protein consisting of two alpha and two beta peptide chains? A. Primary structure. B. Secondary structure. C. Tertiary structure. D. Quaternary structure. E. Linear structure.	
19.	To obtain amylase enzyme in its pure form from the pancreas of animals, the method of affinity chromatography with a ligand attached to the carrier is used. What substance is used as a ligand? A. Glucose B. Starch C. Sucrose D. Cellulose E. Lactose	
20.	The patient is in the "artificial kidney" department. State the method used to purify his blood from low molecular weight compounds: A. Denaturation B. Salting out S. Dialysis D. Hydrolysis E. Electrophoresis	
21.	Hemoglobin transports oxygen in the body and removes carbon dioxide from it. Indicate to which class of complex proteins it belongs: A. Metalloproteins	

	B. Nucleoproteins C. Lipoproteins D. Glycoproteins E. Chromoproteins	
22.	The spatial conformation of proteins is studied by using a certain method. Specify it. A. Salting out B. Electrophoresis C. X-ray structural analysis D. Dialysis E. Isoelectric focusing	
23.	In order to prevent thrombosis, the patient is prescribed the anticoagulant heparin. The non-protein part of this proteoglycan is represented by: A. Heteropolysaccharides B. Oligosaccharides S. Homopolysaccharides D. Monosaccharides E. Lipids	
24.	Specify the principle underlying the classification of complex proteins: A. Chemical nature of the protein component B. Amino acid composition C. Solubility D. Chemical nature of the prosthetic group E. Ability to renaturation	
25.	Different methods of fractionation of protein mixtures are used in biochemical laboratories. Specify the method that is based on the difference in the magnitude and sign of the surface charge of protein	

	molecules: A. Gel filtration B. Affinity chromatography C. Ion chromatography D. Electrophoresis E. Ultracentrifugation	
26.	Most of the protein factors of blood coagulation by chemical nature are: A. Glycoproteins B. Hemoproteins C. Flavoproteins D. Phosphoproteins E. Metalloproteins	
27.	Specify a complex protein that performs a protective function against viral infection and tumor lesions: A. Ferritin B. Transferrin C. Apoferritin D. Hemosiderin E. Interferon	
28.	The formation of several levels of structural organization is characteristic of protein molecules as biopolymers. State the level of structural organization for hemoglobin: A. Quaternary structure B. β-pleated sheet C. Tertiary structure D. Primary structure E. Secondary structure	

4. Literature. Look pic. 229.

1. TOPIC:STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF ENZYME PROTEINS. CLASSIFICATION AND NOMENCLATURE OF ENZYMES. MECHANISM OF ACTION OF ENZYMES AND KINETICS OF ENZYMATIC REACTIONS. REGULATION OF ENZYME ACTIVITY.

2.INFORMATION MATERIAL.

DETERMINATION OF ENZYMES. FEATURES OF THE STRUCTURE ENZYMES

Enzymes are proteins that have catalytic activity,which depends on the degree of preservation of the native (natural) structure of the protein molecule. ***Simple enzymes***- proteins consisting only of polypeptide chains (one or more). ***Complex enzymes (holoenzymes***) in addition to polypeptide chains (this part is called an apoenzyme) contain a non-protein fragment called a cofactor. A cofactor is called a prosthetic group if the cofactor is connected to the apoenzyme due to strong covalent bonds.

A cofactor that can dissociate freely from an apoenzyme is called a coenzyme.

Examples of the biological role of cofactors:

1) ***perform the function of particle carriers between enzymes*** (use of NAD+ and NADH coenzymes in glycolytic reduction with the participation of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase enzymes);
2) ***play the role of a prosthetic group*** (heme, FMN, FAD);
3) ***binding to the enzyme molecule, some cofactors change its conformation*** (Mg^{2+} ions in the pyruvate kinase of glycolysis);
4) ***cause aggregation of enzyme subunits*** (coenzyme NADP+ and four protomers of glucose-6-phosphate dehydrogenase of human erythrocytes);
5) ***stabilize the enzyme*** (selenium ions stabilize the active center of glutathione peroxidase, which participates in the neutralization of organic peroxides and the destruction of hydrogen peroxide together with the catalase enzyme);
6) ***participate in enzymatic catalysis*** (cations);
7) ***play the role of a matrix*** (maternal DNA is a cofactor and matrix for DNA-dependent RNA polymerase that transcribes the primary transcript).

The high specificity of the action of enzymes is due to the presence of unique fragments in their structure: ***active centers*** are consisting of parts of the native molecule that carry out:

- binding to substrate molecules is ***the adapter site of the active center***;
- conversion of substrates into enzymatic reaction products is ***the catalytic site of the active center.***

The active center of a simple enzyme consists only of amino acid residues (mainly ***Ser, Tre, Glu, Asp, Gln, Asn, Arg, Lys, His, Tyr***). They are located in different places of the polypeptide chain, but when the native molecule of the enzyme is formed, they approach each other so much that they are able to perform the function of contact with the substrate and its transformation into reaction products.

The catalytic site of the active center of a complex enzyme, as a rule, contains a non-protein part, if it is represented by a vitamin derivative (see table.1, column “active forms of vitamins”).

The spatial structure of the active center and protein molecule of the enzyme is studied using the ***method of X-ray structural analysis.***

Human tissues contain ***isoenzymes***, they are multiple, genetically determined isoforms of the same enzyme. Any enzyme isoenzyme catalyzes the same reaction, this means that ***the active center of each of the isoenzymes does not differ in structure.*** Isoenzymes are distinguished by their physical and chemical properties and tissue localization in the body. Information about the tissue localization of isoenzymes is useful in the differential diagnosis of diseases accompanied by damage to tissues and organs in which the disease develops (for example: necrotic phenomena in severe forms of viral hepatitis, in liver cirrhosis; in the development of muscular dystrophy; in cardiomyopathies and myocardial infarction ; see the basic preparation textbook: analysis of the example on lactate dehydrogenase isozymes).

The difference of isoenzymes in terms of physical and chemical properties allows them to be isolated from the mixture and determined quantitatively ***(electrophoresis methods followed by densitometry).***

Table 1

Some vitamin coenzymes and their biological role

Vitamin	Vitamers	Active forms of vitamins	Specific functions of vitamins
Vitamin C	**Ascorbic acid, dehydroascorbic acid**	Not known	Participates in the hydroxylation of proline and lysine residues of preprocollagen in the process of collagen maturation, in the hydroxylation of many substrates
Thiamine (vitamin B_1)	***Thiamine***	Thiamine diphosphate(TPP, thiamine pyrophosphate, cocarbo-xylase)	TPP is a coenzyme of the enzymes pyruvateDH, alpha-ketoglutarateDH TCA, trans-ketolase HMP Shunt
Riboflavin (vitamin B_2)	**Riboflavin**	Flavin mononucleotide (FMN), flavin-denine dinucleotide (FAD)	FMN and FAD – prosthetic groups of flavin oxidoreductases – succinate-DH and α-ketoglutrate-DH-complex in TCA, acyl-CoA-DH, L-/D-alanine oxidase, NADH-DH of the inner membrane of mitochondria
Pantothenic acid (old name – vitamin B5)	***Pantothenic acid***	**Coenzyme A (coenzyme A; CoA); 5-phosphopantotheine**	CoA takes part in the processes of the exchange of fatty acids and sterols (cholesterol, steroid hormones), in the processes of acetylation, synthesis of acetylcholine; 5-phosphopantetheine – the prosthetic group of the acyl-

Vitamin	Vitamers	Active forms of vitamins	Specific functions of vitamins
			transporting protein of the palmitate synthase complex
P_{yridoxine,} vitamin B6	***Pyridoxal, pyridoxamine***	Pyridoxal phosphate (PLP)	PLP is a coenzyme of a large number of enzymes of nitrogen metabolism (aminotransferases, decarboxylases of amino acids) and enzymes involved in the exchange of sulfur-containing amino acids, tryptophan, in the synthesis of heme.
Vitamin B_{12} (cobalamin)	***Cyanocobalamin, oxycobalamin***	Methylcobalamin (CH_3-B_{12}), deoxyadenosylcobalamin (dA-B_{12})	In the form of CH_3-B_{12}, it participates in the resynthesis of methionine from homocysteine; in the form dA-B_{12} participates in the breakdown of fatty acids and amino acids with a branched chain or an odd number of carbon atoms; in folic acid metabolism.
Niacin; Nicotinic acid (vitamin B3)	***Nicotinamide (vitamin PP)***	Nicotinamide adenine dinucleotide (NAD+); nicotinamide-adenine dinucleotide-phosphate (NADP+)	NAD+/NADF+ - coenzymes of dehydrogenases involved in the oxidation of substrates; NADPH is the primary donor of electrons and protons for monooxygenase systems, reductases involved in the synthesis of cholesterol.
Folic acid, vitamin B9 (obsolete	***Folic acid, polyglutamates folic acid***	Tetrahydrofolic acid (THFA), its derivatives	Derivatives of THFA participate in the transfer of one-carbon fragments during

Vitamin	Vitamers	Active forms of vitamins	Specific functions of vitamins
name – vitamin Bc)			the biosynthesis of purine bases and thymidine.
Biotin (the old name is vitamin H)	***Biotin***	Carboxybiotin linked to the ε-amino group by a lysine residue in the apoenzyme molecule	It is part of carboxylase, which carries out the initial stage of fatty acid biosynthesis, and part of pyruvate carboxylase, which participates in gluconeogenesis.

CLASSIFICATION AND NOMENCLATURE OF ENZYMES

The modern classification of enzymes, the main principle of which is "classification by the type of catalyzed reaction", was approved in 1961 at the Vth International Biochemical Congress, and includes six main classes:

1. OXIDOREDUCTASES : participate in the process of transferring protons and electrons from the donor, regardless of the type of acceptor. They include all dehydrogenases, oxidases (cofactors: NAD+, FAD, FMN), heme-containing: cytochromes, catalase and peroxidase.

2. TRANSFERASES: participate in the intermolecular transfer of functional fragments of the structure: methyl, acyl, glycosyl, aldehyde, ketone residues, nitrogenous, phosphate groups, etc., usually have at least two substrates (donor-acceptor).

3. HYDROLASES: catalyze the breaking of a bond in the substrate with the addition of fragments of the structure of the water molecule (H-OH) to the structure of the reaction products. They include esterases, glycosidases, phosphatases, peptidases, amidases, etc. Hydrolases usually have one substrate and at least two products, their reactions are mostly irreversible. Hydrolases in living systems often exhibit invasive properties: they are able to penetrate tissue cells, destroying membrane lipids, proteoglycans and other proteins (for example: hyaluronidase, lecithinase), so they are sometimes called enzymes of aggression.

4. LYASES: participate in the breaking of C-O, C-C, C-N bonds, in the reactions of cleavage of various groups from substrates without the participation of

water, with the formation of a double bond (enolase), or joining at the site of a double bond "fumarate hydratase"; decarboxylation enzymes.

5. ISOMERASES: participate in the intramolecular transfer of fragments of the substrate structure with the formation of an isomer product.

6.LIGASES: take part in the synthesis of a reaction product from several substrates (two or more) using the energy of bonds of a macroergic substance (ATP or other nucleoside triphosphates), usually called «synthetase».

In connection with the increase in the number of discovered enzymes, by 1972 the International Union of Biochemists recommended introducing a decimal nomenclature of enzymes, based on the principle of classification by type of chemical reaction. In 1973, the IUPAC Commission on Biochemical Nomenclature published a new appendix to enzyme nomenclature. When forming the name of the enzyme, the name of the reaction substrates, the type of chemical reaction or the name of the enzyme class is usually taken into account, and the suffix -ase is added to the end of the name. For some enzymes, historically developed names have been retained, for example: trypsin, pepsin, catalase, etc. The enzyme code number has four positions. The first digit of the code corresponds to the class number of the enzyme. Other numbers are related to the peculiarities of the course of the chemical reaction and to the structure of the enzyme substrates. As an example, the decoding of the tyrosine aminotransferase enzyme code is offered below:

Tyrosine Aminotransferase:

E.C. 2. 6. 1 . 5. → **serial number of this enzyme in the sub-sub-class**

Sub-sub-class:
Nature of the substrate (donor)

Class: **Subclass:**
Transferase **The type of group being migrated**

PHYSICO-CHEMICAL PROPERTIES OF ENZYMES

Most simple and complex enzymes are classified as globular proteins. They perform their function in biological fluids:

in the cell cytoplasm are enzymes of glycolysis;

in blood plasma is kallikrein, which destroys biologically active kinins;

in the nucleoplasm are enzymes of transcription, post-transcription processing and replication;

in saliva is salivary amylase (takes part in the digestion of starch and glycogen polysaccharides); lysozyme (has a bactericidal effect, causing lysis of the polysaccharide complex of the shell of staphylococci, streptococci);

in gastric and other juices of the gastrointestinal tract (pepsin, trypsin, chymotrypsin, etc.).

The physical and chemical properties of globular proteins, which have already been studied, can be considered for enzymes as well. Membrane-related enzymes occupy a special place in terms of properties. Such enzymes are characterized by a quaternary structure consisting, as a rule, of hydrophobic subunits, which are immersed in the lipid bilayer of the membrane, and globular subunits that perform a catalytic function. The active centers of such enzymes are turned either toward the cytoplasm, if the enzyme is in the cell membrane, or toward the internal contents of the organelle, if the enzyme is placed in the organelle membrane.

Properties that condition its catalytic function and distinguish enzymes from inorganic catalysts are very important for enzymes. It:

1. Specificity of action is the property of an enzyme to have an affinity only for certain organic substances, which are called its substrates (S). The high specificity of the action of the enzyme (E) is due to the conformational and electrostatic complement of the S molecules and the active center of the E molecule.

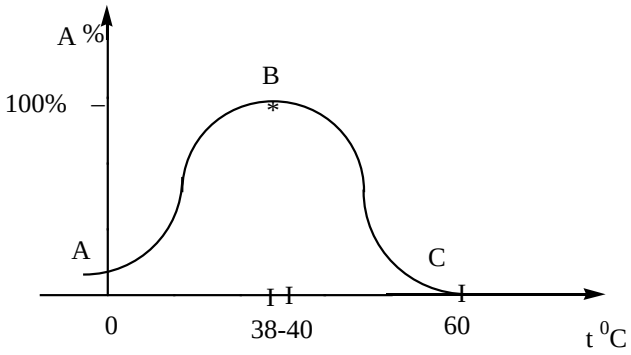
The specificity of the action is called absolute, if the enzyme catalyzes the conversion of only one substance into reaction products. Such enzymes are significantly fewer in number (examples: arginase of the urea formation cycle; urease, which destroys the structure of urea; glucokinase, which phosphorylates only glucose) than enzymes with ***relative group specificity.***

The enzyme has relative group specificity, if it has a group of substrates that are organic compounds similar in a certain structural fragment. Usually, an enzyme with group specificity catalyzes a reaction in which a chemical transformation occurs precisely in this structural fragment. This type of specificity is widely represented in the enzymes of the gastrointestinal tract (GI), which are involved in the digestion of food components. For example, all peptidases of the GI tract destroy only the peptide bond, which may belong to polypeptide chains of proteins

with different amino acid sequences. Salivary amylase destroys only the alpha-1,4-glycosidic bond in the composition of any polysaccharide that has this type of bond and enters the oral cavity.

Another type of enzyme specificity is stereochemical specificity, in which the enzyme catalyzes a reaction with a substrate of only a certain stereoisomeric series (see the basic textbook: oxidative deamination of alanine under the action of L- and D-oxidases). Krebs cycle fumarase has stereochemical specificity, its substrate is only trans-fumaric acid, this enzyme does not catalyze the hydration of cis-fumaric acid.

2. Thermolability of enzymes (change in enzyme activity under the influence of ambient temperature).The graphical dependence of enzyme activity on temperature has the form (Pic. 17):



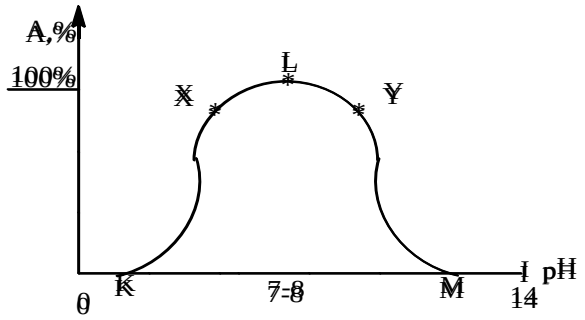
Where the section of the AB curve is explained as follows: with increasing temperature, the rate of chaotic movement of enzyme and substrate molecules increases, thus increasing the probability of ES complex formation. At the temperature of the environment corresponding to point B, the transition state for ES is most quickly reached, which contributes to the formation of enzymatic reaction products.

Pic. 17. Effect of temperature on tissue enzyme activity of the human body.

At higher temperatures, thermal denaturation of the enzyme begins. At temperatures above 60°C, enzymes of the human body are completely denatured. Section BC is at a temperature higher than the optimum, thermal denaturation of the protein molecule of the enzyme begins. At 0 ºC, the activity of human tissue enzymes is low, but not equal to zero.

It should be noted that most human blood plasma and tissue enzymes have little activity at 0°C. Therefore, blood samples (plasma, serum) sometimes (in case of need for preservation and use not immediately) should be stored in a freezer at a temperature of -4-12° C, samples can be thawed once.

3.Behavior of the enzyme at different pH values of the environment. The dependence of the tissue enzyme activity on the pH of the environment is represented by a graphic dependence (Pic. 18):



Pic. 18. The influence of the pH of the environment on the activity of the enzyme of the cytoplasm of human tissue cells.

1) at point L, the highest activity of the enzyme is achieved - the pH value of the medium for this point is optimal. Functional groups of amino acid residues of the active center of the enzyme at this pH value have the most favorable charge for the formation of the ES complex and carrying out acid-base catalysis;

2) at points X and Y, there is a decrease in enzyme activity in relation to point L, because functional groups in the active center are recharged under the action of an excess of protons (point X) or under the action of an excess of hydroxide ions (point Y). The same thing happens in the molecules of the substrate. As a result, the ES complex formation time and ES→EP→E+P conversion time increase;

3) at pH values at points K and M, denaturation of the protein molecule of the enzyme is observed (very acidic or very alkaline environment).

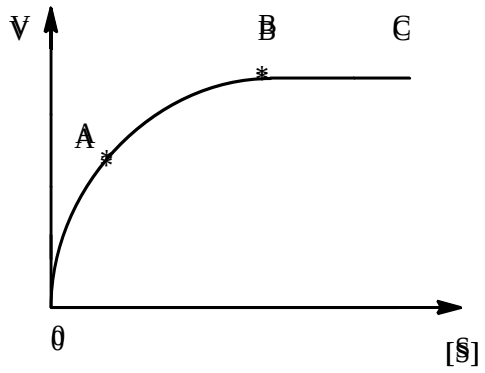
KINETICS OF ENZYMENTAL REACTIONS

This section of enzymology studies the influence of various factors on the rate of an enzymatic reaction. Considering the general equation of enzymatic catalysis of the reversible reaction of the transformation of one substrate into one product (1)

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \rightleftharpoons EP \underset{K_{-2}}{\overset{K_{+2}}{\rightleftharpoons}} E + P \qquad \textbf{(1)}$$

The main factors affecting the speed of the enzymatic reaction should be named: the concentration of the substrate [S], the concentration of the enzyme [E] and the concentration of the reaction product [P].

The interaction of some enzymes with their substrate can be described by a hyperbolic curve of the dependence of the rate of the enzymatic reaction V on the concentration of the substrate [S] (Pic. 19):



Pic. 19. Dependence of the speed of the enzymatic reaction on the concentration of the substrate.

Three areas can be distinguished on this curve, which can be explained by the positions of the mechanism of interaction of the enzyme with the substrate: OA is the area of direct proportional dependence of V on [S], the active centers of the enzyme are gradually filled with substrate molecules with the formation of an unstable ES complex; section AB is the curvilinear dependence of V on [S], complete saturation of the active centers of the enzyme with substrate molecules has not yet been achieved. The ES complex is unstable before reaching the transition state, the probability of reverse dissociation to E and S is still high; BC section is the dependence is described by a zero-order equation, the section is parallel to the [S] axis, complete saturation of active enzymes with substrate molecules is achieved, V=Vmax.

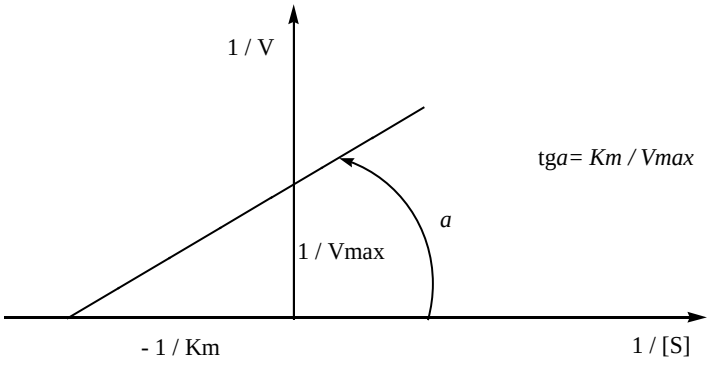
The characteristic shape of the curve is mathematically described by the Briggs-Haldane equation:

$$V=V_{max} \bullet [S]/ Km + [S] \qquad (2),$$

where Km is the Michaelis-Menten constant, numerically equal to the concentration of the substrate at which the rate of the enzymatic reaction is equal to half of Vmax.

The smaller the Km of the enzyme, the higher its affinity to the substrate, and the faster the transition state is reached for the substrate, and it turns into a reaction product. Searching for Km values for each enzyme substrate with group specificity is important in determining the biological role of this enzyme in the cell.

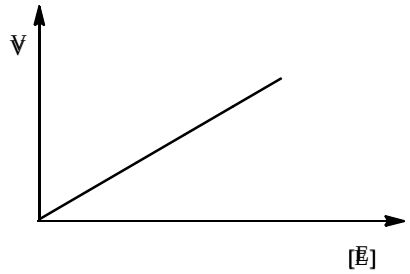
For most enzymes, it is impossible to construct a hyperbolic curve (Pic. 19). In this case, the method of double inverse values (Lineweaver-Burke) is used, that is, a graphical dependence of 1/[V] on 1/[S] is constructed (Pic. 20). The method of constructing such curves in the experiment is very convenient when studying the effect of different types of inhibitors on the activity of enzymes (presented below).



Pic. 20. The graph of the dependence of 1/[V] on 1/[S] (Lineweaver-Burke method) where y is the cut-off section, $-\frac{1}{V_{max}}$, and x is the area to be cut off, $-\frac{1}{K_m}$, the tangent of the angle α is $\frac{K_m}{V_{max}}$.

Dependence of the rate of the enzymatic reaction V on the concentration of the enzyme [E].

This graphic dependence (Pic. 21) is considered at the optimal temperature and pH of the environment, at substrate concentrations that significantly exceed the saturation concentration of the active centers of the enzyme.



Pic. 21. Effect of enzyme concentration on the rate of enzymatic reaction.

Dependence of the speed of the enzymatic reaction on the concentration of the cofactor or coenzyme.For complex enzymes, it should be taken into account that the deficiency of coenzyme forms of vitamins in hypovitaminosis, the violation of the entry of metal ions into the body necessarily lead to a decrease in the concentration of the corresponding enzymes necessary for the flow of metabolic processes. Therefore, we should conclude that the activity of the enzyme is directly dependent on the concentration of the cofactor or coenzyme.

The influence of the concentration of products on the rate of the enzymatic reaction.

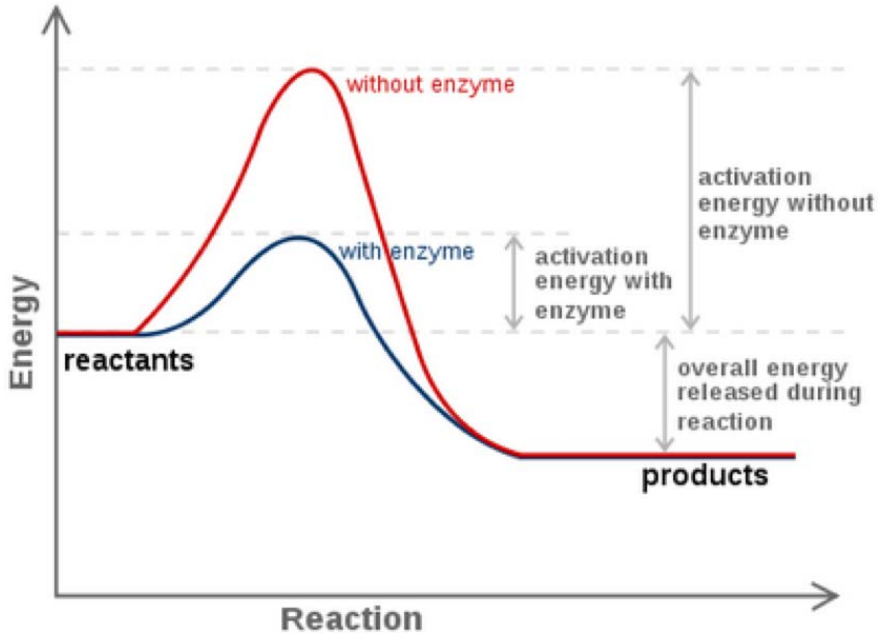
For reversible reactions taking place in the human body, it is necessary to take into account that the products of the direct reaction can be used by the enzyme as substrates of the reverse reaction. Therefore, the flow direction and the moment of reaching Vmax are dependent on the ratio of the concentrations of the initial substrates and reaction products. So, for example, the activity of alanine aminotransferase, which catalyzes the transformation:

Alanine + Alpha-ketoglutarate ↔ Pyruvate + Glutamate depends on the concentration ratio in the cell:

[alanine + alpha-ketoglutarate] / [pyruvate+glutamate].

MECHANISM OF ENZYME ACTION. THEORIES OF ENZYMATIC CATALYSIS

Enzymes, like non-protein catalysts, increase the rate of a chemical reaction due to the ability to lower the activation energy of this reaction.The activation energy of the enzymatic reaction is calculated as the difference between the energy value in the system of the ongoing reaction that has reached the transition state and the energy determined at the beginning of the reaction (see the graphical dependence in pic. 22).



Pic. 22. Graphic dependence of the energy state of a chemical reaction without an enzyme (1) and in the presence of an enzyme (2) on the reaction time. (https://www2.nau.edu/lrm22/lessons/enzymes/reaction.png)

The works of V. Henry and, especially, L. Michaelis, M. Menten on the study of the mechanism of monosubstrate reversible enzymatic reactions made it possible to postulate that the enzyme E initially reversibly and relatively quickly combines with its substrate S c to form an enzyme-substrate complex (ES):

$$E + S <=> ES \quad (1)$$

The formation of ES occurs due to hydrogen bonds, electrostatic, hydrophobic interactions, in some cases covalent, coordination bonds between side radicals of amino acid residues of the active center and functional groups of the substrate. In complex enzymes, the non-protein part of the structure can perform the function of contact with the substrate.

The enzyme is substrate complex then disintegrates in a second slower reversible reaction with the formation of the reaction product P and free enzyme E:

$$ES <=> EP <=> E + P \quad (2)$$

At this day, thanks to the works of the above-mentioned scientists, as well as Keilin D., Chance B., Koshland D. (theory of "induced compliance"), there are theoretical statements about four main points in the mechanism of enzyme action

on the substrate, which determine the ability of enzymes to accelerate chemical reactions :

1. ***Orientation and approaching.*** The enzyme is able to bind the substrate molecule in such a way that the bond attacked by the enzyme is not only located in close proximity to the catalytic group, but also correctly oriented in relation to it. The probability that the ES complex will reach a transition state due to orientation and approaching greatly increases.

2. Tension and strain: induced accordance. Addition of the substrate can cause conformational changes in the enzyme molecule, which lead to the tension of the structure of the active center, and also slightly deform the bound substrate, thereby facilitating the ES complex to reach the transition state. A so-called induced fit between E and S molecules occurs.

3. General acid-base catalysis. In the active center of the enzyme, there are always groups of specific amino acid residues that are good proton donors and acceptors. These groups are powerful catalysts for many organic reactions occurring in the aqueous phase:

GROUPS	
DONORS:	ACCEPTORS:
-COOH	$-COO^-$
-NH	$-NH_2$
-SH	$-S^-$

4. Covalent catalysis. In some enzymatic reactions, enzyme E replaces the functional group R in the substrate R - X, as a result of which a covalent complex E - X is formed. The complex is unstable and prone to hydrolysis much faster than R - X. An example of such an enzyme is chymotrypsin. The reaction catalyzed by it:

RX + E-OH --> R-OH + EX

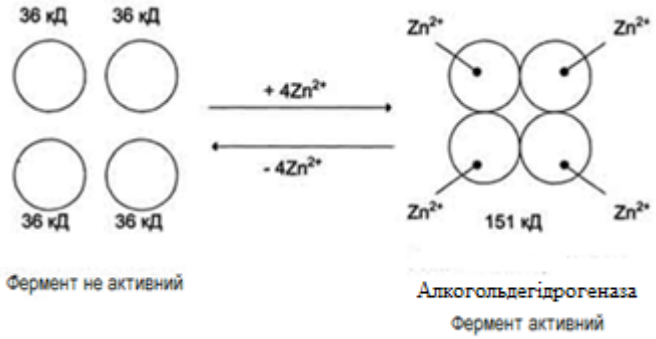
EX + H O --> E-OH + HX

Total equation: RX + H O --> R-OH + HX (3)

Participation of metal ions in electrophilic catalysis

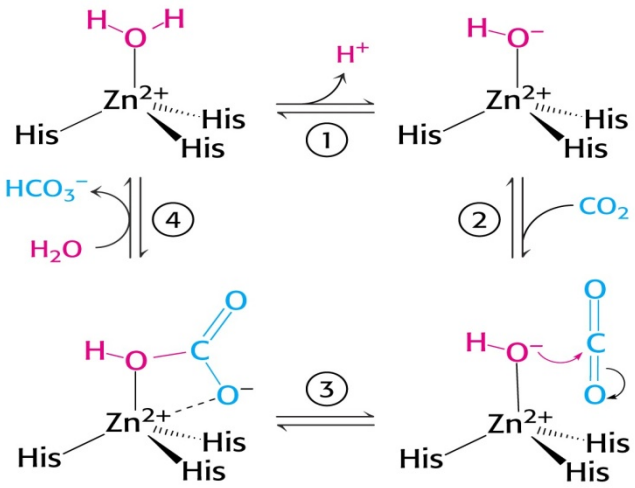
Most often, this function is performed by metal ions with variable valence, which have a free d-orbital and act as electrophiles. These are primarily metal ions

such as Zn^{2+},Fe^{2+}, Mn^{2+}, Cu^{2+}. Alkaline earth metal ions, such as Na^{+} and K^{+}, do not have this property. Zinc ions stabilize the quaternary structure of the enzyme alcohol dehydrogenase (Pic. 23), while the conformation of the molecule changes with the formation of the contact area of the active center. At the same time, the catalytic part of the active center is formed, thanks to the coenzyme NAD^{+}, which is responsible for the dehydrogenation of alcohol.



Pic. 23. The role of zinc ions in the stabilization of the quaternary structure of alcohol dehydrogenase.

The carbonic anhydrase enzyme, which catalyzes the reversible reaction of the formation (decay) of carbon (carboxylic acid) from carbon dioxide and water, by the mechanism of catalysis, also belongs to the enzymes that use metal ions. Zinc ions are present in the active center of the enzyme, the positions of which are stabilized by the residues of the amino acid histidine (His) (pic. 24). It is Zn^{2+} cations that come into contact with water molecules (step 1) and then with a carbon dioxide molecule (step 2) with the formation of a bicarbonate-enzyme complex (step 3), which is destroyed with the participation of the next water molecule to bicarbonate ion and water-enzyme- complex:



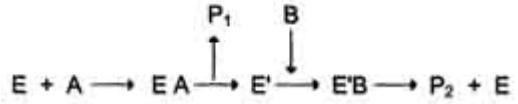
Pic. 24. The mechanism of action of the carbonic anhydrase enzyme.

Mechanism of action of enzymes in multisubstrate reactions

Most enzymes catalyze reactions involving more than one substrate. If the coenzyme is not a prosthetic group, it can also be considered as another substrate. Therefore, there can be several participants in the enzymatic reaction: the enzyme itself, several substrates and a coenzyme.

In these cases, the mechanism of the enzymatic reaction, as a rule, can follow one of two paths: ***by the "ping-pong" mechanism (double substitution mechanism) or sequentially.*** Let's consider both mechanisms.

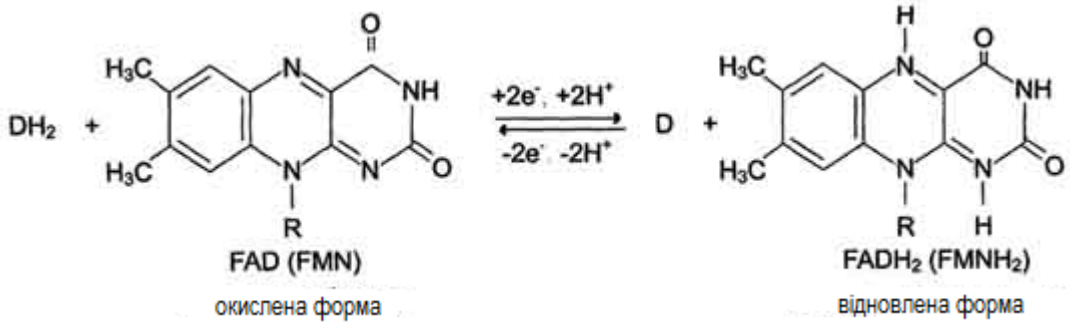
1. The "ping-pong" mechanism.The schematic "ping-pong" mechanism can be represented as follows:



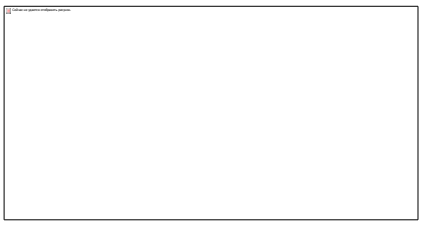
Substrate A, interacting with enzyme (E), turns into product (P1). As a result of this transformation, the enzyme remains not in its native form, but in a changed (E') as a result of coenzyme modification. Next, the substrate B is attached to the active center E', which can be transformed into the product (P2) with the release of the native form of the enzyme €. A good example of the "ping-pong" mechanism is transamination reactions with the participation of aminotransferase enzymes (pyridoxal phosphate coenzyme). Aminotransferases discovered by the domestic scientist A.E. Braunstein, catalyze reversible reactions of the transfer of an amino group from an amino acid to a keto acid. The "ping-pong" mechanism of this reaction is schematically presented in pic. 25. Coenzyme pyridoxal phosphate (PLP), associated with the enzyme, accepts an α-amino group from the first amino acid (AA_1), which at the same time turns into α-ketoacid 1 (KA_1) and is released from the active center of the enzyme. Next, ketoacid 2 (KA_2) is added to the active center of the enzyme, which takes an amino group from the coenzyme and turns into an α-amino acid (AA_2).

Pic. 25. Events in the active center of aminotransferase as an example of the “ping-pong” mechanism.

Another example of the "ping-pong" mechanism is dehydrogenation reactions involving the coenzyme FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide), which are strongly associated with the enzyme and, therefore, cannot be considered as a second substrate. FMN and FAD participate in redox reactions by accepting 2 e- and 2 H^+ in the isoalloxazine ring (see diagram below):



The scheme of the dehydrogenation reaction (as an example of the "ping-pong" mechanism involving FMN and FAD) can be presented in the following form:

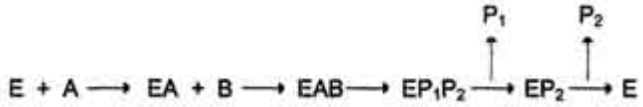


where AH_2 is a hydrogen donor, oxidized substrate 1; A is an oxidized form of substrate 1; B is a hydrogen acceptor what is substrate 2; BH_2 is reduced form of substrate 2; E (FAD), E ($FADH_2$) are oxidized and

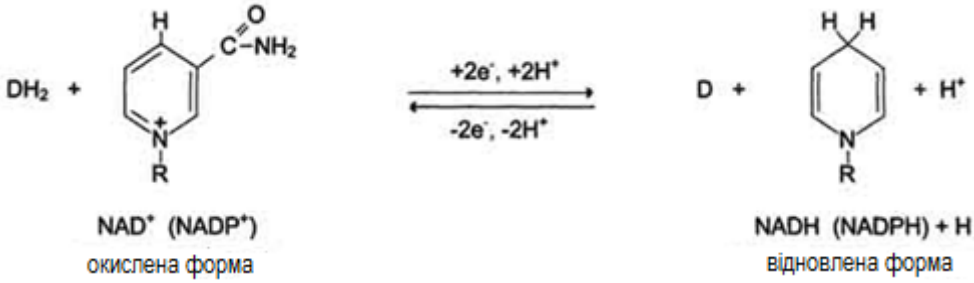
reduced forms of the coenzyme FAD, which is part of the enzyme E. As an example of a FAD-dependent reaction, we can cite the succinate dehydrogenase reaction of the Krebs cycle.

2. Sequential mechanism. In the case of a sequential mechanism, the simultaneous interaction of two substrates is required for the enzymatic reaction to proceed. In this case, it is possible to attach substrates in two different ways:

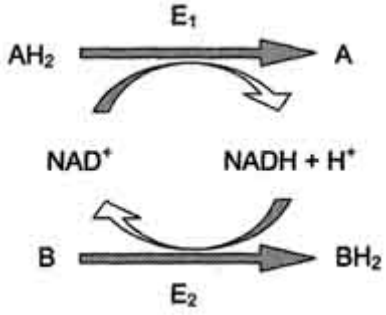
- The mechanism of the ordered interaction of the substrate with the active center of the enzyme:



Substrate A is the first to be added to the active center of the enzyme, facilitating the addition of substrate B. After chemical modification, a certain order of release of reaction products is also observed. An example of a sequential, ordered mechanism can be a dehydrogenation reaction with the participation of coenzymes NAD^+, $NADP^+$.

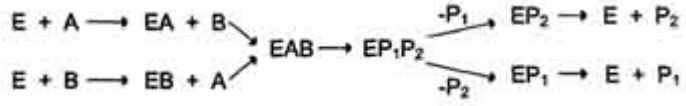


Two enzymatic reactions catalyzed by enzymes E_1 and E_2 are connected to each other by the coenzyme NAD^+, which serves as a substrate in each of these cases. For the first enzyme, the substrate is the oxidized form of NAD^+, the second substrate is a hydrogen donor is an example of sequential reactions, the product is the reduced form of NADH, for the E2 enzyme is the opposite.



where AH_2 is a hydrogen donor, the reduced form of substrate 1; A is an oxidized form of substrate 1; B is a hydrogen acceptor is the second substrate; BH_2 is reduced form of substrate 2; NAD^+, NADH are an oxidized and reduced forms of the coenzyme; E_1 and E_2 are enzymes. As an example, we can consider the following linked reactions of glycolysis, where E_1 is glyceraldehyde phosphate dehydrogenase; E_2 is a lactate dehydrogenase.

- The mechanism of random interaction of the substrate with the active center of the enzyme:



There is no priority for the interaction of substrates A and B in the active center of the enzyme (each substrate has its own binding center in the active center). There is also no clear pattern of release of reaction products.

REGULATION OF ENZYME ACTIVITY

Most enzymes are synthesized in an inactive state in the form of proenzymes (zymogens), in this form enzymes exist for part of their life. The activity of the enzyme is manifested only when certain conditions are met in the cell:

1. presence of cofactors (metal ions, synthesized coenzymes, prosthetic groups);
2. presence of enzyme substrates;
3. presence of enzyme effectors: allosteric activators, enzymes of phosphorylation (protein kinases), dephosphorylation (protein phosphatases);
4. absence of inhibitors of the active form of the enzyme;
5. the presence of energy sources or phosphate group donors (this function is mainly performed by ATP);
6. creation of optimal conditions (appropriate temperature and pH of the environment);
7. permanent removal of enzymatic reaction products from the environment surrounding the enzyme, if the reaction is reversible.

In the conformation of the ***zymogen*** protein molecule ***(proenzyme; inactive form of the enzyme)***, there are no active centers, their formation can occur through ***activation mechanisms***:

1.Cleavage of the oligopeptide from the proenzyme (limited proteolysis; examples: formation of pepsin, trypsin);

2.Formation of additional – S – S – connections, which makes the active center accessible;

3.Formation of a complex with metal ions;

4. ***Covalent modification of zymogen by phosphorylation (function of protein kinase) or dephosphorylation (function of protein phosphatase)*** is

actively used to regulate key enzymatic processes in the cell (examples: glycogen synthase and glycogen phosphorylase in glycogen exchange, mitochondrial pyruvate dehydrogenase, beta-hydroxy-beta-methylglutaryl-CoA reductase in cholesterol synthesis);

5. Allosteric activation of zymogen.

The last type of activation mechanism is the most common in living organisms and allows to regulate the activity of key (main) enzymatic metabolic processes. These enzymes, along with active centers, also have allosteric (one or more) centers.

