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BIOCHEMISTRY

Educational and methodical manual for laboratory work performance by II-year students in the specialty 31.05.01 «General Medicine»

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Б63 **Джашеева, З.А-М.** Биохимия: Учебно-методическое пособие для иностранных студентов 2 курса, обучающихся по специальности 31.05.01 «Лечебное дело» / З. А-М. Джашеева, А.Х. Батчаева, О.М. Батчаева, Ф.У. Айбазова. – Черкесск: БИЦ СКГА, 2023. – 64 с.

В учебно-методическом пособии кратко изложены теоретические положения, касающиеся строения и роли биологически активных молекул в процессах жизнедеятельности. Приведены методики определения и исследования аминокислот, белков, ферментов, углеводов, витаминов, гормонов. К каждой теме предлагаются контрольные работы, задачи и тестовые задания по основным классам соединений.

Учебно-методическое пособие предназначено для использования во время проведения лабораторных и практических работ.

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CONTENT

INTRODUCTION

This workshop sets out the methods of educational laboratory works and some modern biochemical research methods performed by students in biochemistry classes. Conducting laboratory work will make it possible to comprehend the theoretical material, to consider the structural features and properties of the substance

Methodological instructions can be used by students of the Faculty of Medicine for independent, educational and research work

Features of work in a biochemical laboratory and safety briefing:

1. Work is allowed only in a long-sleeved medical robe.
2. Long hair should be carefully hidden under a medical cap.
3. Perform laboratory work is allowed only on the chemical table. On the chemical table can not put clothes, textbooks and other valuables.
4. Work with biological material should be carried out in medical gloves.
5. Work with aggressive liquids (concentrated acids, alkalis, etc.) is allowed only in a strictly designated place (fume hood).
6. Use reagents marked on the label.
7. Such liquids should be measured with great care in the following ways:

a) by means of a measuring cylinder;

b) Using a pipette with a rubber pear;

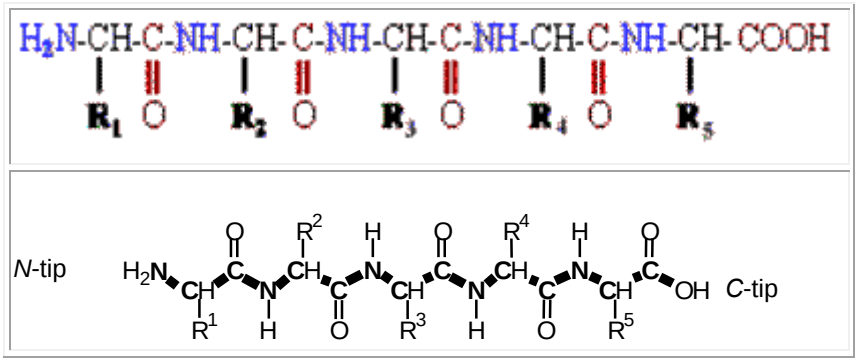
c) Using a glass pipette. Lower the pipette into the liquid, after filling the pipette with liquid tightly close the upper end of the index finger, move the pipette with liquid into the tube. Carefully open the end of the pipette and measure out the liquid.

Attention! When measuring aggressive liquids, it is forbidden to fill the pipette with them by sucking in the mouth. So you can cause damage to tooth enamel, burn the mucous membrane of the oral cavity and esophagus.

8. If the skin, mucous membranes or eyes got aggressive liquid, immediately rinse it with plenty of water and contact the laboratory service of the Department.
9. Do not heat dishes made of simple chemical glass on an open flame.
10. Make sure that water and other liquids do not get into electrical appliances.
11. In laboratory centrifuges, place a pair (even) number of carefully balanced tubes. The rotary chamber can only be opened after the rotor has stopped completely.
12. Work on the measuring equipment should be carried out in the presence of a teacher or a responsible laboratory assistant of the Department.
13. It is necessary to handle laboratory utensils carefully, so that in case of damage, you do not get injured.
14. After the work is finished, clean the workplace, turn off the electricity, and make sure that the cold and hot water taps are closed.

1. PROTEINS STRUCTURE AND PROPERTIES

Proteins are high-molecular polymer organic compounds built from α-amino acids (AA). Amino acids are connected by peptide bonds (-CO-NH-) and forming polypeptide chains. Peptide bond is formed only due to α–NH_2 groups of one AA and COOH- groups other AA at α - carbon atom.



In proteins distinguish the primary structure (a sequence of amino acid residues, interconnected by peptide bonds), the secondary structure (occurs due to the formation of hydrogen bonds between the groups =N-H и O=C= this polypeptide chain, which leads to the location of the polypeptide chain in the form of a spiral or folded structure), tertiary structure (occurs as a result of interaction between the side chains of amino acid residues polypeptide chains), Quaternary structure (intermolecular interactions between individual polypeptide chains).

Laboratory work 1.
Topic: QUALITATIVE REACTIONS

To conduct a qualitative reaction to the test substance, a reagent is added that can interact with a certain chemical group. If the test substance contains such a group, a reaction occurs, as a result of which there is either a change in color, or precipitation, or the appearance of a characteristic smell. In this case, the reaction can be considered positive and the conclusion about the presence of this group in the composition of the test substance. If no changes occur (negative reaction), the substance does not contain the desired group.

Qualitative reactions to proteins and amino acids

The presence of protein in biological liquids can be detected by qualitative (color) reactions. You can select a universal color reaction (Biuret reaction, ninhydrin reaction) that give substance containing at least two peptide bonds, and specific reactions, which are caused by certain amino acids in the protein molecule (xanthoprotein, Millon, and Folic reactions.)

BIURET REACTION ON PEPTIDE BONDS

Principle of method: Groups -CO-NH-, included in the peptide bond, in an alkaline solution form complex compounds with copper ions (Cu^{+2}). The resulting

compounds are colored purple with red or blue tint depending on the length of the polypeptide chain. The protein solution gives a blue-purple coloring, and the products of its incomplete hydrolysis-pink or red.

Reagents:

1) 1 % egg white solution (the white part of the chicken egg is filtered through cheesecloth and diluted with water 1:10);

2) 1 % gelatin solution; (Gelatin or gelatine is a translucent, colorless, brittle (when dry), flavorless food ingredient that is derived from collagen obtained from various animal body parts).

3) 10 % NaOH solution;

4) 1 % $CuSO_4$ solution.

Progress of testing. Take 3 tubes: in the first poured 5 drops 1 % egg white solution, the second-5 drops 1 % gelatin solution, the third-5 drops of water. Then in each test tube add 5 drops 10% NaOH and 2 drops 1 % $CuSO_4$. Shake the tubes. Observe the change in color.

Results:

Tube №	Result
1	
2	
3	

Conclusion:

NINHYDRIN REACTION TO α- AMINO ACIDS

Principle of method. The protein solution by heating with a dilute solution of ninhydrin turns blue or purple color. The reaction is due to the presence of protein residues a-amino acids. In the interaction of ninhydrin with a-amino acids and peptides are oxidized and break down to form NH_3, R-COH и CO_2. Ninhydrin is restored and interacts with the second molecule of ninhydrin and ammonia. The result is a complex compound murexide buildings painted in blue or purple color. The ninhydrin reaction can be used to quantify a-amino acids in amino acid analyzers.

Reagents:

1) 1 % egg white solution;

2) 1 % gelatin solution;

3) 0,5 % ninhydrin solution.

Progress of testing. Take 3 test tubes; in the first add 5 drops 1 % egg white solution, the second-5 drops 1 % gelatin solution, the third-5 drops of water. To each tube add 5 drops of 0.5% ninhydrin solution. Boil 2 minutes in a water bath. Monitor the change in color.

Result:

Tube №	Result
1	
2	
3	

Conclusion:

XANTHOPROTEIN REACTION TO CYCLIC AMINO ACIDS

Principle of method. When mixing the protein solution with concentrated nitric acid, the protein first precipitates, upon subsequent heating, dissolves and the liquid is colored yellow. Amino acids, having in its structure the benzene ring (Phe, Tyr, Trp), starts with nitric acid the nitration reaction with the formation of yellow nitro compounds.

Reagents:

1) 1 % egg white solution;
2) 1 % gelatin solution;
3) азотная кислота концентрированная (HNO_3).

Progress of testing. Take 3 test tubes: in the first add 5 drops 1 % egg white solution, the second-5 drops 1 % gelatin solution, in the third-5 drops of water. Then, 5 drops of concentrated nitric acid are added to each tube (HNO_3 – carefully!). Boil 2 minutes in a water bath. Write the color change.

Result:

Tube №	Result
1	
2	
3	

Conclusion:

FOLIC REACTION TO CYSTEINE

Principle of method. The amino acids cystine and cysteine present in the protein, containing weakly bound sulfur, give a positive folic reaction. Methionine, this reaction does not, because the sulfur in it is bound firmly. By boiling the

protein with the Folic reagent (plumbic sodium in excess NaOH) under the action of alkali, sulfur in the form of sodium sulfide is easily separated from cysteine or cystine, which with lead salts gives a black or brown precipitate of lead sulfide.

Reagents:

1) 1 % egg white solution;
2) keratin (light hair);
3) the Folic reagent.

Progress of testing. Take 2 tubes: one add 5 drops 1 % egg white solution, at the bottom of the second tube will put hair (light hair). Each tube is added to 5 drops of the Foli reagent (lead acetate solution). Boil until the appearance of the black staining.

Result:

Tube №	Result
1	
2	

Conclusion:

Laboratory class № 2
Protein deposition reactions

Protein deposition reactions can be reversible and irreversible. In the first case, proteins are not subject to profound changes, so the resulting precipitation can be re-dissolved in the original solvent while maintaining their native properties. When irreversible reactions precipitated proteins undergo profound changes-denaturation.

Deposition reactions enable:

1) study the properties of proteins;
2) release the liquid from the presence of protein;
3) establish the presence of protein in biological fluids, such as urine;
4) salting out using different concentrations of neutral salts or alcohol to separate the protein fractions.

(1) *PROTEIN DEPOSITION BY BOILING*

Method principle. To precipitate the protein, it is necessary to destroy its hydrate shell and neutralize its charge. When boiling, denaturation occurs, so the hydrate shell is destroyed. The protein loses charge if the pH of the solution is close to the isoelectric point of this protein.

Reagents:

1) 1 % egg white solution;

2) 1 % acetic acid solution;
3) 10 % NaOH solution;
4) saturated solution NaCI;

Progress of testing. Pour 5 drops of 1% egg white solution into 5 tubes. Then add:

in the second tube-1 drop of acetic acid;

the third-10 drops of acetic acid;

in the fourth – 5 drops of acetic acid and 2 drops of saturated NaCI solution;

in the fifth-2 drops of NaOH.

All tubes are boiled for 5 minutes in a water bath. Results make out in the form of a table

Result:

№ tube	The analysis of the reaction	The presence of Sediment (Yes/ No)	Denaturation occurred (Yes/ No)	The presence of stabilization factors	
				Hydrated shell (Yes/ No)	Charge (if there is a charge, indicate its sign)
1	Neutral / heat till boiling				
2	Slightly acidic / heat till boiling				
3	Strongly acidic / heat till boiling				
4	Acid +electrolyte / heat till boiling				
5	Alkaline / heat till boiling				

Conclusion:

THE PROTEIN PRECIPITATION BY CONCENTRATED MINERAL ACIDS

Method principle. Mineral acids cause dehydration of protein particles and form complex salts with proteins. The protein precipitate is dissolved in excess acid except nitric acid. This allows the use of nitric acid in clinical studies to quantify proteins.

Reagents:

1) concentrated HNO_3;
2) concentrated H_2SO_4;
3) 1 % egg white solution.

Progress of testing. 10 drops of concentrated nitric acid are pour into the test tube, then carefully layer about 10 drops of protein solution from the pipette along the test tube wall so that the liquids do not mix. Watching on the border of two liquids form a white cloudy ring. Shake the tube and add excess nitric acid. The precipitate does not dissolve.

Do the same with sulfuric acid. The precipitate is dissolved.

Result

Conclusion:

(2) *THE PRECIPITATION OF PROTEINS BY ORGANIC ACIDS*

Method principle. Like inorganic acids, trichloroacetic, sulfosalicylic and other organic acids, causing dehydration and forming complex salts, denaturate proteins.

Reagents:

1) 1 % egg white solution;
2) 10 % CCI_3COOH solution;
3) 20 % a solution of sulfosalicylic acid $C_6H_3(OH)(COOH)SO_3H$.

Progress of testing. In a test tube pour 5 drops of a egg white solution, then add 2 drops of trichloroacetic acid 10 % solution of CCl_3COOH (aggressive liquid!) or 2 drops of 20% solution of sulfosalicylic acid $C_6H_3(OH)(COOH)SO_3H$ and observe the appearance of protein precipitate.

Result
Conclusion:

(3) PROTEIN SOLUBILITY

Method principle. Many proteins are highly soluble in water, due to the presence on the protein molecules surface of free hydrophilic groups (-OH, -NH_2, -COOH etc.). Различные белки растворяются по-разному; proteins of supporting tissues (Cartilage. Bone tissue) keratin, procollagen, collagen, elastin, etc. insoluble in water.

The solubility of the protein in water depends on the nature of the protein, the reaction of the medium and the presence of electrolytes. In an acidic environment, proteins with acidic properties (albumins, globulins, prolamins, glutelins) are better soluble, alkaline proteins (protamins and histones) are better soluble in an alkaline environment.

The differences in solubility observed among acid- and alkaline-reacting proteins. For example, albumins dissolve in distilled water, and globulins dissolve in water only in the presence of electrolytes.

Reagents:

1) egg white solution;
2) 5 % NaCI solution;
3) Keratin.

Progress of testing. In one tube poured 2 drops of not diluted egg white, 20 drops of water, the contents are mixed. In this case, egg albumin dissolves, and egg globulin falls in the form of a small precipitate. In another test tube add 2 drops of egg white protein and 20 drops of 5% solution of sodium chloride. In a slightly salt solution, both albumins and globulins are dissolved

In the other two tubes poured a small amount of keratin (hair). In one tube make 20 drops of water, the other-20 drops of 5% sodium chloride solution. Keratin does not dissolve in water or in salt solution.

Result:

Protein name	H_2O	5 % NaCI
Egg albumin		
Egg globulin		
Keratin		
Conclusion:		

Questions for self-study and for the control the topic mastering:

1. Amino acids: structure, properties, classifications. Write the formulas of cysteine and methionine, to indicate their position in the classifications.

