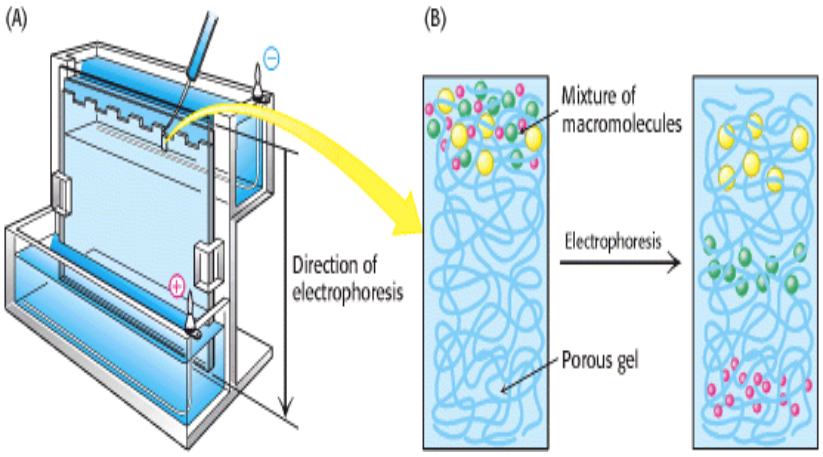
MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA
STATE UNIVERSITY OF MEDICINE AND PHARMACY
"NICOLAE TESTEMIŢANU"

GAVRILIUC Ludmila, TAGADIUC Olga

BIOCHEMISTRY

GUIDE FOR LABORATORY LESSONS

(Methodic material)



Chişinău
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DESCRIEREA CIP A CAMEREI NAŢIONALE A CĂRŢII

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Introduction

A central function of the Clinical Biochemistry or Chemical Pathology laboratory is to provide biochemical information for the management of patients. Biochemical tests are used extensively in medicine, both in relation to diseases that have an obvious metabolic basis (diabetes mellitus, hypo- or hyperthyroidism, etc.) and those in which biochemical changes are a consequence of the disease (malabsorption, renal failure, etc.).

Biochemical tests are used in diagnosis, prognosis, monitoring and screening. Tests used primarily for diagnosis may also provide prognostic information and some are used specifically for this purpose. A major use of biochemical tests is to follow the course of an illness and to monitor the effects of treatment. Biochemical tests can also be used to detect complications of treatment and are extensively used to screen for possible drug toxicity, particularly in trials, but also in some cases when a drug is in established use. Biochemical tests are widely used to determine whether a condition is present subclinically. The best-known example is the mass screening of all newborn babies for phenylketonuria, which is carried out in many countries.

This book includes the Program, Systematic Indications and Biochemical indices (parameters) of the blood serum, urine and saliva. Systematic Indications have been composed according to the Program and the new modern methods used in clinical laboratories. Also in this book we included the special biochemical information (Special Biochemistry) for the students of Dentistry department.

BIOCHEMISTRY PROGRAM

Introduction

Subject of Biochemistry. Biochemistry importance for medicine. The greatest discoveries in Biochemistry in the XX^{th} century. Peculiarities of the alive matter, levels and methods of its study.

CHAPTER I. Protein structure

Amino acids: classes, structure, amphoteric and electro-chemical properties. Utility of amino acids in pharmacy and therapy. Peptides and proteins, pro-tein's classifications. Simple and conjugated proteins, brief characteristics. Peptide bond, genetic changes of the amino acids sequence, hereditary proteinopathies (molecular diseases). Levels of proteins organization: primary, secondary, tertiary, quaternary structures and domains. Analysis of protein conformation: Sanger's, Edman's methods, sequencing of peptide fragments, X-ray crystallography, ultraviolet light spectroscopy, nuclear magnetic resonance. Properties of proteins: molecular mass, thermolability, solubility, amphoteric and charge. Structure-function relationships in proteins, selective interaction with ligands, ligand's recognition. Simple proteins: histones, albumins, globulins, their functions and physico-chemical characteristics. Conjugated (complex) proteins: nucleo-, chromo-, phospho-, glyco-, metallo-, lipoproteins (brief characteristics). Hemoglobin, collagen, immunoglobulins, Ca^{2+}-binding proteins: peculiarities of structure and function. Peculiarities of amino acid composition in collagen, structure, properties and types of collagens. Proteins solutions: types, properties and peculiarities. The purification and analysis of proteins: salting, dialysis, ultracentrifugation, chromatography, electrophoresis.

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Molecular diseases associated with abnormal amino acid metabolism (molecular diseases): Hartnup's disease, phenylketonuria, tyrosinemia, alkaptonuria, maple syrup urine disease, etc.

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Parathyroid hormones: structure, biosynthesis, mechanism of action, release, transport, metabolic effects. Interrelation of parathyrin with other compounds, that regulate calcium and phosphate metabolism. Role of 1,25-dihydroxycholecalciferol for parathyroid hormone action. Disorders of parathyroid glands functions.

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Blood enzymes: classifacation, practical utility. Indicator (organ specific) blood enzymes in liver and heart diseases. Mechanism of enzymes' level increasing in the blood serum.

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Acid-base equilibrium: buffer systems and physiological mechanisms of buffering. Disorders of acid-base equilibrium of the blood: alkaloses and acidoses, types, diagnostics and mechanisms of buffering correction.

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Chemical components of the blood as drugs (albumin, gamma-globulin, interferon, etc.).

CHAPTER X. Stomatological biochemistry: connective, bone and dental tissues. Biochemistry of saliva

Types and functions of the connective tissue. Main components of the connective tissue: cells (representatives, biological role) and extracellular matrix (structural elements and their biological role).

Fibrilar proteins of the connective tissue (collagen, elastin): the particularities of chemical composition, structure and properties. The peculiarities of collagen biosynthesis, post-synthetic (post-translation) modifications and degradation.

Glucosamine glycans (heteropolysaccharides or mucopolysaccharides) of the extracellular matrix of the connective tissue: main representatives, their structure, biological role. Proteoglycans and glycoproteins, their biological role.

Physiological and pathological modifications of the connective tissue metabolism: age-dependent modifications, acquired disorders (hypovitaminosis C, diabetes mellitus). Genetic disorders: collagenoses, mucopolysaccharidoses, biochemical manifestations.

Structure and role of the bone tissue. The cells of the bone tissue: the representatives, their biological role. Organic compounds of the bone tissue: fibrilar and non-fibrilar proteins, proteoglycans, carbohydrates, lipids.

Mineral components of the bone tissue: main anions, cations, their structural organization, minor ions, olygoelements. Appatite: components, structure and properties.

Bone formation and resorbtion: cells, that participate in these processes, the stages and mechanisms of the processes. Regulation of the bone tissue metabolism in the human organism. Role of the thyroid and parathyroid glands' hormones and vitamins D and C for the bone metabolism. Pathology of the bone metabolism: age-dependent, osteoporosis.

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Biochemical processes that take place during the dental caries development.

Periodontium: the main chemical compounds, their characteristics and biological role. Physiological biochemical processes

of the periodontal tissues. Biochemical processes that take place in the pathology of the periodontium, role of the inflammation mediators and immune system. Dental plaque: chemical composition, main biochemical processes in the plaque, the correlation with the periodontal pathologies (gingivitis, parodontitis, parodontosis).

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Saliva: sources, chemical composition, physico-chemical properties and role. Clinical diagnostic means of the salivary components for medicine and dentistry.

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Biological role of water in the human organism (functional and structural one). Physico-chemical properties of water. Water as solute for natural substances and xenobiotics.

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Conjugation of xenobiotics: I type (glucuronide, sulphate, acetyl conjugations) and II type (a prior activation of the substrate-xenobiotic) conjugation. Enzymes involved in the metabolism of xenobiotics in the human organism. Metabolism of drugs and toxins (poisons).