Allosteric center is a structural fragment of the protein molecule of the regulatory enzyme, which is spatially separated from the active center and performs the task of contacting substances-inhibitors or -activators (they are also called allosteric effectors). As a result of such contact, conformational changes occur in the enzyme protein molecule. The meaning of allosteric inhibition or activation is outlined on picture 26.

The role of an allosteric inhibitor can sometimes be performed by a substrate at a higher concentration and complete saturation of the active centers of the enzyme. As an example, consider the main regulatory enzyme of glycolysis - phosphofructokinase I (PFK I), which catalyzes the reaction:

Fructose-6-F + ATP → fructose-1,6-di-F + ADP, where F is phosphate

Phosphofructokinase I is a phosphotransferase that transfers the remainingphosphoric acid from ATP to fructose-6-phosphate: at a low concentration of ATP in the cytoplasm, ATP is a substrate that attaches to the active site of the enzyme.

When the concentration of ATP increases, the active centers of the enzyme are fully saturated with substrate molecules, and therefore ATP begins to attach to the allosteric center of PFK I, stopping the action of the enzyme and the entire process of glycolysis.

A similar function can be performed by the reduced form of the dehydrogenase coenzyme: as an example, consider isocitrate dehydrogenase of the Krebs cycle. This enzyme has several allosteric centers:

-For activators are cations $Mg2^{+}$, $Mn2^{+}$ and ADP

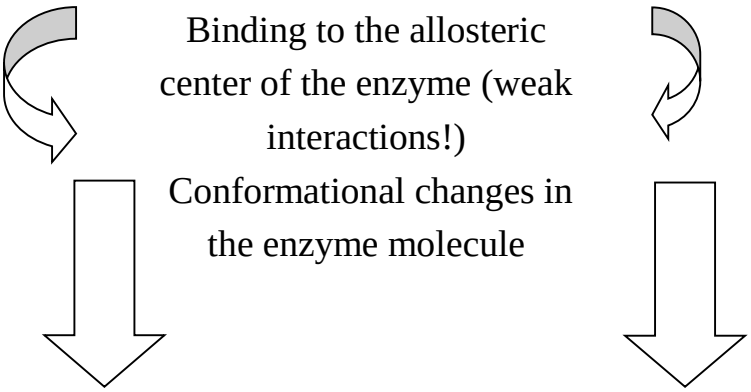
-For inhibitors are ATP and NADH.

The latter substances perform the function of an allosteric inhibitor of isocitrate dehydrogenase only if they accumulate in the mitochondrial matrix, where the enzyme is localized.

It should also be noted that often the zymogen concentration can be changed at the level of regulation of such processes as transcription and translation. This is due to the fact that the rate of formation of polypeptide chains of the proenzyme (zymogen) may depend on many exogenous (external) factors acting on the living cell, to which it must be adapted. If the concentration of the enzyme is regulated at the level of transcription and translation, it is called an induced enzyme.

ALLOSTERIC

ACTIVATORS INHIBITORS



The conformation of the active center changes, or the active center is completely destroyed

Functional groups of the active center occupy the most favorable position in relation to the substrate molecule

The orientation time of E and S increases, their contact time (partial inhibition), or the substrate cannot join the active center

ES formation time is reduced
and the time to reach the transition state

Decreased activity (partial inhibition), or complete absence of activity

Appearance (increase) of enzyme activity

Pic. 26. Mechanism of action of allosteric effectors

Retroinhibition. The final product of the reaction (or process) can perform the function of an allosteric inhibitor. Examples:

- The synthesis of heme, which is the non-protein part of hemoglobin, or cytochromes, begins with the function of δ-aminolevulinate synthetase, which synthesizes δ-aminolevulinic acid from active acyl succinic acid (succinyl-CoA) and glycine. Heme is formed from this product through a series of successive transformations. An excess of heme molecules binds to the allosteric center of the above-mentioned enzyme, changes its conformation and stops the action of δ-aminolevulinate synthetase.

- Cholesterol - a product of cholesterol synthesis, under the condition of its accumulation in the cell, begins to perform the function of an allosteric inhibitor of the key synthesis enzyme - beta-hydroxy-beta-methyl-glutaryl-CoA-reductase.

- Product of oxidative decarboxylation of pyruvic acid is acetyl-CoA. When it accumulates in the mitochondrial matrix, it acts as an allosteric inhibitor of the pyruvate dehydrogenase complex.

- Synthesis of pyrimidine nucleotides stops when UTP and CTP accumulate, as they begin to perform the function of allosteric inhibitors of carbamoyl phosphate synthetase II, the function of which this synthesis begins.

Other types of inhibition are presented in Table 2.

The study of various factors regulating the activity of enzymes is the primary basis for understanding the behavior of any enzyme in vivo in normal conditions, as well as in the occurrence of various diseases. The doctor's choice of ways to treat the pathological process and bring it to normal largely depends on knowledge about the role and regulation of the activity of those enzymes whose function is impaired during the disease. For specialists in the field of pharmacy, this section is fundamental in the development of a strategy for the selection of synthetic organic compounds that can be used as potential drugs. The mechanism of action of such drugs can be aimed at:

- induction of the synthesis of certain enzymes in the body, if this synthesis is reduced in the patient (example: phenobarbital - induction of the synthesis of UDP-glucuronyltransferase in newborns with physiological jaundice);

- inhibition of the enzyme activity of the disease-causing microorganism (sulfanilamide drugs - competitive inhibitors of folic acid synthesis enzymes from para-aminobenzoic acid in bacteria);

- providing an anti-vitamin function - (dicumarol, warfarin, tromexan - antagonists of vitamin K, they reduce the rate of gamma-carboxylation of glutamic

acid residues in the reactions of the synthesis of blood coagulation factors II, VII, IX, X, since the carboxylation enzyme mistakenly uses these substances as a prosthetic group instead of vitamin K);

- inhibition of human tissue enzyme activity in order to reduce the formation of a reaction product, the accumulation of which is associated with the development of the disease (allopurinol is a competitive suicidal inhibitor of xanthine oxidase, which catalyzes the formation of uric acid, the accumulation of which in human tissues in the form of urate salts provokes the development of gout).

Table 2

Types of inhibition of enzyme activity

<table>
<tr><th colspan="3">REVERSIBLE
Inhibitors can change Km, or Vmax, or Km and Vmax when diluting the reaction medium, it is possible:
EJ → E + J, where J is an inhibitor</th><th>IRREVERSIBLE
when diluting the reaction medium, it’s impossible:
EJ → E + J</th></tr>
<tr><td>COMPETITIVE

The inhibitor (J) is similar to the substrate (S) in structure. The interaction of J with the active center of enzyme E is mandatory. EJ is formed, when [S] increases, J is displaced from the active center of the enzyme.

Only</td><td>NON-COMPETITIVE

The inhibitor (J) is not similar to the substrate (S) in structure. It is possible, but not necessary, to join J with the active center of the enzyme. EJS is formed due to weak or covalent interactions.
Vmax decreases, but Km does not change.
COMPLETE</td><td>NON-COMPETITIVE

The inhibitor (J) is not similar to the substrate (S) in structure. The inhibitor interacts only with the ES complex. EJS is formed due to weak or covalent interactions. J changes both values: Km and Vmax.

COMPLETE inhibition: EJS does not break down into</td><td>Inhibitor J can be different in structure. The interaction of J with the active center of enzyme E takes place in two stages: E+ J <=> EJ → (E - J*)

(1) (2)

(1) - EJ- complex is unstable; (2) - (E - J*) - modification of the inhibitor took</td></tr>
</table>

Km changes	inhibition: EJS does not break down into reaction products. PARTIAL inhibition: EJS decays more slowly than ES.	reaction products. PARTIAL inhibition: EJS decays more slowly than ES	place in J* with strong binding by covalent bonds to the active center. The substrate cannot join. This is the so-called competitive suicidal type of inhibition. J changes both values: Km and Vmax
Example:	**Example:**	**Example:**	**Example :**
E is acetylcholinesterase; J is the drug proserin	E is an enzymes containing cysteine residues in the active center; J means ions of heavy metals: Pb^{2+}, Hg^{2+}, Cu^{2+}.	E - alkaline phosphatase; J – L- are phenylalanine	E is a xanthine oxidase; J is an allopurinol

3. TASKS FOR INDEPENDENT WORK..

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test:	Пояснення:
1.	Five isoenzyme forms of lactate dehydrogenase were isolated from human blood serum and their properties were studied. What property proves that the isolated isoenzyme forms of the same enzyme? A. The same molecular weight B. They catalyze the same reaction C. Uniform electrophoretic mobility D. Tissue localization E. Same physical and chemical properties	
2.	In the lungs, carbonic acid (H2CO3) is decomposed with the help of an enzyme into water and carbon dioxide, which is released during breathing. What enzyme catalyzes this reaction? A. Carbonic anhydrase B. Catalase C. Peroxidase D. Cytochrome C E. Cytochrome oxidase	
3.	Deficiency of the trace element selenium in the body is manifested by cardiomyopathy. The likely cause of this condition is a decrease in the activity of this selenium-containing enzyme: A. Lactate dehydrogenase B. Cytochrome oxidase C. Succinate dehydrogenase D. Catalase E. Glutathione peroxidase	
4.	As a result of vitamin B1 deficiency, the oxidative decarboxylation of pyruvic acid is disturbed. The synthesis of which of the following coenzymes is disrupted in this case?	

	A. Coenzyme A. B. Lipoic acid. S. Nicotinamide adenine dinucleotide. D. Thiamine pyrophosphate (TPF) E. Flavinadenine dinucleotide.	
5.	A 32-year-old patient has hypovitaminosis B2. The cause of the occurrence of specific symptoms (damages to the epithelium, mucous membranes, skin, cornea of the eye), most likely, is a deficiency: A. Flavin coenzymes. B. Cytochrome a1. C. Cytochrome c. D. Cytochrome c. E. Cytochrome oxidases	
6.	The pesticide contains sodium arsenate, which blocks lipoic acid. Indicate which enzymes are affected by this pesticide. A. Glutathione peroxidases. B. Glutathione reductases. C. Methemoglobin reductases. D. Microsomal oxidation. E. PVC - dehydrogenase complex	
7.	Reactions of intermolecular transport of one-carbon radicals are necessary for the synthesis of proteins and nucleic acids. From which of the vitamins listed below the coenzyme necessary for the above reactions is formed? A. Ascorbic acid. B. Pantothenic acid. C. Riboflavin. D. Thiamine. E. Folic acid.	
8.	In the blood serum of a patient with complaints of chest pain, a significant increase in the activity of enzymes: MV creatine phosphokinase and aspartate aminotransferase was detected.	

	These changes indicate the development of a pathological process in which tissue? A. In smooth muscles. B. In the heart muscle. C. In skeletal muscles. D. In lung tissue. E. In liver tissue.	
9.	Synthesis of one of the above coenzymes is impaired during oxidative decarboxylation of α-ketoglutaric acid as a result of vitamin B1 deficiency. Name him. A. Coenzyme A (HS-CoA) B. Lipoic acid (LC). C. Nicotinamide (NAD). D. Thiamine pyrophosphate (TPF). E. Flavinadenine dinucleotide (FAD).	
10.	When lipoic acid was excluded from the diet of experimental animals, inhibition of the pyruvate dehydrogenase complex was observed. What is lipoic acid to this enzyme? A. Allosteric regulator. B. Inhibitor. C. Prosthetic group. D. The product. E. The substrate.	
11.	Alkaline phosphatase is an important salivary enzyme. What class of enzymes does it belong to? A. Acid reductase. B. Transferase. C. Hydrolase D. Lyase. E. Ligaza	

12.	Alkaline phosphatase is an important salivary enzyme. What class of enzymes does it belong to? A. Acid reductase. B. Transferase. C. Hydrolase D. Lyase. E. Ligaz.	
13.	In the regulation of enzyme activity, an important role belongs to their postsynthetic covalent modification. What mechanism is used to regulate glycogen phosphorylase and glycogen synthetase? A. Phosphorylation-dephosphorylation B. Methylation C. Adenylation D. Limited proteolysis E. ADP-ribosylation	
14.	Teturam, an aldehyde dehydrogenase inhibitor, is widely used in medical practice to prevent alcoholism. The increase in the blood of which metabolite causes an aversion to alcohol? A. Acetaldehyde B. Ethanol C. Malonaldehyde D. Propionic aldehyde E. Methanol	
15.	When administered to the human body, potassium cyanide, which is a poison, causes death in a few minutes. The most likely cause of its toxic effect is a violation of activity: A. ATP synthetases. B. Catalases. C. NADPH dehydrogenases. D. Methemoglobin reductases.	

	E. Cytochrome oxidases	
16.	Postsynthetic covalent modification has an important place in the regulation of enzyme activity. Which of the types of this modification is used to regulate the activity of glycogen phosphorylase and glycogen synthetase? A. Adenylation B. ADP-ribosylation C. Methylation. D. Limited proteolysis. E. Phosphorylation-dephosphorylation.	
17.	A patient with stomatitis was prescribed a drug from the group of sulfonamides. What is the mechanism of its antibacterial action? A. Decrease in permeability of membranes. B. Protein coagulation. C. Competitive antagonism with PABA. D. Violation of cell wall protein synthesis. E. Suppression of sulfhydryl groups of thiol enzymes.	
18.	For the treatment of some infectious diseases caused by bacteria, sulfonamide drugs that block the synthesis of the bacterial growth factor are used. What is the mechanism of action of these drugs? A. Take part in redox processes. B. They are allosteric enzyme inhibitors. C. Are allosteric enzymes. D. They are p-aminobenzoic acid antivitamins. E. Inhibit the absorption of folic acid.	
19.	When studying the properties of the enzyme, an unknown substance was added to the enzyme-substrate system, as a result of which the Michaelis constant increased by 2 times. What phenomenon took place? A. Allosteric activation. B. Noncompetitive inhibition.	

	C. Competitive inhibition. D. Irreversible inhibition. E. Noncompetitive inhibition.	
20.	In E. coli cells, the synthesis of pyrimidine nucleotides is carried out according to the scheme of the metabolic pathway from the initial substrates CO2, NH3, ATP. With the formation of UTF in the end and then from it - CTF. When the concentration of CTP in the cell increases, the synthesis of pyrimidine nucleotides stops. What type of enzyme activity regulation is described: A. Allosteric B. Partial proteolysis of the enzyme C. Phosphorylation of the enzyme molecule D. Attachment of inhibitory proteins E. Cleavage of the inhibitor protein	
21.	Pharmaceutical drugs proserin, physostigmine are used for myasthenia, paralysis, and intestinal atony. They increase the duration of action of the neurotransmitter, as they are competitive inhibitors of the enzyme: A. Diamine oxidases B. Monoamine oxidases C. Sucrase D. Acetylcholinesterase E. Histidine decarboxylase	
22.	Proteolytic enzymes (pepsin, trypsin) are synthesized in an inactive form, the form of proenzymes (pepsinogen, trypsinogen), and are activated during food digestion by limited proteolysis. The mechanism of their activation: A. Dissociation of the inhibitory peptide from the proenzyme B. Phosphorylation C. Dephosphorylation D. Action of an allosteric effector E. Substrate activation	
23.	Aspirin is used as an anti-inflammatory agent that blocks COX (cyclooxygenase). Indicate how this drug exerts its inhibitory effect? A. By acetylation of the OH group of serine in the active center of the enzyme	

	B. By phosphorylation of the OH-group of serine in the active center of the enzyme C. By methylation of its prosthetic group D. By decarboxylation of glutamate radicals in the active center of the enzyme E. By phosphorylation of the OH-group of tyrosine in the active center of the enzyme	
24.	An employee of the sanitation station was poisoned with an organophosphorus insecticide while processing the facility. Determine the mechanism of action of this substance on the human body: A. Irreversible inhibition of acetylcholinesterase B. Hydrolysis of acetylcholine C. Irreversible inhibition of tryptophanylpyrrolase D. Competitive inhibition of acetylcholinesterase E. Allosteric inhibition of acetylcholinesterase	
25.	Proserin was used to treat myasthenia gravis and other diseases of the muscular system. This drug is a competitive enzyme inhibitor of: A. Lactate dehydrogenases B. Acetylcholinesterase C. Citrate synthases D. Succinate dehydrogenases E. Arginase	

4. Literature. Look pic. 229.

1. TOPIC:DETERMINATION OF ENZYME ACTIVITY IN BIOLOGICAL ENVIRONMENTS. UNITS OF ENZYME ACTIVITY. ENZYMOPATHY. MEDICAL ENZYMOLOGY.

2. INFORMATION MATERIAL.

Achievements of enzymology are increasingly used in medicine, in particular in the prevention, diagnosis and treatment of diseases. A new direction of enzymology is successfully developing what is medical enzymology, which has its own goals and objectives, specific methodological approaches and research methods. One of the most important achievements of modern medicine is the widespread use of enzymes in clinical laboratories around the world. There are three main areas of development of medical enzymology: enzymopathology, enzymodiagnostics and enzymotherapy.

The field of enzymopathology research is a theoretical, fundamental part of pathology. She studies the molecular basis of pathological processes, the cause of which is a violation of the mechanisms of regulation of the activity or synthesis of an individual enzyme or a group of enzymes. Thanks to the achievements of biochemical genetics, it has been established that the molecular basis of congenital metabolic disorders can be enzyme defects caused by mutations in genes responsible for the synthesis of certain enzyme proteins. For a number of diseases, it has been established that the development of the disease can be caused by a hereditary deficiency or a complete lack of synthesis of a single enzyme in the patient's body. Such diseases are called enzymopathies.

Enzymopathies are divided into:

I. Congenital disorders of the metabolism of simple and complex carbohydrates:

glycogenoses: Gierke disease (absence of glucose-6-phosphatase), disease Pompe (absence of acid maltase), Corey and Forbes disease (absence 1,6-glucosidases); mucopolysaccharidosis (deficiency of lysosomal hydrolases glycosaminoglycans); essential fructosuria (deficiency of fructokinase); lactose intolerance (lactase deficiency), galactosemia. Galactosemia is a hereditary disease in which there is an abnormally high concentration of galactose in the blood. The disease develops as a result of a hereditary defect in the synthesis of the enzyme hexose-1-phosphateuridyltransferases, which catalyzes the conversion of galactose into glucose, which is easily metabolized.

II. Congenital disorders of sphingolipid metabolism: sphingolipidoses:

Niemann-Pick disease (lack of sphingomyelinase), Fabry disease (lack of α-galactosidase).

III.Congenital disorders of amino acid metabolism:

phenylketonuria (lack of phenylalanine hydroxylase), alkaptonuria (lack of homogentisic acid oxidase), tyrosinemia, homocystinuria.

The cause of the hereditary disease of phenylketonuria, which is accompanied by a disorder of mental activity, is the loss of liver cells' ability to synthesize an enzyme that catalyzes the conversion of phenylalanine into tyrosine. As a result, there is a deficiency of tyrosine and a number of specific products that are derivatives of tyrosine, and phenylpyruvate and phenylacetate accumulate in the body of patients. The concentration of phenylalanine in the blood of patients also increases significantly (tens of times).

IV. Congenital disorders of porphyrin metabolism:

Porphyria (lack ofuroporphyrinogen synthetases, ferrochelatases, etc.).

V. Congenital disorders of purine and pyrimidine metabolism:

Lesch-Nyhan syndrome (lack of hypoxanthineguaninephos-phoribosyltransferases); orotaciduria (lack of bifunctional enzyme fromorotate phosphoribosyltransferaseand orotidine-5- monophosphate decarboxylaseactivities).

Currently, about 150 hereditary enzymopathies are known. In the form of hereditary pathology, enzymopathies sometimes manifest themselves only in certain physiological conditions. The consequences of the defect at the level of the organism depend on the role of the blocked pathway in metabolism, the presence of alternative pathways, the level of residual enzyme activity, and the toxicity of the metabolite that accumulates as a result of the blockade of the metabolic process. In some cases, the enzyme defect only leads to a decrease in the body's adaptive capabilities and serves as a cause of susceptibility to disease. Enzymopathology also successfully solves the problems of the pathogenesis of somatic diseases. Work is underway to elucidate the molecular basis of atherosclerosis, malignant growth, rheumatoid arthritis, etc. The existence of this direction is due to the awareness of the huge role of enzyme systems or even individual enzymes, the violation of the regulation of activity and synthesis of which can lead to the formation or development of pathological processes.

DETERMINATION OF ENZYME ACTIVITY. TYPES AND UNITS OF ENZYME ACTIVITY

The methods used in modern enzymology to determine the activity of

enzymes necessarily take into account the factors of activity regulation and, as a result, optimal conditions are chosen: temperature, enzyme concentration (determined by the volume of the research object), substrate concentration (chosen equal to [S] of full saturation of the active centers of the enzyme or is taken in excess), the pH of the medium, enzyme activators are present, and its inhibitors are excluded.

Enzyme activity can be determined by:

• rate of decrease in concentration of reaction substrates (examples: determination of ALT and AST activity in blood serum) - [-ΔS / Δt];

• rate of formation of reaction products (example: determination of serum cholinesterase activity; an indicator of the appearance of a reaction product in the incubation medium is additionally used) - [ΔP / Δt];

• the rate of transition of the oxidized form of the coenzyme to the reduced form (examples: determining the activity of lactate dehydrogenase and malate dehydrogenase in blood serum).

In modern enzymology, two units of measurement of ***total enzyme activity (T.A.)*** are used:

1. International unit of activity (IU) - the amount of enzyme required to convert 1 μmol of substrate in 1 minute into a reaction product under standard measurement conditions.
2. Catal (SI) - the amount of enzyme required to convert 1 mole of substrate in 1 second into a reaction product under standard measurement conditions.

If the activity of the enzyme cannot be expressed in the above-mentioned units, conventional units of activity are used (example: Wolgemuth's method for determining urine amylase activity).

When conducting scientific research on the activity of enzymes in tissues, it is more important for a scientist to know ***the specific activity (S.A.) of an enzyme***, which is calculated after previously measuring the protein concentration in the sample: [C] = [mg/ml] or [g/l]; then by the formula: S.A. = O.A ./ [C].

In the literature on biochemistry for some enzymes,***turnover number (N)*** is presented - it is the number of molecules of the substrate that is converted by one molecule of the enzyme per unit of time. As an example, we can cite the enzyme carbonic anhydrase, which catalyzes the reaction: $H_2CO_3 \leftrightarrow H_2O + CO_2$. For this enzyme, N = 36000000 / s.

In the methods of researching the activity of enzymes in plasma (blood serum), for the most part, units of enzyme activity take into account the volume of the

research object and the reaction time. For example, the normal activity of cholinesterase (ChE) in blood serum is in the range of 45-92 μmol / l * s. How to understand this value? This means that the amount of ChE enzyme in 1 liter of blood serum of a healthy person is able to catalyze the formation of 45-92 micromoles of acetic acid from acetylcholine (substrate of the enzyme) in 1 second under standard reaction conditions.

Methods of researching the activity of enzymes in biological fluids in clinical laboratories require:

• proper storage and transportation of patient fluid samples, which is always agreed upon in the experimental methodology;

• use of auxiliary research methods such as spectrophotometry, immunoelectrophoresis, densitometry, etc.

USE OF ENZYMES IN HUMAN LIFE ACTIVITIES

An important area of enzymology is ***enzyme diagnostics***. Diagnostic enzymology can be the basis not only for making a correct and timely diagnosis of the disease, but also for checking the effectiveness of the used treatment method.

1. ***At present, enzymes are actively used in medicine, primarily for the diagnosis of diseases. Examples:***

• When diagnosing acute pancreatitis, amylase activity is determined in blood plasma and urine; the quality of treatment can be controlled by trypsin activity in blood plasma.

• Determination of the activity of lactate dehydrogenase isoenzymes: the activity of LDH1 and LDH2 isoforms is determined in the blood plasma during myocardial infarction, the activity of LDH4 and LDH5 isoforms in the blood plasma - in the differential diagnosis of liver diseases.

• Diagnosis of prostatitis is carried out using the method of determining the activity of acid phosphatase in blood plasma.

• In case of damage to bone tissue (with osteoporosis in adults, with developed rickets in children), the activity of alkaline phosphatase in the blood plasma is determined.

• For the early diagnosis of muscular dystrophies, the most informative is an increase in the activity of creatine kinase in the blood plasma.

• Determination of the activity of organ-specific enzymes, such as arginase,ornithylcarbamoyltransferasehelps in the diagnosis of liver lesions.

Enzymotherapy is the use of enzyme preparations for the purpose of treating

enzymopathy or inhibiting or accelerating chemical reactions in the cells and tissue fluid of the body.

2. Enzymes and their inhibitors are actively used as medicines. Examples:

• After burns, patients are left with keloid scars. To eliminate them, the enzyme preparation "Lidase" (a pharmaceutical form of the enzyme hyaluronidase) is used, which destroys hyaluronic acid, a component of connective tissue in scars.

• Bandages with enzyme immobilized on them are used in the treatment of purulent wounds in patients (the enzyme is bound to a polymer carrier by means of a special chemical reaction). This function is usually performed by proteolytic enzymes trypsin, chymotrypsin.

• The pharmaceutical drug "Asparaginase" is used in the treatment of leukemia. This drug destroys the amino acid asparagine, the concentration of which in the affected cells of the bone marrow is considered as a growth factor of cancer tumors.

• To avoid autolysis of the pancreas in acute pancreatitis, patients are prescribed drugs like inhibitors of proteolytic enzymes, for example, trasylol is a trypsin inhibitor.

• Patients with myocardial infarction are prescribed fibrinolytic drugs Streptodekaza, Streptokinase. Fibrinolytic drugs are able to dissolve already formed thrombi in the human body.

• Pharmaceutical drugs proserin, physostigmine are used for myasthenia, paralysis, and intestinal atony. They increase the duration of action of the neurotransmitter acetylcholine, as they are competitive inhibitors of the enzyme acetylcholinesterase.

• Aspirin is used as an anti-inflammatory agent that blocks cyclooxygenase. This drug exerts its inhibitory effect by acetylating the OH group of serine in the active center of the cyclooxygenase enzyme.

• Patients diagnosed with viral conjunctivitis are prescribed eye drops containing DNase.

3. Enzymes are used as reagents for biotechnological operations and in diagnostic methods for determining certain substances in biological fluids. Examples:

• New antibiotics are synthesized by enzymatic transformations of natural

antibiotics, for which immobilized enzymes are used as synthesis tools

• Glucose oxidase enzyme is the main reagent in the kit for quantitative determination of glucose in blood plasma (serum).

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test:	Explanation:
1.	There are several groups of molecular mechanisms that play an important role in the pathogenesis of cell damage, which contributes to the development of pathology. What processes provide protein damage mechanisms? A. Osmotic stretching of membranes B. Inhibition of enzymes C. Activation of phospholipases D. Acidosis E. Peroxidation of lipids	
2.	An increase in the activity of lactate dehydrogenase isoenzymes LDH4, LDH5, alanine aminotransferase (ALT), carbomoylornithine transferase was found in the patient's blood. In which organ can the development of the pathological process be predicted? A. Heart muscle (possible myocardial infarction) B. Kidneys C. Skeletal muscles D. Connective tissue E. Liver (possible hepatitis)	
3.	Saliva contains an enzyme that has a strong bactericidal effect due to its ability to destroy the peptidoglycans of the bacterial wall. Specify this enzyme:	

	A. Trypsin B. Alpha-amylase C. Lysozyme (muramidase) D. Phosphatase E. Ribonuclease	
4.	A 46-year-old patient has been suffering from progressive muscular dystrophy (Duchenne muscular dystrophy) for a long time. A change in the activity level of which blood plasma enzyme is a diagnostic test in this case? A. Lactate dehydrogenases B. Creatine phosphokinase C. Pyruvate dehydrogenases D. Glutamate dehydrogenases E. Adenylate kinases	
5.	The protective function of saliva is determined by several mechanisms, including the presence of an enzyme that has a bactericidal effect, causing lysis of the polysaccharide complex of the shell of staphylococci and streptococci. Specify this enzyme: A. Lysozyme B. Alpha-amylase C. Oligo-1,6-glucosidases D. Collagenase E. Beta-glucuronidase	
6.	An increase in the activity of LDH1 and LDH2, AST, and creatine phosphokinase was found in the patient. In which organ(s) is the most likely development of the pathological process? A. In the heart muscle (initial stage of myocardial infarction)	

	B. In skeletal muscle (dystrophy, atrophy) C. In the kidneys and adrenal glands D. In connective tissue E. In the liver and kidneys	
7.	The patient was given a preliminary diagnosis: myocardial infarction. A characteristic feature of this disease is a significant increase in activity: A. Alpha amylases B. Catalases C. Glucose-6-F-dehydrogenases D. Creatine phosphokinase E. Arginase	
8.	When examining the patient's blood, a significant increase in the activity of the MV isoform of CPK (creatine phosphokinase) and LDH1 was revealed. Make an assumption about a possible pathology: A. Cholecystitis B. Hepatitis C. Rheumatism D. Pancreatitis E. Myocardial infarction	
9.	During pathological processes accompanied by hypoxia, oxygen molecules are restored to hydrogen peroxide in the respiratory chain. Name the enzyme that destroys this cytotoxic substance: A. Cytochrome oxidases B. Catalase C. Succinate dehydrogenase D.Alpha-ketoglutarate dehydrogenase	

	E. Aconitase	
10.	Based on clinical data, the patient was diagnosed with acute pancreatitis. Specify a biochemical test that will confirm this diagnosis. This definition is: A. Creatinine level in the blood B. Blood acid phosphatase activities C. Blood alkaline phosphatase activities D. Blood aminotransferase activities E. Blood amylase activity	
11.	A 47-year-old man was admitted to the intensive care unit with a diagnosis of myocardial infarction. Concentration of which of the fractions of lactate dehydrogenase (LDH) will be higher in the blood for the first two days? A. LDH1 B. LDH2 C. LDH3 D. LDH4 E. LDH5	
12.	A 50-year-old woman with a diagnosis of myocardial infarction was brought to the intensive care unit. The activity of which enzyme in the patient's blood plasma will be significantly increased in the first two days? A. Alanine aminopeptidases B. Alanine aminotransferases C. Aspartate aminotransferases D. LDH4 E. LDH5	

13.	While examining a patient, 12 hours after an acute attack of chest pain, a sharp increase in AST activity in blood serum was detected. Specify the pathology in which the following changes occur in the blood: A. Myocardial infarction B. Viral hepatitis C. Collagenosis D. Diabetes E. Diabetes insipidus	
14.	An 18-year-old boy with damaged liver parenchyma has the most likely increased level of activity in the blood serum: A. Alanine aminotransferases B. Lactate dehydrogenase-1 C. Creatine kinases D. Acid phosphatase E. Alpha amylases	
15.	A high activity of the LDH1 isoenzyme was detected in the patient's blood serum. What organ does the pathological process take place in? A. Liver B. Heart C. Skeletal muscles D. Pancreas E. Kidneys	
16.	A diagnostic test for acute pancreatitis is to determine the activity of the following enzymes in the urine: A. Lactate dehydrogenases B. Amylases	

	C. Creatine kinases D. Aldolase E. Alanine aminopeptidase	
17.	The patient was taken to the hospital with a preliminary diagnosis of acute pancreatitis. Determine the activity of which enzyme should be investigated in blood and urine to confirm the previous diagnosis? A. ALAT B. Alpha amylases C. AsAT D. Lactate dehydrogenase E. Cholinesterases	
18.	The activity of which enzymes should be determined for diagnostic and prognostic purposes, if a patient with heart muscle pathology was admitted to the clinic? A. Neuraminidase, hexokinase, pyruvate kinase B. Arginases, peptidases, phosphatases C. Lysozyme, citrate synthase, aldolase D. Creatine kinases, ALT, AST E. EPDC, MDH, IDH, a-ketoglutarate dehydrogenase	
19.	A blood and urine analysis of a patient with acute pancreatitis, revealed high activity of one of the specified enzymes: A. Lactase B. Pepsin C. Dipeptidases D. Sucrase E. Alpha amylases	

20.	Name the enzyme, the determination of which activity is the most informative test in the first hours of the development of a myocardial infarction: A. Creatine phosphokinase B. Aspartate aminotransferase C. Alanine aminotransferase D. Lactate dehydrogenase E. Glutamate dehydrogenase	
21.	The patient has acute pancreatitis. What drugs should the doctor prescribe to the patient to avoid autolysis of the pancreas? A. Protease inhibitors B. Protease activators C. Trypsin D. Chymotrypsin E. Amylase	
22.	The patient has a hemorrhagic stroke. The patient's blood has an increased concentration of kinins. The doctor prescribed the drug Kontrikal to the patient. To inhibit which proteinase did the doctor prescribe that drug? A. Kallikrein B. Pepsin C. Trypsin D. Chymotrypsin E. Collagenasesи	
23.	A 49-year-old patient (a driver by profession) complains of unbearable chest pains that radiate to the neck and started 2 hours ago. The condition is severe, the face is pale, the heart tones are weakened. Laboratory examination	

	showed high activity of blood enzymes creatine kinase and LDH1. What disease is characterized by such changes? A. Angina B. Acute pancreatitis C. Acute myocardial infarction D. Gallstone disease E. Diabetes	

4. Literature. Look pic. 229.

1. TOPIC: GENERAL REGULATIONS OF SUBSTANCE AND ENERGY EXCHANGE. TRICARBONIC ACID CYCLE

2. INFORMATION MATERIAL.

General patterns of metabolism of substances and energy

Exchange of substances (metabolism) is a set of chemical reactions involving organic and inorganic compounds (**metabolites**) that occur in a living organism. The constant exchange of substances and energy with the environment is the main difference of a living cell, which determines its thermodynamic state and homeostasis.

Metabolism in the human body consists of five successive phases:

a) ***digestion of nutrients (destruction):*** proteins, lipids, carbohydrates, vitamins, minerals and water in the composition of food in the gastrointestinal tract (GI) to simple compounds what are amino acids, monosaccharides, fatty acids, glycerol (mineral components and vitamins are not destroyed in the gastrointestinal tract);

b) ***absorption of*** the above-mentioned digestion products by the epithelium of the mucous membrane of the small intestine using various mechanisms: ***active transport, passive diffusion, pinocytosis, receptor-mediated endocytosis, etc.;***

c) ***transport of products of digestion of nutrients with the bloodstream and lymphatic system***, their entry through blood vessel membranes and cell membranes into certain organs and tissues (liver, muscles, brain, kidneys, adipose tissue, etc.);

d) ***intracellular metabolism*** of organic molecules in organs and tissues (***intermediate exchange***), which is represented by a set of various chemical reactions of synthesis and decay of substances in the cell;

e) ***allocation (excretion)*** from the body(through the kidneys, lungs, skin, intestines) end products of metabolism: carbon dioxide, ammonium salts, urea, uric acid, creatinine, water, products of conjugation foreign compounds (***xenobiotics***), etc.

Reactions of intracellular metabolism of biomolecules include the following biochemical transformations:

a) splitting of organic molecules (glucose, fatty acids, amino acids, glycerol, etc.) into the final products of metabolism (CO_2, NH_3, H_2O) with the release of energy and its accumulation in the form of adenosine triphosphate (ATP), other macroergic phosphates or in the form of proton

potential, which provide needs of the energy of the basic life processes of the cell and the whole organism as a whole. ***The set of processes of splitting biomolecules into simple compounds with the release of energy was called catabolism;***

b) synthesis of specific biomolecules (proteins, nucleic acids, polysaccharides, lipids, hormones, etc.) that are genetically characteristic of a given organism, which are necessary for the formation of its own cellular and extracellular structures. ***The set of processes of synthesis of complex substances from simpler compounds, proceeding with the consumption of energy (mostly in the form of ATP), was called anabolism;***

c) the use of energy (in the form of ATP or proton potential) to ensure the processes of cellular physiology: the functioning of the contractile system, the activity of elements of the cytoskeleton, villi, flagella, etc., exo - and endocytosis, neurochemical transmission of impulses, active transport of metabolites and inorganic ions, etc.

In addition to anabolic and catabolic processes, ***amphibolic pathway*** take place in the cell, these are processes whose intermediate metabolites can be included in both catabolic and anabolic pathways of substance transformations.

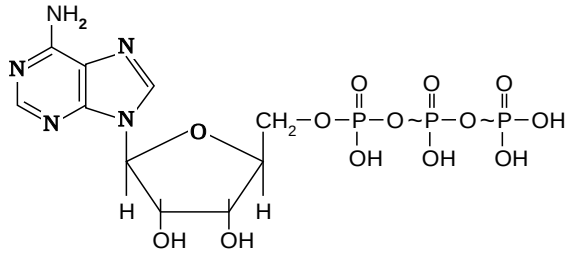
There are three main stages in the catabolism of complex i

Stage 1. In the first stage of catabolism, complex molecules (polysaccharides, proteins, nucleic acids, lipids) are broken down into simple components:

- Polysaccharides to monosaccharides (mainly glucose, fructose, galactose);
- Lipids (triacylglycerol) to fatty acids and glycerol;
- Proteins to amino acids;
- Nucleic acids to nucleosides.

Reactions of the first stage of catabolism are localized in the gastrointestinal tract, in the cytoplasm and lysosomes of tissue cells. Enzymes that catalyze these reactions belong to the class of hydrolases, the energy released during the hydrolysis of chemical bonds of compounds cannot be stored by the cell.

Stage II. In the second stage of catabolism, the metabolites formed in the first stage undergo destruction with the release of energy, one part of which is accumulated in high-energy (macroergic) bonds of ATP, the other part of energy is released in the form of heat energy.



Adenosine triphosphate acid (ATP)

- Almost all classes of enzymes, with the exception of the “ligase” class, participate in the transformations of this stage. Stage II reactions occur mainly in the cytoplasm and mitochondria of cells. The main ones are:
- **for monosaccharides is an aerobic glycolysis**(localization is in the cytoplasm of the cell), the final products of this process are pyruvic acid (**pyruvate**). Glycolysis can also occur under anaerobic conditions, in which case instead of pyruvate, the final product of the process is lactic acid (lactate). Under aerobic conditions, pyruvate is included in oxidative decarboxylation with the formation of **the active form of acetic acid that is acetyl-CoA** (localization is only mitochondria);
- **for fatty acids** is ß-oxidation, the final product of which is acetyl-CoA (localization is only mitochondria);
- **for glycerol is splitting to pyruvate**, which turns into acetyl-CoA;
- **for amino acids is transamination or direct deamination with release of ammonia** and cleavage of their nitrogen-free molecular products to the corresponding carboxylic acids, most of these metabolites are also ultimately converted to acetyl-CoA.

Thus, acetyl-CoA is a common end product of the second stage of intracellular catabolism of carbohydrates, lipids, and amino acids.

Stage III. In the third stage of catabolism, acetyl-CoA is oxidized to end products that are CO_2 and H_2O. This stage is localized in the mitochondria and consists of two processes:

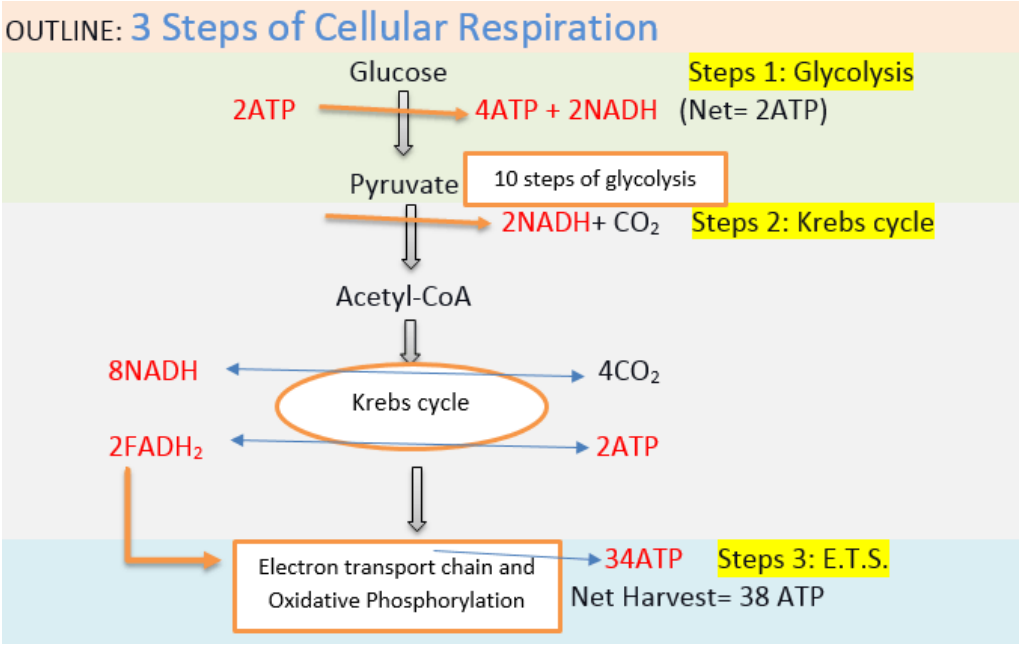
- **Tricarboxylic acid cycle (TCA, Krebs cycle**), as a result of which two moles of CO_2 are formed for one mole of used acetyl-CoA and reduced forms of the coenzymes NAD^+ and FAD;
- **Respiratory chain of electron transfer from reduced forms of coenzymes to molecular oxygen**.

It should be noted that the formation of the above-mentioned reduced forms of coenzymes is also possible in the second stage of catabolism (in aerobic glycolysis, during ß-oxidation of higher fatty acids, during the destruction of

glycerol, etc.).