2. Types of bonds between amino acids in a protein molecule. The origin and function of peptides in the body. Write the formula of a tetrapeptide: PHE – PRO – LYS – TYR

3. Spatial organization of protein molecules. Stress protein.

4. Conformation of a protein molecule. Functional role of conformational transitions. The concept of domains.

5. Physical and chemical characteristics of aqueous solutions of globular proteins. Write the formula of tetrapeptide, an isoelectric point, which lies in a weakly acidic medium.

Tasks to the topic: Structure and properties of proteins

Task # 1

A drop of a solution containing a mixture of amino acids Gly, Ala, Glu, Arg, Gis was applied to the middle of electrophoretic paper, moistened with a buffer pH 6.0 and applied an electric voltage. Specify in which direction (to the cathode, anode or remain at the start) will move the individual amino acids.

Task # 4

How to explain the possible decrease in the solubility of proteins in the cleavage of peptides from them (as in the case of fibrinogen)?

Task # 5

According to the quantitative amino acid analysis in serum albumin contains 0.58% tryptophan, the molecular weight of which is 204. Calculate the minimum molecular weight of albumin.

Task # 6

How to explain that milk protein casein when boiling coagulates (precipitates), if the milk is sour?

2. ENZYMES

ENZYMES are highly effective proteins that act as biological catalysts.

A CATALYST is a substance that accelerates a chemical reaction, but is not consumed during the reaction itself.

A CATALYST is a substance that directs a reaction in a way in which energy barriers are lower.

FEATURES OF ENZYMES AS CATALYSTS BIOLOGICHESKIH

Enzymes have all the common properties of conventional catalysts, but compared to them, all enzymes are proteins, which causes their differences. Features of enzymes as biological catalysts are sometimes common properties of enzymes. These include:

1 high efficiency of action. Enzymes can accelerate the reaction 10-8 10-12 times

2 high selectivity of enzymes to substrates (substrate specificity) and to the type of catalyzed reaction (specificity of action)

3 high sensitivity of enzymes to non-specific physical and chemical factors of the medium-temperature, pH, ionic strength of the solution, etc

4 high sensitivity to chemical reagents

5 high selective sensitivity to the physical and chemical effects of certain chemicals, which thus can interact with enzymes improving or hindering its operation.

Enzyme classes

Class 1-oxidoreductase

Class 2-transfers
Class 3-hydrolase
Class 4 – LiAZ
Class 5 isomerase
Class 6-ligases (synthases and synthetases)

The same physical reaction in the body can be catalyzed by different proteins-enzymes. In this case, they are called isoenzymes – they are different molecular forms of the same enzyme.

The set of enzymes catalyzing successive reactions of a single process is called a multi-enzyme system

The enzyme with a low rate is called the limiting enzyme of the multi-enzyme system. It is this enzyme that determines the speed of the process as a whole.

If the limiting enzyme is both regulatory, it is called a KEY ENZYME of the multi-enzyme system.

Laboratory work №3
Topic: properties of enzymes

The temperature effect on the enzymes activity

Method principle. Enzymes are specific proteins of all living cells that act as biological catalysts. The property of enzymes to break down during boiling is a characteristic feature that distinguishes enzymes from other catalysts. Enzymes are thermolabile and exhibit optimal activity at a temperature of 35-45C. With an increase in temperature above 50C, their activity decreases, and then the structure of the enzyme molecule is destroyed.

Reagents:

1. saliva solution 1: 10
2. 1% starch solution
3. lugol solution

Progress of work:

In 4 tubes, pour 1 ml of diluted saliva and place the tubes in different temperature conditions:

1 tube-ice (0C)
2 the tube is left at room temperature (20C)
3 vial – in thermostat (37S)
4 tube-in boiling water (100C)

After 5 minutes in all tubes add 1 ml of starch solution and leave for 10 minutes for incubation under the same conditions. Further, the result of the action of amylase on starch was evaluated using the qualitative reactions of starch with iodine IN the PRESENCE of STARCH APPEARS dark BLUE, AND in the ABSENCE of BLUE THERE REMAINS the YELLOW COLOR of IODINE.

Thus, after incubation, all the tubes are cooled under the tap and 2 drops of Lugol solution (contains iodine) are added.

The color of the tubes and the findings indicated in the table

Tube №	Color
1	
2	
3	
4	
Summary	

Effect of pH on enzyme activity

Method principle. For saliva amylase, the optimal pH value is 6.8. In acidic and alkaline medium, the activity of amylase is reduced and starch cleavage goes to the stage of dextrins, which with a solution of Lugol give a red-purple or red-brown color.

Reagents:

1 saliva solution 1:10
2 1% starch solution
3 1% Na2HPO4
4 1% NaH2PO4
5 Lugol's iodine

Progress of work:

In 3 tubes, pour 1 ml of saliva solution and create an environment with a different pH value by adding:

1 tube – 1 ml of distilled water (neutral medium)
2 tube-1 ml Na2HPO4 (alkaline medium)
3 vial – 1 ml NaH2PO4 (acidic environment)

Then add 1 ml of starch solution to each scouring, the contents are mixed and placed in a thermostat at a temperature of 37C for 10 minutes. After incubation, the wipes are cooled.

Add 2 ml of NaH2PO4 to 2 scouring as starch in alkaline medium does not give color with iodine.

In all 3 tubes add 2 drops of Lugol solution, the results and conclusions are recorded in the table

Tube №	Color
1	
2	
3	
Summary	

Specificity of the action of amylase and sucrose

Method principle. Specificity is the ability of enzymes to accelerate individual chemical reactions only between strictly defined substances. The result of the action of amylase and sucrose enzymes on starch and sucrose substrates is

evaluated using a qualitative felling reaction. Starch and sucrose do not have free semi-acetal hydroxyl and therefore do not restore Cu+2 copper sulfite (reagent Felinga1) which give the solution a blue color, to Cu2o (brick-red precipitate) that is, give a negative reaction of felling, and the products of starch and sucrose splitting (maltose and glucose) have their own semi-acetal hydroxyl and give a positive felling reaction.

Reagents:
1 1% starch solution
2 2% sucrose solution
3 saliva solution 1:10
4 sucrose from yeast
5 10% NaOH
6 Lugol's iodine

Progress of work:
Take 4 tubes and add reagents as described below:
1 vial – 2 ml sucrose plus 0.5 ml of saliva
2 tube – 2 ml sucrose + 0.5 ml sucrose
3 tube – 2 ml starch + 0.5 ml saliva
4 vial – 2 ml starch + 0.5 ml of casarez

The contents of the tube mixed well and put for 10 min in a thermostat at 37° C deg. Polykovichi in all test tubes add 5 drops of feling's reagent 1, 5 drops of feling's reagent 2 and put in boiling bath for 2 minutes.

The results and conclusions write in the table

Tube №	color
1	
2	
3	
4	
Summary	

Laboratory work №4
Topic: Qualitative determination of enzyme activity

Effect of amylase on starch

Method principle. The amylase enzyme hydrolyzes starch (glycogen) through intermediate decomposition products (dextrins) to maltose and then to glucose.

Undiluted starch with iodine gives a blue color, and dextrins, depending on the size of their particles give iodine purple, red-brown, orange color or not painted at all.

In this regard, if a solution of saliva is added to the starch solution and, at certain intervals, a 1% solution of iodine is added to the sample, the liquid first

acquires a color of Tits, and then – purple, red, orange and yellow. This is due to the fact that saliva amylase breaks down the starch forming a number of intermediate products. Amylodextrin and altodextrine give with iodine the range of colors from purple to orange. The final products of starch hydrolysis – maltose and glucose-can be detected by the reaction of a Trommer.

Progress of work:

(1) prepare amylase solution: a small amount of saliva (about 0.5 ml) diluted with distilled water. Diluted saliva is used as a source of amylase.

(2) take 7 tubes. Each tube is poured from the burette to 1 ml of starch solution and add 1 ml of saliva. Then, 1-2 drops of Lugol solution (contains iodine) are added to the tubes with an interval of 5 minutes. The tube is shaken and indicate the color of the solution in the table.

Tube №	**Time**	**Color**
1	0 min	
2	5 min	
3	10 min	
4	15 min	
5	20 min	
6	25 min	
7	30 min	
Summary		

Questions for independent work and control of mastering the topic:

1. Features of enzymes as biocatalysts. Types of specificity of enzymes (give specific examples).
2. Features of the enzymes functional centers structure. Write the formula of amino acids, which often form the catalytic center of the enzyme.
3. Characteristics of the main stages of enzymatic catalysis.
4. The dependence of the reaction rate of the enzyme concentration. Unit activity and unit quantity of the enzyme. Write the oxidation reaction of glucose-6-phosphate.
5. Classification and indexing of enzymes. Give examples of reactions catalyzed by enzymes of each class of enzymes.

Tasks to topic: Enzymes

Task № 1

Glutamate decarboxylase catalyzes the reaction:

$$\text{Glutamic acid} \xrightarrow{B6} \text{GABA} + CO2$$

1. By changing the concentration of which substances can be characterized activity of the enzyme?
2. How can the reaction’s speed be increase?

Task № 2

Trypsin enzyme is able to break down peptide bonds of proteins. Why does trypsin treatment lead to inactivation of many enzymes?

To justify the answer remember:

1. What are enzymes?
2. What class of enzymes does trypsin belong to?

Task № 3

The inhibitor reduces the activity of the enzyme to 30% of the initial level. Increasing the substrate concentration of the catalyzed reaction restores 80% of the enzyme activity. What type of inhibitor is this?

For the answer:

1. Remember the types of inhibition.
2. The action of an inhibitor depends on the concentration of the substrate?

Task № 4

What can be evidenced by a sharp increase in the blood activity of aspartate aminotransferase (AST), if it is known that this enzyme is localized mainly in the heart?

For answer remember:

1. What class does AST belong to?
2. Why is the pathology in the blood increases the activity of intracellular enzymes?

Task № 5

1. What is substrate?
2. What is the enzymatic chain?
3. What are the basic principles of regulation of enzymatic chains.

3. BIOLOGICAL OXIDATION

BIOLOGICAL OXIDATION – a set of oxidative processes in a living organism, occurring with the obligatory participation of oxygen. Synonym – TISSUE RESPIRATION. Oxidation of one substance is impossible without the reduction of another substance. Oxidation-reduction processes in wildlife very much. Part of the redox processes involving oxygen refers to biological oxidation.

The mitochondrial oxidation system is a multi-enzyme system that gradually transports protons and electrons to oxygen to form a water molecule.

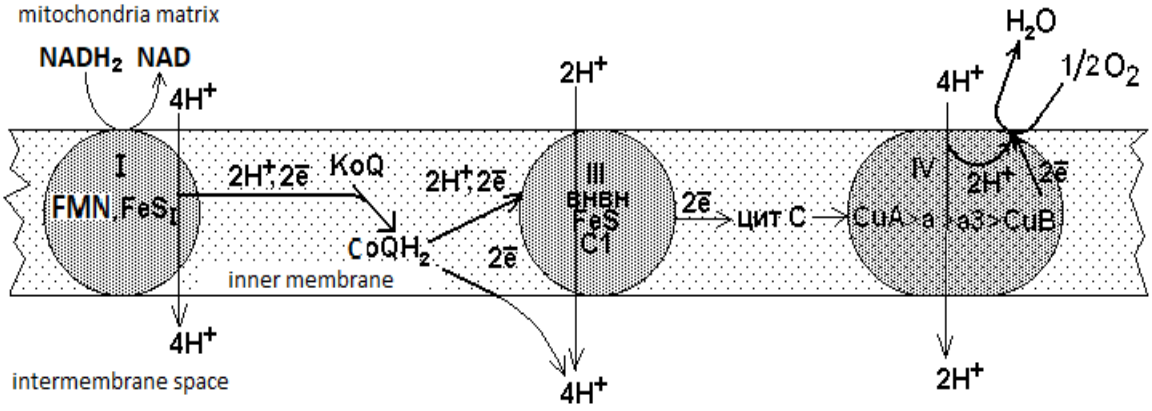
All mitochondrial oxidation enzymes are embedded in the inner membrane of the mitochondria. Only the first carrier of protons and electrons - nicotinamide dehydrogenase-is located in the mitochondrial matrix. This enzyme takes hydrogen from the substrate and transfers it to the next carrier. A complete complex of such enzymes forms a "breathing ensemble" ("respiratory chain"), within which hydrogen atoms are taken away from the substrate, then transferred sequentially from one carrier to another, and finally transferred to the oxygen of the air to form water.

Currently, there are three variants of respiratory chains:
1) MAIN (FULL) CHAIN
2) SHORTENED (ABBREVIATED) CHAIN
3) MAXIMALLY SHORTENED (MAXIMALLY REDUCED) CIRCUIT.

THE MAIN RESPIRATORY CHAIN

The main respiratory chain consists of three multi-enzyme complexes built into the inner membrane of the mitochondria. They are denoted by Latin numerals – I, III and IV.

SCHEME OF THE MAIN (FULL) THE RESPIRATORY CHAIN MITOCHONDRIAL OXIDATION



Complex I – NADH-KoQ-reductase, complex III – KoQH2 reductase complex IV – cytochrome oxidase. There is also a complex of II – succinate-KoQ-reductase, but it exists separately from other complexes and is not part of the main chain.

UNEMOTIONALLY OXIDATION

It accounts for 5-10% of oxygen entering the body. ATP in extra-mitochondrial oxidation is never formed.

There are 2 types of extra-mitochondrial oxidation:

I. OXIDATION OKSIDNOGO TYPE.

Enzymes – OXIDASE. The structure is metalloflavoproteins. Contain metals with variable valence – iron (Fe), copper (Cu), molybdenum (Mo). There are oxidases in peroxisomes – special formations of endoplasmic reticulum, as well as in the outer membrane of mitochondria. Take away the hydrogen from the substrate and transfer it to oxygen with the formation of H2O2 – hydrogen peroxide. General scheme:

II. OXIDATION OKSIGENATINGO TYPE

Occurs on the membranes of the endoplasmic reticulum and in the inner membrane of the mitochondria.

Enzymes – OXYGENASE. They activate the oxygen molecule and then inject one or two oxygen atoms into the oxidized substance molecule.

Oxygenase, consisting of one atom of oxygen to the oxidizable substance, called MONOOXYGENASE (HYDROXYLASE).

Oxygenase, including the two atoms of oxygen to the oxidizable substance, called DIOXYGENASE.

