Nizhny Novgorod State Medical Academy
The department of Biochemistry

LABORATORY MANUAL on BIOCHEMISTRY

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The given manual is designed according to the curriculum on biochemistry for the students of the Faculty of Overseas Admissions (general medicine and dentistry specialities). It is intended to save the student's time and optimize their practical work.

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Настоящее руководство составлено в соответствии с рабочей программой по биохимии для иностранных студентов лечебного и стоматологического факультетов академии. Оно предназначено для экономии времени студентов и оптимизации их практической работы.

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Рекомендован Учебно-методическим объединением по медицинскому и фармацевтическому образованию вузов России в качестве учебного пособия для иностранных студентов медицинских вузов, обучающихся на английском языке.
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Рекомендован к публикации Методической комиссией по естественно-научным дисциплинам.

Introduction

Biochemistry is one of the fundamental medico-biological subjects which is very important for general medical education independently on certain physician speciality. The modern notions about pathogenesis of human diseases are based on molecular aspects of the development of the pathological states. Therefore the detailed comprehension of the essence of diseases is impossible without serious research of biochemistry.

At the same time the modern physician has to know the principles of the biochemical methods of analysis used to prove the main theoretical statements of biochemistry and a number of clinico-diagnostic biochemical methods applied in practical medicine. For it medical students have to get the minimum of manual skills during a research of biochemistry, eg. measuring out solutions and biological liquids, centrifugation, colorimetry of coloured solutions, determination of pH, peculiarities of the technique of enzyme investigations etc. The given manual contains the descriptions of the biochemical methods of analysis which all the skills are required in.

All material of the manual is divided into 29 lessons according to the curriculum of practical lessons adopted in the academy. The 30th lesson is intended for the students of stomatological faculty only.

Besides the basic questions for self-preparation of home tasks and the examples of the control tests are contained in the description of each topic of the manual. It has to facilitate the process of preparation of home tasks by the students and to reduce the elements of stress during student's testing.

The list of examination questions is printed at the end of the manual (Appendix). All examination tickets are compiled according to the list. We hope that it will facilitate a pre-examination training of students.

The authors

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We are indebted to the scientific editor of the manual Academic of RAMS T.T.Beresov, Professor of the Department of Biochemistry of the Russian University of Freindship. We acknowledge with gratitude the generous help we have received from T.G. Shirokogorova, the English editor of this manual.

Further suggestions towards improvement of this manual are welcome.

WARNING!
(The instruction for student's safe work at the department of Biochemistry)
Caustic bases and acids

Caustic (concentrated) bases and acids can provoke burns of skin, mucous membranes and eyes. Therefore if you use the caustic bases or acids (NaOH, KOH, H_2SO_4, HNO_3, trichloracetic acid etc), you should observe the following rules:

1. Do not measure solutions of the bases and acids with a mouth pipette. Do it only with a measuring test tube or a burette.
2. Do not keep a vessel with the base or acid near your eyes.
3. Do not pour water to acid (especially to H_2SO_4) when you prepare an acid solution.
4. If base or acid gets on your skin, mucous membrane or especially eyes you should bathe the affected place in running water for 10 - 15 minutes and then treat this place with the diluted solution of boric acid or ammonia.

Electric equipment and apparatus

Be careful when you work with electric equipment to avoid an electric shock. Observe the following rules:

1. Do not touch the naked electric wires.
2. Do not work with unearthed apparatus.
3. Do not pull out an electric wire, when you switch off an electric apparatus from the electric network. Do it only with an electric plug.
4. Do not touch a water pipe, a tap, and a heating radiator when you work with electric apparatus.
5. If somebody gets an electric shock you should immediately switch off electric power and only after that help him.

Fire-fighting rules

1. Do not smoke in a laboratory, a corridor, or other places of the department.
2. Be careful when you work with explosive or inflamed substances (spirits, ketones, ethers etc.).
3. If fire begins, you should use a fire-extinguisher or water (only for water soluble inflamed substances!).

Call the fire-brigade quickly. Its phone number is 01.

The FIRST TERM

LESSON 1
PHYSICO-CHEMICAL METHODS of ANALYSIS in BIOCHEMISTRY. ELECTROMETRY.

BIOMEDICAL SIGNIFICANCE

Biological liquids of the body have different values of pH (see below). Most of them have approximately neutral pH, but only the gastric juice is characterized as the most acidic liquid; pH of other digestive liquids is slightly alkaline. The specific values of pH are maintained with mineral and organic buffer systems. Blood has the most constant level of pH due to the high buffer capacity of its buffer systems.

Average values of pH of the body

Liquid	pH
Blood	**7.4±0.05**
Urine	**5.0-8.0**
Saliva	**6.4-7.0**
Gastric juice	**1.2-3.0**
Pancreatic juice	**7.4-8.3**
Bile	**6.6-7.6**
Small intestine secretion	**7.8-8.0**
Synovial liquid	**7.3-7.4**
Perspiration	**4.5-7.5**
Tear	**7.0-7.4**
Milk	**6.6-6.8**

Determination of pH of biological liquids is applied for diagnose and correction of some pathological states accompanied by acid-base state disorders. Excessive accumulation of H^+ ions resulting in a decrease in pH of biological liquids is called ***acidosis***, and the opposite phenomenon of excessive accumulation of OH^--ions is marked as ***alkalosis.***

The electrometric method of pH determination allows to measure pH of biological liquids with accuracy of 0.01 units of pH.

Work 1. Measurement of pH of biological liquids with pH-meter (ion meter).

THE EQUIPMENT: an ion meter.

PRINCIPLE of METHOD.

The apparatus consists of two electrodes - glass and chlorine-silver, placed in a researched solution, and electronic mill voltmeter, which scale is graduated in terms of pX and in mill volts of a potential difference of the electrode pair.

The glass electrode is a measuring one. Its potential depends on concentration (activity) of ions of hydrogen in an analytic solution. The chlorine-silver electrode has constant potential.

COURSE of WORK.

Plug the apparatus in the electric network. Press the switch "сеть"(“network”), then the button "анионы/катионы" ("anions/cations"), and the next button of ranges of measurement " -1 - + 19 ".

Wash out the apparatus electrodes carefully with distilled water and wipe them off with a filter paper. Pour the solution for analysis or a biological liquid into a glass. The volume of the solution has to be sufficient enough to completely close the working surfaces of the electrodes with the layer of a liquid.

Read the result on the lower (bottom) scale of the apparatus in terms of pX (pH). Choose the necessary range for more precise measurement of pH and press the appropriate button on the right panel of the apparatus. Read and write down the result from the upper (top) scale of the apparatus. For doing it, mentally divide the distance between two points (lines) of the upper scale into ten equal parts. Count the amount of these parts from the left point of the distance up to the needle position. Thus, the results may be registered precisely up to the third sign (0.01 pH units).

RESULTS:

CONCLUSIONS:

Work N 2 Measurement of pH of biological liquids with universal indicator paper.

Take a piece of indicator paper. Then immerse its small part in a biologic liquid. Next put the wet piece of indicator paper on a white sheet of paper and quickly compare its colour with a standard colour scale marked on the indicator paper case.

RESULTS:

CONCLUSIONS:

LESSON 2
PHYSICO-CHEMICAL METHODS of ANALYSIS in BIOCHEMISTRY. PHOTOMETRY.

BIOMEDICAL SIGNIFICANCE

The photoelectrocolorimetric method of analysis serves for determination of substances concentrations in coloured solutions, biological liquids or tissue extracts. It may be also used for determination of concentrations of colourless substances if they can be transformed into coloured state with the specific reagents. The method is one of the most widespread in biochemistry and clinical medicine.

Work N 1. Quantitative determination of iron ions in solution by a photoelectrocolorimetric method.

The EQUIPMENT: a photoelectrocolorimeter (FEC).

PRINCIPLE of METHOD.

There is a linear dependence between the concentration of substance in a solution and the size of optical density (extinction). This dependence is described by the equation:

$$D = \alpha \cdot l \cdot c,$$

Where:

D - optical density of a coloured solution

α- the molar coefficient of light absorption,

l - the thickness of a layer of the solution,

c - the molar concentration

The COURSE of WORK.

Construction of a calibrating graph.

Pour 10 мl of one of standard solutions with the concentration of iron: 0.1 mg/ml; 0.2 mg/ml; 0.4 mg/ml respectively in three 25 ml measuring flasks. Add 1 ml of salicylic acid in each flask to form the coloured complex and lead up the volume of the liquid in the flasks by distilled water to a label. Wash the experimental cuvette of a FEC with the prepared solutions (the thickness of cuvettes for the analysis is 10 mm). Measure the optical density of the solution against water with a photoelectrocolorimeter (FEC) according to the description below. Plot the values of concentration and optical density of the standard solutions on the calibrating graph axes, and build the calibrating graph.

The ORDER of ANALYSIS with a PHOTOELECTROCOLORIMETER (FEC).

1.Warm up FEC with an open cover for 15 minutes.
2. Establish a light filter (green for this analysis, 540 nm).
3. Put the cuvettes into a FEC cuvette compartment (the distant cuvette, containing distilled water, is marked as "control". The nearer cuvette contains the solution for analysis and is marked as "experimental".
4. Establish the lever to the left position.
5. Press the button "ПУСК" ("START-UP").
6. Press the button "Ш0".
7. Close the cover.
8. Press the button "К1".

9. Move the lever to the right position.
10. Press the button "D5"("OPTICAL DENSITY").
11. Read the result on the window of the apparatus and write it down.
12. Move the lever to the left position.
13. Open the cover.
14. Take out the "experimental" cuvette.
15. Wipe carefully the cuvette compartment of the FEC. After that the apparatus is ready for the next measurement.

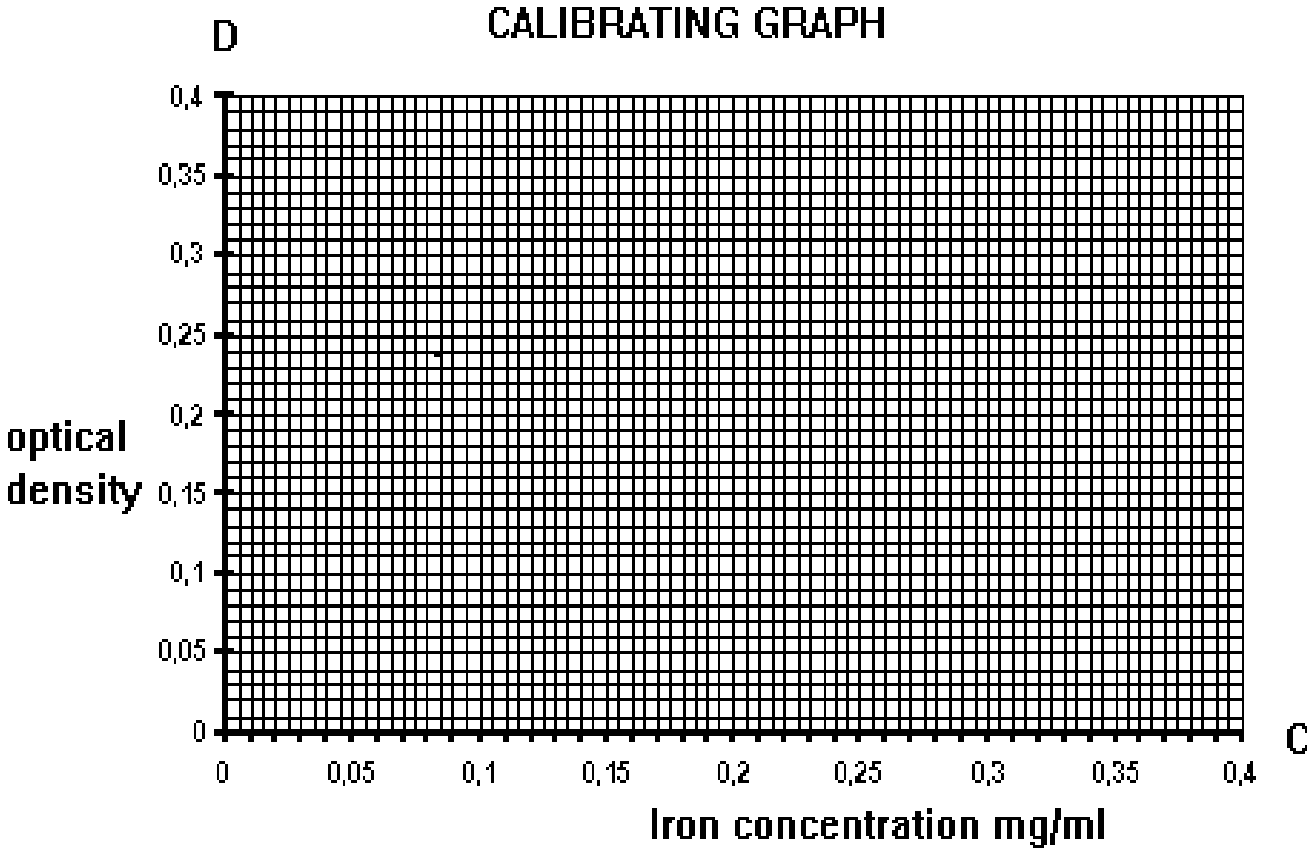
The analysis of a liquid which iron ion concentration is unknown (solution of a task).

Take a bottle containing the liquid for the analysis (write down the number of a bottle).

Measure out 10 ml of a researched solution in a 25 ml measuring flask, add 1 ml of salicylic acid and lead up the volume of the flask contents to a label with distilled water. Mix it carefully. Measure optical density (D) of the coloured solution with a FEC and determine the iron ion concentration of the solution with the calibrating graph.

It is also possible to determine the concentration by the simpler manner, by a choice of a standard solution, which optical density (Dst) is the closest to the optical density of a sample for analysis (Dx), and by the solution of a proportion:

Dst --------- Cst
Dx ---------- Cx



RESULTS (solution of a task):

CONCLUSIONS:

LESSON 3
STRUCTURE and PROPERTIES of PROTEINS. PRIMARY STRUCTURE of PROTEINS.

BIOMEDICAL SIGNIFICANCE

Proteins are high molecular mass substances consisting of 20 kinds of amino acids joined with each other by peptide bonds. There are four levels of the structural organization of the protein molecule. The primary structure is an unbranched polymer chain containing the certain amino acid sequence. The primary structure of each protein is absolutely unique. It is encoded for in DNA and stored as the nucleotide sequence of DNA genes. There are many inherited pathological states caused by the affection of the primary structures of proteins (eg. hemoglobinopathies, enzymopathies and other so called ***molecular diseases***).

The ***chromatographic method*** of determination of amino acids may be used for analysis of the primary structure of proteins and peptides. The determination of free amino acid content in serum, urine, liquor and other biological liquids is applied to estimate protein metabolism in the patient's body.

Work 1. The analysis of chromatograms of protein hydrolysates.

The EQUIPMENT: a FEC, chromatograms of various proteins, test tubes with an ethanol solution for extraction.

PRINCIPLE of METHOD.

Amino acid composition of protein hydrolysates is investigated with the distributive chromatography on paper. The method is based on the ability of substances to be differently adsorbed on various adsorbents, and to be differently dissolved in polar and unpolar solvents. In the present method chromatographic paper is used as an adsorbent. The division of amino acids is carried out by a mixture of solvents (butanol + ice acetic acid + water at the ratio 4:1:5). Thus water is kept by a paper, whereas the organic solvents move along the paper with certain speed. The substances (in this case amino acids), which are soluble in an organic solvent, move on the paper together with a solvent. The water-soluble substances, occupy intermediate positions on a chromatogram between a start line and a front line of a solvent. After termination of a division and drying, the chromatogram is treated by ninhydrin solution and dry up again. After that the stains of the divided amino acids become visible and accessible to the further qualitative and quantitative analysis.

COURSE of WORK.

Ready made chromatograms of various proteins have to be analyzed.

1. Qualitative analysis of chromatograms (identification of amino acids stains) is carried out by account of R_f coefficient for each stain:

$$\mathbf{R_f = L_{aa} / L_{fs}}$$

Where:

Rf- the coefficient of distribution (see the table below),

$\mathbf{L_{aa}}$ - the distance from the start line to the centre of the amino acid stain,

$\mathbf{L_{fs}}$.- the distance from the start line to the front line of a solvent.

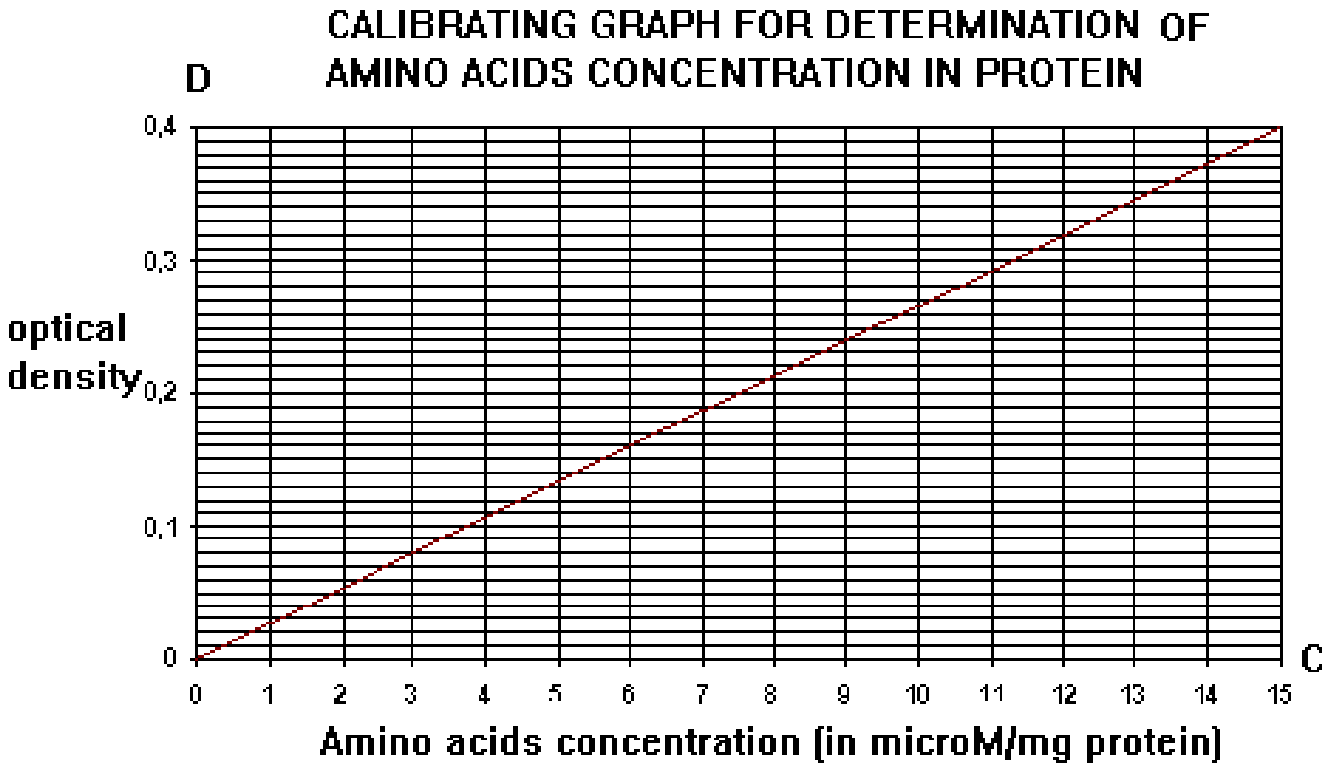
Using tabulated values of R_f for each amino acid it is possible to identify every kind of amino acids on the researched chromatogram. For account of R_f it is necessary to measure the distances from the start line to the centre of each stain and the distance from the start line to the front line of a solvent.

The calculated values of R_f coefficient is compared to the tabulated data and thus every stain is identified on the chromatogram.

Amino acids	R_f	Amino acids	R_f
alanine	0.45	threonine	0.35
arginine	0.20	tryptophan	0.55
aspartic acid (aspartate)	0.24	phenylalanine	0.68
glycine	0.25	methionine	0.55
serine	0.27	cysteine	0.07
glutamic acid (glutamate)	0.30	cystine	0.08
proline	0.43	lysine	0.12
valine	0.60	leucine	0.73

2. Quantitative analysis of chromatograms.

Cut out the coloured stains which belong to certain amino acids with scissors, cut in these stains slightly as a brush and put them into the test tubes, containing an ethanol solution, for extraction of the coloured product. Close the test tubes by fuses and put them in darkness for 30 minutes for complete extraction. Stir the tubes from time to time. Then measure the optical density of the coloured liquids by a FEC with a green light filter (the thickness of cuvettes is 5 mm). Find out the concentration of each amino acid on the received sizes of optical density (D) with a calibrating graph.



RESULTS:

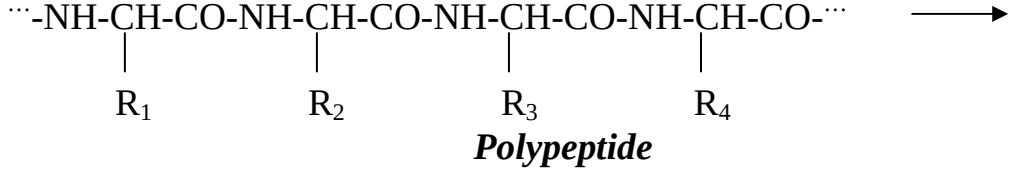
CONCLUSIONS:

Work 2. Biuretic reaction for detection of peptide bond.

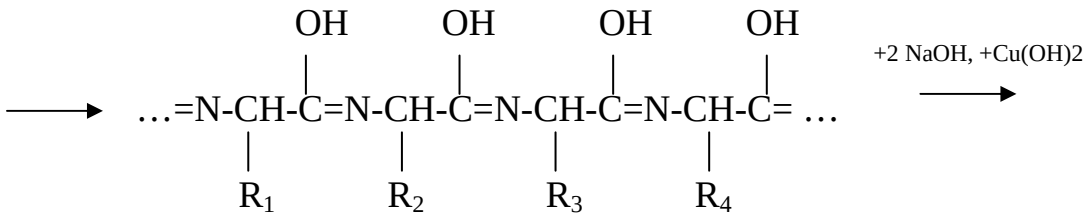
PRINCIPLE of METHOD.

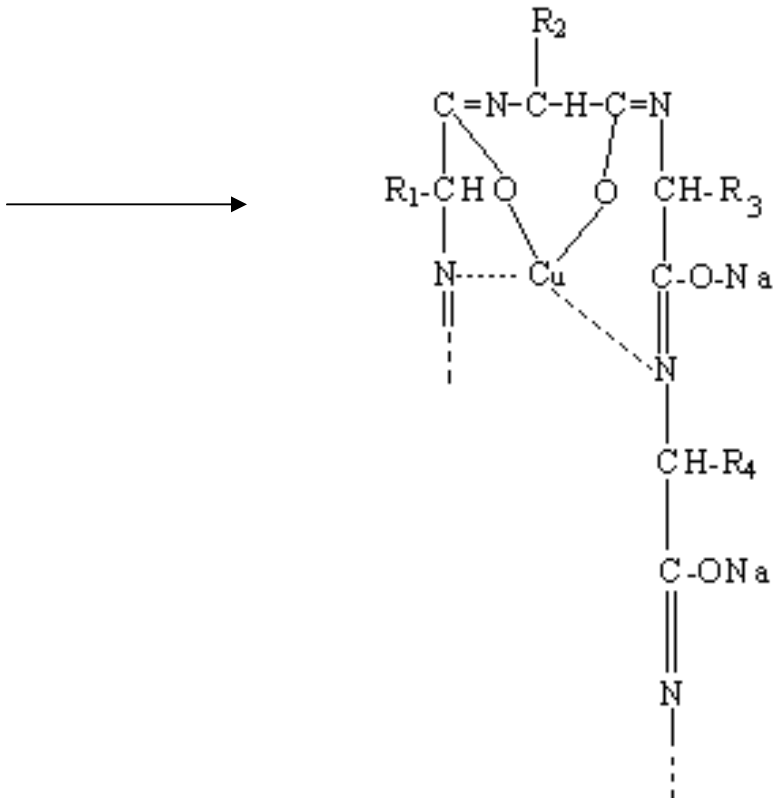
The reaction is caused by the presence of peptide bonds forming the complexes with ions of copper salt in the alkaline medium.

Schematically this reaction may be presented as:



Polypeptide





Depending on the length of polypeptide chain, the solution becomes blue-violet or pink coloured.

COURSE of WORK.

Add 5 drops of 10 % NaOH and 1-2 drop of 1 % $CuSO_4$ to 10 drops of a protein solution and mix it. The contents of a test tube appears to be blue-violet. Carry out the same reaction with a polypeptide solution and a mixture of amino acids. Then solve a task: analyze the unknown solution (protein, polypeptide or mixture of amino acids) with the biuretic reaction.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Proteins as the most important macromolecules of the body. The variety of proteins and their functions. Classification of proteins.
2. Biological functions of proteins.
3. Amino acids as structural units of a protein molecule, their structures, classification, nomenclature. Essential and non-essential amino acids.
4. The primary structure of a protein molecule. The polypeptide chain, peptide bond.
5. Inherited alterations of the primary structure of proteins. Examples of change in protein functioning and properties as the result of change in amino acid sequence.

Examples of the task:

Write the formula and call the tetrapeptide, consisting of the following amino acids:

a) cyclic charged,
b) polar uncharged,
c) heterocyclic uncharged,
d) imino acid.

What charge will this peptide have in a neutral medium?

LESSON 4
SPATIAL ORGANIZATION of PROTEIN MOLECULE.

BIOMEDICAL SIGNIFICANCE

The spatial arrangement of a protein molecule includes the spatial relationships of neighbouring amino acid residues of a polypeptide chain, such as the alpha-helix or the beta-pleated sheet (the secondary structure), the spatial relationships of more distant residues (the tertiary structure), and the spatial relationships between the individual polypeptide chains in oligomeric and multimeric proteins (the quaternary structure). All above kinds of the spatial organization of a protein molecule depend on its primary structure.

Proteins are typical high molecular mass substances having gigantic sizes in comparison with low molecular mass substances (simple sugars, amino acids, fatty acids, mineral substances etc). Therefore proteins can be purified by ***dialysis***. This principle lies in the basis of operating of the ***"artificial kidney"*** or ***hemodialysis*** apparatus intended for cleaning of patient's blood from low molecular weight toxic end products of metabolism.

Noncovalent bonds of a protein molecule may be broken down with some strong physical agents (high temperature, sonication, different kinds of irradiation etc) and with concentrated mineral and organic acids, heavy metals and other high reactive compounds. This phenomenon is called ***irreversible denaturation*** of proteins. It is a reason of toxic effects of these agents. The ability of proteins to bind covalently heavy metals is used as the antitoxic treatment principle in oral ***heavy metal intoxication.***

Work 1. Dialysis of proteins.

THE EQUIPMENT: dialysers.

PRINCIPLE of METHOD.

Dialysis is a division of substances with semi-penetrable membranes which have little pores and are impenetrable for the molecules of high-molecular weight compounds. The molecules of proteins having the significant sizes do not penetrate across the semi-penetrable membranes, while the molecules of low-molecular weight substances pass across them freely.

COURSE of WORK.

Pour a solution of protein with an impurity of any low-molecular substance (glucose, chlorides, phosphates, sulfates etc.) in the internal dialyser vessel.

Immerse the filled internal vessel in the external vessel (glass) of a dialyser, containing distilled water, so that the level of water after the immersing would be a bit higher than the level of a membrane of the internal vessel. In 30-60 minutes carry out the biuretic reaction with the contents of internal and external vessels and qualitative reaction for a low-molecular substance which is in the composition of a researched solution.

The biuretic reaction: (see the protocol of lesson 3).

Test for chlorides: 1 ml of the researched solution + 1-2 drops of 1 % $AgNO_3$.

Test for phosphates: 1 ml of the researched solution + 1-2 drops of magnesial mix.

Test for sulfates : 1 ml of the researched solution + 1-2 drops of 1% $BaCl_2$.

Test for glucose (the Trommer reaction): 1 ml of the researched solution + 0.5 ml of 10 % NaOH + 5 drops of 1 % $CuSO_4$. Heat up a test tube in boiling water for 1-2 minutes.

RESULTS (fill in the table):

Before dialysis		
	Internal vessel	External vessel
Protein		
Low-molecular substance		
After dialysis		
Protein		
Low-molecular substance		

CONCLUSIONS:

Work 2. Detection of the isoelectric point of casein.

PRINCIPLE of METHOD.

In the isoelectric point proteins have the least stability because of loss of a charge. Thus molecules of proteins are getting aggregated and form larger particles, that results in increased turbidity of a solution.

COURSE of WORK.

Measure out 9 ml of a buffer solution with certain meaning pH in five tubes. Add 1 ml of casein solution in each test tube. Mix the contents of the test tubes. In 10-15 minutes estimate a degree of turbidity in all tubes. Note a test tube with the greatest degree of turbidity (coagulation).

RESULTS: Fill in the right column of the table:

№№ of the test tubes	pH of a buffer solution	Volume of a buffer solution (ml)	Volume of casein solution	Degree of turbidity ("-", "+" ,"++", " +++")
1	3.8	9	1	
2	4.4	9	1	
3	4.7	9	1	
4	5.1	9	1	
5	5.7	9	1	

CONCLUSIONS:

Work 3. Ways of irreversible precipitation of protein from solutions.

PRINCIPLE of METHOD.

Precipitation of proteins from solutions and biological liquids can be induced by the physical and chemical agents causing irreversible denaturation of proteins with a loss of both factors of stability of the protein molecule (hydrate coat and an electric charge) that leads to a protein sedimentation.

COURSE of WORK.

The work is carried out according to the conditions specified in the table:

№№ of the test tubes	Denaturating agents	Order of the work	Results
1	High temperature (boiling)	1 ml of a protein solution + boiling for 5-10 min.	
2	Pb ions (a solution of lead acetate)	1 ml of a protein solution + 2-3 drops of lead acetate	
3	Concentrated mineral acids	1 ml of concentrated HNO_3 + 1 ml of a protein solution (pour slowly and carefully without mixing)	
4	Concentrated organic acids	1 ml of protein + 1 ml of 50% trichloracetic acid	

CONCLUSIONS:

BASIC QUESTIONS:

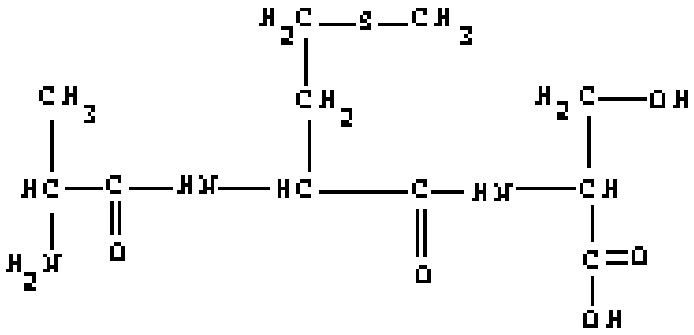
1. The conformation of protein molecules (secondary and tertiary structures). Dependency of protein conformation on the primary structure.
2. Kinds of intramolecular bonds in proteins. Formation of domains and clusters. Their role in proteins functioning. The role of three dimentional organization of a peptide chain in formation of the enzyme active centers.
3. The quaternary structure of proteins. Cooperative alterations of the protomer conformation. Examples of the structure and functions of the oligomeric proteins (hemoglobin in comparison with myoglobin), allosteric enzymes, polyenzyme complexes.
4. Biological functions of proteins. Specific interactions of proteins with ligands. Kinds of natural ligands and peculiarities of their interactions with proteins (prosthetic groups, cofactors, protomers, substrates, transported substances, allosteric effectors). Conformation alterations during protein functions.
5. Factors of protein molecule stability in solutions. Denaturation of proteins. Use of denaturating agents in medicine and biochemical tests.
6. Physico-chemical properties of proteins. Point Isoelectric. Molecular weight. Influence of pH on proteins charge.

Examples of the task:

1. Which of the following bonds takes part in the formation of a-helix structure of a protein molecule?

a) Hydrophobic (nonpolar bonds)
b) Hydrogen bond (H-bond)
c) Peptide bond
d) Ionic

2. Choose the name for the peptide:



a) alanyl-cysteyl-serine
b) seryl-cysteyl-alanine
c) alanyl-methionyl-serine
d) seryl-methionyl-alanine

3. Native functional properties of oligomeric proteins are realized as a result of forming:

a) α-helix
b) quaternary structure
c) β-structure
d) tertiary structure

4. Primary structure of protein is:

a) a configuration of a polypeptide chain
b) a result of folding of a polypeptide chain
c) a polymer chain consisting of the certain amino-acid sequence
d) a product of polymerization of 20 amino-acids

5. Which of the following amino-acids contains the hydroxyl group within its radical?

a) serine
b) lysine
c) histidine
d) leucine

6. PI of chymotrypsin is 9.5. What direction will this protein move to during electrophoresis at pH 5.0?

a) to anode
b) to cathode
c) stays on start line
d) to the both directions

7. Which of the following amino-acid residues may form hydrophobic bond with each other?

a) Alanine-valine
b) Alanine-serine
c) Alanine-glutamic acid
d) Alanine-lysine

8. Which of the following properties of proteins are due to the presence of amino- and carboxyl groups in their structure?

a) Hydrophilia, high permeability across membranes
b) Thermolability, solubility
c) Ability to sedimentation
d) Amphoteric properties, ability to electrophoresis

9. Which of the following amino-acids contains sulfhydryl group within its radical?
a) arginine
b) alanine
c) cysteine
d) tryptophan

10. What part of the protein structure is termed "domain"?
a) The monomer of oligomeric protein
b) A part of protomer which is repeated in different proteins and fulfills the same function
c) The unprotein part of the complex proteins
d) The site of the protein molecule consisting of the similar groups and taking part in the binding function

11. The velocity of protein sedimentation depends on:
a) the size of the molecules and their molecular weight
b) electric charge of the molecules and temperature
c) ionic strength of the solution
d) the shape of the molecules

12. Irreversible denaturation may be caused by:
a) addition of strong acids (TCA, HNO_3)
b) transitory action of acetone
c) transformation to isoelectric state
d) salting out

13. What part of protein structure is called "cluster"?
a) The monomer of oligomeric protein
b) The site of the protein molecule consisting of the similar groups and taking part in the binding function
c) the catalytic centre of enzyme
d) the unprotein part of the complex proteins

14. What is the average range of molecular weight of single-chain proteins?
a) 5-7 kdal
b) 150-200 kdal
c) 10-50 kdal
d) 300-400 kdal

15. Which of the following ionic species of glutamic acid would be the prevalent at pH 10?

a) $^{-}COO\text{-}CH(NH_2)\text{-}(CH_2)_2\text{-}COO^{-}$

b) $^{-}COO\text{-}CH\text{-}(CH_2)_2\text{-}COO^{-}$

NH_3^+

c) $^-COO\text{-}CH\text{-}(CH_2)_2\text{-}COOH$
NH_3^+

d) $HCOO\text{-}CH\text{-}(CH_2)_2\text{-}COOH$
NH_3^+

16. With the exception of glycine, all amino-acids found in proteins are:
a) optically inactive
b) L-configuration
c) levorotatory
d) dextrorotatory

17. Which of the following α-amino-acids is diaminomonocarboxylic?
a) Proline
b) Glutamate
c) Lysine
d) Proline

18. Which of the following α-amino-acids is formed as a result of post-translational modification of its precursor?
a) Isoleucine
b) Threonine
c) γ-carboxyglutamate
d) Methionine

19. Quaternary structure of proteins is:
a) a single polypeptide chain that has unique conformation
b) several plypeptide chains joined to the common molecule
c) a polypeptide chain that has α-helix configuration
d) a polypeptide chain that has β-structure configuration

20. The peptide bonds have a "backbone" of atoms in which of the following sequences:
a) C-N-N-C
b) C-O-C-N
c) N-C-C-C
d) C-C-N-C

21) Specificity of proteins is due to:
a) peptide bonds between amino-acids
b) amount of amino-acids in the protein
c) amount of protomers in oligomeric proteins
d) amino-acid composition and sequence of amino-acids in the polypeptide chain

LESSON 5
ENZYMES. SPECIFICITY of ACTION.