A mandatory condition for the III stage of catabolism and the use of reduced forms of coenzymes formed in the II stage of catabolism is the provision of molecular oxygen to the cell, that is, the creation of aerobic conditions in the cell.

The set of reactions of aerobic oxidation of substrates in tissue cells is called tissue respiration. The II and III stages of catabolic pathways are called the I and II stages of tissue respiration, respectively (they can take place in the cytoplasm, endoplasmic reticulum, and mitochondria). The third stage of tissue respiration is localized on the inner membrane of mitochondria and is represented by the function of the respiratory chain of electron transfer from reduced forms of coenzymes and prosthetic groups to molecular oxygen (pic. 27).



Pic. 27. Stages of tissue respiration according to Lehninger A. (https://www.microbialfacts.com/wp-content/uploads/2020/05/3-Steps-of-Cellular-Respiration2.png)

The function of the respiratory chain of the inner membrane of mitochondria is related to ***oxidative phosphorylation, as a result of which the energy of aerobic oxidation reactions is used for the synthesis of ATP that is the main supplier of energy in all endergonic processes*** (processes that consume energy).

The normal course of tissue respiration is ensured primarily due to the activity of the external respiratory system and the oxygen transport function of blood hemoglobin. Molecular oxygen enters cells by simple diffusion (pinocytosis), where it is used in the following processes:

1) ***Mitochondrial oxidation of organic substrates***, which ends with the inclusion atoms of molecular oxygen in H_2O molecules with the formation of thermal energy and chemical energy of ATP bonds. The carbon of the substrates is oxidized to CO_2;
2) ***Microsomal oxidation with the inclusion of molecular oxygen atoms in the structure of organic substrates for the purpose of its modification***. Energy is not released during the oxidation of substrates.

The set of mitochondrial and microsomal oxidation reactions, as well as oxidation reactions occurring in the cell cytoplasm, is called biological oxidation.

Cycle of tricarboxylic acids

The cycle of tricarboxylic acids (TCA, Krebs cycle, citric acid cycle) is the most important supplier to the respiratory chain of reduced forms of coenzymes and prosthetic groups formed during the utilization of acetyl-CoA (1), keto acids, monosaccharide oxidation products, higher fatty acids (HFA) and amino acids (see pic. 28).

All enzymes of the process are localized in the mitochondrial matrix, with the exception of succinate dehydrogenase (6*, pic. 28). The rate of TCA flow depends primarily on the rate of formation of acetyl-CoA in the matrix of mitochondria (pic. 28, (1)), the supply of its precursors (pyruvate, HFA) and a number of other factors that must be considered in relation to each of the eight reactions of the Krebs cycle:

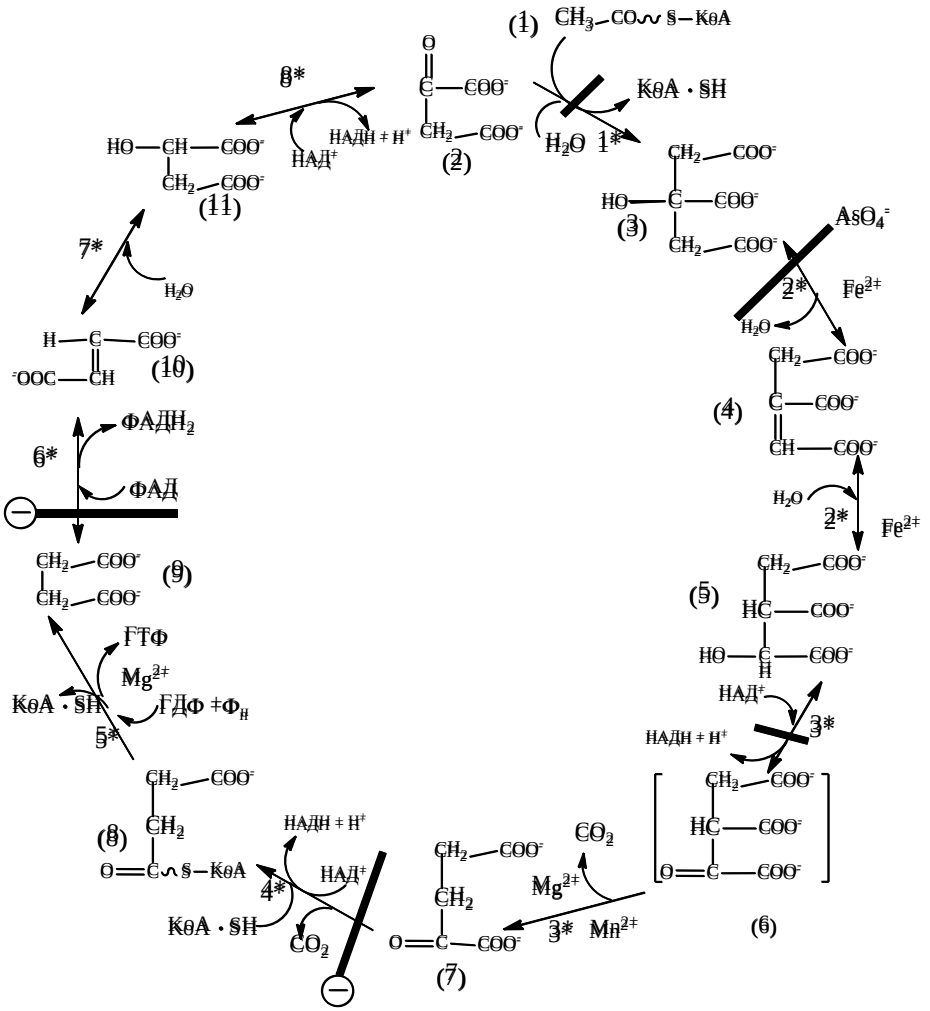
1)Condensation of acetyl-CoA (1) with oxaloacetate (oxaloacetic acid), 2) is carried out by the enzyme citrate synthase (1*). The activity of citrate synthase is inhibited by the accumulation of ATP, NADH, succinyl-CoA, and acyls of HFA in the matrix;

2)Isomerization of citrate (3) into isocitrate (5) is carried out by the enzyme aconitase (Fe^{2+} containing protein, 2*) in two stages:

1st stage is dehydration of citrate with the formation of cis-aconic acid (4);

2nd stage is hydration of cis-aconic acid along the double bond with the formation of isocitrate (5).

The enzyme is inhibited by arsenic acid derivatives.



pic. 28. Krebs cycle. In the process diagram, all enzymes are indicated by a number with an asterisk, metabolites are indicated by a number in parentheses (see names in the text).

3)During the action of NAD^+ that is dependent isocitrate dehydrogenase (3*), oxidative decarboxylation of isocitrate (5) occurs with the formation of products: á-ketoglutarate (7), CO_2 and NADH (respiratory chain electron donor). The reaction proceeds in two stages: 1) dehydrogenation with the formation of oxalic succinic acid (6); 2) decarboxylation of this substance to ά-ketoglutaric acid. Isocitrate dehydrogenase is rate-limiting for the entire Krebs cycle. The enzyme is activated by ADP, Mg^{2+} and Mn^{2+} ions; inhibited by the accumulation n the matrix of ATP, NADH;

4)Oxidative decarboxylation of ά-ketoglutarate is carried out by the ά-ketoglutarate dehydrogenase complex (4*). It is a polyenzyme system in terms of composition (three enzymes) and vitamin supply: vitamins B_1 (coenzyme TPP), B_2 (prosthetic group FAD), B_5 (coenzyme CoASH), B_3 (coenzyme NAD^+), amide of lipoic acid). As a result of the work of the complex, CO_2, succinyl-CoA (macroergic substance, 8) is formed; NADH

(donor of electrons in the respiratory chain);

5)Succinyl-CoA-thiokinase (synthase, 5*), using the energy of breaking the macroergic bond in succinyl-CoA, phosphorylates GDF with the formation of GTP, while succinic acid (by anion what's succinate, 9) is simultaneously formed. This reaction is called substrate phosphorylation. The formed GTP can further be converted into ATP under the action of nucleoside diphosphate kinase according to the equation:

$$GTP + ADP \rightarrow ATP + GDP$$
$$Mg^{2+}$$

6) Succinate dehydrogenase (the only TCA enzyme localized on the inner membrane of mitochondria, 6*) oxidizes succinic acid (9) to trans-fumaric acid (10) thanks to the FAD prosthetic group. Succinate dehydrogenase in the inner membrane of mitochondria forms a complex with iron-sulfur-containing proteins, which is called complex II of the respiratory chain. Malonic acid is a competitive enzyme inhibitor;

7) The fumarase enzyme (7*) hydrates only the trans-form of fumaric acid with the formation of L-malic acid (according to the anion what's called L-malate, 11) by the double bond. The reaction is reversible, fumarase is stereospecific only for L-malate.

8) At the last stage of the cycle of the NAD+ is dependent malate dehydrogenase (8*) catalyzes the oxidation of L-malate into oxaloacetic acid with the formation of NADH (electron donor in the respiratory chain). The reaction is reversible, but the rapid use of oxaloacetic acid in the citrate synthase reaction shifts the equilibrium to the right.

Thus, for eight reactions of the Krebs cycle, due to the formation of three tricarboxylic acids (citric, cis-aconite, isolimonic), in the course of four dehydrogenase reactions, two of which were accompanied by decarboxylation (3*, 4*), the formation of 2 moles of CO_2, 3 NADH, 1 $FADH_2$ and 1 GTP equal to 1 ATP. These substances are called end products of the Krebs cycle per cycle. Oxaloacetic acid is constantly regenerated and re-included in the citrate synthase reaction, so this substance cannot be called the end product of the cycle.

The main regulatory reactions of TCA are citrate synthase and isocitrate dehydrogenase. The principle of feedback metabolic communication takes place in the regulation of TCA. The intensity of oxidation of substrates in it increases under conditions of increased concentration of ADP and NAD^+. Under conditions of increased concentration of ATP and NADH, the rate of oxidation of substrates

in the Krebs cycle decreases. Such regulation allows to adequately change the intensity of functioning of TCA in conditions that require an urgent change in the level of energy supply to the cell.

The intensity of the TCA course can be determined by the value of respiratory control, which is expressed by the concentration ratio [ATP]/[ADP]. At values of [ATP]/[ADP]<1, the rate of incorporation of reduced forms of NADH coenzymes into the respiratory chain increases, while the rate of TCA increases.

The Krebs cycle is an amphibolic process, since, although this is a catabolic process, some of its metabolites can be used by the cell for synthetic purposes. Succinyl-CoA is used by the cell as a starting substrate for the first reaction of heme synthesis. Oxaloacetate and its precursors in the cycle can be used in the synthesis of glucose (the process of gluconeogenesis). Keto acids that are oxaloacetate and alpha-ketoglutarate, thanks to transamination reactions, can be used to form substitute amino acids: aspartic and glutamic acids, respectively.

3. TASKS FOR INDEPENDENT WORK..

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	How many ATP molecules can be synthesized during the complete oxidation of acetyl-CoA in the tricarboxylic acid cycle? A.12 B.1 C.5 D.8 E.3	
2.	The central intermediate product of all exchanges (proteins, lipids, carbohydrates) is: A. Acetyl CoA B. Succinyl-CoA C. Oxalic acetic acid D. Lactate E. Citrate	
3.	Macroergic compounds are necessary for normal cell metabolism. Which of the following substances is classified as macroergic? A. Creatine phosphate B. Creatine C. Creatinine D. Glucose-6-phosphate. E. Adenosine monophosphate	
4.	The Krebs cycle plays an important role in the implementation of the glucoplastic effect of	

	amino acids (participation in the synthesis of glucose). This is due to the mandatory conversion of some amino acids into: A. Oxaloacetate B. Malate C. Succinate D. Fumarate E. Citrate	
5.	Which carboxylic acid is an intermediate product of the tricarboxylic acid cycle and is involved in calcium binding: A. α-ketoglutaric acid (alpha-ketoglutarate). B. Citric acid (citrate). C. Acetic acid (acetate). D. Malic acid (malate). E. Succinic acid (succinate).	
6.	In which of the listed tissues is the content of citric acid, which has a high complexing ability and participates in the process of calcium mobilization, is the highest? A. epithelial tissue. B. bone tissue. C. muscle tissue. D. nervous tissue. E. parenchymatous tissue.	
7.	What process provides erythrocytes with the	

	necessary amount of energy in the form of ATP for their vital activity? A. Aerobic oxidation of glucose. B. Glycolysis. C. B-Oxidation of fatty acids. D. The pentose cycle. E. Cycle of tricarboxylic acids.	

4. Literature. Look pic. 229.

1.TOPIC: **MOLECULAR FUNDAMENTALS OF BIOENERGETICS (SEMINAR)**

2. INFORMATION MATERIAL.

CONCEPT OF TISSUE BREATHING. STAGES OF TISSUE RESPIRATION. COMPOSITION AND FUNCTION OF THE RESPIRATORY CHAIN OF THE INNER MEMBRANE OF MITOCHONDRIA

Tissue respiration is a set of reactions of aerobic oxidation of organic molecules in the cell, in which molecular oxygen is a mandatory substrate for the formation of oxidation products. However, the oxygen cell can be used for various tasks:

1. In the inner membrane of mitochondria, oxygen is the final acceptor of electrons from oxidizing substrates (NADH·H+ or FADH2) with the possibility of incorporating its active form (oxide-anion; atomic oxygen) into a water molecule that is one of the final products of the oxidation of organic molecules in aerobic cells type;

2. ***monooxygenase systems of the inner membrane of mitochondria or membranes of the endoplasmic reticulum (ER)*** use one atom of molecular oxygen to incorporate it into the molecules of organic substrates in order to modify their structure and the appearance of such functional groups as hydroxyl, keto-, aldehyde, carboxyl groups;

3. ***ER dioxygenase systems*** use two atoms of molecular oxygen to form peroxide compounds of the R_2O_2 type. The cell utilizes such peroxides thanks to antioxidant enzymatic systems: glutathione peroxidase, etc.

Task 1 performed by an aerobic type cell mainly when substances-energy sources appear in the cell, and there is a need for energy production by including these substances-energy sources in catabolic pathways. Tissue respiration of cell can be presented in the form of three stages:

The 1st stage of tissue respiration is 2^{nd} stage of catabolic processes;

2nd stage of tissue respiration is Tricarboxylic Acid Cycle (TCA);

The 3rd stage of tissue respiration is the function of the respiratory chain of the internal mitochondrial membrane.

The 1st and 2nd stages of tissue respiration produce reduced forms of

coenzymes and prosthetic groups in the cytosol and matrix of mitochondria that are potential electron donors to the respiratory chain of the inner mitochondrial membrane. It is in this membrane that there is a special complex of enzymes and lipophilic substances (ubiquinone; coenzyme Q), which transfers electrons from reduced forms of coenzymes (NADH) and prosthetic groups ($FADH_2$) to atomic oxygen.

In the structure of mitochondria, there is an outer membrane, an inner membrane, a matrix, and an intermembrane space. The processes of the first and second stages of tissue respiration are localized in the matrix and, partially, in the inner membrane: beta-oxidation of higher fatty acids, reactions of amino acid metabolism what are oxidative deamination, transamination, Krebs cycle (TCA) with the exception of the succinate dehydrogenase reaction.

Both membranes permeate the transport systems responsible for:

1. Transport of amino acids;
2. ATP/ADP transport;
3. Transport of ions;
4. Shuttle systems (malate-aspartate, glycerol phosphate) that transport electrons and protons from cytosolic forms of reduced coenzymes to the matrix and the inner membrane;
5. Transport of tricarboxylic acids;
6. Transport of acyls of high fatty acids;
7. Transport of cations and anions.

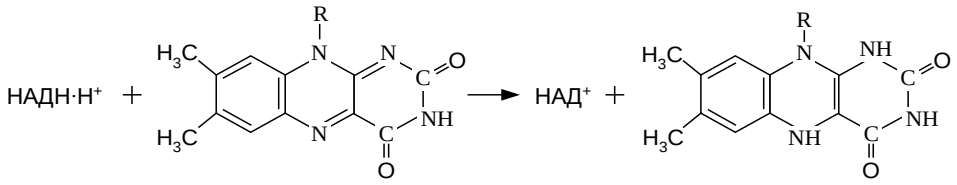
Transport systems ensure the constancy of the composition of the matrix of mitochondria, the exchange of substances with the cytoplasm, the delivery of substrates formed in the matrix to the cytoplasm for the needs of the cell.

The most important from the energy point of view is the third stage of tissue respiration, that is, the function of the respiratory chain of the inner membrane of mitochondria. The respiratory chain consists of electron carriers from reduced forms of coenzymes to oxygen. Electron carriers are combined into complexes of the respiratory chain. The division of the participants of the respiratory chain into complexes (I-IV) arose during experimental studies on the isolation and separation of the components of the respiratory chain in order to study their structure and function.

Complex I of the respiratory chain consists of the transmembrane protein-enzyme NADH-dehydrogenase (non-protein part is FMN) and iron-sulfur-containing proteins (FeS-proteins). From the matrix, NADH forms migrate to the

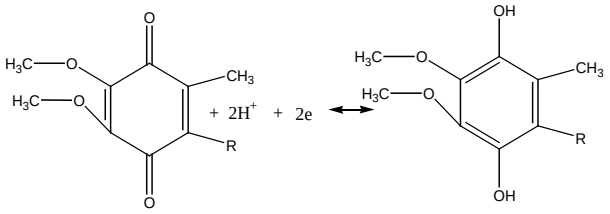
inner membrane of mitochondria, where they are captured by the flavoprotein NADH dehydrogenase. An oxidation-reduction reaction takes place:

$$NADN \cdot H+ + FMN \cdot DHase \rightarrow NAD^+ + FMNN2 \cdot DHase$$



FMN **FMNH2**

The reduced form of NADH-DHase through the FeS-proteins of complex I transfers electrons to ubiquinone (CoQ), and ubiquinone can capture protons from the matrix:



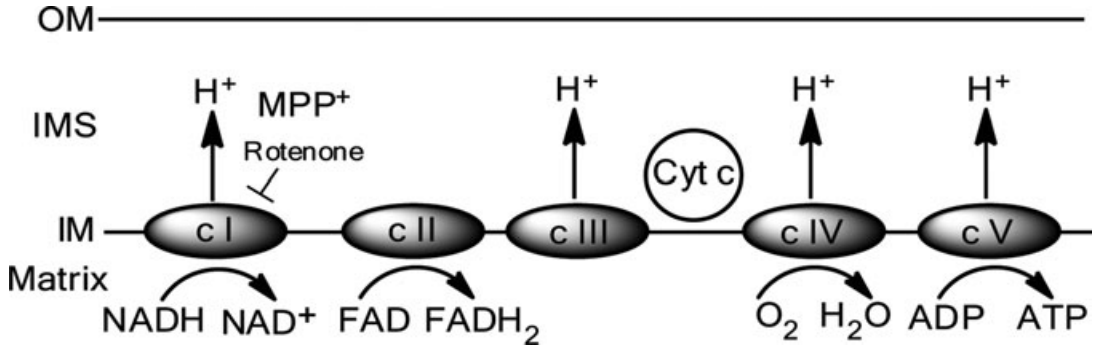
CoQ **CoQH2**

Ubiquinone is a highly lipophilic structure that moves freely in the direction from the surface of the inner membrane facing the matrix ($CoQH_2$) to the surface of the inner membrane facing the intermembrane space (IMS) and back (CoQ). The reduced form of ubiquinone donates electrons to complex III of the respiratory chain, which contains cytochromes c, c1 and FeS-proteins. Cytochromes c and c1 are hemoproteins of the tertiary structure. The peculiarity of hemes is the presence of iron cations in them, which change the degree of oxidation of Fe^{2+}/Fe^{3+}. The heme of cytochromes b, c1 or c is able to accept only 1 ē, therefore, two cytochromes of each type are needed for the transfer of 2ē, which is transported by the respiratory chain from the oxidized substrate (reduced form of the coenzyme). Cytochromes b, c1 and c are not capable of accepting H^+ ions into their structure. The next electron acceptor is cytochrome c (the most mobile cytochrome in the inner membrane; it is not included in any complex), it is also a hemoprotein of the tertiary structure.

The reduced form of cytochrome c (Fe^{2+}) further donates electrons to cytochrome c oxidase. Cytochrome c oxidase is a transmembrane protein, a quaternary hemoprotein consisting of six subunits: 4a and $2a_3$, the latter containing only $Cu^{2+}/Cu+$. This protein is also called complex IV of the respiratory chain. Cytochrome c-oxidase, receiving 4ē from cytochromes C (Fe^{2+}), acquires a high affinity for molecular oxygen. Each pair of

electrons transfers to 1 atom of molecular oxygen with the formation of an oxide anion, which combines with four protons to form endogenous water: $4H^{+}+4\,\bar{e}+O_2 \rightarrow 2H_2O$

Ubiquinone is able to take electrons from the reduced form of $FADN_2$·SuccinateDHase, which together with FeS-proteins and cytochrome b_{560} forms complex II of the respiratory chain in the inner membrane of mitochondria. Thus, ubiquinone is a collector that collects electrons from oxidizing substrates and passes them on to cytochromes (pic. 29):



Pic. 29. Complexes of the respiratory chain of the inner membrane of mitochondria. The diagram shows two respiratory chains: one is long, starting with the function of NADH-DHase; the second is short, starting with the function of SDHase.

Side effects of stimulation of the function of the respiratory chain

It should be noted that with an incomplete transfer of electrons to an oxygen atom, or with a very intensive transport of electrons to molecular oxygen, the formation of reactive radicals $O2^{-}$, $O2^{\cdot}$, $^{\cdot}OH$ and toxic hydroperoxide H_2O_2 occurs, which can interact with the radicals of unsaturated VLC in the phospholipids of membranes , violating their integrity. Therefore, there are a number of protective antioxidant systems in the cell: enzymatic (catalase, peroxidase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic (antioxidant substances: vitamins E, A, K, C, carotene, melatonin, selenium derivatives, etc.)

OXIDATIVE PHOSPHORYLATION

For each pair of electron carriers (oxidizing agent-reducing agent) in the respiratory chain, an indicator called the redox potential of the oxidizing agent-reducing agent pair can be measured using the polarography method. In the "respiratory chain" system, a gradual drop in the redox potential is observed, which confirms the release of energy during the transfer of electrons to molecular oxygen. Part of the energy is released in the form of thermal energy. Another part of it is transformed into the electrochemical

potential of the inner membrane of mitochondria ($\Delta\mu H^+$). How does this happen?

In 1961, Mitchell proposed the chemiosmotic theory as a hypothesis, which has not yet been disproved, but only receives more and more evidence of its validity. The chemiosmotic theory explains the possibility of storing energy released during the functioning of the respiratory chain in the form of energy of macroergic bonds of ATP.

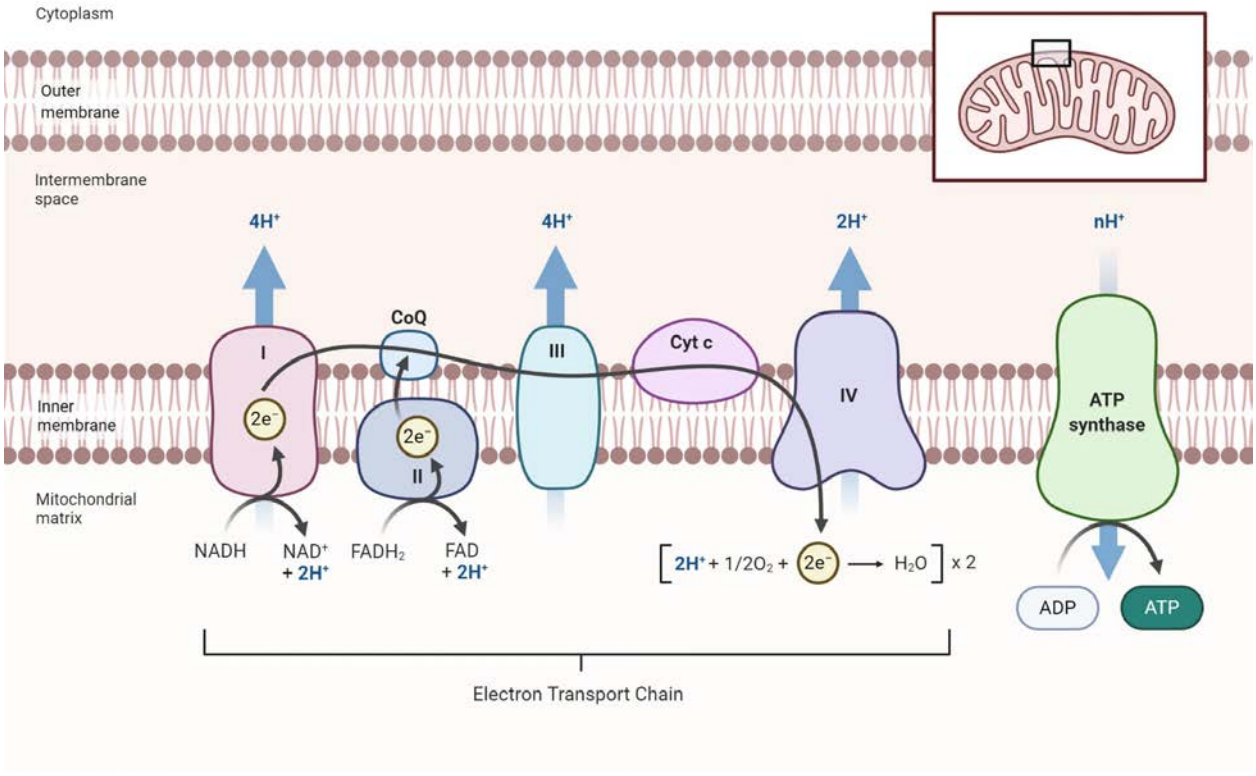
The main provisions of the chemiosmotic theory (P. Mitchell, 1961):

1. The inner membrane of mitochondria is impermeable to ions in general (especially to protons) in the direction from the IMS to the matrix;

2. When electrons are transferred from NADH-DHase to molecular oxygen, protons are pulled from the matrix to the outer surface of the inner membrane.

3. Participants of the respiratory chain, transferring ē, form three oxidation-reduction loops in motion. The first loop is related to the function of reconstituted NADH-DHase. This is an integral protein, which, when transferring electrons to FeS-proteins, pulls 2 H^+ to the outer surface of the inner membrane. The 2nd and 3rd redox loops are related to the function of ubiquinone. Ubiquinone, receiving electrons from FeS-proteins of the I complex of the respiratory chain, captures 2 H^+ in the matrix. The second ubiquinone molecule receives electrons from cytochrome b, captures $2H^+$ from the matrix. Moving again to the outer surface of the inner membrane, passing ē to cytochrome c1, the reduced ubiquinone again releases 2 H^+ into the IMS. Thus, according to P. Mitchell's hypothesis, when the ē pair is transferred from $NADH \cdot H^+$ to one atom of molecular oxygen, 6 H^+ is drawn to the outer surface of the inner membrane.

Modern experimental data confirm that the extraction of H+ from the matrix in the IMS occurs due to the I, III and IV complexes of the respiratory chain, which work as proton pumps (pic. 30).



Pic 30. The mechanism of oxidative phosphorylation in combination with the function of complexes I, III, IV of the respiratory chain. (https://microbenotes.com/wp-content/uploads/2020/06/Electron-Transport-Chain-ETC.jpeg)

4. The impermeability of the inner membrane to H+ determines the possibility of an electrochemical potential on the inner membrane due to the created proton concentration gradient (ΔH+) and charge gradient (ΔΨ; the membrane is charged):

$$\Delta\mu H^+ = \Delta H^+ + \Delta\Psi$$

5. The creation of an electrochemical potential on the inner membrane of mitochondria is the main factor in stimulating the activity of H^+-ATP synthetase, ***the main enzyme of oxidative phosphorylation.***

Oxidative phosphorylation is the synthesis of ATP from ADP and inorganic phosphate under the action of H^+-ATP synthetase due to the energy transformed by this enzyme from the electrochemical potential of the inner membrane of mitochondria.

Structure and function of H^+-ATP synthetase

H^+-ATP synthetase is a transmembrane protein with a quaternary structure, in

which factor F_0 (proton channel) and factor F_1 (contains active centers for attachment of substrates) are isolated (pic. 30). Factors F_0 and F_1, combining in space, resemble a mushroom (F_0 is a leg, F_1 is a cap).

According to modern data, ATP synthesis is not the main energy-consuming stage, rather, such a stage is the detachment and release of the ATP molecule from the F1 factor, in which conformational changes occur. These conformational changes occur due to the discharge of the membrane during the passage of H^+ through the proton channel of the F_0 factor in the direction from the IMS to the matrix. Calculations of biophysicists on the rate of ATP synthesis, thanks to the action of H^+-ATP synthetase, give the following average values: when one pair of electrons from NADH passes to an atom of molecular oxygen, the maximum formation of 3 ATP is possible.

Conjugation points of oxidation with phosphorylation. Phosphorylation coefficient

Experimental biophysics provides data on three possible points of conjugation of oxidation in the respiratory chain with oxidative phosphorylation, in which a drop in the red-ox potential allows the creation of an electrochemical potential $\Delta\mu H$ is sufficient for the synthesis of ATP. The redox potential drop must be at least 0.22 in order for one ATP molecule to be synthesized.

There are three such points in the respiratory chain: 1st is at the place of electron transfer from complex I to KoQ; 2nd is at the place of electron transfer from cytochrome to c1; 3rd is during the transfer of electrons from cytochrome c-oxidase to O_2. This does not contradict the data on the functions of the corresponding complexes of the respiratory chain as proton pumps. These points are called points of conjugation of oxidation with phosphorylation.

To evaluate the efficiency of oxidative phosphorylation, the P/O indicator that is the phosphorylation coefficient was introduced. It can take the following values: 3, 2, 1, 0 based on one molecule of the oxidizing organic substrate.

P/O is the number of molecules of inorganic phosphate that joined with ADP during the synthesis of ATP at the moment of transfer of one pair of electrons to one atom of molecular oxygen.

The P/O value correlates with the number of conjugation points. For example: when one molecule of isocitrate is oxidized, P/O = 3, i.e. the respiratory chain is long and has three points of conjugation.

When one molecule of succinic acid is oxidized, the respiratory chain is

shorter, the first point of conjugation is missing, therefore P/O = 2. However, P/O can take values less than 2 and 0 under the influence of a number of factors or on the function of participants in the respiratory chain (inhibitors of tissue respiration), or on the process of oxidative phosphorylation.

REGULATION OF TISSUE RESPIRATION AND OXIDATIVE PHOSPHORYLATION

Inhibitors of tissue respiration

Each of the complexes of the respiratory chain can be inhibited or completely blocked.

Inhibitors of complex I of the respiratory chain:

1)barbiturates;

2) rotenone; P/O = 0 for NAD-dependent oxidation reactions

3) in the matrix: $NADH \cdot H^+/NADH^+ > 1$

4) piericidin A;

Inhibitors of complex II of the respiratory chain:

1) malonic acid;
2) carboxin; P/O<2 for FAD-dependent oxidation reactions
3) thenoyltrifluoroacetone;

Carboxin and thenoyltrifluoroacetone block the transfer of electrons from $FADN_2$*SDHase to KoQ.

Inhibitors of complex III of the respiratory chain:

1)dimercaprol;

2) antimycin A; P/O<3; <2 for oxidation reactions

Inhibitors of complex IV (cytochrome c-oxidase ***) of the respiratory chain:***

1) Hydrogen sulfide H2S;

2) CO carbon monoxide;

3) Cyanides (CH^-)

4) Organic azides

If the cytochrome c-oxidase is blocked, tissue respiration is blocked completely, the living system dies. Calculating P/O makes no sense.

Inhibitors of oxidative phosphorylation

H+-ATP synthetase inhibitor: Oligomycin blocks the F_0 proton channel, thus

stopping both oxidation and phosphorylation.

ATP/ADP translocase inhibitor:Atractyloside blocks the transport of ADP from the cytoplasm to the matrix and in the reverse direction the transport of ATP from the matrix to the cytoplasm, thus disrupting oxidative phosphorylation.

Disconnectors of tissue respiration and oxidative phosphorylation

A substance capable of reducing the electrochemical potential of the inner membrane of mitochondria is called a disconnector. For the most part, these substances are very lipophilic and are capable of attaching either protons (protonophores) or Na+, K+ cations (ionophores).

Protonophores: The substance lowers the gradient ΔH^+ on the inner membrane of mitochondria, taking H^+ into its structure and, being lipophilic, diffuses into the matrix$\Delta\mu H$ also decreases. Protonophores: 2,4-dinitrophenol, dinitrocresol, pentachlorophenol, carbonyl cyanide-m-chlorophenylhydrazone, thyroxine in concentrations above physiological normal values.

Ionophores: valinomycin transports K^+ through the inner membrane, decreases $\Delta\mu N$. Nigericin is an ionophore for K^+ ions, but in exchange for H^+, it reduces the gradient ΔH^+ on the membrane. The appearance of such substances in a sufficient quantity in an aerobic type cell leads to the removal of the main factor of stimulation of H^+-ATP synthetase -$\Delta\mu H$, and this enzyme stops functioning. In this case: P/O=0 for all aerobic oxidation reactions! However, the respiratory chain continues to more intensively transport electrons to molecular oxygen. Thus, all the energy of tissue respiration is transformed into thermal energy, which can lead to overheating of the living system.

In the brown adipose tissue of animals (humans also have it, but in a smaller mass compared to white adipose tissue) there are special uncoupling proteins (thermogenins) that are able to stimulate the oxidation of substrates with the production of more heat energy, preventing the stimulation of oxidative phosphorylation . Stimulation of the synthesis of such proteins in the brown adipose tissue of animals and humans occurs when the ambient temperature decreases.

Factors affecting speed tissue respiration and oxidative phosphorylation in the cell

1. ***Respiratory control is the ratio of ATP/ADP concentrations in a living system***

If ATP/ADP>1: aerobic oxidation, tissue respiration, and oxidative phosphorylation were actively occurring in the cell until the moment of measurement of respiratory control; at the moment, the speed of these processes is decreasing; however, conditions are created for stimulation of synthetic processes in the cell;

If ATP/ADP<1: by the time respiratory control is measured, aerobic oxidation, tissue respiration and oxidative phosphorylation have been reduced in speed, at this point these processes should be stimulated. The synthesis of substances in the cell is currently impossible! The function of ATP/ADP translocase decreases - the rate of ADP delivery;

If ATP/ADP =0 – the system is dead!

2. ***Rate of supply of substrates for oxidative phosphorylation: ADP and inorganic phosphate. Timely removal of ATP from the mitochondrial matrix into the cell cytoplasm.***

These factors are controlled by the function of the transport system called ATP/ADP – translocase. This system penetrates the outer and inner membranes of mitochondria, is anti-port, transport of ATP and ADP is carried out along a concentration gradient.

3. ***Speed of O_2 supply to the cell***.

This factor is determined by the speed of blood flow in the blood vessels that are in contact with the tissue to which the cell in question belongs, as well as the content of erythrocytes and hemoglobin in the blood. Transport of oxygen into the cell occurs by the mechanism of pinocytosis. The state of local or general hypoxia, the state of anemia, which reduces the supply of oxygen to tissues, is accompanied by a decrease in the rate of tissue respiration and oxidative phosphorylation.

Medicines as components of the respiratory chain

In many disorders of biological oxidation, the therapeutic use of components of the respiratory chain has a positive effect. In most cases, this is metabolic (substrate) therapy. The introduction of glucose, fructose, amino acids, lactic, citric, succinic, and malic acids into the body as energy sources has a positive effect on the state of the body in conditions of increased energy consumption (emotional stress, physical, mental stress, etc.).

Also important is the parallel use of vitamins, in particular, nicotinamide, riboflavin, as well as their cofactors, such as flavinate pharmaceuticals (FAD) and FMN, as agents that are part of enzymes that regulate redox processes in the body.

The positive result of oxygen therapy in hypo- and anoxic conditions has been known for a long time. Recently, attempts at the therapeutic use of some enzymes of the respiratory chain have been of great interest.

Thus, cytochrome c - an enzyme preparation obtained by extraction from cattle heart tissue, increases the use of oxygen in tissues. Cytochrome c is used to improve tissue respiration in newborn asphyxia, asthmatic conditions, chronic pneumonia, heart failure, and anemia.

3.TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	With thyrotoxicosis, the production of thyroid hormones T3 and T4 increases, weight loss, tachycardia, and mental agitation develop. How exactly do thyroid hormones affect the energy exchange in cell of mitochondria? A. Block substrate phosphorylation. B. Separate oxidation and oxidative phosphorylation. C. Activate oxidative phosphorylation. D. Activate substrate phosphorylation. E. Block the respiratory chain.	
2.	A 38-year-old woman complains of increased sweating, palpitations, and a rise in body temperature in the evening. Basic exchange is increased by 60%. The doctor diagnosed thyrotoxicosis. What properties of thyroxine lead to increased heat production? A. Reduces deamination of amino acids B. Increases the coupling of oxidation and phosphorylation C. Promotes the accumulation of acetyl-CoA D. Separates oxidation and phosphorylation E. Reduces β-oxidation of fatty acidsот	
3.	Cyanides are extremely powerful cellular poisons that can cause death if ingested.	

	Blocking of which enzyme of tissue respiration is the basis of this action? A. Cytochrome oxidases B. Glucose-6-phosphate dehydrogenases C. Catalases D. Ferrochelatases E. Hemoglobin reductases	
4.	The medical examiner, upon examination of the corpse of a 20-year-old girl, established that the death occurred as a result of cyanide poisoning. Violation of which process was the most likely cause of the girl's death? A. Tissue respiration B. Hemoglobin synthesis C. Transport of oxygen by hemoglobin D. Urea synthesis E. Transport of hydrogen protons by the malate-aspartate mechanism.	
5.	How does thyroxine affect the processes of tissue respiration and oxidative phosphorylation in patients with thyrotoxicosis? A. Reduces the activity of FAD dehydrogenase B. Blocks electron transport in the cytochrome chain C. Causes ATP hydrolysis. D. Separates the processes of tissue respiration and oxidative phosphorylation E. Reduces the activity of NADH	

	dehydrogenase	
6.	The medical examiner, upon examination of the corpse of a 20-year-old girl, established that the death occurred as a result of cyanide poisoning. Which enzyme is most inhibited by cyanides? A. Malate dehydrogenase B. Cytochrome oxidase C. Hemesynthetase D. Aspartate aminotransferase E. Carbamoyl phosphate synthetase	
7.	The process of ATP synthesis, which is coupled with oxidation reactions with the participation of the mitochondrial respiratory enzyme system, is called: A. Oxidative phosphorylation B. Substrate phosphorylation C. Free oxidation D. Photosynthetic phosphorylation E. Peroxidation	
8.	Potassium cyanide, which entered the body of patient B., caused almost instant death against the background of symptoms of hypoxia. The most likely cause of the toxic effect of cyanide was inhibition of activity: A. Cytochrome oxidases B. NADH dehydrogenases C. ATP synthetases D. NADPH dehydrogenases	

	E. ATPases	
9.	In the process of metabolism in the human body, active forms of oxygen arise, including the radical superoxidanion •O2-. This anion is destroyed by an enzyme: A. Superoxide dismutase B. Catalases C. Peroxidases D. Glutathione peroxidase E. Glutathione reductases	
10.	Potassium cyanide is a poison, death occurs instantly. Name the mitochondrial enzymes on which this poison acts: A. Cytochrome P-450 B. Flavin enzymes C. Cytochrome B5 D. NAD^+-dependent dehydrogenases E. Cytochrome oxidase [cytochrome aa_3]	
11.	A patient with rotenone insecticide poisoning was brought to the hospital. Which part of the mitochondrial electron transport chain is blocked by this substance? A. ATP synthetase B. Coenzyme Q – cytochrome C reductase C. Succinate coenzyme Q-reductase D. Cytochrome C-oxidase E. NADH - coenzyme Q-reductase	
12.	In patients with thyrotoxicosis, hyperthermia,	

	bulimia, and a decrease in body weight are observed, which is associated with a violation... A. Fat synthesis B. Beta - oxidation of fatty acids C. Conjugation of oxidation and phosphorylation D. Citric acid cycle E. ATP breakdown	
13.	A patient was admitted to the intensive care unit in serious condition, unconscious. An overdose of barbiturates, which caused the phenomenon of tissue hypoxia, was diagnosed. At what level did the blocking of electronic transport take place? A. Ubiquinone B. Cytochrome b - cytochrome c C. ATP synthase D. NADH - coenzyme Q-reductase E. Cytochrome oxidase	
14.	The cell was treated with a substance that blocks the phosphorylation of nucleotides in mitochondria. What process of life activity of the cell will be disturbed in the first place? A. Integration of functional protein molecules. B. Oxidative phosphorylation. C. Glycolysis. D. Synthesis of mitochondrial proteins. E. Aerobic oxidation of glucose	

15.	In the presence of 2,4-dinitrophenol, the oxidation of substrates can continue, but the synthesis of ATP molecules is impossible. What is the mechanism of its action? A. Activation of the ATP-ase enzyme. B. Inhibition of cytochrome oxidase enzyme. C. Transfer of substrates outside the mitochondrion. D. Dissociation of oxidation and phosphorylation in mitochondria. E. Stimulation of hydrolysis of the formed ATP.	
16.	In pathological processes accompanied by hypoxia, there is an incomplete recovery of the oxygen molecule in the respiratory chain and the accumulation of hydrogen peroxide. Name the enzyme that breaks down this compound. A. Aconitase. B. Catalase. C. Ketoglutarate dehydrogenase. D. Succinate dehydrogenase. E. Cytochrome oxidase	
17.	Researches of recent decades have established that special enzymes that are called caspases are the direct "executors" of apoptosis in the cell. Cytochrome c is involved in the formation of one of them. State its function in a normal cell.	