Oxygenases work as part of a multi-enzyme complex, TSA

ANTIOXIDANT SYSTEM.

1. ENZYMATIC

a) **CATALASE** – a heme enzyme containing Fe3+ catalyzes the destruction reaction of hydrogen peroxide. This produces water and molecular oxygen.

$2H_2O_2$ ------> $H_2O + O_2$

Many of catalase in erythrocytes where it protects the heme of hemoglobin from oxidation.

b) **SUPEROXIDE DISMUTASE (SOD)** catalyzes the neutralization reaction of two molecules of superoxide anion, turning one of them into molecular oxygen, and the other – in hydrogen peroxide (less strong oxidant than superoxide anion).

$O_2^{\cdot} + O_2^{\cdot} + 2H+$ ------> $H_2O_2 + O_2$

SOD is paired with catalase and is found in all tissues.

C) **PEROXIDASE**.

Peroxidase – a hemic enzyme that restores hydrogen peroxide to water, but it is necessarily the oxidation of another substance, which is a reducing agent. In the human body, such a substance is **GLUTATHIONE** – Tripeptide: gamma-glutamyl-cysteine-glycine. Therefore, the peroxidase of the human body called **GLUTATHIONE PEROXIDASE**.

SH-group of cysteine, which is part of glutathione, can give only 1 atom of hydrogen, and peroxidase reaction requires 2 atoms. Therefore, molecules of glutathione to work in pairs.

Reaction catalyzed by glutathione peroxidase:

$2H_2O_2$ + 2G-SH ------> H_2O + G-S-S-G

Regeneration of glutathione is involving $NADPH_2$, the enzyme catalyzes its glutationreductasa.

G-S-S-G + $NADPH_2$ ---------> 2G-SH + NADF

Glutathione is constantly maintained in a reduced state in red blood cells, where it serves to protect heme hemoglobin from oxidation.

multi-enzyme complex, TSA

2. NON-ENZYMATIC COMPONENTS OF ANTIOXIDANT SYSTEM

1. **Vitamins E (tocopherol) and A (retinol),** which are part of the cell membranes.

2. **Ceruloplasmin** is a plasma protein that takes part in the transport of copper.

3. **Uric acid**.

LABORATORY WORK № 5

Topic: Detect ON in leaps and bounds

The principle of the method: NAD is found in many organs and tissues of humans and animals, are rich in them and yeast. NAD is easily extracted from yeast by hot water (it is thermostable) and can be detected by the formation of a fluorescent complex with acetone. This reaction is typical for N-derivatives of nicotinic acid amide. It is used to determine methylnicotinamide in urine.

Reagents:

1. Yeast;
2. Acetone;
3. 30% NaOH solution;
4. Alcohol solution of phenolphthalein.

Progress of work:

The tube is placed a piece of yeast 4-5 mm in size, add 1/3 of the water tube and boil 20-30s, preventing the ejection of liquid. Filter into an empty tube 5-10 drops of the resulting extract, add 3-5 drops of acetone and 1-2 drops of 30% sodium hydroxide solution. Leave the tube for 2 minutes; then add 1 drop of 5 g/l alcoholic solution of phenolphthalein and drop by drop concentrated hydrochloric acid to discoloration of phenolphthalein. The tube is shaken during the addition of acid. Place the tube for 2 minutes in a boiling water bath, then cool and bring to the included fluorimeter. The acetone complex OVER fluorescent, blue light.

Result:

Conclusion:

LABORATORY WORK № 6

Topic: Detection of catalase in blood

The principle of the method: During oxidative processes, hydrogen peroxide can be formed, which is decomposed by the enzyme catalase (contained in various cells including blood) into water and molecular oxygen.

Reagents:

1. 30 g/l hydrogen peroxide;
2. blood.

Progress of work:

In a test tube to make 10-15 drops of 30 g/l of hydrogen peroxide and 3 drops of blood. Note the release of oxygen bubbles. When introduced into the vial of smoldering splinter, she erupts. Since the released oxygen increases combustion.

Result:

Conclusion:

LABORATORY WORK № 7

Theme: Mapping the ORP (= oxidation / reduction potential) of Riboflavin

The principle of the method: For the study of ORP used indicators that change their color at a certain value of ORP. For example, methylene blue (blue in the oxidized state) turns into leucomethylene blue (colorless in the reduced form).

Reagents:

1) zinc granular;
2) concentrated hydrochloric acid;
3) Riboflavin suspension — 0.25 g/l;
4) methylene blue solution — 0.1 g/l.

Progress of work: 4-6 drops of water, 1 drop of Riboflavin suspension and methylene blue drop – to blue or greenish-blue staining of the solution are introduced into the tube. Add a piece of zinc and 2-3 drops of concentrated hydrochloric acid to the tube (hydrogen bubbles begin to separate). As the solution is saturated with hydrogen, the redox potential of the mixture gradually decreases, and the reduction of Riboflavin and methylene blue occurs. The recovery of the entire volume of methylene blue occurs earlier than the recovery of a significant part of the Riboflavin introduced into the tube, so the color of the liquid passes sequentially into green, yellow, yellow or pink, and finally, the liquid is discolored due to the recovery of Riboflavin in the leuko-compound.

Colorless or pink liquid is poured into another tube and observe the change in color. Hydrogen no longer enters the liquid, and already dissolved in it goes into

the air and is transferred through Riboflavin and methylene blue to oxygen in the air. As a result, there is a gradual increase in the oxidation-reduction potential, after the consumption of hydrogen in the solution, the oxidation of the reduced Riboflavin begins. It transfers hydrogen through the methylene blue indicator to oxygen and turns yellow (part of the hydrogen enters the oxygen, bypassing the indicator). Thereafter, the oxidation leucomethylene blue solution becomes first green (yellow and blue make green) and then the blue.

Result:

Conclusion:

Questions for self-work and control of mastering the topic:

1. Complex 1 of mitochondrial oxidation. Structure and mechanism of FMN (Flavin mononucleotide) participation in electron and proton transport along the respiratory chain.
2. Coenzyme Q. Structure and mechanism of action.
3. Cytochromes. Structure and mechanism of action.
4. General scheme of the complete chain of mitochondrial oxidation. To write the formula of the substrates of this chain.
5. Short circuit of the mitochondrial oxidation of (General scheme). To write the oxidation of substrates.
6. Oxidative and substrate phosphorylation. Coefficient P/O. Give an example of substrate phosphorylation (equation).

Tasks to the topic: Biological oxidation

Task # 1

To the rat liver mitochondria preparation added NAD+. The activity of which Krebs cycle enzymes will increase in this case? To justify the answer:

1. Write a scheme of Krebs cycle reactions.
2. What is the function of NAD+?
3. What enzymes of Krebs cycle does it (NAD+) work with?

Task # 2

To the mitochondria preparation added pyruvate labeled with ^{14}C in the methyl group. What position will take ^{14}C in oxaloacetate after one revolution of the Krebs cycle?

For the answer:

1. Write the reaction of the Krebs cycle.
2. Trace the position of the mark in each metabolite.

Task # 3

In the experiment with isolated mitochondria, the Krebs cycle intensity on NADH accumulation was determined. Will the work of the Krebs cycle change if the outflow of the recovered equivalents stops? To justify the answer, remember:

1. In which Krebs cycle reactions NADH is formed?
2. Which enzymes catalyze these reactions?
3. What determines the speed of the Krebs cycle?

Task # 4

What is the number of ATP formed by the complete oxidation of 5 pyruvate moles in the presence of rotenone and 2,4 – dinitrophenol? To perform calculations:

1. Give the scheme of pyruvate oxidation till CO_2 and H_2O.
2. Specify how to change the energy of pyruvate oxidation in the presence of rotenone and 2,4 – dinitrophenol (complete separation).

Task # 5

What is the number of ATP molecules synthesized during the oxidation of one pyruvate molecule to 2-oxoglutarate; one isocitrate molecule to succinate; one succinate molecule to oxaloacetate, provided that dehydrogenase reactions involve the respiratory chain?

For calculation:

1. Write the course of reactions in these areas of the Krebs cycle.
2. Specify the reactions associated with the respiratory chain.
3. Remember what the number of ATP is formed during the oxidation of NADH and $FADH_2$.

Task # 6

Rotenone (a toxic substance produced by a plant species) sharply inhibits the activity of mitochondrial NADH-dehydrogenase. Toxic antibiotic antimycin strongly inhibits the oxidation of ubiquinol. Let's assume that both of these substances block the corresponding parts of the respiratory chain with equal efficiency. Which one would be the more potent poison? Give a reasoned answer.

To justify the answer remember:

1. What are the blockers of the respiratory chain?
2. What areas of the respiratory chain receives hydrogen from NADH and FADH2?

4. VITAMINS

A complete food must contain:

1. ENERGY SOURCES (CARBOHYDRATES, FATS, PROTEINS).
2. ESSENTIAL AMINO ACIDS.
3. ESSENTIAL FATTY ACID.
4. VITAMINS.
5. INORGANIC (MINERAL) ACID.
6. CELLULOSE
7. H_2O

Vitamins are low molecular weight organic substances with various structure. Combined into one group according to the following criteria:

1. Vitamins are absolutely necessary for the body and in very small quantities.

2. Vitamins are not synthesized in the body and must be supplied from outside or synthesized by the intestinal microflora.

Vitamins play the same role in all forms of life, but higher animals have lost the ability to synthesize them. For example, ascorbic acid (vitamin ”C”) is not synthesized in humans, monkeys and Guinea pigs, as in the process of evolution was lost enzyme system of synthesis of this vitamin from glucose. **BERIBERI** (=avitaminosis, vitamin deficiency) is a disease that develops in the complete absence of a particular vitamin in the body. Currently, beriberi are not usually found, and there are **HYPOVITAMINOSIS** with a lack of vitamin in the body.

THE REASONS FOR THE DEVELOPMENT OF HYPO - AND AVITAMINOSIS

All causes can be divided into external and internal.

EXTERNAL causes of hypovitaminosis:

1. Insufficient vitamin content in food (with improper processing of food, with improper storage of food)

2. The composition of the diet (for example, the lack of vegetables and fruits in the diet)

3. The need for a particular vitamin is not taken into account. For example, a protein diet increases the need for vitamin “PP” (in normal diet, it can be partially synthesized from tryptophan). If a person consumes a lot of protein foods, it may increase the need for vitamin “B_6“ and reduce the need for vitamin PP.

4. Social causes: urbanization of the population, eating extremely high-purity and canned food; the presence of anti-vitamins in food. Social causes of vitamin deficiency exist in the world. For example, in remote areas of the North, in the diet of people there are few vegetables and fruits. Urbanization also matters, because the food consumed a lot of canned and rafinirovannom products. In large cities, people are not sufficiently provided with sunlight – so there may be hypovitaminosis D.

INTERNAL causes of hypovitaminosis:

1. Physiological increased need for vitamins, for example, during pregnancy, with heavy physical labor.

2. Long-term severe infectious diseases, as well as the period of recovery;

3. Violation of the absorption of vitamins in some diseases of the gastrointestinal tract, for example, with gallstone disease, the absorption of fat-soluble vitamins is disturbed;

4. Intestinal dysbacteriosis. It matters, as some vitamins are synthesized completely by the intestinal microflora (vitamins B_3, BC, B_6, H, B_{12} and K);

5. Genetic defects of some enzymatic systems. For example, vitamin D-resistant rickets develops in children with a lack of enzymes involved in the formation of the active form of vitamin D (1,25-dioxycholecalciferol).

CLASSIFICATION OF VITAMINS

1. Soluble vitamin. This group includes vitamins C, P, B1, B2, v3, BC, B6, B12, PP, N.

2. Fat-soluble vitamins a, D, E and K.

Water has special physical and chemical properties:

1. The universal solvent of the body to gases and other substances.
2. Means of transportation of various substances from the place of formation to the place of consumption or excretion.
3. Forms hydrated protein shells, provides colloidal protein solutions.
4. Participates in many chemical reactions.
5. It provides absorption of nutrients in the intestine and excretion of metabolic products.
6. Participates in thermoregulation of the body.

Laboratory work № 8
Topic: Qualitative reactions to vitamins.

The reduction reaction of vitamin B2

Hydrogen, produced by adding zinc metal to a concentrated hydrochloric acid, Riboflavin restores via the intermediate red (roboflavin) to colorless laceflower. In this case, the yellow color of the solution turns into pink, then the solution is discolored:

Research material and reagents:

1. Riboflavin, 0.025 % suspension in water.
2. Hydrochloric acid, concentrated.
3. Zinc metal.

Progress of work:

10 drops of Riboflavin suspension in water (0.025 %) is poured into the tube, 5 drops of concentrated hydrochloric acid and a small piece of metal zinc are added there.

Released hydrogen reacts with Riboflavin and the solution changes color from yellow to red and pink, and then discolored.

Result:

Conclusion:

Ferrichloride test for vitamin B6.

Method principle. A colorless solution of vitamin B6 acquires a red color in the presence of ferric chloride; the reaction is due to the formation of a complex salt such as iron phenolate of red color.

Reagents:

1. Pyridoxine, 5 % solution.
2. Iron chlorine, 5 % solution.

Progress of work: In a test tube mix 5 drops of 5% aqueous solution of vitamin B 6 and 1 drop of 5 % solution of ferric chloride and shake it. The mixture is colored red.

Result:

Conclusion:

Vitamin C

Method principle. Detection of ascorbic acid is based on its ability to enter into redox reactions.

Reagents:

1. Methylene blue, 0.01 % solution
2. Sodium carbonate, 10 % solution
3. Vitamin C, 1 % solution
4. Water
5. Lugol solution (0.1 % iodine solution in 0.2 % potassium iodide solution)

Progress of work:

Reaction with methylene blue.

In two tubes make 1 drop of 0.01 % solution of methylene blue, 1 drop of 10% solution of soda. In one of them add a few drops of ascorbic acid solution, in the other – 1 ml of water. Both tubes are heated and record the result. (Discoloration occurs in a tube with ascorbic acid).

Reaction with Lugol solution.

In one test-tube bring 10 drops of distilled water and 2 drops of Lugol's solution. In one tube add 10 drops of distilled water, in another 10 drops of ascorbic acid solution.

In a tube with ascorbic acid Lugol solution is discolored as a result of the reduction of iodine to iodic-hydrogen acid.

Result:

Conclusion:

Vitamin A

Method principle. Vitamin A in interaction with sulfuric acid is colored in red-purple or red-brown color, in interaction with the ferric chloride – in yellow-green.