BIOMEDICAL SIGNIFICANCE

Each enzyme can catalyze only one reaction with one substrate or a number of substrates having the similar structure. High specificity is one of the most characteristic properties of enzymes. Clinical enzymology is the field of medicine using this property for diagnosis and control of the treatment of pathological states. A great number of diseases are accompanied by the cell damage and death as a result of hypoxia, ischemia or inflammation. The enzymes of the dead cells enter the blood where they can be determined with the specific quantitative methods.

The phenomenon of absolute specificity of enzymes is very often used for quantitative determination of metabolite concentrations in blood and other biological liquids.

Work 1. The specificity of amylase and sucrase action.

The EQUIPMENT: a thermostat for 38ºC, a boiling water bath.

PRINCIPLE of METHOD.

In this work the specificity is studied for two enzymes of the hydrolases class (the subclass of glycosydases) - amylase and sucrase. Amylase catalyses the reaction of the starch hydrolysis and forms the several kinds of dextrins and then maltose. Sucrase catalyses the reaction of the sucrose hydrolysis and forms glucose and fructose as the products of the reaction. The Trommer reaction is applied to reveal the products of reactions having free aldehyde or ketone group.

COURSE of WORK.

The work has to be carried out by *two students* which fill 4 test tubes according to the following description (look at the table):

№№ of the test tubes	**Starch**	**Sucrose**	**Amylase (Saliva 1:10)**	**Sucrase**	**Results of the Trommer reaction**
1	10 drops	-	5 drops	-	
2	10 drops	-	-	5 drops	
3	-	10 drops	5 drops	-	
4	-	10 drops	-	5 drops	

Put all the test tubes into a thermostat at 38ºC for 10 min. After that carry out the Trommer reaction with the contents of each test tubes.

The Trommer reaction is based on the ability of carbohydrates to reduce metals (in this case Cu^{2+}). With heating in the boiling water bath $Cu(OH)_2$ (the light-blue sediment) is reduced to CuOH (yellow sediment) and then to Cu_2O (red sediment).

The order of the reaction.

Add 5 drops of 10% NaOH and 2 drops of 1% $CuSO_4$ to the researched solution. Put the test tubes in the boiling water bath and keep them there until the appearance of yellow or red colour. Do not keep the test tubes in the water bath too long and do not add the excess of $CuSO_4$ because copper oxide -CuO (black sediment), masking positive reaction, can be formed.

RESULTS:

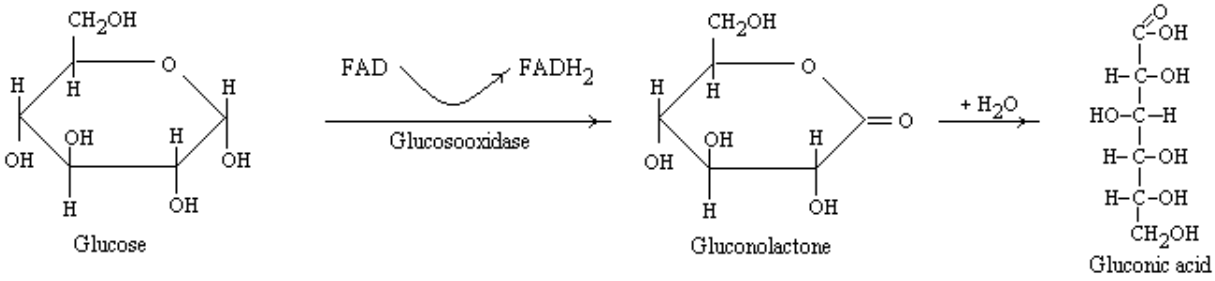
CONCLUSIONS:

Work 2. Quantitative determinaion of glucose in blood by a glucose-oxidase method.

The EQUIPMENT: a FEC, a centrifuge.

PRINCIPLE of METHOD.

The method is based on the specificity of enzyme *glucose-oxidase*. This enzyme catalyses the reaction of oxidation of glucose in the presence of molecular oxygen with the formation of gluconolactone, which is spontaneously hydrolyzed to gluconic acid.



$$FADH_2 + O_2 \longrightarrow FAD + H_2O_2$$

Thus the equimolar quantity of hydrogen peroxide (H_2O_2) is formed. Then hydrogen peroxide is decomposed by peroxidase. Monoatomic oxygen released in peroxidase reaction oxidizes ortho-toluidine. Ortho-toluidine is transformed from a colourless compound to the light-blue or dark blue compound. The colour of the product depends on the quantity of the atomic oxygen and, hence, on the quantity of glucose in researched blood. The quantitative determination of glucose is finished by a measurement of optical density of a sample and a comparison of it with optical density of a sample containing the standard solution of glucose.

COURSE of WORK.

Experimental sample.

1. Measure out 0.4 ml of 5 % $ZnSO_4$ and 0.4 ml of 0.3 N NaOH in a centrifuge test tube, containing 0.1 ml of blood and 1.0 ml of a physiological solution. Mix carefully the contents of the test tube.
2. In 10 min centrifuge it at 1500 rev/min for 10 min in order to sediment proteins (*the rules of centrifugation see in the instruction near a centrifuge*).
3. After centrifugation pour *CAREFULLY* the supernatant into a clean dry test tube.
4. Take another clean dry test tube and measure out 1 ml of supernatant and add 3 ml of "Glucose reagent" for the determination of glucose. This reagent contains glucose-oxidase, peroxidase and ortho-toluidine.
5. In 15 min determine optical density of the coloured solution by a FEC with the red light-filter against water (670 nm, the thickness of a cuvette is 5 mm).

Standard sample.

Prepare precisely the same as the experimental sample, but take 0.1 ml of a standard solution of glucose with the concentration 5.5 mM /l instead of blood.

Account

Make the account of the glucose concentration with a technique, described in the protocol to the Lesson 2:

Dst ---------- 5.5
Dx ----------X

Where:
Dst - the value of optical density of the standard solution of glucose,
Dx - the value of optical density of the experimental sample,
X - the value of glucose concentration in the sample of blood.
Solve the proportion and find the concentration of glucose in the sample of blood. Compare the found result with the normal values. The normal concentration of glucose in blood of an adult healthy person changes within the limits of 3.3 – 5.5 mM/l.
RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. The definition of the term "enzymes". The main differences of enzymes from inorganic catalysts.
2. The structure of enzymes. Apoenzyme, coenzyme and prosthetic group. Vitamins and metal ions as cofactors of enzymes (examples).
3. Congenital (primary) enzymopathies. The reasons for their rise.
4. The active centre of an enzyme. Its structure. Sites of the active centre. Amino-acids included in the active centre.
5. Theories of substrate-enzyme interaction (Fisher, Koshland).
6. The nomenclature and classification of enzymes. The description of each class. The examples of the enzymes of different classes.

7. The principles of the quantitative determination of the enzyme activity.Units of enzyme activity: catal and international unit (IU).
8. Specificity of the enzyme action. The kinds of specificity. The importance of the property for metabolism.

Examples of the task:

1. Which of the following unprotein components is tightly bound with an apoprotein?
    a) Prosthetic group
    b) Coenzyme
    c) Sodium ion
    d) None above
2. What is the name of an enzyme that transfers -NH_2-group?
    a) Acyltransferase
    b) Aminoacyltransferase
    c) Amidinotransferase
    d) Aminotransferase
3. Can enzymes catalyze the reactions which are impossible without an enzyme thermodynamically?
    a) Yes
    b) No
    c) Can, if these reactions are exergonic
    d) Can, if these reactions are endergonic
4. The specific activity of an enzyme is:
    a) the amount of enzyme that produces 1 mol of product per second under standard conditions
    b) the amount of an enzyme causing transformation of 1 micromol substrate per minute under standard conditions
    c) the activity of an enzyme in relation to a standard preparation of the enzyme
    d) the number of enzyme units per milligram of enzyme protein
5. The active centre of an enzyme is being constructed during the formation of:
    a) a primary structure (amino-acid sequence of a protein)
    b) a secondary structure (α-helix, β-structure and amorphous fragments)
    c) a tertiary structure of protomeric enzymes and quaternary structure of oligomeric proteins
    d) It depends on amino-acids which protein is built from.
6. What does it mean "*activation energy*"?
    a) Average kinetic energy of molecules in the system
    b) Energy of reactive molecules
    c) Energy of an exergonic reaction
    d) Additional energy required to reach a transition state.
7. Enzymes, catalyzing the reactions of CO_2 incorporation and releasing, are included into the class of:
    a) ligases
    b) lyases

c) isomerases
d) hydrolases

8. What is the name of a protein part of enzyme molecule that interacts with substrate?
a) Apoenzyme
b) Allosteric centre
c) Active centre
d) Domain

9. What phenomenon of enzyme catalysis occurs under a low temperature (freezing)?
a) Reversible inactivation
b) Irreversible inactivation
c) Activation
d) Sedimentation

10. Enzymes increase the rates of reactions by:
a) increasing in activation energy
b) increasing in the free energy change of the reaction
c) changing in the equilibrium constant of the reaction
d) decreasing in activation energy

11 Enzymes catalyzing the reactions of intramolecular transfer of various groups are included into the class of:
a) hydrolases
b) isomerases
c) transferases
d) oxydoreductases

12. What is meant by the steady-state assumption that underlies the Michaelis-Menten relationship between substrate concentration and reaction velocity?
a) The reaction velocity is linearly related to substrate concentration
b) The reaction velocity is independent of substrate concentration
c) The rate of breakdown of enzyme-substrate complex equals the rate of formation of the complex
d) The rate of formation of product equals the rate of disappearance of substrate

13. What functional group of glutamate is included into the active center of an enzyme?
a) α-carboxyl group
b) α-amino-group
c) γ-carboxyl-group
d) γ-amino-group

14. What phenomenon of enzyme catalysis occurs under a high temperature (boiling)?
a) irreversible denaturation
b) reversible denaturation
c) activation
d) hydrolysis of peptide bonds

15. Enzymes, catalyzing the reactions of dehydrogenation, are included into the class of:

a) hydrolases
b) lyases
c) transferases
d) oxidoreductases

16. What is the curve that describes Michaelis-Menten equation?

a) rectangular hyperbola
b) parabola
c) right line
d) sigmoid curve

17. If the substrate concentration in an enzyme-catalyzed reaction is equal to 1/2 K_m, the initial reaction will be:

a) 0.25 V_{max}
b) 0.33 V_{max}
c) 0.5 V_{max}
d) 0.75 V_{max}

18. The Michaelis-Menten constant (K_m) is:

a) numerically equal to V_{max}
b) the equilibrium constant for the dissociation of ES to E + P
c) increased in value with increasing affinity of the enzyme for its substrate
d) the substrate concentration at 1/2 V_{max}

19. Enzymes, catalyzing the synthetic reactions with ATP utilization, are included into the class of:

a) oxidoreductases
b) transferases
c) lyases
d) ligases

LESSON 6
STRUCTURE of ENZYMES. VITAMINS as PARTICIPANTS of ENZYME REACTIONS.

BIOMEDICAL SIGNIFICANCE

Vitamins are the essential components of food because as a rule they can not be synthesized in the body. Most of vitamins are the cofactors of many complex enzymes, but some vitamins take part immediately in chemical reactions as co substrates.

Dietary foodstuffs have to contain sufficient amounts of vitamins according to the recommended dietary allowances. Both insufficient and excessive intake of vitamins lead to the development of metabolic disorders termed as ***hypo- or***

hypervitaminoses respectively. Therefore it is very important to know the distribution and content of vitamins in various foodstuffs.

Work 1. The detection of an apoenzyme and a coenzyme in aspartate-aminotransferase.

PRINCIPLE of METHOD.

In complex enzymes a protein component (apoenzyme) can be found out with the biuretic reaction. An unprotein component, containing a derivative of any vitamin, can be opened with the appropriate qualitative reactions for each vitamin. The derivative of vitamin B_6 (pyridoxal-phosphate) is the unprotein component of aspartate-aminotransferase. Vitamin B_6 (pyridoxine) and its derivative are colourless, but get red colour in the presence of iron chloride. The reaction is caused by the formation of the complex salt, the same as iron phenolate.

COURSE of WORK.

1. The biuretic reaction. See the protocol of the Lesson 3.
2. The reaction to vitamin B_6. Mix 5 drops of an aspartate-aminotransferase solution and 1 drop of 5% iron chloride solution. Stir up.

RESULTS:

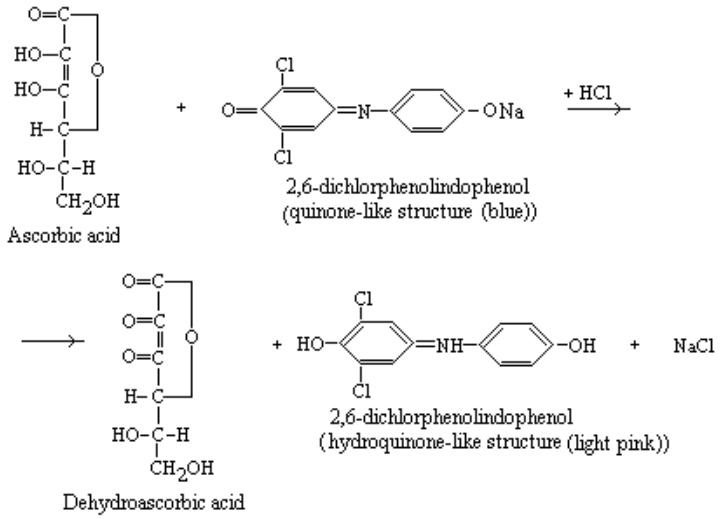
CONCLUSIONS:

Work 2. Quantitative determination of ascorbic acid in various food-stuffs.

The EQUIPMENT: scales with standard weights, burettes for titration.

PRINCIPLE of METHOD.

The method is based on the oxidation of ascorbic acid by blue reagent 2,6 - dichlorphenolindophenol (DCIP). The products of this reaction are dehydroascorbic acid and light pink reduced form of DCIP.



COURSE of WORK.

Prepare an extract of vitamin C according to the table (see below). Measure out the necessary extract volume **in a flask,** add 1 ml of 2 % HCL to the diluted extract and titrate with 2,6-dichlorphenolindophenol until the appearance of light pink colour. If the extract contains chlorophyll and has green colour, titrate this extract, comparing its colour with the colour of the same extract stored without titration, because green colour masks light pink colour. In this case titrate the content of the flask until the change of the colour of the titrated extract in comparison with a control sample (without titration).

Researched foodstuff	**Weight of a sample (grams) C**	**Volume of water for extraction D**	**Volume of extract and water for the analysis B**	**Notes**	**Vitamin C content (mg%)**	**Daily require-ment of the foodstuff (grams or ml)**
Infusion of dog-rose fruits			1 ml of the infusion + 10 ml of water			
Fresh cabbage	5 g	20 ml	10 ml	to filter extract		
Pickled cabbage	10 g	20 ml	10 ml	-.-		
Pickled cabbage juice			1 ml of the juice + 5 ml of water	-.-		
Lemon pulp	1 g	10 ml	5 ml	-.-		
Lemon peel	1 g	10 ml	5 ml	-.-		
Apple juice			1 ml of the juice + 5 ml of water	-.-		
Nettle	1 g	10 ml	5 ml of the extract + 5 ml of water	-.-		
Sorrel	1 g	10 ml	1 ml of the extract+ 5 ml of water	-.-		

Dandelion	1 g	10 ml	5 ml of the extract + 5 ml of water	-.-		
Brown onions	5 g	20 ml	1 ml of the extract+ 5 ml of water	-.-		
Green onions	1 g	10 ml	1 ml of the extract+ 5 ml of water	-.-		
Apple	5 g	10 ml	5 ml of the extract+ 5 мl of water	-.-		
Needles	1 g	10 ml	1 ml of the extract+ 10 ml of water	-.-		
Potatoes	1 g	10 ml	1 ml of the extract+5 ml of water	-.-		
Polyvitamin (pills)	1 pill	50 ml	1 ml of the extract +5 ml of water			

Account. 1 ml of 2,6-dichlorphenolindophenol corresponds to 0,088 mg of ascorbic acid (titer of ascorbic acid for 2,6-dichlorphenolindophenol).

The account of the ascorbic acid content is carried out for 100 g of the dense foodstuff or for 100 ml of the liquid foodstuff (in mg%). For account it is convenient to use the formula:

$$\mathbf{X = 0.088 \cdot A \cdot D \cdot 100 / C \cdot B}$$

Where:

X - the content of ascorbic acid in the foodstuff (in mg/dl),

A - the result of the titration (the volume of 2,6-dichlorphenolindophenol),

B - the volume of the extract for the titration (ml) without the account of water,

C - the weight of a dense sample (g) or the volume of a liquid sample (ml) of a product for the analysis,

D - the total volume of an extract (ml),

100 - the figure necessary for recalculation of the content of ascorbic acid in 100 g (ml) of the food-stuff.

Calculate the quantity of the product needed for satisfaction of the daily requirement of vitamin C (in grams or ml).

RESULTS: write down them in the right column of the table.

CONCLUSIONS:

Work 3 Quantitative determination of vitamin P in the different sorts of tea.
THE EQUIPMENT: burettes for the titration.

PRINCIPLE of METHOD.

The method is based on the ability of vitamin P to be oxidized by potassium permanganate. Indigocarmine is used as an indicator. It reacts with potassium permanganate after the complete oxidation of vitamin P.

COURSE of WORK.

Put 100 mg of dry tea in a glass and add 50 ml of boiling water. Extract it for 5 minutes. Measure out 10 ml of extract of tea in another clean and dry glass, add 5 ml of distilled water of room temperature and 5 drops of indigocarmine. Titrate until the appearance of yellow colour. This colour has not to disappear for 30 sec. Make the account of the content of vitamin P in the tea by the formula:

$$\mathbf{X = 16 \cdot A\ (mg\%)}$$

Where:

X - the content of vitamin P in dry tea (mg%),

A - the result of the titration (volume of potassium permanganate) (ml).

Then calculate the quantity of dry tea needed for satisfaction of the daily requirement of vitamin P (in grams).

RESULTS (fill in the table):

№№	Sort of tea	Content of vitamin P (mg/%)	The quantity of dry tea needed for satisfaction of the daily requirement of vitamin P (in grams)
1			
2			
3			

CONCLUSIONS:

BASIC QUESTIONS:

1. Definition of the term "vitamins". Classification and nomenclature of vitamins.
2. Biological functions of vitamins. Hyper-, hypo- and avitomonosises.
3. Lipid-soluble vitamins - A,D,E,F,K. Their metabolic role.
4. Water-soluble vitamins - B_1, B_2, B_3, B_5, B_6, B_{12}, B_c, C, P, H. Their metabolic functions.
5. The role of vitamins as cofactors of the enzymes:
   a) vitamins B_2 and B_5 as cofactors of oxidoreuctases,
   b) vitamins B_3, B_6, B_c, B_{12} as cofactors of transferases,
   c) vitamin B_{12} as a cofactor of isomerases,
   d) vitamins B_1, B_6 as cofactors of lyases,
   e) vitamins H and K as cofactors of ligases (synthetases).
6. The role of ascorbic acid as a co-substrate for reactions of oxidation-reduction.
7. Vitamin-like substances: choline, inosine, S-adenosylmethyonine, lipoic acid etc.
8. Antivitamins and their metabolic role.
9. Use of vitamins in medicine.

Examples of the task:

1. The main metabolic function of vitamin B_1 is its participation in:
   a) carboxylation of a substrate
   b) decarboxylation of a substrate
   c) dehydratation of a substrate
   d) isomerization of a substrate
2. What vitamins are co-factors of *oxidoreductases*?
   a) B_3, B_6, B_{12}
   b) C, B_2, B_5
   c) A, D, E, F
   d) B_1, B_2, H
3. What coenzyme contains vitamin B_2 in its structure?
   a) Nicotinamide adenine dinucleotide (NAD)
   b) Flavine adenine dinucleotide (FAD)
   c) Coenzyme A (CoA-SH)
   d) Coenzyme Q (ubiquinone)
4. Pyridoxal phosphate is a coenzyme derivative of vitamin:
   a) B_1
   b) B_3
   c) B_6
   d) B_{12}
5. Vitamins are:
   a) essential factors of food
   b) nonessential factors of foods
   c) essential or nonessential (partly) factors of foods
   d) necessary only for a childhood period

6. The main metabolic function of vitamin B_2 is its participation in:
   a) carboxylation of a substrate
   b) decarboxylation of a substrate
   c) dehydrogenation of a substrate
   d) isomerization of a substrate
7. What vitamins are cofactors of *transferases*?
   a) B_3, B_6, B_{12}
   b) B_5, B_6, B_{12}
   c)B_1, B_6, B_{12}
   d) B_2, B_3, B_5
8. What coenzyme contains vitamin B_3 in its structure?
   a) Nicotinamide adenine dinucleotide (NAD)
   b) Flavine adenine dinucleotide (FAD)
   c) Coenzyme A (CoA-SH)
   d) Coenzyme Q (ubiquinone)
9. Coenzyme A is a co-factor containing one of the following vitamins:
   a) B_1
   b) B_2
   c) B_3
   d) B_5
10. What is the cause of the vitamin deficiency?
   a) Inadequate dietary intake
   b) Hypodynamia
   c) Surplus nutrition
   d) Carbohydrate deficiency
11. The main metabolic function of vitamin B_3 is its participation in:
   a) carboxylation of a substrate
   b) transfer of acyl residues
   c) dehydratation of a substrate
   d) isomerization of a substrate
12. What vitamins are cofactors of *isomerases*?
   a) B_1
   b) C
   c) B_{12}
   d) B_5
13. What coenzyme contains vitamin B_5 in its structure?
   a) Nicotinamide adenine dinucleotide (NAD)
   b) Flavine adenine dinucleotide (FAD)
   c) Coenzyme A (CoA-SH)
   d) Coenzyme Q (ubiquinone)
14. What cofactor is formed from vitamin B_c (folic acid)?
   a) Monohydrofolic acid

b) Dihydrofolic acid
c) Trihydrofolic acid
d) Tetrahydrofolic acid

15. Which of the following characteristics would be seen in a patient with a severe deficiency of thiamine?
a) A decreased level of blood pyruvate and lactate
b) An increased clotting time of blood
c) A decreased level of transketolase activity in red blood cells
d) An increased urinary excretion of xanthurenic acid following a tryptophan load

16. The main metabolic function of vitamin B_5 is its participation in:
a) carboxylation of substrate
b) decarboxylation of substrate
c) dehydrogenation of substrate
d) isomerization of substrate

17. What vitamins are cofactors of *lyases*?
a) B_1, B_6
b) B_1, B_2
c) B_6, B_{12}
d) B_3, B_5

18. What coenzyme contains vitamin B_6 in its structure?
a) Nicotinamide adenine dinucleotide (NAD)
b) Flavine adenine dinucleotide (FAD)
c) Coenzyme A (CoA-SH)
d) Pyridoxal phosphate

19. What co-factor is formed from vitamin H (biotin)?
a) ε-N-biotinyl-lysine enzyme complex
b) δ-N-biotinyl arginine enzyme complex
c) γ-carboxy-biotinyl glutamate enzyme complex
d) β-hydroxy-biotinyl-serine enzyme complex

20. The increased protein use is accompanied by an increased dietary requirement for:
a) ascorbic acid (vitamin C)
b) riboflavin (vitamin B_2)
c) Cobalamin (vitamin B_{12})
d) Pyridoxine (vitamin B_6)

21. The main metabolic function of vitamin B_6 is its participation in:
a) carboxylation of substrate
b) transfer of amino-group
c) dehydrogenation of substrate
d) isomerization of substrate

22. What vitamins are cofactors of *ligases*?
a) Biotin(H), E

b) C, P
c) Biotin (H), K
d) B_2, K

23. What vitamin is a participant of the proline and lysine hydroxylation?
a) A
b) B_3
c) C
d) B_1

24. Choose the water-soluble vitamins.
a) A, D, B_1, C
b) B_2, B_3, E, K
c) B_1, B_5, B_6, C
d) A, D, E, C

25. The disease pellagra is due to a deficiency of:
a) vitamin B_6
b) vitamin H (biotin)
c) vitamin B_3
d) vitamin B_5 (PP, niacin)

26. The main metabolic function of vitamin B_{12} is its participation in:
a) transfer of monocarbon groups
b) decarboxylation of substrate
c) dehydratation of substrate
d) transfer of amino-group

27. What is the chemical name of vitamin C?
a) Folic acid
b) Pantothenic acid
c) Ascorbic acid
d) Pangamic acid

28. What vitamins are fat-soluble?
a) A, D, B_2, C
b) K, E, B5, B_3
c) A, D, E, K
d) D, E, B_6, C

29. The absorption of light by cells in the retina of the eye results in the conversion of:
a) β-carotene to retinal
b) cis-retinal to all-trans-retinal
c) all-trans-retinal to cis-retinal
d) retinol to retinal

LESSON 7
SPECIFIC REGULATION of ENZYME ACTIVITY.

BIOMEDICAL SIGNIFICANCE

The specific regulation of enzyme activity is carried out by the substances regulatory action of which is not universal but directed to certain enzymes. The pharmacologist has long recognized that substances having substrate like structures may compete with physiological substrates and induce the phenomenon of competitive inhibition. E.g. anticholinesterase substances (neostigmine) compete with acetylcholine for including into the enzyme active center and reduce the catalytic activity of the enzyme.

Noncompetetive inhibitors are also applied as drugs. Eg substances containing heavy metals inhibit enzyme activity in bacteria by noncompetitive type of inhibition, so they are used as antiseptic drugs. Toxic action of heavy metals on the human body cells is due to their ability to bind covalently with proteins and enzymes. The last form unproductive enzyme-inhibitor (EI) complex. The prevention of the complex formation is possible with chelators (the substances capable of forming the complex compounds with metals).

Work 1. Determination of acetylcholinesterase (ACE) activity of blood by the Hestrin method. Substrate and competitive inhibition.

The EQUIPMENT: a FEC.

PRINCIPLE of METHOD

The method is based on the ability of hydroxylamine to interact with acetylcholine (AC) in alkaline environment. The product of this interaction forms the readily soluble yellow-brown complex with iron chloride in acid environment. The intensity of its colour is proportional to the acetylcholine concentration.

The activity of the enzyme is determined as a difference between the quantity of acetylcholine in experimental **(e)** and control **(c)** test tubes. In the control test tube acetylcholine does not hydrolyze because of absence of acetylcholinesterase (blood) in this test tube.

COURSE of WORK.

The work has to be carried out by a subgroup of the students (6 persons). Each member of the subgroup works with only one test tube using the following order of the work (see the table):

TYPE of EXPERIMENT	REAGENTS								ACE activity mg AC/per h
	№№ of test tubes	Dist. water	Blood	0.9% NaCl (Physiol. sol.)	Proserin (neostigmine) 0.05 %	Acetylcholine 0.5%	Acetylcholin1e 0.5 %	Phosphate buffer	
1. ACE activity without any inhibitors	1c	1.0		0.1		1.0		2.0	
	2e	1.0	0.1			1.0		2.0	
2. Competitive inhibition (addition proserine (neostigmine))	3c	1.0		0.1		1.0		2.0	
	4e	1.0	0.1		0.1	1.0		2.0	
3. Substrate inhibition (excess of a substrate)	5c	1.0		0.1			1.0	2.0	
	6e	1.0	0.1				1.0	2.0	

Put simultaneously all the test tubes into a thermostat (37ºC) for 60 min.

After the incubation:

1. Add 0,5 ml of 50 % solution of trichloracetic acid (TCA) in each test tube for precipitation of proteins.
2. Filter the contents of the test tubes with a paper filter.
3. Measure out 1 ml of the filtrate in another clean test tube and add 2 ml of an alkaline solution of hydroxylamine.
4. In 2 minutes add 1 ml of hydrochloric acid. Mix carefully.
5. Then add 1 ml of 0,37 M solution of iron chloride. Mix again.
6. Measure the optical density (extinction) of the contents of the test tubes by a FEC with a green light-filter against water (540 nm, thickness of a cuvette is 5 mm).

7. Account of ACE activity:

a) First of all solve the proportion:

Dc ---------- 5 mg
De ---------- X мг

as

$$X = De \cdot 5 / Dc$$

Where:

Dc – the optical density of the contents of the control test tube,

5 mg – the amount of acetylcholine in the control test tube before and after the incubation (or in the experimental tube before the incubation),

De – the optical density of the contents of the experimental test tube,

X - the quantity of acetylcholine in the experimental test tube after the incubation.

Calculate the activity **(Y)** of cholinesterase as a difference between the amount of acetylcholine before and after the incubation (the quantity of acetylcholine hydrolyzed during the incubation for an hour):

Y = 5 mg - X mg.

Use this way of calculation only for the 1-st and the 2-nd sorts of experiments!

b) **For the 3-rd sort of experiments solve another proportion**:

Dc ---------- 10mg

De ---------- X mg

as

$$X = De \cdot 10 / Dc$$

Calculate the activity **(Y)** of cholinesterase as a difference between the amount of acetylcholine before and after the incubation (the quantity of acetylcholine hydrolyzed during the incubation for an hour):

Y = 10 mg - X mg ,

because 1% acetylcholine is used as a substrate in this experiment.

RESULTS: include into the right column of the table.

CONCLUSIONS:

BASIC QUESTIONS:

1. The basic principles of enzyme kinetics. The Michaelis-Menten equation. The Michaelis constant (Km) as a measure of substrate-enzyme affinity. Kinetic peculiarities of allosteric enzymes.
2. The ways of the enzyme activity regulation. Modifications of enzymes: partial proteolysis, chemical modification, allosteric regulation. Proenzymes (zymogens).
3. Inhibition of enzyme activity: reversible, irreversible, specific, nonspecific.
4. Competitive and noncompetitive kinds of enzyme activity inhibition.
5. The influence of a substrate concentration on an enzyme activity. Substrate inhibition.
6. Isoenzymes. Clinical importance of isoenzyme determination.
7. Use of enzyme inhibitors as drugs in medicine.

LESSON 8
NONSPECIFIC REGULATION of ENZYME ACTIVITY.

BIOMEDICAL SIGNIFICANCE

Dependency of the enzyme activity on pH is quite important as a factor of nonspecific regulation of the rate of enzyme reactions and body metabolism on the whole. Certain values of pH are necessary as one of the conditions for the normal enzyme activity, because each enzyme has its own ***pH optimum*** providing maximal rate of the enzyme reaction. Declension of pH of biological liquids from the pH-optimum point to the both acidic and alkaline directions leads to a decrease in enzyme activity and to affection of metabolic processes. Determination of the pH-optimum values is quite important for researching of enzyme activity ***in vitro*** and for correction of their activity in disorders of acid-base state of the body.

A property of thermolability of enzymes is also used in physician's practice. E.g. artificial cooling of the body (hypothermia) or of its separate organs is applied for carrying out cardiovascular surgical operations and for organ transplantations. The decrease of the temperature allows to decrease power expenditure and to store body or organ vitality for a longer time.

The increase of body temperature (fever) in pathological states (e.g. in infectious diseases) accelerates biochemical reactions catalyzed by enzymes. Heat-stress-induced hyperthermia is the specific kind of treatment of some chronic infectious and cancer diseases.

Work 1. The influence of pH of environment on enzyme activity (determination of the pH optimum of saliva amylase).

PRINCIPLE of METHOD.

The optimum pH of amylase of human saliva is determined under the conditions of the enzyme interaction with starch in the medium with the various values of pH. A degree of starch splitting is estimated with the coloured products of the reaction (amylodextrins, rubrodextrins, maltodextrins, maltose) found out with the Lugol solution.

COURSE of WORK.

Carry out the experiment using the following table:

№№ of the test tubes	pH of buffer solution	Volume of buffering solution (ml)	Volume of 0,5% starch (ml)	Amylase of saliva diluted as 1:30 (ml)	Results (colour of reaction with the Lugol solution)
1	5.0	1.0	2.0	1.0	
2	6,2	1.0	2.0	1.0	
3	6.8	1.0	2.0	1.0	
4	7.2	1.0	2.0	1.0	
5	8.0	1.0	2.0	1.0	

Mix carefully the contents of each of the test tubes after addition of saliva. At once make a drop test:
-Take 1 drop of the contents of the 3-d tube on the Petry dish and add 1 drop of the Lugol solution.
-If the colour of the reaction is blue, brown or red, you will have to repeat this reaction once again (maybe several times) after 1 minute's interval. It is necessary to repeat it until the appearance of yellow colour. As soon as yellow colour appears, add quickly 1 drop of the Lugol solution in each test tube, and mix their contents.

Compare attentively the colour of the contents of the test tubes with each other. On the basis of the received data characterize a degree of starch hydrolysis depending on pH of medium.

Detect the pH-optimum of saliva amylase.
RESULTS: (fill in the right column of the table):

CONCLUSIONS:

Work 2. Thermolability of enzymes.

The EQUIPMENT: a thermostat for 38^{o}C, a boiling water-bath.

PRINCIPLE of METHOD.

Saliva amylase is used for studying the influence of temperature conditions on the activity of enzymes. Amylase activity is estimated with the coloured products of the reaction (amylodextrins, rubrodextrins, maltodextrins, maltose) found out with Lugol solution.

COURSE of WORK.

1. Measure out 2 ml of starch in four test tubes (see the table below).
2. Measure out 1 ml of saliva diluted 1:10 with distilled water in other four test tubes.
3. Put each pair of the test tubes (one - with substrate /starch/, another - with enzyme /diluted saliva/) in the following temperature conditions:
   - put the 1-st pair into thawing snow (0^{o}C),
   - put the 2-nd pair into a test tube support (room temperature, approximately 20^{o}C),
   - put the 3-d pair into a thermostat (40^{o}C),
   - put the 4-th pair into a boiling water-bath (100^{o}C).
4. In 5 minutes join the substrate with the enzyme, mix quickly and put again all the test tubes in the corresponding temperature conditions.
5. In 1-2 minutes begin to carry out the drop-test with the Lugol solution on the Petry dish (see the previous description). For it use the contents of the 2-nd test tube (room temperature). Repeat the test until the appearance of red-brown colour.

6. Then add 1 drop of the Lugol solution into the 1-st, 2-nd, 3-d, and 4-th test tubes and mix carefully.

(Do not remove the test tubes from the thermostat, the boiling water bath and snow until the Lugol solution addition!).

7. Carry all test tubes in a test tube-support and compare attentively the colour of the contents of the test tubes with each other. On the basis of the received data determine a degree of starch hydrolysis that depends on temperature.