	A. A component of the H^+ ATP-ase system. B. A component of the pyruvate dehydrogenase system. C. Enzyme of the respiratory chain of electron transfer. D. Enzyme β-oxidation of fatty acids. E. TCA enzyme.	
18.	The process of ATP synthesis, which takes place in conjunction with oxidation reactions with the participation of the respiratory enzyme system, takes place in the mitochondria. What is its name? A. Free oxidation B. Oxidative phosphorylation. C. Peroxidation. D. Substrate phosphorylation. E. Photosynthetic phosphorylation.	
19.	Specify the fastest mechanism of ATP formation, which is necessary for the urgent activation of the process of muscle contraction. A. Generation of ATP from creatine phosphate. B. Aerobic glycolysis. C. Glycolysis. D. Glycogenolysis in muscles. E. Oxidation of triglycerides	

20.	Hydrocyanic acid and cyanides are among the strongest poisons. Depending on the dose, death occurs after a few seconds or minutes. Suppression of the activity of which enzyme is the cause of death? A. ATP synthetases. B. Acetylcholinesterase. C. Catalases. D. Methemoglobin reductases. E. Cytochrome oxidases.	

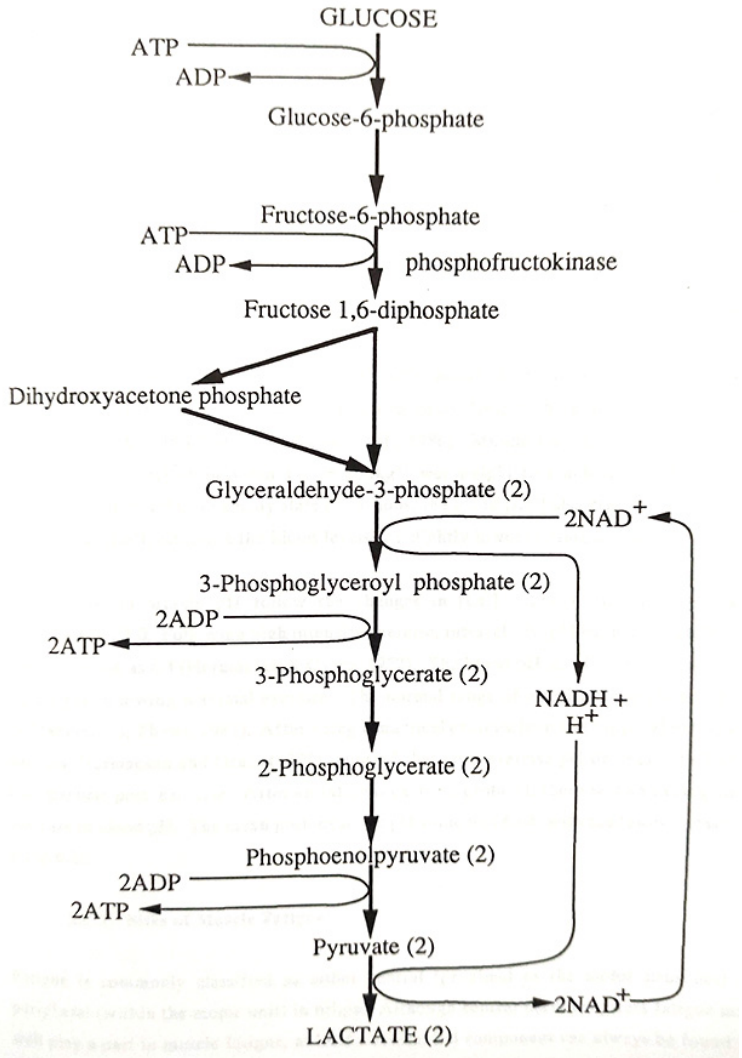
4. Literature. Look pic. 229.

1. TOPIC: ANEAROBIAN OXIDATION OF GLUCOSE - GLYCOLYSIS. GLUCOSE BIOSYNTHESIS - GLUCONEOGENESIS

2. INFORMATION MATERIAL.

Anaerobic glycolysis

In some situations, the supply of acid to textiles may not meet their needs. For example, at the early stages of intensive muscle work under stress, the heart rate may not reach the required frequency and will require muscule from sour for the aerobic breakdown of glucosea. In such cases a process is activated that flowing without oxygen and ends with the formation of lactate of pyruvic acid. This process is called anaerobic decomposition or anaerobic glycolysis (pic.32) Anaerobic breakdown of glucose is energetically ineffective, but this process can become the only source of energy muscle cel in this situation..



Pic.32. Anaerobic glycolysis (Enzymes are indicated by numbers : 1 – hexokinase ; 2 – phosphoglucoisomerase; 3 – phosphofructokinase ; 4 – aldolase.;

5 – Triose phosphate isomerase; 6 – glyceraldehyde-phosphate dehydrogenase ; 7 – phosphoglycerate kinase; 8 – phosphoglyceromutase; 9 – enolase ; 10 – pyruvate kinase; 11 – lactate dehydrogenase

Glycolytic oxidation reduction

Continuous regeneration of NAD+ is necessary for continuous anaerobic breakdown of glucose . It happens in the glycolytic oxidoreduction reaction in which NADH formed in the glyceraldehyde phosphate dehydrogenase reaction is oxidized in the lactate dehydrogenase reaction (during the reduction of PVC to lactate).

Regulation of glycolysis is carried out at the level of "key" enzymes:

- ✓ hexokinase
- ✓ phosphofructokinase
- ✓ pyruvate kinase

These enzymes catalyze irreversible reactions.

Anaerobic glycolysis actively occurs in muscles during intensive work and in erythrocytes.

Biological role of glycolysis

Energy value: 2 ATP is formed due to substrate phosphorylation as well as NADH, which enters to the chain of tissue respiration during aerobic breakdown of glucose, and during anaerobic - restores PVC to lactate.

Anabolic value : intermediate products can be used for the synthesis of other substances.

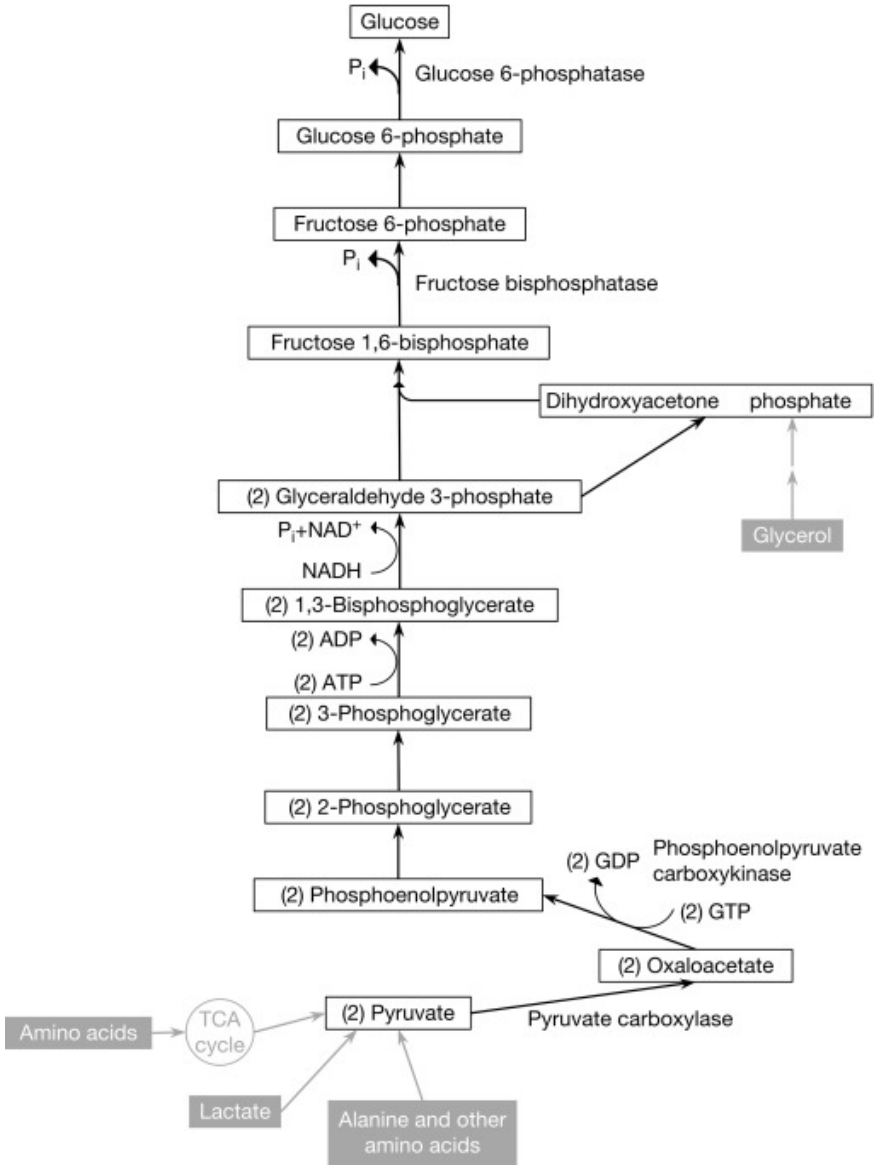
Gluconeogenesis

Gluconeogenesis – synthesis of glucose from non-carbohydrate products. Such products or metabolites are lactic and pyruvic acid, so-called glycogenic amino acids, glycerol and a number of other compounds.. In other words, the predecessors of glucose in gluconeogenesis can be pyruvate or any compound that is transformed in the process of catabolism into pyruvate or one of the intermediate products of the tricarboxylic acid cycle.

In vertebrates, gluconeogenesis occurs most intensively in the liver cells and kidneys (in the cortical substance).

Most stages of gluconeogenesis are reversals of the glycolysis reaction. Only 3 reactions of glycolysis (hexokinase, phosphofructokinase and pyruvate kinase) are irreversible, therefore other enzymes are used in the process of

gluconeogenesis at 3 stages (pic.33).



pic.33. Glycolysis and gluconeogenesis. "Fat" arrows specified the "bypass" pathways of gluconeogenesis during the biosynthesis of glucose from pyruvate and lactate are indicated; numbers in circles mean the the appropriate stage of glycolysis.(https://ars.els-cdn.com/content/image/3-s2.0-B9780123849472001148-f00114-03-9780123849472.jpg)

The Pasteur effect

The Pasteur effect is full proof of the ability of metabolism to switch from one direction to another without any additional intervention.

The Pasteur effect is called the decrease in glucose consumption and the discontinuation of the production of lactic acid by the cell in the presence of oxygen. The biochemical mechanism of the effect consists in the competition for pyruvate between pyruvate dehydrogenase, which converts pyruvate into acetyl-S-CoA, and lactate dehydrogenase, which converts pyruvate into lactate.

Pyruvate dehydrogenase has a much higher affinity and under normal aerobic conditions it oxidizes most of the pyruvic acid (PVAC). As soon as the supply of oxygen decreases (shortage of blood circulation, thrombosis, etc...) the following occurs.:

1 – internal mitochondrial respiration processes do not occur and NADH in the respiratory chain is not oxidized;

2 - instantly accumulates in the mitochondria of NADH, which in turn inhibits the cycle of tricarboxylic acids;

3 - acetyl-S-CoA is not included in CTC and inhibits PVC dehydrogenase.

Due to the formed conditions, PVC has no choice but to turn into lactic acid.

In the presence of oxygen the inhibition of pyruvate dehydrogenase stops and it, having a high affinity for PVC, wins the competition..

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and bring proof it:

№	Test	explanation
1.	A patient with signs of acute alcohol poisoning was brought to the clinic. What changes in carbohydrate metabolism are characteristic of this condition? A. The rate of gluconeogenesis decreases in the liver B. Muscle increases aerobic breakdown of glucose C. Hepatic gluconeogenesis increases D. Anaerobic glucose breakdown prevails in muscles E. Hepatic glycogen breakdown is enhanced	
2.	Some time after intense physical training, gluconeogenesis is activated in the athlete, the main substrate of which is: A. leucine B. oxaloacetate C. lysine D. glycogen E. acetyl-CoA	
3.	As a result of prolonged fasting in the human body, reserves of carbohydrates quickly disappear. Which of the metabolic processes supports the glucose content in the blood? A. gluconeogenesis B. Pentose phosphate cycle C. aerobic glycolysis D. Anaerobic glycolysis E. glycogenolysis	

4.	Some time after intense physical training, gluconeogenesis is activated in an athlete. What is its main substrate? A. aspartic acid B. serine C. α-ketoglutarate D. glutamic acid. E. lactate	
5.	In a patient who is undergoing a course of medical fasting normal blood glucose levels are maintained mainly by gluconeogenesi . From which amino acid is the most actively synthesized glucose in the human liver? A. lysine B. alanine C. glutamic acid D. leucine E. valine	
6.	A large number of metabolites of glucose oxidation are dissolved in the cytoplasm of myocytes. Name one of them that is directly converted into lactate: A. oxaloacetate B. pyruvate C. Fructose-6-phosphate D. glucose-6-phosphate E. glycerophosphate	
7.	A 7-year-old girl has signs of anemia. A deficiency of pyruvate kinase in erythrocytes was installed in the laboratory. Disruption of which process plays the main role in the development of anemia in a girl?	

	A. anaerobic glycolysis B. oxidative phosphorylation C. Deamination of amino acids D. Decomposition of peroxides E. tissue respiration	
8.	People after prolonged physical exertion have intense muscle pain. What muscle changes are the most likely cause of this? A. lactic acid accumulation B. Accumulation of creatinine C. Increasing the breakdown of proteins D. Increased excitability E. Increase in ADP content	
9.	During short-distance running, an untrained person develops muscle hypoxia. To the accumulation of which metabolite in the muscles does this lead? A. acetyl-CoA B. oxaloacetate C. ketone bodies D. lactate E. glucose-6-phosphate	
10.	The experiment showed that with Jensen's sarcoma, the consumption of glucose from the artery leading to the tumor increases significantly, and there is also an increase in the content of lactic acid in the efferent vein. What does this phenomenon indicate? A. On strengthening anaerobic glycolysis B. On strengthening oxidative processes C. On strengthening protein oxidation D. On reduction of oxidative processes E. On the reduction of anaerobic glycolysis	

11.	When there is a lack of blood circulation during intense muscle work, lactic acid accumulates in the muscle as a result of anaerobic glycolysis. What is her future fate? A. It is used in tissues for the synthesis of fatty acids B. Involved in gluconeogenesis in the liver C. It is used in the muscle for the synthesis of amino acids D. It is removed through the kidneys with urine E. It is used in tissues for the synthesis of ketone bodies	
12.	After prolonged physical exertion during physical education classes, the students developed muscular strength. The cause of its occurrence was the accumulation of lactic acid in the skeletal muscles. It was formed after activation in the body of students: A. Glycolysis B. Gluconeogenesis C. Lipolysis D. Glycogenesis E. Pentose phosphate cycle	
13.	During starvation, muscle proteins break down into free amino acids. In what process are these compounds most likely to be involved under such conditions? A. Decarboxylation B. Gluconeogenesis in the liver C. Glycogenolysis D. Gluconeogenesis in muscles E. Synthesis of higher fatty acids	
14.	In some anaerobic bacteria, pyruvate, formed as a result of glycolysis, is converted into ethyl	

	alcohol (alcoholic fermentation). What is the biological meaning of this process? A. Replenishment of the NAD + fund B. Formation of lactate C. Formation of ADP D. Providing the cell with NADPH E. Formation of ATP	
15.	Human erythrocytes do not contain mitochondria. What is the main way of ATP formation in these cells? A. Aerobic glycolysis B. Oxidative phosphorylation C. Adenylate kinase reaction D. Creatine kinase reaction E. Anaerobic glycolysis	

4. Literature. Look pic. 229.

1. TOPIC: AEROBIC OXIDATION OF CARBOHYDRATES. PENTOSOPHOS-PHATE CYCLE OF GLUCOSE OXIDATION. FRUCTOSE AND GALACTOSE METABOLISM

2. INFORMATION MATERIAL.

AEROBIC OXIDATION OF CARBOHYDRATES

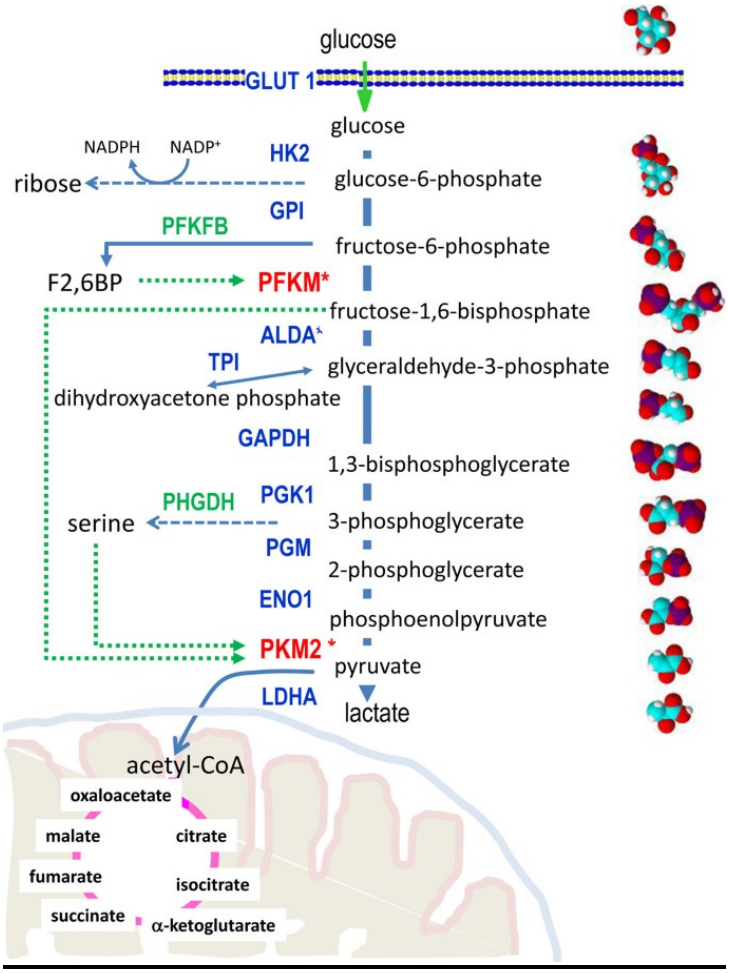
There are three stages in the process of aerobic disintegration of glucose :

I. Breakdown of glucose to pyruvate (aerobic glycolysi);

II. Oxidative pyruvate decarboxylation

III. Krebs cycle..

I stage. Aerobic glycolysis- the process of glucose oxidation to pyruvic acid, which occurs in the presence of oxygen. . All enzymes that catalyze the reactions of this process are localized in the cytoplasm of the cell (pic. 34).



Pic. 34. Aerobic glycolysis (https://media.springernature.com/lw685/springer-static/image/art%3A10.1186%2F1741-7007-11-3/MediaObjects/12915_2013_Article_622_Fig1_HTML.jpg)

Enzymes: 1. hexokinase ; 2. Glucose Phosphate Isomerase;3. phosphofructokinase ; 4. Fructose Bis Phosphataldolase (aldolase); 5. Triose phosphate isomerase; 6. glyceraldehyde-3-phosphate dehydrogenase 7. Phosphoglycerate kinase; 8. Phosphoglyceromutase; 9. Enolase;10 pyruvate kinase.

II stage. Oxidative pyruvate decarboxylation occurs in the mitochondrial matrix. Transport of pyruvate into the mitochondrial matrix through the inner membrane of mitochondria is carried out with the participation of a special carrier protein by the mechanism of symport with H +.

Oxidative decarboxylation is catalyzed by the pyruvate dehydrogenase complex, which includes 3 enzymes and 5 coenzymes

enzyme		number of monomers	coenzyme	vitamin
1.pyruvate decarboxylase(pyruvate dehydrogenase)	E1	120(30 tetramers)	TDF	B1
2.dihydrolipoyl transacetylase	E2	180(60 tetramers)	KoA	lipoic acid pantothenic acid
3.dihydrolipoyl dehydrogenase	E3	12 (6 dimers)	FAD. NAD+	B2. PP

The conversion of pyruvate into acetyl-CoA involves 5 stages:

stage I. At this stage, pyruvate is connected with TDF as part of E1 and undergoes decarboxylation

pyruvate + E1-TDF → hydroxyethyl - TDF + CO2

stage II. Dihydrolipoyltransacetylase (E2) catalyzes the transfer of a hydrogen atom and an acetyl group from TDF to the oxidized form of lipolysine groups with the formation of lipoic acid acetylthioester.

stage III. In stage III, CoA interacts with the acetyl derivative of E2. The result- acetyl-CoA and a completely reduced lipoyl residue of the E2 prosthetic group are formed..

stage IV. At stage IV, dihydrolipoyl dehydrogenase (E3) catalyzes the transfer of hydrogen atoms from reduced lipoyl groups to FAD - the prosthetic group of the E3 enzyme.

stage V . In stage V, reduced FAD H2 transmits hydrogen to NAD with the

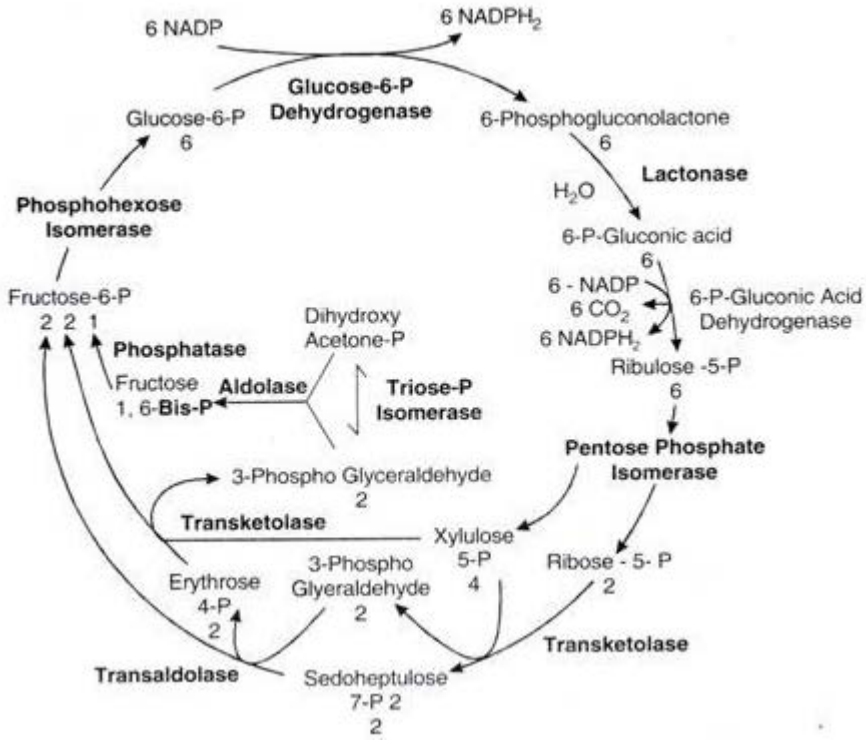
formationNAD H.

III stage. Acetyl~SKoA is oxidized in tricarboxylic acid cycle to CO2 and H2O.

PENTOSOPHOSPHATE (APOTOMIC) WAY (PFSh), is an alternative pathway of glucose oxidation (pic. 35).

All PFSh enzymes are localized in the cytosol.

It is most active in the liver, adipose tissue, mammary gland, adrenal cortex, erythrocytes.



Pic. 35. Pentose phosphate pathway of carbohydrate oxidation

Value of pentose phosphate route of glucose oxidation.

1. Synthesis of ribose-5-phosphate, which is used for the synthesis of nucleotide coenzymes (NAD, FAD, FMN), nucleic acids (DNA, RNA), mononucleotides (AMP, HMF, UMF, TMF, TMF).

2. Synthesis in the cell cytoplasm of reduced forms of NADPH, which are used for:

- synthesis of fat acids, cholesterol;
- synthesis of steroid hormones;
- synthesis of thyroid hormones;
- bile acid synthesis in the liver;

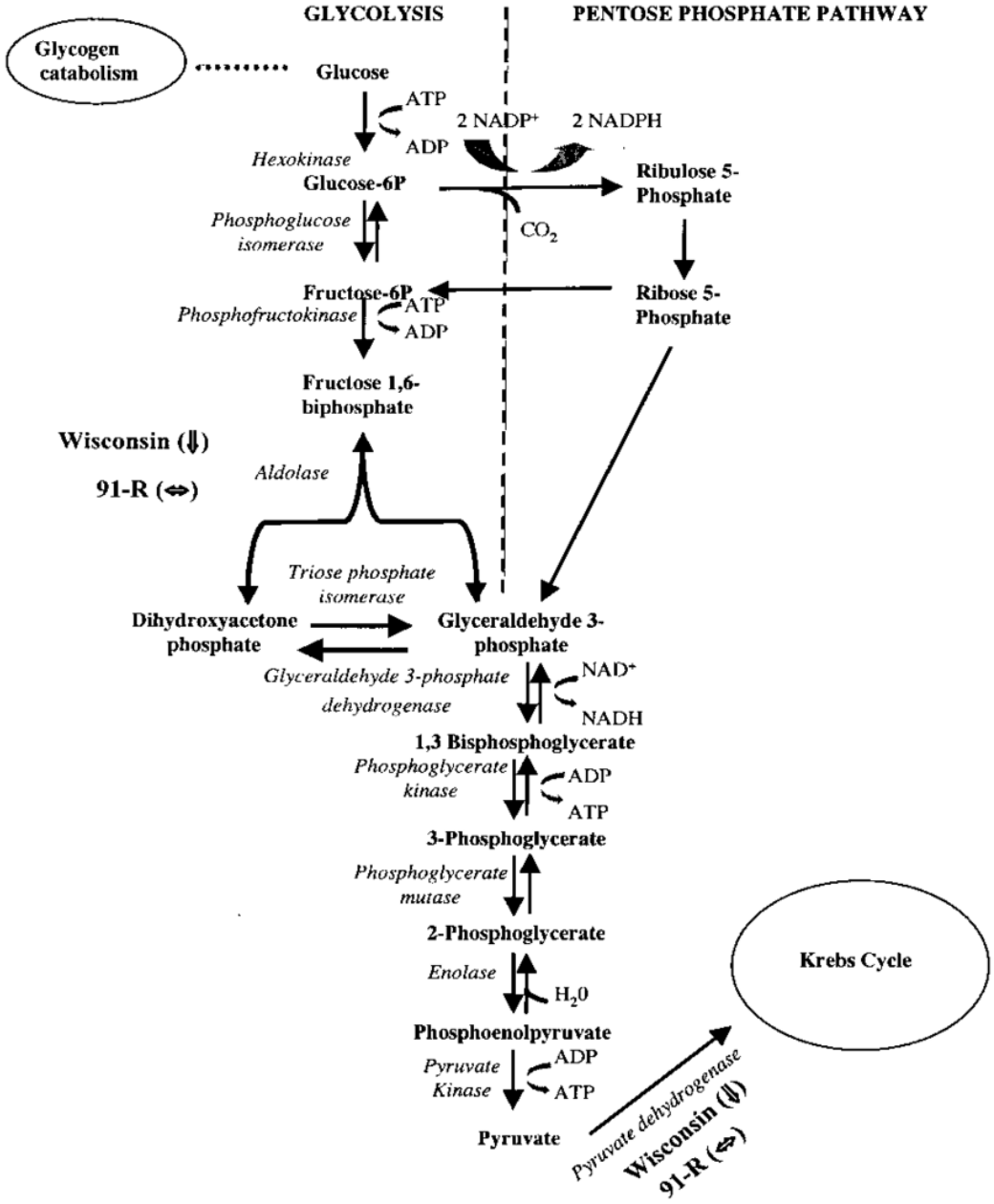
• activation of vitamin D3;

• inactivation of medicines;

• neutralization of toxins,

3. Intermediate products (fructose-6-phosphate, glyceraldehyde-3-phosphate) can be included in the path of aerobic and anaerobic oxidation and serve as a source of energy for the synthesis of ATP (pic. 36).

4. The not oxidative stage of formation of pentoses is reversible and can be used to form hexoses from pentoses (pic. 36).



Pic. 36. A modern diagram of the pentose phosphate pathway of carbohydrate oxidation, reflecting its connection with glycolysis (according to Hers).

1 - transketolase; 2 - transaldolase; 3 - aldolase; 4 - phosphofructokinase; 5 -

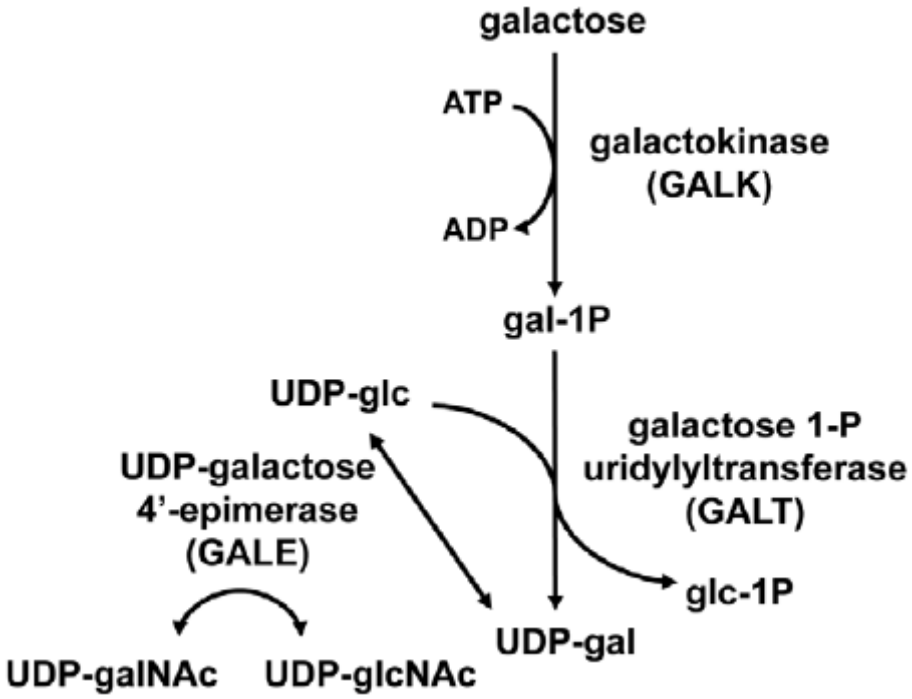
fructose-1,6-bisphosphate.; 6 - hexokinase; 7 – glucose phosphate isomerase; 8 - Triose phosphate isomerase; 9 - glucose-6-phosphate dehydrogenase ; 10 - 6-phosphogluconolactone; 11- 6-phosphogluconate dehydrogenase; 12 - isomerase; 13 - epimerase; 14 – lactate dehydrogenase.

Metabolism of fructose and galactose in the human body

involving other carbohydrates in the process of glycolysis is of great importance in the realization of the body's energy needs.

Galactose metabolism (pic. 37)

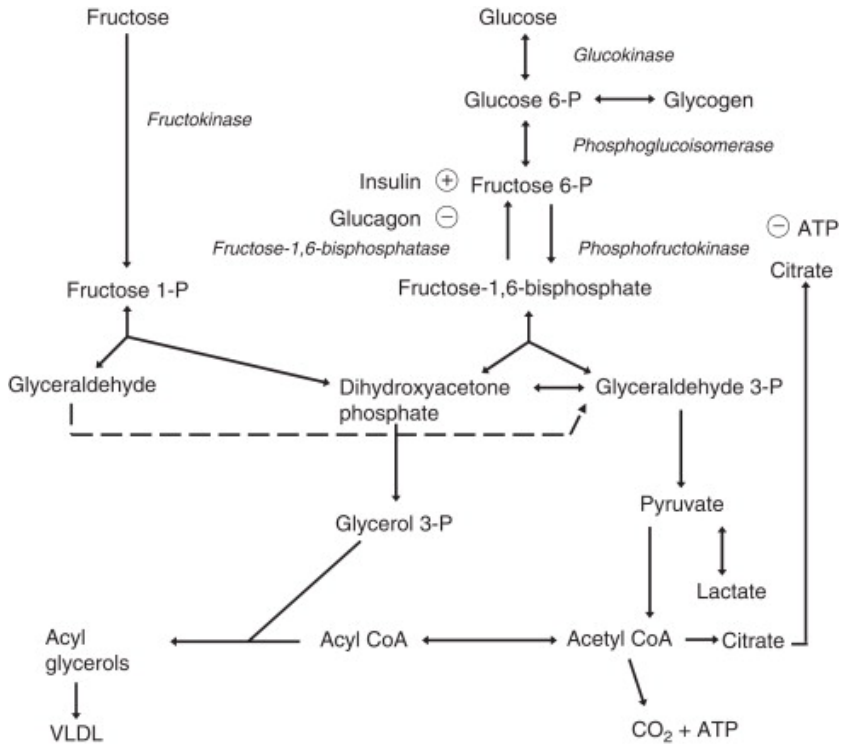
The main source of galactose is lactose in food, which is split into galactose and glucose in the digestive tract.



Pic. 37. *Galactose metabolism*

***Metabolism of fructose (pic.** 38*)

It has been established that fructose is present in free form in many fruits and is formed in the small intestine from sucrose, absorbed in tissues, can be phosphorylated into fructose-6-phosphate with the participation of the enzyme hexokinase and ATP:



Pic. 38. Metabolism of fructose.

Enzymes: 1 - hexokinase; 2 - 6-phosphofructokinase; 3 - fructose-bisphosphate aldolase; 4 - ketohexokinase; 5 - ketose-1-phosphate-aldolase; 6 - triokinase; 7 - glucose phosphate isomerase; 8 glucose-6-phosphatase ; 9-triosephosphate isomerase

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and bring proof it:

№	test	explanation
1.	During long-distance running, the skeletal muscles of a trained person use glucose to generate ATP energy for muscle reduction.. Indicate the main process of utilization of glucose under these conditions: A. Anaerobic glycolysis B. Glycogenolysis S. Aerobic glycolysis D. Gluconeogenesis E. Glycogenesis	
2.	Galactosemia was found in the child. The concentration of glucose in the blood has not changed significantly. Deficiency of which enzyme causes this disease? A. Hexokinase B. Phosphoglucomutase C. Amylo-1,6-glucosidase D. Galactokinase E. Galactose-1-phosphate-uridyltransferase	
3.	A 2-year-old boy has an increase in the size of the liver and spleen, cataracts. The concentration of sugar in the blood is increased, but the glucose tolerance test is normal. Hereditary metabolic disorder of which substance is the cause of this condition?	

	A. Sucrose B. Maltoses C. Glucose D. Fructose E. Galactose	
4.	The baby has vomiting and diarrhea, general dystrophy, hepato- and splenomegaly. Symptoms decrease when breastfeeding is stopped. What main hereditary defect will be noted in the pathogenesis? A. Hypersecretion of glands of the external secretion B. Disorders of phenylalanine metabolism S. Disorders of galactose metabolism D. Disorders of tyrosine metabolism E. Deficiency of glucose-6-phosphate dehydro collagenase	
5.	A high content of galactose was found in the child's blood, and the concentration of glucose was reduced. Cataracts, mental retardation, and fatty degeneration of the liver develop. What disease is there? A. Galactosemia B. Fructosemia C. Lactosemia D. Steroid diabetes E. Diabetes	
6.	In a 38-year-old patient, after taking aspirin and sulfonamides, increased hemolysis of erythrocytes caused by glucose-6-phosphate dehydrogenase deficiency is observed. Violation of the formation of which coenzyme is caused by this pathology? A FADN2 V. NADFN	

	S. Ubihinon D. FMNN2 E. Pyridoxal phosphate	
7.	In a 3-year-old child with a high body temperature, increased hemolysis of erythrocytes is observed after taking aspirin. Congenital insufficiency of which enzyme could cause hemolytic anemia in a child? A. Glucose-6-phosphate dehydrogenases B. Glucose-6-phosphatases C. γ-glutamyltransferases D. Glycogen phosphorylases E. Glycerol phosphate dehydrogenase	
8.	The sick child was found to have delayed mental development, enlarged liver, and impaired vision. The doctor associates these symptoms with a deficiency of galactose-1-phosphate-uridyltransferase in the body. What pathological process is taking place in the child? A. Hypoglycemia B. Galactosemia C. Hyperlactatacidemia D. Hyperglycemia E. Fructosemia	
9.	A large amount of galactose was found in the patient's blood, and the concentration of glucose was reduced. Mental retardation, opacification of the lens was noted. What disease is there? A. Fructosemia B. Diabetes C. Steroid diabetes D. Lactosemia E. Galactosemia	

10.	A worker at a chemical plant came to the hospital with signs of poisoning. An increased concentration of arsenate, which blocks lipoic acid, was found in this woman's hair. Violation of which process is the most likely cause of poisoning? A. Oxidative decarboxylation of PVC B. Microsomal oxidation C. Restoration of methemoglobin D. Reduction of organic peroxides E. Neutralization of superoxide ions	
11.	Beriberi disease (polyneuritis) occurs with a deficiency of thiamine - vitamin B1, and a violation of carbohydrate metabolism. What metabolite accumulates in the blood? A. Lactate B. Malate C. Pyruvate D. Succinate E. Citrate	
12.	An 8-month-old child has vomiting and diarrhea after taking fruit juices. Fructose loading led to hypoglycemia. Hereditary deficiency of which enzyme is the cause of the child's condition? A. Hexokinase B. Phosphofructokinase C. Fructose-1-phosphate aldolase D. Fructose-1,6-diphosphatases E. Fructokinase	

4. Literature. Look pic. 229.

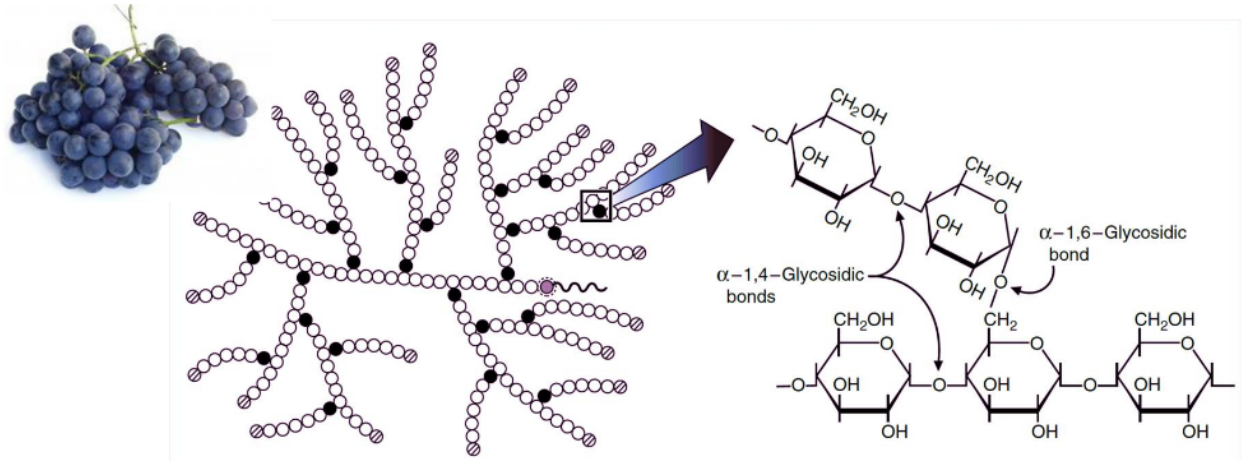
1. TOPIC: METABOLISM OF POLYSACCHARIDES AND ITS REGULATION. REGULATION AND PATHOLOGIES OF CARBOHYDRATE METABOLISM.

2 INFORMATION MATERIAL..

METABOLISM OF POLYSACCHARIDES (on the example of glycogen).

regulation of metabolism *of glycogen*

Glycogen - a branched homopolymer of glucose in which the glucose impairment residues are connected in linear regions by an α-1,4-glycosidic connection . The monomers are connected by α-1,6-glycosidic connection at the branching points. These bonds are formed with approximately every tenth glucose residue. Therefore, branch points in glycogen occur approximately every ten glucose residues. This results in a tree-like structure with a molecular weight > 107 D, which corresponds to approximately 50,000 glucose residues.