Reagents:

1) oily solution of retinol acetate – 34,4 g/l; 2) concentrated sulfuric acid; 3) chloroform; 4) trichloride antimony, 23% of the chloroform solution.

Progress of work:

Reaction to vitamin A by concentrated sulfuric acid. In a dry tube make 1 drop of retinol acetate solution and 5 drops of chloroform, stir and add 1 drop of concentrated sulfuric acid. Note the result of the reaction.

Reaction to vitamin A with ferric chloride. In a dry tube make 1 drop of retinol acetate and 5 drops of chloroform. Stir, add 3 drops of iron chloride. Note the result of the reaction.

Reaction to vitamin a with antimony trejderskoj. In a dry test tube add I drop of a solution of retinol acetate and add 2-3 drops of 23% chloroform solution of antimony trichloride. When mixing, the contents of the tube is colored blue

Result:

Conclusion:

Vitamin D

Method principle. Vitamin D when interacting with aniline reagent when heated is colored red, when interacting with a solution of bromine in chloroform – in greenish-blue.

Reagents:

1) aniline reagent (15 parts aniline and 1 part concentrated hydrochloric acid);

2) chloroform;

3) bromine solution in dehydrated chloroform in a ratio of 1:60;

4) oil solution of ergocalciferol – 1,25 g/l.

Progress of work:

Reaction to vitamin D with aniline. In a dry tube make 2 drops of oil solution of ergocalciferol, 10 drops of chloroform and 1-2 drops of aniline reagent, gently heated with constant stirring. Note the result of the reaction.

Reaction to vitamin D bromkamfora. In a dry test tube add 2-3 drops of a solution of ergocalciferol and 2-4 drops of solution of bromine in chloroform. Mark the result

Result:

Conclusion:

Vitamin E

Method principle. An alcohol solution of vitamin E in the presence of concentrated nitric acid is oxidized to a quinoid compound, colored red.

Reagents:

1) vitamin E, 0.1 % alcohol solution;

2) concentrated nitric acid;

3) sucrose powder.

Progress of work:

In a dry tube make 6 drops of 0.1 % alcohol solution of vitamin E, add a few grains of sucrose. Gently on the side of the tubes was added 10 drops of concentrated nitric acid. The test tube is slightly shaken. After 1-2 minutes, red or yellowish-red staining is observed.

Result:

Conclusion:

5. HORMONES

HORMONES are biologically active substances that are synthesized in small quantities in specialized cells of the endocrine system and through circulating fluids (for example, blood) are delivered to target cells, where they have their regulatory effect.

Hormones, like other signaling molecules, have some common properties.

GENERAL PROPERTIES OF HORMONES

1. are isolated from the cells that produce them into the extracellular space;

2. are not structural components of cells and are not used as a source of energy.

3. are able to specifically interact with cells that have receptors for this hormone.

4. have very high biological activity - effectively act on cells in very low concentrations (about 10^{-6} - 10^{-11} mol/l).

MECHANISMS OF ACTION OF HORMONES

Hormones influence target cells.

Target CELLS are cells that specifically interact with hormones using special protein receptors. These receptor proteins are located on the outer membrane of the cell, or in the cytoplasm, or on the nuclear membrane and other cell organelles.

BIOCHEMICAL MECHANISMS OF SIGNAL TRANSFER FROM HORMONE IN A CELL-TARGET.

Any protein receptor consists of at least two domains (sites) that provide two functions:

– "recognition" of the hormone;

– conversion and transmission of the received signal to the cell.

One of the domains of the receptor protein has in its composition a site complementary to some part of the signal molecule. The process of binding the receptor to the signal molecule is similar to the process of formation of the enzyme-substrate complex and can be determined by the value of the affinity constant.

If the violation of the synthesis or the ability of proteins-receptors contact signaling molecules that arise disease – endocrine disorders. There are three types of such diseases:

1) Associated with insufficiency of protein-receptor synthesis.

2) Associated with changes in the structure of the receptor - genetic defects.

3) Associated with blocking protein-receptor antibodies.

MECHANISMS OF ACTION OF HORMONES ON TARGET CELLS

Depending on the structure of the hormone, there are two types of interaction. If the hormone molecule is lipophilic (e.g. steroid hormones), it can penetrate the lipid layer of the outer membrane of the target cells. If the molecule is large or polar, its penetration into the cell is impossible. Therefore, for lipophilic hormones, the receptors are inside the target cells, and for hydrophilic – receptors are in the outer membrane.

To obtain a cellular response to the hormonal signal in the case of hydrophilic molecules, an intracellular mechanism of signal transmission acts. This occurs with substances, which are called the "SECOND owner". The hormone molecules are very diverse in form, and the "second mediators" are not.

The reliability of signal transmission provides a very high affinity of the hormone to its protein receptor.

What are the mediators that participate in the intracellular transfer of humoral signals? These are cyclic nucleotides (camp and cGMP), inositoltriphosphate, calcium-binding protein – calmodulin, calcium ions, enzymes involved in the synthesis of cyclic nucleotides, and protein kinases – enzymes of protein phosphorylation. All these substances are involved in the regulation of the activity of individual enzyme systems in the target cells.

Let us examine in more detail the mechanisms of action of hormones and intracellular mediators. There are two main ways of **signal transmission to target cells from signal molecules with membrane mechanism of action:**

1. ADENYLYL CYCLASE (OR GUANILATZIKLAZY) SYSTEM

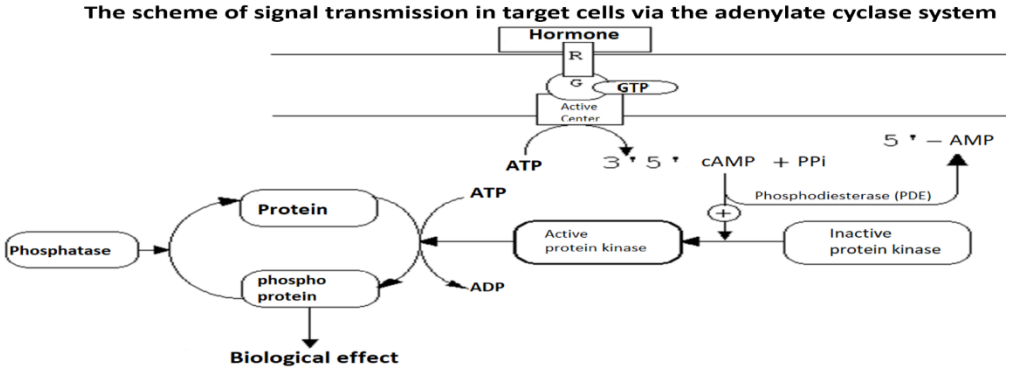
2. PHOSPHOINOSITIDE MECHANISM

ADENYLYL CYCLASE SYSTEM.

Main components: membrane protein receptor, G-protein, adenylate cyclase enzyme, guanosine triphosphate, protein kinases.

In addition, for the normal functioning of the adenylate cyclase system, ATP is required.

The scheme of adenylate cyclase system is shown in the figure:



As can be seen from the figure, protein-receptor, G-protein, which are located next to the GTP and enzyme (adenylate cyclase) are embedded in the cell membrane.

Before the action of the hormone, these components are in a dissociated state, and after the formation of a complex signal molecule with a receptor protein, changes in the conformation of the G-protein occur. As a result, one of the g-protein subunits acquires the ability to bind to GTP.

Complex "G-protein-GTP" activates adenylate cyclase. Adenylate cyclase begins to actively convert ATP molecules into C-AMP.

C-AMP has the ability to activate special enzymes – protein kinases, which catalyze the phosphorylation reaction of various proteins involving ATP. In this case, the protein molecules include phosphoric acid residues. The main result of this phosphorylation process is a change in the activity of phosphorylated protein. Proteins with different functional activity are exposed to phosphorylation in different types of cells as a result of adenylate-cyclase system activation. For example, it can be enzymes, nuclear proteins, membrane proteins. As a result of phosphorylation reaction proteins can become functionally active or inactive.

Such processes will lead to changes in the rate of biochemical processes in the target cell.

Activation of adenylyl cyclase Sistemi lasts for a very short time because G-protein after binding to adenylyl cyclase begins to show the GTP-asnow activity. After hydrolysis of GTP, G-protein restores its conformation and ceases to activate adenylate cyclase. As a result, the reaction of camp formation stops.

In addition to the participants of the adenylate cyclase system, some target cells have receptor proteins associated with G-proteins that lead to inhibition of adenylate cyclase. The complex "GTP-G-protein" inhibits adenylate cyclase.

When camp formation stops, phosphorylation reactions in the cell do not stop immediately: as long as the camp molecules continue to exist, the process of protein kinase activation will continue. In order to stop the action of camp, in the cells there is a special enzyme – phosphodiesterase, which catalyzes the hydrolysis reaction 3',5'-cyclo-AMP to AMF.

Laboratory work № 9
Topic: Qualitative reactions to hormones

Reaction to adrenaline with chlorine iron

The principle of the method: Adrenaline has a slightly alkaline reaction, easily oxidized in air to form adrenochrome, whereby the solution with ferric chloride is colored green.

Reaction with ferric chloride is characteristic of the pyrocatechin ring entering the adrenaline molecule.

Reagents, test material:

1) epinephrine solution – 1 g/l;
2) ferric chloride solution – 10 g/l;
3) caustic soda solution – 100 g/l.

Progress of work: In a test tube pour 10 drops of epinephrine solution and add 1 drop of ferric chloride. There is a green staining due to the presence of pyrocatechin in the adrenaline molecule. When 1drop of sodium hydroxide solution is added, cherry-red staining is observed.

Result:

Conclusion:

Buyretova reaction and reaction to insulin Foll

The principle of the method: With the help of color reactions to proteins, it can be proved that insulin is a protein having in its composition SH-groups of cysteine.

Progress of work:

1. In the test tube, add 10 drops of insulin solution, 5 drops of NaOH and 1 drop of $CuSO_4$. Note the change in color.
2. In the test tube, add 5 drops of insulin solution, 5 drops of the folic reagent. Boil 2 minutes. Mark the result.

Result:

Conclusion:

Diazo reaction on the adrenaline

The principle of the method: In the interaction of diazoreaktiva with adrenaline liquid is colored red due to the formation of complex compounds such as azo dye.

Reagents, test material:

1) sulfanilic acid, 0.5% solution;
2) sodium nitrite, 10% solution;
3) caustic soda, 10% solution;
4) adrenaline – 1 g/l.

Progress of work:

To 6 drops of sulphanilic acid solution add 6 drops of a solution of sodium nitrite (diazoreaktivom), 10 drops of epinephrine solution and 3 drops of sodium hydroxide solution. The liquid is colored red.

Result:

Conclusion:

Qualitative reactions to cortisol

With Felling reagent. Cortisone and prednisolone are able to recover copper oxide from oxide salts.

Reagents:

1) felling reagent;
2) a solution of prednisolone.

Progress of work:

To 1 ml of prednisolone solution, 1 ml of felling reagent is added, heated on an alcohol lamp. The red precipitate of copper oxide falls.

Conclusion:

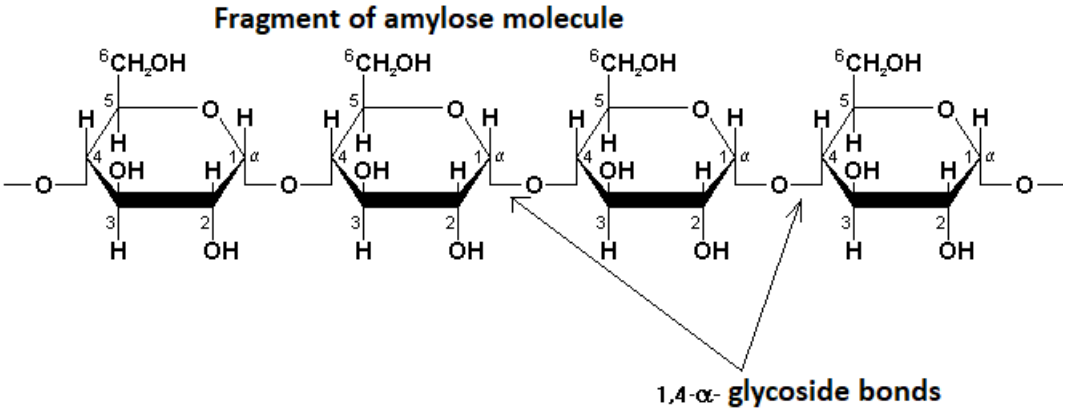
Questions for independent work and control of mastering the topic:

1. Hormones – General characteristics. Structure, classification, mechanisms of action. Adenylate cyclase and its catalyzed reaction.
2. Hormones of the hypothalamus and pituitary. Their structure and mechanisms of action.
3. Thyroid hormone. Biosynthesis of thyroxine. Biochemical mechanisms of hyperthyroidism. Hypofunction of the thyroid gland.
4. Parathyroid hormones, their regulatory functions.
5. Hormones of the pancreas. Molecular mechanisms of their action and biochemical consequences.
6. Hormones of the adrenal medulla. Biosynthesis and molecular mechanisms of their action.
7. Adrenal cortex hormones. Molecular mechanisms of action.

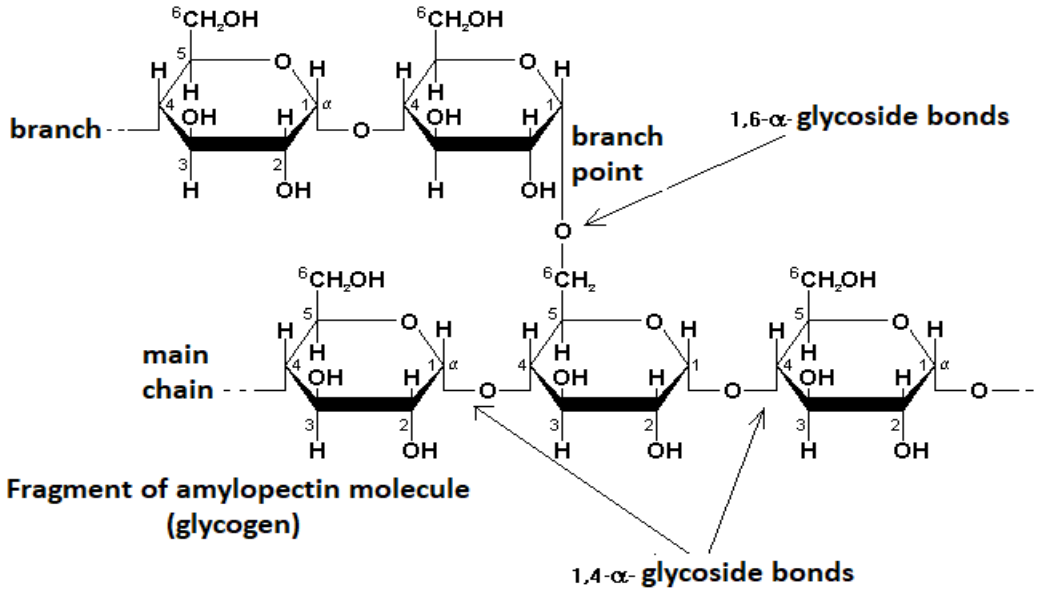
6. CARBOHYDRATES

Carbohydrates a person receives from food mainly in the form of polysaccharides (vegetable starch, fiber (cellulose), less – glycogen), in smaller quantities in the form of disaccharides, and quite a bit – monosaccharides. Digestion of carbohydrates in the gastrointestinal tract (GIT) of a person does not refer to metabolism, since the gastrointestinal tract is considered as part of the external environment.