№№ of the test tubes	**Volume of starch solution (ml)**	**Volume of saliva diluted as 1:10 (ml)**	**t^{o}C**	**Results of a test with the Lugol solution (the character of colour)**
1	2.0	1.0	0^{o}	
2	2.0	1.0	20^{o}	
3	2.0	1.0	40^{o}	
4	2.0	1.0	100^{o}	

RESULTS (fill in the right column of the table):

CONCLUSIONS:

BASIC QUESTIONS:

1. Biological catalysis. Energy aspects of enzyme action (initial, transition and final states, energy of activation).
2. Dependency of enzyme activity on temperature and pH. Temperature and pH as nonspecific factors of enzyme activity regulation.
3. Specificity of enzyme action. Different kinds of specificity. Biological importance of the specificity.

Examples of the task:

1. Specific inhibition of enzymes occurs under the influence of:
   a) temperature
   b) ionic strength
   c) pH
   d) hormones
2. The site of the competitive inhibitor binding is called:
   a) the active centre
   b) the allosteric centre

c) coenzyme
d) a prosthetic group

3. For the activation of a proenzyme it is necessary:
a) binding of the activator to the allosteric centre
b) binding the activator to the active centre
c) removal of the peptide blocking the activity centre
d) oxidation of sulfhydryl groups of the active centre

4. The excess of a substrate causes:
a) increase of enzyme activity
b) reversible inhibition of enzyme activity
c) irreversible inhibition of enzyme activity
d) constant velocity of enzyme reaction

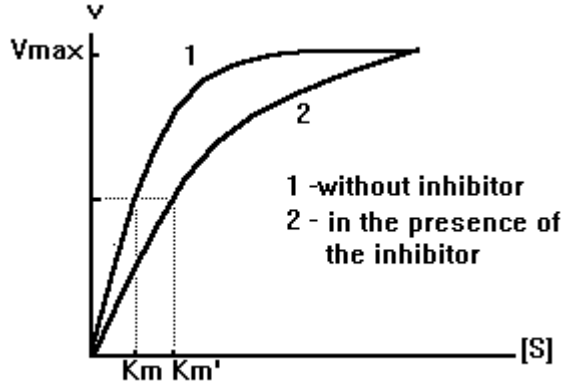
5. Which of the following mechanisms of allosteric enzymes action is correct?
a) They induce the denaturation of an apoenzyme
b) They block the enzyme active centre
c) They destroy the spatial configuration of the enzyme active centre
d) They oxidize sulfhydryl groups of the enzyme active center

6. What is the inactive form of a proteolytic enzyme?
a) Apoenzyme
b) Coenzyme
c) Proenzyme
d) Isoenzyme

7. What is the mechanism of competitive inhibitors action?
a) They block the enzyme active centre
b) They induce the denaturation of an apoenzyme
c) They alter spatial configuration of an enzyme active centre
d) They oxidize sulfhydryl groups of an enzyme active centre

8. Which of the following inhibitors induce irreversible inhibition of enzymes?
a) Hormones
b) Salts of alkaline metals
c) Salts of heavy metals
d) Excess of a substrate

9. What kind of the inhibition is drawn on the graph?



a) Allosteric
b) Competitive

c) Noncompetitive
d) Mixed

10. Isoenzymes are:
a) oligomeric enzymes which have different physico-chemical properties and catalyze the same reactions
b) protomeric enzymes which have different physico-chemical properties and catalyze the same reactions
c) oligomeric enzymes which have the same physico-chemical properties and catalyze different reactions
d) protomeric enzymes which catalyze the reactions of isomerization

11. Negative allosteric effector (inhibitor):
a) influences on the active center of the enzyme and accelerates the reaction
b) induces the deformation of the active center of the enzyme and slows down the reaction c) induces the reversible denaturation of apoenzyme
d) induces the irreversible denaturation of apoenzyme

12. Enzymopathies are diseases which are due to the insufficient function of:
a) structural proteins
b) substrates
c) enzymes
d) hormones

13. What kinds of the inhibition occur under the action of inhibitors that have the structural similarity with the substrate?
a) Noncompetitive
b) Competitive
c) Allosteric
d) Mixed

14. The substrate inhibition may be induced by:
a) insufficient concentration of a substrate
b) optimal concentration of a substrate
c) high concentration of a substrate
d) any concentration of a substrate

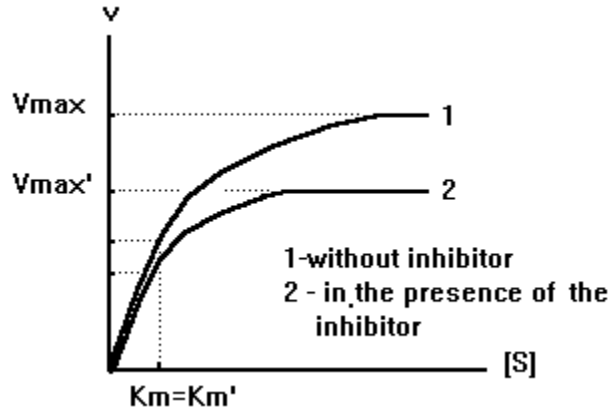
15. Reversible inhibition of an enzyme is possible as the result of:
a) the inheritable disturbance of its primary structure
b) the action of heavy metal salts
c) its contact with an allosteric inhibitor
d) action of high temperature

16. Malonic acid attenuates succinate dehydrogenase (SDH) activity because:
a) it is a competitive inhibitor of SDH
b) it is an allosteric inhibitor of SDH
c) a substrate inhibition occurs
d) a feedback inhibition occurs

17. The amount of an enzyme that is present in a cell depends on the following factors:
a) rate of its synthesis only
b) rate of its degradation only

c) both
d) neither

18. What kind of inhibition is drawn on the graph?



a) Allosteric
b) Competitive
c) Noncompetitive
d) Mixed

19. Which of the following processes is the example of covalent modification of an enzyme?

a) Association of protomers with formation of an oligomeric enzyme
b) Conjugation of a coenzyme with an apoenzyme
c) Phosphorylation of serine OH-groups of an apoenzyme
d) Binding of allosteric effector to the regulatory site

20. The rate of enzyme reaction 2 in the polyenzyme system

$$A \xrightarrow{1} B \xrightarrow{2} C \xrightarrow{3} D$$ depends on:

a) the rate of the product B formation in the reaction 1 only
b) the rate of the product C utilization in the reaction 3 only
c) both the rate of the product B formation and the product C utilization
d) concentration of a starting substrate A only

21. The end-product (feedback) inhibition is:

a) inhibition of an allosteric enzyme at the beginning of a metabolic sequence by the end product of the sequence.
b) inhibition of an allosteric enzyme at the end of a metabolic sequence by the end product of the sequence.
c) inhibition of an allosteric enzyme at the middle of a metabolic sequence by the end product of the sequence.
d) competitive inhibition of an enzyme at the beginning of a metabolic sequence by the end product of the sequence.

22. Allosteric enzymes are:

a) regulatory oligomeric enzymes whose catalytic activity is modulated by the noncovalent binding of a specific metabolite (effector) at a site other than the catalytic site (allosteric site).
b) regulatory protomeric enzymes whose catalytic activity is modulated by the

noncovalent binding of a specific metabolite (effector) at a site other than the catalytic site (allosteric site).

c) regulatory oligomeric enzymes whose catalytic activity is modulated by the covalent binding of a specific metabolite (effector) at the catalytic site.

d) enzymes that have multiple forms of an oligomeric enzyme

23. How are kinetic parameters modified under the competitive inhibition?

a) Vmax does not change, Km increases

b) Vmax increases, Km does not change

c) Both V max and Km increase

d) Both Vmax and Km decrease

24. Adipose tissue lipase may be as two forms with the different activity: either the simple protein or the phosphoprotein. Explain the mechanism of alteration of its activity.

a) It is subjected to partial proteolysis

b) It is subjected to the allosteric modulation

c) It is subjected to the co-operative transformation

d) It is subjected to the chemical modification (phosphorylation)

LESSON 9
ENERGY METABOLISM. TRICARBOXYLIC ACID CYCLE.

BIOMEDICAL SIGNIFICANCE

The main function of the TCA cycle is integrative one: it is the common terminal oxidative pathway of tissue carbohydrate, lipid and protein metabolism. Besides the TCA cycle takes part in many specific metabolic processes such as gluconeogenesis, transamination, deamination, lipogenesis etc. The enzymes of the TCA cycle are located in the mitochondrial matrix. The single exclusive enzyme of the cycle is succinate dehydrogenase (SDH) that is tightly bound to the inner mitochondrial membrane.

The appearance of the TCA cycle enzymes in serum may be registered as a result of severe pathological states accompanied by cell damage or death. E.g. the activity of mitochondrial malate dehydrogenase isoenzyme increases in these diseases. High activity of isocitrate dehydrogenase takes place in hepatitis and obstructive jaundice as a result of hepatocyte break down.

Vitamins of B-group (B_1, B_2, B_3, B_5) and some vitamin like substances (E.g. lipoic acid) are cofactors of the number of the TCA cycle enzymes. Therefore dietary insufficiency of the substances leads to inhibition of the TCA cycle on the whole. The phosphorylated derivative of vitamin B_1- thiamine pyrophosphate - is applied as a medicine for treatment of myocardium diseases.

There are no genetically damaged enzymes of the TCA cycle because the mutations of the genes encoding these enzymes are absolutely lethal.

Work 1. The determination of activity of the Krebs cycle dehydrogenases in the liver.

The EQUIPMENT: a thermostat for 38^{o}C, porcelain mortars.

PRINCIPLE of METHOD.

The determination of dehydrogenase activity is carried out by a tetrazolium method. Dehydrogenases are the enzymes oxidizing the appropriate substrates. 2,3,5-triphenyl-tetrazolium chloride (tetrazolium) is used as a hydrogen acceptor which is reduced and forms the red coloured product. The intensity of the colour depends on the amount of the formed product and consequently on dehydrogenase activity. Pyruvate, isocitrate, 2-oxoglutarate, succinate, and malate are used as the substrates. The general chemical equation of the reaction is the following:

Substrate + tetrazolium ⟶ ***Dehydrogenated product + reduced tetrazolium***
(colourless) ***(red coloured)***

COURSE of WORK.

1. A liver homogenate is used in this work as a source of the TCA cycle enzymes.
2. Make incubation mixtures for experimental /1/ and control /2/ test tubes according to the table:

№№ of the test tubes	**Liver homo-genate (ml)**	**Phos-phate buffer (ml)**	**Substrate (ml)**	**Tetra-zolium (ml)**	**Distilled water (ml)**	**Results of the experiment (estimate the intensity of colour as "-", "+", "++", or "+++")**
1	2	1	0,5	0,5	-	
2	2	1	0,5	-	0,5	

Mix the contents of the test tubes, and then place them into a thermostat at 38^{o}C and keep for 30 minutes.

After the incubation compare the colour intensity in the experimental and in the control test tubes containing pyruvate, isocitrate, 2-oxoglutarate, succinate, and malate as the substrates.

RESULTS: fill in the right column of the table.

CONCLUSIONS

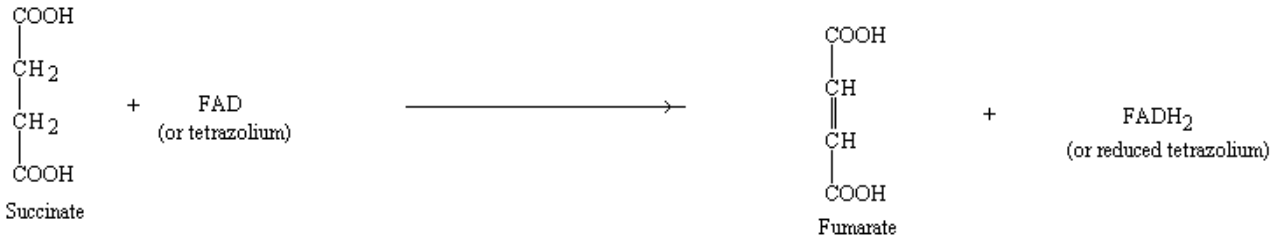
Work 2. Determination of succinate dehydrogenase activity in various tissues. Inhibition of succinate dehydrogenase by oxaloacetate.

EQUIPMENT: a thermostat for 38ºC, porcelain mortars.

PRINCIPLE of METHOD.

The determination of dehydrogenase activity by a tetrazolium method is described above (see the previous description).

The succinate dehydrogenase reaction is illustrated by the following equation:



COURSE of WORK.

1. Homogenates of the brain, the heart, kidneys, the liver, a skeletal muscle, the spleen, the lung are used in the work as a source of succinate dehydrogenase.
2. Prepare the incubation mixtures for 3 test tubes according to the table:

№№ of the test tubes	**Tissue homo-genate (ml)**	**Phos-phate buffer (ml)**	**Tissue homo-genate (ml)**	**Succi-nate (ml)**	**Tetra-zolium (ml)**	**Oxa-loace-tate (ml)**	**Distilled water (ml)**	**Results of the experiment (estimate the intensity of colour as "-", "+", "++", or "+++")**
1 (experimental without oxaloacetate)	0,5	2,5	0,5	0,5	0,5	-	-	
2 (experimental with addition of oxaloacetate)	0,5	2,5	0,5	0,5	0,5	0,5	-	
3 (control)	0,5	2,5	0,5	0,5	-	-	0,5	

Stir the contents of the test tubes, then place them into a thermostat at 38^{O}C and keep for 45 minutes.
RESULTS: fill the right column of the table.

CONCLUSIONS:

BASIC QUESTIONS:

1. The mechanism of oxidative decarboxylation of pyruvate. The enzyme composition of the pyruvate dehydrogenase complex. The regulation of the pyruvate dehydrogenase activity.
2. A tricarboxylic acid part of the TCA cycle and enzymes catalyzing this process and the regulation of their activity.
3. A dicarboxylic acid part of the TCA cycle and enzymes catalyzing this process and the regulation of their activity.
4. Oxidative decarboxylation of 2-oxoglutarate. The enzyme composition of 2-oxoglutarate dehydrogenase complex. The regulation of 2-oxoglutarate dehydrogenase activity.
5. Substrate phosphorylation in the TCA cycle. The mechanism of substrate phosphorylation. Succinyl CoA as a participant of substrate phosphorylation.
6. Metabolic and energy significance of the TCA cycle. The energy yield of the pyruvate and acetyl CoA oxidation in the TCA cycle. Regulation of the TCA cycle.

LESSON 10
BIOLOGICAL OXIDATION.

BIOMEDICAL SIGNIFICANCE

Tissue respiration is the main provider of energy in a body. Hypoxia (decrease in oxygen distribution to cells) is one of the most common reasons of cell death. That is why recovery of oxygen supply is a key principle of reanimation. Application of oxygen mask can save life of patients with respiratory and blood circulation disorders. Oxygen therapy is used in some diseases. It is necessary to remember, that prolonged oxygen therapy can cause oxygen intoxication.

Clinicians should know that there are some substances inhibiting tissue respiration. They are: 1) inhibitors of dehydrogenase activity (antituberculous drugs are competitive inhibitors of NAD-linked dehydrogenases, heavy metal ions and arsenates block SH-groups of dehydrogenases); 2) inhibitors of electron transport between CoQ and complex I of a respiratory chain (barbiturates, rotenone,

progesterone); 3) inhibitors of complex III of a respiratory chain (antimysin A); 4) inhibitors of complex IV (CO, azides, cyanides).

Work 1. The determination of NADH-dehydrogenase activity in various tissues.

The EQUIPMENT: a thermostat for 38ºC, porcelain mortars.

PRINCIPLE of METHOD.

Principle of the tetrazolium method of the determination of dehydrogenase activity is described above (see the lesson 10).

COURSE of WORK.

1. Homogenates of the brain, the heart, kidneys, the liver, a skeletal muscle, the spleen, a lung are used in the work as a source of succinate dehydrogenase.
2. Prepare the incubation mixtures for the experimental (1,2) and control (2) test tubes according to the table:

№№ of the test tubes	**Tissue homo-genate (ml)**	**Phos-phate buffer (ml)**	**$NADH_2$ (ml)**	**Tetra-zolium (ml)**	**Distilled water**	**Results of the experiment (estimate the intensity of colour as "-", "+", "++", or "+++")**
1	0,5	2,5	0,5	0,5	-	
2	0,5	2,5	0,5	-	0,5	

Mix carefully the contents of the test tubes and put them into a thermostat at 38º and keep them for 45 minutes.

RESULTS: fill in the right column of the table.

CONCLUSIONS:

Work 2. The determination of redox-potentials of components of the electron transfer chain.

The EQUIPMENT: an ionometer with a platinum redox-electrode.

PRINCIPLE of METHOD.

A potential, got by a noble metal electrode (redox-electrode) which is placed into a redox-system, depends on a ratio of oxidized and reduced forms of a substance and is called **redox-potential ($E_{red/ox}$).**

It may be determined by measuring of a difference of potentials **(DP)** of a redox electrode and a standard electrode such as a chlorine-silver electrode having the constant potential **(E_{st})**.

COURSE of WORK.

An ionomer may be used as a potentiometer for the determination of a redox-potential. The electrode system consists of a platinum electrode (measuring electrode) and a chlorine-silver electrode (the standard electrode having the constant potential $\mathbf{E_{st}}$ **=+0.222v**).

The registration of the values of potential differences (in mv) is carried out with the bottom scale of the apparatus. This scale is graduated into hundreds millivolts.

IONOMETER OPERATING PROCEDURE

1.Switch on the apparatus and warm it for 15 minutes

2. Wash out the electrodes with water, dry them with a piece of filter paper, and immerse them into a glass containing a solution (redox-system) for analysis.

3. Press the button "mv" and the button of an interval of measurement " -1 - 19 "

4. Register the value of the difference of potentials **(DP)** on the bottom scale of the apparatus and multiply it by **100**.

5. If the pointer of the apparatus is deviated out of the scale, it is necessary to press the button "анионы/катионы" (“anions/cations”) and register the results with sign **"-"**.

6. Calculate the value of redox-potential using the value of DP:

$$\mathbf{E_{red/ox} = DP + E_{st}},$$

Where:

$\mathbf{E_{st}}$ **=+0.222 v**

The TASK:

1. Determine a normal redox-potential of the following systems:

- $NAD/NADH_2$,
- $CoQ/CoQH_2$,
- Ferricytochrome C/Ferrocytochrome C,
- Ferrycytochrome A_3/Ferrocytochrome A_3

2. Distribute these systems according to the increase of the values of their redox-potentials.

3. Calculate a difference of potentials between the components of a respiratory chain.

4. Calculate the yield of energy /ATP/ in these sites using the formula:

$$\mathbf{\Delta G = - n F \Delta E\ red/ox,}$$

Where

ΔG -a change of free energy of a redox-system in kJ/м,

n - a number of electrons,

F - the Faraday constant (96.5 kJ/M),

$\mathbf{\Delta E_{red/ox}}$ - a difference of redox-potentials of two redox-systems.

RESULTS (fill in the table):

Redox-system	DP (v)	E red/ox (v)	ΔEo/в (v)	ΔG (кJ)
NAD/NADH				
$CoQ/CoQH_2$				
Ferri/ferro-cytochrome C				
Ferri/ferro-cytochrome A_3				

CONCLUSIONS:

BASIC QUESTIONS:

1. The definition of the terms: "oxidation", reduction", "biological oxidation".
2. Redox-systems. The examples of biological redox-systems.
3. The ways of oxygen consumption in the body: (oxidase and oxygenase kinds of the reactions).
4. A substrate oxidation by dehydrogenation. The structures of dehydrogenases coenzymes: NAD^+, NADH, $NADP^+$, NADPH, FMN, $FMNH_2$, FAD, $FADH_2$.
5. NADH and succinate as substrates of a respiratory chain. The reactions of their formation.
6. Flavoproteins. The structure of their prosthetic group.
7. Ubiquinone (coenzyme Q). Its structure and its role in a respiratory chain.
8. An electron transfer system of cytochromes. The structure of the cytochrome prosthetic groups. Electron transfer function of cytochromes.
9. Functional complexes of a respiratory chain, their names, composition and role in a respiratory chain. Inhibitors of the respiratory chain complexes.
10. Redox-potential as a quantitative measure of energy yield of oxidation-reduction reactions.

LESSON 11
OXIDATIVE PHOSPORYLATION.

BIOMEDICAL SIGNIFICANCE

Each cell of the body constantly requires ATP. The daily renewing (synthesis and decomposition) of ATP is about 60 kg. The main pathway of the ATP synthesis is oxidative phosphorylation. The decrease of the ATP synthesis leads to the development of low energy state. This state arises under hypoxia, starvation, hypovitaminosis etc.

Hypoxia may be developed as a result of oxygen deficiency in atmospheric air, lung ventilation disorders, heart diseases, bleeding, shock, anemia, intoxications by hemoglobin-binding toxins and inhibitors of the mitochondrial respiratory chain.

A lot of enzymes containing vitamins B_1, B_2, B_3, B_5 take part in the power metabolism. The deficiency of these vitamins results in low energy state of the body tissues.

Oxidative phosphorylation is a vital process, and its disorders are quite dangerous for human life.

Work 1. The detection of ATP in various tissues.

EQUIPMENT: a centrifuge, porcelain mortars

PRINCIPLE of METHOD.

ATP will be insoluble if it forms barium salt with barium acetate in an alkaline medium.

COURSE of WORK.

1. Homogenates of the brain, the heart, kidneys, the liver, a skeletal muscle, the spleen, a lung are used in the work as a source of succinate dehydrogenase.
2. Add 4 ml of 25 % TCA to 4 ml of the tissue homogenate and mix it carefully.
3. In 5 minutes pour the liquid into centrifuge test tubes and centrifuge for 10 minutes at 1500 rev/min.
3. Transfer the supernatant liquid into the usual dry glass test tubes.
4. Take 5 ml of supernatant liquid into another clean test tube , then add 2 drops of phenolphthalein and 10% NaOH **(by drops)** until the appearance of light-pink colour, next add 25 % barium acetate **(by drops)** up to the complete sedimentation of ATP, watching that the solution remained alkaline after the addition of barium acetate.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Oxidative phosphorylation, its differences from substrate phosphorylation.
2. Coupling of oxidative phosphorylation. The sites of coupling. The chemiosmotic mechanism of coupling (Mitchel's theory).
3. Uncoupling. Different kinds of uncouplers. The mechanisms of their action.
4. High energy compounds. The structure and energy properties of ATP.
5. Regulation of electron and protone transport in the respiratory chain.

Examples of the task:

1. It is possible for a variety of substrates to use a common pathway for the transfer of electrons to oxygen because:

a) the substrates are oxidized in the mitochondria

b) many substrates are oxidized by enzymes linked to NAD and FAD

c) the substrates are all oxidizes by the same enzymes
d) the electrons from all substrates are transferred to the common acceptor, adenosine triphosphate

2. What chemical compound is the universal accumulator of energy in an organism?
a) GDP
b) 1,3-bis-phosphoglycerate
c) Succinyl CoA
d) ATP

3. By what enzymes are the reactions of biological oxidation catalyzed under the immediate participation of oxygen?
a) By dehydrogenases
b) By oxidases
c) By cytochromes
d) By NAD-linked enzymes

4. Where are the enzymes of oxidative phosphorylation located?
a) In the mitochondrial matrix
b) In the endoplasmic reticulum
c) In the inner mitochondrial membrane
d) In the outer mitochondrial membrane

5. Choose the III complex of a respiratory chain.
a) FMN-linked protein + nonheme FeS-protein
b) cytochromes b, c_1 + nonheme FeS-protein
c) FAD-linked protein + nonheme FeS-protein
d) cytochromes a, a_3 + Cu-protein

6. The mitochondrial electron transport chain carriers are located:
a) in the inner mitochondrial membrane
b) in the mitochondrial matrix
c) in the intermembrane space
d) on the inner surface of the outer mitochondrial membrane

7. What is the value of the P:O ratio under the NAD-dependent substrates oxidation?
a) P:O=4
b) P:O=3
c) P:O=2
d) P:O=1

8. What part of the NAD structure provides the possibility of its reversible oxidation-reduction transformation?
a) Adenine
b) Nicotinamide
c) ribose
d) phosphate

9. Which of the following poisons is an uncoupler?
a) 2,4-dinitrophenol
b) Antimycin A
c) Cyanide

d) Rotenone

10. Choose the proton-transporting fragment of the mitochondrial respiratory chain.

a) From $NADH_2$ to oxygen

b) From cytochrome b to cytochrome c

c) From $NADH_2$ and succinate to coenzyme Q (ubiquinone)

d) From coenzyme Q (ubiquinone) to oxygen

11. All of the following electron carriers are components of the mitochondrial electron transport chain EXCEPT:

a) nicotinamide-adenine dinucleotide

b) nicotinamide-adenine dinucleotide phosphate

c) flavine mononucleotide

d) flavine-adenine dinucleotide

12. Show the TCA-cycle substrate that is oxidized by the FAD-dependent dehydrogenase.

a) malate

b) isocitrate

c) α-ketoglutarate

d) succinate

13. Choose the inhibitor of the complex III of a respiratory chain.

a) Oxaloacetate

b) Oligomycin

c) Antimycin A

d) Amytal

14. The process of ATP synthesis coupled to the oxidation reactions in a respiratory chain is called:

a) substrate phosphorylation

b) oxidative phosphorylation

c) photosynthetic phosphorylation

d) transphosphorylation

15. If the ratio P:O was 2.0, which of the following substrates would be used?

a) Malate

b) α-ketoglutarate

c) Succinate

d) Pyruvate

16. Electrons from pyruvate enter the mitochondrial electron transport chain at the level of:

a) coenzyme Q (complex II)

b) NADH-dehydrogenase (complex I)

c) QH_2-cytochrome c oxidase (complex III)

d) cytochrome c oxidase (complex IY)

17. Why do electrons flow along a respiratory chain towards oxygen?

a) It depends on the difference of the electron carriers redox potentials

b) It depends on the arrangement of electron carriers in the inner membrane of mitochondria

c) It depends on high concentration of oxygen in the mitochondrial matrix

d) It depends on high concentration of NAD and succinate in the mitochondrial matrix

18. Which of the following cytochromes is water-soluble and can be extracted from mitochondria?

a) cytochrome c_1

b) cytochrome c

c) cytochrome b

d) cytochrome a_3

19. What reaction do monooxygenases catalyze?

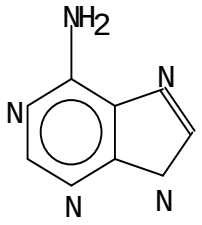
a) Transfer of H^+ and e^- to oxygen

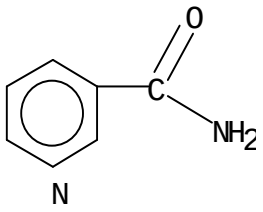
b) Incorporation of two oxygen atoms into the substrate

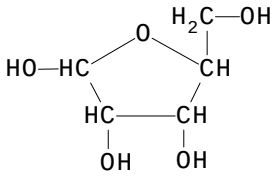
c) The reaction of the hydrogen peroxide synthesis

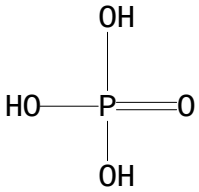
d) Incorporation of an oxygen atom into the substrate

20. What component of the NAD structure takes part in the binding of hydrogen?

a) 

b) 

c) 

d) 

21. The P:O ratio is the best described by which of the following statements?

a) The ratio of adenosine monophosphate to ATP present in respiring cells

b) The ratio of moles of ADP phosphorylated to moles of oxygen consumed

c) The ratio of moles of phosphate incorporated into ATP to moles of oxygen consumed

d) The ratio of moles of phosphate incorporated into adenosine diphosphate (ADP) to gram atoms of oxygen consumed

22. Complex III of the respiratory chain is:

a) the fragment where the reduction of ubiquinone occurs

b) the fragment where the reduction of FAD occurs

c) the fragment where the reduction of NAD occurs

d) the fragment where the reduction of cytochrome c occurs

23. What is the value of the P:O ratio under malate oxidation?

a) P:O=1

b) P:O=2

c) P:O=3

d) P:O=4

24. What is the NADH-dehydrogenase cofactor?

a) FAD
b) NAD
c) FMN
d) NADP

25. What is the mechanism of the action of uncouplers on oxidative phosphorylation?

a) They inhibit the electron transport along the mitochondrial respiratory chain
b) They inhibit ATP-synthase
c) They inhibit ATP/ADP-antiporter (adenine nucleotide translocase)
d) They remove a proton membrane potential ($\Delta\mu H^+$) of the inner mitochondrial membrane

LESSON 12
BIOCHEMISTRY of HORMONES.

BIOMEDICAL SIGNIFICANCE

Hormones function as regulators of enzyme activity of target cells. Both increase and decrease in hormone production lead to the development of endocrine diseases. E.g. low production of insulin results in diabetes mellitus. Hormone-producing tumors of adrenal medulla termed as pheochromocytoma are characterized by the increased blood epinephrine level that is accompanied by high blood pressure, basal metabolism, blood glucose and fatty acids levels and appearance of glucosuria.

Many hormones are used as drugs for treatment of endocrine diseases. Besides a number of medicines can modulate the hormone action. Some of xanthine derivatives such as caffeine, theophyllin, or euphylline inhibit phosphodiesterase and promote the accumulation of cAMP, but on the contrary, trental (pentoxyfylline) raises cGMP concentration in the cytosol of the target cells. The administration of these medicines enhances the regulatory effects of the cAMP and cGMP mediated hormones respectively.

Work 1. Qualitative reactions for detection of hormones.

1. The detection of insulin with sulfosalicylic acid

PRINCIPLE of METHOD.

The strong organic acids cause the denaturation of proteins, therefore the sediment of denaturated protein drops out.
The sedimentation of proteins with sulfosalicylic acid is a specific and sensitive reaction for the protein detection.

COURSE of WORK.

Pour 1 ml of an insulin solution into a test tube and add 3-5 drops of sulfosalicylic acid. In the presence of insulin white sediment drops out.

2. Biuretic reaction for detection of protein and polypeptide hormones.

PRINCIPLE of METHOD.

The reaction is caused by the presence of peptide bonds forming the complexes with copper ions in alkaline media. The solution becomes blue-violet or pink coloured that depends on the length of a polypeptide chain. The chemical essentiality of the reaction is described above (see lesson 3).

COURSE of WORK.

Measure out 4-5 drops of ACTH solution (adrenocorticotropic hormone) into a test tube, then add 5 drops of 10% NaOH solution and 1 drop of 1% $CuSO_4$. The occurrence of pink-violet colour is observed.

3. Detection of epinephrine with iron chloride.

PRINCIPLE of METHOD.

Epinephrine (adrenaline, N-methylaminoethanolpyrocatechol) may be determined by the reactions characteristic for pyrocatechols. It forms phenolate-like compound which has green colour in the presence of iron ions. Epinephrine is easily oxidized that also is characteristic for pyrocatechols. If to add alkali to an epinephrine solution or to its complex with iron, red coloured adrenochrome will be formed.

COURSE of WORK.

Take 10 drops of 0.1% epinephrine (adrenaline) solution into a test tube and add 1 drop of 1 % $FeCl_3$ solution . Blue-green colour appears. After that add 1 drop of 10% NaOH to the received solution. The colour of the liquid becomes dark-red.

4. Detection of iodine in thyroxine.

PRINCIPLE of METHOD.

Thyroid hormones (thyroxine, triiodothyronine) contain iodine in their structure. If to hydrolyze this hormones with alkali (boiling in the presence of $KHCO_3$), potassium iodite will be formed, from which iodine is released with the addition of potassium iodate and sulfuric acid.

$$5\,KJ + 3\,H_2SO_4 \rightarrow 3J_2 + 3KH_2SO_4 + 3H_2O$$

Free iodine is found out by the colour reaction with starch.

$$J_2 + \text{Starch} \rightarrow \text{Blue colour}$$

COURSE of WORK.

Add sulfuric acid (4-5 drops) to 1 ml of alkaline hydrolysate of thyroid hormones in order to create acidic pH, then add 2 drops of 1 % starch and 5 drops of 20% potassium iodated. After that you can see dark blue colour.

RESULTS:

Write down the results in the table:

Hormone liquid	Result of the reaction			
	Precipi-tation of protein	Biuretic reaction	Pyrocate-chol reaction	iodine reaction
1. Solution of insulin for injections				
2. Solution of ACTH (or prolactin)				
3. Solution of epinephrine (adrenaline)				
4. Alkaline solution of thyroid hormones				

CONCLUSIONS (classify hormones according to their chemical structures):

Work 2. The study of the insulin, epinephrine, and cortisol influence on the blood glucose level.

PRINCIPLE of METHOD.

Hormones of adrenal cortex (cortisol), adrenal medulla (epinephrine) and two hormones of the pancreas (insulin, glucagon) are the specific regulators of carbohydrate metabolism.

For the estimation of influence of these hormones on the blood glucose level it is necessary to take blood of an animal before the injection of hormone and in every 30 minutes after an injection of hormone (insulin, adrenaline) or in every 6 hours (cortisol). Glucose concentration in blood is determined by a glucose oxidase method (see lesson 5).

COURSE of WORK:

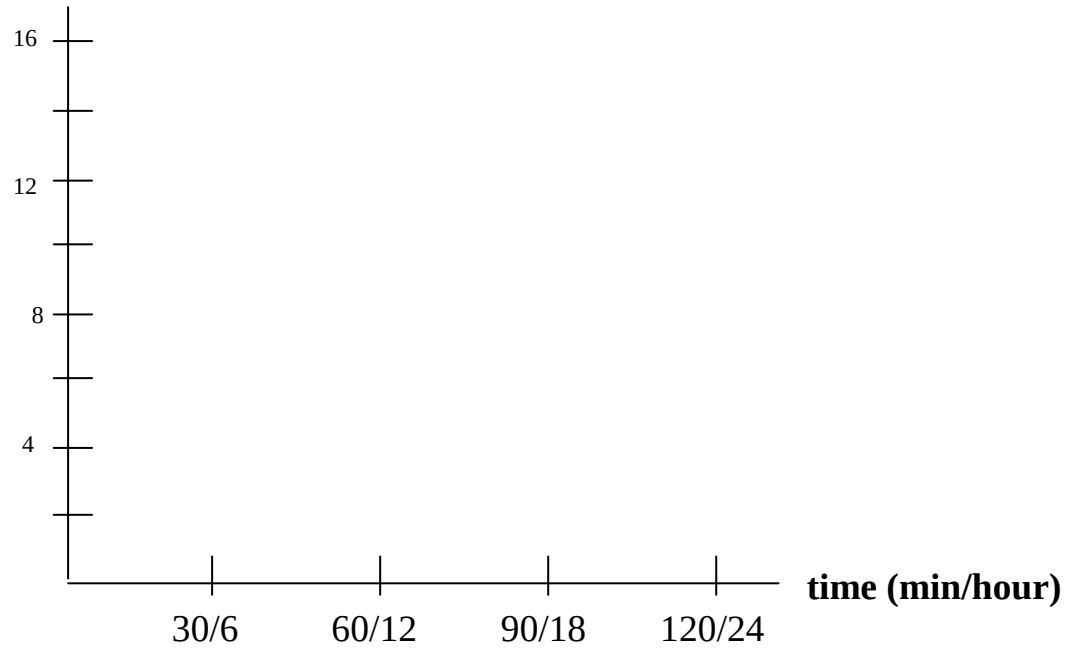
1. Take 1ml of supernatant liquid for determination of glucose (received before or after the injection of certain hormone) in a test tube.
2. Take 1 ml of supernatant liquid received from a standard solution of glucose (5,5 mM/L) in another test tube.
3. Add 3 ml of "glucose reagent" for determination of glucose into each test tube and place all the test tubes at the room temperature for 15 minutes for the development of colour.
3. In 15 minutes determine the optical density of the solutions by FEC with the red light-filter against water (670 nm, the thickness of a cuvette is 5 mm). Calculate the glucose concentration by the equation (see lesson 5).