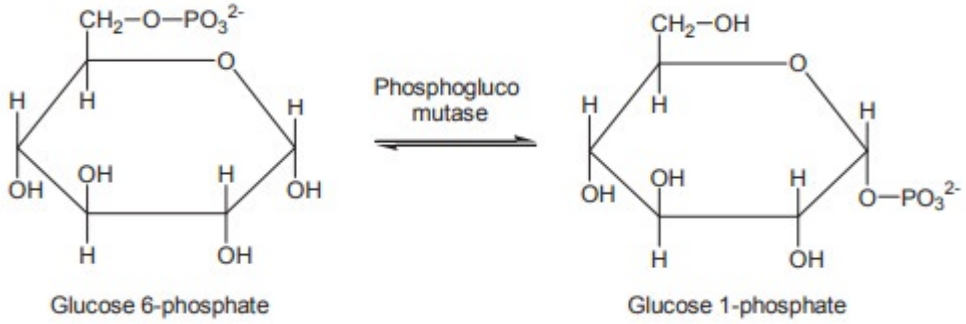
(https://bookdown.org/jcog196013/BS2003/_book/_main_files/figure-html/glycogen.PNG)

The need to convert glucose into glycogen is due to the fact that the accumulation accumulation of a large number of glucose in the cell would lead to an increase in osmotic pressure, since glucose is a highly soluble substance.. However, glycogen is contained in the cell in the form of granules and is slightly soluble

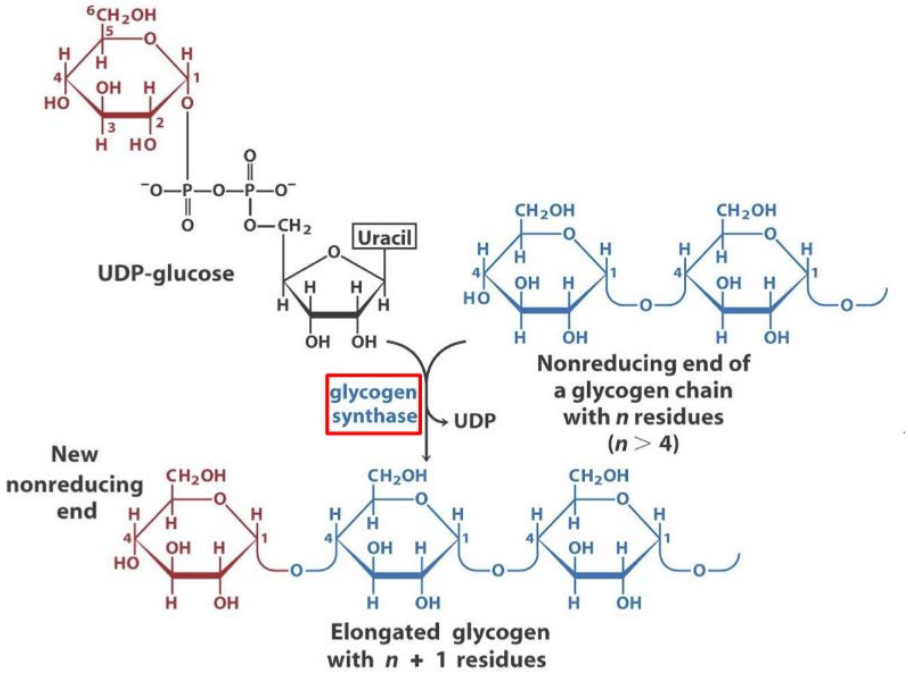
glycogen synthesis (glycogens)

First of all, glucose is subjected to phosphorylation with the participation of the enzyme hexokinase, and in the liver - glucokinase. . Then, under the

influence of the enzyme phosphoglucomutase, glucose-6-phosphate turns into glucose-1-phosphate:



Glucose-1-phosphate is already directly is involved in glycogen synthesis At the first stage of synthesis, glucose-1-phosphate interacts with UTP (uridine triphosphate), forming uridine diphosphate glucose (UDP-glucose) and pyrophosphate. This reaction is catalyzed by the enzyme glucose-1-phosphate-uridyltransferase (UDPH-pyrophosphorylase). In the second stage, the glucose residue, which is part of UDP-glucose, is transferred to the glycosidic chain of glycogen ("seed" amount). At the same time, an α-1,4-glycosidic bond is formed between the first carbon atom, the remaining glucose and the 4-hydroxyl group of the remaining glucose chain are added. This reaction is catalyzed by the enzyme glycogen synthase. Reminder, that the reaction catalyzed by glycogen synthase is possible only if the polysaccharide chain already contains more than 4 D-glucose residues.



The formed UDP is then again phosphorylated into UTP for the account of ATP, therefore the entire cycle of transformations of glucose-1-phosphate begins again.

The enzyme amyl-α-1,4-α-1,6-glycosyltransferase catalyzes the formation of α-1,6-glycosidic bonds present at the branching points of glycogen. In this reaction, the final oligosaccharide fragment, consisting of 6 or 7 glucose units., is transferred from the non-reduced end of one of the side chains, which has at least 11 residues, to the 6-hydroxyl group of the glucose residue of the same or another glycogen chain. As a result, a new side chain is formed.

The breakdown of glycogen. (glycogenolysis)

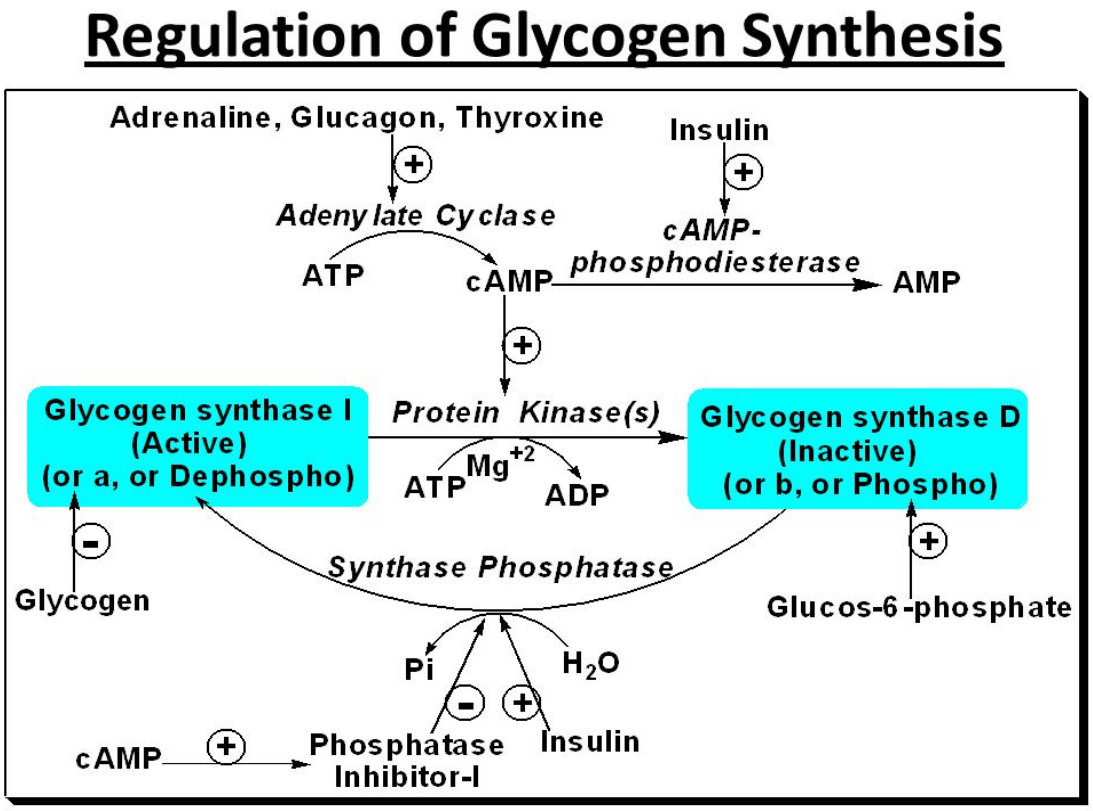
Occurs between meals.

The glucose release in the form of glucose-1-phosphate from the glycogen molecule occurs as a result of phosphorolysis catalyzed by glycogen phosphorylase (pyridoxal phosphate coenzyme). The enzyme splits off final residues one by one, shortening the glycogen chain. However, this enzyme cleaves only the α-1,4-glycosidic bond.

Branch Point Links are hydrolyzed by the enzyme amyl-α-1,6-glycosidase which splits off the glucose monomer in free form.

Hormonal regulation

With an increase in energy consumption in the body as a result of excitation of the central nervous system, there is usually an increase in the breakdown of glycogen and the formation of glucose In addition to the direct transmission of nerve impulses to effector organs and tissues, when the central nervous system is stimulated, the functions of a number of endocrine glands (adrenal medulla, thyroid gland, pituitary gland, etc.) increase, the hormones of which activate the breakdown of glycogen, primarily in the liver and muscles. The effect of catecholamines is largely mediated by cAMP, which activates tissue protein kinases (pic. 39).



Pic. 39. Hormonal regulation of phosphorolytic cleavage of glucose residues from glycogen.(https://slideplayer.com/slide/4450252/14/images/8/Regulation+of+Glycogen+Synthesis.jpg)

With the participation of protein kinases, a number of proteins are phosphorylated, including glycogen synthase and phosphorylase b - enzymes involved in carbohydrate metabolism. The phosphorylated glycogen synthase enzyme itself is weakly active or completely inactive, but is significantly activated by the positive modulator glucose-6-phosphate, which increases the Vmax of the enzyme. This form of glycogen synthase is called the D-form or dependent form because its activity depends on glucose-6-phosphate. The dephosphorylated form of glycogen synthase, the so-called I-form or independent form, is active even in the absence of glucose-6-phosphate.

Thus, adrenaline has a double effect on carbohydrate metabolism: it inhibits the synthesis of glycogen from UDP-glucose and accelerates the breakdown of glycogen, as it promotes the formation of active phosphorylase a. In general, the total effect of adrenaline consists in accelerating the conversion of glycogen into glucose.

DISORDERS OF GLYCOGEN METABOLISM

Glycogen diseases are a group of hereditary disorders, the basis of which is a decrease or lack of activity of enzymes that catalyze reactions of synthesis or breakdown of glycogen, or a violation of the regulation of these enzymes.

1. **glycogenesis** - diseases caused by a defect in enzymes involved in the breakdown of glycogen. They are manifested either by an unusual structure of glycogen, or by its excess accumulation in the liver, cardiac or skeletal muscles, kidneys, lungs, and other organs. Below are described some types of glycogenoses, which differ in the nature and localization of the enzyme defect.

It should be noted that the term "glycogenosis" was first proposed by K.F. Corey and G.T. Corey. They proposed a numbering system for these diseases. However, at present, the division of glycogenosis into 2 groups prevails: hepatic and muscular. Hepatic forms of glycogenosis lead to a violation of the use of glycogen to maintain the level of glucose in the blood.

Girke's disease (type I) is most often observed . A description of the main symptoms of this type of glycogenosis and their causes can serve as a basis for understanding the symptoms of all other types. The cause of this disease is a hereditary defect of glucose-6-phosphatase - enzyme which provides glucose output to blood after its release from the glycogen of liver cells. **Girke's disease** manifested by *hypoglycemia*, *hyper triacylglycerol mia* (increasing the content of triacylglycerols), *hyperuricemia* (increasing the content of uric acid).

hypoglycemia - the consequence of a violation of the reaction of the formation of free glucose from glucose-6-phosphate . In addition, as a result of a glucose-6-phosphatase defect, the substrate glucose-6-phosphate accumulates in the liver cells, which is involved in the catabolism process, where it is converted into pyruvate and lactate. The amount of lactate in the blood increases, so acidosis is possible. In severe cases, the result of hypoglycemia can be convulsions. Hypoglycemia is accompanied by a decrease in the content of insulin and a decrease in the ratio of insulin / glucagon which in turn leads to the acceleration of lipolysis of adipose tissue as a result of the action of glucagon and the release of fatty acids into the blood.

hyper triacylglycerol mia occurs as a result of a decrease in the activity of LP-lipase of adipose tissue - an enzyme that is activated by insulin and ensures the assimilation of TAG by adipose tissue cells.

hypoglycemia occurs as a result:

•increasing the content of glucose-6-phosphate in cells and its use in the pentose phosphate pathway with the formation of ribose-5-phosphate, a substrate for the synthesis of purine nucleotides;

•increased formation of uric acid as a result of excessive synthesis and, therefore, catabolism of purine nucleotides, the end product of which is uric acid;

•reduce excretion of uric acid due to an increase in lactate production and a change in the pH of urine to the acidic side, which complicates the excretion of urates - sparingly soluble salts of uric acid determine the activity of glucose-6-phosphatase in liver biopsies when diagnosing this pathology. In addition, a test with glucagon or adrenaline stimulation is used, which gives a negative result in case of illness

That is, after hormone injection, blood glucose levels change slightly.

Treatment consists in limiting the use of products containing glucose. It is recommended to exclude from the diet products containing sucrose and lactose, because galactose and fructose are formed from them after conversion into glucose-6-phosphate, leading to further accumulation of glycogen. Frequent feeding is used to prevent hypoglycemia. This can prevent symptoms of hypoglycemia.

Glycogenosis type I inherited by autosomal recessive type. Already in the early period the most noticeable sign is hepatomegaly. Sick children have a short body, a large stomach and enlarged kidneys. Sick children lagging in physical development.

The described disease is sometimes referred to as glycogenosis type 1a, as there is a variant of it - type Ib. Glycogenosis type Ib is rare, characterized by the fact that the defective enzyme is glucose-6-phosphate translocase which ensures the transport of phosphorylated glucose in the ER. Therefore, despite sufficient activity glucose-6-phosphatases cleavage of inorganic phosphate and the release of glucose into the blood is disturbed. The clinical picture of glycogenosis type Ib is the same as in glycogenosis Ia.

Forbes disease, Corey (type III) is very common. It accounts for 1/4 of all cases of liver glycogenosis. Anomalous glycogen in structure accumulates because amylo-1,6-glucosidase, which hydrolyzes glycosidic bonds at branching points is the defective enzyme (Debranching enzyme). Deficiency of glucose in the blood manifests itself quickly, since glycogenolysis is possible, but in a small amount. Unlike glycogenosis type I, lactic acidosis and hyperuricemia are not noted. The disease has a milder course.

Andersen's disease (type IV) - extremely rare autosomal recessive disease whichoccurs due to a branched enzyme defect - amyl-1,4-1,6-glucosyltransferases.

The content of glycogen in the liver is not greatly increased but its structure is changed and this prevents its breakdown. A glycogen molecule has few branching points and very long and rare side branches. At the same time, hypoglycemia is expressed moderately. The disease develops quickly is aggravated by early cirrhosis of the liver and is practically untreatable. The defect of the branching enzyme is found not only in the liver, but also in leukocytes, muscles, fibroblasts, but the early and predominant manifestations of the disease are due to impaired liver function.

McArdle's disease (type V) - autosomal recessive pathology, in which the activity of glycogen phosphorylase is completely absent in skeletal muscles. Since the activity of this enzyme in hepatocytes is normal, hypoglycemia is not observed (the structure of the enzyme in the liver and muscles is coded by different genes).

Heavy physical activity Heavy physical exertion is poorly tolerated and may be accompanied by convulsions, however, hyperproduction of lactate is not observed during physical exertion, which emphasizes the importance of extramuscular energy sources for muscle contraction, for example, such as fatty acids, which replace glucose in this pathology. Although the disease is not linked to gender a high frequency of the disease is characteristic of men.

Hers disease (type VI) is also manifested by symptoms caused by liver damage. This type of glycogenosis - a consequence of a glycogen phosphorylase defect. Glycogen of normal structure accumulates in hepatocytes. The course of the disease is similar to type I glycogenosis, but the symptoms are less pronounced. Decreased activity of glycogen phosphorylase is also found in leukocytes. Hers disease is a rare type of glycogenosis; is inherited according to the autosomal recessive type.

The phosphofructokinase defect is characteristic of **glycogenosis VII** type . Patients can perform moderate physical activity. The course of the disease is similar to glycogenosis type V but the main manifestations are less pronounced.

Phosphorylase kinase defect **(type IX)** occurs only in boys, as this trait is linked to the X-chromosome.

Protein kinase A defect **(type X),** as well as a phosphorylase kinase defect, manifests itself in symptoms similar to Hers disease.

Muscle forms of glycogenosis (unnumbered according to Cory's classification) are characterized by a disturbance in the energy supply of skeletal

muscles. These diseases manifest themselves during physical exertion and are accompanied by pains and cramps in the muscles, weakness and rapid fatigue.

Muscular forms of glycogenosis are characterized by a defect in phosphoglyceromutase and a defect in the M-subunit of LDH. Manifestations of these pathologies are similar to McArdle's disease. Defect of phosphoglyceromutase in muscles is described only in one patient.

2. AGlycogenoses

Aglycogenoses (aglycogenosis 0 according to classification) - a disease resulting from a defect in glycogen synthase. Very low glycogen is observed in the liver and other tissues of patients. This is manifested by severe hypoglycemia in the post-absorptive period. A characteristic symptom is convulsions which appear especially in the morning. The disease is compatible with life, but sick children need frequent feeding.

3. Diabetes mellitus

Diabetes mellitus - a widespread disease which is observed with absolute or relative deficiency of insulin. It occurs in two forms.

In type I diabetes (insulin-dependent diabetes) insulin-synthesizing cells die already at an early age . In type I diabetes (insulin-independent form) usually manifests itself in old age. It manifests itself as a persistent increase in the level of glucose in the blood, with subsequent damage to all systems, organs, and tissues of the body.

One of the main mechanisms of tissue damage in diabetes is glycosylation of proteins, which leads to changes in their conformation and functions. One of the first signs of diabetes is a 2-3 times increase in the amount of glycosylated hemoglobin (norm HbA1c 5,8-7,2%), dry mouth, thirst, frequent urination, increased appetite, development of diabetic cataract, significant change in body weight, ketoacidosis, glycosuria.

4. Disorders of glycosaminoglycan metabolism (mucopolysaccharidoses)

mucopolysaccharidoses - hereditary serious illness, which are manifested by significant disorders in the mental development of children, damage to blood vessels, opacification of the cornea, deformations of the skeleton, and a decrease in life expectancy. Mucopolysaccharidoses are based on hereditary defects of any lysosomal hydrolases involved in the catabolism of glycosaminoglycans. These diseases are characterized by excessive accumulation of glycosaminoglycans in tissues, which leads to deformation of the skeleton and enlargement of organs. Tissues that normally synthesize the largest amount of glycosaminoglycans are

usually affected. At the same time, incompletely destroyed glycosaminoglycans accumulate in lysosomes, oligosaccharide fragments of glycosaminoglycans are detected in the urine.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	A one-year-old child lags behind his peers in mental development. In the morning: vomiting, convulsions, loss of consciousness. Fasting hypoglycemia in the blood. Which enzyme defect can this be associated with? A. Sucrase B. Glycogen synthases C. Arginase D. Phosphorylases E. Lactases	
2.	In the patient's blood, the glucose content on an empty stomach was 5.65 mmol/l, after 1 hour after the sugar load it was 8.55 mmol/l, and after 2 hours - 4.95 mmol/l. Such indicators are typical for: A. A healthy person B. A patient with thyrotoxicosis C. A patient with hidden diabetes D. A patient with insulin-dependent diabetes mellitus E. A patient with non-insulin-dependent diabetes mellitus	
3.	During the examination of the patient's blood, pronounced fasting hypoglycemia was detected. When examining a liver biopsy, it was found that glycogen synthesis does not occur in liver cells. Insufficiency of which enzyme is the cause of the disease?	

	A. Aldolases B. Fructose diphosphatases S. Glycogen synthetases D. Phosphorylases E. Pyruvate carboxylase	
4.	Mucopolysaccharidosis refers to accumulation diseases. Due to the lack of enzymes, the breakdown of polysaccharides is disrupted. In patients, there is an increase in their excretion in urine and their accumulation in one of the cell organoids. In which organelles are mucopolysaccharides accumulated? A. B lysosomes B. In the Golgi complex C. In the cell center D. In EPR E. In mitochondria	
5.	A 62-year-old woman developed cataracts (clouding of the lens) against the background of diabetes. Enhancement of which process in diabetes is the cause of clouding of the lens? A. Glycosylation of proteins B. Proteolysis of proteins C. Ketogenesis D. Lipolysis E. Gluconeogenesis	
6.	A 46-year-old patient complains of dry mouth, thirst, frequent urination, general weakness. Biochemical examination revealed hyperglycemia and ketonemia. In urine - glucose, ketone bodies. The electrocardiogram	

	shows diffuse changes in the myocardium. The patient has a probable diagnosis: A. Diabetes B. Alimentary hyperglycemia C. Acute pancreatitis D. Diabetes insipidus E. Ischemic heart disease	
7.	A patient with diabetes lost consciousness after the injection of insulin, and convulsions are observed. What is the result of a biochemical blood sugar analysis? A. 3.3 mmol / l B. 5.5 mmol / l C. 1.5 mmol / l D. 10 mmol / l E. 8 mmol / l	
8.	In humans, the glucose content in the blood is 15 mmol/l (the reabsorption threshold is 10 mmol/l). The consequence of this will be: A. Reduction of diuresis B. Decreased vasopressin secretion C. Reduction of aldosterone secretion D. Reduction of glucose reabsorption E. Glucosuria	
9.	In the patient's blood, the fasting glucose content is 5.6 mmol/l, one hour after the sugar load - 13.8 mmol/l, and after 3 hours - 9.2 mmol/l. For which pathology are these indicators characteristic? A. Hidden form of diabetes B. Thyrotoxicosis C. Typical for a healthy person D. Acromegaly	

	E. Itsenko-Cushing's disease	
10.	The child is sluggish, apathetic. The liver is enlarged, its biopsy revealed a significant excess of glycogen. The concentration of glucose in the blood is below normal. What is the reason for the reduced concentration of glucose in the blood of this child? A. Deficiency of the gene responsible for the synthesis of glucose-1-phosphate-uridyltransferase B. Decreased (absent) activity of glucose-6-phosphatase in the liver C. Decreased (absent) glucokinase activity in the liver D. Decreased (absent) activity of hexokinase in the liver	
11.	The child has a delay of physical and mental development, deep disorders of the connective tissue of internal organs, keratan sulfates were detected in the urine. The exchange of which substances is disturbed? A. Elastin B. Collagen C. Fibronectin D. Hyaluronic acid E. Glycosaminoglycans	
12.	A characteristic sign of glycogenosis is muscle pain during physical work. Hypoglycemia is registered in the patient's blood. Congenital deficiency of which enzyme causes this pathology? A. Glycogen phosphorylases B. Glucose-6-phosphate dehydrogenases C. Alpha amylases	

	D. Gamma amylases E. Lysosomal glycosidase	
13.	During the transformation of glucose in the pentose cycle, phosphates of various monosaccharides are formed. Which of these substances can be used for the synthesis of nucleic acids? A. Erythrose-4-phosphate B. Xylulose-5-phosphate C. Ribose-5-phosphate D. Ribulose-5-phosphate E. Sedoheptulose-7-phosphate	
14.	The level of which blood plasma protein allows retrospectively (for the previous 4-8 weeks before the examination) to assess the level of glycemia, if the patient has diabetes, which is accompanied by fasting hyperglycemia above 7.2 mmol / l? A. Albumin B. Glycosylated hemoglobin C. C-reactive protein D. Ceruloplasmin E. Fibrinogen	
15.	Define the type of pathology that is characterized by such signs as the absence of glucose-6-phosphatase, hypoglycemia and hepatomegaly in a child with a point mutation of genes: A. Addison's disease B. Bitter's disease C. Cory's disease D. McArdle's disease E. Parkinson's disease	
16.	In a 30-year-old pregnant woman, insufficient activity of β-glucuronidase was detected during	

	the study of enzymes in amniotic fluid cells. What pathological process is observed: A. Aglycogenosis B. Glycogenosis C. Collagenosis D. Lipidosis E. Mucopolysaccharidosis.	
17.	Ketonemia developed as a result of starvation after a jaw injury. How to quickly and effectively eliminate this phenomenon? A. Enter protein hydrolyzate B. Administer glucose C. Administer insulin. D. Introduce a complex of vitamins E. Enter physiological solution	
18.	What pathological components were detected during the laboratory examination of the urine of a patient treated in the endocrinology department with a diagnosis of diabetes mellitus, complaining of thirst and increased appetite? A. Bilirubin, urobilin B. Protein, amino acids C. Protein, creatine D. Glucose, ketone bodies E. Blood	
19.	Hyperglycemia develops with a chronic overdose of glucocorticoids in a patient. Specify the process of carbohydrate metabolism due to which the concentration of glucose increases: A. Aerobic oxidation of glucose B. Glycogenesis C. Glycogenolysis D. Gluconeogenesis	

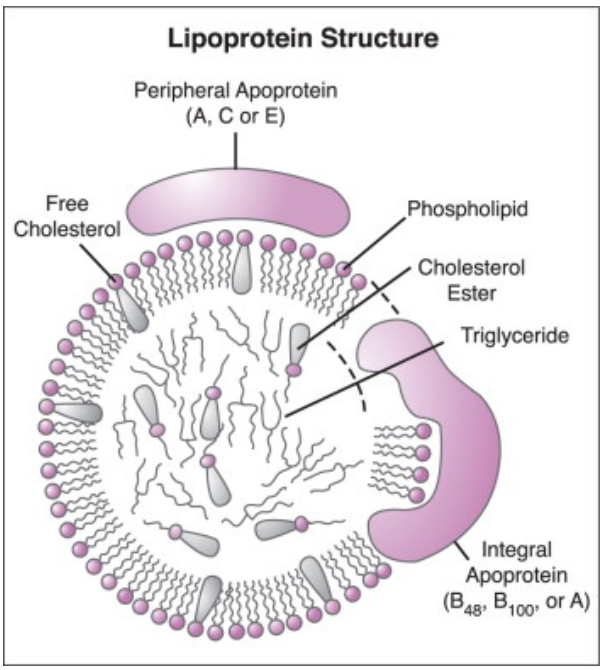
	E. Pentose phosphate cycle	
20.	Characteristic signs of steroid diabetes (Itsenko-Cushing's disease) are hyperglycemia and hypochloremia. Which of the following processes is activated first? A. Glycogenolysis B. Glycolysis C. Gluconeogenesis D. Reabsorption of glucose E. Transport of glucose into the cell	
21.	A 58-year-old patient was admitted in a serious condition: her consciousness was clouded, her skin was dry, her eyes were inflamed, cyanosis, and the smell of rotten apples from her mouth. Blood glucose 15.1 mmol/l, 3.5% glucose in urine. What is the cause of this condition? A. Anaphylactic shock B. Hyperglycemic coma C. Hypovolemic coma D. Hypoglycemic coma E. Uremic coma	
22.	A 2-year-old child was diagnosed with Gierke's disease, which is manifested by severe hypoglycemia. The reason for this condition is the absence of the glucose-6-phosphatase enzyme. What process is this pathology associated with? A. Glycolysis B. Gluconeogenesis C. Ketogenesis D. Mobilization of glycogen E. Glycogen synthesis	

4. Literature. Look pic. 229.

1. TOPIC: LIPOPROTEINS OF BLOOD PLASMA. EXCHANGE OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS

2. INFORMATION MATERIAL

Transport of lipids in the blood is carried out with the help of lipoproteins. Lipoproteins - spherical particles, in which a hydrophobic core consisting of triglycerides (TRG) and cholesterol esters (ECS) and an amphiphilic shell consisting of phospholipids, glycolipids and proteins can be distinguished (pic.40).



Pic.40 (https://ars.els-cdn.com/content/image/3-s2.0-B9780123919090500633-f63-03-9780123919090.jpg)

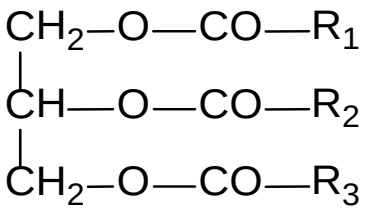
Shell proteins are called apoproteins. Cholesterol usually occupies an intermediate position between the shell and the core. The components of the particle are connected by feeble types of bonds and are in a state of constant diffusion - able to move relative to each other. Depending on the density during ultracentrifugation, blood lipoproteins are divided into:

Chylomicrons - provide transport of triacylglycerols of exogenous origin. A high concentration of chylomicrons in the blood plasma is a consequence of the insufficiency of lipoprotein lipase, which breaks down triacylglycerols, and indicates the development of type 1 hyperlipoproteinemia.

- ***Very low density lipoproteins (VLDL)*** **-** tolerate mainly triacylglycerols synthesized in the liver (endogenous).
- ***Low-density lipoprotein (LDL)*** **-** they mainly transport free and esterified cholesterol**.** Studies conducted in different countries of the world on large groups of people have shown that an increase in the content of total cholesterol in the blood is an independent risk factor for the development of coronary heart disease in both men and women. It has also been proven that a high level of LDL cholesterol, as well as VLDL and hypertriglyceridemia are significant risk factors for atherosclerosis and CHD
- ***High-density lipoprotein (HDL)*** - transfer mainly phospholipids and esters of sterols. They promote the release of cholesterol from the vascular wall and prevent its accumulation in cells, therefore they are considered as anti-atherogenic lipoproteins.

Higher fatty acids, due to their insolubility in water, are practically not found in cells in free form. They are part of various lipid molecules: triacylglycerols, phospholipids, glycolipids, etc.

Triacylglycerols (TAG) are complex esters of glycerol and higher fatty acids:



where R_1, R_2, R_3. – fatty acid residues

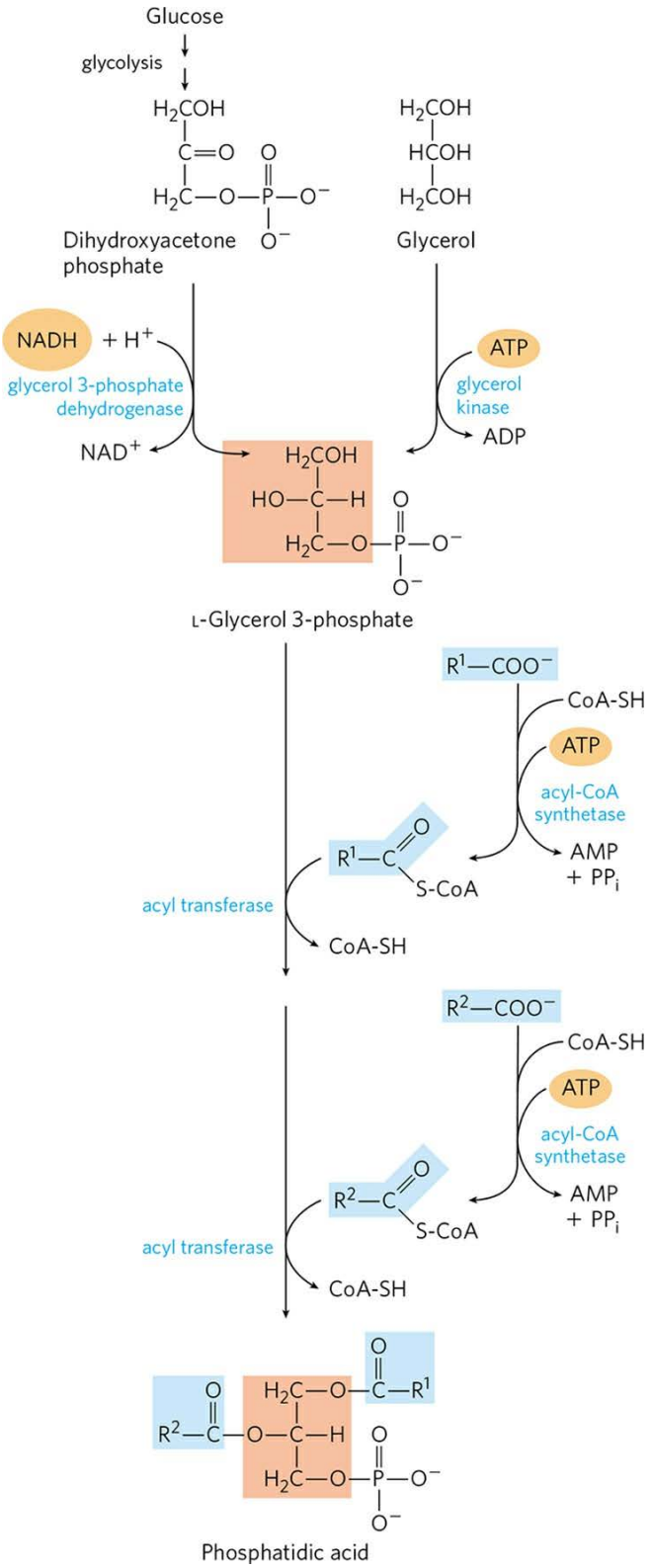
TAG in large quantities are contained in fat depots of the body and are a reserve form of fats. They are in the cytoplasm of cells in the form of inclusions - fat droplets. In human adipose tissue cells (adipocytes), a fat droplet can fill most of the cytoplasm.

In the molecules of neutral fats and phospholipids, glycerol and higher fatty acids are connected to each other by a complex ester bond. These bonds also connect cholesterol with higher fatty acids in cholesterol esters.

The synthesis of fats in the liver and adipose tissue is stimulated by insulin. Fat mobilization is activated in those cases when glucose is not enough to meet the body's energy needs: in the post-absorptive period, during starvation and physical work under the influence of the hormones glucagon, adrenaline, and somatotropin. Fatty acids enter the blood and are used by tissues as sources of energy.

The synthesis of triacylglycerols in the liver and adipose tissue is presented

below in the diagram (pic.41).



Pic.41. Synthesis of triacylglycerols (TAG). DAF (dihydroxyacetone phosphate), DAG (diacylglycerol), TAG (triacylglycerol). (https://onlinesciencenotes.com/wp-content/uploads/2021/05/TAG-

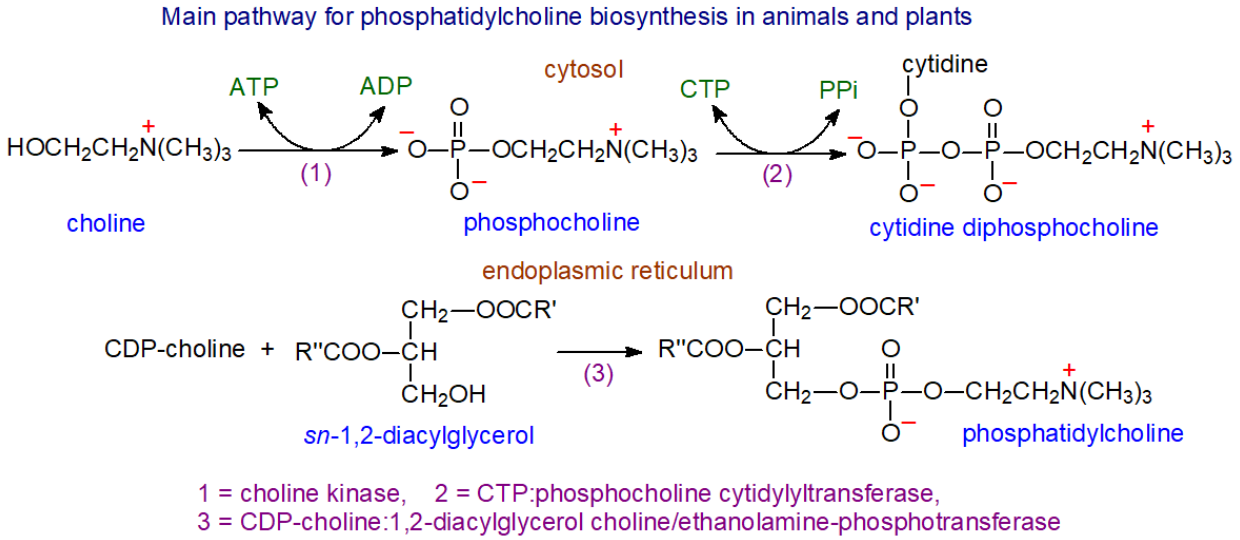
biosynthesis.png)

Synthesis of phospholipids

The biosynthesis of phospholipids in comparison with the synthesis of TAG has significant features. They consist in additional activation of PL components - phosphatidic acid or choline and ethanolamine. There are several variants:

1 variant

Choline and ethanolamine are reused and are not catabolized. Activation of choline (or ethanolamine) occurs through the intermediate formation of phosphorylated derivatives followed by the addition of CMF. In the next reaction, phosphocholine (or phosphoethanolamine) is transferred to DAG. This pathway is particularly characteristic of the lungs and intestines, but also occurs in other tissues (pic.42).

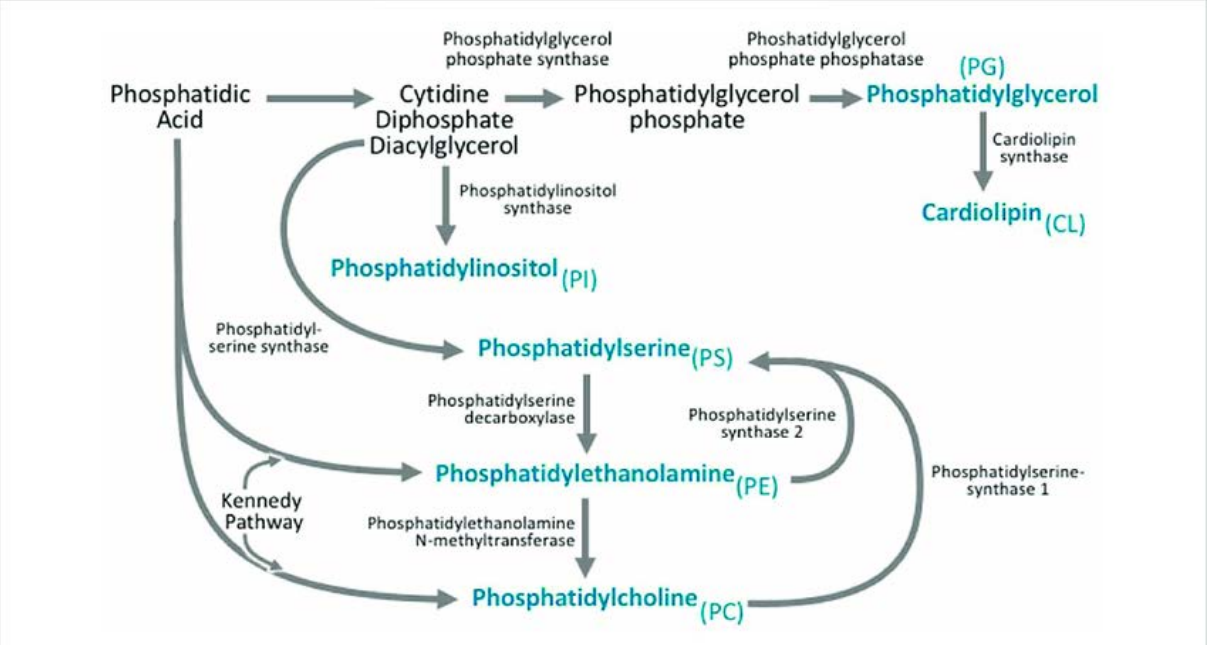


Pic.42. Reactions of phospholipid synthesis using 1,2-DAG on the example of phosphatidylcholine.
(https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/complex/pc/Figure2.png)

2 variant

In this case, choline is not incorporated in a ready-made form, but is formed in a number of reactions . The activation of phosphatidic acid consists in the addition of CMF to it with the formation of CDF-DAG. Next, the six-atom alcohol inositol or serine is added to it to form phosphatidylinositol and phosphatidylserine. Synthesized phosphatidylserine undergoes decarboxylation to form phosphatidylethanolamine. The latter is methylated with the participation of

S-adenosylmethionine into phosphatidylcholine (pic.43).



Pic.43. Phospholipid synthesis reactions using phosphatidic acid (https://www.researchgate.net/publication/372349375/figure/fig3/AS:1143128117 4783398@1689338565362/Phospholipid-Biogenesis-Phosphatidic-acid-is-involved-in-phospholipid-biogenesis-through.png)

3 variant

A reaction can occur between phosphatidylethanolamine and serine with formation as a result of the reaction of phosphatidylserine and free ethanolamine.

Lipotropic substances

Triacylglycerols are deposited in liver cells and cause its fatty degeneration. Lipotropic substances are used to prevent this. For example, the amino acid methionine, which is a donor of methyl groups for the synthesis of choline and, thus, promotes the formation of phosphatidylcholine and prevents the synthesis of triacylglycerols.

Any substances that promote the synthesis of PL and prevent the synthesis of TAG are called lipotropic factors. They include:

1. Structural components of phospholipids: polyunsaturated fatty acids, inositol, serine, choline, ethanolamine.

2. Methionine is a donor of methyl groups obtained in the exchange of serine and glycine for the synthesis of choline and phosphatidylcholine.

3. Vitamins:

Pyridoxine (B6), which contributes to the formation of FEA from FS.