Microsomal oxidation of substances and xenobiotics (monooxigenase and reductase chains). Role of cytochrome P_{450} in this process. Drugs-activators of cytP_{450} production in the organism. Conditions for drug metabolism. Biotechnology of drugs. Bio-

chemical methods used in drug standardization and quality control.

Photosynthesis and characteristics of the photosynthetic structures – chloroplasts. Chlorophyll (major structural characteristics) and its role in photosynthesis.

Photosynthesis basis: mechanism of the light (photochemical) and dark (synthetic) stages. Cyclic and noncyclic transport of electrons in the photochemical stage of the photosynthesis. Water photolysis, Hill reaction. The role of photosystems I and II in the photosynthesis.

Photophosphorylation (ATP production in chloroplasts) and oxidative phosphorylation in mitochondria, common characteristics and differences. The role of the Calvin cycle in the synthesis of polysaccharides. Photosynthesis as a way for the buildup of the products with therapeutic properties (alkaloids, flavonoids, polyphenols, steroids, etc.).

CHAPTER I
Proteins

Proteins of the human organism are linear, unbranched polymers constructed from 20 different L,α-amino acids linked by peptide bond and encoded in the DNA of the genome. Proteins are involved in enzymatic catalysis, transport and storage of small molecules and ions, systematic movements, immune defense, hormonal regulation, control of genetic expression, etc.

Every protein in its native state has a unique three-dimensional structure that is referred to as its conformation. Protein structure can be classified into four levels of organization: primary, secondary, tertiary and quaternary structures. Based on conformation proteins are divided into two classes: globular and fibrous proteins. Based on their composition proteins are divided into simple and complex (conjugated) proteins: hemo-, glyco-, lipo-, phospho-, nucleo- and metaloproteins.

Proteins are large molecules, with molecular weight ranging from 10 to 50 kDa for singlechain proteins and from 150 to 200 kDa for multichain (oligomeric) proteins. Proteins have amphoteric properties, are optically active and soluble.

Separation of a protein from other proteins or from smaller molecules is achieved by applying a combination of several methods based on properties such as molecular size, electrical charge, solubility and specific binding of the protein to a specific substance. Separation procedures are salting, dialysis, ultracentrifugation, chromatography, and electrophoresis. Analyses of proteins begin with determination of the amino acid composition by hot acid hydrolysis and ion exchange chromatography separation. The amino acid sequence is identified by Edman reaction or sequencing of peptide fragments. The secondary, tertiary and quaternary structures are studied by X-ray crystallography, ultraviolet light spectroscopy, nuclear magnetic resonance, etc.

LESSON 1
Introduction to Biochemistry. Biochemistry in medical and pharmaceutical education. Amino acids

Experiment 1. Biuret reaction (Piotrovskii).

Method principle. Peptidic bonds react with $CuSO_4$ in alkaline conditions to form red-violet complex compounds

Procedure: Mix the reagents according to the table.

№	Reagents	Test tube
1	Ovalbumine, 1%	5 drops
2	NaOH, 10%	5 drops
3	$CuSO_4$, 1%	2 drops
Shake thoroughly		

Conclusion:

Experiment 2. Ninhydrin reaction.

Method principle. Ninhydrin reacts with α-amino groups of amino acids and proteins to form a blue-violet compound.

Procedure: Mix the reagents according to the table.

№	Reagents	Test tube
1	Ovalbumine, 1%	5 drops
2	Ninhydrine 0,5%	5 drops
Boil the solution for 1-2 min		

Conclusion:

Experiment 3. Xantoproteic reaction (Mulder).

Method principle. Boiling the solution of the aromatic amino acids with concentrated HNO_3 produces the nitrates' yellow-colored complex. The addition of alkali changes the coloured complex to orange.

Procedure: Mix the reagents according to the table.

№	Reagents	Test tube
1	Ovalbumine, 1%	5 drops
2	HNO_3 concentrated	5 drops
Mix and boil for a few minutes		
Cool the solution		
3	NaOH, 20%	10 drops

Conclusion:

Experiment 4. Unoxidized sulfur test (Fol).

Method principle. This is a test for the presence of sulfurcontaining amino acids. The protein or amino acid is boiled with strong alkali to split out sulfur as sodium sulfite (Na_2S), which is detected by addition of sodium leaden (Na_2PbO_2), which causes the formation of brown to black lead sulfide (PbS).

The sequence of reactions may be represented as follows:

1. CH_2-SH
   |
   CH-NH_2 + NaOH → $+t^o$
   |
   COOH
   cysteine

   CH_2-OH
   |
   CH-NH_2 + Na_2S + H_2O
   |
   COOH

1. $Na_2S + N_2PbO_2 + 2\ H_2O \rightarrow PbS\downarrow + 4\ NaOH$
   black colour

This reaction is a test for cystine and cysteine, but not for methionine, which contains firm-binded sulfur.

Procedure: 5 drops of protein solution (1%) and 5 drops of Fol's solution carry into test-tube, mix and boil for 1-2 min.

Conclusion:

Self-training questions

1. Biochemistry, role of the biochemical investigations for medicine.
2. Biological role of proteins.
3. Chemical structures and classifications of amino acids.
4. Polypeptide theory of the protein structure. "N"-end and "C"-end of amino acids of the polypeptides.
5. Peptides and their therapeutic utility (anserine, carnosin, glutathione, anhiotensin, kinins, secretin, endothelin, hormons).
6. Antibiotics of protein nature.

Self-testing questions:

1. Write and name the following peptides: his-phe-cys; tyr–pro-arg. Which of the coloured reactions will be positive or negative?
2. Is the compound a protein? Explain, please.
   a) its reaction with ninhydrine is negative and with biuret reagent is positive;

b) it gives the positive Fol reaction.

3. Is it possibly to identify an amino acid in solution only by ninhydrin reaction? Explain.
4. A mixture contains the amino acids: gly, ala, glu, lys, arg, ser. Which electrode each of them will move to at pH= 6,0? Explain.
5. Which electrode histidine will move to at pH=4.0 and pH=12.0? Explain.

LESSON 2

Proteins: chemical structure and biological role. Chromatographycal method for identification of amino acids

Experiment 1: Chromatographycal identification of the amino acids.

Method principle: Amino acids have different distributive coefficient in water and organic solvent (butanol). Amino acid's velocity of migration is directly proportional to their solubility in butanol.

Procedure:

Mark the take-off line on the chromatographycal paper. Fall a drop of amino acids mixture in the middle of the starting line (the diameter of the spot must be less then 5 mm), dry the spot. Introduce the chromatographycal strip in a vessel with the solvents mixture (water-butanol). The strip must be in vertical position and not touch the vessel.

Take out the strip after 90 min, mark the distance passed by the solvent and dry the chromatogram (10 min at 70-100° C). Pass the strip through 0,1-0,2% ninhydrin solution and dry it at 100º C. On the strip were develop several coloured spots.

Measure the following distances:

a - from the take-off line to the middle of each spot;

b - from the take-off line to the solvent's front.

Calculation:

Calculate the distributive coefficients for each amino acid using the next formula:

$R_f = a/b.$

$R_{f1} = a_1 : b =$ $R_{f2} = a_2 : b =$ $R_{f3} = a_3 : b =$

Conclusion:

Clinical-diagnostic means: This method allows to determine quality and quantity of amino acids in different biological samples (blood serum, urine, etc.). Assays for amino acids content and composition are indispensable in clinical diagnosis of numerous inborn errors of metabolism, liver and kidneys diseases, etc.