Write down the received results in the table:

Blood glucose level														
insulin					**epinephrine**					**cortisol**				
befor	30'	60'	90'	120'	befor	30'	60'	90'	120'	befor	6 h	12 h	16 h	24 h

Plot the results on the graph:

Blood glucose level (mM/L)



CONCLUSIONS:

BASIC QUESTIONS:

1. The meaning of the term “hormone”. The neuroendocrine system of an organism. Examples of target cells regulated by various hormones.
2. The chemical classification of hormones. Give examples of hormones belonging to peptide or proteins, amino acid derivatives, cholesterol derivatives. Where are they formed?
3. Hormones of hypothalamus and hypophysis. Their role in regulation of other hormones production.
4. Hormone synthesis and transport.
5. The mechanism of a hormone action. Kinds of hormone-binding receptors.
6. A membrane mechanism of a hormone action. The character of a membrane receptors. Kinds of messengers, their formation by membrane enzymes.
7. Cyclic nucleotides as hormone messengers. An example of a “cascade” mechanism of hormone signal transmission.
8. The role of Ca^{++}-ions in the mechanism of the hormone regulatory action.
9. Inositol-3-phosphate and diacylglycerols as hormone messangers.
10. An intracellular mechanism of the hormone action. The character of intracellular receptors. The event sequence of intracellular transmission of a hormone signal.
11. The peculiarities of insuline action, structure of an insuline receptor.
12. Eucosanoids, their synthesis and regulatory role.

The examples of the control task:

1. The role of guanidine nucleotide-binding protein (G protein) in adenylate cyclase activation is the best described by which of the following statements?
    a) The G protein, when complexed with guanosine triphosphate (GTP), is able to activate adenylate cyclase
    b) The G protein forms a complex with different hormones, and the hormone-G protein complex is responsible for activation of adenylate cyclase
    c) The complex formation between the G protein and adenylate cyclase is sufficient to activate the latter
    d) Active adenylate cyclase is inactivated by the creation of the GTP form of the G protein in a reaction catalyzed by the hormone-receptor complex
2. Hormones are yielded by
    a) all kinds of cells of an organism
    b) the cells of hypothalamus only
    c) the cells of hypophysis only
    d) the cells of endocrines
3. cAMP concentration in a target cell cytosol depends on
    a) the adenylate cyclase activity only
    b) the amount of receptor proteins only
    c) the phosphodiesterase activity only
    d) both adenylate cyclase and phosphodiesterase activities
4. Which of the following hormones is a storage one?
    a) Insulin
    b) epinephrine
    c) nor-epinephrine
    d) glucagon
5. Active proteinkinase catalyses the reaction of
    a) a phosphorylation of the target cell proteins
    b) the cAMP formation
    c) the cGMP formation
    d) a phosphorylation of adenylate cyclase
6. Insulin is released from the β cells of the pancreatic islets when
    a) the concentration of amino acids in blood is below normal
    b) the concentration of free fatty acids in blood is below normal
    c) the rate of free fatty acid release from adipose tissue is elevated
    d) blood sugar levels are elevated above normal
7. Which of the following hormones is a mobilizing one?
    a) Insulin
    b) Vasopressin
    c) Melanoliberin
    d) Glucagon
8. What cell protein binds Ca^{++} ions in the target cell cytosol?
    a) adenylate cyclase
    b) guanylate cyclase
    c) calmodulin

d) calsequestrin

9. Hormones interact with their target cells by

a) the binding to receptors on the outer surface of the cell membrane only

b) the binding to intracellular receptors only

c) the binding to the receptors on the outer surface of the cell membrane or to intracellular receptors (it depends on the hormone properties)

d) the binding to receptors on the endoplasmic reticulum membrane

10. Adenylate cyclase catalyses the reaction:

a) ATP→cAMP + PP

b) ATP→AMP+ PP

c) GTP→cGMP+PP

d) GTP→GMP+PP

11. All following statements regarding steroid hormones are correct EXCEPT:

a) the synthesis and secretion of aldosterone by adrenal glomerulosa cells is modulated by Na^+ and K^+

b) cortisol increases the production of glucose in the liver, the deposition of glycogen in the liver, and protein synthesis in the liver

c) 1,25-dihydroxy-cholecalcipherol acts on mucosal cells of the small intestine to increase the rate of Ca^{2+} absorption

d) steroid hormones interact with receptors on the outer surface of the plasma membrane of target cells, and the complex is then transferred to the nucleus, where it modulates gene expression

12. Guanylate cyclase catalyses the reaction:

a) ATP→cAMP + PP

b) ATP→AMP+ PP

c) GTP→cGMP+PP

d) GTP→GMP+PP

13. Which of the following hormones acts by the slowest mechanism?

a) epinephrine

b) cortisol

c) glucagon

d) insulin

14. Which of the following substances are the hormone messengers?

a) cAMP, cGMP, ADP

b) olygoadenylates, Ca^{++}, ATP

c) GTP, cAMP, inositol phosphates

d) cAMP, cGMP, Ca^{++}

15. Choose the hormones that have intracellular receptors.

a) epinephrine, ACTH (corticotropin), estradiol

b) testosterone, thyroxine, cortisol

c) testosterone, thyroxine, glucagon

d) cortisol, estriol, oxytocin

16. All following statements regarding insulin are correct EXCEPT:

a) it is produced by the β cells of the islets of Langerhans in the pancreas

b) the active hormone is made up of one B chain linked by disulfide bonds to a C chain

c) preproinsulin is clipped to form proinsulin in the cisterna of the endoplasmic reticulum

d) proinsulin is converted to insulin in storage vesicles in the β cell

17. Phosphodiesterase catalyses the reaction:

a) cAMP+H2O→5'AMP

b) GDP+H2O→GMP+Pi

c) ATP→cAMP+PP

d) GTP→cGMP+PP

18. Insulin is synthesized in

a) adrenal cortex

b) adrenal medulla

c) the α-cells of the Langerhans islets

d) the β-cells of the Langerhans islets

19. The G protein is capable to bind

a) cAMP

b) ATP

c) cGMP

d) GTP

20. Hormones that modulate gene expression are the following:

a) nor-epinephrine, serotonin, testosterone, estradiol

b) aldosterone, cortisol, testosterone, thyroxine

c) progesterone, insulin, epinephrine, oxytocin

d) estriol, cortisol, vasopressin, glucagon

21. All following statements are true for both the β_1 and β_2 catecholamine receptors EXCEPT:

a) they are linked to adenylate cyclase by the G protein

b) they are located on the outer surface of the plasma membrane

c) the order of potency for activating the cell response is isoproterenol> norepinephrine>epinephrine

d) the catecholamine stimulus is relayed via the increased cAMP formation

22. The G protein can catalyze the reaction of

a) the ATP hydrolysis

b) the cAMP synthesis

c) the GTP hydrolysis

d) the cGMP synthesis

23. The cAMP-dependent protein kinase is

a) a tetramer having two kinds of subunits (R_2C_2)

b) a dimer having two kinds of subunits (RC)

c) an octamer having two kinds of subunits (R_4C_4)

d) a trimer consisting of two R and one C subunits (R_2C)

LESSON 13
The BASIC CARBOHYDRATES of an ORGANISM. GLYCOGEN: STRUCTURE, SYNTHESIS and DECOMPOSITION.

BIOMEDICAL SIGNIFICANCE

The disturbances of dietary carbohydrates digestion are induced by either deficiency of digestive enzymes or by disorder of absorption of monosaccharides in the small intestine. The inherited or acquired deficiency of pancreatic amylase leads to the disturbance of starch digestion. Dietary lactose intolerance may be inherited or developed as a result of breast feeding of infants for a long time. The syndrome of malabsorption of dietary mono- and disaccharides is one of the most widespread pathological states in babies.

Glucose is the most important carbohydrate of human body. Most of dietary carbohydrates are digested in the small intestine with the formation of free glucose; stored carbohydrate of the liver - glycogen is transformed into blood glucose; glucose is used for the synthesis of all other carbohydrates of the body. Glucose is the universal metabolic fuel for all tissues of the body. The excess of dietary glucose may be converted into liver and skeletal muscle glycogen and adipose tissue neutral fat. A number of congenital diseases termed as ***glycogenoses*** are due to the disturbance of glycogen metabolism. They appear as a result of deficiency or full absence of the enzymes catalyzing the processes of decomposition and synthesis of glycogen. All varieties of glycogenoses are characterized by the excessive accumulation of glycogen in the tissues. 9 types of glycogenoses are distinguished (I-IX).

Work 1. The influence of saliva, gastric juice and pancreatic juice on starch.

The EQUIPMENT: a thermostat for 38^{o}C, a boiling water bath.

The PRINCIPLE of a METHOD.

Starch is digested in the gastrointestinal tract and is transformed into the products which have free hemiacetal hydroxyl (maltose, glucose).
The Trommer reaction is applied for revealing the products of digestion (see the Lesson 5).

The COURSE of WORK.

Measure out the following liquids into 4 test tubes according to the table:

№ of the test tube	Solution of starch (ml)	Saliva 1:10 (ml)	Gastric juice (ml)	Pancreatic juice (ml)	Results (colour of the Trommer reaction)
1.	1.0	1.0	-	-	
2.	1.0	-	1.0	-	
3.	1.0	1.0	1.0	-	
4.	1.0	-	-	2.0	

Put all the tubes into a thermostat at 38º for 30 minutes. After the incubation the contents of each test tube is analyzed to detect the products of the polysaccharide splitting with the Trommer reaction.
Make a conclusion about the influence of various digestive juices on starch.
RESULTS: (fill in the right column of the table)

CONCLUSIONS:

Work 2. Isolation of glycogen from the liver of a replete and hungry animal.
The EQUIPMENT: porcelain mortars.

The PRINCIPLE of a METHOD.

The method is based on glycogen water-solubility and stability in slightly acidic media. Therefore an isolation of glycogen is carried out by the following manner: a homogenate of the liver is prepared and glycogen is extracted with trichloracetic acid (TCA). Proteins are denaturated and precipitated by TCA, so they may be easily removed from a solution by filtration. In the experiment it is necessary to use the liver of replete and hungry animals. The liver of animals is quickly cut to form thin plates and they are immediately put into a glass with a boiling physiological solution for 10-15 of minutes to inactivate glycogen-phosphorylase. The further experimental procedure is carried out with the liver of replete and hungry animals by the same manner.

The COURSE of WORK.

1. Weight out 0.5 g of the replete and hungry animal liver. Place each sample in a porcelain mortar, pour there 3 ml of 5% TCA and pound it carefully for 10 minutes. Then add 3 ml of distilled water to the homogenate, mix and filter through a paper filter to the clean test tubes.
2. Make the qualitative reaction for the detection of glycogen in the filtrates:
-pour 1 ml of distilled water in the first test tube,
-pour 1 ml of the filtrates received from the liver of a replete and a hungry animal in the second and third test tube respectively.
-add 1-2 drops of the Lugol solution in each test tube and compare the colour of the test tube contents with each other.
RESULTS:

CONCLUSIONS:

Work 2. Phosphorolysis of glycogen in the muscular tissue.

The EQUIPMENT: porcelain mortars, a thermostat for 38ºC.

The PRINCIPLE of a METHOD.

Phosphorolysis of glycogen in the muscular tissue is carried out according to the equation:

$$\underset{\text{glycogen}}{[\text{Glucose}]_n} \xrightarrow[\text{phosphorylase}]{+\ H_3PO_4} \text{Gucose-1-phosphate} + \underset{\text{glycogen}}{[\text{Gucose}]_{n-1}}$$

A change in the inorganic phosphate concentration is a measure of phosphorylase activity and phosphorolysis of glycogen on the whole.

The COURSE of WORK.

1. Pound 300 mg of the tissue in a mortar, and then add 2 ml of phosphate buffer pH = 7.2 and 2 ml of sodium fluoride (sodium fluoride is added for inhibition of the further transformations of glucoso-1-phosphate).
2. Transfer the contents of the mortar to a test tube, then add there 2 ml of a glycogen solution. This test tube is marked as *"experimental"*.
3. Pound again 300 mg of the tissue with 2 ml phosphate buffer and 2 ml sodium fluoride. Transfer the contents of the mortar to another test tube, but do not add glycogen to this test tube. This test tube is marked as *"control"* (This test tube is used for the comparison, as the reaction does not occur because of the absence of a substrate in this test tube).
4. Put the both test tubes into a thermostat at 38oC for 60 minutes.
5. After the incubation add 2 ml of a glycogen solution in the control test tube to make the volumes of the contents of the experimental and control test tubes the same.
6. Add 2 ml of 20% trichloracetic acid (TCA) in the both test tubes in order to stop the reaction and precipitate proteins.
7. In 5 minutes filter the contents of the both test tubes through paper filters.
8. Measure out the aliquots (2 ml) of the unprotein filtrates in the other clean test tubes.
7. Then add the reagents for the colour reaction to detect phosphoric acid: 2 ml of molybdic acid, 0.5 ml of hydroquinone, and 4 ml of a carbonate-sulfite mixture **(Warning! Add the mixture of carbonate and sulfite only by drops and mix the contents of the test tubes carefully).**

In 10 minutes compare the colour intensity of the contents of the experimental and control test tubes.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Common character of carbohydrates. Their classification and structure.
2. Proteoglycans and glycoproteins. Glucosaminglycans. Classification. The principle structure of a glucosaminglycan monomer. Functions and distribution or proteoglycans and glycoproteins in a human body.
3. Digestion of polysaccharides. The digestive effect of the saliva, pancreatic, and intestine enzymes on dietary polysaccharides. Digestionable (starch, glycogen) and non-digestionable polysaccharides (dietary fibers). Absorption of carbohydrates in the intestine. Disorders of carbohydrates digestion.
4. Glycogen as the main storage polysaccharide. The ways of a glycogen mobilization: amylolytic and phosphorolytic. Hormonal regulation of the process.
5. The glycogen synthesis in the liver. The energy and enzyme provision of the process. The character of key enzyme of the glycogen synthesis. The hormone regulation of the process.
6. Glycogen diseases, the reasons of their rise.

LESSON 14
GLYCOLYSIS.

BIOMEDICAL SIGNIFICANCE

Glycolysis is a process in which glucose is enzymatically decomposed either into two molecules of pyruvate (in aerobic conditions) or two molecules of lactate (in anaerobic conditions). The tissues characterized by high activity of glycolysis (liver, skeletal muscle) are functionable in the period of ***oxygen deficiency (hypoxia)***. In fast-growing malignant tumor cells glycolysis proceeds with the higher rate than the TCA cycle, so the formation of pyruvate exceeds its utilization in the TCA cycle, lactate is accumulated and local increase in acidity develops. In the myocadium the glycolytic capacity is limited, so the heart can not be tolerant to prolonged hypoxia. There are a number of diseases due to inherited insufficiency of activity of glycolytic ezymes (eg pyruvate kinase). ***Hemolytic anemia*** develops in these pathological states.

Work 1. Determination of lactate dehydrogenase activity in different tissues.

The EQUIPMENT: porcelain mortars, a thermostat for 38^{o}C.

The PRINCIPLE of a METHOD.

Dehydrogenase of lactic acid (lactate dehydrogenase, LDH) oxidizes lactic acid and forms pyruvic acid in the presence of hydrogen acceptor - 2,3,5-triphenyltetrazolium chloride (tetrazolium) which is reduced into a red coloured product. The intensity of colour depends on the amount of the formed product and consequently on dehydrogenase activity.

The COURSE of WORK

The work is carried out by a subgroup of students, each of which determines LDH activity in certain tissue.

1. Homogenates of the brain, the heart, kidneys, the liver, a skeletal muscle, the spleen, a lung are used in the work as a source of succinate dehydrogenase.
2. The experiment order.

Test tube № 1 (experimental).

Measure out 1 ml of sodium lactate, 2 ml of phosphate buffer pH = 7.4, 1 ml of tetrazolium solution, and 1ml of the tissue homogenate.

Test tube № 2 (control).

Measure out 1ml of sodium lactate, 2 ml of phosphate buffer pH = 7.4; 1 ml of distilled water instead of tetrazolium solution, and 1 ml of the tissue homogenate.

Stir the both test tubes and place them into a thermostat at 38^{o}C for 1 hour.

In an hour compare the intensity of colour in the experimental and control test tubes

RESULTS:

CONCLUSIONS:

Work 2. Detection of lactic acid in muscular tissue.

The PRINCIPLE of a METHOD.

Iron chloride and phenol form iron phenolate which has dark violet colour. In the presence of lactic acid the yellow complex substance is formed.

The COURSE of WORK.

Pound a piece of muscular tissue together with 5ml of phosphate buffer (pH = 7.4) in a mortar. Transfer the received homogenate into a test tube. Measure out 2-3 ml of a phenol solution in another test tube and add several drops of iron chloride until the appearance of dark violet colour. Next add the same volume of homogenate (about 5 ml) in this test tube, and then add 10% NaOH by drops until the appearance of yellow colour.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS**:**

1. Definition of the terms “Glycolysis, glycogenolysis”. Biological significance of the processes.
2. Glucose-6phosphate as the main metabolite of the carbohydrate metabolism, its formation and different pathways of its metabolism.
3. The sequence of the glycolytic reactions. The enzymes, cofactors, substrates and products of the reactions. Regulation of this process.
4. The glycolytic oxidoreduction, the significance of this process.
5. Substrate phosphorylation in the glycolysis.
6. The energy effect of glycolysis and glycogenolysis. The energy effect of glucose oxidation to CO_2 and water.
7. The entry of other carbohydrates into the glycolytic pathway.
8. The aerobic and anaerobic glycolysis. Lactate as the end metabolite of anaerobic glycolysis.

LESSON 15
The GLUCONEOGENESIS and The PENTOSE PHOSPHATE PATHWAY of a GLUCOSE TRANSFORMATION .

BIOMEDICAL SIGNIFICANCE

The pentose phosphate pathway produces $NADPH_2$ and ribose phosphates for reductive synthesis (synthesis of fatty acids, steroids, nucleotides etc), and CO_2 for the reactions of carboxylation. It provides a route for the use of pentoses and for their conversion to hexoses and trioses. The inherited deficiency of the pentose phosphate pathway enzymes is one of the reasons of erythrocytes hemolysis.

Gluconeogenesis allows to maintain the optimal blood glucose level when dietary intake of carbohydrates is insufficient. The mechanism of gluconeogenesis includes the removal of the products of tissue metabolism (e.g. lactate formed in skeletal muscles and erythrocytes or glycerol formed as a result of stored neutral fat decomposition) from blood for the synthesis of glucose in the liver.

Work 1. Quantitative determination of phosphoenolpyruvate in the liver and muscles.

The EQUIPMENT: a FEC

The PRINCIPLE of a METHOD.

The method is based on the ability of phosphoenolpyruvate to be oxidized by iodine in alkaline medium. Inorganic phosphate is released as a result of this oxidation. The content of phosphoenolpyruvate is calculated by the determination of the amount of inorganic phosphate released in these conditions.

The COURSE of WORK.

The work is carried out by two students: one of them investigates the muscular tissue, the other - the liver tissue.

1. Preparation of homogenates.

Pound carefully 0.5 g of muscle or liver tissue together with 5 ml of 2.5 % trichloracetic acid (TCA). Transfer the homogenate into the test tubes and put them in ice or snow for 10 minutes. ***(Mix constantly the contents of the test tubes with a glass stick)***. Then add 5 ml of distilled water in each test tube and filter their contents through a paper filter.

2. The order of the experiment.

Pour 2 ml of filtrated homogenate of the muscle or liver into a 10 ml measuring test tube, add 1 ml of 2M NaOH, and 1 ml of an iodine solution. Mix the contents of the test tubes and keep them at room temperature for 15 minutes.

After that pour 2.5 ml of 2M HCL and then add 2M HCL by drops until the appearance of dark yellow colour. Next add 2 ml of sodium thiosulfate by drops and mix constantly until the appearance of light yellow colour. Finally add distilled water up to 10 ml of the total volume of the contents of the test tube.

Take 2 ml of the received solution in another 10 ml measuring test tube, add 0.5 ml of ammonium molybdate, then 0.5 ml of ascorbic acid, and finally distilled water up to 10 ml of the total volume of the test tube.

In 10 minutes measure the optical density of the both samples with FEC against water. Use the red light-filter. The thickness of a cuvette is 5 mm.

Calculate the amount of phosphoenolpyruvate in muscle and liver tissue with a calibrating graph.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. The glucose biosynthesis (gluconeogenesis): the probable precursors, the reaction sequence.
2. The significance and regulation of gluconeogenesis. The glucose-lactate cycle (The Cory cycle).
3. The pentose phosphate pathway of the glucose transformation (pentose cycle, oxidative pathway of the glucose transformation).
4. The oxidative portion of the pentose cycle (the equations of the reactions).
5. The non-oxidative portion of the pentose cycle (without the formulas).
6. The role of the pentose phosphate pathway.

LESSON 16.
REGULATION of CARBOHYDRATE METABOLISM.

BIOMEDICAL SIGNIFICANCE

There’re two levels of metabolism regulation: neuroendocrine and metabolic. The activity of carbohydrate metabolism is under the control of a variety of hormones

(ACTH, glucagon, insulin, epinephrine, glucocorticoids, thyroid hormones, etc.). A lot of them act via cAMP and protein kinases. On the other hand, concentration of substrates and products of reactions, oxygen supply, cofactors concentrations can influence carbohydrate metabolism. In this case metabolic regulation take place mainly via change in ATP, ADP, NAD, $NADH_2$ and intermediate products concentrations and is due to allosteric regulation and chemical modification.

The level of glucose in blood is one of the diagnostic criteria for estimation of carbohydrate metabolism in patients. Increase or decrease of the blood glucose content can result in the development of hypo- or hyperglycemic coma. A number of pathological states may be accompanied by deviation of the normal limits of this parameter, but ***diabetes mellitus*** is the most widespread of them. Oral glucose tolerance test (OGTT) is applied for the diagnosis of latent diabetes mellitus. Under significant increase in blood glucose concentration (up to 9-10 mM/L) it may be detected in urine. This phenomenon is marked ***glucosuria.*** The determination of glucose in urine is also used for the diagnosis and prognosis of the course of diabetes mellitus and its further treatment .

Work 1. Research of a blood glucose level before and after a glucose load. Oral glucose tolerance test.

The EQUIPMENT: a FEC.

The PRINCIPLE of a METHOD.

It is impossible to get the reliable information about the state of carbohydrate metabolism by an unitary determination of the glucose concentration in blood. Therefore it is necessary to research the glucose concentration during certain interval of time after an oral glucose load. Although the oral glucose tolerance test (OGTT) is contraindicated for patients who clearly have diabetes mellitus, it may be used for diagnosis of latent diabetes mellitus in patients with the normal blood glucose level after night fasting. In this test, a nonpregnant patient has fasted overnight drinks 75 g of glucose (approximately 1 g/kg of body mass) or 100 g of sucrose (approximately 1.5 g/kg of body mass) in an aqueous solution. Blood samples are analyzed before the oral glucose load and at 15, 30, 45, 60, 90, and 120 min thereafter.

With the received data the diagram is drawn. On an X-axis of the diagram time (min) is pointed, and on Y-axis - glucose concentrations in blood (mM/l). The received diagram represents glycemic curve after the glucose load.

The COURSE of WORK

A team of 6 students carries out this work.

Experimental samples.

Take a test tube containing a supernatant liquid received before or at certain time after a glucose load and add 3 ml of "glucose reagent" (see the description of the glucose oxidase method of determination of blood glucose concentration, the Lesson 5).

In 15 minutes measure the optical density of the contents of the test tubes ($\mathbf{D_X}$) with a FEC against water. A red light-filter is used for this work (670 nm).

Standard sample.

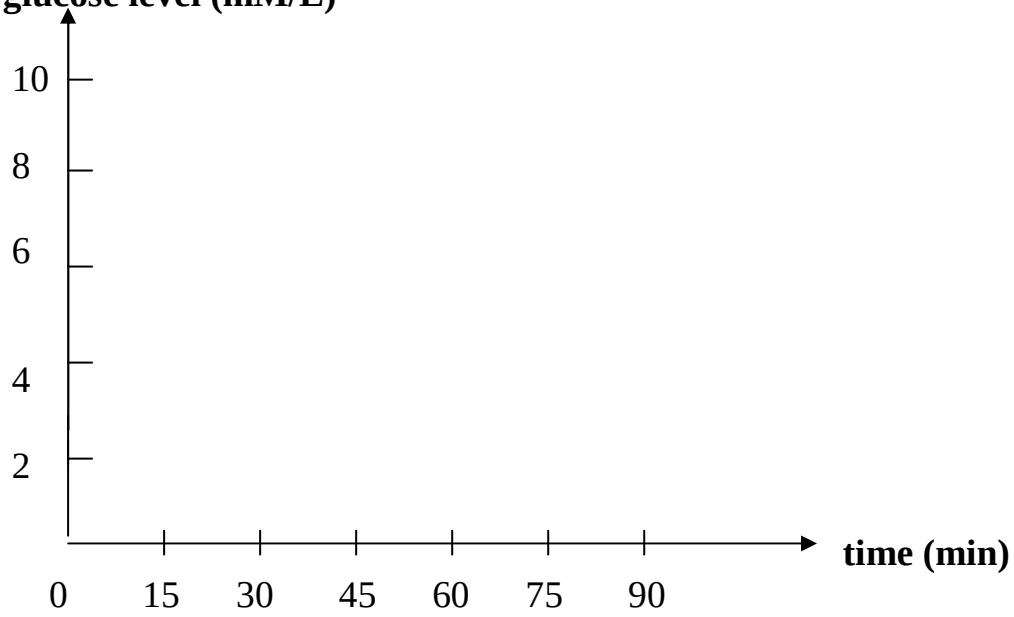
The level of glucose in blood is calculated with the analysis of a standard solution. It is known that the concentration of glucose in the standard solution is 5.5 mM/l. Take 1ml of the standard solution and add 3 ml of the "glucose reagent". In 15 minutes measure the optical density ($\mathbf{D_{st}}$) of the standard solution and solve the proportion:

D_{st}----------5.5 mM/l

Dx----------X mM/l

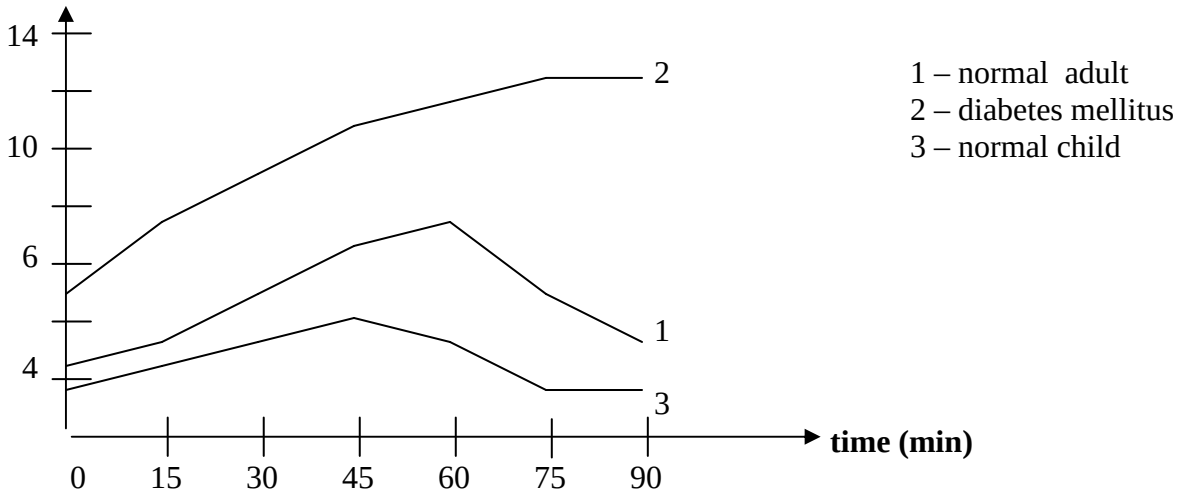
RESULTS (build a sugar curve):

Blood glucose level (mM/L)



Compare your graph with the following typical normal and abnormal graphs (see below)

Blood glucose level (mM/L)



CONCLUSIONS:

Work 2. Qualitative detection of glucose in urine (the Trommer reaction).

The PRINCIPLE of a METHOD.

See Lesson 4, work 1 " Dialysis of proteins".

The COURSE of WORK

Add 5 drops of 10 % solution NaOH to 5 drops of the researched urine, and then add 1-2 drops of 1 % $CuSO_4$ and heat up the test tube in a boiling water bath. In the presence of carbohydrates the yellow or red colour of sediment develops. Carry out the work with normal urine and with the urine containing sugar.

RESULTS:

CONCLUSIONS:

Work 3. Quantitative determination of sugar in urine with Altghausen's method.

The PRINCIPLE of a METHOD.

The method is based on the polymerization of aldehydes when they react with diluted alkali. The products of this reaction are called aldols and are detected with the reaction of aldol condensation. As a result of heating aldehydes together with the concentrated alkali are transformed to the brown colored substances.

The COURSE of WORK.

Carry out this work only with the urine containing sugar.

Mix 4 ml of urine and 1 ml 10 % NaOH in a test tube.

Heat the test tube in the **boiling water** bath for 1 min *(exactly!)*.

In 10 minutes compare the developed colour with a standard colour scale. Choose the most suitable standard and determine the content of sugar in the urine (in percentages). If the received colour of a liquid is darker than the nearest standard strip of the scale but weaker than the next standard strip, the content of sugar will be calculated as the average size between designated on these strips. If the urine sample contains sugar more than 4 %, it is necessary to dilute it with water by 2 times and repeat the analysis with already diluted urine. In this case the received amount of sugar has to be multiplied by 2.

RESULTS:

CONCLUSION:

BASIC QUESTIONS:

1. The origin of blood glucose.
2. The regulation of the blood glucose concentration.
3. The role of the liver in maintaining a blood glucose level.
4. The role of the nervous system in the regulation of a blood glucose level.
5. The hormone regulation of a blood glucose level. The role of cyclic AMP.
6. Participation of epinephrine and glucagon in the regulation of carbohydrate metabolism. Role of fructose-2,6-diphosphate in transduction of hormones signal.
7. The influence of glucocorticoids on carbohydrate metabolism.
8. The role of insulin in maintaining a blood glucose level. The insulin receptor structure. Insulinresistance.
9. The influence of ATP and ADP concentration on the main pathways of carbohydrate metabolism.
10. Disorders of carbohydrate metabolism (starvation, diabetes mellitus, glycogen diseases).
11. A sugar tolerance. The method of investigation of a sugar tolerance for revealing the disorders of carbohydrate metabolism.
12. Hypo- and hyperglucosemia, their reasons. Glucosuria. Its reasons and mechanisms.
13. The oral glucose tolerance test (OGTT) and its importance for a diabetes mellitus diagnosis.

The examples of the control task:

1. Caffeine inhibits 3',5'-cAMP phosphodiesterase, which converts cAMP to AMP. Which of the following effects would be observed if cells were treated with caffeine?

   a) Decreased activity of liver protein kinase A
   b) Decreased activity of muscle protein kinase
   c) Increased activity of liver pyruvate kinase
   d) Decreased activity of liver glycogen synthase

2. Which of the following bonds is broken down by saliva amylase?
   a) 1,4-glycoside
   b) 1,6-glucoside
   c) ester
   d) 1,2-glucoside

3. Phosphorylase catalyses the following reaction:
   a) UDP-glucose + $(C_6H_{10}O_5)n \rightarrow (C_6H_{10}O_5)n\text{-}_1$ + UDP
   b) $(C_6H_{10}O_5)n$ + H3PO4 → glucoso-1-phosphate + $(C_6H_{10}O_5)n\text{-}_1$
   c) UDP-glucose ↔ UDP-galactose
   d) UDP-glucose + fructose → UDP-sucrose

4. Glycogen synthase catalyses the following reaction:
   a) removal of glucose from the point of the branching of a glycogen molecule

b) transfer of 6-8 glucose residues from an amylose chain to the point of the branching

c) formation of 1,6 glycoside bond in the glycogen molecule

d) formation of 1,4 glycoside bond in the glycogen molecule

5. Muscle glycogen cannot contribute directly to blood glucose levels because:

a) muscle lacks glucose 6-phosphatase

b) muscle contains no glucokinase

c) muscle contains no glycogen phosphorylase

d) muscle glycogen cannot be converted to glucose 6-phosphate

6. In a glucose tolerance test, an individual in the basal metabolic state ingests a large amount of glucose. If this individual is normal, this ingestion results in

a) enhanced glycogen synthase activity in the liver

b) an increased ratio of phosphorylase A to phosphorylase B in the liver

c) an increased rate of lactate formation by enterocytes

d) inhibition of glycogen synthase phosphatase activity in the liver

7. Which of the following substances are needed to the cAMP synthesis?

a) Adenylate cyclase and GTP

b) Adenylate cyclase and CTP

c) Guanylate cyclase and ATP

d) Adenylate cyclase and ATP

8. Choose the hormone which inhibits adenylate cyclase.

a) Insulin

b) Epinephrine

c) Glucagon

d) ACTH

9. Synthesis of 1,6-glycoside bonds is catalyzed by which of the following enzymes:

a) amylase

b) amylo-1,6-glucosidase

c) “branching” enzyme

d) glycogen synthase

10. Hexokinase catalyses which of the following reactions:

a) Glucose + ATP $\rightarrow$ glucoso-6-phosphate + ADP

b) Glucoso-1-phosphate + ATP$\rightarrow$ Glucoso-1,6-diphosphate + ADP

c) $(C_6H_{10}O_5)$n + H3PO4 $\rightarrow$ glucoso-1-phosphate + $(C_6H_{10}O_5)n\text{-}_1$

d) Glucoso-6-phosphate $\rightarrow$ Glucose + H_3PO_4

11. An infant with the enlarged liver has a glucose 6-phosphatase deficiency. This infant

a) cannot maintain blood glucose levels either by glycogenolysis or by gluconeogenesis

b) can use liver glycogen to maintain blood glucose levels

c) can use muscle glycogen to maintain blood glucose levels

d) can convert both alanine and glycerol to glucose to maintain blood glucose levels

12. Allosteric activator of the cell protein kinase is

a) ATP
b) ADP
c) cAMP
d) AMP

13. Which of the following enzymes catalyzes the synthesis of 1,4-glycoside bonds in a glycogen molecule?
a) 1,4-glycosidase
b) 1,6-glycosidase
c) "Branching" enzyme
d) Glycogen synthase

14. Phosphoglucomutase catalyses which of the following reactions:
a) Glucose-1-phosphate $\leftrightarrow$ Glucoso-6-phosphate
b) Glucose + ATP$\rightarrow$ Glucose-6-phosphate + ADP
c) Glucoso-6-phosphate $\rightarrow$ glucose + H_3PO_4
d) $(C_6H_{10}O_5)n$ ++$H_3PO_4 \leftrightarrow$ $(C_6H_{10}O_5)n\text{-}_1$ +Glucoso-1-phosphate

15. The reaction of the conversion of glucose 1-phosphate to glucose-6-phosphate is catalyzed by which of the following enzymes:
a) hexosophosphate isomerase
b) phosphoglucomutase
c) phosphodiesterase
d) phosphatase

16. A 16-year-old patient with Type 1 diabetes mellitus was admitted to the hospital with a blood glucose level of 12 mM/L. One hour after an insulin infusion was begun, her blood glucose level had dropped to 9 mM/L One hour later, it was 7 mM/L The patient's glucose level dropped because insulin
a) stimulated the transport of glucose across cell membranes of the liver and brain b) stimulated the conversion of glucose to glycogen and triacylglycerol in the liver c) inhibited the synthesis of ketone bodies from blood glucose
d) stimulated glyconeogenesis in the liver

17. Activators of adenylate cyclase are the following hormones:
a) ACTH, STH, TTH, prostaglandins, insulin
b) epinephrine, glucagon, insulin, STH
c) epinephrine, glucagon, ACTH, STH, TTH
d) ACTH, glucagon, insulin, STH, TTH

18. Which of the following substances are the glycosyl residue donor in the glycogen synthesis?
a) UDP-glucose
b) CDP-glucose
c) Glucose-6-phosohate
d) Glucose1,6-diphosphate

19. The reversible reaction of the interconversion of glucose-1-phosphate and glucose-6-phosphate is catalyzed by:
a) phosphorylase
b) hexokinase

c) phosphatase
d) phosphoglucomutase

20. Which of the following hormones induces hypoglucosemia?
a) Insulin
b) Epinephrine
c) Glucagon
d) Cortisol

21. Epinephrine and glucagon have the following effects on glycogen metabolism in the liver:
a) the net synthesis of glycogen is increased
b) glycogen phosohorylase is activated while glycogen synthase is inactivated
c) both glycogen phosphorylase and glycogen synthase are activated but at markedly different rates
d) glycogen phosphorylase is inactivated while glycogen synthase is activated

22. The reaction

$$\textbf{Glucose-6-phosphate} + H_2O \rightarrow \textbf{Glucose} + H_3PO_4$$

is catalyzed by:
a) phosphoglucomutase
b) glucose-6-phosphatase
c) phosphorylase
d) phosphodiesterase

23. Transformation of inactive glycogen synthase D into active glycogen synthase I requires:
a) ATP and glycogen synthase kinase
b) H_2O and glycogen synthase phosphatase
c) ATP and glycogen synthase phosphatase
d) H_3PO_4 and glycogen synthase kinase

24. Amylo-1,6-glucosidase (“debranching” enzyme) catalyses which of the following reactions:
a) hydrolysis of 1,4 glycoside bonds oh the glycogen molecule
b) synthesis of 1,4 glycoside bonds
c) synthesis of 1,6 glycoside bonds
d) hydrolysis of 1,6-glycoside bonds

25. Choose the main sources of blood glucose.
a) Proteoglycans, glycoproteins, muscle glycogen
b) Muscle glycogen, non-carbohydrate substances (lactate, pyruvate, amino acids) c) Liver glycogen, food carbohydrates, proteoglycans
d) Liver glycogen, food carbohydrates, non-carbohydrate substances (lactate, pyruvate, amino acids)

The SECOND TURM

LESSON 16
MAIN LIPIDS of the BODY. LIPID DIGESTION.