Cyanocobalamin (B12) and folic acid, which participate in the formation of active form of methionine, and therefore in the synthesis of phosphatidylcholine.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	A dry cleaner's worker was diagnosed with fatty liver disease. Violation of the synthesis of which substance in the liver can lead to this pathology? A. Cholic acid B. Phosphatidylcholine C. Tristearin D. Phosphatidic acid E. Urea	
2.	The patient's examination revealed an increased content of low-density lipoproteins in the blood serum. What disease can be expected in this patient? A. Atherosclerosis B. Kidney damage C. Inflammation of the lungs D. Acute pancreatitis E. Gastritis	
3.	With fatty infiltration of the liver, the synthesis of phospholipids is disturbed. Indicate which of the following substances can enhance methylation processes in the process of phospholipid synthesis? A. Ascorbic acid B. Citrate S. Methionine D. Glycerin E. Glucose	
4.	A 65-year-old patient with signs of general	

	obesity and the risk of fatty liver dystrophy is recommended a diet enriched with lipotropic substances. The content of which substance in the diet is the most important in this case? A. Glucose B. Glycine C. Vitamin C D. Cholesterol E. Methionine	
5.	A 35-year-old man has pheochromocytoma. An increased level of adrenaline and norepinephrine is determined in the blood, the concentration of free fatty acids increased 11 times compared to the norm. Indicate which enzyme activation under the influence of adrenaline increases the rate of lipolysis? A. Triacylglycerol lipases B. Lipoprotein lipases C. Phospholipases A2 D. Phospholipases Z E. Cholesterolesterases	
6.	Which of the listed hormones reduces the rate of lipolysis in adipose tissue? A. Hydrocortisone B. Adrenaline C. Insulin D. Somatotropin E. Norepinephrine	
7.	An experimental animal on a protein-free diet developed fatty infiltration of the liver, in particular, as a result of a deficiency of methylating agents. Name the metabolite, the formation of which is disturbed in the experimental animal:	

	A. Kholin B. DOFA C. Cholesterol D. Acetoacetate E. Linoleic acid	
8.	In the human body, the main place of deposition of triacylglycerols (TG) is adipose tissue. However, their synthesis can also take place in the liver. In the form of which transport form are TG transferred from the liver to adipose tissue? A. HDL B. Chylomicrons C. LDL D. LPDNSH E. Complex with albumins	
9.	A man was bitten by a snake. He begins to suffocate, increased hemolysis of erythrocytes in the blood. The effect of toxic components of snake venom leads to: A. Formation of lysolecithin B. Acidosis C. Polyuria D. Development of alkalosis E. Formation of triglycerides	
10.	Fatty infiltration of the liver develops when the synthesis of phospholipids is disturbed. Indicate which of the listed substances can increase the synthesis of phospholipids by stimulating the methylation process? A. Methionine B. Ascorbic acid C. Glucose D. Glycerin E. Citrate	

11.	With insufficient intake of lipotropic factors in the human body, fatty infiltration of the liver develops. Which of the following substances should be classified as lipotropic substances? A. Kholin B. Cholesterol C. Triacylglycerides D. Fatty acids E. Riboflavin	
12.	An increase in the content of free fatty acids is observed in the blood of patients with diabetes. The reason for this may be: A. Accumulation of palmitoyl-CoA in the cytoplasm B. Increase in the activity of triglyceride lipase of adipocytes C. Activation of utilization of ketone bodies D. Activation of synthesis of apolipoproteins A-1, A-2, A-4. E. Decreased activity of lecithin-cholesterol-acyltransferase in blood plasma	

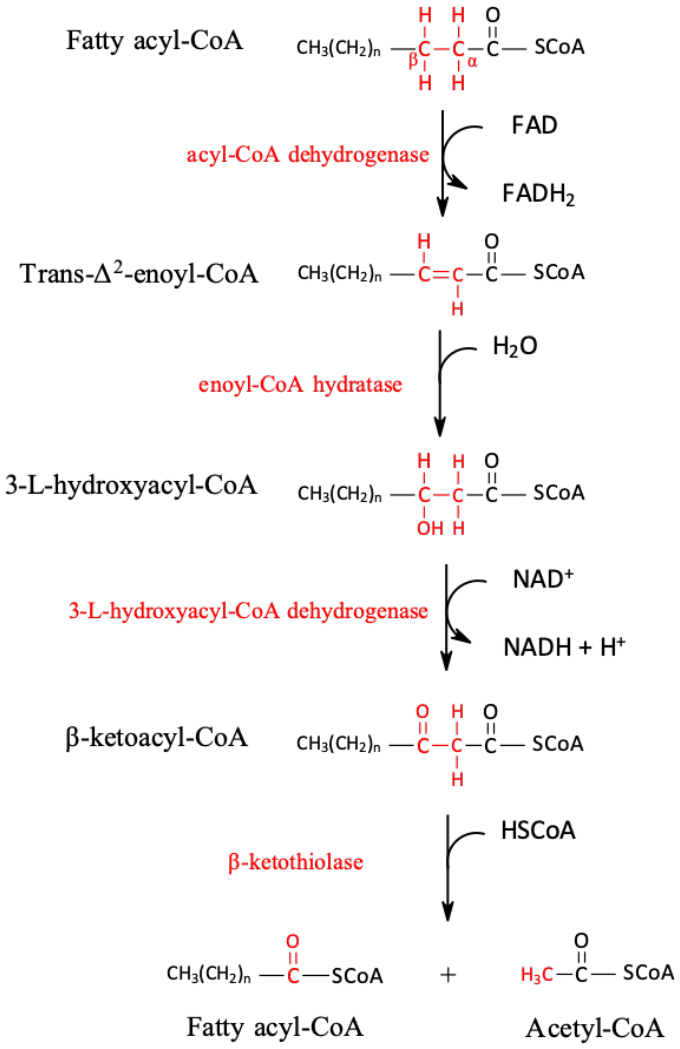
4. Literature. Look pic. 229.

1. TOPIC: METABOLISM OF HIGHER FATTY ACIDS AND KETONE BODIES

2. INFORMATION MATERIAL.

b-OXIDATION OF FATTY ACIDS.

b-OXIDATION OF FATTY ACIDS occurs in the mitochondria, where acids are transported from the cytosol with the help of carnitine. The process of b-oxidation of VHF is cyclic. For each turn of the cycle, 2 carbon atoms are split off from the fatty acid in the form of an acetyl residue (pic.44).



Pic.44. β-oxidation of fatty acids (https://files.mtstatic.com/site_4334/314600/0?Expires=1698230898&Signature=Asv1Eo2651FSzQiN8LfTEq2eRDZqt63P~~RM-QpoiRFZvtfm5Ra-3iWi6e5gZiI9su7VlO8w3sBnPkmnV4YCBQ-pxjyaNxZDGSc1xgjDPv2~iwbYblya5atLdJ9OG8uNnFxTivTNsQaZnh0lPqSgmL

sL8C0B2H5XHLpVG5E0l5w_&Key-Pair-Id=APKAJ5Y6AV4GI7A555NA)

After that, acyl-CoA, shortened by 2 carbon atoms, undergoes oxidation again (enters a new cycle of b-oxidation reactions). The formed acetyl-CoA can further enter the cycle of tricarboxylic acids, synthesis of VLDL, synthesis of ketone bodies and cholesterol.

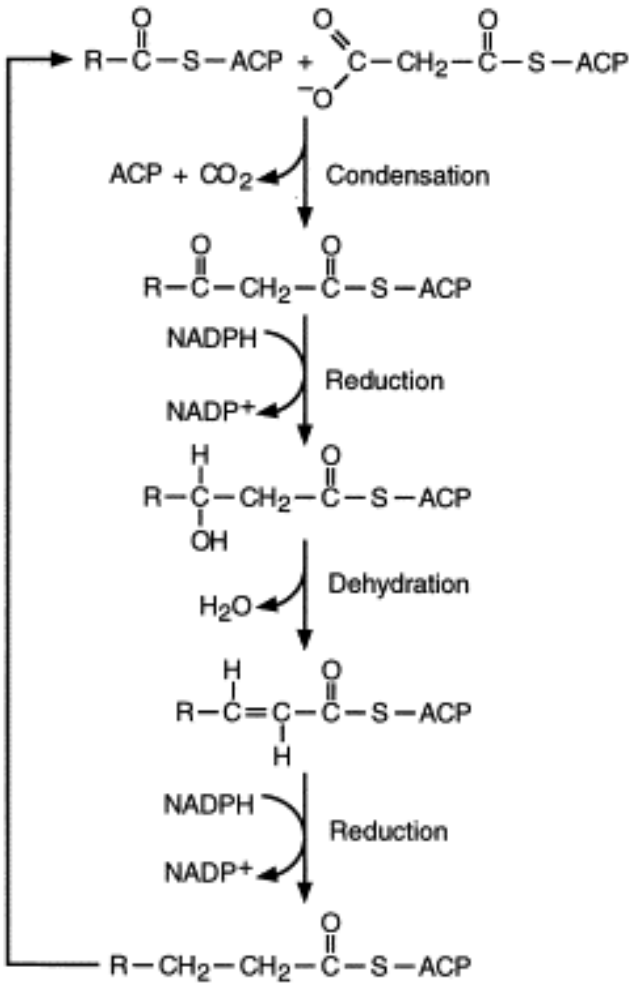
SYNTHESIS OF HIGHER FATTY ACIDS

Acetyl-CoA is the substrate for the synthesis of VHL. However, during the synthesis of fatty acids (FA) in each elongation cycle, not acetyl-CoA itself is used, but its derivative -malonyl-CoA.

This reaction is catalyzed by the enzyme acetyl-CoA-carboxylase - a key enzyme in the multienzyme system of the synthesis of fatty acids. Enzyme activity is regulated by a type of negative feedback. The inhibitor is a synthesis product: acyl-CoA with a long chain (n=16) - palmitoyl-CoA. The activator is citrate. The non-protein part of this enzyme includes vitamin H (biotin).

Further, in the course of the synthesis of fatty acids, the acyl-CoA molecule is successively elongated by 2 carbon atoms for each stage, due to malonyl-CoA, which loses CO2 in this lengthening process.

After the formation of malonyl-CoA, the main reactions of fatty acid synthesis are catalyzed by one enzyme - fatty acid synthetase (fixed on EPR membranes). Fatty acid synthetase contains 7 active sites and APB (acyl transfer protein). The area that binds malonyl-CoA contains a non-protein component - vitamin B3 (pantothenic acid). The sequence of one cycle of reactions of the synthesis of VLC on pic .45.



Pic.45. Reactions of the synthesis of higher fatty acids

After the end of the acyl-APB cycle, it enters the next cycle of synthesis. A new malonyl-CoA molecule is attached to the free SH-group of the acyl-transfer protein. Then the acyl residue is split off, it is transferred to the malonyl residue (with simultaneous decarboxylation) and the cycle of reactions repeats.

Thus, the carbohydrate chain of the future fatty acid gradually grows (for each cycle - by two carbon atoms). This happens until it lengthens to 16 carbon atoms (in the case of the synthesis of palmitic acid) or more (the synthesis of other fatty acids). This is followed by thiolysis, and the active form of fatty acid - acyl-CoA - is formed in its finished form. The following conditions are necessary for the normal synthesis of higher fatty acids:

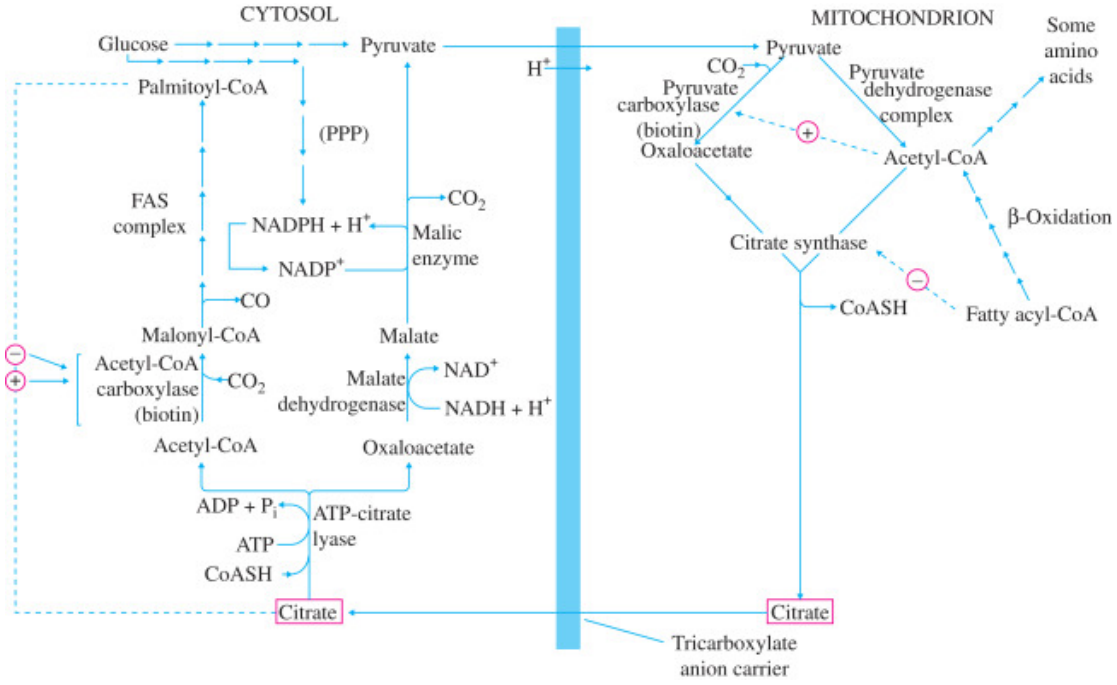
1) The arrival of carbohydrates, during the oxidation of which the necessary substrates and NADPH2 are formed.

2) The high energy charge of the cell is the high content of ATP, which

ensures the release of citrate from the mitochondria into the cytoplasm.

Comparative characteristics of b-oxidation and synthesis of higher fatty acids:

1. b-oxidation takes place in the mitochondria, and the synthesis of fatty acids takes place in the cytoplasm on EPR membranes. However, the acetyl-CoA formed in the mitochondria cannot pass through the membrane itself. Therefore, there are mechanisms for the transport of acetyl-CoA from the mitochondria to the cytoplasm with the participation of enzymes of the Krebs cycle (pic.46).



Pic.46. Mechanism of transport of acetyl-CoA from mitochondria to cytoplasm. (https://ars.els-cdn.com/content/image/3-s2.0-B9780124166875000166-f16-08-9780124166875.jpg)

The key enzymes of CTC are citrate synthase and isocitrate dehydrogenase. The main allosteric regulators of these enzymes are ATP and ADP. If there is a lot of ATP in the cell, the ATP acts as an inhibitor of these key enzymes. However, isocitrate dehydrogenase is more strongly inhibited by ATP than citrate synthetase. This leads to the accumulation of citrate and isocitrate in the mitochondrial matrix. When citrate accumulates, it leaves the mitochondrion and enters the cytoplasm. In the cytoplasm there is an enzyme citrate lyase. This enzyme splits citrate into PIKE and acetyl-CoA.

Thus, a condition for the release of acetyl-CoA from the mitochondria into the cytoplasm is a good supply of ATP to the cell. If there is little ATP in the cell, then acetyl-CoA is split into CO2 and H2O.

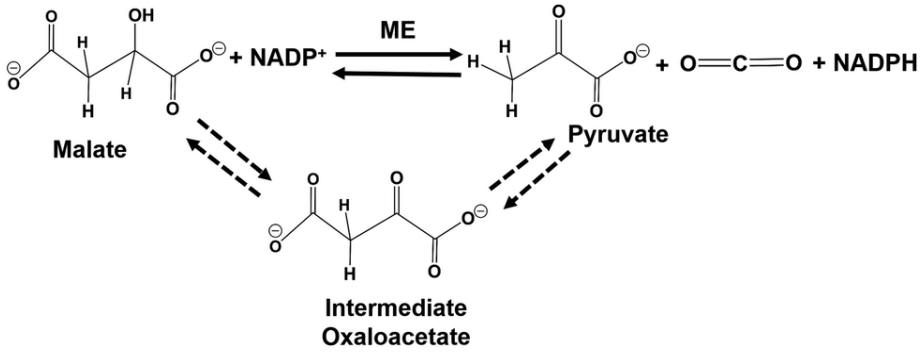
2. In the course of b-oxidation, intermediate products are associated with HS-CoA, and during the synthesis of fatty acids, intermediate products are associated with a special acyl transfer protein (APB). It is a complex protein, its non-protein part is similar in structure to CoA and consists of thioethylamine, pantothenic acid (vitamin B3) and phosphate.

. In b-oxidation, NAD and FAD are used as oxidants. A reducing agent is required for the synthesis of FA - NADP*H2 is used.

There are 2 main sources of NADPH*H2 for the synthesis of fatty acids in the cell:

a) pentose phosphate pathway of carbohydrate breakdown;

b) malate oxidation reactions:



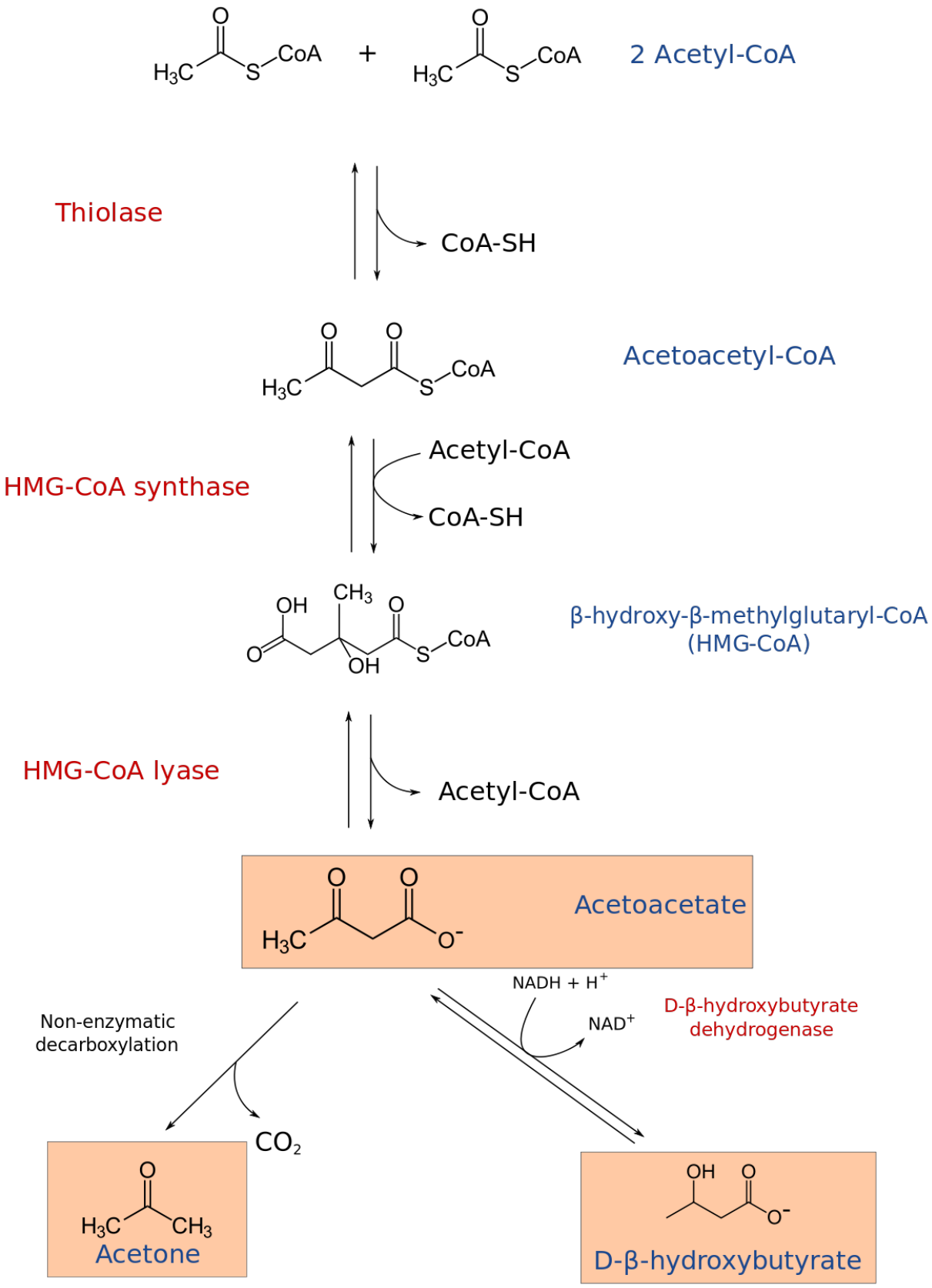
This reaction takes place in the cytoplasm and is catalyzed by the enzyme malate dehydrogenase.

Thus, carbohydrate metabolism and fat metabolism are very closely related.

Carbohydrates can easily be converted into fats, but the conversion of fats into carbohydrates is impossible, since acetyl-CoA cannot be converted into pyruvate.

SYNTHESIS OF KETONE BODIES

In addition to the synthesis of VLDL, ketone bodies are synthesized from acetyl-CoA in the liver, which are a special transport form of acetyl-CoA, because cell membranes are impermeable to it (pic.47).



Pic 47. Reaction of synthesis of ketone bodies. (https://upload.wikimedia.org/wikipedia/commons/thumb/e/e6/Ketogenesis.svg/12

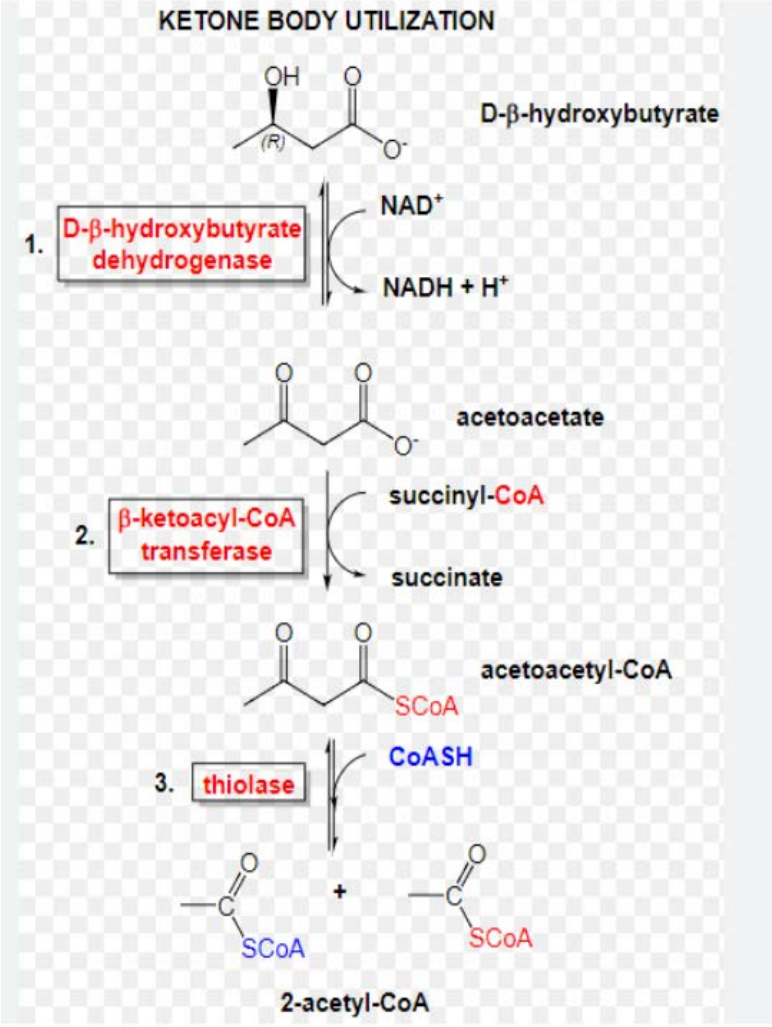
00px-Ketogenesis.svg.png)

Acetone, which is formed during the sudden (non-enzymatic) decarboxylation of acetoacetate, is not used in the body. It is excreted with exhaled air, secretion of sweat glands and urine. Normally, the concentration of acetone in the blood is low and cannot be determined by normal reactions.

Ketone bodies (acetoacetate and β-hydroxybutyrate), synthesized in the liver, are not used in it, easily pass through mitochondrial and cell membranes and enter the blood. They are transported by blood to all other tissues.

DISPOSAL OF KETONE BODIES

Occurs in mitochondria (except liver cells). β-hydroxybutyrate turns into acetoacetate, and acetoacetate reacts with an intermediate product of CTC - succinyl-CoA.



(https://files.mtstatic.com/site_4463/51283/0?Expires=1698232585&Signature=Rt

avikR33HqrBJG9zb6kPQJHz~4tQp7T7orceEjCvSWD1p6P8T3Cr5fBz5BqnxAOz tfxUNFLHHwHsHf~9si5llwgfLuuvvLY7c6H1HanG1jWjhOBHcjD9kOq1vt07kK n6-sdExGQWdbD3EsMzAvSYrGOGZSI8t9MN1vBQ9TTv8_&Key-Pair-Id=APKAJ5Y6AV4GI7A555NA)

Ways of using acetyl-CoA formed from ketone bodies depend on the functional state of the cell and its specificity.

However, most often acetyl-CoA is used in the central nervous system for energy.

Normally, the processes of synthesis and use of ketone bodies are balanced, so the concentration of ketone bodies in the blood and in the tissues is usually very low, and is 0.12 - 0.30 mmol / l.

However, with general or carbohydrate starvation, the balance between the formation and utilization of ketone bodies may be disturbed. This is due to the fact that the rate of formation of ketone bodies depends on the rate of b-oxidation of fatty acids in the liver, and the process of b-oxidation is accelerated with increased lipolysis (fat breakdown) in adipose tissue. Enhancement of lipolysis can occur under the influence of the hormone adrenaline, during muscle work, during starvation, and when there is a lack of insulin (diabetes). In the future, as lipolysis increases, the rate of disposal of ketone bodies, which are important sources of energy, increases.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	A diabetic patient was brought to the hospital in an unconscious state. Kussmaul type breathing, blood pressure 80/50 mm Hg, the smell of acetone from the mouth. Accumulation of what substances in the body can explain the occurrence of these disorders? A. Ketone bodies B. Lactic acid C. Modified lipoproteins D. Complex carbohydrates E. Carbonic acid	
2.	In patients with diabetes and during starvation, the content of ketone bodies, which are used as energy material, increases in the blood. From what substance are they synthesized? A. Citrate B. Malatu C. Ketoglutarate D. Acetyl-CoA E. Succinyl-CoA	
3.	To improve the results, the athlete was recommended to use a drug containing carnitine. What process is activated to the greatest extent by carnitine? A. Synthesis of ketone bodies B. Synthesis of steroid hormones C. Synthesis of lipids D. Transport of fatty acids in mitochondria E. Tissue respiration	

4.	A patient with a high degree of obesity is recommended carnitine as a dietary supplement to improve fat "burning". What is the direct participation of carnitine in the process of fat oxidation? A. Transport of UVC from cytosol to mitochondria B. Activation of VLC C. Transport of UVC from fat depots to tissues D. Takes part in one of the reactions of beta-oxidation of VHLK E. Activation of intracellular lipolysis	
5.	A 1-year-old child came to the clinic with signs of muscle damage. After the examination, carnitine deficiency in the muscles was revealed. Violation of which process is the biochemical basis of this pathology? A. Transport of fatty acids in mitochondria B. Utilization of lactic acid C. Synthesis of actin and myosin D. Substrate phosphorylation E. Regulation of Ca2 + level in mitochondria	
6.	An experimental animal was given an excessive amount of carbon-labeled glucose for a week. In which connection can the label be found? A. Arachidonic acid B. Methionine C. Holin D. Palmitic acid E. Vitamins A	
7.	With a lack of biotin, there is a violation of the synthesis of higher fatty acids. The formation of which of the mentioned metabolites can be	

	disturbed in this case? A. Alanine B. Pyruvate C. Serotonin D. Succinyl-CoA E. Malonil-CoA	
8.	A 28-year-old man consumes an excessive amount of carbohydrates (600 g per day), which exceeds his energy needs. What process will be activated in this case? A. Lipogenesis B. Glycolysis C. Lipolysis D. Gluconeogenesis E. Oxidation of fatty acids	
9.	Cardiac muscle is characterized by an aerobic pathway of oxidation of any substrate. The main one is oxidation: A. Fatty acids B. Triacylglycerols C. Glycerin D. Glucose E. Amino acids	
10.	A 40-year-old man ran 10 km in 60 minutes. How will the metabolism in muscle tissue change? A. Gluconeogenesis will increase B. Glycolysis will increase C. The rate of fatty acid oxidation will increase D. Glycogenesis will increase E. Proteolysis will increase	
11.	The patient fasts for 48 hours. What substances are used by muscle tissue as sources of energy in these conditions?	

	A. Ketone bodies B. Triglycerides C. Pyruvate D. Lactate E. Amino acids	
12.	Metabolic acidosis is observed in patients who suffer from a severe form of diabetes and do not receive insulin. An increase in the concentration of which metabolites lead to this? A. Ketone bodies B. Fatty acids C. Unsaturated fatty acids D. Triacylglycerols E. Cholesterol	

4. Literature. Look pic. 229.

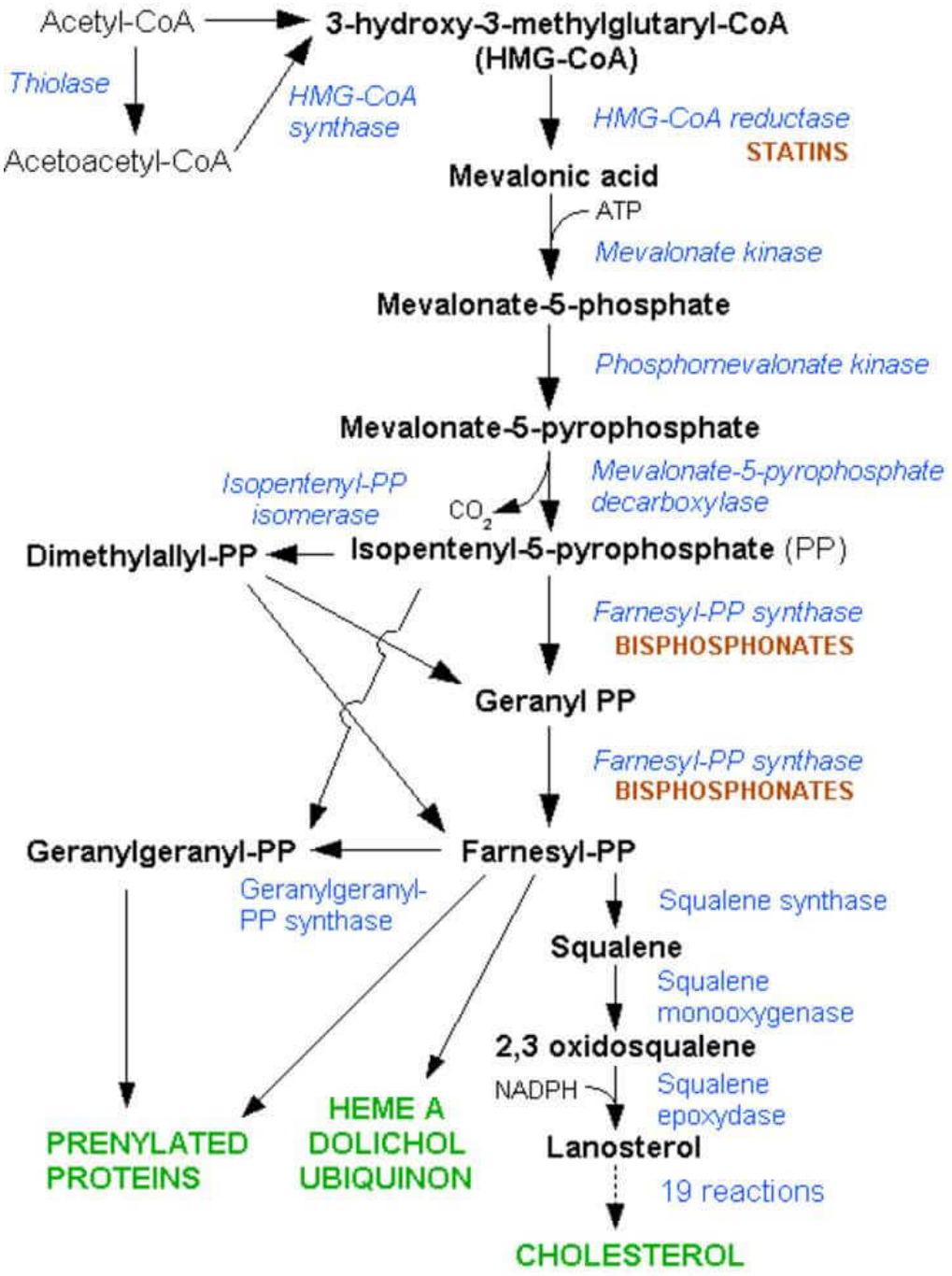
<u>TOPIC:</u> CHOLESTEROL METABOLISM IN THE BODY. LIPID METABOLISM DISORDERS: ATHEROSCLEROSIS, OBESITY.

2. INFORMATION MATERIAL.

CHOLESTEROL - a steroid characteristic only of animal organisms. The main place of its formation in the human body is the liver, where 50% of cholesterol is synthesized, 15-20% of it is formed in the small intestine, the rest is synthesized in the skin, cortex of the adrenal glands and gonads

Dietary cholesterol is transported by chylomicrons and enters the liver. Therefore, the liver is a source of both dietary cholesterol (got there as part of chylomicrons) and endogenous cholesterol for tissues.

Cholesterol synthesis occurs mainly in the liver on the EPS membranes of hepatocytes. This cholesterol is endogenous. There is a constant transport of cholesterol from the liver to the tissues. Dietary (exogenous) cholesterol is also used to build membranes. The key enzyme of cholesterol biosynthesis is HMG-reductase (β-hydroxy-β-methyl-glutaryl-CoA reductase). This enzyme is inhibited by the principle of negative feedback by the final product - cholesterol (pic.49).



Pic.49. Cholesterol synthesis scheme (https://microbenotes.com/wp-content/uploads/2018/08/Cholesterol-Synthesis-Steps.jpg)

The liver synthesizes and then enters the blood VLDL - very low-density lipoproteins (consisting of 75% cholesterol), as well as LDL - low-density lipoproteins, which include the apoprotein apoB100. Almost all cells have receptors for apoB100. Therefore, LDL is fixed on the surface of cells. At the same time, the transfer of cholesterol to cell membranes is observed. Therefore, LDL is able to deliver cholesterol to tissue cells.

In addition, cholesterol is released from tissues and transported to the liver.

Cholesterol is transported from tissues to the liver by high-density lipoproteins (HDL). They contain very little lipid and a lot of protein. Synthesis of HDL occurs in the liver. HDL particles have the shape of a disc, and they contain apoproteins apoA, apoC and apoE. In the bloodstream, the protein enzyme lecithin cholesterol acyltransferase (LCAT) attaches to LDL.

ApoC and apoE can transfer from HDL to chylomicrons or VLDL. Therefore, HDL are donors of apoE and apoC. ApoA is an activator of LHT.

LHT catalyzes the following reaction:

This is the reaction of the transfer of a fatty acid from the R2 position to cholesterol.

The reaction is very important, because the formed cholesterol ester is a very hydrophobic solution and immediately passes into the core of HDL cells - thus, upon contact with the membranes of HDL cells, excess cholesterol is removed from them. Next, HDL go to the liver, where they are destroyed, and excess cholesterol is removed from the body.

Pathologies of lipid metabolism include: obesity, atherosclerosis, hyperlipoproteinemia, Tay-Sachs disease - in which there is an accumulation of a significant amount of lipids in lysosomes.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it::

№	Test	Task
1.	A 65-year-old patient with signs of general obesity and the risk of fatty liver dystrophy is recommended a diet enriched with lipotropic substances. The content of which substance in the diet is the most important in this case? A. Glucose B. Glycine C. Vitamin C D. Cholesterol E. Methionine	
2.	Complaints and objective data suggest that the patient has an inflammatory process in the gallbladder, a violation of the colloidal properties of bile, and the possibility of gallstone formation. What can mainly cause the formation of stones? A. Cholesterol B. Chlorides C. Phosphates D. Urates E. Oxalates	
3.	When examining a teenager suffering from xanthomatosis, familial hypercholesterolemia was detected. The concentration of which lipoproteins is significantly increased in the blood in this pathology? A. HDL	

	B. LPDNSH C. LDL D. NEZHK E. Chylomicrons	
4.	In the culture of cells obtained from a patient with lysosomal pathology, the accumulation of a significant amount of lipids in lysosomes was revealed. In which of the listed diseases does this disorder occur? A. Wilson-Konovalov disease B. Gout C. Phenylketonuria D. Tay-Sachs disease E. Galactosemia	
5.	A 70-year-old man suffers from atherosclerosis of the vessels of the lower extremities and coronary heart disease. During the examination, a violation of the lipid composition of the blood was revealed. An excess of which lipoproteins is the main link in the pathogenesis of atherosclerosis? A. Chylomicronov B. Low density S. High density D. Very low density E. Intermediate density	
6.	A 56-year-old woman suffers from atherosclerosis of cerebral vessels. During the examination, hyperlipidemia was detected. The content of which class of lipoproteins in the blood serum of this patient is most likely to be increased? A. High-density lipoproteins B. Chylomicrons	

	C. Low-density lipoproteins D. Cholesterol E. Complex of fatty acids with albumins	
7.	The mother contacted the doctor about the child's poor health - lack of appetite, restless sleep, irritability. A blood test revealed the absence of the glucocerebrosidase enzyme. What pathology is this characteristic of? A. Pompe disease B. Bitter disease C. Tay-Sachs diseases D. Niemann-Pick diseases E. Gaucher diseases	
8.	The patient's examination revealed an increased content of low-density lipoproteins in the blood serum. What disease can be expected in this patient? A. Atherosclerosis B. Kidney damage C. Inflammation of the lungs D. Acute pancreatitis E. Gastritis	
9.	The patient has hypertension, atherosclerotic vascular damage. Indicate which lipid consumption should be reduced in the patient's diet? A. Monooleate glyceride B. Oleic acid C. Arachidonic acid D. Cholesterol E. Phosphatidylserine	
10.	In diabetes mellitus, ketosis occurs as a result of the activation of higher fatty acid oxidation processes. What disturbances in the acid-	

	alkaline balance of the blood can lead to an excessive accumulation of ketone bodies in the blood? A. Metabolic acidosis B. Metabolic alkalosis C. No changes D. Respiratory acidosis E. Respiratory alkalosis	
11.	A sick 12-year-old boy has a serum cholesterol level of up to 25 mmol/l. In the anamnesis - hereditary familial hypercholesterolemia, the cause of which is a violation of the synthesis of protein receptors to: A. Low-density lipoproteins B. Chylomicrons C. Intermediate density lipoproteins D. Very low density lipoproteins E. High-density lipoproteins	
12.	For the prevention of atherosclerosis, coronary heart disease and disorders of cerebral blood circulation, a person should receive 2-6 g/day of polyunsaturated fatty acids in foods. These acids are necessary for the formation of: A. Prostaglandins B. Bile acids C. Steroids D. Vitamins of group D E. Neurotransmitters	
13.	During the examination of the patient, stagnation of bile in the liver and stones in the gall bladder were found. Name the main component of gallstones that form in this condition: A. Cholesterol	

	B. Triglycerides C. Calcium bilirubinate D. Protein E. Mineral salts	
14.	An increase in the level of HDL leads to a decrease in the risk of atherosclerosis. What is the mechanism of antiatherogenic effect of HDL? A. Cholesterol is removed from tissues B. Deliver cholesterol to tissues C. Take part in the breakdown of cholesterol D. Activate the transformation of cholesterol into bile acids E. Contribute to the absorption of cholesterol in the intestines	
15.	To prevent fatty liver dystrophy, the doctor prescribed a lipotropic drug - a donor of methyl groups. This is likely: A. Glucose B. Cholesterol C. Bilirubin D. Valin E.S	
16.	Hereditary hyperlipoproteinemia was detected in the sick child according to a blood test. A genetic defect in the synthesis of which enzyme can cause this pathological condition? A. Lipoprotein lipases B. Glycosidases C. Proteinases D. Hemesynthetases E. Phenylalanine hydroxylases	
17.	Violation of the processes of myelination of	

	nerve fibers leads to neurological disorders and mental retardation. Such symptoms are characteristic of hereditary and acquired metabolic disorders: A. Phosphatidic acids B. Neutral fats S. Cholesterol D. Higher fatty acids E. Sphingolipids	
18.	Steatosis occurs due to the accumulation of triacylglycerols in hepatocytes. One of the mechanisms of the development of this disease is a decrease in the utilization of neutral VLDL fat. What lipotropic substances prevent the development of steatosis? A. Methionine, B6, B12 B. Isoleucine, B1, B12 C. Valin, B3, B2 D. Arginine, B2, B3 E. Alanin, B1, RR	

4. Literature. Look pic. 229.

<u>TOPIC:</u> GENERAL PATHWAYS OF AMINO ACID CATABOLISM. GLUCOGENIC AND KETOGENIC AMINO ACIDS.

2. INFORMATION MATERIAL

There are general and specific pathways of amino acid metabolism. Common pathways of catabolism of amino acids include:

1) transamination;

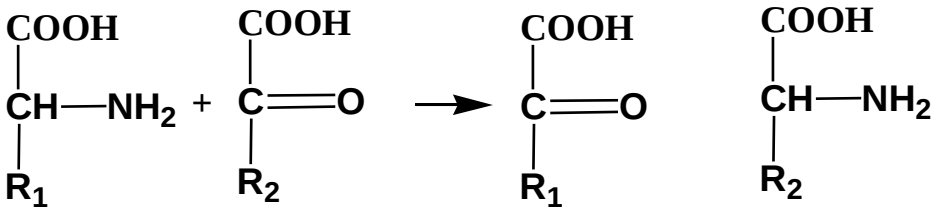
2) demining;

3) decarboxylation.

1. **transamination** amino acids - the main way of deamination of amino acids, which occurs without the formation of free NH3. This is the reverse process of transferring the NH2 group from an amino acid to an -keto acid. The process was opened by A.E. Braunstein and M.B. Kritzman (1937).

All amino acids except threonine, lysine, proline and hydroxyproline can participate in transamination.