Self-training questions

1. Protein levels of organization: primary, secondary, tertiary and quaternary structures. Specific chemical bonds for stabilization of each structure. Domains, folding and refolding of proteins molecules. Biological active peptides.
2. Analysis of protein conformation (determination of the amino acid composition, N- and C-terminal amino acids, the amino acid sequence, secondary, tertiary and quaternary structures).
3. Proteins classification.
4. Simple proteins: general characteristic, structural peculiarities.
5. Collagen: peculiarities of amino acids composition and structure.
6. Conjugated (complex) proteins: nucleo-, phospho-, lipo-, glyco-, chromo- and metalloproteins; their characteristics.
7. Ca^{2+}-binding proteins.

Self-testing questions:

1. Fill in the table the bonds that are characteristic to each structural level of the protein.

Primary	Secondary	Tertiary	Quaternary
Peptidic bond between α-amino and α-carboxyl groups			

2. Why histones are basic proteins and albumins – acidic? What are their charges (positive or negative) at physiological pH?
3. Write down the formulas of untypical amino acids that are present in the collagen primary structure. Which are their specific functions in this protein?
4. How Ca^{2+} ions are attached to the Ca^{2+}-binding proteins?

LESSON 3
Physico-chemical properties, purification and analysis of proteins

Experiment 1. Dialysis of proteins.

Method principle. Dialysis (from the greek *dialisis* – separation) is a method of separation based on the molecular size. Small molecules can be removed from solutions because they pass through semipermeable membranes. Proteins are larger than the pores of the membrane and don't cross it.

Procedure: Mix in a retort 20 ml of ovalbumin and 20 drops of $(NH_4)_2SO_4$ solution (saturated one). Put the solution in a cellophane bag and immersed it into water in a glass. In 60 min pull out the bag and transfer the solution into a test-tube. Identify protein and $(NH_4)_2SO_4$ in both solutions and write the result in the table.

Solution	Are proteins present?	Are SO_4^{2-} ions present?
From the bag		

From the glass		

Note:

a) The presence of protein is determined by biuretic reaction.

Conclusion:

b) The presence of $(NH_4)_2SO_4$ is determined with $BaCl_2$ solution. Transfer 5 drops of solution from the glass into the test-tube and add 3-4 drops of $BaCl_2$, 5%. Formation of insoluble $BaSO_4$ certified the presence of SO_4^{2-} ions.

$$BaCl_2 + (NH_4)_2SO_4 \rightarrow BaSO_4\downarrow$$

Conclusion:

Experiment 2: Protein precipitation (sedimentation).

Protein can be precipitated by denaturation. Proteins are denaturated by action of physical and chemical factors such as heat, radiation, concentrated acids or bases, etc. Denaturated proteins loose its three dimensional structures and therefore are insoluble.

a) *Method principle:* Proteins are precipitated by metals because they form insoluble complex.

Procedure:

№	Reagents	Test-tube	
		1st	2nd
1	Ovalbumin, 1%	5 drops	5 drops
2	$CuSO_4$, 1%	2 drops	–
3	$Pb(CH_3COO)_2$, 5%	–	2 drops

Conclusion:

b) *Method principle:* Proteins are precipitated by acids.

Procedure: Put in the 1st test–tube 10 drops of HNO_3 concentrated and in the 2nd test-tube – 10 drops of H_2SO_4 concentrated. Warm up the test-tubes to 45° C and ***carefully*** add 10 drops of ovalbumin in each ones.

Conclusion:

Experiment 3: Separation of albumins and globulins from ovalbumin solution.

Method principle. Adding bivalent salts, such as ammonium sulfate, to a solution of proteins mixture will precipitate some proteins at a given salt concentration, but not the others. The procedure is named as salting-out. Globulins are salted out by semisaturated solution of ammonium sulfate and albumins – by the saturated solution.

Procedure: To 20 drops of undiluted solution of ovalbumin add 20 drops of saturated $(NH_4)_2SO_4$ solution. The solution will be semisaturated and therefore globulins will precipitate from it. In 5 min incubation at the room temperature this solution filtrate using a moisten paper-filter. After that add crystalline ammonium sulfate to the filtrated solution and mix it until saturation and albumins precipitation. The solution is filtrated again. Carry out the biuretic reaction with the filtrate-solution, to prove the absence of proteins.

Conclusion:

Self-training questions:

1. Physical and chemical properties of proteins: molecular mass, protein solubility, colloidal, acid-basic (amphoteric), osmotic.
2. Protein solubility. Correlation between solubility of a protein and its amino acids composition and conformation.
3. Protein colloidal solutions: properties, factors affecting the protein solubility. Variants of colloidal solutions: gel, zol, xerogel.
4. Electric charge of the protein. The isoelectric point and isoelectric state of proteins.
5. Denaturation of proteins. Factors causing denaturation. Coagulation of proteins.
6. Methods used for isolation and purification of proteins: dialysis, ultrafiltration, salting-out, electrophoresis,

chromatography, gel-filtration.

Self-testing questions:

1. Why is the iso-electric point of the majority proteins in acidic medium, except histones?
2. Which is electrode for histones migration loading in the middle of the strip and buffer solutions with pH 4,0 and 9,5?
3. What happenes to a protein molecule in hydrolysis, coa gulation, and denaturation?
4. Why are alcohol, iodine solution used for disinfecting the surgical field?
5. What methods of protein purification and analysis are based on: a) molecular size; b) molecular charge; c) specific affinity binding?

CHAPTER II
Enzymes

Enzymes are biological catalysts. They are neither consumed nor produced during the course of a reaction. They speed up reactions that would ordinarily proceed, but at a much slower rate in their absence. In other words, they do not alter the equilibrium constants of reactions they catalyze.

Enzymes are invariably globular simple or complex (conjugated) proteins. If the enzyme is a simple protein, only the native conformation of a protein is required for activity. If the enzyme is a conjugated protein, activity will depend upon both the protein's conformation and the availability of cofactors. As cofactors for enzymes may be used metals (particularly transition elements), organic compounds (nucleotides, hem, glutathione, etc.) or

active forms of vitamins. If the cofactor is strongly bounded to the enzyme proteinic portion it is called prosthetic group. If it can reversibly dissociate from the enzyme proteinic portion, it is called coenzyme.

Each enzyme has an active site that turns the given reactants, or substrates, to products. The enzyme decreases the energy of activation of the reaction so a certain proportion of substrate molecules are sufficiently energized to reach the so-called transition state, in which there is a high probability that a chemical bond will be made or broken to form a product.

The rate of a reaction depends on the substrate and enzyme concentrations, pH and temperature conditions, presence of different regulatory factors (allosteric modulators, acceptors, products of reaction, hormones, etc.).

Enzymes activity is inhibited by compounds that are structurally related to the substrate and capable of preventing the formation of an enzyme-substrate complex (competitive inhibition), or alternatively compounds that can modify the active site (non-competitive inhibition), or the conformation of the whole molecule (allosteric inhibition).

The assay of enzymes in the serum of peripheral blood is used as an important aid to diagnosis. The level of activity of a number of enzymes is raised in different pathological conditions as a result of cells damage. The rate of enzyme activity depends on the composition of the cells and the state of the tissues.

LESSON 4
Enzymes nature and structure. Classifications. Vitamins as coenzymes. Mechanism of enzyme catalysis

<u>Experiment 1:</u> Examination of saliva α-amylase's chemical properties.

For execution of this task it is necessary to know:

a) α-amylase hydrolyzes starch and glycogen to maltose through a series of dextrins, which may be coloured by iodine. Maltase acts on maltose to form glucose only. Glucose in alkaline solution is a very powerful reducing agent. This reducing action of glucose in alkaline solution is utilized for both the qualitative and quantitative determination of glucose. Reagents containing Cu-ions are the most commonly used (Trommer reagent).