Digestion begins in the oral cavity. The salivary glands secretes the enzyme "α-amylase of saliva". This enzyme is able to break down □-1,4-glycosidic bonds in the molecules of vegetable starch (a mixture of polysaccharides of amylose and amylopectin) and glycogen (animal starch). The structure of starch and glycogen is shown in the figures:



Unlike the linear structure of amylose, consisting only of 1,4-α-glycosidic bond, the molecules of amylopectin and glycogen branched. Communication at the point of branching – 1,6-α-glycoside bond.

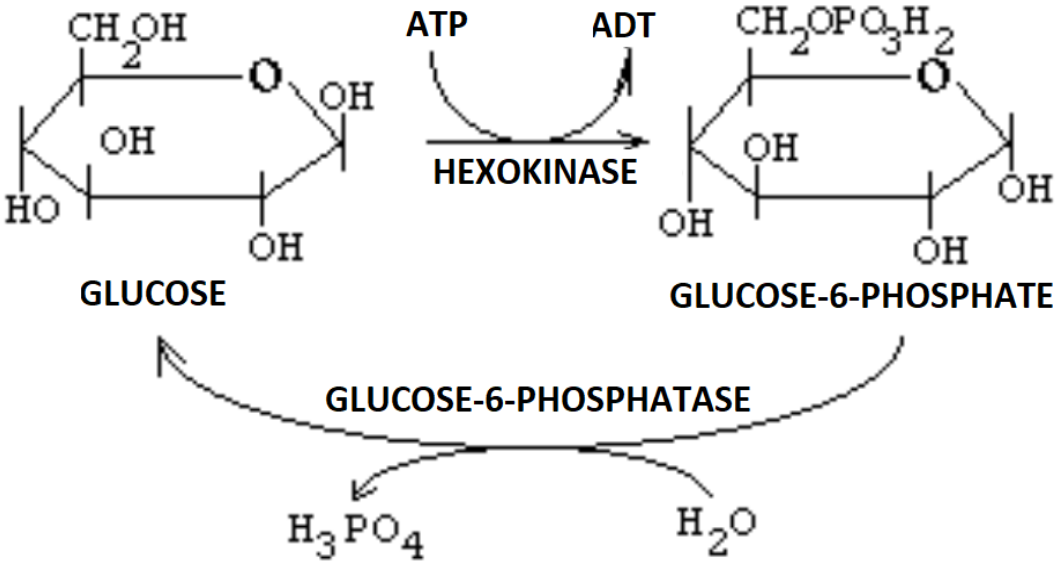


In the day an adult with a balanced diet receives about 500 grams of carbohydrates. After absorption of glucose in the portal vein system enters the liver. In the liver, the main amount of glucose is deposited stored in the form of

glycogen, and the rest of the glucose goes into the General bloodstream to feed other cells. This happens after eating at the height of digestion.

In a state of "fasting" (out of meals) glycogen in the liver gradually disintegrates to glucose, and glucose from the liver goes into the General blood flow to other tissues.

These mechanisms maintain blood glucose concentration at a constant level: 3.9-6.1 mmol/l.



Under the influence of insulin glucose enters the cells of the tissues. What happens to glucose in the cell?

The first reaction, which enters the glucose in the cell, is the only one. This phosphorylation reaction of glucose at the expense of ATP. The enzyme that catalyzes this reaction is in every cell. It is called hexokinase (ha).

After the formation of glucose-6-phosphate begins branching further ways of glucose metabolism. These three main ways.

1. Glycogen synthesis.
2. Geksozofosfatov the path of the breakdown of carbohydrates (GMF-path)
3. Exoterically the path of the breakdown of carbohydrates (GBF-way).

There are minor pathways (they use a small fraction of glucose coming to the cell). These paths do not play an energy role, and are used to build oligo – and polysaccharide chains of glycoproteins, that is, perform a structural role

Laboratory work № 10
Topic: Qualitative reactions to carbohydrates

The Fehling's reaction for monosaccharides

Method principle. Monosaccharides – are composed of semi-acetal hydroxyl, due to which they are able to restore Cu+2 copper sulfate (CuSO4) to Cu+1 copper oxide (Cu_2O). This reaction takes place in an alkaline environment

(in the presence of NaOH) when heated. The result is the disappearance of the blue color of copper sulfate and the appearance of brick-red sediment (copper oxide).

Reagents:

Glucose solution 10%

Fehling's solution 1 and 2

Progress of work:

2 drops of glucose solution are poured into the test tube, 1 drop of felling reagent I (copper sulfate solution) and 2 drops of felling reagent II (contains NaOH) are poured to it. Heat in a boiling bath for 5 minutes.

Result:

Conclusion:

Selivanov's reaction to fructose

Method principle. Fructose (ketosis) when interacting with concentrated acid loses 3 molecules of water and forms hydroxymethylfurfural, which, when heated with resorcinol gives a cherry red coloration, Aldose practically do not react Selivanov.

Reagents:

A solution of fructose 10%

Concentrated hydrochloric acid HCl

The Selivanov Reagent

Progress of work:

In a test tube pour 2 drops of fructose solution, pour 1 drop of concentrated hydrochloric acid NSI (aggressive liquid!) and 2 potassium Selivanov reagent (contains resorcinol). Copasa heated in the bath at techenia.5 minutes.

Result:

Conclusion:

Felling's reaction to disaccharides

The principle of the method: Only those disaccharides that have free semi-acetal hydroxyl, are able to restore copper sulfate to copper oxide, t. (e). give a positive felling reaction.

Reagents:

A solution of maltose 10%

Lactose solution 10%
The solution of sucrose 10%
The Feling's Reagent 1
The Feling's Reagent 2

Progress of work:

2 drops of maltose 2 drops of lactose 2 drops of sucrose
+ 1 drop of Fehling's reagent I + 1 drop of Fehling's reagent I + 1 drop of Fehling's reagent I
+ 2 drops of feling's reagent II + 2 drops of feling's reagent II + 2 drops of feling's reagent II
Heat in a boiling bath for 5 minutes

Result:

Conclusion:

Hydrolysis of sucrose and determination of hydrolysis products

Progress of work: 10 drops of sucrose solution and 10 drops of 10% sulfuric acid solution are poured Into the tube. Heat for 10 minutes in a boiling water bath. Sucrose hydrolysate is formed.

Reagents:

The solution of sucrose 10%
The Feling's Reagent 1
The Feling's Reagent 2
10% sulfuric acid solution

Progress of work:

hydrolysate hydrolysate
+ 1 drop of felling reagent I + 1 drop of HCI conc.
+ 2 drops of feling's reagent II +2 drops of Selivanov reagent
Boil for 5 minutes Boil for 5 minutes

Result:

Conclusion:

The Fehling's reaction with starch

Method principle: There are practically no free semi-acetal hydroxyl molecules in polysaccharides, so they can not restore Cu+2 to Cu+1

Reagents:

Starch solution 10%
The Feling's Reagent 1
The Feling's Reagent 2

Progress of work:

In the test tube to make 2 drops of starch solution, add 1 drop of the reagent felling I and 2 drops of the reagent felling II. Heat in a boiling bath for 5 minutes

Result:

Conclusion:

The reaction of starch with iodine.

The principle of the method: starch Amylose forms a complex with iodine, painted in blue. When heated, the complex is destroyed, after cooling formed again.

Reagents:

Starch solution 10%

The Feling's Reagent 1

The Feling's Reagent 2

lugol

Progress of work:

In the tube make 1 ml of distilled water and 1 drop of starch. After mixing, add 1 drop of Lugol (contains iodine). Mark the result. Heated in a boiling water bath, and then cooled. Observe the change in color.

Result:

Conclusion:

Laboratory work № 11
Topic: the Discovery of a dehydrogenase of 3-phosphoglyceraldehyde aldehyde

Method principle

3-phosphoglycerin aldehyde during anaerobic conversion of glucose by dehydrogenase 3-phosphoglycerin aldehyde is oxidized to 1,3-diphosphoglyceric acid. When this occurs, the recovery is OVER, which can be detected by the restoration of methylene blue in lycosoidea.

Reagents:

1) glucose, 2% solution;

2) yeast;

3) methylene blue, 0.002% solution.

Progress of work:

In the test tube make a piece of yeast (the size of a pea), a few drops of glucose solution, RUB the contents of a glass rod to obtain a uniform suspension. Add a drop of methylene blue solution, mix the contents of the tube with a glass rod. Put the tube in a tripod and observe the discoloration of methylene blue, starting from the bottom of the tube.

Result:

Conclusion:

Detection of lactic acid

Method principle

Lactic acid in the presence of the phenolate of iron (the reagent Uffelmann), painted in purple color, forming lactate of iron of yellow-green.

Progress of work:

1 g of muscles are ground to a homogeneous state in a mortar with a small amount of quartz sand for 3 minutes, adding 5 drops of water. Then pour 3 ml of distilled water and filtered through water-soaked cotton wool.

To 15 drops of filtrate, the Uffelmann reagent is added drop by drop (20 drops of 1% phenol solution + 2 drops of 1% ferric chloride solution) until a purple color appears. In the presence of lactic acid, the violet color of the liquid turns into yellow-green, since iron lactate is formed. For comparison, the reaction of Uffelmann with a solution of lactic acid is carried out and the development of yellow-green staining is observed.

Result:

Conclusion:

7. LIPID

LIPIDS are organic substances characteristic of living organisms, insoluble in water, but soluble in organic solvents and in each other.

Digestion of exogenous fat necessarily requires prior **emulsification**. Some edible fats enter the body in an emulsified form, such as milk fat. For the rest it is necessary to emulsify with the help of special substances – emulsifiers (detergents).

Emulsifiers are substances of amphiphilic nature. They reduce surface tension and stabilize the emulsion. General in the structure of emulsifiers: the presence of hydrophilic and hydrophobic sites. The hydrophilic portion of the emulsifier molecule dissolves in water, hydrophobic – in fat. This creates a large area of fat contact with the aqueous phase in which the enzyme is located. Proteins can act as emulsifiers. Infants do not need emulsifiers: they get already emulsified milk fat.

In the human body emulsifiers are BILE ACIDS. These are substances of steroid nature. Synthesized in the liver from cholesterol by oxidation at monooksigenazna type in two primary bile acids: CHOLIC and CHENODESOXYCHOLIC, which then bind with amino acid residues glycine and taurine. So conjugated bile acids are formed - GLYCOCHOLIC acid (in which the hydrophilic site is represented by the glycine residue) and TAUROCHOLIC acid (in it the hydrophilic site is represented by taurine). The hydrophobic component of all bile acids is a cholesterol derivative. Formed and other bile acids – their diversity is large enough. In the composition of bile acids enter in the 12-duodenum and allosteric activate pancreatic lipase.

Actually digestion of fats is hydrolysis of ester bonds.

There are three enzymes:

1. **Lingual lipase**. It is produced by the cells of the mucous membrane of the back of the tongue. The action of this enzyme is manifested only in the stomach (previously thought that it is – gastric lipase). Lingual lipase can digest already emulsified fat. Its pH-optimum is 4-5. Therefore, in the stomach of an adult, lingual lipase is inactive. Actually fats are digested by lingual lipase only in infants.

In adults, the digestion of fat is only in the intestine according to the scheme: "bile secretion ⇒ emulsification of fat ⇒ the action of pancreatic lipase."

2. **Pancreatic lipase**. By itself, this enzyme has a very low activity. But the pancreas produces protein, which, getting into the intestine, is able to activate pancreatic lipase. The name of this protein is "colipase". Colipase is produced as an inactive precursor – procolipase, which is activated by trypsin in the intestine. Colipase is not a classic activator, it only binds the substrate and brings it closer to the active center of lipase.

The resulting fatty acids and monoacylglycerols can be absorbed into the intestinal wall.

3. **Lipid esterase**. Under the action of this enzyme, part of the monoacylglycerols can be hydrolyzed to form glycerol and fatty acids.

Thus, the products of digestion of fat are glycerol, fatty acids and monoacylglycerol. The products of digestion are absorbed by pre-formation of mixed MICELLES with bile acids.

So, bile acids perform 2 functions: emulsification of fat and absorption of fatty acids.

Micelles into the enterocytes. There are the components of the micelles are formed again triacylglycerols, and bile acids via the portal vein back to the liver, and may again enroll in the bile. This process is called bile acid recycling.

The synthesis of fat in the enterocytes of the components of the micelles is called RESYNTHESIS of fat. In the process of resynthesis is the formation of fats, similar in composition to body fats. Then resynthesizing of fat, other lipids and Atabekov formed lipoprotein particles: **CHYLOMICRONS**.

The chylomicron is constructed in the same way as other lipoproteins (see page 2). This is a small fat BLOB: in the center of it are triacylglycerols, which are the predominant component of the particles is 80% of the mass of chylomicrons. On the periphery there are layers of phospholipids (8% by weight) and layers of apobelks (2% by weight), two of which – A and B48 are synthesized on enterocyte ribosomes, which alternate. The remaining 10% of the mass falls on cholesterol and its esters. The surface of the chylomicron is hydrophilic: hydrophilic parts of proteins and phospholipids are on the surface of the particle. The size of the chylomicron is so large that it can not pass through the pores, available in the walls of blood capillaries, by exocytosis. Therefore, by exocytosis chylomicrons enter the lymph. Through it they get into a large circle of blood circulation, bypassing the liver. After eating fat in the blood there is an increased content of chylomicrons. In the blood stream is carried in chylomicrons two Atabekov: "C" and "E". The walls of the capillaries of fat, muscle and other tissues, as well as the membranes of such cells contain an enzyme – lipoproteinlipase. It hydrolyzes the triacylglycerols of the chylomicrons. Apos is a potent activator of lipoprotein lipase.