BIOMEDICAL SIGNIFICANCE

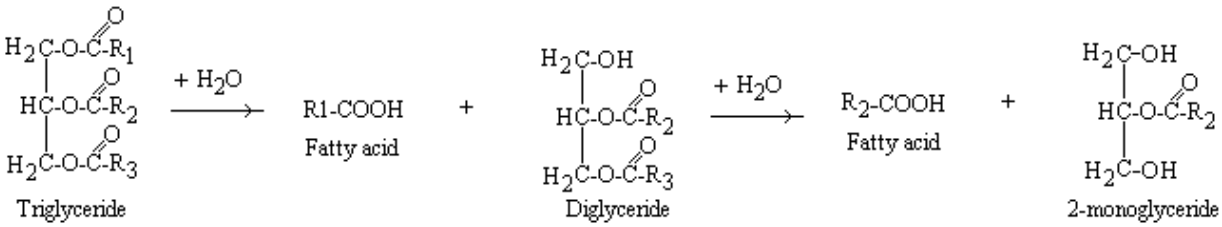
The disturbances of digestion and absorption of dietary lipids may be due to many reasons such as inherited or acquired disorders of the synthesis of pancreatic lipase, obstruction of bile excretory tract and the other reasons leading to the disturbance of emulsification of dietary lipids, inflammation processes in the small intestine etc.

Work 1. The determination of lipase activity in duodenal contents.

The EQUIPMENT: an ionometer with a glass electrode, a thermostat for 38°C.

The PRINCIPLE of a METHOD.

Pancreatic lipase catalyses the consecutive hydrolysis of neutral fat (triglyceride) according to the following equation:



The method is based on the electrometric determination of pH-gradient formed because of the change in the concentration of fatty acids released by a hydrolysis of emulsified fat with pancreatic lipase.

The COURSE of WORK.

Prepare a reaction mixture in three test tubes (1 - control, 2 and 3 - experimental) according to the table:

	1. Control	**2. Experimental (without bile acids)**	**3 Experimental (in the presence of bile acids)**
1. Boiled milk (substrate)	1.5 ml	1.5 ml	1.5 ml
2. Distilled water	1.0 ml	1.0 ml	0.5 ml
3. Tris-buffer	0.5 ml	0.5 ml	0.5 ml
4. Duodenal contents	-	0.5 ml	0.5 ml
5. Bile acids	-	-	0.5 ml

Measure the pH_o value of the control test tube contents before the incubation and write down the initial result for all test tubes in the table RESULTS (see below).

Put all the tubes into a thermostat and incubate them at the temperature 37°C for 1 hour.

After the incubation add 1.5 ml of ethyl alcohol in all test tubes for inhibition of the enzyme action. Add 0.5 ml of duodenal contents in the control test tube.
Then determine the value of pH (pH_1) of each test tube contents. Write down the received results in the table and make the accounts.

RESULTS:

	Control	**Experiment 1 (without bile acids)**	**Experiment 2 (in presence of bile acids)**
Before incubation	pH_o=	pH_o=	pH_o=
After incubation	pH_1=	pH_1=	pH_1=
	$\Delta pH_{contr.}$=pH_o - pH_1 =	ΔpH=pHo - pH_1 =	ΔpH=pHo - pH_1 =
Lipase activity	-	**ΔpH-ΔpHcontr.= =**	**ΔpH-ΔpHcontr= =**

Subtract the value of ΔpH of the control from the value of ΔpH calculated for the 1-st and the 2-nd test tubes.
Find the value of lipase activity with the calibrating graph: plot the received difference on the calibrating graph to find the lipase activity in the 1-st and the 2-nd tubes. The lipase activity is expressed by the standard units. A standard unit is the amount of the enzyme which produces 1 micromole of fatty acids per 1 minute in 1 ml of duodenal contents. The normal pancreatic lipase activity in duodenal contents is 400-700 un./ml.

CONCLUSIONS:

BASIC QUESTIONS:

1. The main lipids of the body, their structures, classification and functions.
2. Digestion of lipids in the stomach. The significance of the stomach lipase for children.
3. Digestion of lipids in the intestine. The effect of the pancreatic enzymes (lipase, phospholipase) on dietary lipids. The role of bile as an emulsifier and activator of the pancreatic lipase.
4. Absorption of the products of the lipid digestion in the intestine. The role of bile. The disorders of the absorption of lipids.
5. The lipid resynthesis in the enterocytes.

LESSON 17
LIPID CATABOLISM. KETONE BODIES.

BIOMEDICAL SIGNIFICANCE

Ketone bodies are normal products of lipid metabolism which are formed in the liver and serve as metabolic fuel for many tissues (myocardium, kidney, skeletal muscle etc). Abnormal accumulation of ketone body (***ketonemia***) occurs in the pathological states characterized by carbohydrate deficiency in the tissues (diabetes mellitus, starvation, malabsorption of dietary sugars in infants, pregnancy toxicoses etc). Ketonemia is accompanied by tissue acidification that leads to the disturbance in tissue metabolism and appearance of ketone bodies in urine (***ketonuria***).

Work 1. The formation and oxidation of ketone bodies in the body tissues.

The EQUIPMENT: porcelain mortars, a thermostat.

The PRINCIPLE of a METHOD.

The synthesis of ketone bodies takes place in the liver, and their oxidation proceeds in different tissues, except the liver. Hungry white rats are used as experimental animals, because the excessive accumulation of ketone bodies takes place after fasting.

The COURSE of WORK.

1. Preparation of homogenates of the liver, kidneys, heart, skeletal muscle, and brain.

Pound a sample of each tissue about 2 grams (except the liver) in cooled mortars (use snow or ice). *Liver is used as a whole organ.* Pound the tissue; add gradually 15 ml of distilled water (50 ml for the liver). Filter the homogenates through a cotton filter to the previously cooled test plugs.

The liver homogenate serves as a source of ketone bodies, while the peripheral tissues homogenates are used as the sources of the enzymes oxidizing ketone bodies.

2. The experiment order (see the table below):

№№ of the test tubes	Type of the test tube	Volume of homogenate (ml)					Phosphate buffer (ml)	Conditions of incubation	Results
		liver	kidneys	heart	skeletal muscle	brain			
1	experimental	1	1	-	-	-	1	45°C	
2	experimental	1	-	1	-	-	1	45°C	

3	**expe-ri-men-tal**	1	-	-	1	-	1	45ºC	
4	**expe-ri-men-tal**	1	-	-	-	1	1	45ºC	
5	**con-trol**	2	-	-	-	-	1	0ºC	

Place the test tubes №№ 1- 4 into a thermostat at 45ºC for an hour.

Place the control test tube (№ 5) in thawing ice (temperature OºC). Close the test tubes by fuses.

After incubation determine visually the contents of ketone bodies in the experimental and control test tubes.

3.. The reaction of detection of ketone bodies (acetone and acetoacetate)

After incubation add 7 drops of the Imbert reagent (mixture of equal volumes of 10 % sodium nitroprusside and concentrated acetic acid) in each test tube, mix carefully, and then pour cautiously about 1 ml of 25 % of ammonia (**do not mix and keep the test tube in the inclined position**). After that return carefully the test tube in a vertical position and put it in the test tube support. You can see a crimson ring on the border of two liquids if ketone bodies are present in the liquid.

The intensity of the colour is proportional to the ketone bodies concentration in the incubation mixture.

The intensity of the colour may be marked as (+, ++ or +++). The absence of a colour ring is marked as (-). Write down the results in the right column of the table.

CONCLUSIONS:

Work 2. Detection of the ketone bodies in urine.

The PRINCIPLE of a METHOD.

The detection of acetone in urine is based on its interaction with sodium nitroprusside and formation of the violet coloured product. The qualitative revealing of acetoacetic acid is based on the formation of complex compound of iron with the enol form of acetoacetic acid.

The COURSE of WORK:

1. Express method of definition of acetone in urine (Lestrade's reaction).

Put small quantity of powder consisting of sodium nitroprusside, ammonia sulfate and sodium carbonate on a surface of the Petry dish placed on a sheet of white paper. Then add 2-3 drops of the researched urine. The maximal cherry colour will appear in 1-2 minutes if acetone is present in the urine. The test will be estimated as negative if the change of the urine colour does not occur and as positive if the distinct violet colour appears in 1-3 minutes.

2. The Legal reaction with sodium nitroprusside for the detection of acetone .

Measure out 1 ml of urine in a test tube, then add 1-2 drops of sodium nitroprusside solution, next 3-4 drops of 10 % NaOH solution. The red colour will be formed. Then add 1-2 drops of 10 % acetic acid. Red cherry colour will appear if acetone is present in the urine. If acetone is absent in the urine, red colour will disappear after the addition of acetic acid.

3. The Gerhardt reaction for the detection of acetoacetic acid.

Add 4-5 drops of iron chloride to 1-2 ml of the researched urine. The cherry-red colour will appear, if acetoacetic acid is present in the urine.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Lipolysis. The hormonal control of this process.
2. Glycerol oxidation to glyceralaldehyde-3-phosphate. ATP yield from glycerol oxidation to carbon dioxide and water.
3. Oxidation of fatty acids. The role of carnitine. The pathway of β-oxidation, the reaction equations, the stoichiometry of β-oxidation, the net ATP yield.
4. Oxidation of unsaturated fatty acids.
5. Biosynthesis of ketone bodies.
6. The reactions of ketone bodies activation. Ketone bodies oxidation. The net energy yield of ketone bodies oxidation.
7. Ketonemia, ketonuria, their reasons.

LESSON 18
LIPID ANABOLISM.

BIOMEDICAL SIGNIFICANCE

In a human body most of lipids are triglycerides. They are the main lipids of food and adipose tissue. Phospholipids are the main components of biological membranes. Also they have some special functions. For instance, dipalmitolecithin is the main phospholipids of lung surfactant. Phosphatidylinositols fulfill messenger function in hormone signal transduction. Glycosphingolipids form glycocalyx of cell surfaces. Phospholipids are known to be lipotropic substances protecting liver against fat liver dystrophy. Phosphatidylcholines take part in blood plasma lipoprotein metabolism. They activate lipoproteinlipase as well as are present in the hydrophilic shell of lipoproteins.

Work 1. Quantitative determination of phospholipids concentration in serum.

The PRINSIPLE of a METHOD.

Phospholipids are precipitated by trichloracetic acid together with proteins. Then the sediment is subjected to mineralization and inorganic phosphate can be detected in the mineralizate. An amount of phospholipids is calculated according to the phosphoric acid concentration that can be measured by a photoelectroclorimetric method.

The COURSE of WORK:

Experimental sample.

1. Add 2.5 ml of distilled water into a measuring test tube containing 0.2 ml of serum, mix its content carefully;
2. Add 3ml of 10% TCA, stir it with a glass stick and keep for 2 minutes at room temperature;
3. Centrifuge the test tube for 10 minutes;
4. Pour out supernatant liquid and make the further experiment with the sediment;
5. Add 1ml of 57% of chloric acid and stir its content carefully with a glass stick;
6. Add 6 ml of distilled water, mix and then filter the liquid through a paper filter;
7. Add 1 ml of 4% solution of ammonium molybdate to the filtrate, mix carefully and then add 1 ml of amino-naphthol-sulfonic acid;
8. Increase the volume of the test tube up to 10 ml by adding distilled water;
9. In 10 minutes measure optical density of the solution with a FEC (red light filter - 670nm; the thickness of a cuvette is 10 mm).

Standard sample.

1. Add 2ml of the standard solution of monopotassium phosphate and add 0.8 ml of 57 % chloric acid and stir it carefully with a glass stick;
2. Increase the volume of the test tube up to 7 ml by adding distilled water;
3. Add 1ml of 4% solution of ammonium molybdate, mix carefully and then add 1 ml of amino-naphthol-sulfonic acid;
4. Increase the volume of the tube up to 10 ml by adding distilled water;
5. In 10 minutes measure optical density of the solution with a FEC (red light filter - 670nm; the thickness of a quvette is 10 mm).

<u>Calculation.</u>

Calculate the lipid phosphate concentration according to the following equation:

$$X = \frac{E_{exp} \cdot 0.02 \cdot 100}{E_{st} \cdot 0.2} = \frac{E_{exp}}{E_{st}} \times 10$$

Where:

X - lipid phosphate concentration in mg /dl;

E_{st} - optical density of the standard sample;

E_{exp} - optical density of the experimental sample;

0.02 - the amount of phosphorous contained in 2ml of the standard sample (mg);

0.2 - the volume of serum taken for a experiment.

The normal range of serum phospholipids content equals 6.1-14.5 mg/dl or 1.97-4.68 mM/l for adults.

BASIC QUESTIONS:

1. Transport of acetyl CoA from mitochondria to cytoplasm. Regulation of this process.
2. The structure of fatty acid synthase complex. The fatty acid synthesis, reactions. Regulation of this process. Elongation of fatty acids. Desaturation of fatty acids.
3. Synthesis of triacylglycerols. Synthesis of triacylglycerols from glucose (a scheme).
4. Synthesis of phospholipids. Regulation of the process. Lipotropic and antilipotropic substances.
5. Synthesis of cholesterol. Regulation of this process.
6. The role of acetyl CoA in the integration of carbohydrate and lipid metabolism.

LESSON 19
LIPOPROTEINS of BLOOD PLASMA. CHOLESTEROL TRANSPORT.

BIOMEDICAL SIGNIFICANCE

The determination of blood plasma lipoproteins is used for revealing the familiar disorders of lipid metabolism including both hyper- and hypolipidemias. Blood of normal individuals contains three main classes of lipoproteins, such as HDL (high density lipoproteins), LDL (low density lipoproteins) and VLDL (very low density lipoproteins). Chylomicrons are absent in blood plasma after 12-hr fasting. They appear in blood just after meal and have been utilized for the fasting time. Some pathological states may be accompanied by the appearance of IDL (intermediate density lipoproteins). ***Hyperlipoproteinemia*** is a phenomenon of increase in certain lipoprotein content in blood plasma. The development of atherosclerosis is due to increase in VLDL and LDL content in blood plasma. Both of the fractions are termed ***atherogenic lipoproteins***. HDL fraction is responsible for the excess cholesterol reverse transport to the liver, and this fraction is marked as ***antiatherogenic***.

The excess of dietary cholesterol and abnormal activation of its synthesis from dietary carbohydrates lead to the development of atherosclerosis accompanied by cholesterol deposit under the big blood vessel intima and atherosclerotic plugs forming.

Work 1. Determination β- and pre-β-lipoproteins in blood plasma.

The EQUIPMENT: a FEC

The PRINCIPLE of a METHOD.

The method is based on the ability of heparin to form the complex with β- and pre- β-lipoproteins which drops out in a sediment after the interaction with calcium chloride.

Concentration of β- and pre-β-lipoproteins of blood plasma is determined as a degree of turbidity which is proportional to the concentration of the lipoproteins..

The COURSE of WORK.

Add 2 ml of 0.05 M calcium chloride into a test tube containing 0.2 ml of blood plasma .

Determine the initial size of optical density **/D_1/** with a FEC (use a red light-filter and 0.5 cm cuvettes). Pour back the contents of the cuvette in the same test tube. Then add there 0.04 ml of heparin **(precisely, with the special micropipette!)**, washing out a pipette with the contents of the test tube 2-3 times .

In 5 minutes **(precisely!)** measure the value of optical density /**D_2**./ with a FEC.

ACCOUNT:

Calculate the content of β- and pre-β-lipoproteins in blood plasma by the formula:

$$(D_2 - D_1) \cdot 12$$

Where **12-** is the empirical factor for an expression the lipoprotein concentration in g/L.

The normal content of blood plasma β- and pre-β-lipoproteins is 3.0-7.2 g/L.

RESULTS:

CONCLUSIONS:

Work 2. Quantitative determination of cholesterol in serum.

The EQUIPMENT: a FEC.

The PRINCIPLE of a METHOD.

Cholesterol gives green colour in the presence of both acetic anhydride and sulfuric acid (The Ilk method).

The COURSE of WORK.

Add very slowly (**Warning! See the instruction for students' safe work**) 0.1 ml of serum to 2.1 ml of the Ilk reagent in a test tube with a micropipette or dosator. Stir the test tube contents 10-12 times and put the test tube into a thermostat at 37ºC for 20 minutes. Then determine the optical density with FEC. Use a red-light filter (630-690 нм). The thickness of a cuvette is 5 mm. The colorimetry has to be carried out against distilled water.

Calculate the concentration of cholesterol in the serum with a calibrating graph.

Normal concentration of cholesterol in serum is 3,0-8,8 мМ/l.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Resynthesis of neutral fat in enterocytes. Formation of chylomicrons.
2. The transport blood lipoproteins, specificity of their structure, composition, and function.
3. CM, VLDL, LDL, HDL. Their role in the fat and cholesterol metabolism.
4. Apoproteins, their classification, participation in the formation and metabolism of lipoproteins.
5. Hypercholesterolemia and lipoproteinemias as the factors of atherosclerosis development.

The examples of the control task:

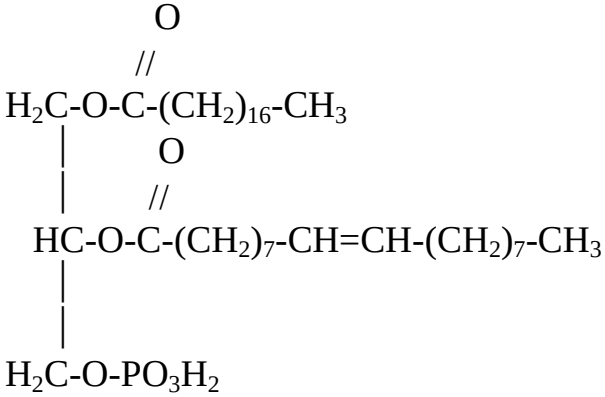
1). Which of the following substances are phospholipids?

1. Phosphatidylserine
2. Lecithin
3. Sphingomyelin
4. Phosphatidylcholine
5. Plasmalogen
6. Prostaglandin
7. Cardiolipin

2). Which of the following substances are the derivatives of cholesterol?

1. Vitamin D_3
2. Vitamin A
3. Bile acids
4. Corticosteroids
5. Prostaglandins
6. Alpha-tocopherol
7. Sex hormones

3). What is the name of the compound?



1. Diacylglycerol
2. Lysophospholipid
3. Phosphatidic acid
4. 3-phosphodiacylglycerol
5. Phosphoglycerol

4). Chylomicrons are synthesized in the...

1. liver
2. small intestine
3. blood plasma
4. liver and blood plasma
5. liver and small intestine

5). Metabolism of chylomicrons proceeds with the participation of

1. liver triacylglycerol lipase
2. lipoprotein lipase
3. phospholipase
4. diacylglycerol lipase

6). In VLDL metabolism which of the following metabolic processes take part:

1. Hydrolysis of triglycerides with liver triacylglycerol lipase
2. Hydrolysis of phospholipids with liver phospholipase
3. Interaction with HDL and formation of IDL
4. Endocytosis of a portion of VLDL remnants (IDL) in the liver with BE-receptors

7). Choose the HDL functions.

1. Transport of cholesterol from the liver to the peripheral tissues
2. Reverse transport of cholesterol from the cells of the extrahepatic tissues to the liver
3. Transport of endogenous triglycerides
4. "Scavenger" of excessive cholesterol

8). LCAT is activated by

1. apo-C_2
2. apo-E
3. apo-A1
4. apo-A_1 and apo-C_2
5. apo-B_{100} and apo-E

9). HDL particles contain

1. apo-B100
2. apo-A
3. apo-B48
4. apo-C
5. Maximal amount of protein
6. Maximal amount of cholesterol
7. Excess of triacylglycerides
8. Average amount of phospholipids about 20-30%.

10). Apo-E

1. is synthesized in the small intestine
2. is synthesized in the liver
3. is a marker of HDL and CM
4. is a marker of VLDL and LDL
5. activates LCAT
6. is a ligand for the cell receptors

11). Which of the following apoproteins take part in a control of enzymes of lipoprotein metabolism?

1. Apo-A1
2. Apo-B48
3. Apo-B100
4. Apo-C2
5. Apo-E

12). Choose the triglyceride (neutral fat) functions.

1. Structural
2. Power
3. Storage
4. Thermoregulation
5. Transport
6. Regulative
7. Protective (defense of hurts)

13). Which of the following substances belong to **bile acids**?

1. Cholic acid
2. Chenocholic acid
3. Taurocholic acid
4. Taurine
5. Glycocholic acid

14). Choose the substances which are involved into the group of **minor lipids.**

1. Arachidonic acid
2. Dolichols
3. Vitamin A
4. Vitamin E
5. Cholic acid

15). Metabolism of chylomicrons in blood plasma is characterized by the following processes:

1. Hydrolysis of phospholipids
2. Hydrolysis of triglycerides
3. Incorporation of Apo-C into HDL
4. Absorption of CM remnants by HDL
5. Absorption of CM remnants by the liver with Apo-E-receptors

16). Choose the VLDL functions.

1. Transport of cholesterol from the liver to the peripheral tissues
2. Transport of exogenous triglycerides
3. Transport of endogenous triglycerides
4. Transport of cholesterol from the peripheral tissues to the liver

17). LCAT (lecithin:cholesterol acyltransferase)

1. catalyses the reactions of phospholipid hydrolysis
2. catalyses the reaction of esterification of cholesterol
3. catalyses the reaction of transfer of an acyl residue to cholesterol
4. catalyses the reaction of formation of lysolecithin
5. catalyses the reaction of protein hydrolysis
6. is activated by apo-C_2
7. is activated by apo-A_1

18). In blood plasma HDL particles are associated with

1. lipoprotein lipase
2. triglyceride lipase
3. lecithin:cholesterol acyltransferase
4. phospholipase

19). Which of the following plasma lipoproteins contains the largest particles?

1. HDL
2. LDL
3. VLDL
4. CM

20). Which of the following factors promote to the development of atherosclerosis?

1. High level of LDL in blood
2. High level of triglycerides in blood
3. High level of HDL in blood
4. Low level of HDL in blood
5. High level of VLDL in blood
6. Excess of cholesterol

21). Apoprotein C_2

1. is synthesized in the liver
2. activates LCAT
3. is transferred from HDL to CM and VLDL
4. is transferred from HDL to VLDL and LDL
5. activates lipoprotein lipase

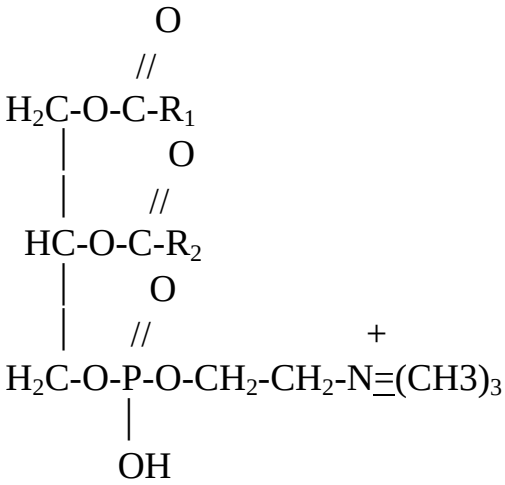
22). Choose the functions of cholesterol.

1. Power
2. Transport
3. Structural
4. Regulative
5. Serves as a source to the synthesis of the biologically active molecules (vitamins, hormones)

23). Which of the following substances belong to sphingosine derivatives?

1. Sphingomyelin
2. Plasmalogens
3. Prostaglandins
4. Cerebrosides
5. Glycolysis
6. Lysophospholipids
7. Gangliosides

24). What is the name of the compound?



1. Cardiolipin
2. Plasmalogen
3. Phosphatidic acid
4. Phosphatidylinositol
5. Phosphatidylcholine

25). Which of the following substances are incorporated into the nuclei of lipoprotein particles?

1. Free fatty acids
2. Triglycerides
3. Phospholipids
4. Cholesterol esters
5. Free cholesterol

26). The density and electrophoretic classification of lipoproteins allow to separate the following fractions:

1. CM - β-LP
2. VLDL - preβ-LP
3. HDL - β-LP
4. LDL - α-LP
5. CM - CM
6. HDL - α-LP
7. LDL - β-LP

27). Chylomicrons contain

1. endogenous triglycerides mainly
2. exogenous triglycerides mainly
3. apo-B_{48}
4. apo-A
5. cholesterol (about 40%)
6. small amount of protein
7. large amount of protein

28). Lipoprotein metabolism proceeds with the participation of

1. liver triglyceride lipase
2. diglyceride lipase
3. lipoprotein lipase
4. phospholipase

29). LDL particles are formed in the...

1. liver
2. small intestine
4. liver and blood plasma
5. liver and small intestine

3. blood plasma

30). The main function of LDL is

1. transport of exogenous triglycerides
2. transport of endogenous triglycerides
3. transport of cholesterol from the liver to the peripheral tissues
4. transport of phospholipids
5. transport of cholesterol from the peripheral tissues to the liver

31). Apo-A_1

1. is synthesized in the liver
2. is synthesized in the small intestine
3. activates lipoprotein lipase
4. activates LCAT
5. is a ligand for the cell receptors
6. is transferred from CM to HDL

32). Choose the functions of apoproteins.

1. Catabolic
2. Structural
3. Regulative (regulation of enzyme activity)
4. Protective
5. Storage
6. Secretion
7. Ligands for the cell receptors

3). Which of the following substances are the head group of phospholipids?

1. Inositol
2. Glycine
3. Serine
4. Choline
5. Inosine
6. Ethanolamine
7. Guanine
8. Adenine

34). Choose the statements which describe the structures of sphingosine derivatives.

1. Cerebrosides consist of the following constituents: sphingosine, phosphoric acid, fatty acid, choline, oligosaccharide.
2. Cerebrosides consist of the following constituents: sphingosine, fatty acid (C_{24}), hexose.
3. Gangliosides consist of the following constituents: sphingosine, fatty acid, monosaccharide.
4. Gangliosides consist of the following constituents: sphingosine, fatty acid, oligosaccharide.
5. Sphingomyelins consist of the following constituents: sphingosine, choline, fatty acid

35). Choose the functions of chylomicrons.

1. Transport of cholesterol from the liver to the peripheral tissues
2. Transport of exogenous triglycerides
3. Transport of endogenous triglycerides
4. Transport of cholesterol from the peripheral tissues to the liver

36). Lipoprotein lipase is activated by

1. apo-C_2
2. apo-E
3. apo-A_1
4. glucagon
5. epinephrine
6. norepinephrine

37). VLDL particles are synthesized in the...

1. liver
3. liver and small intestine

2. small intestine 4. blood plasma

38). LDL particles contain

1. apo-B100 4. about 50% of cholesterol
2. apo-B48 5. minimum of protein
3. apo-E 6. maximal amount of triglycerides

39). Utilization of free cholesterol released in the LDL decomposition includes the following processes:

1. Cholesterol is converted into cholesterol esters
2. Cholesterol is used to the synthesis of steroid hormones in adrenal cortex
3. Excess of cholesterol activates the synthesis of endogenous cholesterol
4. Excess of cholesterol inhibits the synthesis of endogenous cholesterol
5. Cholesterol is incorporated into the structure of biological membranes

40). Which of the following apoproteins carry out the function of the specific ligands for the cell receptors?

1. Apo-A 4. Apo-C2
2. Apo-B48 5. Apo-E
3. Apo-B100

41). In the inherited deficiency of lipoprotein lipase the following phenomena take place:

1. increase of CM content in blood
2. increase of LDL content in blood
3. increase of HDL content in blood
4. increase of triglyceride contents in blood
5. increase of cholesterol content in blood
6. appearance of a cream-like layer over transparent serum

42). Human neutral fat is characterized by the following properties:

1. liquid consistency at the body temperature
2. solid consistency at the body temperature
3. low melting temperature ($t^{o}_{m}=15^{o}C$)
4. contains only saturated fatty acids
5. contains only unsaturated fatty acid
6. contains prevalent amount of oleic acid (nearly 70%)
7. contains prevalent amount of arachidonic acid

43). Phospholipids and sphingolipids carry out the following function

1. Power 4. Protective
2. Transport 5. Storage
3. Structural

44). In blood plasma fatty acids circulate

1. in the content of nuclei of plasma lipoprotein particles
2. in the content of an outer shell of plasma lipoprotein particles
3. in the complex with serum albumin
4. freely without formation of any complexes

45). The outer shell of a lipoprotein particle contains

1. phospholipids oriented to the nucleus of a lipoprotein particle by their hydrophylic groups

2. phospholipids oriented to the nucleus of a lipoprotein particle by their hydrophobic groups
3. free cholesterol oriented to the nucleus by its 3-OH group
4. superficial proteins
5. cholesterol esters
6. integral apoproteins
7. free cholesterol oriented to the nucleus by its hydrophobic sites

46). Chylomicrons exchange their apoproteins with the other blood plasma lipoproteins by the following manner:

1. They give apo-A to VLDL
2. They receive apo-C from HDL
3. They give apo-B_{48} to LDL
4. They give apo-A to HDL
5. They receive apo-E from LDL
6. They receive apo-E from HDL

47). The following factors prohibit from the excessive accumulation of cholesterol:

1. inhibition of endogenous synthesis of chololesterol
2. hyperchylomicronemia
3. activation of lipoprotein lipase
4. inhibition of the formation of BE-receptors
5. catalytic action of LCAT
6. hyperglucosemia

48). VLDL particles contain

1. apo-B48
2. apo-B-100
3. exogenous triglycerides (about 60%)
4. apo-E
5. Endogenous triglycerides (about 60%)
6. small amount of cholesterol
7. protein (50% and more)

49). Metabolism of LDL includes:

1. hydrolysis of triglycerides by lipoprotein lipase
2. binding of LDL with a plasmatic membrane
3. endocytosis of an LDL-receptor complex
4. formation of cholesterol esters by LCAT
5. hydrolysis of LDL by lysosomal enzymes and accumulation of cholesterol in the cells
6. partial endocytosis with BE-receptors

50). Choose the atherogenic fractions of plasma lipoproteins.

1. CM and LDL
2. CM and VLDL
3. VLDL and HDL
4. VLDL and LDL
5. LDL and HDL
6. CM and HDL

51). HDL particles are synthesized in:

1. the liver
2. blood plasma
3. the small intestine
4. the liver and blood plasma

LESSON 20
MEMBRANE METABOLISM.
LIPID PEROXIDATION (LPO).

BIOMEDICAL SIGNIFICANCE

Biological membranes play very important role in the structural arrangement of the cell and in the functioning of the cell and intracellular organelles. The inadequate activation of lipid peroxidation leads to the destruction of membranes and disturbances of cell metabolic processes. The increased rate of free radical lipid peroxidation is one of the key factors of the pathogenesis of atherosclerosis, rheumatoid arthritis, radiolesion, aging etc. Many pathological states are accompanied by either decrease in antioxidant enzymes activity or deficiency of antioxidant vitamins (especially E and C).

Work 1. Quantitative determination of malonic dialdehyde in the liver tissue.

The EQUIPMENT: a centrifuge, a thermostat for 38ºC, a boiling water bath, a FEC.

The PRINCIPLE of a METHOD.

The method is based on the determination of malonic dialdehyde (MDA) as a parameter characterizing peroxidation of lipids under the incubation of tissue homogenate in the presence of oxygen. In the presence of pro-oxidants MDA is determined by the specific colour reaction with 2-thiobarbituric acid (TBA) in an acidic medium.