Trommer chemical reaction:

1. $Cu_2SO_4 + 2\ NaOH \rightarrow Cu(OH)_2 + Na_2SO_4$
2. $R\text{-}COH + 2Cu(OH)_2 \rightarrow R\text{-}COOH + 2CuOH + H_2O$
   aldehyde (glucose) — blue colour — acid — cuprous hydroxide (yellow)
3. $2CuOH \rightarrow Cu_2O + H_2O$
   yellow — red precipitate

b) pepsin is a peptidase, which hydrolyzes α-amilase as any other protein in the strong acidic medium (HCl).

c) Starch with iodine forms a blue-coloured complex.

Procedure:

Step 1		
Reagents (ml)	**Control**	**Test**
1. Saliva (α-amylase)	1	1
2. Pepsin (in HCl solution)	–	1
3. Solution of HCl	1	–
Incubation: at temperature +38° C, 10 min		
Step 2		
4. Solution of starch	1	1
Incubation: at temperature +38° C, 10 min		
5. Iodine solution (drops)	1	1
Colour with iodine:		

Conclusion (about saliva α-amylase nature):

Experiment 2: Vitamin B_2 identification.

Method principle: Solution of the oxidized form of riboflavin is yellow-coloured. At the beginning of the reduction reaction the colour turns rose and the solution becomes colourless when all the molecules are reduced.

Procedure:

№	Reagent	Test-tube
1	Riboflavin solution	10 drops
2	Concentrated HCl	5 drops
3	Metallic Zn	1-2 granules

Note:

H_2 gas is eliminated at the beginning of the reaction.

Conclusion:

Experiment 3: Vitamin PP identification.

Method principle: When nicotinamide and nicotinic acid are treated with copper acetate at a high temperature, the blue sediment of the copper salt of niacin is formed.

Procedure:

№	Reagent	Test-tube
1	Vitamin PP solution	20 drops
2	Boil the solution so the turbidity will disappear	
3	Solution of $Cu(CH_3COO)_2$,5%	20 drops
4	Boil the mixture	
5	Cool the mixture in a glass of cold water	

Conclusion:

Self-training questions:

1. Enzymes: definition, function. Properties of the enzymes, biological role.
2. Enzymes nature, evidences of proteic nature. Structure of enzyme: active and allosteric sites. Proenzymes.

3. Simple and conjugated enzymes. Isoenzymes.
4. Cofactors for enzymes: coenzymes and metal ions. Vitamins as coenzymes.
5. Structure and biological role of vitamins B_1, B_2, B_6, B_{12} (only notions about its structure), C, PP, biotin, pantothenic and folic acids.
6. Vitamins as pharmaceutic preparations, use in medicine.
7. Mechanism of enzyme catalysis.
8. Enzyme nomenclature, classifications. Systematic classification: code number, overview of classes.

Self-testing questions:

1. Is it possible to isolate the active site in functional state from an enzyme structure? Explain.
2. Which is the difference between the active center of the simple and conjugated enzymes?
3. Name one reaction where vitamins B_1, B_2, B_6, B_{12}, C, PP, biotin, folic and panthotenic acids act as coenzymes.
4. What are the consequences of hypo- and avitaminoses?
5. Determine the class and subclass of the α-amylase and pepsin.

LESSON 5
Regulation of the enzyme activity. Measures of the enzyme activity. Factors affecting the rate of the enzyme action

Experiment 1: General properties of enzymes.

Method principle: Succinate dehydrogenase (SDH, EC 1.3.99.2) catalyzes the following oxido-reduction reaction of the tricarboxilic acid cycle (Krebs cycle):

$$\underset{\textit{succinate}}{HOOC - CH_2 - CH_2 - COOH} + FAD^+ \xleftrightarrow{SDH}$$

$\leftrightarrow$ HOOC – CH = CH – COOH + $FADH_2$
fumarate

$FADH_2$ + oxidazed H^+ - acceptor $\leftrightarrow$ FAD^+ + reduced H^+ - acceptor
methylenazure (blue) *methylenazure (colorless)*

Procedure:

a) Enzyme specificity:

№	Reagents	Test-tubes		
		1	2	3
1	Tissue homogenate	2 ml	2 ml	2 ml
2	H_2O dist	1 ml	–	–
3	Succinate solution, 1%	–	1 ml	–
4	Malate solution, 1%	–	–	1 ml
5	Methylenazure, 1%	3 drops	3 drops	3 drops

Incubation for 5-10 min at 38°C			
Notice the colour of the solutions			

Conclusion:

b) Specificity of α-amylase:

N	Saliva α-amylase (1:5)	Substrate, 1% (10 drops)	Trommer reaction	Starch hydrolysis	Conclusion
1.	10 drops	Starch			
2.	10 drops	Sucrose			

c) Termolability of α-amylase:

N	α-amylase (1:5)	t°C=5 min	Substrate (starch, 1%)	Colour with I_2	Starch hydrolysis	Conclusion
1.	10 drops	100° C	10 drops			
2.	10 drops	38° C	10 drops			

d) Enzyme competitive inhibitions:

№	Reagents	Test-tubes

		1	2
1	Tissue homogenate	2 ml	2 ml
2	Succinate solution, 1%	1 ml	1 ml
3	Malonate solution, 1%	–	1 ml
4	Methylenazure, 1%	3 drops	3 drops
5	Paraffin liquid	3 drops	3 drops
6	Incubation for 5-10 min at 38°C		
7	Notice the colour of the solutions		

Conclusion:

e) Modification of α-amylase activity (factors affecting rate of enzyme action: activators and inhibitors):

N	α-amylase (1:5) (saliva) drops	Substrate, starch, 1%	Modificator (2 drops)	Colour with I_2	Conclusion
1.	10	5 drops	NaCl, 1%		
2.	10	5 drops	$CuSO_4$, 1%		
3.	10	5 drops	H_2O dist		

Experiment 2: Blood serum or urinary α-amylase assay with stabile starch substrate (Caravei method).

Method principle: α-Amylase breaks down starch to products (dextrines) that don't give a positive iodine reaction. α-Amylase activity is proportional to the rate of starch break down.

Procedure:

Use graduated test-tubes (10ml).

№	Reagents	Test-tubes	
		Test	Control
1	Starch solution (0,004 g/ml)	1 ml	1 ml
2	Heat the solution at 37°C	5 min	–
3	Blood serum (or urine)	0,02 ml	–
4	Heat the solution at 37°C	7,5 min	–
5	Iodine solution	1 drop	1 drop
6	Blood serum (or urine)	–	0,02 ml
7	H_2O dist	up to 10 ml	up to 10 ml

The extinction (optical density) of the solutions are measured immediately using dest water as a comparative solution, 10 mm cuvettes and red filter (λ = 630-690 nm).

Calculation: α-Amylase activity is calculated by the formula:

A = (Ec-Et) / Ec·0,0004·8·50000 = (Ec-Et) / Ec·160 g/l·h=

Where:

Ec – extinction of the control solution

Et – extinction of the test solution

0,0004 – starch amount used for the assay

8 – the coefficient for recalculation of time to an hour of incubation (60 min/7,5 min = 8)

50 000 – the coefficient for the recalculation of serum or urine quantity to 1 liter (1000 ml/0,02 ml = 50 000)

Normal values:

in blood serum: 12-32 g/h·l

in urine: 20-160 g/h·l.

Conclusion:

Clinical–diagnostic significance: α-amylase's activity increases in blood serum and/or urine in pancreatic (acute pancreatitis) and parotid glands diseases.