So after this interaction the amount of triacylglycerols in the chylomicrons decreases and he loses apalisok "s", and apoE is a good ligand for receptors of the liver. The mass of the chylomicron decreases. This leads to a change in its conformation, it turns into a "residual chylomicron". Residual chylomicrons interact with receptors of the liver and is absorbed by the hepatocytes by endocytosis. The liver is composed of residual chylomicrons receives dietary (exogenous) cholesterol.

Therefore, the functions of chylomicrons are:

1) delivery of food (exogenous) fat from the intestine to other tissues (mainly to adipose tissue).

2) Transport of exogenous cholesterol from the intestine to the liver.

Therefore, chylomicrons are a transport form of exogenous fat and exogenous cholesterol.

In adipose tissue from the products of hydrolysis of triacylglycerols, fat resynthesis occurs again (second), and it is deposited there until it is claimed.

β-FATTY ACID OXIDATION

Process β-oxidation is cyclic. For each cycle revolution, 2 carbon atoms in the form of an acetyl residue are cleaved from the fatty acid.

After that, the acyl-COA shortened by 2 carbon atoms is again subjected to oxidation (enters a new cycle of β-oxidation reactions). The resulting Acetyl-COA can further enter the tricarboxylic acid cycle.

β-oxidation occurs most intensively in muscle tissue, kidneys, liver.

The result β-LCD-oxidation produces Acetyl-COA. Oxidation rate is determined by the rate of lipolysis processes. The acceleration of lipolysis is typical for the state of carbohydrate starvation and intensive muscle work. Acceleration of β-oxidation is observed in many tissues, including the liver. The liver produces more Acetyl-COA than it needs. The liver is an "altruistic organ" and therefore the liver sends glucose to other tissues. The liver tends to direct to other tissues and its own Acetyl-COA, but can not, as for Acetyl-COA cell membranes are impervious. Therefore, special substances called "**KETONE BODIES**" are synthesized from Acetyl-COA in the liver.

KETONE BODIES – IS a SPECIAL TRANSPORT FORM of ACETYL-COA!

CHOLESTEROL SYNTHESIS

It occurs mainly in the liver on the membranes of the endoplasmic reticulum of hepatocytes. This cholesterol is endogenous. There is a constant transport of cholesterol from the liver to the tissue. Food (exogenous) cholesterol is also used to build membranes. The key enzyme of cholesterol biosynthesis is HMG reductase (beta-hydroxy, beta-methylglutaryl-COA reductase). This enzyme is inhibited by the principle of negative feedback of the final product – cholesterol.

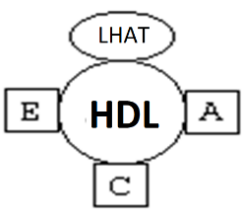
THE TRANSPORT OF CHOLESTEROL Food cholesterol is transported by chylomicrons and enters the liver. Therefore, the liver is a source of tissues and food cholesterol (got there in the composition of chylomicrons), and endogenous cholesterol.

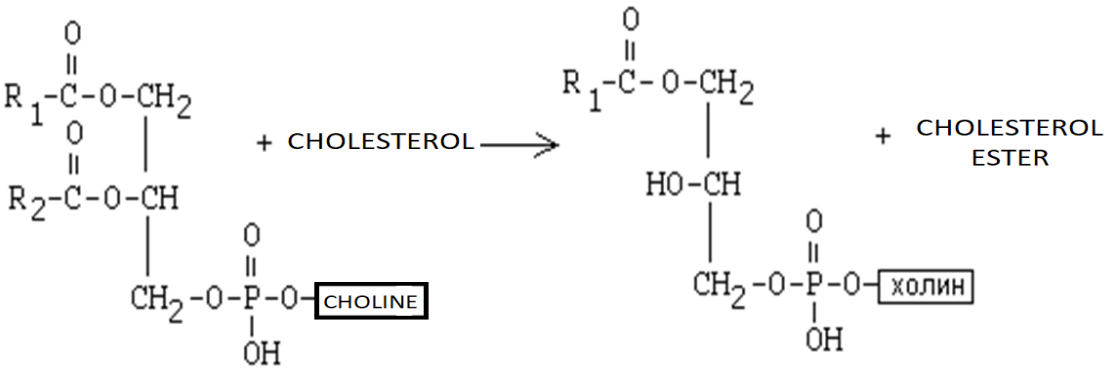
Synthesized in the liver and then released into the bloodstream, lonp – very low density lipoproteins (composed of 75% cholesterol), and LDL - low density lipoproteins(they have some of apalloc апоВ100.

Almost all cells have receptors for apov100. Therefore, LDLs are fixed on the cell surface. In this case, there is a transition of cholesterol into cell membranes. Therefore, LNP is able to supply cholesterol to tissue cells.

In addition, there is a release of cholesterol from the tissues and transport it to the liver. Cholesterol is transported from tissues to the liver by high-density lipoproteins (HDL). They contain very few lipids and a lot of protein. Synthesis of HDL occurs in the liver. HDL particles are disc-shaped, and in their composition are apalaci, apoa, apos and apoE. In the bloodstream attached to LDL protein, the enzyme lecithincholesterolacyltransferase (LAT) (see figure).

Apos and apoE can go from HDL to chylomicrons or lonp. Therefore, HDL are donors of apoE and apos. Apoa, is an activator of LHAT.
LHAT catalyzes the following reaction:





LECITHIN LYSOLECITHIN

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

The reaction is very important, because the resulting cholesterol ester is a very hydrophobic substance and immediately passes into the nucleus of HDL – so in contact with the membranes of HDL cells remove excess cholesterol from them. Further HDL go to the liver, there are destroyed, and excess cholesterol is removed from the body.

Violation of the ratio between the amount of LDL, LDL and HDL can cause cholesterol retention in tissues. This leads to atherosclerosis. Therefore, LDL is called atherogenic lipoproteins, and HDL – antiatherogenic lipoproteins. With hereditary HDL deficiency, early forms of atherosclerosis are observed.

Laboratory work № 12
Topic: Effect of bile on lipase activity.

Method principle. If the milk to add a bit of lipase and alkalize the resulting mixture until a pink color to phenolphthalein, while standing in a water bath at 37°C, the liquid gradually becomes colourless. This is due to the fact that under the action of lipase higher fatty acids are released and the pH is shifted towards lower values. Discoloration in this experience is accelerated by the addition of bile. This is because lipase is activated by bile acid salts.

Reagents:

1. Pancreatin (extract from the pancreas containing lipase), 5% solution;
2. Phenolphthalein, 0.5% alcohol solution;
3. 0.05 I. Naon solution;
4. Milk, boiled and pre-diluted with distilled water in a ratio of 1:1.

Progress of work:

In 2 tubes make 1 ml of milk and 1 drop of phenolphthalein solution. In each tube, add a drop of sodium hydroxide solution to a slightly pink color of phenolphthalein. In each tube, make 2 drops of lipase extract, and in the tube 1, in addition, and a drop of bile. The tubes are placed in a water bath at 37 C and note

the time of discoloration of phenolphthalein in the experiment with and without bile.

Result:

Conclusion:

Emulsification of fat

The principle of the method Fats to be subjected to lipase cleavage in the gastrointestinal tract, must be pre-emulsified. The main emulsifiers of fat in the digestive tract are bile acids. Proteins, Soaps, salts of carbonic acid, contained in a certain amount in the duodenum, also emulsify fats.

Shaking the fat with water, you can observe the formation of a delaminating, unstable emulsion. Adding surfactants, it is possible to obtain a stable emulsion. Emulsifiers are easily adsorbed on the interface of the two phases, forming a thin film that prevents the fusion of emulsion droplets.

Progress of work:

In 5 tubes, pour 3 drops of vegetable oil. In the first tube, add 20 drops of distilled water, in the second – 20 drops of bile, in the third – 20 drops of 1 % egg white solution, in the fourth – 20 drops of I % soap solution, and in the fifth – 20 drops of 1 % sodium carbonate solution. All tubes are thoroughly shaken. After 5 minutes, the preservation of the emulsion is evaluated.

valuation	Soap	Water	Bile	Protein	Soda
Emulsion stability					
The degree of dispersion of the emulsion					

Result:

Qualitative reactions to cholesterol

By chemical nature, cholesterol is a cyclic unsaturated secondary alcohol. The reactions are based on its ability to form colored compounds in the presence of sulfuric acid and acetic anhydride. This ability is used to quantify cholesterol in the blood.

Reagents:

1. Cholesterol, 1% chloroform solution;
2. Sulfuric acid, concentrated;

3. 1% chloroform solution;
4. Bromine water.
5. Glacial acetic acid.

Progress of work:

The detection of unsaturated bonds in a molecule of cholesterol. An unsaturated bond in a cholesterol molecule can be detected by the discoloration of bromine water.

In the tube make 10-15 drops of chloroform cholesterol solution, add 2 drops of bromine water. There is a discoloration of bromine water when shaking the contents of the tube.

Obtaining cholesterol crystals. Cholesterol in interaction with acids forms esters – steroids.

In the test tube make 10 drops of acetic acid and at the tip of the blade a little cholesterol. Heated to a boil. After cooling the tube in the air, observe the appearance of sediment. A few drops of the resulting suspension transferred to a glass slide, cover with coverslip and examined under a microscope the crystals azetilholinesterzu

Result:

Conclusion:

8. NUCLEIC ACID

Nucleic acids are heteropolymers, their monomers are mononucleotides. Mononucleotide consists of a nitrogen base+ribose in RNA (or deoxyribose in DNA) - together they make up a nucleoside, and the remainder of phosphoric acid.

NOMENCLATURE OF NUCLEOTIDES

Nitrogenous base	Nucleoside	Nucleotide
adenine	adenosine	adenosine monophosphate(AMP)
guanine	guanosine	guanosine monophosphate(GMP)
uracil	uridine	uridine monophosphate (UMP)
thymine	thymidine	thymidine monophosphate (TMP)
cytosine	cytidine	cytidine monophosphate (CMP)

TMP is found only in DNA, and the UMF - only in RNA.

As part of nucleic acids, mononucleotides are bound by 3',5'-diester bonds between riboses (d-riboses) of neighboring mononucleotides through the phosphoric acid residue.

THE BIOLOGICAL ROLE OF NUCLEIC ACIDS.

1. DNA: storage of genetic information.

2. RNA:

(a) storage of genetic information in some viruses;

(b) implementation of genetic information: I-RNA (m-RNA) – information (matrix), t-RNA (transport), R-RNA (ribosomal)

(c) some RNA molecules are able to catalyze hydrolysis reactions of 3',5'-phosphodiester bond in the RNA molecule itself. Such RNA is called ribozyme.

FUNCTION MONONUCLEOTIDES.

1. **Structural**. Constructed of mononucleotides, nucleic acids, several coenzymes and prosthetic groups of enzymes.

2. **Power**. The mononucleotides hold macroergic connections – are the energy accumulators. ATP is a universal energy accumulator, UTP energy is used for glycogen synthesis, CTP – for lipid synthesis, GTP – for ribosome movement during translation (protein biosynthesis).

ATP synthesis from ADP occurs in two ways: oxidative and substrate phosphorylation, synthesis of any other nucleotide triphosphates (NTP) from diphosphate forms - through ATP:

NMP + ATP <-----> NDP + ADP

Enzyme: nucleosidephosphorylases

NDP + ATP <-----> NTF + ADP

Enzyme: nucleotide diphosphokinase

3. **Regulatory**. Mononucleotides – allosteric effectors of many key enzymes, camp and cGMP are intermediaries in the transfer of hormonal signal under the action of many hormones on the cell (adenylate cyclase system).

The nitrogen base of adenine is more universal than the others: it has such a mutual arrangement of the amino group with phosphate that it is possible to synthesize ATP from ADP and non-enzymatic way.

CATABOLISM OF NUCLEIC ACIDS

Starts with the hydrolysis of 3',5'-fosfodiesterazy connection under the action of enzymes **nucleases**:

– DNA**ases** – break down DNA

– RNA**ases** – digested RNA

Among DNA**ases** and RNA**ases** distinguish:

– exonucleases (5' and 3');

– endonucleases – specific to the mononucleotide sequence, there are highly specific: restrictive – used in genetic engineering.

Next is the cleavage of phosphate from the mononucleotide with the participation of enzymes of nucleotides with the formation of nucleosides.

Nucleoside can be broken down by hydrolysis under the action of the enzyme nucleosidase on a nitrogen base and pentose, but more often there is phosphorolysis – while nucleoside is split into a nitrogen base and phosphoribose.

Further, pentoses can be disposed of in the II-m stage of the GMF pathway.

Differences in catabolism of purine and pyrimidine nitrogenous bases

Pyrimidine nitrogenous bases undergo total destruction to CO2, H2O and NH3.

Purine nitrogenous bases retain the cyclic structure of purine. Final product: uric acid – purine substance

THE ANABOLISM OF NUCLEIC ACIDS

Nucleic acids (NC) are polymers. Therefore, their synthesis is a chain of polymerization reactions of mononucleotides. In the course of these reactions there is a gradual lengthening of the polinukleotid.

Substrates for the synthesis are mononucleotides in triphosphate form - nucleoside triphosphates (NTP).

NTP + NCP -------> NCP+1 + PP

The resulting pyrophosphate (PP) destroyed pyrophosphatases.

In the synthesis of RNA as NTP uses ATP, GTP, CTP, UTP. For the synthesis of DNA – dATP, dGTP, DTP, TTP (always synthesized with deoxyribose).

The synthesis goes in the direction of 5'--->3'.

NK synthesis is a matrix process, the order of addition of mononucleotides is determined by the structure of the parent NK.

NK synthesis ferments are called polymerases. Polymerases belong to the class of synthetases. Their biosynthesis is controlled by the substrates themselves – NC.

In the human body there are the following polymerases:

DNA POLYMERASE:

– alpha polymerases are responsible for the synthesis of the main chain;

– beta-polymerases eliminate defects that occur in the synthesis of DNA;

– gamma polymerases - mitochondrial enzymes.