The COURSE of WORK

Prepare a mixture of the reagents in three centrifuge test tubes according to the table:

REAGENTS	**the 1-st test tube (experimen-tal)**	**the 2-nd test tube (control)**	**the 3-d test tube (experimental with the addition of prooxidants)**
Tris-buffer 0.15 M	3.4 ml	3.4 ml	1.4 ml
Iron sulfate ($FeSO_4$)	-	-	1.0 ml
Ascorbic acid	-	-	1.0 ml
Trichloracetic acid (TCA) with etylenediaminetetraacetic acid (EDTA)	-	1.0 ml	-
Liver homogenate	1.0 ml	1.0 ml	1.0 ml

Put all the test tubes into a thermostat at 37ºC for 15 minutes. After the incubation stop the reaction in the 1-st and 3-d test tubes by the addition of 1 ml of TCA with EDTA. Then centrifuge all the test tubes for10 minutes at 3000 rev/min.
After the centrifugation transfer all volume of the supernatant liquid into a clean glass test tube. Next measure out 4 ml of the supernatant liquid in other clean test tubes, add 2 ml of the fresh-prepared solution of TBA, and place the test tubes in the boiling water bath for 15 minutes. After that cool the test tubes by cold water and then determine the values of optical density with a FEC at 540 nm (a green light filter). The thickness of a cuvette is 10 mm. The colorimetry has to be carried out against distilled water.
The LPO activity is expressed by the quantity of micromoles of MDA, formed for a period of the incubation per 1 ml of homogenate. Use the formula for the calculation:

$$X = (D_{exp} - D_{cont})/\alpha ,$$

Where:
X - the quantity of micromoles MDA formed for a period of the incubation per 1 ml of homogenate,
$\mathbf{D_{exp}}$ and $\mathbf{D_{cont}}$ - the values of optical density of "experimental" and "control" test tubes respectively.
α- the molar coefficient of extinction for malonic dialdehyde in the TBA reaction. It is equal to 0.156.
Calculate the LPO activity for the 1-st and 3-d test tubes and make a conclusion about the influence of ions Fe^{2+} and ascorbate on LPO.
RESULTS:

CONCLUSIONS:

Work 2. Quantitative determination of catalase activity in blood.
The EQUIPMENT: burettes for titration.

The PRINCIPLE of a METHOD.

Catalase is one of the most active enzymes of the body. This enzyme is a typical hemoprotein accelerating the decomposition of hydrogen peroxide:

$$H_2O_2 \rightarrow O_2 + H_2O$$

The amount of hydrogen peroxide which has been broken down by the enzyme for certain interval of time is a basis of the quantitative determination of the catalase activity in blood. The amount of broken down hydrogen peroxide is determined as the difference between the amount of potassium permanganate spent for the titration of hydrogen peroxide before and after the incubation.

The action of catalase in an acidic medium is stopped because the pH-optimum for this enzyme is 7.4. As in the control sample sulfuric acid is added before the addition of hydrogen peroxide, all amount of hydrogen peroxide in the control glass remains unsplitted.

Catalase activity is expressed with the *"catalase number"*.

"Catalase number" **is the quantity of milligrams of hydrogen peroxide, which is splitted under the catalytic action of 1 microliter of blood.**

The COURSE of WORK.

Take two glasses and fill them in according to the table:

Reagents	Glass № 1 (experimental)	Glass № 2 (control)
Blood	1 ml	1 ml
Distilled water	7 ml	7 ml
Hydrogen peroxide 1%	2 ml	-
Sulfuric acid (H_2SO_4) 10%	-	5 ml
Both glasses stay for 30 minutes at the room temperature. Shake them from time to time **After 30 minutes add into the glasses:**		
Hydrogen peroxide	-	2 ml
Sulfuric acid (H_2SO_4) 10 %	5 ml	-

Then titrate the contents of each glass with the solution of potassium permanganate until the appearance of pink colour which has not to disappear within 30 sec.

Next calculate the catalase number (CN) by the formula:

$$\mathbf{CN = (A - B) \cdot 1.7} \quad ,$$

Where:

A - Quantity of milliliters of 0.1 N $KMnO_4$ used for the titration of a control sample.

B - Quantity of milliliters of 0.1 N $KMnO_4$ used for the titration of an experimental sample.

1.7 - is a factor which shows how many mg of H_2O_2 is contained in 1ml of 0.1N solution H_2O_2.

The normal values of CN are changed from 10 to 15 units for adults and 7,5 - 9,9 units for children.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Structural organization of the biological membrane. The liquido-mosaic crystalline theory of membrane structure.
2. The role of lipids in the membrane organization.
3. The common characteristic of the membrane proteins.
4. The biological functions of membranes.
5. Different kinds of transport through the membrane.
6. Membrane metabolism. LPO. Physiological and pathological role of LPO.
7. Regulation of LPO. Antioxidants and prooxidants.
8. Interaction of carbohydrate and lipid metabolism.

LESSON 21
DIGESTION of PROTEINS.

BIOMEDICAL SIGNIFICANCE

Many gut diseases are accompanied by disorders of hydrochloric acid and pepsinogen secretion in the stomach. In these pathological states the acidity of the gastric juice may be increased, decreased or equal to zero. The absence of hydrochloric acid and pepsin in the gastric juice (achylia) is frequently observed in severe atrophic gastritis and malignant tumors of the stomach. The decreased acidity of the gastric juice (hypochlorhydria) occurs in hypoacidic gastritis and stomach cancer. Increased acidity of the juice (hyperchlorhydria) is characteristic of hyperacidic gastritis and stomach or duodenum ulcer. Achylia is very often complicated by the development of pernicious anemia because of the absence of the Castle's intrinsic factor (gastromucoprotein) needed for the absorption of vitamin B_{12}.

Newborn infants have the low activity of proteolytic enzymes and high permeability of small intestine mucosa, so some native dietary proteins may be absorbed without hydrolysis; that is one of the reasons of dietary allergy.

Work 1. Determination of free, bound, total hydrochloric acid, and total acidity of the gastric juice separately and in a common sample.

The EQUIPMENT: burettes for titration.

The PRINCIPLE of a METHOD.

For the determination of the parameters of the gastric juice acidity the method of titration by 0.1 N NaOH solution in the presence of the specific indicators (phenolphthalein and *p*-dimethylaminoazobenzene) is used. Acidity of the gastric juice is estimated with "*units of acidity*". **One *unit of acidity* is the quantity of milliliters of 0.1 N NAOH solution used for neutralization of 100 ml of the gastric juice.**

1. Quantitative determination of total acidity of the gastric juice

The COURSE of WORK

Wash up carefully a flask or glass with water, then with hydrochloric acid and finally with water again in order to remove the trace of sodium ions adsorbed on the glass wall.
Measure out 10 ml of the gastric juice in a vessel for titration, add 1-2 drops of 0.5% phenolphthalein (the zone of its colour transition lies within the limits of pH = 8.2-10.0).
Then titrate it by 0,1 N NaOH until the appearance of the stable light-pink colour.
Before the titration put the flask or glass on a sheet of white paper.
RESULTS:

2. Quantitative determination of a free hydrochloric acid

COURSE of WORK

Measure out 10 ml of the gastric juice in a clean flask or a glass, add 1-2 drops of *p-dimethylaminoazobenzene* (the zone of its colour transition lies within the limits of pH = 2.9-4.2).
Titrate it by 0.1N NAOH solution until the appearance of the orange colour. A free hydrochloric acid is fully neutralized at pH = 3.0.
RESULTS:

3. Quantitative determination of free, bound, total hydrochloric acid, and total acidity of the gastric juice in a common sample.

The COURSE of WORK

Determination of the listed kinds of acidity of the gastric juice in a common sample is carried out by a titration with 0.1N NaOH in the presence of the both indicators - dimethylaminoazobenzene and phenolphthalein.

Measure out 10 ml of the gastric juice into a clean flask or a glass, add 1-2 drops of dimethylaminoazobenzene and 2 drops of phenolphthalein.

Titrate the contents of the flask by 0,1N NaOH solution until the appearance of the orange colour.
Fix the volume of NaOH used for the titration (the first level).

Then continue the titration until the appearance of the lemon-yellow colour. Fix once more the volume of NaOH used for the titration(the second level).

Finally continue the titration until the appearance of the stable pink colour. Fix again the volume NaOH used for the titration (third level).

The first level is used for a calculation of free hydrochloric acid.

The average value between the second and the third levels is used for the calculation of the total amount (free and bound) hydrochloric acid.

The third level is used for the calculation of total acidity.

The example:

the first level (orange colour) - 3.2 ml,
the second level (lemon-yellow colour)-4.5 ml,
the third level (pink colour) - 5.5 ml.

Calculation:

1.Free hydrochloric acid.

The value of the first level is multiplied by 10*:*

3.2. 10 = 32 units.

2.*Total hydrochloric acid*

The average value between the second and third levels

(4.5 + 5.5)/ 2 = 5

is multiplied by 10:

5.0. 10 = 50 units.

3. *Bound hydrochloric acid* is calculated as the difference between the values of total and free hydrochloric acid:

50 -32 = 18 units.

4. *Total acidity.* The value of the third level is multiplied by 10*:*

5.5. 10 = 55 units.

RESULTS: Write the results of titrations in the table (see below)

Quantity of ml of 0.1 N NaOH used for the titration	**Gastric juice with normal acidity**	**The task №.... (gastric juice with unknown acidity)**
The first level		
The second level		
The third level		
R E S U L T S of the C A L C U L A T I O N		
Free HCL		
Total HCL		
Bound HCL		
Total acidity		

CONCLUSIONS:

Work 2. Detection of indican in urine.

The PRINCIPLE of a METHOD.

The method is based on the oxidation of indoxyl to dark blue indigo with hydrochloric acid in the presence of Fe^{3+} ions .

The COURSE of WORK.

Take 1 ml of urine in a test tube, add the equal volume of hydrochloric acid, mix carefully. Then add 1-2 drops of iron chloride and shake up for 1-2 minutes. Iron chloride oxidizes indoxyl to dark blue indigo.
RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Nitrogen balance of the body. Biological significance of proteins.
2. Common character of digestive enzymes. The peculiarities of their action and activation.
3. Digestion of proteins in the stomach. Biological functions of hydrochloric acid of the gastric juice.
4. Digestion of proteins in the intestine as a result of the action of the pancreatic and intestine enzymes.
5 Absorption of amino acids in the intestine.
6. Rotting of proteins in the intestine. Detoxication of the products of protein rotting in the liver.
7. Decomposition of nucleoproteins in the intestine. Enzymes taking part in this process.

The examples of the control task:

1. All following statements are applied to the digestive enzyme α-amylase EXCEPT:

a) glycogen and hydrated starch are normal substrates
b) the form of α-amylase in the human pancreas is the most important isoenzyme
c) it catalyses the hydrolysis of α-1,4-glycosidic linkages except those of glucose units that serve as branch points
d) glucose is the major product of α-amylase action on starch

2. Pancreatic juice contains the following proteolytic enzyme EXCEPT:
a) trypsin
b) chymotrypsin
c) pepsin
d) elastase

3. An emulsification of dietary lipids is carried out with the participation of
a) bile acid salts and phosphatidylcholine
b) bile acid salts and hydrochloric acid
c) phosphatidylcholine and hydrochloric acid
d) pancreatic lipase

4. The major products of the dietary proteins hydrolysis by pepsin are
a) large peptides

b) amino acids
c) olygopeptides
d) dipeptides

5. What is the mechanism of the glucose absorption in the small intestine?
a) Passive diffusion with a concentration gradient
b) Active , carrier-mediated transport (Na^+ cotransport)
c) Endocytosis with the participation of an enterocyte membrane
d) Facilitated diffusion with the participation of bile acids

6. All following statements regarding the digestion of protein are correct EXCEPT:
a) pepsinogen is activated by autoactivation (pH=2.0) or autocatalysis
b) the major products of peptic hydrolysis are large peptides and some free amino acids
c) trypsin and chymotrypsin are secreted by the pancreas as inactive zymogens
d) most of digested proteins are absorbed in the intestine in the form of polypeptides

7. Pancreatic lipase cleaves triacylglycerols into
a) glycerol and three fatty acid molecules
b) 1,2-diacylglycerol and a fatty acid molecule
c) 2-monoacylglycerol and two fatty acid molecules
d) glyceraldehyde and three fatty acid molecules

8. The activation of pepsinogen in the gastric juice is carried out by
a) partial hydrolysis
b) phosphorylation
c) attaching of a Fe^{2+} ion
d) none above

9. Cholesterol esterase catalyses the reaction of the
a) cholesterol esters hydrolysis
b) formation of an ester bond with the 3-OH group of the cholesterol molecule
c) hydrolysis of cholesterol derivatives (vitamin D, bile acids)
d) non above

10. Which of the following proteolytic enzymes is contained in the gastric juice?
a) trypsin
b) chymotrypsin
c) pepsin
d) elastase

11. A convalescent patient is in the positive nitrogen balance if
a) the amount of nitrogen ingested equals the amount of nitrogen excreted in the urine, feces and sweat
b) the amount of nitrogen excreted in the urine, feces, and sweat is less than the nitrogen ingested
c) the amount of nitrogen excreted in urine, feces, and sweat is greater than the amount of nitrogen ingested
d) the patient is on a protein-free diet

12. Phospholipase A_2 hydrolyzes dietary phospholipids according to the equation:

a) Phospholipid+ $H_2O \rightarrow$phosphatidate + a nitrogen base

b) Phospholipid + $H_2O \rightarrow$1,2-diacylglycerol + a phosphorylated nitrogen base

c) Phospholipid + $H_2O \rightarrow$lysophospholipid + a fatty acid

d) None above

13. All digestive enzymes belong to

a) oxidoreductases

b) hydrolases

c) lyases

d) transferases

14. The absorption of end products of the lipid digestion (free cholesterol, fatty acids, and 2-monoacylglycerols) is carried out by

a) the passive diffusion

b) the active transport

c) the transport through the membrane canals

d) the cotransport with Na^+ ions

15. Choose the combination of essential amino acids.

a) Thyrosine, serine, valine

b) Lysine, glycine, thryptophan

c) Phenylalanine, histidine, isoleucine

d) Phenylalanine, histidine, cysteine

16. Which of the following constitute essential amino acids; that is, which must be ingested on a daily basis by humans?

a) leucine, phenylalanine

b) cysteine, serine

c) glycine, arginine

d) valine, alanine

17. What enzymes of the digestive tract are disaccharidases?

a) Sucrase, amylase, maltase

b) Sucrase, lactase, maltase

c) Sucrase, dipeptidase, maltase

d) Lactase, maltase, amylase

18. What enzymes catalyses the reaction of the RNA and DNA digestion in the small intestine?

a) Polypeptidases, nucleotidases

b) Endopeptidases, nucleosidases (nucleoside phosphorylases)

c) Exopeptidases, nucleotidases

d) Polynucleotidases, nucleosidases (nucleoside phosphorylases)

19. What is the mechanism of trypsinogen activation?

a) Trypsinogen is converted to active trypsin by enterokinase of the intesine at pH 5.2 - 6.0 and autocatalytic at pH 7.9

b) Trypsinogen is converted to active trypsin by pepsin at pH 5.2 - 6.0 and autocatalytic at pH 7.9

c) Trypsinogen is converted to active trypsin by hydrochloric acid at pH 3.0 - 5.0 and autocatalytic at pH 7.9

d) Trypsinogen is converted to active trypsin by autocatalytic mechanism at pH 7.9

20. Call the products of the lipid digestion by gastric lipase.

a) Glycerol, fatty acids
b) 2-monoacylglycerols, fatty acids
c) 1,2-diacylglycerols, fatty acids
d) 1,3-diacylglycerols, fatty acids

21. Which of the following monosaccharides are the major end products of the digestion of carbohydrates?

a) Glucose, fructose, xylose
b) Glucose, fructose, galactose
c) Galactose, glucose, ribose
d) Deoxyribose, glucose, ribulose

22. Call the products of the lipid digestion by pancreatic lipase.

a) 1-monoacylglycerols, fatty acids
b) 2-monoacylglycerols, fatty acids, glycerol
c) 1,2-diacylglycerols, fatty acids
d) 1,3-diacylglycerols, fatty acids

23. Carboxypeptidase catalyses the reaction of

a) protein hydrolysis at the free carboxyl end of a chain
b) polynucleotide hydrolysis at the free 3-OH end of a chain
c) polypeptide hydrolysis at the free amino end of a chain
d) polypeptide hydrolysis at the free carboxyl end of a chain

LESSON 22
METABOLISM of AMINO ACIDS.

BIOMEDICAL SIGNIFICANCE

Disorders of amino acid metabolism are divided into:

1. Inherited syndromes (enzymopathies) of amino acid metabolism. The absence or deficiency of the enzymes result in the abnormal accumulation of the intermediates of amino acid metabolism. This accumulation induces the affections of the central nervous system and the development of various toxic syndromes. The typical enzymopathies of amino acid metabolism are phenylketonuria, alkaptonuria, albinism, tyrosinosis, Hartnup's disease etc.
2. The disturbances of the synthesis and inactivation of biologically active amines are the following:

-the disturbance of the synthesis of acetylcholine in the synapses induces myasthenia (muscle weakness),

- the disturbance of the synthesis of dopamine in Parkinson's disease,

-hypersecretion of dopamine in temporal lobe of the brain is characteristic for schizophrenia.

3. The syndromes accompanied by increase in aminotransferase activity in blood. E.g. in acute and chronic liver diseases the significant increase of alanine aminotransferase activity in blood is observed. So this enzyme is tissue specific for the liver. On the other hand, aspartate aminotransferase is a tissue specific enzyme for the heart. Its activity increases in myocardium infarction.

Work 1. Determination of alanine aminotransferase activity in serum and tissues homogenates.

The EQUIPMENT: a FEC, a thermostat.

The PRINCIPLE of a METHOD

The process of transamination can be observed as an example of a reversible transfer of an amino group between alanine and 2 -oxoglutarate. The reaction is catalyzed by alanine aminotransferase. A product of the reaction is pyruvate. The reaction is stopped with an addition of 2.4 dinitrophenylhydrazine (2,4 DNPH). Pyruvate hydrazone is accumulated, which forms the specific colour product with 2,4 DNPH in an alkaline medium. The intensity of its colour is changed from yellow to brown, and it is proportional to the amount of pyruvic acid formed during the incubation.

The COURSE of WORK

1. **An experimental sample.**

Measure out 1 ml of a mixture of substrates (alanine + 2 oxoglutarate) in a test tube containing 0.2 ml of a tissue homogenate or serum and place the test tube into a thermostat at 37ºC for 30 minutes.

After the incubation add 1 ml of DNPH to the test tube and keep it at the room temperature for 20 minutes.

2. **A control sample.**

Measure out 1 ml of DNPH in a test tube containing 0.2 ml of a tissue homogenate or serum and 1 ml of a mixture of substrates (alanine + 2-oxoglutarate). Keep the test tube at the room temperature for 20 minutes.

3. Determine the concentration of pyruvate in the experimental and control test tubes. For it add 10 ml of 0.4 N NaOH into the control and experimental test tubes. In 5 minutes measure the optical density of the contents of the both test tubes with a FEC against water. Use a green light-filter (540nm).

RESULTS:

First of all calculate the difference between the values of the optical density of the experimental (D_{ex}) and control (D_C) samples:

$$\mathbf{D = D_{ex} - (D_C)}$$

Then find the value of the pyruvate concentration with a calibrating graph.

Finally calculate the activity of alanine aminotransferase (AlAT) in blood or tissues.

<u>The AlAT activity in serum is calculated according to the formula:</u>

A = [Pyr]/0.2·88 ,

Where:
A - the activity of AlAT in micromoles / ml for 30 minutes of incubation,
[Pyr] - the pyruvate concentration in the sample,
0.2 - the volume of serum (ml),
88 - the factor of recalculation for the translation into the System SI.

The AlAT activity in tissues is calculated according to the formula:

$$\mathbf{A = [Pyr]\cdot DH/0.2\cdot 88}\ ,$$

where:
DH - the value of the tissue homogenate dilution.

CONCLUSIONS:

Work 2. Detection of phenylpyruvate in urine (The Feling test).

The PRINCIPLE of a METHOD.

Phenylpyruvic acid forms a blue-green colour complex compound with iron (Fe^{3+})ions.

The COURSE of WORK.

Add 4-5 drops of 10 % iron chloride to 2 ml of urine. Blue-green colour will appear if the urine contains phenylpyruvate.
RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Transamination of amino acids: donors and acceptors of amino-group, the characteristics of the enzyme and the biological importance of the process. Clinico-diagnostic significance of the determination of alanine- and aspartate aminotransferase activity in serum.
2. Oxidative deamination of amino acids, the characteristics of oxidases of amino acids. Biological significance of the process.
3. Transdeamination (the inderect transamination), the stages of the process, the characteristics of enzymes, the biological significance.
4. Reductive amination of oxoglutarate, characteristic of glutamate dehydrogenase.

5. Decarboxylation of amino acids and the formation of the biologically active amines: histamine, serotonin, GABA. Inactivation of the amines, the role of MAO and DAO. Functions of biologically active amines.
6. Phenylalanine and tyrosine metabolism:
a) the synthesis of epinephrine and norepinephrine,
b) the transformation of phenylalanine and tyrosine into fumarate and acetoacetate,
c) the synthesis of melanine,
d) the formation of thyroid hormones.
Enzymopathy of phenylalanine and tyrosine metabolism.
7. The peculiarities of sulfur-containing amino acid metabolism and metabolism of branched chain amino acids.

LESSON 23
END PRODUCTS of PROTEIN METABOLISM.

BIOMEDICAL SIGNIFICANCE
Ammonia formed in protein decomposition is quite toxic for the human tissues, especially for CNS. The most frequent reason of ***hyperammoniemia*** is the disturbance of functioning of the ornithine cycle enzymes. The correct diagnosis of the hyperammoniemia type is established with the ornithine cycle metabolites in blood and in urine. The phenomenon of appearance of creatine in urine – ***creatinuria*** – may be found in adult men and it is due to the disturbance of the creatine conversion into creatinine. This phenomenon appears in skeletal muscle diseases such as myopathies, progressive muscle dystrophy, muscle convulsions etc.

Creatinine – is the end product of creatine decomposition. The daily urine excretion of creatinine is very individual and constant for each person. It depends on the individual muscle mass. Creatinine can not be reabsorbed from the primary urine in the kidney, so the determination of the amount of excreted creatinine is used for estimation of renal filtration capacity.

The determination of residual nitrogen and its fractions, especially urea, is quite clinically significant. A number of pathological states are accompanied by the increase in residual nitrogen level in blood. This phenomenon is termed ***azotemia.*** It is divided into pre-renal, renal and post-renal. The renal and post-renal azotemia are developed as a result of insufficient renal excretion of nitrogen-containing substances although their intake into blood may be absolutely normal. Pre-renal azotemia is due to the excessive intake of the nitrogen-containing substances into blood because of enhanced tissue proteolysis occurring in severe inflammatory processes, wounds, burns, and starvation.

Work 1. The influence of hemodialysis on the content of residual nitrogen in blood.
The EQUIPMENT: a FEC

The PRINCIPLE of a METHOD.

Hemodialysis is the diffusive cleaning of blood (removal of low-molecular weight nitrogen-containing substances such as urea, uric acid, creatinine, ammonia salts etc from blood) with the
semi-permeable membranes. The efficiency of hemodialysis is estimated according to the content of residual nitrogen in blood before hemodialysis, during hemodialysis, and after its termination.

Residual nitrogen is the nitrogen of all unprotein substances of blood which remains after a precipitation of proteins. For determination of residual nitrogen of blood it is necessary:

1. to precipitate blood proteins with trichloracetic acid and to filter them.
2. to mineralize the received filtrate (i.e. to burn down the organic substances with concentrated sulfuric acid and a catalyst). Thus all nitrogen-containing organic substances are transformed to the single universal product - ammonia sulfate.
3. to determine the ammonia sulfate content by a photoelectrocolorimetric method. The interaction of ammonia sulfate with the Nessler reagent, which contains the mercury salt, leads to the appearance of the yellow-orange product. The intensity of its colour is directly proportional to the concentration of residual nitrogen in the sample of blood.

The COURSE of WORK.

Add distilled water to 0.8 ml of a mineralizate of an unprotein filtrate of blood up to approximately a half of volume of a 10 ml measuring test tube.

Pour 0.4 ml of the Nessler reagent into the test tube, mix carefully, and add distilled water up to the label of total volume 10 ml. Mix it carefully again.

In 10 minutes measure the optical density of the coloured solution with a FEC against water. Use a dark-blue light-filter (440nm) and 10 mm cuvettes.

Use a calibrating graph for the calculation of the content of residual nitrogen in blood.

Multiply the result received with the calibrating graph by 10, because the mineralizate was obtained as a result of burning of 3 ml of an unprotein filtrate that corresponds to 0.1 ml of blood.

The content of residual nitrogen in blood is expressed in mM/L.

RESULTS: write down in the table:

CONTENT OF RESIDUAL NITROGEN (mM/L)					
Before hemodia-lysis	**In process of hemodialysis**			**After hemodialysis**	
	in an hour	**in 3 hours**	**in 5 hours**	**in a day**	**in 5 days**

CONCLUSIONS

Work 2.. Determination of creatinine in urine.
The EQUIPMENT: a FEC

The PRINCIPLE of a METHOD.

Creatinine forms a dark yellow or light brown product as a result of its reaction with picric acid in an alkaline medium. The optical density of the received product depends on the urine creatinine concentration.

The COURSE of WORK.

Prepare two samples of the reaction medium:

a) the experimental sample

Measure out 0.5 ml of urine into a measuring flask with total volume of 25 ml. Then add 0.25 ml of 10% sodium hydroxide, next add 0.5 ml of picric acid. Finally fill the flask with distilled water up to the label 25 ml.

b) the control sample

Measure out 0.5 ml of distilled water (instead of urine) into a measuring flask with total volume 25 ml. The further details of the preparations are the same as in the experimental sample (see above).

The content of the experimental flask has to be colorimetried with blue light filter (400 nm) against the content of the control flask.

CALCULATION: The amount of creatinine is determined with a calibrating graph. Plot the determined value of optical density on the calibrating graph and find the creatinine concentration in the urine sample. Then solve the proportion:

X=[creatinine] · **(**daily diuresis)/0.5

Where: **X** - the amount of creatinine excreted per day,

daily diuresis - the volume of daily urine,

0.5 - the volume of the urine sample.

The normal range of creatinine excretion with urine is 1.0 -2.0 gr/day.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS**:**

1. The main pathways of the ammonia formation.
2. The main pathways of the temporary detoxication of ammonia.
3. The final detoxication of ammonia. The ornithine cycle. The sources of nitrogen in the urea . The daily excretion of urea. Enzymopathies of the ornithine cycle.

4. Residual nitrogen of blood. The clinico-diagnostic significance of its detection.
5. Synthesis of creatine and creatine phosphate. Creatinine as the end product of nitrogen metabolism. The medical significance of cratine and creatinine detection in blood and urine.
6. Formation and metabolism of nitrogen oxide (NO). Its physiological and pathological role.

LESSON 24
METABOLISM of NUCLEOTIDES.

BIOMEDICAL SIGNIFICANCE

The disturbances of purine metabolism appear to be ***hyperuricemia***, that may be due to either a metabolic (primary) reason or the other pathological states (secondary reasons). The metabolic hyperuricemia may be induced by either hyperproduction of uric acid or a decrease in its excretion. The hyperproduction of uric acid is usually a consequence of enzyme defects connected with the nucleotide synthesis, accelerated erythrocyte hemolysis, sickle cell anemia etc. The secondary hyperuricemia is due to some pathological states of blood, kidney, diabetes insipidus, lead intoxication, overdoses of diuretic drugs etc. Hyperuricemia is the main reason of ***podagra (gout)***. Allopurinol is a structural analogue of hypoxanthine, which is applied for treatment of podagra because it can inhibit xanthine oxidase reaction by competitive mechanism and reduce the accumulation of uric acid in blood and tissues.

Depositing uric acid salts in the urine excretory tract leads to the development of ***urolithiasis*** and to kidney insufficiency in severe cases of this pathological state.

Work 1. Quantitative determination of uric acid in urine.

The EQUIPMENT: burettes for a titration.

The PRINCIPLE of a METHOD.

The quantitative determination of uric acid is based on its ability to reduce tungsten-phosphate reagent to the product which has dark blue colour. The amount of the product is determined by a titration with potassium ferricyanide: 1 ml of potassium ferricyanide corresponds to 0.66 mg uric acid.

The COURSE of WORK.

1. Measure out 5 ml of urine into a flask, add 2 ml of tungsten-phosphate reagent, then 10 ml of 20 % sodium carbonate, and carefully mix the contents of the flask which has dark blue colour.
2. Titrate carefully the sample by potassium ferricyanide solution (by drops) with a burette until the appearance of light yellow colour.

Make the calculation by the formula:

$$x = 0.66 \cdot A \cdot B / 5,$$

Where:

x - the daily excretion of uric acid (mg),

A - the quantity of potassium ferricyanide solution used for titration (ml),

B - the quantity of urine excreted for day (ml),
5 - the quantity of titrated urine (ml),
0.66 - the quantity of uric acid (mg), that corresponds to 1 ml of potassium ferricyanide.
RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Hydrolisis of nucleoproteins in the intestine and tissues.
2. The purine and pyrimidine nucleotides biosynthesis: the origin of C- and N-atoms of a purine and pyrimidine rings and the role of 5'-phosphoribosyl-1-pyrophosphate (PRPP). Regulation of nucleotide synthesis.
3. Biosynthesis of deoxyribonucleotides.
4. Catabolism of purine nucleotides. Physico-chemical properties of uric acid.
5. Hyperuricemia and podagra (gout).
6. Catabolism of pyrimidine nucleotides.

LESSON 25
NUCLEIC ACIDS. SYNTHESIS of PROTEINS.

BIOMEDICAL SIGNIFICANCE

Inherited alterations of the primary structure of DNA result in either stopping of the protein synthesis encoded by the affected gene or the synthesis of "incorrect" proteins. Gene mutations may be either useful or harmful. The useful mutations provide more successful adaptation of the organism to the alterating conditions of the environment in the evolution process. Harmful mutations lead to the development of molecular pathology or death of a cell or even the organism on the whole. E.g. sickle cell anemia (sicklemia), hemophylia, enzymopathies of metabolism of amino acid, carbohydrate, and lipid belong to typical inherited diseases. About 90% of human malignant tumors are induced by harmful chemical substances or physical mutagen agents. Many antibiotics are used as the specific inhibitors of the processes of DNA replication, transcription or translation of genetic information in prokaryotic cells (bacteria, Protista) for treatment of infectious diseases. In malignantly transformed eukaryotic cells antibiotics are used for chemotherapy of cancer.

Work 1. Quantitative determination of DNA in various tissues.
The EQUIPMENT: a FEC.

The PRINCIPLE of a METHOD.

The method is based on the determination of the amount of phosphorus which is incorporated in the structure of DNA. Free inorganic phosphate is released from DNA after its mineralization. DNA is previously extracted from 10 mg of certain tissue (the spleen, liver, pancreas, skeletal muscle) and then is subjected to mineralization.

The inorganic phosphorus of the mineralizate is determined by a colorimetric method. The colour reaction with ammonium molybdate in the presence of any reducing agents is used in this method. The product of the reaction has dark blue colour. The intensity of the colour is directly proportional to the amount of phosphorus in a test.

The COURSE of WORK.

1. Add the reagents for the colorimetric determination of inorganic phosphate (0.5 ml of 2.5% molybdenic acid and 0.5 ml of 10% ascorbic acid) to the whole volume of the ready mineralizate from the spleen, liver, pancreas or muscle.
2. In 10 minutes measure the optical density of the coloured solution with a FEC (the thickness of cuvettes is 5 mm) with the red light-filter (670 nm).
3. Calculate the amount of phosphorus with a calibrating graph built with the data of determination of optical density of the standard solutions of chemically pure KH_2PO_4. The value of the result, found with the calibrating graph, multiply by 10 because DNA concentration is expressed as mg of phosphorus per 100 mg of the tissue.

RESULTS:

CONCLUSIONS:

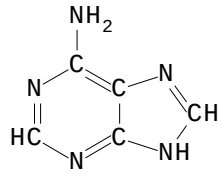
BASIC QUESTIONS:

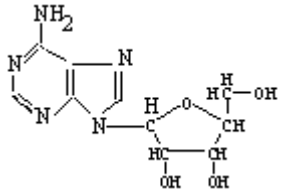
1. Nucleic acids. The definition of the term. The chemical composition of nucleic acids, differences between DNA and RNA.
2. The primary and secondary structure of DNA. Nucleotides as monomers of a polynucleotide chain. The double helix. Bonds consolidating the double helix structure of DNA. Complementarity of the nitrogen bases.
3. The structural organization of DNA in chromosomes. The chemical composition of chromatin. Proteins of chromatin. Histones, their classification, common physico-chemical properties (charge, ability to form oligomers). Nucleosomes as the kind of DNA packing in chromosomes.
4. The primary and spatial structure of RNA. Functional classification of RNA and the distribution of different kinds of RNA in the cell compartments.
5. Replication of DNA in eukaryotes. Formation of the replication fork. Peculiarities of the leading and lagging strand replication. Okazaki fragments. Replication enzymes: DNA polymerase, DNA ligase.

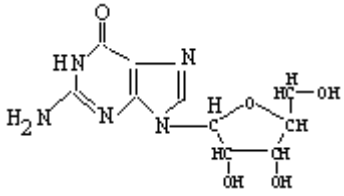
6. Eukaryotic transcription of DNA: RNA synthesis. Stages of transcription. RNA polymerase.
7. Posttranscriptional processing of nuclear RNA precursors: the cap structure, polyA tail, removal of introns and join of exons.
8. Translation of mRNA: protein synthesis. Genetic code and its properties. Stages of translation.
9. The recognition of amino acids: formation of aminoacyl-tRNAs.
10. The eukaryotic ribosomal cycle. Stages of the ribosomal cycle: initiation, elongation, termination. GTP requirement of the ribosomal cycle.
11. Posttranslational processing of proteins: partial proteolysis, chemical modification
12. Antibiotics as inhibitors of protein synthesis.
13. Regulation of protein synthesis in eukaryotes and prokaryotes. Induction and repression of genes expression.

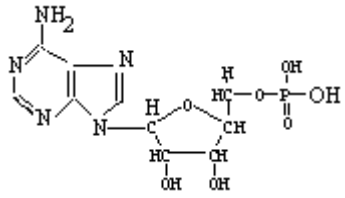
The examples of the control task:

1. Which of the following substances is adenosine?

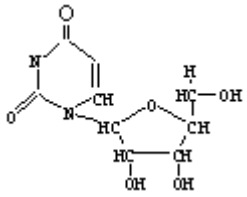
a) 

b) 

c) 

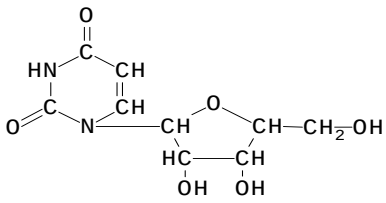
d) 

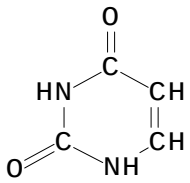
2. This compound is called:

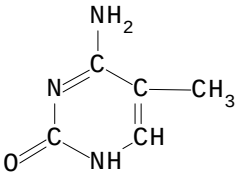


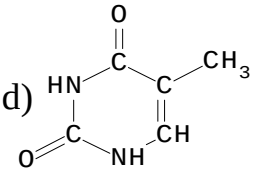
a) uridine
b) cytidine
c) thymidine
d) dioxycitidine

3. According to the Chargaff rules in DNA molecule the amount of adenine is equal to the amount of the following nitrogenous base (choose the suitable formula):

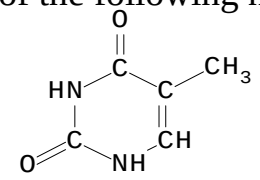
a) 

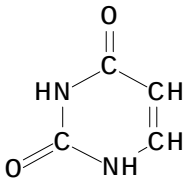
b) 

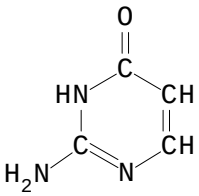
c) 

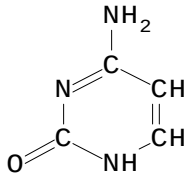
d) 

4. The synthesis of mRNA occurs during the stage of :
   a) translation
   b) replication
   c) transcription
   d) recognition
5. The genetic code refers to the:
   a) number of chromosomes in the cells of a species
   b) nucleotide sequences that correspond to common amino acids
   c) ratios of mendelian inheritance
   d) hierarchy of DNA, RNA and protein
6. Which of the following nitrogenous bases is contained in RNA but not in DNA?