Self-training questions:

1. Properties of the enzymes: thermolability, specificity, etc. Temperature and pH influence on enzyme activity and reaction rate.
2. Kinetics of the enzyme reactions. Enzyme-substrate complexes. Dependence of enzymic reaction rate on substrate and enzyme concentrations.
3. Enzymes specificity: absolute, relative, stereochemical.
4. Enzyme inhibition: competitive (isosteric), noncompetitive, uncompetitive, allosteric, by end product (retroinhibition). Reversible and irreversible inhibition.

Therapeutical application of competitive inhibition (sulfanilamides).

5. Enzyme activation: proenzymes proteolysis, covalent modification, quaternary auto-folding, allosteric.
6. Organization of multi-enzymic systems. Compartmentalization.
7. Tissue diversity of enzymes. Organospecific enzymes. Genetic diversity of enzymes -iso-enzymes (lactate dehydrogenase, creatine kinase). Usage of enzymes and iso-enzymes assay in clinical diagnostics.
8. Practical utility of enzymes. Immobilized enzymes. Enzymodiagnostics and enzymotherapy.
9. Purification and analysis of enzymes. Affinity chromatography. Enzyme activity assay. Measures of enzyme activity (units of enzyme activity, specific activity, katal).

Self-testing questions:

1. How to prove that enzyme specificity is depended only on apo-enzyme?
2. How can tissue diversity of the enzymes be explained?
3. Name at least one organospecific enzyme (or iso-enzyme) for pancreas, liver, heart, brain and kidneys.
4. "Pancreatin" is a medicine contained enzymes of the pancreatic gland. How to find out which one?
5. Which are the standart conditions for enzyme activity assay?

LESSON 6
Colloqium: Proteins and enzymes

1. Chemical structure and classifications of proteogenic amino acids.
2. Protein primary structure, the peptide bond.
3. Secondary and tertiary structures of proteins: their cova-

lent and noncovalent specific bonds, conformation – function correlations.

4. Quaternary structure of proteins. Co-operative movement of protomers (on the example of hemoglobin).
5. Protein folding, conformational changes correlated with protein activity.
6. Proteins solubility, factors that influence it. Protein colloi dal solution, properties and factors for its stability.
7. Amphoteric and electro-chemical properties of proteins. Isoelectric state and point.
8. Protein coagulation and precipitation in solution. Salting-out.
9. Denaturation (denativation) and renaturation (renativati-on) of proteins.
10. Proteins classification. Simple and complex (conjuga-ted) proteins. Characteristics and functions of proteins.
11. Collagen: peculiarities of amino acid composition, struc-ture and properties.
12. Enzymes. Classifications and nomenclature of enzy-mes. Functions of the enzyme.
13. Structural and functional organization of enzymes. Active and allosteric sites, nature and role. Notions of substrate and allosteric modulator (effector).
14. Simple and complex enzymes. Notions of cofactor, co-enzyme and prosthetic group.
15. Water-soluble vitamins as coenzymes. Structure and metabolic function of vitamins B_1, B_2, B_6, PP, C, biotin, folic and pantothenic acids.
16. Provitamins and antivitamins: notions, examples and role. Utility in medicine (sulphanilamides, etc.).
17. Mechanism of enzymic action, enzyme kinetics. Influ-ence of temperature, pH, substrate and enzyme concentrations on the reaction rate.
18. Regulation of enzyme activity. Activation and inhibiti-on types. Enzyme activation: partial proteolysis,

quaternary auto-folding, covalent modification, allosteric mechanism.

19. Inhibition of enzyme activity: competitive (isosteric), noncompetitive and allosteric inhibition. Medical utility of competitive inhibitors.
20. Enzymes specificity: types, characterictics and examples.
21. Genetic diversity of enzymes. Multiple molecular forms of enzymes (isoenzymes), examples. Use of isoenzymes assay in diagnostics (LDH, CPK, etc.).
22. Methods of enzyme separation and purification. Affinity chromatography.
23. Practical utility of enzymes: immobilized enzymes, enzymodiagnostics and enzymotherapy.
24. Enzyme's activity assay. Units of enzyme activity.
25. Biochemical adaptation. Enzyme induction and repression, inductive and constitutive enzymes.

CHAPTER III
Nucleic acids: structure and biosynthesis. Protein biosynthesis and its regulation

There are two types of nucleic acids in the human organism: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are polymers-polynucleotides. Monomeric unit is a nucleotide including three components: nitrogenous base (adenine, guanine, cytosine, thymine, uracil), pentose (ribose, deoxyribose), phosphate. The nucleotides are linked in polynucleotides through 3',5'-phosphodiester bonds. Four nucleotides in the polynucleotide chains are specific for each type of nucleic acid: A, T, G, C for DNA and A, U, G, C for RNA. Both DNA and RNA have a characteristic conformation that evolves from the primary structure to a nucleoproteic complex.

DNA is the carrier of genetic information (genome) in all organisms except certain viruses (e.g. tobacco mosaic virus, polio-

virus, influenza virus), which use RNA for this purpose. The biosynthesis of either DNA or RNA, where the parental nucleic acid serves as a template to direct the structure of the product, is named replication. Transcription (synthesis of RNA) means the transport of genetic information from DNA to RNA, and reverse transcription means the reverse process (from RNA to DNA). When DNA is transcribed into RNA, three different kinds of RNA can be identified: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). Replication and transcription are important sites for the action of drugs, either to protect eukariotic cells from action of bacteria and viruses or to inhibit the growth of neoplasic cells. Changes in base sequence of DNA are called mutations. There are several kinds of mutations: substitutions (transition and transversion), deletion, insertion. Mutations can be spontaneous, can arise from DNA polymerase malfunction and can be induced by mutagens. Mutations can be repaired or can evolve in genetic diseases.

Translation (synthesis of protein) means the transport of genetic information from mRNA to the polypeptide chain, the mRNA serving as a template which directs the primary structure of the polypeptide. The code for the translation of the sequence of four different nucleotides in mRNA into the order of the 20 amino acids in a polypeptide is known as genetic code. The cod is triplet, degenerate, universal and collinear. It has chain start and termination signals and, also, reading frames. Post-translational modification takes place after termination of polypeptide chain synthesis.

LESSON 7
Structure of nucleic acids. Biosynthesis of DNA (replication)

Experiment 1. Protein identification (Biuret reaction).

Mix 5 drops of hydrolysate, 10 drops of 10% NaOH, and 2 drops of 1% $CuSO_4$.

Conclusion:

Experiment 2. Pentose identification (Molisch reaction).

Method principle: H_2SO_4 turns ribose in furfurole which condense with thymol to a purpule complex compound.

Procedure:

Mix 10 drops of hydrolysate and 3 drops of alcoholic thymol solution, 1%. Shake the mixture, bend the test-tube and add 20-30 drops of concentrated H_2SO_4.

Conclusion:

Experiment 3. Molybdenum reaction for phosphoric acid identification.

Method principle: When phosphoric acid is treated with molybdenum reagent the yellow sediment is produced.

Procedure:

Mix 10 drops of hydrolysate and 20 drops of molybdenum reagent. Boil the mixture, and cool it in flowing water.

Conclusion:

Experiment 4. Ribose and deoxyribose identification (diphenylamine reaction).

Method principle: When ribose is created with diphenylamine a green colour is produced, while deoxyribose gives a blue colour.

Procedure:

5 drops of hydrolysate are mixed with 20 drops of diphenylamine solution, 1%. The mixture is boiled for 15 minutes.

Conclusion:

Self-preparing questions:

1. Nucleic acids: types, functions and intracellular location.
2. Nucleic acid's components: nitrogenous bases, pentoses, phosphate.
3. Nucleosides, nucleotides, cyclic nucleotides: structure, functions. Usage of nucleotides and natural or synthetic

analogs in therapy.
4. Primary, secondary and tertiary structures of DNA. Nucleosomes. Chromosomes.
5. RNA structure. Peculiarities of tRNA, mRNA, rRNA structure.
6. Denaturation and hybridization of nucleic acids, utility in medicine.
7. Structure of the genes (introns, exons).
8. The central dogma of genetics. Genetic code and its characteristics.
9. Replication (duplication) of DNA: template, enzymes, substrates. Telomeres and telomerases, biological role in the cells.