RNA POLYMERASE:

I – participate in the synthesis of ribosomal RNA(rRNA);

II – involved in the synthesis of information (matrix) RNA(mRNA, mRNA);

III – involved in the synthesis of transport RNA (tRNA).

Inhibitor of RNA-polymerase-II is the peptide L-amanitin. Found in poisonous mushrooms Amonyta (pale toadstool).

DNA synthesis is called **replication**. The direction of phosphodiester bonds of one of the synthesized DNA polynucleotide chains coincides with the direction of synthesis (5'--->3'), therefore, it is synthesized continuously and immediately as a whole. And the other is not the same (3'--->5'). Therefore, it is synthesized by parts. These parts are called "Okazaki fragments". To synthesize the fragments

Okazaki de novo (from scratch) DNA polymerase can not, therefore, for the synthesis of each fragment need "seed" – primer. Primer is a piece of RNA chain. Synthesis of primers catalyze special enzymes – primase (this is one of the variants of RNA polymerases). RNA synthesis occurs in certain areas of the DNA molecule and is called transcription. In the DNA chain, there are special areas: promoters, which indicate the beginning of transcription and terminators, indicating the end of transcription. During transcription, a high – molecular precursor of RNA, the primary transcript, is formed. Then here, in the cell nucleus, there is a postsynthetic modification of RNA splicing. This process is catalyzed by enzymes of endonuclease - from the primary transcript cut introns. The remaining exons are crosslinked by RNA ligases. Next to the 5'-end of the RNA molecule is attached 7-methyl-GTP (cap-fragment) – this process is called "captioning". To the 3'-end is attached polyadenylate "tail" (polyamp) – reaction catalyzes polyadenylation.

A feature of post-transcriptional modification of ribosomal RNA (R-RNA) is methylation of nitrogenous bases.

In the synthesis of transport RNA (t-RNA) to the end of each molecule is attached a sequence of three mononucleotides: CMF-CMF-AMF (CCA). This sequence is necessary to attach the amino acid to t-RNA.

Laboratory work №13
Topic: Acid hydrolysis of nucleoproteins.

Method principle. Nucleoproteins – complex proteins, prosthetic group of which are nucleic acids. In an acidic environment, there is a weakening of the ionic interaction between the protein and nucleic acids and their separation. Upon subsequent heating, hydrolysis proceeds to amino acids, purine or pyrimidine bases, ribose or deoxyribose, phosphoric acid.

Reagents:

1. Baker's yeast;
2. 10% H_2SO_4 solution;
3. 10% NaOH solution;
4. 1% solution of $CuSO_4$;
5. concentrated ammonia;
6. 1% silver nitrate solution;
7. 1% alcohol solution of thymol (or naphthol);
8. concentrated H_2SO_4;
9. 30% NaOH solution;
10. 7% solution of Ciso4;
11. molybdenum reagent;
12. 1% solution of diphenylamine.

Progress of work:

1 g of baking yeast is placed in a round-bottomed flask. Add 20 ml of 10% sulfuric acid solution, 20 ml of water and close the plug with a reflux. Boil 40-50 min. Cooled, poured into a cylinder. Bring to the initial volume and filter.

With the filtrate is done qualitative reactions to the components of nucleoproteins:

Peptide sample: 5 drops of hydrolysate are mixed with 10 drops of 10% NaOH solution and 1 drop of 1 % CuSO4 solution. The contents are painted pink.

Sample for purine bases: 1 drop of concentrated ammonia and 5 drops of 1% solution of silver nitrate are added to 10 drops of hydrolysate. After 3-5 amines, a brown precipitate of silver derivatives of purine bases appears.

Pentose test (Molish reaction): 2-3 drops of 1% alcohol solution of thymol (or naphthol) are added to 10 drops of hydrolysate. The contents are mixed and 20 drops of concentrated sulfuric acid are poured along the wall.

When shaking, a red staining of the condensation product of furfural with thymol formed from pentose is formed.

Trial of Trommer on ribose and deoxyribose: the 5 drops of hydrolysate, add 10 drops of 30% NaOH solution and 1 to 3 drops of 7% solution CиSO4 before the advent of Muti-stable Cu (OH) 2 mixed. When heated to a boil, a yellow precipitate of copper hydroxide (I) or a red precipitate of copper oxide (I) falls.

Qualitative reaction for ribose and deoxyribose with diphenylamine: diphenylamine gives dark blue coloring with deoxyribose, and green with ribose. To 5 drops of hydrolyzate, add 20 drops of 1% diphenylamine solution and put the tube in a boiling water bath for 15 minutes. Blue-green colouring develops.

Phosphoric acid test: 20 drops of molybdenum reagent are added to 3-5 drops of hydrolysate (7.5 g of ammonium molybdenum is dissolved in 100 ml of distilled water and 100 ml of 32% nitric acid solution with a relative density of 1.2) and boiled for several minutes. Develops a yellow coloration. The tube is immediately cooled in a stream of cold water. At the bottom of the tube appears crystalline lemon-yellow precipitate of phosphoric-molybdenum-acid ammonium.

Result:

Conclusion:

Teoretic the basics of PCR

PCR is used for molecular genetic study of tissue samples. PCR method allows to selectively synthesize in vitro small areas of DNA with a length of several tens to several hundred pairs of nucleotides, using as a matrix any DNA samples.

Reagents:

1) thermophilic DNA polymerase (Tad polymerase);

2) special device - thermal cycler(DNA amplifier), which allows you to set the temperature (90 °C, 30-50 °C, 60--70 °C); time parameters; number of cycles.

The process is fully automated. The choice of optimal operation mode is determined by the length and specificity of the amplified area.

Progress of work:

For the preparation of the reaction mixture is necessary:

• DNA under study;
• substrates: dATP, DSTF, dCTP, dTTP;
• two primers;
• thermophilic DNA polymerase (TA^-polymerase);
• the buffer containing Mg2+.

DNA is extracted from the test material (blood, urine, histological sections, amniotic fluid, etc.). Artificially synthesized primers are oligodeoxyribonucleotides (20-30 nucleotide residues). For the synthesis of primers it is necessary to know the nucleotide sequence of the amplified DNA region (primary structure). Primers should be complementary to the 3'ends of the amplified area.

Progress of work:

To 1.5 ml urine add 1 ml of a 20% solution of sodium carbonate and 1 ml of photovoltage reagent, stirred and titrated with 0,01 n solution of K3[Fe(CN)6] to disappearance of blue color.

Calculation: (uric acid, mmol/day) = 0.8*A*B*0.0059/1.5, where 0.8 – milligrams of uric acid corresponding to 1 ml K3[Fe(CN)6]; A – the amount of red blood salt, gone to titration; B – daily diuresis, ml; 1.5 – the amount taken for urine analysis, ml; 0.0059 – conversion factor in si units.

Result:

Conclusion:

The theoretical basis of the PCR

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STEPS OF CYCLIZATION

Stage I – denaturation. The reaction mixture is heated to a temperature of 90°C for several minutes. Heating leads to the rupture of weak bonds between the bases of the DNA molecule and the formation of single-stranded molecules from the studied double-stranded matrix DNA.

Stage II – DNA hybridization with primers. The temperature is reduced to 30-50 °C. At this temperature, a specific hybridization of DNA chains with primers is possible.

Stage III – elongation (polymerization). It's a synthesis of sequences, complementary matrix DNA. At a temperature of 60-70°C, optimal for the operation of Tad polymerase, polymerase chain reaction begins. Moving along the matrix in the direction of the 5'-end, Tad polymerase extends the primer, attaching to it one after another nucleotide residues complementary to the nucleotides of the matrix.

The further PCR process consists of alternating cycles. The duration of each cycle depends on the length and primary structure of the amplified area, but, as a rule, does not exceed 3-5 minutes.

For each cycle there is a twofold increase in the number of synthesized copies, i.e. the content of amplification products in the reaction mixture increases exponentially.

This three-stage cycle can be repeated many times, until enough material is formed for further research.

The resulting material is fractionated by electrophoresis in agarose or polyacrylamide gel. Special polyacrylamide gels have been developed, with the help of which it is possible to divide DNA fragments up to 500 nucleotides, differing only by one nucleotide.

Compared with traditional methods of diagnosis, this method allows for rapid, sensitive and specific identification of genetic material.

In medical diagnosis with this method, you can solve many questions:

- Identify viral and bacterial DNA in human DNA.
- Carry out prenatal diagnosis of hereditary diseases.
- Identify heterozygous carriers of defective genes.
- Determine the exact location of genes in human DNA.
- Identify defective genes that disrupt the regulation of cell proliferation and cause the development of tumor diseases.
- Analyze and identify drugs that contain highly degraded DNA.

9. SPECIFIC BIOCHEMISTRY

Laboratory work №14
Subject: Benzidine blood test

The principle of the method Hemoglobin has the ability to catalyze the oxidation of benzidine with hydrogen peroxide, the Resulting products of oxidation of benzidine have a blue or green color. The reaction is very sensitive ' and serves to detect small amounts of blood in biological fluids.

Reagents:

1) benzidine solution – 50 g/l (in glacial acetic acid, freshly prepared); 2) hydrogen peroxide solution – 30 g/l; 3) defibrinated blood diluted with water.

Progress of work:

5 drops of diluted defibrinated blood and the same amount of benzidine are poured into the tube, then 5 drops of hydrogen peroxide solution are added. Appears blue or green coloring.

Result:

Conclusion:

Obtaining crystals of hemin from hemoglobin (sample of Tahmina)

The principle of the method When heated blood with glacial acetic acid hemoglobin breaks down into heme and globin. If blood hydrolysis is carried out in the presence of acetic acid containing halogen salts, heme turns into a hydrochloric salt – hemin, forming characteristic diamond-shaped crystals. Teichman's sample is used in forensic practice to prove the presence of blood spots.

Reagents, test material:

1) glacial acetic acid containing halogen salts; 2) blood.

Progress of work:

A glass stick is applied to the slide a drop of blood, smearing it on the glass. The resulting smear should not be very thin, however, and not too thick. The smear is dried, holding the slide high above the flame of the burner (if the glass with blood is kept above the flame itself or introduced into the flame, the experience will not succeed). On the dried smear, 2 drops of acetic acid are applied, covered with a cover glass and carefully heated to boil the liquid under the glass (not.boil!). After that, 2 more drops of acetic acid are introduced under the glass and the drug is examined under a microscope.

Gemin crystals under a microscope have the form of diamond-shaped sticks, painted in brown. Gemin crystals are sketched in a notebook.

Result:

Conclusion:

Qualitative reactions to tetracycline

With ferric chloride. Tetracycline with ferric chloride forms compounds such as iron phenolates.

Reagents:

1) tetracycline solution, 0.5% solution; 2) ferric chloride, 5% solution.

Progress of work:

To 10 drops of tetracycline solution add 1-2 drops of ferric chloride solution. Appears brown color.

Conclusion:

With concentrated sulfuric acid.

With the addition of concentrated sulfuric acid to the dry preparation of tetracycline, a red staining appears as a result of its water-absorbing action.

Reagents: 1.tetracycline, powder; 2.concentrated sulfuric acid.

Progress of work:

To 5-10 mg of tetracycline powder add 10 drops of concentrated sulfuric acid. The liquid is colored red.

Conclusion:

Tests for residual knowledge check

1. Proteins consisting of more than one polypeptide chain are called:

- Polyfunctional;
- Oligomeric;
- Polymeric;
- Synthetic.

2**. The sequence of amino acid residues in polypeptide chains determines _____ protein structure:**

- Tertiary;
- Secondary;
- Primary;
- Quaternary.

3. The state of the protein, in which the number of major functional groups is equal to the number of acidic, called:

- Amphoteric;
- Isoelectric;
- Isoelectronic;
- Isostatic.

4. In the formation of α-helix polypeptide chain in proteins hydrogen bonds occur between the fragments:

- =C- H and O=C= ;
- =N-H and O=C=;
- =C=N-H and H-C=;
- =N andH-C=.

5. Acid-base properties of polypeptides are determined by the presence of functional groups in them:

- =N и COOR.;
- =N и SR;
- =N и COOR;
- –NH2 и –COOH

6. What is the optimum temperature for the action of most enzymes:

- 50-60 C;
- 15-20 C;
- 80-100 C;
- 35-40 C.

7. What is the name of the site of the enzyme molecule is responsible for both the addition of the substance^ subjected to enzymatic action^ and for the implementation of enzymatic catalysis:

- Hydrophobic center;
- A catalytic center;
- Active center;
- Adsorption center;
- Allosteric center.

8. Where over-dependent dehydrogenases are localized:

- in the mitochondrial matrix;

• in the inner membrane of mitochondria;
• in intermembrane space;
• in the outer membrane of the mitochondria.

9. What vitamin is part of the coenzymes NAD and NADP:

- P;
- PP;
- B2;
- B1.

10. What vitamin is part of FMN and FAD:

- B1;
- B2;
- B3;
- B5.

11. The end product of a complex oxidizing system is:

• peroxide;
• water;
• superoxide ion.

12. What class of proteins are cytochromes:

• lipoproteins;
• glycoproteins;
• chromoproteins.

13. What vitamin is needed for hydroxylation of Proline and lysine:

• ascorbic acid;
• pantothenic acid;
• nicotinic acid.

14. What vitamin contributes to the formation of connective tissue:

- C;
- B12;
- H.

15. Choose from the following hormone protein-peptide nature:

• adrenaline;
• insulin;
• testosterone.

16. Choose from the following amino acid derived hormone:

• thyroxine;
• glucagon;
• estriol.

17. Choose from the following steroid nature hormone

• oxytocin;
• thyrotropin;
• progesterone.

18. Which enzyme catalyzes the transformation of fructose-1,6-bisphosphate into 2 trioses:

• Triazolothiadiazoles;

• Fructose-1,6-bisphosphate-aldolase;
• Hexokinase.

19. What is the final product synthesized by oxidative decarboxylation of pyruvate:

• Citrate;
• Ketoglutarate;
• Acetylphosphate;
• Acetyl-CoA;
• Propionate.

20. What is the number of ATP molecules formed by oxidation of 1 molecule of glucose to CO2 and water

• 2;
• 8;
• 10;
• 24;
• 38.

21. What lipoprotein complexes transport cholesterol from the intestine:

• chylomicrons
• LDL;
• VLDL;
• HDL.

22. What lipoprotein complexes transport cholesterol from the liver:

• Chylomicrons
• LDL;
• VLDL;
• HDL.