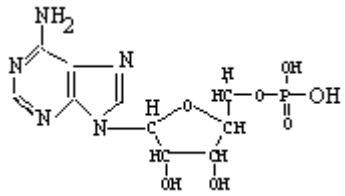
a) 

b) 

c) 

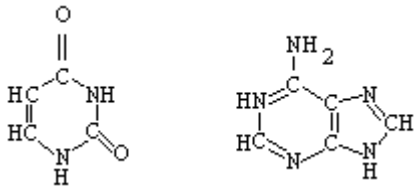
d) 

7. This nucleotide is called:

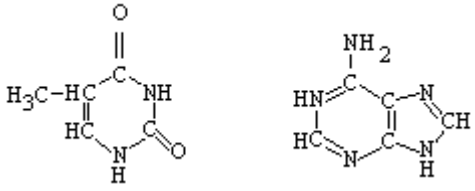


a) guanosine
b) adenosine
c) guanosine monophosphate
d) adenosine monophosphate

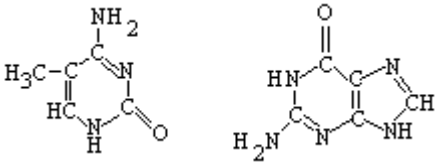
8. In RNA hydrogen bonds are arisen between (choose the duplex formula):



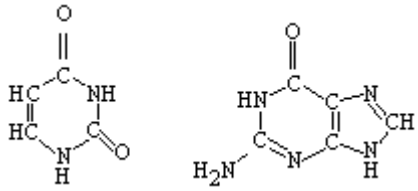
a)



b)



c)



d)

9. What is the name of the enzyme that catalyses the amino acid activation under the protein synthesis?
a) Aminoacyl-tRNA-synthetase
b) Aminoacyl-tRNA-transferase
c) Aminoacyl-mRNA-synthetase
d) Aminoacyl-mRNA-transferase

10. Which of the following statements about DNA polymerase is correct?
a) It joins together Okasaki fragments to complete the strand of DNA
b) It functions as a DNA repair enzyme but is not involved in the DNA replication processc) Its presence is not necessary for normal replication
c) It requires a template and a primer to polymerize deoxyribonucleoside triphosphates

11. What kind of nucleic acids contains an anticodone in its structure?
a) tRNA
b) mRNA
c) rRNA
d) DNA

12. What DNA duplex is supported by 3 hydrogenous bonds?
a) A-T c) A-U
b) G-C d) G-T

13. DNA contains sequences of bases known as promoters which:

a) are recognized by DNA polymerase

b) occur in the chain just before the message sequence that defines the messenger RNA

c) are single stranded regions of duplex DNA

d) are transcribed into RNA sequences

14. Choose the right definition of the term "codon":

a) A DNA fragment that codes for a specific polypeptide chain

b) A sequence of three adjacent nucleotides in a m-RNA that codes for a specific amino acid

c) A nucleotide triplet of tRNA that serves for the binding with mRNA in A-site of a ribosome

d) A nucleotide sequence of mRNA that codes for a specific polypeptide chain

15. Which of the following conditions are necessary and sufficient for DNA replication?

a) The presence of RNA polymerase and ribonucleoside triphosphates

b) The presence of histons and high energy phosphates

c) The presence of a DNA-template, DNA polymerase and deoxyribonucleoside triphosphates

d) The presence of a DNA-template, RNA polymerase and ribonucleoside triphosphates

16. What compartment of a cell does the amino acid activation and recognition take place in?

a) In mitochondria

b) In nuclei

c) In ribosomes

d) In cytosol

17. Translation of a synthetic polyribonucleotide containing the repeating sequence CA A in a cell-free protein synthesizing system produced three homopolypeptides: polyglutamine, polyasparagine, and polythreonine. If the codons for glutamate and asparagine are CAA and AAC, respectively, which of the following triplets is a codon for threonine?

a) ACA

b) AAC

c) CAA

d) CCA

18. Post-transcriptional processing includes the following events:

a) the formation of the "cap" and polyA-"tail", splicing, and modification of nitrogenous bases

b) partial proteolysis, chemical modification of a polypeptide chain after the ribosomal cycle

c) regeneration of ATP, GTP, CTP, and UTP with oxidative phosphorylation

d) transport of mRNA from a nucleus to cytosol

19. What reaction does DNA polymerase catalyze?

a) The template synthesis of complementary polynucleotide chain
b) The primer synthesis
c) The binding of 5-'end and 3'-end of the adjacent Okazaki fragments
d) The hydrolytic removal of 5'-nucleotide from Okazaki fragments

20. DNA ligase catalyzes the following reaction:
a) the linkage of polynucleotide fragments and the formation of 3'-5'-phosphodiester bonds
b) the linkage of mononucleotides and the formation of 3'-5'-phosphodiester bonds
c) the temporary linkage of DNA and mRNA
d) the linkage of DNA and histons and the formation of nucleosomes

21. Gene expression is called:
a) recognition
b) translation
c) replication
d) transcription

22. Can nucleic acids form nucleoprotein complexes?
a) No
b) Only RNA
c) Only DNA
d) All kinds of nucleic acids

23. The energy source for the elongation stage of the ribosomal cycle is:
a) ATP
b) GTP
c) CTP
d) UTP

24. Choose the right definition of the term "intron".
a) The part of an eukaryotic gene that has no information function and is removed by splicing
b) The part of eukaryotic gene that has the amino acid sequence information and is retained in the final mRNA
c) The highly repetitive sequence of a DNA molecule
d) The unique sequence of a DNA molecule

25. Which of the following nitrogenous bases pairs are the pyrimidine derivatives?
a) Cytosine and adenine
b) Guanine and thymine
c) Uracil and cytosine
d) Guanine and uracil

26. Which of the following energy sources is used for amino acid activation?
a) ATP
b) GTP
c) TTP
d) UTP

27. The site of a mRNA molecule, that is bound with aminoacyl-tRNA during the ribosomal cycle, is called:

a) cap
b) codon
c) gene
d) anticodon

28. Genetic code contains:
a) 64 codons to encode 20 amino acids
b) 64 coding codons and 3 nonsense codons
c) 61 codons to encode 20 amino acids and 3 nonsense codon
d) 63 coding codons and 1 nonsense codon

29. All of the following statements about tRNA molecules are true EXCEPT:
a) they have extensive intrachain hydrogen bonding
b) they contain a number of bases that have been modified post-transcriptionally
c) the amino acid is attached to the 3'-terminal adenosine
d) they contain an anticodon triplet of the bases at the 5'-end of the molecule

30. What substances are called ***nucleosides***?
a) Nitrogenous bases
b) Nitrogenous bases joined with pentose
c) Nitrogenous bases joined with phosphate
d) Nitrogenous bases joined with pentose and phosphate

31. What kind of the bond is formed under the binding of nucleotides and formation of polynucleotide chain?
a) 3'-5'-phosphodiester bond
b) 5'-5'-phosphodiester bond
c) peptide bond
d) hydrogen bond

32. As a result of transcription in eukaryotes the following molecule is formed:
a) heterogeneous nuclear RNA
b) messenger RNA
c) transfer RNA
d) ribosomal RNA

33. Which of the following nitrogenous bases pairs are the purine derivatives?
a) Guanine and uracil
b) Guanine and cytosine
c) Adenine and thymine
d) Adenine and guanine

34. Which of the following statements about chain termination in peptide synthesis is true?
a) The formation of peptide bonds by the ribosomal mRNA complex continues until a nonsense codon on mRNA is reached
b) The formation of peptide bonds by the ribosomal mRNA complex continues until a tRNA with an anticodon for UAA, UAG, or UGA interacts with the A-site on the ribosome
c) Peptide bond formation ceases when the ribosome reaches the 5'-end of the mRNA

d) Peptide bond formation continues until the ribosome dissociates into large and small subunits

35. Which of the following statements about DNA polymerase is correct?

a) It functions as a DNA repair enzyme but is not involved in the DNA replication process

b) Its presence is not necessary for normal replication

c) It requires a template and a primer to polymerize deoxyribonucleoside triphosphates

d) It joins together Okasaki fragments to complete the strand of DNA

36. DNA contains sequences of nitrogen bases known as promoters which:

a) are recognized by DNA polymerase

b) occur in the chain just before the message sequence that defines the messenger RNA

c) are single stranded regions of duplex DNA

d) are transcribed into RNA sequences

LESSON 26
BIOCHEMISTRY of BLOOD.

BIOMEDICAL SIGNIFICANCE

Biochemical analysis of blood is the most universal kind of laboratory examinations in clinical medicine. It is carried out in almost all diseases. The determination of blood level of the specific metabolites is used for estimation of the effects of regulatory systems controlling the state of ***homeostasis*** and also for diagnosing of many pathological states.

Work 1. Determination of total blood plasma protein content with the biuretic method.

The EQUIPMENT: a FEC.

The PRINCIPLE of a METHOD.

Proteins react with copper sulfate in an alkaline medium and form the compound that has pink-violet colour. The intensity of the received colour is directly proportional to the protein concentration in a liquid. The optical density is measured with a FEC.

The COURSE of WORK.

1. Pour 5 ml of the biuretic reagent into the experimental test tube containing 0.1 ml of blood plasma, mix, and keep it at the room temperature for 30 minutes.
2. Pour 0.1 ml of 0.9% sodium chloride into the control test tube, add 5 ml of the biuretic reagent, mix, and keep it at the room temperature for 30 minutes.
3. After that measure the optical density of the experimental test tubes contents **against the control sample** in 10 mm cuvettes. Use a green light-filter (540-560 нм).
4. Determine the concentration of total protein in the sample of blood plasma with a calibrating graph.

The normal total blood plasma protein content is 65-85 g/l.

RESULTS:

CONCLUSIONS:

Work 2. Division of proteins of blood plasma with a method of paper electrophoresis.

The PRINCIPLE of a METHOD.

Under the influence of a constant electrical current proteins, being negatively charged in alkaline environment at pH -8.6-8.9, move along the paper, moistened with a buffer solution, to the anode. The speed of the electrophoresis depends on the size of electric charge and molecular mass of the protein.

The serum proteins may be divided into 5 fractions with the paper electrophoresis and the relative content of each protein fraction may be determined.

The normal range of blood plasma proteins:

Albumins----------52-65%

α 1-globulins----------2.5-5%

α_2- globulins----------7-13%

β- globulins----------8-14%

γ –globulins----------12-22%

The COURSE of WORK.

1. Identify 5 protein fractions on the electroforegram – albumins, α 1, α_2-, β-, and γ -globulins.
2. Cut out the stains of the fractions and place them in the marked test tubes.
3. Add 5 ml of 0.01 N NaOH into the test tube for the extraction of albumins.
4. Add 2.5 ml of the same solution into the test tubes for the extraction of each of the globulin fractions.
4. Shake up all the test tubes and keep them for 30 minutes for the complete extraction of the coloured compounds.
5. Next measure the optical density of each sample **(D_X)** at 500-560 nm (green light-filter) against 0.01 N NaOH in cuvettes with the thickness of 5 mm.
6. Calculate the total sum of the values of optical density **(D_{tot})** of all the fractions and adopt it for **100 %**. Then calculate the percentage of the optical density of each protein fraction (and consequently the percentage content of each protein fraction **(X)**). Use the formula:

$$X = D_X \cdot 100 / D_{tot}$$

Remember, that the value of optical density for albumins is necessary to multiply by 2, as this fraction was extracted by the double volume of NaOH.

RESULTS:

CONCLUSIONS:

Work 3. Benzidine test for heme group of hemoglobin.

The PRINCIPLE of a METHOD.

The reaction is caused by a hemoglobin ability to catalyze the oxidation of benzidine (colourless substance) with hydrogen peroxide to the dark blue coloured product.

The COURSE of WORK.

Add 5 drops of 0.2% alcohol solution of benzidine to 5 drops of diluted blood and then several drops of hydrogen peroxide. The liquid becomes dark blue or green coloured.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Serum proteins, their functions.
2. The properties of serum proteins:
   a) albumin, its role in the organism,
   b) globulins, their features.
3. Synthesis of the blood proteins. The diagnostic significance of determination of protein concentration in blood. Pathological deviations of the normal blood plasma protein content: hyperproteinemia, hypoproteinemia, disproteinemia, paraproteinemia.
4. Hemoglobin as the main protein of erythrocytes, its molecular organization.
5. The role of hemoglobin in the transport of oxygen, carbon dioxide and H -ions. The mechanism of regulation of the affinity of hemoglobin to oxygen.
6. Abnormal varieties of hemoglobin, hemoglobinopathies.
7. Biosynthesis of heme.
8. Blood plasma enzymes, the diagnostic significance of enzymes activity detection in blood.

The examples of the control task:

1). What kinds of hemoglobin are contained in the human erythrocytes?

1. Hb A_1
2. Hb A_2
3. HbL
4. HbF
5. HbC
6. HbN

2). Which of the following substances belong to the class of heme proteins?

1. Myoglobin
2. Cytochromes
4. Transferrin
5. Hemoglobin

3. Catalase 6. Peroxidase

3). The dependency between the extent of the hemoglobin saturation and pO_2 is graphically characterized by

1. a hyperbola 4. a right line
2. an S-like curve
3. a parabola

4). Choose the post-translational modifications of hemoglobin.

1. Glycosylated Hb 5. Caboxyhemoglobin
2. Phosphorylated Hb 6. Sulfated Hb
3. Methemoglobin
4. Complex with glutathione

5). αβ-subunits of hemoglobin are bound by

1. hydrophobic bonds 3. ionic bonds (salt bridges)
2. disulfide bonds 4. hydrogen bonds

6). The phenomenon of *paraproteinemia* is

1. an appearance of abnormal proteins in blood
2. an increase of the blood protein concentration
3. an appearance of the Bence-Jones protein in blood
4. a decrease of the blood protein concentration

7). Which of the following oligomeric combinations is characteristic for the fetal hemoglobin?

1. $\alpha_2\gamma_2$ 3. $\beta_2\gamma_2$
2. $\alpha_2\beta_2$ 4. $\alpha_2\alpha_2$

8). In the synthesis of heme succinyl CoA interacts with glycine and is transformed into

1. γ-aminobutyrate 4. oxaloacetate
2. acetoacetate 5. β-aminopropionate
3. δ-aminolevulinate 6. α-hydroxybutyrate

9). The ability of hemoglobin to bind oxygen depends on

1. pH 3. pO_2
2. temperature 4. presence of 2,3-diphosphoglycerate

10). The central cavity of deoxygenated hemoglobin contains

1. CO_2 3. 2,3-diphosphoglycerate
2. bicarbonate 4. Cl^- anion

11). Which of the following oligomeric combinations are characteristic for HbA1, HbA2, and Hb F?

1. $\alpha_2\beta_2$ 4. $\beta_2\gamma_2$
2. α_2s_2 5. $\alpha_2\delta_2$
3. $\alpha_2\gamma_2$ 6. $\alpha_2\alpha_2$

12). The maintaining of blood osmotic pressure is carried out by

1. Na^+ cathions 3. albumins
2. Cl^- anions 4. blood concentration of cyclic nucleotides

13). What is the source of the ferrous ions for the synthesis of heme?

1. Cytochromes 4. Catalase

2. Myoglobin 5. Peroxidase
3. Ferritin

14). The fraction of α_1-globulins contains the following proteins:
1. ceruloplasmin 4. thyroxin-binding protein
2. haptoglobin 5. transcortin
3. antitrypsin 6. transferrin

15). Choose the organospecific (marker) enzymes of blood which may be determined to diagnose the liver disorders.
1. Lactate dehydrogenase (isoenzymes LDH1 and LDH2)
2. Lactate dehydrogenase (isoenzymes LDH_4 and LDH_5)
3. Amidinotransferase
4. Alanine aminotransferase
5. Aspartate aminotransferase
6. Creatine kinase
7. Arginase

16). Which of the following properties of hemoglobin and erythrocytes are alterated in the sickle cell anemia (sicklemia)?
1. Solubility in water decreases
2. An affinity to oxygen increases
3. Deformation of erythrocytes takes place
4. Solubility in water increases
5. An affinity to oxygen decreases

17). Hemoglobin fulfils the following functions:
1. transport of oxygen from the lung to the tissues
2. transport of oxygen inside the cell to the mitochondria
3. accumulation of oxygen in the tissue
4. transport of CO_2 from the tissue to the lung
5. maintenance of pH of blood (buffer function)
6. formation of tissue pigments

18). β-globulins contain the following proteins:
1. transferrin 4. albumins
2. hemopexin 5. immunoglobulins
3. low density lipoproteins (LDL)

19). In the deoxygenated state the iron ion of heme is situated
1. in the plane of a heme ring
2. 0.06 nm over the plane of a heme ring
3. at any point of a hemoglobin molecule
4. none of the above

20). In the oxygenated state the iron ion of heme is situated
1. in the plane of a heme ring
2. 0.06 nm over the plane of a heme ring
3. in any point of a hemoglobin molecule
4. none of the above

21). The ability of hemoglobin to bind oxygen depends on the following factors
1. pH 4. concentration of ATP

2. temperature 5. concentration of ADP
3. pO_2 6. pCO_2

22). The transport of CO_2 from the tissues to the lung is carried out by:
1. solubilization of CO_2 in blood plasma
2. temporary formation of carbohemoglobin
3. temporary formation of a complex compound with plasma albumin
4. temporary formation of blood plasma bicarbonates
5. temporary formation of a complex compound with transferrin

23). Hyperproteinemia is developed as a result of
1. diarrhea (the loss of water through the intestine)
2. cirrhosis of the liver
3. burn of the body (the loss of water through the skin)
4. vomiting (the loss of water with the gastric juice)

24). Which of the following blood plasma proteins are mainly synthesized in the liver?
1. Albumins 4. β-globulins
2. α_1-globulins 5. γ-globulins
3. α_2-globulins

25). Which of the following blood plasma proteins are synthesized in the spleen and lymphoid tissue?
1. Albumins 4. β-globulins
2. α_1-globulins 5. γ-globulins
3. α_2-globulins

26). Which of the following disturbances occur in thalassemia?
1. The synthesis of α or β chains of a hemoglobin molecule is affected
2. The cooperativity of an oxygen binding disappears
3. The total blood hemoglobin concentration decreases
4. The solubility of hemoglobin decreases

27). Which of the following substances are used for heme synthesis?
1. alanine 3. acetyl CoA
2. glycine 4. succinyl CoA
3. valine 5. acetoacetyl CoA

28). The main function of ceruloplasmin is:
1. transport of Fe^{2+} ions
2. transport of Cu^{2+} ions
3. transport of Zn^{2+} ions
4. transport of Mg^{2+} ions

29). The main function of transferrin is:
1. transport of Fe^{2+} ions
2. transport of Cu^{2+} ions
3. transport of Zn^{2+} ions
4. transport of Mg^{2+} ions

30). Haptoglobin is the blood plasma protein responsible for transport of:
1. free heme 3. free hemoglobin

2. free bilirubin 4. free Fe^{2+} ions

31). The phenomenon of *disproteinemia* is
1. an appearance of abnormal proteins in blood
2. an increase of the blood protein concentration
3. disturbance of the normal ratio between blood plasma proteins
4. a decrease of the blood protein concentration

32). Choose the abnormal fragment of a β-chain in the structure of HbS
1. val-his-leu-tre-pro-val-glu-lys
2. val-his-leu-tre-pro-glu-glu-lys
3. val-his-leu-ala-pro-glu-glu-lys
4. val-his-leu-tyr -pro-glu -glu-lys

LESSON 27
BIOCHEMISTRY of the LIVER.

BIOMEDICAL SIGNIFICANCE

The liver is "***the chemical laboratory of the body***" that maintains the normal blood concentrations of many metabolites by controlling protein, lipid and carbohydrate metabolism. One of the most important functions of the liver is the ***detoxication*** of the endogenous biologically active substances and exogenous poisons (***xenobiotics***). The disturbance of bilirubin detoxication in the liver leads to the development of various pathological states characterized by the term ***jaundice***.

Work 1. Quantitative determination of total, direct-reacting and indirect-reacting bilirubin in serum with the Iendrassik method.

The EQUIPMENT: a FEC.

The PRINCIPLE of a METHOD.

Bilirubin is transformed to a soluble form after the addition of the caffeine reagent to serum. Bilirubin becomes reactionable and forms pink-violet coloured product in the presence of the diazoreagents. After the addition of the Feling reagent the liquid acquires green colour. The concentrations of bilirubin fractions are determined by the measuring the colour intensity with a photoelectrocolorimeter.

The COURSE of WORK.

Fill in the test tubes the following reagents (look at the table):

№ of a test tube	Reagents	Total bilirubin (A)	Direct-reacting bilirubin (B)	Control (C)
1.	Serum	0.5 ml	0.5 ml	0.5 ml
2.	Caffeine reagent	1.75 ml	-	1.75 ml
3.	Physiological solution (0.9% sodium chloride)	-	1.75 ml	0.25 ml
4.	Mixture of diazoreagents	0.25 ml	0.25 ml	-
I n 10 m i n u t e s				
5.	Feling reagent	0.5	0.5	0.5

Then measure the optical density of each sample with a FEC against water. Use a red light-filter (670 nm) and 5 mm cuvettes. Subtract the value of optical density of sample C (control) from the values of optical density of the samples A and B (experimental) correspondingly.

Find the values of concentrations of total and direct reacting bilirubin with a calibrating graph. For determination of the indirect bilirubin level subtract the value of direct bilirubin from the total one.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. The role of the liver in protein, lipid and carbohydrate metabolism.
2. The most important mechanisms of detoxication of poisoning substances in the liver.
3. The microsomal oxidation of endogenous toxins and xenobiotics.
4. The unmicrosomal oxidation of toxic substances.
5. The reactions of conjugation in the liver.
6. The degradation of heme. The formation of bilirubin and bilirubin glucuronide.
7. The ways of bilirubin and other bile pigments excretion.
8. The significance of bile pigments determination for a diagnosis of the liver diseases, bile duct obstruction, and blood hemolysis.

The examples of the control task:

1). Which of the following functions are carried out by the liver?

1. Homeostatic
2. Digestive
3. Storage
4. Excretory
5. Antitoxic
6. Contractive

2). Bilirubin glucuronide is marked as

1. conjugated bilirubin
2. direct reacting bilirubin
3. indirect reacting bilirubin
4. hemobilirubin
5. choleic bilirubin
6. unconjugated bilirubin

3). The maximal blood total bilirubin level in healthy adults is

1. 21.5 mM/L
2. 42.5 microM/L
3. 5.5 microM/L
4. 42.5 microM/L
5. 21.5 microM/L
6. 5.5 mM/L

4). Estimate the results of the analysis:

a) total blood bilirubin---80 microM/L,
b) "direct"/"indirect" ratio-----1:3,
c) "di"/"mono" ratio------1:10,
d) urine bilirubin----------present,
e) urine urobilinogen-------present
f) fecal stercobilinogen----traces

1. Normal
2. Higher than normal
3. Less than normal

5). Choose the metabolic pathways which belong to homeostatic function of the liver in carbohydrate metabolism.

1. Synthesis and mobilization of glycogen
2. Gluconeogenesis
3. Synthesis of bile acids
4. Synthesis of urea
5. Glucose-lactate cycle

6). The rapid toxic effects of the endogenous toxins and xenobiotics are the following:

1. Mutagenic effect
2. Inhibition of enzymes
3. Effect of denaturation
4.Tumorigenic effect
5. Alteration of pH
6. Blocking of the cell receptors

7). Choose the examples of non-microsomal oxidation of toxic substances

1. Oxidation of biogenic amines by MAO and DAO
2. Hydroxylation of xenobiotics
3. Decomposition of hydrogen peroxide with catalase and peroxidase
4. Synthesis of bilirubin diglucuronide

8). The oxidation of ethanol with alcohol dehydrogenase leads to the formation of

1. acetyl CoA
2. acetaldehyde
4. $NADH_2$
5. pyruvate

3. free acetate 6. $NADPH_2$

9). Bilirubin formed in the reticulo-endothelial system (RES) and entered the blood is called

1. bilirubin glucuronide
2. bilirubin-albumin
3. choleic bilirubin
4. direct reacting bilirubin
5. indirect reacting bilirubin
6. hemobilirubin
7. conjugated bilirubin
8. unconjugated bilirubin
9. biliverdin
10. verdoglobin

10). The sequence of the chemical transformations of bilirubin in the intestine is the following:

1. bilirubin→ stercobilinogen→ mesobilinogen→mesobilirubin
2. bilirubin→ mesobilinogen→ mesobilirubin → stercobilinogen
3. bilirubin→ mesobilirubin→ mesobilinogen → stercobilinogen
4. bilirubin→ mesobilinogen→ stercobilinogen → mesobilirubin

11). Which of the following substances are excreted by the liver?

1. sterols
2. bile acids
3. bile pigments
4. urea
5. uric acid
6. some of xenobiotics

12). Which of the following reactions are catalyzed by bilirubin glucuronyltransferase?

1. Bilirubin + UDP-glucuronate→Bilirubin monoglucuronide + UDP
2. Bilirubin monoglucuronide + UDP-glucuronate→Bilirubin diglucuronide +UDP
3. Bilirubin + glucuronate→Bilirubin monoglucuronide
4. Bilirubin monoglucuronide + glucuronate→Bilirubin diglucuronide

13). The normal value of the ratio "direct":"indirect" bilirubin in blood is approximately

1. 1:10
2. 1:1
3. 10:1
4. 1:3
5. 4:1
6. 5:1

14). Estimate the results of the analysis:

a) total blood bilirubin---80 microM/L,
b) "direct"/"indirect" ratio-----3:1,
c) "di"/"mono" ratio------4:1,
d) urine bilirubin----------present,
e) urine urobilinogen-------absent
f) urine stercobilinogen----absent
g) fecal stercobilinogen----absent

1. Normal
2. Higher than normal
3. Less than normal

15). Choose the metabolic pathways which belong to the homeostatic function of the liver in lipid metabolism.

1. Gluconeogenesis
2. Synthesis of non-essential fatty acids
3. Synthesis of ketone bodies
4. Synthesis of cholesterol
5. Synthesis of bile acids
6. Pentose cycle

16). The slow toxic effects of the endogenous toxins and xenobiotics are the following:

1. Mutagenic effect
2. Inhibition of enzymes
3. Effect of denaturation
4. Teratogenic
5. Tumorigenic effect
6. Alteration of pH
7. Blocking of the cell receptors

17). Which of the following reactions is marked as *conjugation*?

1. Attachment of oxygen to a toxic substance
2. Attachment of protons and electrons to a toxic substance
3. Binding of one or several molecules of endogenous hydrophylic residues to a toxic substance
4. Any reactions of a synthesis

18). Ethanol can be oxidized by

1. alcoholdehydrogenase way only
2. catalase way only
3. microsomal oxidation only
4. all three above presented ways
5. non above presented ways

19). The chemical transformations of bilirubin in the bile excretory system and in the intestine proceed with

1. the digestive enzymes
2. the bacterial enzymes
3. the both digestive and bacterial enzymes
4. a non enzymatic mechanism

20). Which of the following components are involved into a microsomal respiratory chain?

1. Cyctochrome a_3
2. $NADH_2$
3. $NADPH_2$
4. Cytochrome P_{450}
5. Flavoprotein
6. Cytochrome c_1

21). The verdoglobin formation reaction

1. is catalyzed by heme oxygenase
2. requires $FADH_2$ and O_2
3. requires $NADPH_2$ and O_2
4 requires $NADH_2$ and O_2
5. leads to the formation of CO_2
6. leads to the formation of CO

22). Conjugated bilirubin formed in the hepatocytes is secreted into

1. the blood capillaries

2. the bile capillaries
3. the lymphatic capillaries

23). Jaundice is classified into

1. prehepatic
2. posthepatic
3. inflammatory
4. malignant
5. hepatic
6. inherited

24). Choose the metabolic pathways which belong to homeostatic function of the liver in protein metabolism.

1. Synthesis of non-essential fatty acids
2. Synthesis of non-essential amino acids
3. Synthesis of bile acids
4. Synthesis of urea
5. Synthesis of blood plasma proteins
6. Gluconeogenesis

25). The conjugation of bilirubin in the liver requires which of the following substances?

1. S-adenosyl methionine
2. 3'-phosphoadenosine-5'-phosphosulfate
3. UDP-glucuronate
4. HS-CoA
5. Glutathione-SH

26). What hepatocyte compartments do the oxidative transformations of toxic substances proceed with?

1. Lysosomes
2. Mitochondria
3. Endoplasmic reticulum
4. Peroxisomes
5. Cytosol
6. Plasmatic membrane

27). The reaction: R-OH + UDP-glucuronate→→R-O-glucurunate + UDP is marked as

1. microsomal oxidation
2. non-microsomal oxidation
3. conjugation
4. microsomal reduction

28). Which of the following endogenous biologically active substances can be detoxified in the liver?

1. Caffeine
2. Methanol
3. Steroid hormones
4. Bilirubin
5. Neurotransmitters
6. Strong oxidizig agents
7. Vitamin D
8. Free ammonia

29). Stercobilinogen undergoes to the partial reabsorption in the...

1. stomach
2. proximal portion of the small intestine
3. distal portion of the small intestine

4. proximal portion of the large intestine
5. distal portion of the large intestine
6. gallbladder

30). What is the colour of verdoglobin and biliverdin?

1. Red
2. Yellow
3. Green
4. Light brown

31). What is the colour of bilirubin?

1. Red
2. Blue
3. Green
4. Light brown

32). Mesobilinogen undergoes the partial reabsorption in the...

1. stomach
2. small intestine
3. large intestine
4. gallbladder

33). The reaction: R-OH + PAPS$\rightarrow$R-OSO_3H +PAP is marked as

1. glucuronate conjugation
2. sulfate conjugation
3. thiolytic break down
4. glutathione conjugation

34). The process of detoxicatication of toxic substances in the liver includes which of the following two phases?

1. Oxidation, reduction or hydrolysis + conjugation with hydrophylic substances
2. Oxidation, reduction or hydrolysis + conjugation with hydrophobic substances
3. Oxidation, reduction or hydrolysis + polymerization of the products and their excretion with bile
4. Oxidation, reduction or hydrolysis + conjugation with hydrophylic or hydrophobic substances

35). Estimate the results of the analysis:

a) total blood bilirubin---80 microM/L,
b) "direct"/"indirect" ratio-----1:10,
c) "di"/"mono" ratio------3:1
d) urine bilirubin----------absent,
e) urine urobilinogen-------present
f) urine stercobilinogen----present
g) fecal stercobilinogen----present

1. Normal
2. Higher than normal
3. Less than normal

36). Which of the following functions belong to a microsomal respiratory chain?

1. Oxidative phosphorylation
2. Hydroxylation of steroid hormones
3. Oxidation of drugs

4. Oxidation of endogenous toxic substances
5. Oxidation of xenobiotics
6. Generation of a proton membrane potential

37). A reducing microsomal chain consists of
1. $NADPH_2$, flavoprotein, cytochrome b_5, cytochrome a_3
2. $NADPH_2$, flavoprotein, cytochrome b_5, cytochrome P_{450}
3. $NADH_2$, flavoprotein, cytochrome b_5, cytochrome P_{450}
4. $NADPH_2$, flavoprotein, cytochrome b_5, cytochrome c

38). The reaction: R-OH + CH_3-COS-CoA→ R-O-$COCH_3$ + HS-CoA is marked as
1. glucuronate conjugation
2. sulfate conjugation
3. acetyl conjugation
4. glutathione conjugation

39). Which of the following substances are xenobiotics?
1. Caffeine
2. Alcohol
3. Residual pesticides of food
4. Food carbohydrates
5. Dioxins

LESSON 28.
BIOCHEMISTRY of CONNECTIVE and MUSCLE TISSUE.

BIOMEDICAL SIGNIFICANCE

The connective tissue is account for about 50% of body mass. It provides the maintenance of the tissue structure of many organs and systems of the body by forming their stroma. The bone tissue is the mineralized kind of the connective tissue. The connective tissue pathological states (***collagenoses***) are characterized by generalized affection of the extracellular components of the connective tissue, collagen fibers mainly. The typical collagenoses are systemic lupus erythematosis (SLE), rheumatism, sclerodermia, etc. Mucopolysaccharidoses are the diseases due to congenital defects of the enzymes hydrolyzing glucosaminoglycans and accompanied by their pathological accumulation in the connective tissue. Alkaline phosphatase is a tissue specific enzyme of the bone tissue. It is derived from the osteoblasts, so the catalytic action of this enzyme in blood depends on their activity.

Work 1. Quantitative determination of alkaline phosphatase activity in blood.

EQUIPMENT: a FEC, a centrifuge.

The PRINCIPLE OF A METHOD.

PHOSPHATASES are the enzymes which catalyze the reaction of hydrolytic releasing of phosphate from its organic esters:

$$R\text{-}O\text{-}PO_3H_2 + H_2O \rightarrow R\text{-}OH + H_3PO_4$$

There are 2 types of the enzymes: the acid phosphatase which is active at pH 4.4-6.2 and the alkaline phosphatase which has maximal activity at pH 6.6-10.0. We use glycerophosphate as a substrate for the determination of alkaline phosphatase activity. Free inorganic phosphate, released during the reaction, serves as a measure of the enzyme activity. The amount of inorganic phosphate is determined by a colorimetric method based on the colour reaction with the molybdate reagent in the presence of ascorbic acid as the reducing agent. The product of the reaction has blue colour, the intensity of the colour is directly proportional to the amount of phosphorus in a sample.

The COURSE OF WORK.

Prepare the reaction mixture according to the following table:

№	Glycerophosphate buffer	Serum	Temperature for incubation
1	5.0	0.5	37^o
2	5.0	0.5	Room t^o

The test tube 1 (experimental) serves for the determination of total inorganic phosphate, including phosphate hydrolyzed by alkaline phosphatase. The test tube 2 (control) is used for the determination of unhydrolyzed inorganic phosphate.

After the incubation add 4.5 ml of 10% TCA into the both test tubes. Centrifuge the test tubes at 3000 rev/min for 10 min. Then determine the content of inorganic phosphate in the both samples of supernatant liquid. For it:

1.Take 3 ml of an unprotein supernatant liquid into the control and experimental test tubes.

2. Add 1 ml of molybdate reagent and 1 ml of 1% ascorbic acid in each test tube. Wait for 15 min.

3. Measure the optical density of the coloured solution with a FEC. Use a red light filter (670 nm).

4. Calculate the difference between the experimental and control test tubes and find the amount of phosphorus with a calibrating graph. Multiply this value by 200 to express the result in the Bodansky units (BU).