Self-testing questions:

1. Write the formulas: a) adenine, adenosine, adenylic acid, adenosine triphosphate; b) thymine, thymidine, thymidylic acid, thymidine triphosphate.
2. Write the structure of the tetranucleotides: a) 5'–ATGC-3' ; b) 5' – UGCA - 3'.
3. Find the difference between the dinucleotides of a nucleic acid and of a nucleotidic coenzyme (NAD).
4. Name all types of bonds that stabilize nucleic acid's structure.
5. Estimate how many pairs of nucleotides are in: a) 1 μm of the DNA double helix; b) 1 mln Da of the DNA double helix.
6. The content of adenine in the DNA of two unidentified types of bacteria is correspondingly 32% and 17%. Which is the amount of guanine, cytosine and thymine in each one? Which DNA belongs to the bacteria that were discovered in a thermal spring? Explain.

LESSON 8
Biosynthesis of nucleic acids. Genes: mutations, repair of DNA

Experiment 1: Quantitative analysis of DNA.

Method principle: When DNA is treated with diphenylamine deoxyribose interacts with the reagent to produce a blue-coloured compex. The intensity of the coloration is proportional to the DNA quantity.

Procedure:

№	Reagents	Test-tubes	
		Test	Control
1	Yeast hydrolysate	1 ml	–
2	H_2O dist	–	1ml
3	Diphenylamine reagent	2 ml	2 ml
4	Boil the test-tubes for 10 minutes		
5	Cool the solutions in a water flow		
6	Extinction of the test solution is measured in comparison with the control solution, using 5 mm cuvettes and red filter (λ=630-690 nm). DNA quantity is estimated using the calibration graph.		

Calculation: E = C =

Experiment 2: Quantitative analysis of RNA.

Method principle: When RNA is treated with orcinol reagent (orcinol ferric choride reagent, Bial's reagent) ribose interacts with the reagent to produce a green-coloured complex. The intensity of the coloration is proportionate to the quantity of RNA.

Procedure:

№	Reagents	Test-tubes	
		Test	Control
1	Yeast hydrolysates	1 ml	–
2	Orcinol reagent	1 ml	1 ml

3	H_2O dist	–	1 ml
4	Boil the test-tubes for 20 minutes		
5	Cool the solutions in a water flow		
6	Extinction of the test solution is measured in comparison with the control solution, using 5 mm cuvettes and red filter (λ=630-690 nm). RNA quantity is estimated using the calibration graph.		

Calculation: E = C =

Self-preparing questions:

1. Transcription or RNA biosynthesis: the template, substra tes, enzymes, mechanism.
2. Post-transcriptional alterations (modifications) of the RNA. Maturation of pre-mRNA, pre-tRNA and pre-rRNA.
3. Reverse transcription, mechanism.
4. Molecular mutations: mutagens, role of mutations.
5. Reparation of DNA, mechanism.
6. Inhibitors of nucleic acids synthesis. Antibiotics: chemical structure, mechanism of antibiotics and antimetabolites action that influence on replication and transcription; action of antibiotics on the level of bacteria cell membrane.
7. Genetic engineering. Its means for synthesis of the natural preparations (drugs).

Self-testing questions:

1. What enzymes and compounds are necessary for the synthesis of the:
   a) leading strand;
   b) lagging strand of DNA?
2. Which will be the composition of the new-DNA synthesized by DNA-polymerase using as a template single-strand DNA of the bacteriophage 174? It contains: G-24,1%, C-18,5%, A-24,6%, T-32%.

3. Which is the composition of the DNA that was synthesized on the DNA template of the bacteriophage 174 ((+) and (-) strands)?
4. What factors are ensured the exactness of replication?
5. Is the lagging strand synthesized with the same exactness as the leading one?

LESSON 9
Protein biosynthesis and its regulation. Biosynthesis of antibodies

Experiment 1: Quantitative assay of blood serum proteins (Biuret method).

Method principle: The assay is based on biuret reaction.

Procedure:

№	Reagents	Test-tubes	
		Test	Control
1	Blood serum	0,1 ml	-
2	H_2O dist	-	0,1 ml
3	Biuret reagent	4,9 ml	4,9 ml
4	Incubation for 30 min at the room temperature		
5	The extinction of the experimental solution is measured in comparison with the control solution using 10mm cuvettes and green filter (λ=540-560 nm). Protein quantity is estimated using the calibration graph.		

Normal values: C= 65 - 85 g/l.

Calculation: E = C =

Conclusion:

Clinical-diagnostic significance: Protein content in the blood serum decreases (hypoproteinemia) in nephrites, cirrhosis, starvation, hemorrhage, etc. Increased protein content in the blood serum is called hyperproteinemia and is specific in myelom, vomiting, diarrhea, dehydration of the human organism, etc.

Self-preparing questions:

1. Ribosomes: composition, structure.
2. Protein synthesis:
   a) activation of amino acids;
   b) initiation;
   c) elongation;
   d) termination;
   e) post-synthetic changes of proteins (post-translational modifications).
3. Regulation of protein biosynthesis. Enzyme induction and repression (lactose operone).
4. Preparations affecting protein biosynthesis: activators and inhibitors. Protein synthesis inhibitors. Antibiotics: inhibitors of translation, mechanism of action.
5. Structure and peculiarity of antibodies production, antibodies usage as medicines in therapy.
6. Proteins diversity (hemoglobin, antibodies and enzyme isoforms).
7. Inherited disorders and their clinico-biochemical diagnostics.

Self-testing questions:

1. What difference is there between RNA-polymerase and polynucleotide-phosphorylase?
2. (+) Single-stranded DNA (A-21%, G-29%, T-21%, C-29%) is replicated by DNA-polymerase to form the (-) strand. The (-) strand is served as a template for RNA-polymerase. Which will be the RNA composition?
3. Bacteriophage 174 DNA contains the 5386 nucleotides. This amount is not enough to encode all the proteins that

are synthesized by it. How to explain this phenomenon?

CHAPTER IV.
METABOLISM. BIOENERGETICS

The vital activity of any living organism is determined by the specific organization of biological structures, metabolic processes, energy metabolism, genetic information transfer, and regulatory mechanism. Damage of any of these links develops a pathological process and a disease in the organism.

In the overall metabolism of the living organism are distinguished: *exogenous metabolism,* which comprises extracellular transformations of materials on the way to their uptake and excretion by the cells, and *intermediary metabolism,* which occurs in the cells. The intermediary metabolism is conceived as the sum of chemical reactions that occur in the living cell.

The sequences of chemical reactions involved form *metabolic pathways,* or cycles, each of these performing a definite function. Conventionally, *central* and *special* metabolic pathways are distinguished.

In the metabolism, two oppositely directed processes, or phases, are commonly distinguished: catabolism and anabolism. *Catabolism* is the sum of degradative processes leading to the cleavage of large molecules into smaller ones. *Anabolism* is the sum of metabolic processes leading to the synthesis of complex molecules from simpler ones. Catabolism is accompanied by a release of energy that can be stored as energy-rich ATP. Anabolic processes proceed through consumption of ATP and decomposition of the latter into ADP and H_3PO_4.

The biological activity of the cell is closely associated with the continuous redistribution of the energy delivered by the compounds that enter the cell. The storage of energy in the specific phosphate bonds of ATP constitutes the basis for the energy transfer mechanism in the living cell. The energy resources stored in

the cells are used to provide for the cells energetic requirements. They include monosaccharides, amino acids, glycerol, fatty acids.