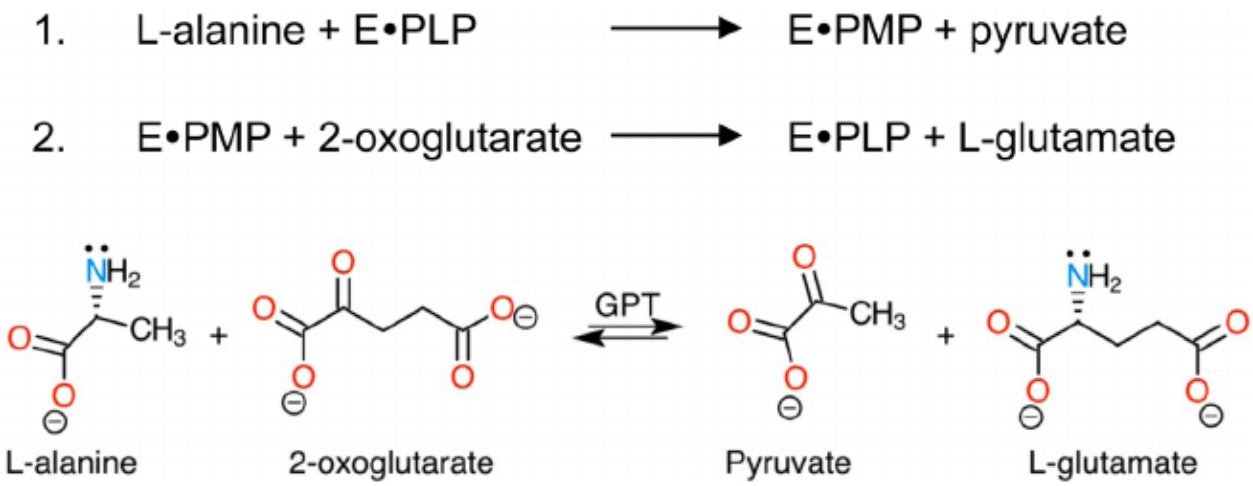
The transamination reaction generally looks as follows:



amino acid *α-keto acid* *α-keto acid* *amino acid*

Enzymes that catalyze reactions of this type are called aminotransferases (transaminases). L-amino acid aminotransferases function in the human body. The acceptor of the amino group in the reaction is α– keto acids - pyruvate, oxaloacetate, α-ketoglutarate. The most common aminotransferases are ALT (alanine aminotransferase), AST (aspartate aminotransferase), tyrosine aminotransferase.

The reaction catalyzed by the enzyme AlT is presented below:



The reaction catalyzed by the AST enzyme can be schematically depicted as follows:

Asp + α- ketoglutarate ⇔ Oxaloacetate + Glu.

Coenzyme transaminase - pyridoxal phosphate (vitB6 derivative)- is part of the active center of the enzyme. In the process of transamination, the coenzyme acts as a carrier of the amino group, and two coenzyme forms of PALF (pyridoxal-5-f) and PAMP (pyridoxamine-5-f) are interconverted:+ NH_2–группа

PALF ⇔ PAMP

- NH2 group

Transamination takes place actively in the liver. This allows you to regulate the concentration of any amino acids in the blood, including those that came with food (with the exception of tre, lyse, pro). Thanks to this, the optimal mixture of amino acids is carried with the blood to all organs.

Some clinical aspects

In some cases, transamination of amino acids may be disturbed:

1) with hypovitaminosis B6;

2) in the treatment of tuberculosis with transaminase antagonists - ftivazid and its analogs;

3) with starvation, cirrhosis and steatosis of the liver, there is a lack of synthesis of the protein part of transaminases.

Determination of aminotransferase activity in blood plasma is important for diagnosis. In pathological conditions, there is an increase in cytolysis in one or another organ, which is accompanied by an increase in the activity of these

enzymes in the blood.

Transaminases are found in different tissues in different amounts. Asat is more in cardiomyocytes, liver, skeletal muscles, kidneys, pancreas. ALT - in a record amount in the liver, to a lesser extent - in the pancreas, myocardium, skeletal muscles. Therefore, an increase in the activity of AST in the blood is more characteristic of a myocardial infarction (MI), and an increase in the activity of ALT may indicate cytolysis in hepatocytes. So, with acute infectious hepatitis, there is activity in the blood ↑ ALT > ↑ AST but with cirrhosis of the liver - ↑ AST > ↑ ALT. A slight increase in ALT activity also occurs in MI. Therefore, determining the activity of two transaminases at once is an important diagnostic test. The ratio of activities is normal AST / ALT (de Ritis coefficient) is 1,33±0,42. With MI, the value of this coefficient increases sharply, in patients with infectious hepatitis, on the contrary, this indicator decreases.

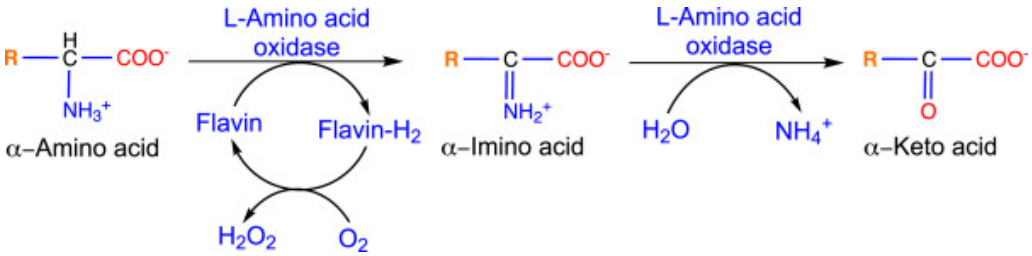
2. Deamination of amino acids.

Direct demining

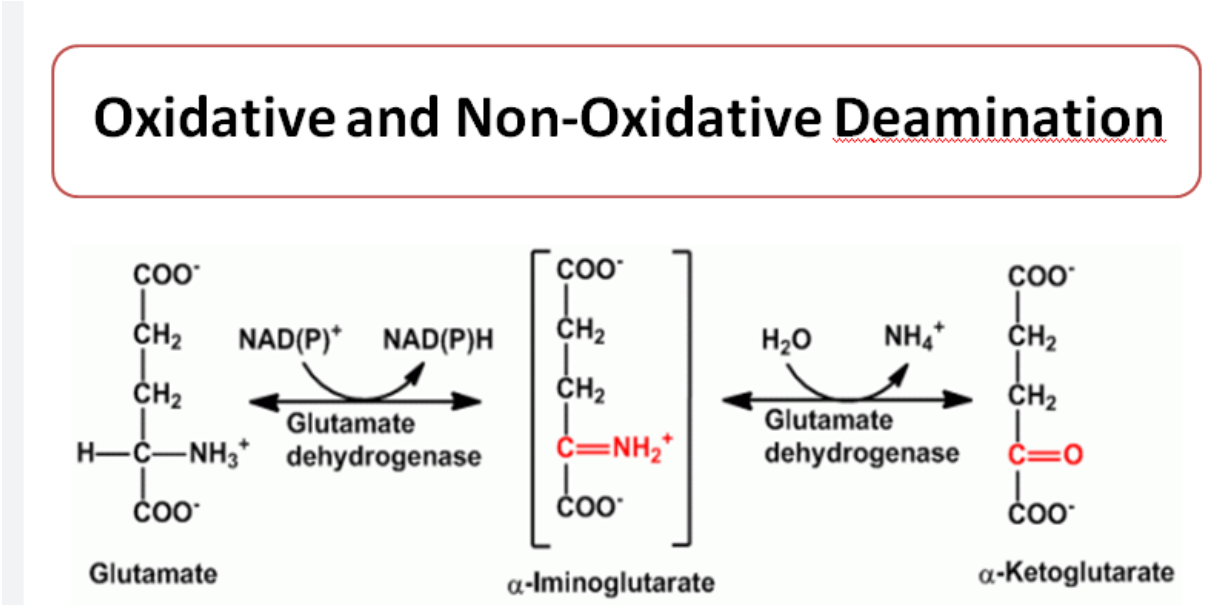
The human body is characterized by a direct process *oxidative deamination*, as a result of which the NH2-group is split off with the formation of NH3, NADH · H + and α- keto acids. Deamination of amino acids occurs most actively in the liver and kidneys.

The process is catalyzed by enzymes *oxidases*, which are flavoproteins. There are oxidases of L- and D-amino acids. L-amino acid oxidases are FMN-dependent, and D-amino acid oxidases are FAD-dependent.

The reaction of oxidative deamination of L-amino acids can be represented schematically as follows:



In the human body, the activity of most amino acid oxidases is extremely low, and the direct oxidative deamination of only L-glutamic acid under the action of glutamate dehydrogenase occurs most actively in cells:



1 - *glutamate dehydrogenase* (can use both NAD+ and NADPH +);

2 - the stage is ongoing *non-enzymatic*.

Schematically, the general reaction equation (this reaction is reversible):

L- glutamate dehydrogenase - the enzyme that catalyzes this reaction has high activity and is widespread in mammalian tissues.

Liver glutamate dehydrogenase is a regulatory enzyme localized in mitochondria. The activity of this enzyme depends on the energy status of the cell. With a lack of energy, the reaction occurs in the direction of the formation of -ketoglutarate and NADH.H +, which are sent to the CTC and oxidative phosphorylation, respectively. As a result, ATP synthesis in the cell increases. Therefore, for glutamate dehydrogenase, the inhibitors are ATP, GTP, NADH, and the activator is ADP.

Indirect demining

Most amino acids are deaminated by indirect deamination - this is a process of combining 2 reactions:

1) transamination of any amino acid with α-ketoglutarate to form glutamate;

2) glutamate dehydrogenase reaction.

In this case, the biological meaning of transamination (1) is to collect the amino groups of all decomposing amino acids in the form of one type of amino acid - glutamate. Next, glutamic acid is transported into mitochondria, where it

undergoes oxidative deamination under the action of glutamate dehydrogenase (2).

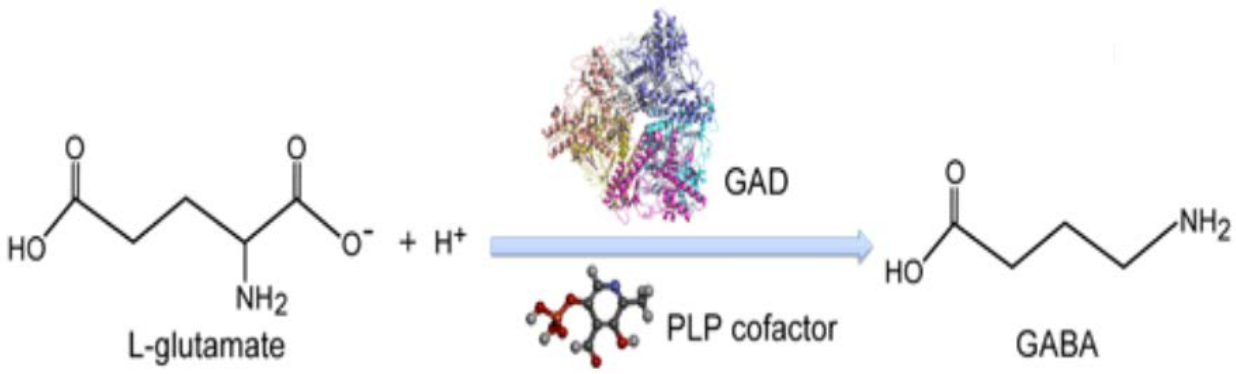
The most active indirect deamination occurs in the liver. NH3 is formed here and enters the urine cycle for elimination.

The directionality of the equilibrium processes of transamination and indirect deamination largely depends on the presence and concentration of amino acids and α- keto acids. With an excess of amino nitrogen, the transformation of amino acids into the corresponding keto acids with their subsequent energetic and plastic utilization is enhanced.

3. Decarboxylation of amino acids

This is the process of splitting off the carboxyl group, which is in α-position of the amino acid, with the formation of amines and CO2. These reactions are catalyzed by decarboxylase enzymes, the coenzyme of which is a derivative of vitB6. As a result of decarboxylation of amino acids, the following are formed:

1) biogenic amines (histamine, dopamine, tyramine,γ- aminobutyric acid - GABA, etc.).



Decarboxylation of amino acids with the formation of biogenic amines occurs most actively in the liver, brain and chromaffin tissue.

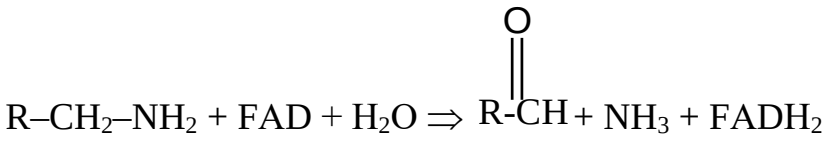
2) products of "protein decay in the intestine", which are the result of decarboxylation of amino acids under the action of intestinal microflora. Toxic products are formed from amino acids.

In total, more than 40 different amines are formed in the human body. An increase in the synthesis of amines is observed during hypoxia and starvation. A local increase in the rate of synthesis, release and inactivation of catecholamines,

histamine and serotonin is characteristic of the focus of inflammation.

Malignant tumors of apodocyte origin, located in the intestines, bronchi, pancreas, can synthesize a large amount of serotonin (using for this purpose up to 60% of the daily need for tryptophan).

Biogenic *amines are inactivated* under the action of oxidative FAD-dependent enzymes - monoamine oxidase (MAO). Oxidative deamination of amines to aldehydes occurs:



Deamination products of biogenic amines - aldehydes - are oxidized to organic acids with the help of aldehyde dehydrogenases. These acids are excreted in the urine or undergo further oxidative degradation. In addition, catechol-O-methyltransferase is involved in the degradation of catecholamines.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

№	Test	Explanation
1.	A 7-year-old child was brought to the emergency hospital in a state of allergic shock that developed after a wasp bite. Increased concentration of histamine in the blood. As a result of which reaction is this amine formed? A. Hydroxylation B. Decarboxylation C. Deamination D. Recovery E. Dehydrogenation	
2.	Hospitalized patient diagnosed with intestinal carcinoid. The analysis revealed increased production of serotonin. It is known that this substance is formed from the amino acid tryptophan. What is the biochemical mechanism underlying this process? A. Formation of paired connections B. Microsomal oxidation C. Deamination D. Transamination E. Decarboxylation	
3.	Decarboxylation of glutamate in the central nervous system produces an inhibitory mediator. Name it: A. Glutathione B. GABA C. Histamine D. Serotonin	

	E. Asparagine	
4.	A biogenic amine is formed from histidine, which has a powerful vasodilating effect. Name it: A. DOFA B. Serotonin C. Dopamine D. Histamine E. Norepinephrine	
5.	During starvation, muscle proteins break down into free amino acids. In what process are these compounds most likely to be included under such conditions? A. Gluconeogenesis in muscles B. Decarboxylation C. Glycogenolysis D. Gluconeogenesis in the liver E. Synthesis of higher fatty acids	
6.	The patient turned to the doctor with complaints of dizziness, memory impairment, periodic convulsions. It was established that the cause of such changes in the patient's body is a decrease in the concentration of the decarboxylation product of glutamic acid. Name it: A. PALF B. GABA C. TDF D. ATP E. THFK	
7.	When testing for hypersensitivity, an allergen was injected subcutaneously into the patient, after which the patient experienced redness, swelling, and pain as a result of the action of histamine. As a result of which transformation	

	of histidine is this biogenic amine formed? A. Decarboxylation B. Methylation C. Phosphorylation D. Isomerization E. Deamination	
8.	According to clinical indicators, the patient was prescribed a drug containing pyridoxal phosphate. What processes is this drug recommended for correction? A. Protein synthesis B. Oxidative decarboxylation of keto acids C. Deamination of purine nucleotides D. Synthesis of purine and pyrimidine bases E. Transamination and decarboxylation of amino acids	
9.	Indicate which of the compounds is an acceptor of amino groups in transamination reactions of amino acids: A. Alpha-ketoglutarate B. Argininosuccinate C. Lactate D. Citrulline E. Ornithine	
10.	An 18-year-old boy with lesions of the liver parenchyma in the blood serum most likely has an increased level of activity: A. Acid phosphatase B. Lactate dehydrogenase (LDH1) C. Creatine kinases D. Alanine aminotransferases E. Alpha amylases	
11.	A newborn has convulsions that pass after	

	taking a drug containing vitamin B6. The described clinical symptom is most likely due to the fact that vitamin B6 is part of the enzyme: A. Glutamate decarboxylase B. Pyruvate dehydrogenases C. Alpha-ketoglutarate dehydrogenases D. Delta-aminolevulinate synthases E. Glycogen phosphorylases	
12.	In the patient, after an attack of acute chest pain within 12 hours, a sharp increase in the activity of AST in the blood serum is determined. Specify the pathology for which this change is characteristic: A. Diabetes B. Viral hepatitis C. Collagenosis D. Myocardial infarction E. Diabetes insipidus	
13.	A 50-year-old woman was admitted to the intensive care unit with a diagnosis of myocardial infarction. The activity of which enzyme will be the most increased during the first two days? A. Aspartate aminotransferases B. Alanine aminotransferases C. Alanine aminopeptidases D. LDH4 E. LDH5	
14.	Biogenic amines: histamine, serotonin, dopamine and others are biologically active substances that affect various physiological functions of the body. As a result of which reaction are biogenic amines formed in body	

	tissues? A. Deamination of amino acids B. Decarboxylation of amino acids C. Transamination of amino acids D. Oxidation of amino acids E. Regenerative amination of amino acids	
15.	The pharmacological effects of antidepressants are associated with their blocking (inhibition) of an enzyme that catalyzes the breakdown of such biogenic amines as norepinephrine and serotonin in the mitochondria of brain neurons. What enzyme is involved in this process? A. Decarboxylase B. Monoamine oxidase C. Transaminase D. Peptidase E. Lyase	
16.	Depression and emotional disorders are the result of a lack of norepinephrine, serotonin and other biogenic amines in the brain. An increase in their content in synapses can be achieved due to antidepressants that inhibit such an enzyme: A. Monoamine oxidase B. D-amino acid oxidases C. L-amino acid oxidases D. Diamine oxidases E. Phenylalanine-4-monooxygenase	
17.	During the examination in the clinic, the man was diagnosed with acute radiation sickness. A	

	sharp decrease in the serotonin content in platelets was established in the laboratory. Violation of the metabolism of which substance is a possible cause of a decrease in platelet serotonin? A. Tyrosine B. Phenylalanine C. Histidine D. 5-oxytryptophan E. Serina	
18.	During the examination of a 56-year-old woman suffering from type 1 diabetes, a violation of protein metabolism was detected, which was manifested by aminoacidemia during a laboratory blood test, and clinically by a delay in wound healing and a decrease in the synthesis of antibodies. Which of the listed mechanisms causes the development of aminoacidemia? A. Decrease in the concentration of amino acids in the blood B. Hyperproteinemia C. Increase in low-density lipoproteins D. Increase in proteolysis E. Increase in oncotic pressure in blood plasma	

4. Literature. Look pic. 229.

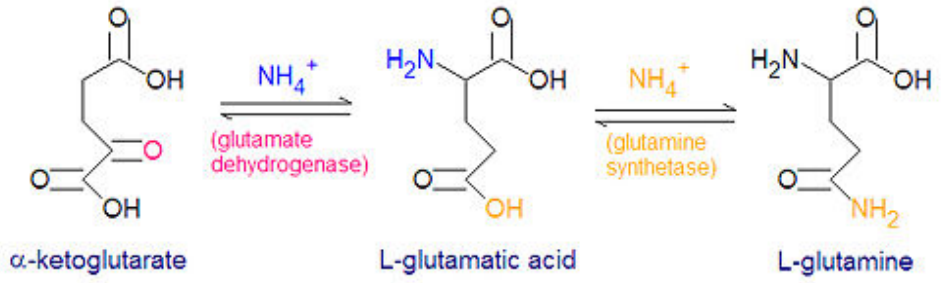
1. TOPIC: WAYS OF AMMONIA DISPOSAL IN THE BODY. EXCHANGE OF INDIVIDUAL AMINO ACIDS. MOLECULAR PATHOLOGIES OF AMINO ACID METABOLISM.

2. INFORMATION MATERIAL.

The result of the processes of deamination and catabolism of amino acids, nucleotides, biogenic amines is the formation of ammonia. In addition, a large amount of ammonia is formed in the intestines during protein decay, as well as in skeletal muscles during increased physical exertion. Ammonia is a toxic substance, therefore there are special ways of its detoxification in the body.

1. Reductive amination.

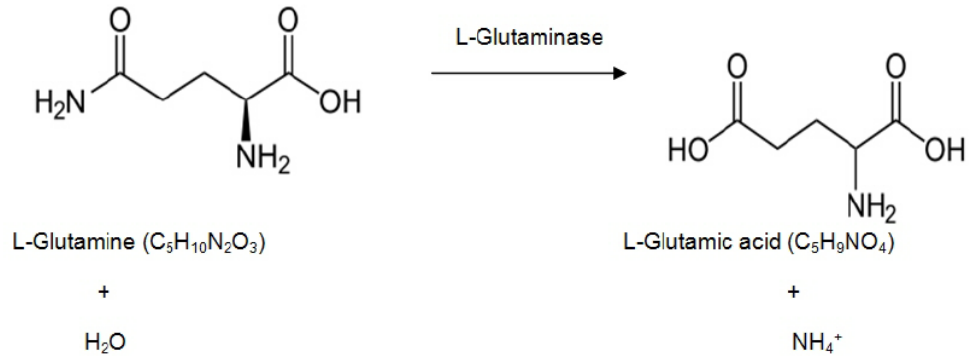
One of the ways of binding and neutralizing ammonia in the body, in particular in the brain, retina, kidneys, liver and muscles, is the biosynthesis of amides of glutamic and aspartic acids (glutamine and asparagine):



This reaction occurs in many tissues, but it is most important for the nervous system, which is especially sensitive to the toxic effects of ammonia. The first reaction is the rotation of the glutamate dehydrogenase reaction (the reverse of the oxidative deamination of HLU).

The neutralization of ammonia by the synthesis of glutamine also has an anabolic value, since glutamine is used for the synthesis of a number of compounds. First of all, it should be noted that glutamine is one of the 20 amino acids included in proteins. In addition, the amide group of glutamine is used for the synthesis of asparagine, glucosamine and other amino sugars, purine and pyrimidine nucleotides. Thus, in these reactions, ammonia nitrogen is included in various structural and functional components of the cell.

Glutamine can then enter all tissues, where it is hydrolyzed with the participation of glutaminase:



In a similar way, the formation of asparagine occurs (through CHOK).

2. Formation of ammonium salts.

Ammonia excretion with urine is normally small - about 0.5 g per day. But it increases several times with acidosis, that is, with an increase in the content of acids in the body.

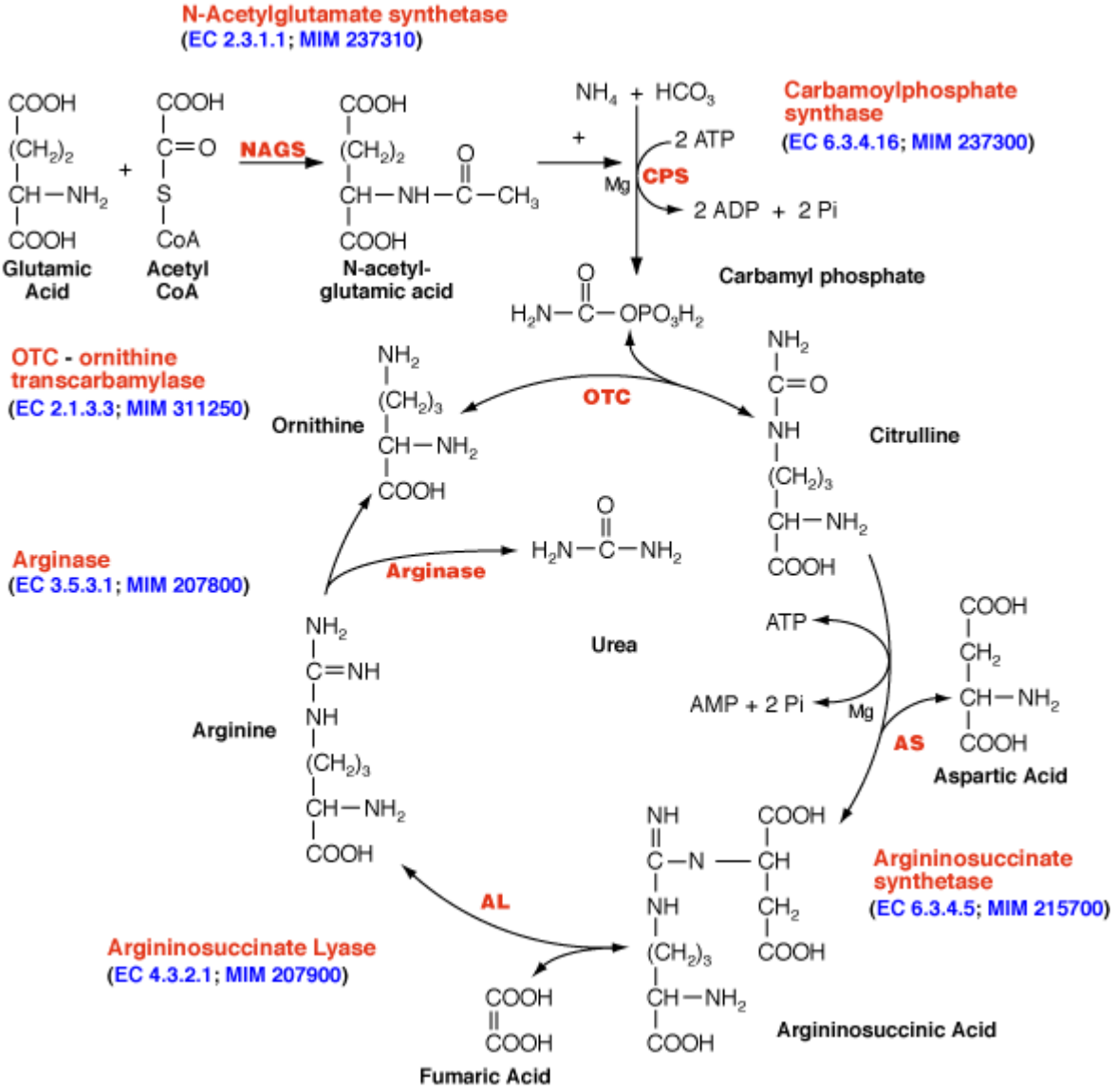
Ammonia in the kidneys is formed mainly due to the amide group of glutamine. Glutamine is hydrolyzed by phosphate-activated glutaminase, which is contained in the epithelial cells of the kidney tubules. Part of ammonia (approximately 30%) is formed in another way - as a result of indirect deamination of amino acids.

Formed ammonia neutralizes acids: $NH_3 + H^+ \rightarrow NH^{4+}$. Non-ionized ammonia and acids in cells are in equilibrium with their ionized forms. Mostly non-ionized ammonia and acids penetrate through the cell membranes, and in the lumen of the renal tubule (that is, already in the urine), ammonia accepts the proton of the acid, forming an ammonium salt, which is excreted from the body. Ammonia excretion by the kidneys serves to remove acids, not nitrogen, which is indicated by a significant rate of excretion in acidosis, a low rate in normal acidity of the intercellular fluid and blood, and the absence of ammonia excretion in alkalosis. At the same time, this process ensures the preservation of ions by the body Na^+, which in the absence of ammonium ions would be removed with acid anions. Losing that much Na+, which is necessary for the removal of acids in acidosis, could cause a decrease in the osmotic pressure of the intercellular fluid and blood, and as a result, a decrease in the volume of the intercellular fluid, that is, tissue dehydration.

3. Urea synthesis.

Urea is excreted from the body with urine as the main end product of protein and, respectively, amino acid metabolism. Urea accounts for 80-85% of all nitrogen excreted from the body. The amount of excreted urea depends on the amount of proteins that come with food. If the daily diet includes 80-100 g of protein, then 25-30 g of urea is formed and excreted per day.

The main site of urea synthesis is the liver. The synthesis of urea is a cyclic metabolic process and is called **ornithine cycle** (pic.50).



Pic 50. Urea synthesis scheme
(https://www.ncbi.nlm.nih.gov/Omim/Images/ureacycleweb.gif)

At the first stage with NH_3 i CO_2 macroergic compound carbamoyl phosphate is synthesized with the participation of ATP.

At the second stage of the urine formation cycle, condensation of carbamoyl phosphate and ornithine occurs with the formation of citrulline. At the next stage, one molecule binds first NH_3 by reductive amination with the formation (with consumption of an ATP molecule) of aspartic acid. Then citrulline and aspartic acid interact with the formation of argininosuccinate, which breaks down into arginine and fumarate with the participation of argininosuccinatlyase. Arginine is split into ornithine and urea by the enzyme arginase.

The formed ornithine can enter the next cycle of urine formation. Although arginine is present in all cells of the human body, the formation of urea occurs exclusively in liver cells - the only organ where the arginase enzyme is localized. Urea from liver cells enters the blood and is excreted from the body through the kidneys.

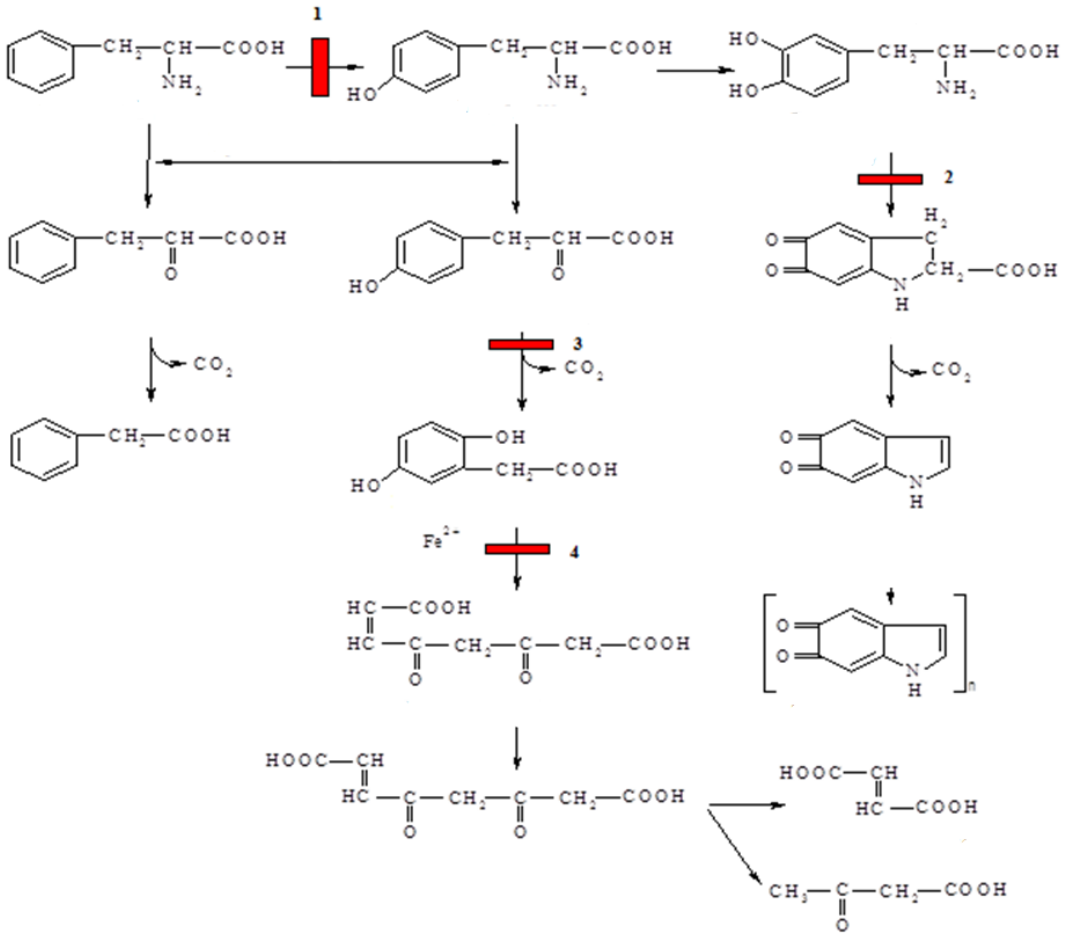
Two molecules are needed to synthesize one molecule of urea NH_3, one molecule CO_2 and three molecules of ATP.

Exchange of individual amino acids

1. Metabolism of aromatic amino acids

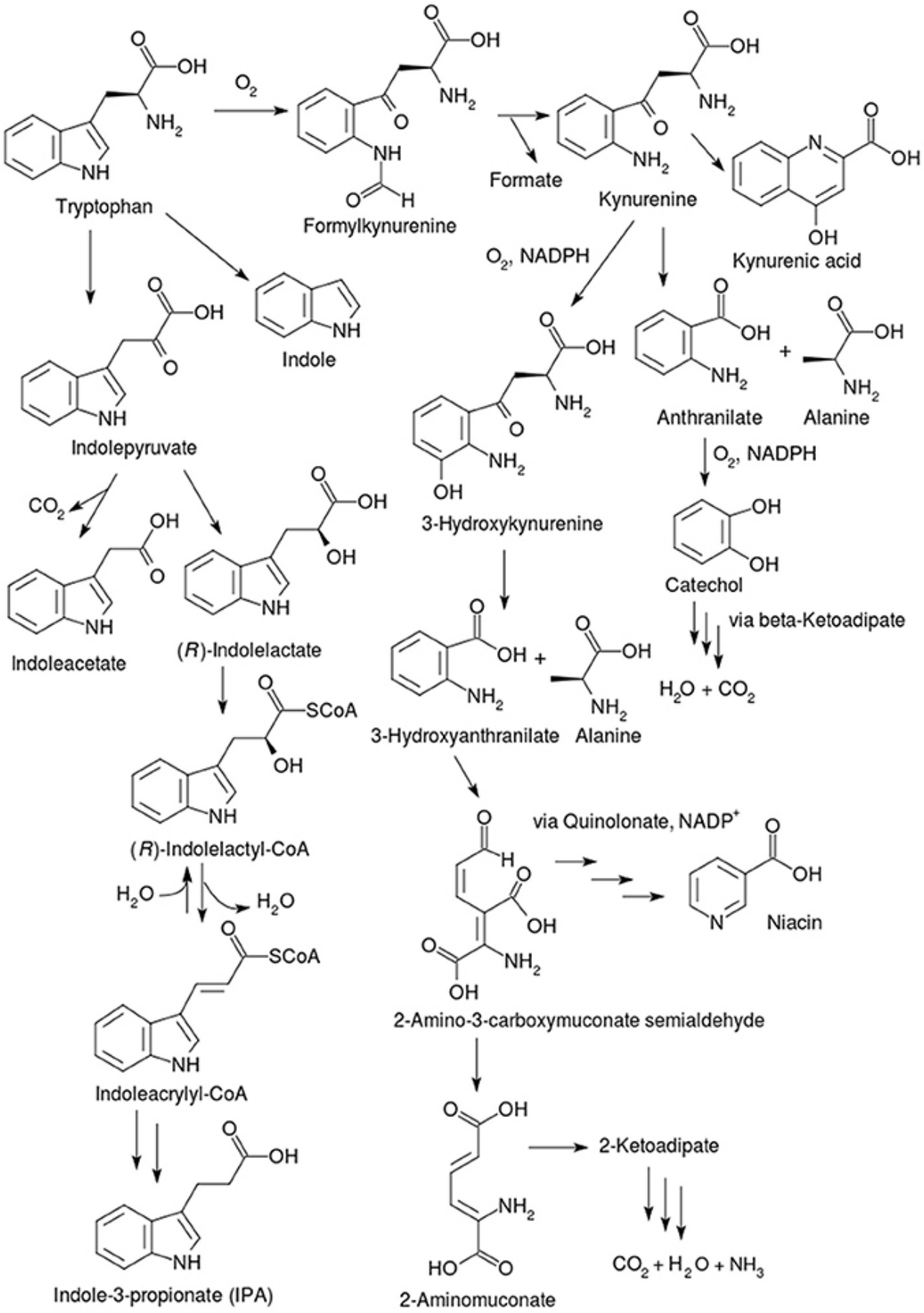
Exchange of natural amino acids

In the body, phenylalanine is used only in the synthesis of proteins. All unused amino acid reserves are converted into tyrosine. The enzyme phenylalanine-4-monooxygenase, which ensures the oxidation of the aromatic ring, is directly involved in this process (pic.51).



pic.51. Basic metabolic transformations of natural amino acids.

The numbers are areas of blocked reactions in phenylketonuria (1), tyrosinosis (2), albinism (3) and alkaptonuria (4).



Hereditary disorders of aromatic amino acids

Phenylketonuria

The cause of the disease is congenital *deficiency of hepatic phenylalanine-4-hydroxylase.* This leads to an increase in the concentration of phenylalanine in the blood and urine, as well as phenylethylamine, phenylpyruvate, phenyllactate, which cause disorders on the part of the central nervous system, which can provoke malformations of the fetus in women. It is considered the simplest method of early diagnosis of phenylketonuria *the Fehling reaction*. The green color of urine when FeCl3 is added is a positive reaction to the presence of phenylpyruvate.

Albinism

This disease is caused by a violation of tyrosine metabolism, and is caused by a defect *tyrosinase* - an enzyme that catalyzes the reaction of melanin pigment formation from tyrosine. The main manifestations of the pathology are depigmentation, accelerated cleavage of rhodopsin, resulting in poor daytime vision, photophobia, photodermatitis.

Alkaptonuria

The cause of the disease is congenital *homogentisic acid oxidase defect*, which takes part in the metabolism of phenylalanine and tyrosine and catalyzes the splitting reaction of homogentisic acid to fumarate and acetoacetate. A characteristic sign is the blackening of urine in the air, this is due to the fact that homogentisic acid is excreted in the urine and upon contact with air turns into alkaptone ("ochronous pigment"). Alkaptone in the body is deposited in the main substance of the cartilages and makes them brittle. Homogentisic acid suppresses *lysyl hydroxylase*, which participates in the synthesis of collagen.

Hartnup's disease

Hereditary pathology caused by a violation of tryptophan metabolism. This disorder refers to a violation of the processes of absorption of tryptophan in the intestine and reabsorption of this and other neutral amino acids in the kidneys. As a result, the following develop: a deficiency of vitamin PP, which can provoke the development of pellagra.

2. Metabolism of sulfur-containing amino acids.

Exchange of methionine

Methionine - it is an essential amino acid that is the main donor of methyl groups in methylation reactions.

The active form is S-adenosylmethionine (SAM), the reaction of which is formed below:

Met + ATP→ S-Adenosylmethionine + FFn + Fn

Enzyme - *methionine adenosyl transferase.*

SAM participates in methylation reactions during the synthesis of: choline, creatine, adrenaline, melanin, nucleotides, plant alkaloids. After transfer CH_3-group SAM turns into S-adenosylhomocysteine, which as a result of successive reactions is reduced to methionine

This cyclic process cannot function without a constant supply of Met because Met is consumed in catabolism reactions.

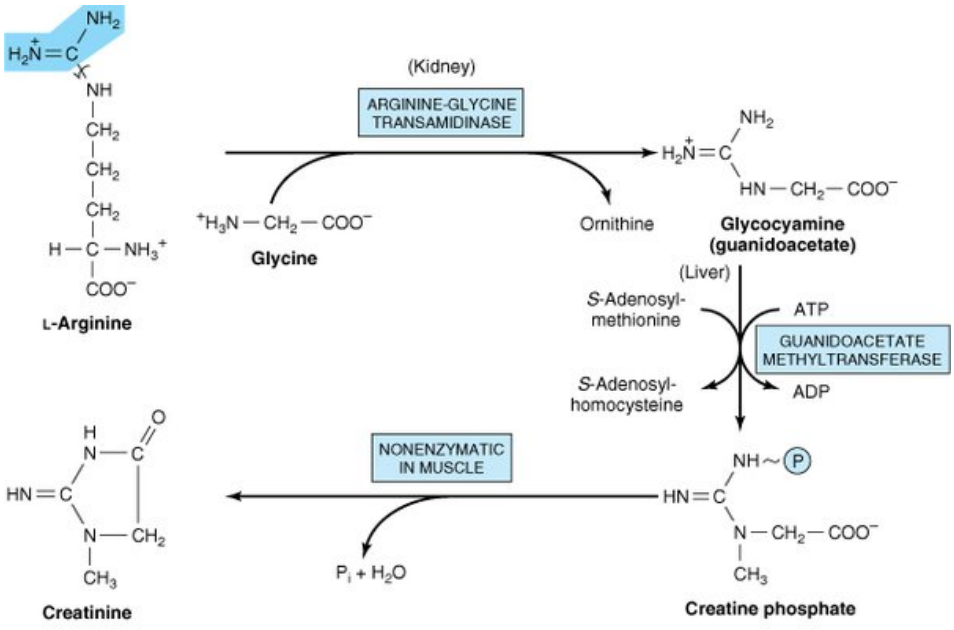
Methionine as a donor of methyl groups participates in the synthesis of creatine.

Creatine synthesis

Creatine is the main substrate for the formation of creatine phosphate in muscles and nervous tissue. Creatine synthesis occurs sequentially in the kidneys and liver (some of it can be synthesized in the pancreas).