The normal activity: 2-5 BU (for adults), 5-15 BU (for children).

RESULTS:

CONCLUSIONS:

Work 2 "Determination of creatine kinase activity in serum and muscle tissue homogenate".

EQUIPMENT: an ionometer, a thermostat for 38ºC.

The PRINCIPLE of a METHOD.

The method of creatine kinase activity detection is based on the fact that in alkaline medium creatine kinase reaction results in release of H^+.

$$\text{Creatine} + ATP^{4-} \Rightarrow \text{Creatine phosphate}^{2-} + ADP^{3-} + \mathbf{H}^{+}$$

Creatine kinase activity can be registered by the detection of change in pH during the reaction (before and after the incubation).

The COURSE of WORK:

1. Create incubation medium. Mix 1.5 ml of creatine, 1.0 ml of ATP and 1.0 ml of buffer solutions with 0.5 ml of skeletal muscle homogenate (or serum).
2. Measure initial pH_0 of the incubation medium.
3. Put the test tube into the thermostat for 45 minutes.
4. In 45 min measure pH_1 of incubation medium.
5. Find $\Delta pH = pH_0 - pH_1$. Then find the value of the enzyme activity with a calibrating graph.

Creatine kinase activity is expressed in micrograms of H^+ released for 45 min incubation period (it's equal to micromoles of ADP released for the same period).

RESULTS:

CONCLUSIONS (compare creatine kinase activity in serum and muscle homogenate):

Work 3. Determination of total nitrogen in tooth enamel and dentine.

EQUIPMENT: a FEC

The PRINCIPLE OF A METHOD.

The determination of total nitrogen is carried out in enamel and dentine mineralizate. Under the mineralization all nitrogen-containing substances are transformed into ammonia sulfate. The interaction of ammonia sulfate with the Nessler reagent result in the formation of yellow-orange product, optical density of which depends on the concentration of total nitrogen.

The COURSE OF WORK.

Add distilled water to 0.8 ml of a mineralizate of enamel or dentine up to approximately a half of volume of a 10 ml measuring test tube.

Pour 0.4 ml of the Nessler reagent into the test tube, mix carefully, and add distilled water up to the label of total volume 10 ml. Mix it carefully again.

In 10 minutes measure the optical density of the coloured solution with a FEC against water. Use a dark-blue light-filter (440 nm)and 10 mm cuvettes.

Use a calibrating graph for the calculation of the content of residual nitrogen in enamel and dentine.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS:

1. Collagen as an unique fibrous protein, its structural characteristic. Biosynthesis of collagen, characteristics of the stages. The collagen types. Diseases caused by the disturbances of the collagen synthesis.
2. Elastin, its characteristic. The major differences between collagen and elastin.
3. Proteoglycans - the main proteins of the ground substance of connective tissue, their composition. Glycosaminoglycans, classification. The formation of the proteoglycan aggregates. Mucopolysaccharidoses.
4. The main myofibrillar proteins of muscle tissue: actin, myosin, tropomyosin, troponin; their role in muscle contraction (according the sliding filaments theory).
5. Biochemical mechanism of muscle contraction and relaxation. The role of calsium ions and ATP in these processes. The ways of ATP regeneration in muscular tissue.
6. The peculiarities of myocardium metabolism.

For the stomatological faculty:

1. Chemical composition of mineralized tissues. Apatites. The peculiarities of the structures and properties of different kinds of apatites.
2. The character of the mineral composition of tooth components (enamel, dentin, cementum).
3. The main bone and tooth proteins. Collagen. Processing of collagen.
4. Uncollagen proteins of bone tissue (proteoglycans, glycoproteins, osteocalcin, osteonectin. The specific bone enzymes.
5. Unprotein organic components of bones and teeth. The role of citrate in bone metabolism.
6. The biochemical character of pulp.
7. Mineralization of bone and tooth tissue. The participation of collagen, uncollagen proteins, and GAG in formation of hydroxyapatite crystals.
8. Regulatory factors of osteogenesis (mitogens, morphogens, chemotactic substances, antagonists of mitogens and morphogens).
9. Hormones controlling the processes of osteogenesis and decomposition of bone tissue. Parathyroid hormone and calcitonin. Their influence on metabolism of calcium and phosphorus.
10. Vitamins of D-group. Provitamins D. The endocrine system of vitamine D and its role in the maintenance of calcium homeostasis.
11. The influence of a diet on the state of teeth. The role of dietary carbohydrates, proteins, microelements, and vitamins in the maintenance of a normal physiological state of teeth.
12.Microelements: fluorine and strontium, their role in mineralization of bone and tooth tissue. Pathological states due to insufficient and excessive intake of fluorine and strontium.

The examples of the control task:

1). The secondary structure of a collagen molecule is

1. α-helix
2. β-pleated sheets
3. triple helix
4. amorphous structure

2). The extracellular processing of collagen includes which of the following events?

1. Phosphorylation of procollagen by ATP and proteinkinase
2. Removal of the fragments of triple helix from N and C terminals with procollagen amino-terminal and carboxy-terminal proteases
3. Spontaneous formation of regular parallel arrays connected by side-to-side cross-links and end-to-end ionic links
4. Oxidative deamination of lysine residues with lysyl oxidase
5. Glycosylation of a procollagen molecule

3). Tropoelastin is synthesized as

1. an unsoluble fibrillar polypeptide chain
2. a soluble protomer of 70 kDa
3. a preproelastin subjected to the further partial proteolysis
4. a complex oligomeric protein

4). Which of the following substances are glycosaminoglycans?

1. Glycogen
2. Gyaluronic acid
3. Glucuronic acid
4. Heparin
5. Heparin sulfate
6. Keratan sulfate
7. Dermatan sulfate
8. Chondroitin sulfate

5). The every third residue of a tropocollagen polypeptide chain is

1. alanine
2. glycine
3. valine
4. proline

6). Preprocollagen is converted into procollagen by

1.the partial hydrolysis with the removal of a signal peptide
2. the phosphorolytic shortening of a preprocollagen molecule
3. an association of two molecules of preprocollagen

7). Choose the prevalent amino acids of tropocollagen.

1. Alanine, glycine, tryptophan
2. Glycine, methionine, tyrosine
3. Proline, hydroxyproline, isoleucine
4. Glycine, proline, hydroxyproline

8). The intracellular processing of collagen includes which of the following events?

1. Partial hydrolysis of preprocollagen with the removal of a signal peptide
2. Hydroxylation of proline and lysine residues
3. Use of ascorbic acid as a reducing cosubstrate
4. Glycosylation of hydroxylysine and arginine

5. Phosphorylation of serine residues

9). Elastin is synthesized as

1. ready made unsoluble fibrillar protein with a molecular mass of 500 kDa
2. a soluble monomer of 70 kDa called tropoelastin
3. a precursor of tropoelastin called protropoelastin

10). Spontaneous side-to-side association of tropocollagen molecules proceeds with the formation of

1. covalent aldol-like bonds
2. covalent Schiff's-base-like bonds
3. non-covalent ionic bonds
4. non-covalent hydrogen bonds

11). Proteoglycans consist of

1. positive charged polysaccharides (~95% of mass) and proteins (~5% of mass)
2. negative charged polysaccharides (~5% of mass) and proteins (~95% of mass)
3. negative charged polysaccharides (~95% of mass) and proteins (~5% of mass)
4. positive charged polysaccharides (~5% of mass) and proteins (~95% of mass)

12). All the statements concerning the peculiarities of connective tissue metabolism are correct EXCEPT

1. Rate of metabolic transformations of the connective tissue constituents (rate of their renewing) is lower than in the other tissues
2. Daily excretion of hydroxyproline with urine is tightly dependent on age
3. Rate of metabolic transformations of the connective tissue constituents (rate of their renewing) is higher than in the other tissues
4. Excessive accumulation of calcium salts takes place in the old human organism

13). Collagen is

1. a predominant protein of connective tissue
2. a minor protein of connective tissue
3. an oligomeric globular protein
4. a multimeric fibrillar protein

14). The triple helix of a collagen molecule is supported by

1. hydrogen bonds
2. disulfide bonds
3. ionic bonds
4. phosphodiester bonds

15). Desmosime and isodesmosine are

1. a product of condensation of three lysyl residues of tropoelastin molecules
2. a product of condensation of four lysyl residues of tropoelastin molecules
3. a product of condensation of three leucine residues of tropoelastin molecules
4. a product of condensation of four lysyl residues of tropoelastin molecules

16). Choose the right principle structure of a proteoglycan monomer.

1. [Uronic acid-O-glucose]
2. [Galactose-O-Glucuronic acid]
3. [Uronic acid (or galactose)-O-Acetylated or (and) sulfated hexosamine]
4. [Uronic acid (or galactose)-O-Acetylated or (and) phosphorylated hexosamine]

17). Proteoglycans are complex proteins consisting of apoproteins and

1. monosaccharides
2. disaccharides
3. oligosaccharides
4. glycosaminoglycans

18). The tropocollagen molecule consists of

1. one polypeptide chain
2. two polypeptide chains
3. three polypeptide chains
4. four polypeptide chains

19). Which of the following bonds is used for end-to-end contacts of tropocollagen molecules?

1. Ionic
2. Disulfide
3. Hydrophobic
4. Hydrogen
5. Phosphodiester

20). Choose the correct properties and functions of collagen.

1. The major protein of bones, ligaments, tendons, cartilages, skin, dentine, cementum
2. The major protein of parenchymal organs
3. Non-extensible rigid protein
4. It is responsible for the structural and mechanic functions of connective tissue
5. Extensible rubber-like protein

21). Extensible rubber-like properties of elastin are due to

1. a presence of a carbohydrate unprotein component in its structure
2. a presence of a large amount of α-helixes in its structure
3. a presence of desmosine and isodesmosine in its structure
4. a presence of a large amount of β-pleated sheets in its structure

22). Which of the following functions of connective tissue belong to proteoglycans?

1. Structural (the formation of intracellular matrix)
2. Storage (the accumulation of water and mineral ions)
3. Barrier (the formation of histo-hematic barriers)
4. Anticlotting (inhibition of clotting)
5. Regulatory (control of the cell metabolism)

23). The [gly-pro-oxypro] sequence is characteristic for which of the following proteins?

1. Fibrillin
2. Laminin
3. Elastin
4. Proteoglycans
5. Collagen
6. Fibronectin

24). A myosin molecule contains

1. two identical heavy chains (230 kDa) and one light chain (20 kDa)
2. two identical heavy chains (230 kDa), two essential, and two regulatory light chains (16 and 20 kDa respectively)
3. four identical heavy chains (230 kDa), two essential, and two regulatory light chains (16 and 20 kDa respectively)
4. two different heavy chains (230 kDa and 250 kDa respectively), two essential, and two regulatory light chains (16 and 20 kDa respectively)

25). Troponin I

1. is responsible for the binding of Ca^{++} ions
2. is responsible for the binding of G-actin
3. is responsible for the inhibition of the actin and myosin interaction
4. is phosphorylated by cAMP-dependent protein kinase

26). The primary signal for the transition of a muscle from the rest state to the contractive state is

1. the formation of cAMP
2. the formation of hormone-receptor complex
3. an increase of the cell ATP concentration
4. a nervous impulse

27). How many isoenzymes of creatine kinase are found in the brain, skeletal muscle and myocardium?

1. Two
2. Six
3. Four
4. Five
5. Seven
6. Three

28). Troponin T

1. is responsible for the binding of Ca^{++} ions
2. is a tropomyosin-binding subunit of a troponin molecule
3. is responsible for the inhibition of the actin and myosin interaction
4. is phosphorylated by cAMP-dependent protein kinase

29). A sliding of the filaments during the muscle contraction is accompanied by

1. ATP hydrolysis
2. GTP hydrolysis
3. the conformational transformations of myosin
4. alteration of the angle between a head and a body of a myosin molecule (45^o)
5. shortening of the sacomer length

30). Choose the specific marker enzymes of the myocardium.

1. Hexokinase
2. Creatine kinase (MM)
3. Creatine kinase (MB)
4. Creatine kinase (BB)
5. Alanine aminotransferase
6. Aspartate aminotransferase
7. Enolase
8. LDH_4 and LDH_5
9. LDH_1 and LDH_2

31). Choose the protein which is incorporated into the thick filaments.

1. Actin
2. Myosin
3. Troponin
4. Tropomyosin
5. Collagen
6. Elastin

32). Tropomyosin is

1. a long, thin, stick-like protein of thin filaments
2. a long, thin, stick-like protein of thick filaments
3. a small globular protein of thin filaments
4. a small globular protein of thick filaments

33). Choose the sarcoplasmic proteins of muscle tissue

1. Hexokinase, enolase, myoglobin
2. Phosphofructokinase, myosin, actin
3. Myoglobin, lactate dehydrogenase, actin
4. Collagen, actin, myosin

34). What enzyme activity is characteristic for the myosin head?

1. Adenylate cyclase
2. Creatine kinase
4. GTP-ase
5. ATP-ase

35). Troponin C

1. is responsible for a binding of Ca^{++} ions
2. is responsible for a binding of G-actin
3. is responsible for the inhibition of the actin and myosin interaction
4. is phosphorylated by cAMP-dependent protein kinase

36). Which of the following events are induced by a nervous impulse in the myocyte?

1. Depolarization of the plasmatic membrane
2. Activation of adenylate cyclase
3. Opening of Ca^{++} channels
4. the formation of cAMP
5. Phosphorylation of I-subunit of troponin
6. Phosphorylation of T-subunit of troponin
7. Binding of Ca^{++} ions with C-subunit of troponin
8. Binding of Ca^{++} ions with I-subunit of troponin

37). Choose the specific peculiarities of myocardium metabolism.

1. Obligatory aerobic type of the metabolism
2. Obligatory anaerobic type of the metabolism
3. Myocardium oxidizes ketone bodies and fatty acids rather than glucose
4. Myocardium oxidizes glucose rather than ketone bodies and fatty acids
5. The activity of lactate dehydrogenase 4 and 5 (LDH_4 and LDH_5) is higher than of lactate dehydrogenase 1 and 2 (LDH_1 and LDH_2)

38). Which of the following reactions is catalyzed by adenylate kinase?

1. ATP + glucose$\rightarrow$ ADP + glucose-6-phosphate
2. ATP + GDP$\rightarrow$ ADP + GTP
3. ATP $\rightarrow$ cAMP + PPi
4. ADP + ADP $\rightarrow$ ATP + AMP

39). The relaxation (a transition to the relaxed state) is accompanied by

1. hydrolysis of acetylcholine
2. repolarization of the plasmatic membrane
3. opening of Ca^{++} channels
4. closing of Ca^{++} channels
5. phosphorylation of I-subunit of troponin
6. dephosphorylation of I-subunit of troponin
7. inhibition of the actin and myosin interaction with tropomyosin
8. activation of cAMP synthesis
9. hydrolysis of cAMP by phosphodiesterase

40). Myosin is
1. a globular oligomeric protein of thin filaments
2. a fibrillar oligomeric protein of thick filaments
3. a fibrillar protomeric protein of thick filaments
3. a globular multimeric sarcoplasmic protein

41). Which of the following stages of a muscle contraction is characterized by the lowest concentration of Ca^{++} in the sarcoplasm ?
1. Relaxed (rest) stage
2. Signal stage
3. The stage of the filament sliding
4. Transition to the relaxed stage

42). Actin is
1. a complex fibrillar protein of thick filaments
2. a simple protein of thick filaments
3. a complex protein of thin filaments
4. a simple protein of thin filaments

43). Choose the main ways of an ATP synthesis in the skeletal muscle and myocardium.
1. Reverse hexokinase reaction
2. Substrate phosphorylation
3. Reverse phosphofructokinase reaction
4. Oxidative phosphorylation
5. Reverse adenylate cyclase reaction
6. Creatine kinase reaction
7. Adenylate kinase reaction

44). The relaxed (rest) state of a muscle is characterized by
1. an absence of any contacts between actin and myosin
2. low concentration of Ca^{++} ions
3. a presence of contacts between actin and myosin
4. high concentration of Ca^{++} ions

45). According to *the sliding filament theory*
1. the cross size of a sarcomer increases during the muscle contraction
2. the cross size of a sarcomer decreases during the muscle contraction
3. the cross size of a sarcomer is not changed during the muscle contraction
4. the distance between z-membranes of a sarcomer decreases

LESSON 29
BIOCHEMISTRY of SALIVA.

BIOMEDICAL SIGNIFICANCE

Normal saliva has approximately ***neutral pH (6.6-7.2).*** The acidic shift of saliva pH disturbs the process of enamel mineralization and promotes to the development of ***caries***. On the other hand, the alkaline shift of saliva pH leads to the formation of ***dental calculus*** and the development of ***parodontitis***. The proteinase activity of saliva is significantly rised in parodontitis, inflammatory processes of the oral cavity, and in the appearance of ***dental plaques***. The normal range of the saliva proteinase activity is 1.5-10.0 micromol/min/l. The abnormally high content of ***rhodanides*** in saliva is a typical phenomenon for ***smokers***. Its reason is the intake of large amount of cyanides with cigarette smoke. Cyanides are detoxified in the tissues with ***rhodanese (thiosulfate-sulfite transferase)***, converted into rhodanides, and excreted from the body through the salivary glands.

Work 1. Determination of saliva pH.

The PRINCIPLE of a METHOD.

The colour of indicator paper depends on pH of a liquid because the paper is impregnated with a mixture of different acid-base indicators.

The COURSE of WORK.

Immerse a strip of indicator paper into a sample of saliva. Immediately compare the colour of the paper with the standard colour scale on the box. Evaluate the result of pH measuring in comparison with the normal values (6.5-7.5 units of pH).

RESULS:

CONCLUSION:

Work 2 Determination of peptidyl peptide hydrolase activity in saliva.

EQUIPMENT: a thermostat, a centrifuge, a FEC.

The PRINCIPLE of a METHOD.

The method is based on the determination of the amount of tyrosine and other cyclic amino acids formed as a result of hydrolysis of hemoglobin by saliva proteases. In an alkaline medium the blue coloured complex products of interaction of the amino acids with the Folin reagent are formed.

The diagnostic meaning of the analysis is the following: the normal saliva protease activity is 1.5-10.0 micromol/min/L. The activity of peptidyl peptide hydrolase is increased in inflammatory processes of the oral cavity and as a result of appearance of dental plaques.

The COURSE of WORK

Carry out the practical work according to the table:

REAGENTS	Experimental test tube	Control test tube	Standard test tube
Hemoglobin denaturated 2% (pH 7.5)	0.5 ml	0.5 ml	-
Saliva	0.25 ml	-	-
I N C U B A T I O N at 38^{o}C for an hour			
TCA	1.25 ml	1.25 ml	-
Saliva	-	0.25 ml	-
C E N T R I F U G A T I O N at 3000 rev/min for 20 min			
Supernatant (into other clean and dry test tubes)	1.0 ml	1.0 ml	-
Solution of tyrosine	-	-	1.0 ml
NaOH 0.5 N	2.0 ml	2.0 ml	2.0 ml
The Folin reagent	0.5 ml	0.5 ml	0.5 ml

In 15 minutes colorimetry the content of the test tubes at red light-filter (670 nm) against the content of the control test tube (the content of the control test tube has to be poured into a distant cuvette).
Calculate the activity of enzyme by the equation:

$$X = \frac{2 \cdot 0.16 \cdot D_{exp} \cdot 1000}{D_{st} \cdot 60 \cdot 0.25} \text{ micromol/min} \cdot L$$

Where:

X - the activity of peptidyl-peptide hydrolase in micromol/min · L;
0.16 - the amount of micromoles of tyrosine in 1 ml of the standard solution of tyrosine;
0.25 - the volume of saliva
2 - the total volume of a sample
D_{exp} and **D_{st}** - the values of optical density of experimental and standard test tubes respectively
1000 - the coefficient for a recalculation per 1 L of saliva
60 - the time of the incubation in min.
RESULTS:

CONCLUSIONS:

Work 3. Isolation of mucin from saliva.

The PRINCIPLE of a METHOD

The method is based on the ability of mucin to form a sediment in the presence of acetic acid. The sediment is unsoluble in the excess of acetic acid. The detection of mucin is based on the phenomenon of a formation of oxymethylfurfurol which is transformed into the coloured product after its condensation with alpha-naphthol.

The COURSE of WORK

Measure out 2 ml of your own saliva into each of two test tubes. Add 4-5 drops of concentrated acetic acid to the both test tubes. The sediment of mucin is formed. One of the test tubes is used for the detection of a protein component in mucin, another - for the detection of an unprotein (carbohydrate) component of the mucin structure.

1. Detection of a protein component. Carry out **the biuretic reaction** with the sediment of mucin (see Lesson 3, p. 8).

2. Detection of an unprotein (carbohydrate) component. Add 2 drops of 1% alcohol solution of alpha-naphthol, mix, then add carefully 10 drops of concentrated sulfuric acid (**Keep the test tube in the inclined position when you add sulfuric acid). Do not mix the content of the test tube**!). You can see a red-violet ring on the border of two liquids.

RESULTS:

CONCLUSIONS:

Work 4. Detection of rhodanides (thiocyanates) in saliva.

The PRINCIPLE of a METHOD

The red coloured complex compound is formed as a result of interaction of CNS^- ions with iron ions.

A content of rhodanides is significantly increased in saliva of smokers that is due to the absorption of large amount of cyanides.

The COURSE of WORK

Add 2 drops of 2% HCL to 5 drops of saliva. Then add 2 drops of 1% $FeCl_3$. Red colour appears.

RESULTS:

CONCLUSIONS:

Work 5. Qualitative reaction for saliva amylase.

The PRINCIPLE of a METHOD

The method is based on the specificity of amylase activity. The substrate of amylase reaction (starch) and the products of the reaction (dextrins, maltose) give the specific colors with iodine (the Lugol solution).

The COURSE of WORK

Put 1 drop of 0.5% solution of starch on a Petry's dish. Then add 1 drop of saliva and mix the both drops. In 5 min add 1 drop of the Lugol solution.

RESULTS:

CONCLUSIONS:

BASIC QUESTIONS

1. Common mouth liquid (mixed saliva) and saliva of separate salivary glands. The peculiarities of their chemical composition, properties, and dependency on the salivation stimulation. The physiological role of saliva.
2. Gingival liquid. The peculiarities of its chemical composition.
Proteins of saliva. Mucin. Immunoglobulins, and other salivary glycoproteins
3. Salivary enzymes: amylase, lysozyme, peroxidase, phosphatase, peptidyl-peptide hydrolase etc. Their origin and physiological meaning.
4. Unprotein low-molecular organic components of saliva: glucose, carbonic acids, lipids, vitamins etc.
5. Inorganic components of saliva: cathion and anion composition of saliva. Calcium and phosphorus. Their distribution in stimulated and unstimulated saliva.
Thiocyanates of saliva.
6. Active reaction of saliva (pH of saliva). Salivary buffers. The reasons and significance of acidic shift of salivary pH.
7. Salivary factors in the protection of teeth. Acquired pellicle. Its chemical composition and physiological role.
8. Biochemical aspects of pathogenesis of tooth caries.
9. Tooth deposit (plaques) and a tooth stone (calculus). Their biochemical character and the role in the pathogenesis of caries and parodontitis.

Appendix

The EXAMINATION QUESTIONS on BIOLOGICAL CHEMISTRY.

Proteins, their types and classification. Amino acids as monomers of a protein molecule. Their kinds. Simple and complex proteins. Chemical composition of nucleoproteins, lipoproteins, glycoproteins, phosphoproteins, chromoproteins, metalloproteins.

The primary structure of proteins. Peptide bond. The methods of analysis of the primary structure. Inherent alterations of the primary structure of proteins. The examples of the alterations of protein properties under alterations of the primary structure.

The conformation of protein molecules (secondary and tertiary structures). Dependency of the protein conformation on a primary structure. The role of chaperons in the formation of unique conformation of proteins.

The kinds of intramolecular bonds in proteins. The role of three dimentional organization of a peptide chain in formation of the enzyme active centers. Conformation alterations during the protein functions. Denaturation of proteins.

The quaternary structure of proteins. Cooperative alterations of the protomer conformation. The examples of the structure and function of the oligomeric proteins (hemoglobin in comparison with myoglobin), allosteric enzymes, polyenzyme complexes.

Biological functions of proteins. Specific interactions of proteins with the ligands. The kinds of natural ligands and peculiarities of their interactions with the proteins (prosthetic groups, cofactors, protomers, substrates, transported substances, allosteric effectors).

Enzymes as biological catalysts. The mechanism of catalytic action of enzymes.

Specificity of enzyme action. The enzyme cofactors. The dependence of the velocity of enzyme reactions on substrate and enzyme concentrations, temperature, and pH. Kinetics of enzyme reactions. The principles of quantitative determination of enzyme activity. The units of enzyme activity.

Activation of enzymes. The enzyme inhibitors: reversible, irreversible, competitive, noncompetitive. The role of enzymes in metabolism.

Diversity of enzymes. The enzyme classification. Isoenzymes. Inherent (primary) enzymopathies. The reasons of their rise. The examples of inherent enzymopathies. Determination of enzyme activities for the diagnosis of the diseases (LDH, creatine kinase, Aspartate aminotransferase).

Regulation of enzyme activity. The enzyme modification: partial proteolysis, chemical modification, allosteric regulation. The examples of the metabolic ways regulated by these mechanisms. Physiological significance of enzyme activity regulation.

Nucleoproteins. The primary and secondary structure of DNA. Chemical composition of chromatin.

DNA replication: the mechanism, biological role, influence of antibiotics. Lesion of DNA. Mutations and repair of DNA.

The primary and secondary structure of RNA. The kinds of RNA: the peculiarities of their structure, size, form, cell location, and function. Biosynthesis of RNA (transcription). Post-transcriptional processing of mRNA.

Aminoacyl-tTRA synthesis. Substrate specificity of aminoacyl-tRNA-synthetases. The structure of ribosomes and polyribosomes. Ribosome function and the reaction sequence under polypeptide chain synthesis. Functions of tRNA and mRNA. The influence of antibiotics on the translation process.

Biochemistry of digestion. The enzymes of digestion of carbohydrates, lipids, proteins, nucleic acids. The enzyme composition of various digestive juices (saliva, gastric, pancreatic intestinal juices). The mechanism of action of digestive enzymes. Absorption of the digestion products.

The notion of catabolism and anabolism and their interrelation. Endergonic and exergonic reactions in metabolism. The notion of energy metabolism. ATP and the other high energy compounds. Basic pathways of ATP phosphorylation and ATP utilization.

NAD-linked dehydrogenases. The structure of oxidized and reduced forms of NAD. The main substrates of NAD-linked dehydrogenases.

NADH dehydrogenase and the electron carriers of an inner membrane of mitochondria.

FAD-linked dehydrogenases: succinate dehydrogenase, acyl-CoA dehydrogenase. The further way of electrons in a respiratory chain.

Oxidative phosphorylation. P:O ratio. The structural organization of a mitochondrial respiratory chain: the oligoenzyme complexes of a respiratory chain.

Coupling of oxidation to phosphorylation in mitochondria. Uncoupling respiration to phosphorylation.

Cytochomes, the common character of their structure. The complexes III and IY of a respiratory chain, their functions.

Oxidative decarboxylation of pyruvate and the tricarboxylic acid cycle (the TCA cycle, the Krebs cycle): the reaction sequence, relation to a respiratory chain, regulation.

Pyruvic acid: the ways of the formation and utilization in the body. The significance of these processes.

Acetyl CoA: the ways of the formation and utilization in the body. The significance of these processes.

The aerobic degradation of glucose: the reaction sequence, physiological significance. The role of aerobic and anaerobic splitting of glucose in a muscle under the muscle contraction. The role of aerobic splitting of glucose in the brain.

The anaerobic splitting of glucose: the reaction sequence, physiological significance. The role of anaerobic splitting in a muscle under the muscle contraction. The further metabolic fate of lactate.

The glucose biosynthesis (gluconeogenesis): the probable precursors, reaction sequence. The significance and regulation of gluconeogenesis. The glucose-lactate cycle (the Cory cycle).

The glycogen synthesis and its degradation till the glucose formation: the reaction sequence, physiological significance. The regulation of the phosphorylase activity and glycogen synthesis. The glycogen metabolism disturbances.

The pentose phosphate pathway of the glucose transformation (the pentose cycle, the oxidative pathway of the glucose transformation). The role of the pentose phosphate pathway.

The digestion and absorption of carbohydrates.

Glycoproteins and proteoglycans, their structure and functions. Glycosaminoglycans.

Regulation of the blood glucose concentration. The origin of blood glucose. The mechanism of the influence of insulin, glucagon, adrenaline, and cortisol on blood glucose concentration. Hypo- and hyperglucosemia, their reasons. The determination of the tolerance to glucose for the diabetes mellitus diagnosis.

The main lipids of the body, their structure. Classification of lipids.

Fatty acid oxidation. The sequence of the oxidation reactions. The connections of the fatty acid oxidation to a TCA-cycle and a respiratory chain. The physiological significance of the fatty acid oxidation. The lipid biosynthesis in the liver and in adipose tissue.

Food lipids. The recommended dietary allowance of fat, digestion of food lipids, absorption of the digestion products. Resynthesis of neutral fat in intestine cells.

Synthesis and degradation of phospholipids. The transport blood lipoproteins, specificity of their structure, composition, and function. CM, VLDL, LGL, HDL. Their role in the fat and cholesterol metabolism. Apoproteins, their classification, participation in the construction and metabolism of lipoproteins. The lipoproteinemias, their kinds and character.

Storage and mobilization of neutral fat in adipose tissue. Physiological significance of the fat storage. Transport and utilization of fatty acids formed under the lipid utilization.

Biosynthesis and utilization of ketone bodies. Ketonemia and ketonuria, their reasons .

Cholesterol, its kinds, functions in an organism. The cholesterol synthesis: the reaction sequence till the formation of mevalonic acid, the notion of the further stages, regulation of the biosynthesis. The role of lipoproteins in the cholesterol transport. The integration of the carbohydrate, lipid, and protein metabolism . The scheme of the glucose transformation to lipids. The role of the pentose phosphate pathway of glucose metabolism to the lipid synthesis.

The role of the basic components (lipids and proteins) in the structural organization and function of membranes. The mechanisms of transport of substances across a membrane.

Oxygen toxicity: the formation of reactive oxygen species (ROS). Physical and chemical oxidants (pro-oxidant factors). Membrane mrtabolism. Lipid peroxidation (LPO). The membrane protection against ROS toxic action: antioxidants, antioxidant enxymes.

Transamination of amino acids. The specificity of aminotransferases. The significance of the reactions of transamination. Transdeamination of amino acids. The reaction sequence, enzymes, biological significance.

Catabolism of amino acid. The formation and detoxication of ammonia. The urea biosynthesis, the reaction sequence, summary equation. Residual nitrogen of blood.

The phenylalanine and thyrosine metabolism. The thyrosine utilization for the catecholamine, melanin and thyroxine synthesis. The degradation of thyrosine up to fumarate and oxaloacetate. The inherent disturbances of phenylalanine and thyrosine metabolism.

Decarboxylation of amino acids and their derivatives. The formation of biologically active amines with the participation of enzymes monoaminooxidase (MAO) and diaminooxidase (DAO).

The formation of creatine and creatine phosphate. The role of creatine phosphate in energy metabolism. Creatine kinase, its isoenzyme forms. A diagnostic significance of determination of creatine, creatinine and creatine kinase activity.

The protein digestion: the specificity of proteolytic enzymes. Absorption of the dietary amino acids. Essential and non-essential amino acids.

A purine nucleotides biosynthesis: the origin of C- and N-atoms of a purine ring and the role of 5'-phosphoribosyl-1-pyrophosphate (PRPP). The catabolism of purine nucleotides . Hyperuricemia and podagra (gout).

The biosynthesis and catabolism of pyrimidine nucleotides. The biosynthesis of deoxyribonucleotides.

The role of hormones in a system of regulation of metabolism and organ functions. The classification of hormones according to their chemical structure: protein and peptide hormones, derivatives of amino acids, steroid hormones (gluco- and mineralocorticoids, androgens, estrogens). The central regulation of the endocrine system: the role of liberins, statins, and hormones of adenohypophysis.

The mechanism of transference of hormone signal in a cell.

The most important mechanisms of detoxication of poisoning substances in the liver: a microsomal oxidation, the reactions of conjugation.

The degradation of heme. the formation of bilirubin and bilirubin glucuronide. The ways of the bilirubin and other bile pigments excretion. The significance of bile pigments determination for the diagnosis of the liver diseases, bile duct obstruction and blood hemolysis.

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The most important proteins of extracellular matrix: collagen and elastin. Post-translational modifications of collagen, the formation of fibrillar structures. The participation of vitamin C in the collagen synthesis.

Proteoglycans, their structure and functions.

The chemical composition of the brain. Lipids and proteins of the brain. Neuropeptides and amino acids of the brain. Energy metabolism. The significance of aerobic split of glucose. The role of glutamic acid in the brain.

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Proteoglycans, their structure and functions.

Chemical composition of mineralized tissues. Apatites. The peculiarities of the structures and properties of different kinds of apatites.

The character of the mineral composition of tooth components (enamel, dentin, cementum).

The main bone and tooth proteins. Collagen. Processing of collagen.

Uncollagen proteins of bone tissue (proteoglycans, glycoproteins, osteocalcin, osteonectin. The specific bone enzymes.

Unprotein organic components of bones and teeth. The role of citrate in bone metabolism.

The biochemical character of pulp.

Mineralization of bone and tooth tissue. The participation of collagen, uncollagen proteins, and GAG in formation of hydroxyapatite crystals.

Regulatory factors of osteogenesis (mitogens, morphogens, chemotactic substances, antagonists of mitogens and morphogens).

Hormones controlling the processes of osteogenesis and decomposition of bone tissue. Parathyroid hormone and calcitonin. Their influence on metabolism of calcium and phosphorus.

Vitamins of D-group. Provitamins D. The endocrine system of vitamine D and its role in the maintenance of calcium homeostasis.

The influence of a diet on the state of teeth. The role of dietary carbohydrates, proteins, microelements, and vitamins in the maintenance of a normal physiological state of teeth.

Undigested carbohydrates of foodstuffs. Substitutes of dietary sugars.

Microelements: fluorine and strontium, their role in mineralization of bone and tooth tissue.

Pathological states due to insufficient and excessive intake of fluorine and strontium.

Common mouth liquid (mixed saliva) and saliva of separate salivary glands. The peculiarities of their chemical composition, properties, and dependency on the salivation stimulation. The physiological role of saliva.

Gingival liquid. The peculiarities of its chemical composition.

Proteins of saliva. Mucin. Immunoglobulins, and other salivary glycoproteins

Salivary enzymes: amylase, lysozyme, peroxidase, phosphatase, peptidyl-peptide hydrolase etc. Their origin and physiological meaning.

Unprotein low-molecular organic components of saliva: glucose, carbonic acids, lipids, vitamins etc.

Inorganic components of saliva: cathion and anion composition of saliva. Calcium and phosphorus. Their distribution in stimulated and unstimulated saliva. Thiocyanates of saliva.

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LABORATORY MANUAL
on BIOCHEMISTRY

ЛАБОРАТОРНЫЕ РАБОТЫ ПО БИОХИМИИ

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МАКЕТ И ТЕХНИЧЕСКОЕ РЕДАКТИРОВАНИЕ - ЗАГОСКИН П.П.,
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