In the process of energy release from various substrates, three conventional stages may be defined. The first stage is necesary for converting biopolimers to a monomeric form suitable for energy extraction. The second stage is partial degradation of monomers to key intermediates, chiefly to acetyl-CoA and to a number of the Krebs cycle acids. The third stage represents an ultimate, oxygen-assisted degradation of the materials to CO_2 and H_2O. This phase comprises the aerobic biological oxidation of materials and proceeds with a complete release of energy (ATP).

LESSON 10

Introduction to metabolism. Bioenergetics. Oxidative decarboxylation of pyruvate to acetyl-SCoA. The tricarboxylic acid cycle (Krebs cycle)

Experiment 1*:* **Quantitative assay of pyruvate in urine.**

Method principle: When pyruvate is treated with 2,4-dinitrophenylhydrazine in alkaline conditions a yellow-orange complex is produced.

Procedure:

№	Reagents	Test-tubes	
		Test	Control
1	Urine	1 ml	–
2	H_2O dist	–	1ml
3	2,5% alcoholic solution	1 ml	1 ml
4	Shake thoroughly for 60 seconds		
5	Dinitrophenylhydrazine solution, 0,1%	0,5 ml	0,5 ml
6	Shake thoroughly and incubate it for 15 minutes at room temperature		

7	Extinction of the experimental solution is measured in comparison with the control solution using 5 mm cuvettes and blue filter. The pyruvate content is estimated by the calibration graph.

Normal values: 113,7-283,9 mM/24 hours
(10-25 mg/24 hours).

Clinical-diagnostic significance. Pyruvate content rises in B_1 deficiency, diabetes mellitus, hyperfunction of hypophysis (pituitary) and/or adrenal glands, introduction of adrenalin, camphor or strychnine.

Results: E = C =

Conclusion:

Self-preparing questions

1. Introduction to metabolism. Catabolism and anabolism. The final common metabolic pathway. Central, cyclic and specific metabolic pathways.
2. Metabolism assay: approaches and methods.
3. Free energy, the standard state. High-energy compounds: structure and functions. ATP-cycle.
4. The energetic state of the cell. Energetic regulation of metabolism.
5. Oxidative decarboxylation of pyruvate to acetyl-CoA: multienzyme complex, coenzymes, reactions, control.
6. The tricarboxylic acid cycle (Krebs cycle): reactions, functions, stoichiometry, control. The biological role of the Krebs cycle. Substrate phosphorylation. Energy balance of the Krebs cycle.
7. Anaplerotic reactions for the Krebs cycle.

Self-testing questions

1. Write the chemical structure of vitamins and their coen zymes:

a) B_1 and TDP (TPP);
b) B_2 and FMN;
c) B_5, nicotinamide and NAD.

2. Why does $NADH^+$ ($NADH_2$) solution have the acidic me dium (pH<7.0)?

LESSON 11
Mitochondria electron transport chain. Oxidative phosphorylation

Experiment 1: Qualitative analysis of catalase activity.

Method principle: Catalase catalyzes the reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2\uparrow.$$

Procedure

№	Reagents	Test-tubes	
		Test	Control
1	H_2O dist	1 ml	1 ml
2	Blood	2 drops	2 drops
3	Incubation for 1-2 min	37°C	100°C
4	H_2O_2, 1%	5-10 drops	5-10 drops
6	Shake thoroughly		
7	Gas elimination (O_2)		

Conclusion:

Experiment 2: Quantitative analysis of catalase activity.

Method principle: Catalase activity is estimated by H_2O_2 amount that is broken down in a time unit. The H_2O_2 quantity is evaluated from the reaction:

$$2\ KMnO_4 + 5H_2O_2 + 4H_2SO_4 \rightarrow 2\ KHSO_4 + 2\ MnSO_4 + 8\ H_2O + 5\ O_2$$

The catalase activity is expressed in conventional units. A unit is the amount of H_2O_2 in mg that is broken down in 1 ml of blood.

Procedure:

Dilute the blood to 1:1000. Shake thoroughly the solution.

№	Reagents	Flasks	
		Test	Control
1	blood (1:1000)	1 ml	1 ml
2	H_2O dist.	7 ml	7 ml
3	H_2O_2, 1%	2 ml	–
4	H_2SO_4, 10%	–	5 ml
5	Incubate 30 minutes at room temperature		
6	H_2SO_4, 10%	5 ml	–
7	H_2O_2, 1%	–	2 ml
8	Titrate both solutions with 0,1N $KMnO_4$ to pink color		

Calculation: the activity of catalase is calculated by the formula:

$$CU = (A-B)\cdot 1,7 =$$ where:

A - quantity of $KMnO_4$ used for titration of the control solution.

B - quantity of $KMnO_4$ used for titration of the test solution.

Normal values: 10 - 15 units.

Clinical-diagnostic significance. The activity of catalase decreases in cancer, anemia, tuberculosis, etc.

Results: A = B = CU= $(A-B)\cdot 1,7=$

Conclusion:

Self-preparing questions

1. Biological oxidation. Dehydrogenases. Dehydrogenation – energy source for ATP biosynthesis.
2. Mitochondria electron transport chain (ETC), or respiratory chain (RC): organization, enzymic complexes. Electrons and protons acceptors, structure, function.
3. Reduction-oxidation (redox) potential of ETC components. Free energy and sites of ETC for ATP production (oxidative phosphorylation).
4. The ATP:O or P:O ratio. Respiratory control. Free respi

ration.

5. Mitochondria: structure, properties, permeability for different compounds. The glycerol-3-phosphate and malate-aspartate shuttle systems.
6. Mechanism of oxidative phosphorylation: chemiosmotic hypothesis (Mitchell hypothesis).
7. Microsomal oxidation: electron transport chains, cytochrome P_{450} function and role in inactivation of xenobiotics and drugs.
8. Oxygen free radicals, free radical oxidation. Lipid peroxidation of the cell membranes. Antioxidative systems defense of the human organism.

Self-testing questions

1. What is the proton potential or H^+ electrochemical gradient?
2. What are the short, middle or long types of Respiratory chain?
3. What do you know about the biorole of cytochrome oxidase? Write the reaction with its participation.
4. Where is the site of rotenone (fish poison) inhibition of the Respiratory chain?
5. Name the respiration inhibitors of cytochrome oxidase.
6. Write the reaction catalyzing of H^+-ATPase.
7. Please, calculate the coefficients P/O values for isocitrate ($NADH^+$) and succinate ($FADH_2$) oxidation.

LESSON 12
Colloqium:INTRODUCTION TO METABOLISM. BIOENERGETICS

1. Metabolism. Anabolism and catabolism. Metabolic pathways: central and specific, cyclic and linear, amphibolic.
2. Stages of anabolism and catabolism. The common path-

way of catabolism and anabolism, its role. Connections of anabolism and catabolism.

3. High-energy phosphate compounds: chemical structure, properties and functions. The ATP cycle. The ways of ATP biosynthesis and utilization.
4. Free energy, standard free energy. Exergonic and endergonic reactions. Coupled reactions.
5. Biological oxidation. Reduction-oxidation reactions. Redox-potential.
6. Oxidative decarboxylation of pyruvic acid: location, multienzymic complex, coenzymes, reactions, connection with the Krebs cycle and respiratory chain (RC). Energetic output (balance) of pyruvic acid oxidation.
7. The Krebs cycle: reactions, enzymes, regulation. Anaplerotic reactions, role, examples. Energetic output (balance) of the acetyl-CoA oxidation in tricarboxylic acid cycle (Krebs cycle). Interrelation with RC.
8. Mitochondria electron transport chain (ETC or RC): localization, structure and function of its components. Their oxidized and reduced states.
9. Sources of protons and electrons. Dehydrogenation reactions: the main energy sources in the human cells. NAD- and FAD-dependent dehydrogenases, their connection with the RC.
10. Mechanism of protons and electrons transport through the respiratory chain. Long, media and short ways of electron transport: sources of electrons for RC and ATP production.
11. Redox-potential. ATP production (oxidative phosphorylation). RC sites of phosphorylation, the P/O ratio. Inter-relation of oxidation (respiration) and phosphorylation.
12. ATP-synthetase, structure and function. Mechanism of oxidative phosphorylation: chemiosmotic coupling Mitchell hypothesis.