23. What enzymes are involved in direct deamination of amino acids?

• L-oxidases;
• transaminases;
• decarboxylase.

24. The patient with acute pain in the heart is determined by the activity in the blood serum:

• AlAT;
• ASAT;
• alkaline phosphatase.

25. What are the final products formed by the oxidation of amino acids?

• CO2^ H2O^ NH3;
• CO2, H2O;
• CO2^ H2O^ pyruvate.

26. The physiological minimum of proteins is:

• 100-120 g/day;
• 30-45 g/day;
• > 120 g/day.

27. Thick filaments of the sarcomeres of the myofibrils of skeletal muscle are composed mainly of:

- Actin's;
- Myosin's;
- Troponin.

28. The main protein of the thin filaments of the sarcomeres of the myofibrils of skeletal muscle is:

- Actin;
- Myosin;
- Troponin.

29. Uric acid is the end product of metabolism:

- purine nucleotides;
- pyrimidine nucleotides;
- neutral lipids.

30 connective tissue is characterized by the presence of:

- lipoproteins';
- metalloproteins;
- production of;
- proteoglycan.

Year-end test questions for "Biological chemistry" discipline

1. Encoded amino acids: structure, properties, classification. Write the formulas of cysteine and methionine, to indicate their position in the classifications.
2. Types of bonds between amino acids in a protein molecule. The origin and function of peptides in the body. Write the formula of a tetrapeptide: HAIRDRYER-PRO-LYS-TYR.
3. Spatial organization of protein molecules. Belkin stress.
4. Conformation of a protein molecule. Functional role of conformational transitions. The concept of domains.
5. Physico-chemical characteristics of aqueous solutions of globular proteins. Write the formula of tetrapeptide, isoelectric point, which lies in a weakly acidic medium.
6. The Nativity of the protein molecule. Deprivations of the protein to its native properties.
7. Modification of the side chains of a protein molecule. Mechanisms and role.
8. Modern methods of protein separation and purification. Their practical significance.
9. Methods for determining the primary and higher structures of the protein molecule.
10. Energy of enzymatic catalysis. Activation energy and energy result of the reaction. General properties of enzymes and non-biological catalysts.
11. Features of enzymes as biocatalysts. Types of specificity of enzymes (give specific examples).
12. Features of the structure of the functional centers of the enzyme. Write the formula of amino acids, which often form the catalytic center of the enzyme.
13. Characteristics of the main stages of enzymatic catalysis.
14. The dependence of the reaction rate of the enzyme concentration. Unit activity and unit quantity of the enzyme. Write the oxidation reaction of glucose-6-phosphate.
15. Classification and indexing of enzymes. Give examples of reactions catalyzed by enzymes of each class of enzymes.
16. Isoenzymes: definition, biological significance. Diagnostic value of identification of isoenzymes in biological fluids.
17. The equation of Michaelis-cement and its graphic expression. The main kinetic constants of the enzyme. Their physical meaning, the practical significance of their definition.
18. Levels of regulation of enzymatic processes. Autonomous self-regulation of enzymes: definition; basic principles; specific manifestations in the simplest system.
19. Features of Autonomous self-regulation of multi-enzyme systems. The concept of key enzymes. Give an example of such an enzyme and write the equation of the reaction catalyzed by it.
20. Activation of enzymes. Interconversion of active and inactive forms of enzymes. Write the formula of cyclic mononucleotide.
21. Enzyme inhibitors: definition and classification. Methods for determining the type of inhibition.

22. Mitochondrial oxidation. General characteristics of the process, biological value.
23. Structure and mechanism of action of nicotinamide dehydrogenases. Give examples of substrates of these enzymes (formula).
24. Complex 1 of mitochondrial oxidation. Structure and mechanism of FMN participation in electron and proton transport along the respiratory chain.
25. Coenzyme Q. Structure and mechanism of action.
26. Cytochromes. Structure and mechanism of action.
27. General scheme of the complete chain of mitochondrial oxidation. To write the formula of the substrates of this chain.
28. Short circuit of the mitochondrial oxidation of (General scheme). To write the oxidation of substrates.
29. Oxidative and substrate phosphorylation. Coefficient P/O. Give an example of substrate phosphorylation (equation).
30. Modern ideas about the conjugation of oxidation and phosphorylation. Mechanisms of separation of these processes.
31. Oxidase and oxygenase types of biological oxidation. Features and biological significance of each type. Give examples of reactions.
32. Active forms of oxygen, the ways of their formation. The role of reactive oxygen species in normal and pathological conditions.
33. Antioxidant system of the body.
34. The production, structure and biological functions. Hemoglobin and other hemoproteins. Structure and biological functions of hemoglobin. Hemoglobin derivatives.
35. The main stages of hemoglobin synthesis. Molecular forms of hemoglobin. The concept of hemoglobinopathies and porphyria.
36. The breakdown of hemoglobin (the scheme). The main products of decay, the place of their formation and ways of excretion. The concept of jaundice.
37. Nucleoproteins. Structure, classification and biological functions of nucleic acids. Write the formula pyrimidine bases.
38. Structure, nomenclature and biological functions of mononucleotides. ATP: structure and biological role,
39. Biosynthesis of purine mononucleotides. Write the formulas of the substrates for synthesis. The biosynthesis of DNA.
40. Decay of nucleic acids in tissues. End products of decay.
41. Biosynthesis of pyrimidine mononucleotides. The biosynthesis of RNA.
42. Stages of protein catabolism. Proteolysis. Proteolysis enzymes, their structure, substrate specificity. Write the formula of a tetrapeptide: lay-Hairdryer-lease-three.
43. Ways to protect proteins from the action of proteinases. Write the formula of tetrapeptide: Glu-Pro-GIS-arg.
44. Digestion of proteins in the gastrointestinal tract. Enzymes that catalyze the digestion of proteins.
45. Putrefaction of protein breakdown products in the intestine. Mechanisms of neutralization in the body of rotting products, as well as other toxic substances.
46. Proteins as an indispensable component of food. The concept of nitrogen balance, the physiological minimum of protein, the coefficient of wear. Essential amino acids (write formulas).

47. The concept of limited proteolysis. Characteristics and role of the process.
48. Mechanism and biological significance of transamination. The most important transaminase.
49. Ways of formation and neutralization of ammonia. The mechanism of the temporary neutralization of ammonia.
50. Biosynthesis of urea. Regeneration of aspartic acid. The biological significance of this process.
51. Mechanism of decarboxylation of amino acids. The biological significance of this process. Write reactions of formation and neutralization of the most important biogenic amines.
52. Methods of deamination of amino acids. The biological significance of this process.
53. Synthesis and biological role of creatine.
54. Synthesis of interchangeable amino acids from the number of negatively charged and hydrophobic.
55. Features of the exchange of sulfur-containing amino acids.
56. Synthesis of interchangeable amino acids from among hydrophilic uncharged. The concept of active (C).
57. Features of metabolism of phenylalanine and tyrosine. Congenital disorders of their metabolism
58. Tricarboxylic acid cycle. The sequence of reactions to the stage of formation of a-ketoglutaric acid. Autonomous self-regulation of CTC.
59. The biological significance of the tricarboxylic acid cycle. The sequence of reactions after the formation of a-ketoglutaric acid.
60. Structure and metabolism of glycogen.
61. Digestion and absorption of carbohydrates. Write the reactions occurring during wall digestion of carbohydrates.
62. Autonomous and hormonal regulation of glycogen metabolism.
63. Path of aerobic breakdown of carbohydrates. General characteristics and biological significance. Write the equations of the first three reactions of this process.
64. Carbohydrate breakdown from fructose-1,6-bisphosphate to pyruvic acid. Write the reaction equations of this process in aerobic conditions.
65. Mechanism of oxidative decarboxylation of a-ketoacids.
66. Shuttle mechanisms of transmembrane transport of substances.
67. Glycolysis, glycogenolysis and alcoholic fermentation. General characteristic. Biological significance. To write reactions of the glycolytic oxidation-reduction.
68. Treatment of glycolysis. Write the equations of irreversible reactions of this process. The concept of glyconeogenesis. Autonomous self-regulation.
69. The pentose phosphate pathway of breakdown of carbohydrates. Chemistry of reactions to ribozo-5-phosphate. Autonomous self-regulation pentozofosfatnogo way.
70. The General concept of pentozofosfatnogo way of the breakdown of carbohydrates. The biological role of pentozofosfatnogo way. Scheme of the non-oxidative stage. The final equation pentozofosfatnogo way.
71. Sources, biological role and ways of using NADPH2 in the cell.

72. Lipids – definition, classification. Triacylglycerols. Structure, physico-chemical properties and biological role. Higher fatty acids. Essential fatty acid.
73. Digestion and absorption of triacylglycerides
74. Mobilization of fat from fat depots. Regulation of this process.
75. b-oxidation of fatty acids (starting with their activation).
76. Biosynthesis of fatty acids.
77. The main ways of formation and utilization of acetyl-COA (scheme).
78. Ways of formation and utilization of ketone bodies
79. The synthesis and breakdown of glycerophospholipids.
80. Possible metabolic pathways of glycerol in the tissues (scheme).
81. Phospholipids – classification, properties, biological role. Write the General formula of glycerophospholipids and sphingomyelins.
82. Glycolipids – structure and biological role. Write the General formula of glycolipids.
83. Steroids – General characteristics, classification. Metabolic pathways and the role of cholesterol.
84. Structure of biological membranes. Write General formulas of lipid components of membranes.
85. Arachidonic acid cascade. Mechanism and role of the process.
86. Disorders of carbohydrate metabolism in diabetes. Biochemical manifestations. Mechanism and consequences of the process of glycation of macromolecules.
87. Disorders of lipid metabolism in diabetes mellitus. Biochemical manifestations.
88. Autonomous regulation of carbohydrate metabolism. Key enzymes of the aerobic path of decay, equation catalyzed by their reactions, and their mechanism of self-regulation.
89. Autonomous self-regulation of carbohydrate metabolism in conditions of intensive muscular work.
90. Autonomous self-regulation of carbohydrate metabolism at rest.
91. Autonomous self-regulation of energy metabolism in conditions of excessive nutrition and sedentary lifestyle.
92. The concept of parameterize. Main types of parameterizes reactions.
93. Hormones – General characteristics. Structure, classification, mechanisms of action. Adenylate cyclase and its catalyzed reaction.
94. Hormones of the hypothalamus and pituitary. Their structure and mechanisms of action.
95. Thyroid hormone. Biosynthesis of thyroxine. Biochemical mechanisms of hyperthyroidism. Hypofunction of the thyroid gland.
96. Parathyroid hormones, their regulatory functions.
97. Hormones of the pancreas. Molecular mechanisms of their action and biochemical consequences.
98. Hormones of the adrenal medulla. Biosynthesis and molecular mechanisms of their action.
99. Adrenal cortex hormones. Molecular mechanisms of action.
100. Sex hormones. Similarities and differences in structure. Biological effect.
101. Vitamins – definition, classification, biochemical functions (examples). Hypo-and hypervitaminosis, their causes.

102. Vitamin A. Structure, food sources, daily requirement. Biochemical function. Manifestations of insufficiency.
103. Vitamin D. Structure, food sources, role in metabolism, daily requirement. Active forms of vitamin D, signs of hypovitaminosis.
104. Vitamin K. Biochemical functions and manifestation of its insufficiency.
105. Vitamin E. Biochemical functions. A manifestation of failure.
106. Vitamin B1 Structure, food sources, role in metabolism. Daily need, manifestation of failure.
107. Vitamin B2. Structure, food sources, role in metabolism. Daily need, manifestation of failure.
108. Vitamin PP. Structure, food sources, role in metabolism. Daily need, manifestation of failure.
109. Vitamin B6. Structure, food sources, role in metabolism. Causes of failure.
110. Vitamin N. Role in metabolism, daily requirement, manifestation of insufficiency.
111. Vitamin B3. Role in metabolism, daily requirement, manifestation of insufficiency.
112. Vitamin B12 and folic acid. Structure (descriptive). Food sources, daily requirement.
113. Biochemical function. Manifestations of insufficiency.
114. Vitamin C. Structure, food sources, role in metabolism, manifestation of insufficiency.
115. Plasma proteins, structural features. Methods of separation of plasma proteins.
116. Hypo - Hyper - and dysproteinemia. Their identification. Acute phase proteins, their diagnostic value.
117. Plasma albumin – structure, biological functions.
118. The main groups of blood plasma globulins. Their biological functions.
119. Transport forms of lipids of blood plasma. Lipoprotein spectrum of blood plasma in normal and pathological conditions.
120. Enzymes of blood plasma. Zymogen. List the enzymes, the definition of which in blood plasma is diagnostic value.
121. Non-protein components of blood plasma. Residual blood nitrogen – the content of this concept, diagnostic value.
122. Blood plasma minerals. Their biological functions and features of distribution between plasma and shaped elements.
123. Features of chemical composition and metabolism of leukocytes
124. Platelet biochemistry.
125. Biological functions of blood. Its physico-chemical properties. Chemical composition of blood plasma.
126. Respiratory function of blood. Molecular mechanisms of oxygen and carbon dioxide transport.
127. The main proteolytic system of blood. General regularities of their functioning.
128. Blood coagulation system. Mechanisms of its functioning. The meaning of the principle of cascading.
129. The system of fibrinolysis. Mechanisms of its functioning, value.

130. Anticoagulants, structure and mechanism of action.
131. Proteolytic system of vascular tone regulation. Formation of vasoactive peptides.
132. General characteristics of blood participation in immune reactions. Immunoglobulins – structure, biological role.
133. Complement system and its role in immunological processes.
134. Kidney function. Features of their metabolism. Hormonal regulation of urine formation.
135. Physico-chemical properties and chemical composition of normal urine.
136. Pathological components of urine.
137. Carbohydrate components of connective tissue - structure, role. Write the formulas of monomers
138. hyaluronic acid and chondroitin-6-sulfate.
139. Proteins of connective tissue - structure, biological role.
140. Chemical composition and features of nervous tissue metabolism.
141. Chemical composition and features of muscle tissue metabolism. Biochemistry of muscle contraction.
142. Features of the structure and metabolism of bone tissue.
143. Water exchange and its regulation.
144. Forms of existence and the role of minerals in the body. Regulation of sodium and potassium metabolism.
145. The role of calcium and inorganic phosphate in the human body. Regulation of phosphorus-calcium metabolism.
146. The role and metabolism of iron in the human body.

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