There are two stages of synthesis:

1. Occurs in the kidneys.



(https://qph.cf2.quoracdn.net/main-qimg-70d1c324fe3a1e240021b4d913350443-

pjlq)

2. Occurs in the liver after transport from the kidneys of glycocyamine.

Next, creatine is phosphorylated to form macroergic phosphate - creatine phosphate, which is a form of energy storage in muscles and the nervous system. An enzyme that catalyzes this reaction, - *creatine phosphokinase (KFC)*

Cysteine metabolism

Cysteine - it is a replaceable amino acid, the main role of which is as follows:

1) takes part in stabilizing the structure of proteins and peptides - forms disulfide bonds;

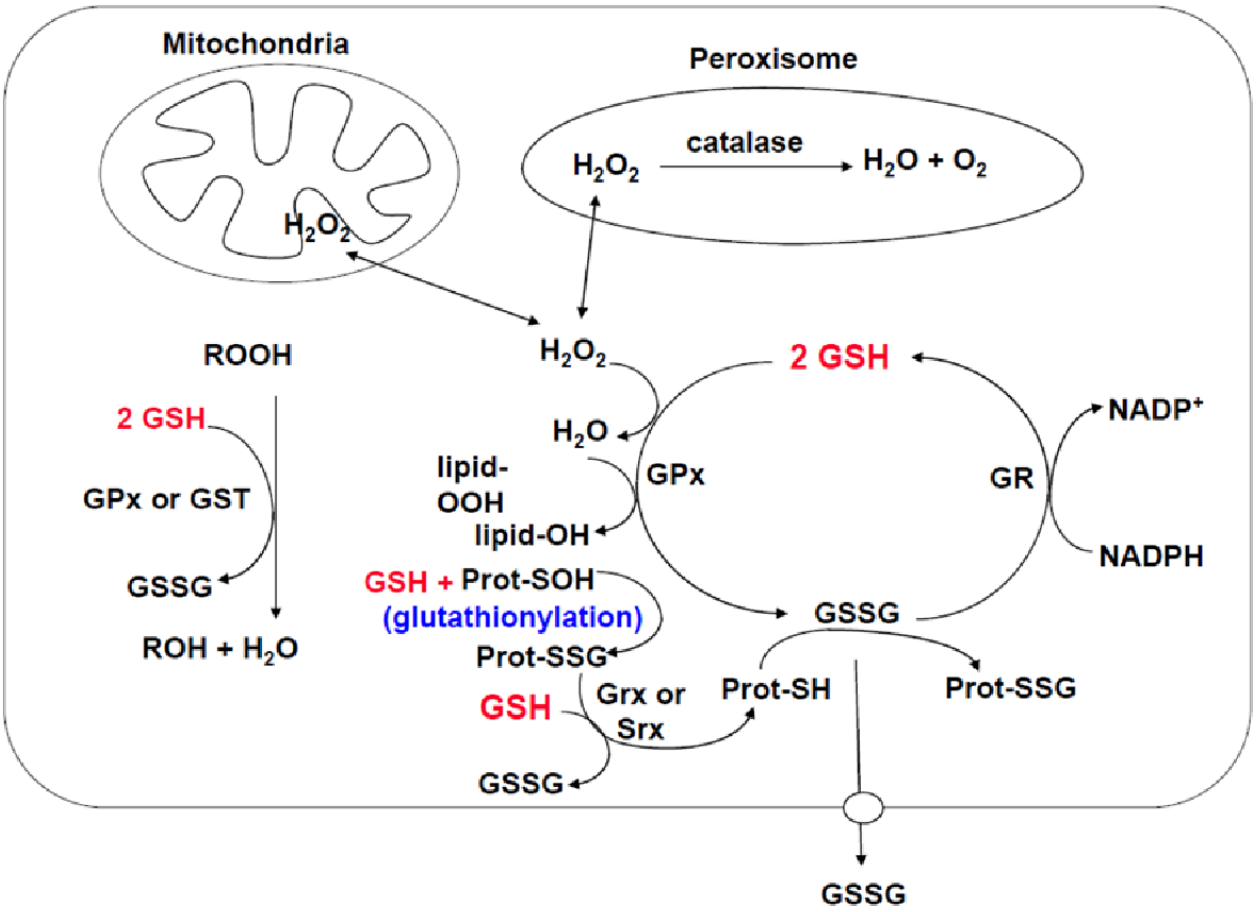
2) is a structural component of the glutathione tripeptide (glu-cis-gly), which as a coenzyme participates in the functioning of the body's antioxidant system, the transport of some amino acids through the membrane, the reduction of ascorbic acid from dehydroascorbic acid, etc.

Glutathione - it is a coenzyme of such an oxidoreductase as glutathione peroxidase. This selenium-containing enzyme catalyzes the reaction *detoxification of organic peroxides.* This is an important mechanism to prevent lipid peroxidation, which can be stimulated by radiation or xenobiotics. glutathione is an intracellular antioxidant;

3) during cis catabolism, pyruvate is formed, which is used as a substrate for gluconeogenesis, i.e. cis is a glycogen amino acid;

4) participates in the synthesis of taurine - a physiologically important compound that is necessary for the formation of paired bile acids, can perform the function of a mediator in the central nervous system and is important in the functioning of the myocardium.

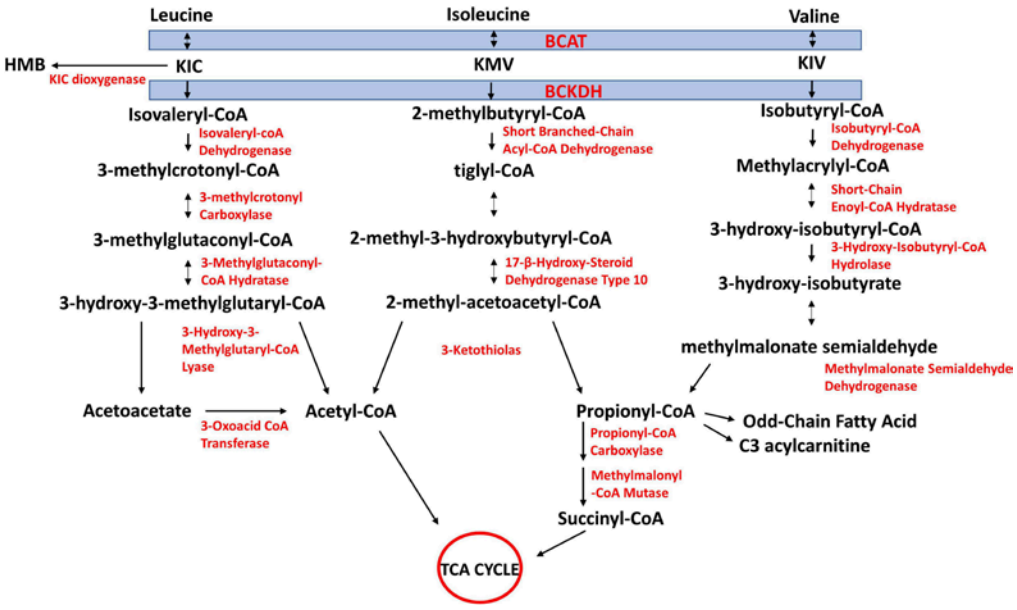
The biosynthesis of glutathione is presented in the diagram



(https://www.researchgate.net/publication/372349375/figure/fig3/AS:11431281174783398@1689338565362/Phospholipid-Biogenesis-Phosphatidic-acid-is-involved-in-phospholipid-biogenesis-through.png)

3. Metabolism of branched chain amino acids

Catabolism of amino acids with a branched chain: leucine, isoleucine and valine - is mainly carried out not in the liver (the place of decomposition of most other amino acids), but in muscle and fat tissues, in the kidneys and brain tissues. First, all three amino acids undergo transamination with α-ketoglutarate under the action of one general and specific enzyme - aminotransferase of amino acids with a branched chain (not contained in the liver) with the formation of the corresponding α-ketoacids. Further oxidative decarboxylation of α-keto acids leads to the formation of acyl-CoA derivatives. Coenzymes are biotin and deoxyadenosylcobalamin.



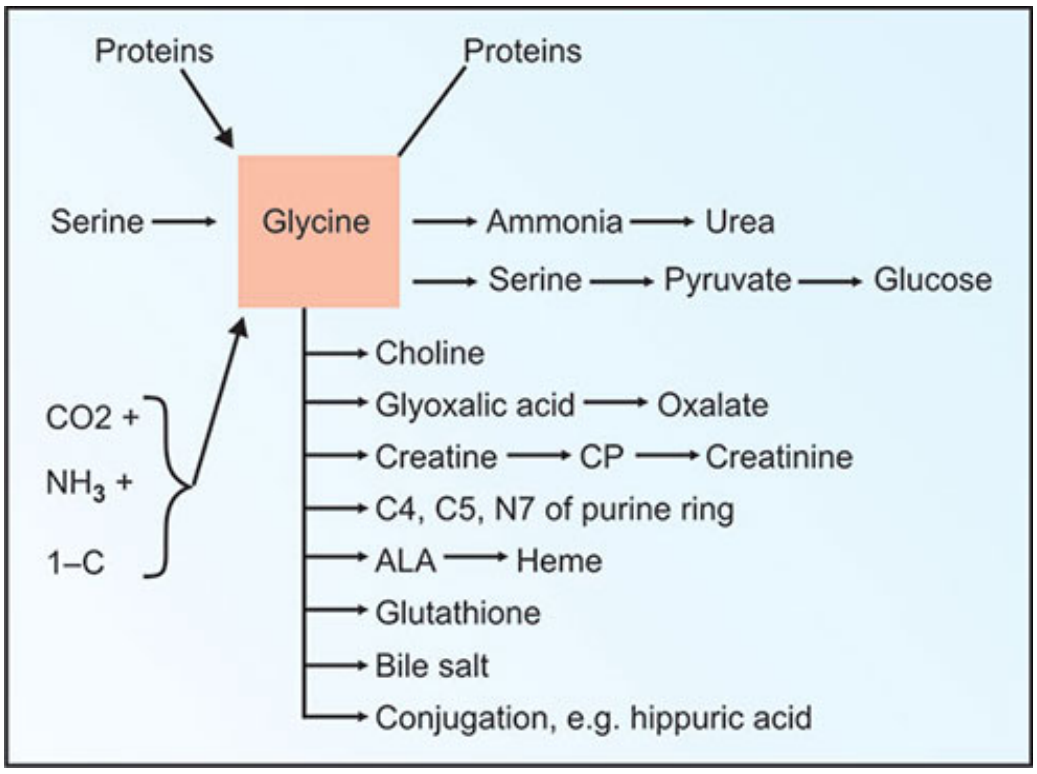
(https://www.frontiersin.org/files/Articles/342220/fmolb-05-00029-HTML/image_m/fmolb-05-00029-g005.jpg)

In case of shortage B_{12} the conversion of methylmalonyl-CoA into succinyl-CoA is disturbed, as a result of which a large amount of methylmalonic acid is excreted in the urine - *methylmalonic aciduria*. Methylmalonic acid is toxic to nervous tissue and, in the absence of treatment, causes degeneration of the posterolateral trunks of the spinal cord. It should also be noted that the enzyme that catalyzes the oxidative decarboxylation of the above α-keto acids, highly specific.

The **hereditary disease "maple syrup disease"** is known, in which the decarboxylation of the specified α-keto acids is disturbed, which leads not only to the accumulation of amino acids in the blood and α- keto acids, and to their excretion with urine, which has the smell of maple syrup.

4. Metabolism of glycine

Glycine - it is a substitute amino acid that can be formed in the human body from serine, threonine, choline, etc. substances Glycine is part of many biologically important peptides and proteins (insulin, thyroglobulin, albumins, globulins, collagen, etc.). In the human body, this amino acid is used for the synthesis of a number of metabolites. Schematically, these processes are depicted below:


Proteins
Proteins
Serine
Glycine
Ammonia
Urea
Serine
Pyruvate
Glucose
Choline
Glyoxalic acid
Oxalate
Creatine
CP
Creatinine
C4, C5, N7 of purine ring
ALA
Heme
Glutathione
Bile salt
Conjugation, e.g. hippuric acid
CO2 +
NH3 +
1–C

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

1.	A brain injury caused an increased formation of ammonia. Which amino acid is involved in the removal of ammonia from brain tissue? A. Tyrosine B. Glutamic acid C. Valin D. Lysine E. Tryptophan	
2.	Ammonia is a poisonous substance, especially for the nervous system. What substance takes an especially active part in the neutralization of ammonia in brain tissues? A. Lysine B. Proline C. Histidine D. Glutamic acid E. Alanine	
3.	A 4-year-old boy has vomiting, loss of consciousness, convulsions after suffering severe viral hepatitis. Hyperammonemia in the blood. Violation of which biochemical process caused the pathological condition of the patient? A. Violation of ammonia neutralization in the liver B. Violation of neutralization of biogenic amines C. Inhibition of transamination enzymes D. Increasing the decay of proteins in the intestines E. Activation of decarboxylation of amino acids	

4.	*A decrease in the intensity of sucking, frequent vomiting, and hypotension are observed in a newborn child. The concentration of citrulline is significantly increased in the urine and blood. What metabolic process is disturbed?* *A. CTC* *B. Gluconeogenesis* *C. Cory cycle* *D. Glycolysis* *E. Ornithine cycle*	
5.	The main mass of nitrogen is excreted from the body in the form of urea. A decrease in the activity of which enzyme in the liver leads to the inhibition of urea synthesis and the accumulation of ammonia in the blood and tissues? A. Aspartate aminotransferases V. Amylase S. Urease D. Pepsin E. Carbamoyl phosphate synthases	
6.	A two-year-old child with delayed mental and physical development, suffering from frequent vomiting after eating, was brought to the hospital. Phenylpyruvic acid was detected in the urine. This pathology is the result of a violation of which exchange? A. Lipid metabolism B. Amino acid metabolism C. Carbohydrate metabolism D. Water-salt exchange E. Phosphorus-calcium metabolism	
7.	Methyl groups (-CH3) are used in the body for the synthesis of such important compounds as	

	creatine, choline, adrenaline, etc. Which of the essential amino acids is the source of these groups? A. Valin B. Leucine C. Tryptophan D. Isoleucine E. Methionine	
8.	Albinos do not tolerate sunburn well, they get burns. Violation of the metabolism of which amino acid is the basis of this phenomenon? A. Histidine V. Tryptophan S. Phenylalanine D. Glutamic acid E. Methionine	
9.	A patient came to the doctor with complaints of intolerance to solar radiation. There are skin burns and visual disturbances. Previous diagnosis: albinism. A violation of the metabolism of which amino acid is noted in this patient? A. Proline B. Tryptophan C. Alanine D. Tyrosine E. Lysin	
10.	When examining the child, the pediatrician noted a lag in physical and mental development. The urine has a sharply increased content of ketoacids, which gives a high-quality color reaction with iron chloride. What metabolic disorder was detected? A. Cystinuria	

	B. Tyrosinemia C. Phenylketonuria D. Alkaptonuria E. Albinism	
11.	A 13-year-old boy complains of general weakness, dizziness, and fatigue. There is a lag in mental development. The examination revealed a high concentration of valine, isoleucine, leucine in the blood and urine. Urine with a specific smell. What is the most likely diagnosis? A. "Maple syrup" disease B. Histidinemia C. Tyrosinosis D. Based's disease E. Addison's disease	
12.	A 6-month-old child has a sharp delay in psychomotor development, seizures, pale skin with eczematous changes, blond hair, blue eyes. This child is most likely to be diagnosed based on the results of determining the concentration in blood and urine: A. Leucine B. Histidine C. Tryptophan D. Valina E. Phenylpyruvate	
13.	In a child with mental retardation, a green color of urine was detected after adding 5% FeCl3 solution. The positive result of this diagnostic test indicates a violation of the metabolism of which amino acid? A. Arginine	

	B. Tyrosine C. Glutamine D. Phenylalanine E. Tryptophan	
14.	The 10-month-old child, whose parents are brunettes, has blond hair, very fair skin, and blue eyes. From the outside, she looked normal at birth, but during the last 3 months there were disorders of cerebral blood circulation, retardation in mental development. The reason for this condition can be: A. Acute porphyria B. Histidinemia C. Glycogenosis D. Phenylketonuria E. Galactosemia	
15.	The baby has discoloration of the sclera and mucous membranes. Urine is secreted, which darkens in the air. Homogentisic acid was detected in the blood and urine. What can be the cause of this condition? A. Cystinuria B. Histidinemia C. Alkaptonuria D. Galactosemia E. Albinism	
16.	Dark spots were found on the newborn's diapers, indicating the formation of homogentisic acid. What substance is it related to a metabolic disorder? A. Cholesterol	

	B. Tryptophan C. Methionine D. Tyrosine E. Galactose	
17.	In a 1.5-year-old child, there is a delay in mental and physical development, lightening of the skin and hair, a decrease in the content of catecholamines in the blood. When adding a few drops of a 5% solution of iron trichloroacetate to fresh urine, an olive-green color appears. For which pathology of amino acid metabolism are these changes characteristic? A. Xanthinuria B. Phenylketonuria C. Alkaptonuria D. Albinism E. Tyrosinosis	
18.	The baby refuses to breastfeed, the breathing is irregular, the urine has the smell of "beer sourdough" or "maple syrup". Congenital defect of which enzyme caused this pathology? A. Dehydrogenases of branched alpha-keto acids B. Aspartate aminotransferases C. UDP-glucuronyltransferases D. Glucose-6-phosphate dehydrogenases E. Glycerol kinases	
19.	A patient with a diagnosis of "malignant carcinoid" has a sharply increased serotonin content in the blood. From which amino acid can this biogenic amine be formed? A. Threonine	

	B. Methionine C. Alanine D. Tryptophan E. Leucine	
20.	The parents of a 3-year-old child noticed the darkening of the child's urine when standing. The child's body temperature is normal, the skin is pink and clean, the liver is not enlarged. Name the probable cause of this condition. A. Alkaptonuria B. Hemolytic anemia C. Itsenko-Cushing syndrome D. Phenylketonuria E. Gout	
21.	The patient was diagnosed with alkaptonuria. Specify the enzyme whose defect is the cause of this pathology: A. Pyruvate dehydrogenase B. Homogentisic acid oxidase C. Glutamate dehydrogenase D. DOPA-decarboxylase E. Phenylalanine hydroxylase	
22.	A high content of all amino acids of the aliphatic series was found in the urine of a 12-year-old boy. At the same time, the highest excretion of cystine and cysteine was noted. In addition, ultrasound of the kidneys showed the presence of stones in them. What pathology is most likely? A. Cystinuria B. Alkaptonuria C. Phenylkeptonuria D. Hartnup's disease E. Cystitis	

23.	A person fell ill with pellagra. During the interview, it became known that for a long time she ate mainly corn, she did not eat much meat. The deficiency of which substance in corn led to the development of the disease? A. Proline B. Alanine C. Histidine D. Tyrosine E. Tryptophan	
24.	In the urine of a newborn, citrulline and a high level of ammonia are determined. Indicate the formation of which substance is most likely disturbed in this child: A. Creatine B. Ammonia C. Uric acid D. Urea E. Creatinine	
25.	The cause of pellagra disease can be a predominant diet of corn and a decrease in the diet of products of animal origin. The absence of which amino acid in the diet leads to this pathology? A. Isoleucine B. Phenylalanine C. Tryptophan D. Histidine E. Methionine	
26.	A five-year-old child's mother found that urine was too dark. The child does not complain about anything. No pathological bile pigments were found in the urine. A diagnosis was made: alkaptonuria. Which enzyme is deficient in the child's body?	

	A. Homogentisic acid oxidases B. Phenylalanine hydroxylases C. Tyrosinases D. Oxyphenylpyruvate oxidases E. Phenylpyruvate decarboxylase	
27.	Ammonia is especially toxic to the human central nervous system. Indicate the main way of its neutralization in nervous tissue: A. Synthesis of ammonium salts B. Synthesis of glutamine C. Urea synthesis D. Transamination E. Formation of paired connections	
28.	With repeated exposure to ultraviolet rays, the skin darkens due to the synthesis of melanin in it, which protects skin cells from damage. The main mechanism of turning on melanin synthesis is: A. Activation of tyrosinase B. Inhibition of tyrosinase C. Activation of homogentisic acid oxidase D. Inhibition of homogentisic acid oxidase E. Inhibition of phenylalanine hydroxylase	
29.	In humans, the feeling of fear is formed in the limbic system of the brain due to the formation of the substance dioxyphenyl-alanine (DOPA). This substance is formed from: A. Tyrosine B. Glutamic acid C. Tryptophan D. Lysine E. 5-oxytryptophan	
30.	When determining the residual nitrogen of the blood, it was established that the nitrogen of urea	

	is significantly reduced in content. For the disease, which organ is this characteristic? A. Liver B. Brain C. Hearts D. Intestines E. Stomach	
31.	A 9-year-old boy with mental and physical retardation is being examined in the hospital. The boy's blood has a significantly increased phenylalanine content. Blocking the activity of which enzyme can lead to such disorders? A. Phenylalanine-4-monooxygenase B. Homogentisic acid oxidases C. Glutamine transaminases D. Aspartate aminotransferases E. Glutamate decarboxylase	
32.	The baby has an increased concentration of phenylpyruvic acid in the blood (phenylketonemia). What type of treatment is necessary for phenylketonemia? A. Vitamin therapy B. Diet therapy C. Enzyme therapy D. Antibacterial therapy E. Hormone therapy	
33.	A 19-year-old boy has clear signs of skin depigmentation due to a violation of melanin synthesis. Indicate which amino acid metabolism is disturbed in the skin? A. Glycine B. Tryptophan C. Histidine D. Proline	

	E. Tyrosine	
34.	In a patient with a long-term seizure of epilepsy, ammonia is formed in the center of excitation as a result of the breakdown of biogenic amines, which is neutralized in the brain with the participation of: A. Serina B. Uric acid C. Aminobutyric acid D. Glutamic acid E. Lipoic acid	
35.	An excess of phenylpyruvate and phenylacetate was detected in the baby's urine on the 6th day of life. The exchange of which amino acid is disturbed in this child? A. Arginine B. Tryptophan C. Methionine D. Histidine E. Phenylalanine	
36.	To prevent fatty infiltration of the liver, the patient was prescribed a drug containing a donor of methyl groups. This is likely: A. S-adenosylmethionine B. Cholesterol C. Bilirubin D. Valin E. Glucose	
37	One form of congenital pathology is accompanied by inhibition of the conversion of phenylalanine into tyrosine. A biochemical sign of the disease is the accumulation of some organic acids in the body, in particular: A. Molochnoi	

	B. Glutaminova	
	C. Pyrovinogradnoi	
	D. Phenylpyruvinogradnoy	
	E. Limonnaya	

4. Literature. Look pic. 229.

1. TOPIC: SPECIALIZED PATHWAYS OF AMINO ACID METABOLISM. MOLECULAR PATHOLOGIES OF AMINO ACID METABOLISM. AMINO ACIDS AS PHARMACEUTICAL AGENTS

2. INFORMATION MATERIAL.

Proteins in the body are not permanent structures—they are continuously synthesized and degraded. The reasons for protein degradation include:

- Protein renewal – removal of old or damaged proteins.
- Regulation of cellular processes – control of enzyme and signaling molecule levels.
- Source of amino acids – for the synthesis of new proteins.
- Adaptation to environmental changes – for example, during fasting.

Main Mechanisms of Protein Degradation

1) Ubiquitin-Proteasome Pathway (UPS)

🔹 This is the primary mechanism for protein breakdown in the cytoplasm and nucleus.

🔹 It is used for the rapid degradation of regulatory, damaged, or abnormal proteins.

Key steps:

1. Ubiquitination – a special protein called ubiquitin attaches to the target protein.
2. Recognition by the proteasome – the ubiquitinated protein enters the 26S proteasome.
3. Degradation – the proteasome cuts the protein into peptides.
4. Ubiquitin recycling – ubiquitin detaches and is reused.

2) Lysosomal Degradation (Autophagy)

🔹 Works for the bulk degradation of organelles, long-lived proteins, and extracellular material.

🔷 Key enzymes – cathepsins (proteolytic lysosomal enzymes).

🔷 Processes:

- Microautophagy – the lysosome engulfs small protein particles.
- Macroautophagy – an autophagosome forms and fuses with the lysosome.
- Chaperone-mediated autophagy – proteins are recognized by specialized chaperones.

What is the intracellular amino acid pool?

🔷 It is the reserve of free amino acids in the cell, used for protein synthesis, energy metabolism, and biosynthetic reactions.

Sources of the Intracellular Amino Acid Pool

1. Degradation of intracellular proteins (ubiquitin-proteasome and lysosomal pathways).
2. Dietary intake of amino acids (protein breakdown in the digestive tract).
3. De novo amino acid synthesis (for non-essential amino acids).
4. Transamination – transfer of amino groups between molecules to create new amino acids.

Phenylalanine Metabolism

Phenylalanine is an essential amino acid used for:

1. Tyrosine synthesis (enzyme: phenylalanine hydroxylase, cofactor – tetrahydrobiopterin).
2. Neurotransmitter synthesis via tyrosine.
3. Melanin and thyroid hormone synthesis.

🔷 Phenylalanine → Tyrosine → Catecholamines (dopamine, adrenaline)

Genetic Disorders of Phenylalanine and Tyrosine Metabolism

1. Phenylketonuria (PKU)
    - Defect in phenylalanine hydroxylase → accumulation of phenylalanine → toxic effects on the brain.
    - Treatment: Low-phenylalanine diet.
2. Albinism

- Deficiency of tyrosinase → impaired melanin synthesis → lack of pigmentation.

3. Alkaptonuria
    - Deficiency of homogentisate oxidase → dark urine, joint damage.

Main Pathways of Tryptophan Metabolism

1. Serotonin Synthesis
    - Tryptophan → 5-Hydroxytryptophan → Serotonin
    - Serotonin regulates mood, sleep, and appetite.
2. Melatonin Synthesis
    - Serotonin → Melatonin
    - Melatonin regulates circadian rhythms and sleep.
3. Nicotinamide (Vitamin B3) Synthesis
    - Tryptophan → Kynurenine

Genetic Disorders of Tryptophan Metabolism

1. Pellagra
    - Vitamin B3 deficiency → symptoms: dermatitis, diarrhea, dementia.
2. Hartnup Disease
    - Impaired tryptophan transport in the intestines → nicotinamide deficiency.

Methionine Metabolism

Methionine is an essential amino acid that plays a key role in methylation reactions (via S-adenosylmethionine, SAM).

1. Activation of Methionine

- Methionine + ATP → S-Adenosylmethionine (SAM) – a major methyl group donor in biochemical reactions.
- SAM transfers a methyl group to other molecules (e.g., in adrenaline synthesis, DNA methylation).

2. Conversion of Methionine to Homocysteine

- After losing a methyl group, SAM is converted to S-adenosylhomocysteine, then to homocysteine.

3. Two Possible Pathways for Homocysteine Metabolism

1. Recycling back to methionine (requires vitamins B9, B12)
    - Homocysteine → Methionine (enzyme: methionine synthase, cofactor: vitamin B12).
2. Conversion to cysteine
    - Homocysteine + Serine → Cystathionine (enzyme: cystathionine synthase, cofactor: vitamin B6).
    - Cystathionine → Cysteine.

Cysteine Metabolism

🔻 Cysteine is used for the synthesis of:

- Glutathione – a powerful antioxidant.
- Taurine – important for bile and cardiovascular function.
- Keratin – a structural protein in hair and nails.

Glycine Metabolism

Glycine is the simplest amino acid and is involved in:

- Porphyrin synthesis (heme in hemoglobin).
- Purine synthesis (building blocks of DNA and RNA).
- Creatine synthesis (important for muscles and energy metabolism).
- Inhibitory neurotransmission in the CNS (glycine is an inhibitory neurotransmitter).

Genetic Disorders

1. Hyperhomocysteinemia – high blood homocysteine due to methionine cycle enzyme defects.
    - Complications: thrombosis, atherosclerosis, neurological disorders.
    - Treatment: Vitamin B6, B9, B12 supplements.
2. Cystathioninuria – defective cystathionine synthase → impaired cysteine formation.

Metabolism of Branched-Chain Amino Acids (BCAAs)

Valine, leucine, and isoleucine are essential amino acids primarily used in skeletal muscles for energy metabolism.

Main Steps of BCAA Catabolism

1. Transamination (conversion to α-keto acids)
    - Enzyme: branched-chain aminotransferase.
2. Oxidative decarboxylation (conversion to acyl-CoA compounds)
    - Enzyme: branched-chain α-keto acid dehydrogenase.
3. Further conversions:
    - Valine → Succinyl-CoA (enters the Krebs cycle).
    - Leucine → Acetoacetate and Acetyl-CoA (ketogenic pathway).
    - Isoleucine → Succinyl-CoA and Acetyl-CoA.

Genetic Disorders

1. Maple Syrup Urine Disease (MSUD)
    - Deficiency of branched-chain α-keto acid dehydrogenase.
    - Accumulation of unmetabolized BCAAs → neurotoxicity.
    - Symptoms: Urine smells like maple syrup, seizures, lethargy.
    - Treatment: BCAA-restricted diet.

Glutathione Synthesis and Function

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine, and glycine.

Functions:

1. Antioxidant defense – neutralizes free radicals.
2. Detoxification – removes heavy metals and toxins.
3. Immune regulation – supports lymphocyte function.
4. Protection of red blood cells – prevents oxidative stress.

Amino Acids Used in Dentistry

1. Arginine – Reduces tooth sensitivity, improves gum circulation.
2. Glycine – Used in powder-based dental cleaning products.
3. Cysteine – Antioxidant for gum protection, helps remove dental plaque.

3. TASKS FOR INDEPENDENT WORK.

In the table with test tasks, underline the key words, choose the correct answer and justify it:

1.Examination of a patient suffering from cancer of urinary bladder revealed high rate of serotonin and hydroxyanthranilic acid. It is caused by excess of the following amino acid in the organism: A. Tryptophan. B. Alanine. C. Histidine. D. Methionine. E. Tyrosine.	
2.Ammonia is a very toxic substance, especially for nervous system. What substance takes the most active part in ammonia detoxication in brain tissues? A. Glutamic acid. B. Lysine. C. Proline. D. Histidine. E. Alanine	
3.A child has an acute renal failure. What biochemical factor found in saliva can confirm this diagnosis? A. Increase in urea concentration.	

B. Increase in glucose concentration. C. Decrease in glucose concentration. D. Increase in concentration of higher fatty acids. E. Increase in nucleic acid concentration.	
4.After severe viral hepatitis a 4 year old boy presents with vommiting, occasional loss of consciousness, convulsions. Blood test revealed hyperammoniemi Such condition is caused by a disorder of the following biochemical hepatic process: A. Disorder of ammonia neutralization. B. Disorder of biogenic amines neutralization. C. Protein synthesis inhibition. D. Activation of amino acid decarboxylation. E. Inhibition of transamination enzymes.	
5.A 4 y.o. boy has had recently serious viral hepatitis. Now there are such clinical presentations as vomiting, loss of consciousness, convulsions. Blood analysis revealed	

hyperammoniemia. Disturbunce of which biochemical process caused such pathological condition of the patient? A. Disturbed neutralization of ammonia in liver. B. Disturbed neutralization of biogenic amines. C. Increased putrefaction of proteins in bowels. D. Activation of aminoacid decarboxylation. E. Inhibition of transamination enzymes.	
6.The greater amount of nitrogen is excreted from the organism in form of urea. Inhibition of urea synthesis and accumulation of ammonia in blood and tissues are induced by the decreased activity of the following liver enzyme: A. Carbamoyl phosphate synthetase. B. Aspartate aminotransferase. C. Urease. D. Amylase. E. Pepsin.	
7.After a serious viral infection a 3-	

year-old child has repeated vomiting, loss of consciousness, convulsions. Examination revealed hyperammoniemia. What may have caused changes of biochemical blood indices of this child? A. Disorder of ammonia neutralization in ornithinic cycle. B. Activated processes of aminoacids decarboxylation. C. Disorder of biogenic amines neutralization. D. Increased purtefaction of proteins in intestines. E. Inhibited activity of transamination enzymes.	
8..A newborn child was found to have reduced intensity of sucking, frequent vomiting, hypotonia.Urine and blood exhibit increased concentration of citrullin What metabolic process is disturbed? A. Ornithinic cycle. B. Tricarboxylic acid cycle. C. Glycolysis. D. Glyconeogenesis. E. Cori cycle.	
9.Cerebral trauma caused increase of ammonia formation. What	

aminoacid takes part in removal of ammonia from cerebral tissue? A. Glutamic. B. Tryptophan. C. Lisine. D. Valine. E. Tyrosine.	
10.A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B6 concentration. The infant presents with pellagral dermatitis, convulsions, anaemia. Convulsion development might be caused by the disturbed formation of: A. GABA. B. Histamine. C. Serotonin. D. DOPA. E. Dopamine.	
12.A male patient has been diagnosed with acute radiation disease. Laboratory examination revealed a considerable reduction of platelet serotonin level. The likely cause of platelet serotonin reduction is the disturbed metabolism of the following substance: A. 5-oxytryptofane.	

B. Tyrosine. C. Histidine. D. Phenylalanine. E. Serine.	
13.Pharmacological effects of antidepressants are connected with inhibition of an enzyme catalyzing biogenic amines noradrenaline and serotonine in the mitochondrions of cerebral neurons. What enzyme participates in this process? A. Monoamine oxidase. B. Transaminase. C. Decarboxylase. D. Peptidase. E. Lyaze	
14. During hypersensitivity test a patient got subcutaneous injection of an antigen which caused reddening of skin, edema, pain as a result of histamine action. This biogenic amine is generated as a result of transformation of the following histidine amino acid: A. Decarboxylation. B. Methylation. C. Phosphorylation. D. Isomerization. E. Deaminization.	

15. A patient with suspected diagnosis "progressing muscular dystrophy" got his urine teste. What compound will confirm this diagnosis if found in urine? A. Creatine. B. Collagen. C. Porphyrin. D. Myoglobin. E. Calmodulin.	
16.A patient complained about dizziness, memory impairment, periodical convulsions. It was revealed that these changes were caused by a product of decarboxylation of glutamic acid. Name this product: A. GABA. B. Pyridoxal phosphate. C. TDP. D. ATP. E. THF.	
17.Glutamate decarboxylation results in formation of inhibitory transmitter in CNS. Name it: A. GABA. B. Glutathione. C. Histamine. D. Serotonin.	

E. Asparagine.	
18.In course of histidine catabolism a biogenic amine is formed that has powerful vasodilatating effect. Name it: A. Histamine. B. Serotonin. C. Dioxyphenylalanine. D. Noradrenalin. E. Dopamine.	
19.A newborn child has convulsions that have been observed after prescription of vitamin B6. This most probable cause of this effect is that vitamin B6 is a component of the following enzyme: A. Glutamate decarboxylase. B. Pyruvate dehydgenase. C. Ketoglutarate dehydrogenase. D. Aminolevulinate synthase. E. Glycogen phosphorylase.	
20.A patient with serious damage of muscular tissue was admitted to the traumatological department. What biochemical urine index will be increased in this case?	

A. Creatinine.
B. Common lipids.
C. Glucose.
D. Mineral salts.
E. Uric acid.

21.A patient diagnosed with carcinoid of bowels was admitted to the hospital. Analysis revealed high production of serotonin. It is known that this substance is formed of tryptophane aminooacid. What biochemical mechanism underlies this process?

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

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

A. Transamination and decarboxylation of aminoacids.
B. Desamination of purine nucleotide.
C. Synthesis of purine and pyrimidine bases.
D. Protein synthesis

23.Depressions and emotional insanities result from the deficit of noradrenalin, serotonin and other biogenic amines in the brain. Their concentration in the synapses can be increased by means of the antidepressants that inhibit the following enzyme: A. Monoamine oxidase. B. Diamine oxidase. C. L-amino-acid oxidase. D. D-amino-acid oxidase. E. Phenylalanine-4-monooxygenase.	
24. A patient with serious damage of muscular tissue was admitted to the traumatological department. What biochemical urine index will be increased in this case? A. Creatinine. B. Common lipids. C. Glucose. D. Mineral salts. E. Uric acid.	
25. A13-year-old boy complains of general weakness, dizziness, tiredness. He is mentally retarded. Increased level of valine, isoleucine,	

leucine is in the blood and urine. Urine has specific smell. What is the diagnosis? A. Maple syrup urine disease. B. Addison's disease. C. Tyrosinosis. D. Histidinemia. E. Graves' disease.	
26.Examination of a patient suffering from cancer of urinary bladder revealed high rate of serotonin and hydroxyanthranilic acid. It is caused by excess of the following amino acid in the organism: A. Tryptophan. B. Alanine. C. Histidine. D. Methionine. E. Tyrosine.	
27. A patient has pellagra. Interrogation revealed that he had lived mostly on maize for a long time and eaten little meat. This disease had been caused by the deficit of the following substance in the maize: A. Tryptophan. B. Tyrosine.	

C. Proline. D. Alanine. E. Histidine.	
28. A 2-year-old child with mental and physical retardation has been delivered to a hospital. He presents with frequent vomiting after having meals. There is phenylpyruvic acid in urine. Which metabolism abnormality is the reason for this pathology? A. Amino-acid metabolism. B. Lipidic metabolism. C. Carbohydrate metabolism. D. Water-salt metabolism. E. Phosphoric calcium metabolism.	
29.Nappies of a newborn have dark spots that witness of formation of homogentisic acid. Metabolic imbalance of which substance is it connected with? A. Thyrosine. B. Galactose. C. Methionine. D. Cholesterine. E. Tryptophane.	
30.A 1,5-year-old child presents with	

both mental and physical lag, decolorizing of skin and hair, decrease in catecholamine concentration in blood. When a few drops of 5% solution of trichloroacetic iron had been added to the child's urine it turned olive green. Such alteration are typical for the following pathology of the amino acid metabolism: A. Phenylketonuria. B. Alkaptonuria. C. Tyrosinosis. D. Albinism. E. Xanthinuria.	
31. Albinos can't stand sun impact - they don't aquire sun-tan but get sunburns. Disturbed metabolism of what aminoacid underlies this phenomenon? A. Phenylalanine. B. Methionine. C. Tryptophan. D. Glutamic acid. E. Histidine.	
32. A patient has been diagnosed with alkaptonuria. Choose an enzyme whose deficiency can be the reason for this pathology:	

A. Homogentisic acid oxidase. B. Phenylalanine hydroxylase. C. Glutamate dehydrogenase. D. Pyruvate dehydrogenase. E. Dioxyphenylalanine decarboxylase.	
33.Laboratory examination of a child revealed increased concentration of leucine, valine, isoleucine and their ketoderivatives in blood and urin Urine smelt of maple syrup. This diseaseis characterized by the deficit of the following enzyme: A. Dehydrogenase of branched keto acids. B. Aminotransferase. C. Glucose-6-phosphatase. D. Phosphofructokinase. E. Phosphofructomutase.	
34.Patient with encephalopathy was admitted to the neurological in-patient department. Correlation of increasing of encephalopathy and substances absorbed by the blood stream from the intestines was reveale What substances that are created in the intestines can cause	

endotoxemia? A. Indole. B. Butyrate. C. Acetacetate. D. Biotin. E. Ornithine.	
35.A patient diagnosed with carcinoid of bowels was admitted to the hospital. Analysis revealed high production of serotonin. It is known that this substance is formed of tryptophane amino acid. What biochemical mechanism underlies this process? A. Decarboxylation. B. Desamination. C. Microsomal oxidation. D. Transamination. E. Formation of paired compounds.	

4. Literature. Look pic. 229.

RECOMMENDED BOOKS

Basic

1. Lehninger principles of biochemistry (8th edition). / by Cox, Michael M.; Hoskins, Aaron A. - New York, Macmillan Learning, 2021. – 1248 p.
2. Biological chemistry / Yu. I. Gubsky [et al.] ; ed. by: Yu. I. Gubsky, I. V. Nizhenkovska. - 2nd ed. - Kyiv : AUS Medicine Publishing, 2021. - 544 p.
3. Gubsky, Yu. I. Biological chemistry : textbook for students of medical and pharmaceutical faculties / Yu. I. Gubsky ; ed. by.: Yu. I. Gubsky. - 2nd ed. - Vinnytsya : Nova Knyha, 2018. - 488 p.
4. Biological and Bioorganic Chemistry. In 2 books. Book 1. . Edited by B.S. Zimenkovskyi, I.V.Nizhenkovska . Kyiv, AUS Medicine Publishing, 2018. – 288 p
5. Biochemistry / Lubert Stryer, Jeremy M.Berg, John I. Tymoczko, Gatto Jr.,Gregory J. – Ninth Edition – New York.W.H. Freeman, 2019. – 1296 p.
6. Lehninger Principle of Biochemistry / by David L.Nelson and Michael M. Cox – New York, W.H. Freeman and Company, 2017. – 1312 p.

Additional

1. Lippincott Illustrated Reviews. Biochemistry / Denise Ferrier Seventh, North American Edition, 2017. – 560 p.
2. Biochemistry (7th edition). / Ferrier, Denise R. – Philadelphia, Wolters Kluwer, 2017. 553 p.
3. Murray R. K. Harper's Illustrated Biochemistry / R. K. Murray, D. K. Granner, V. W. Rodwell. - 27th ed. - Boston [etc.] : McGraw Hill, 2006. - 692 p.
5. Stryer L. Biochemistry / L. Stryer : W.H. F & C: N.Y. - 1995 - 1064 p.

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