13. Regulation of respiratory chain function: inhibitors of electrons transport, uncouples of oxidative phosphorylation and phosphorylation inhibitors, role, examples and consequences of their influence.
14. Chemical structure of vitamins and vitamin-like compounds of the respiratory chain (PP, B_2, ubiquinone). Notion about chemical structure of cytochromes (b, c, a), their role, oxidized and reduced state.
15. Glycerol-3-phosphate and malate-aspartate shuttle systems: role, intracellular location, reactions, enzymes and connection with RC.
16. Microsomal electron transport chains (monooxigenase and reductase): structure, functions and location. Cytochrom P_{450}, role in inactivation of xenobiotics, drugs.
17. Oxygen toxicity. Oxygen free radicals: mechanism of formation and toxicity. Lipid peroxidation. Antioxidative defense systems: enzymes, non-enzymic compounds.

CHAPTER V
Carbohydrates structure and metabolism

Carbohydrates are polyhydroxy aldehydes, polyhydroxy ketones, or compounds that can be hydrolyzed to them. Monosaccharides are simple sugars which cannot be broken down into smaller molecules by hydrolysis (glucose, fructose, ribose, etc.). Their nomenclature indicates the number of carbons (triose, tetrose, pentose, hexose, etc.) or the reactive groups (aldoses and ketoses). Disaccharides (sucrose, maltose, lactose) can be hydrolyzed into two monosaccharides (glucose, galactose). Oligosaccharides are polymers made up of two to ten monosaccharide units. Polysaccharides are polymers with many monosaccharide units (amylose, amylopectin, starch, glycogen and cellulose). Carbohydrates have a number of derivatives: phosphoric acid

esters, amino sugars, sugar acids, deoxysugars, etc. Carbohydrates may be considered as having two distinct and vital functions. First – as substrates which oxidation provides energy for biosynthetic reactions and work; second – as structural compounds.

Glucose is of special importance as an energy source. It acts as a substrate for glycolysis, one of the main energy-rich oxidative pathways. Glucose derived from the energy-storage polymers-starch, in plants, and liver are major sites for the storage of glycogen. The glycogen stores allow us to eat intermittently by provisional an immediate source of blood glucose for use as a metabolic fuel. During starvation or during periods of limited carbohydrate intake, when the levels of liver glycogen are low, gluconeogenesis maintains adequate blood sugar concentrations.

Several alternate pathways of carbohydrate metabolism serve as sourse of compounds for synthesis or detoxication: pentose phospate pathway, uronic acid pathway, fructose and lactose metabolism.

Insulin and glucagon are the major regulators of carbohydrates metabolism. Also, the important role play adrenalin, glucocorticoids and thyroid hormones.

LESSON 13

Carbohydrate chemistry. Carbohydrate digestion and absorption. Glycogen metabolism. Aerobic and anaerobic pathways of glycolysis.

Experiment 1: Trommer reaction.

Method principle: When monosaccharides are treated with $Cu(OH)_2$ in alkaline solution, a brick-red precipitate of Cu_2O is formed.

$CuSO_4 + 2NaOH \rightarrow Na_2SO_4 + Cu(OH)_2$

blue

$Glucose + 2Cu(OH)_2 \rightarrow gluconic\ acid + H_2O + 2CuOH$

yellow

$2CuOH \rightarrow H_2O + Cu_2O$

brick-red

Procedure:

№	Reagents	Test-tubes			
		I	II	III	IV
1	Glucose	1 ml	–	–	–
2	Sucrose	–	1 ml	–	–
3	Starch	–	–	1ml	–
4	Urine	–	–	–	1 ml
5	NaOH, 10%	1 ml	1 ml	1 ml	1 ml
6	$CuSO_4$, 2%, the blue colour the solution	0.2 ml	0.2 ml	0,2 ml	0.2 ml
7	Heat carefully the mixtures, $t^o = 100^o$ C (2 min)				
8	The colour of the solution				

Conclusion:

Experiment 2: Fehling reaction.

Method principle: It is the same as in experiment 1.

Procedure:

№	Reagents	Test-tubes			
		I	II	III	IV
1	Glucose	1 ml	–	–	–
2	Sucrose	–	1 ml	–	–
3	Starch	–	–	1ml	–
4	Urine	–	–	–	1 ml
5	Fehling solution	1 ml	1 ml	1 ml	1 ml
6	Boil the mixtures (2 min)				
7	The colour of the solution				

Conclusion:

Experiment 3: Seliwanoff's reaction.

Method principle: When ketose (fructose) is heated with HCl and resorcinol, the solution colour turned to cherry-red.

Procedure:

№	Reagents	Test-tubes	
		I	II
1	Seliwanoff's reagent	1 ml	1 ml
2	Glucose solution	2 drops	–
3	Fructose solution	–	2 drops
4	Heat the mixtures (t^o=100^{o}C, 2 min)		
5	Colour of the solution		

Conclusion:

Experiment 4: **Salivary α-amylase activity assay (Wolghemuth's method).**

Method principle: Method is based on the determination of the minimum quantity of α-amylase which hydrolyzes the added starch completely.

Procedure: Dilute saliva 1:10 (1 ml saliva + 9ml H_2O dest). Put 1 ml of distilled water in six test-tubes. Add 1 ml of saliva to the water in the 1st test-tube and mix thoroughly. After that, 1 ml of mixture transfer from the 1st test-tube to the 2nd one. Mix the content of the 2nd test-tube and repeat the procedures to the 6th test-tube. Mix the solution of the 6th test-tube and take away (remove) 1 ml from it.

Put in every test-tube 2 ml of starch solution, 0,1%, mix and put them in a bath (38^{o}C) for 30 min. Cool the test-tubes with solutions in a cold water flow. After that put 1 drop of iodine solution, 0,1%, in each test-tube, mix the solutions and note their colour.

Calculation: Note the test-tube with the lowest amount of α-amylase which completely hydrolyzed the starch. Knowing the dilution calculate the activity of α-amylase using the ratio:
1 ml of diluted saliva breaks up 2 ml of starch solution, 0,1%;
1 ml of undiluted saliva breaks up **X** ml of starch solution, 0,1%.

$$X = \frac{2 \cdot 1}{1 \cdot (a)} = 2{:}a =$$

where: **a** – dilution of the saliva in the test-tube, where the starch was completely hydrolyzed

Note: Dilutions of the solutions are the following:

№	1	2	3	4	5	6
Dilution (a)	1:20	1:40	1:80	1:160	1:320	1:640

Conclusion:

Self-preparing questions

1. Carbohydrate definition, physiologic importance and classification.
2. Monosaccharides, disaccharides, polysaccharides: structure, properties and functions.
3. Glucosamine glycans (heteropolysaccharides): hyaluronic acid, chondroitin sulphates, heparin, structure and functions in organism.
4. Digestion and absorption of carbohydrates.
5. Glycogen metabolism: glycogenogenesis and glycolgenolysis pathways, regulation.
6. Glycolysis: aerobic and anaerobic pathways. The aerobic pathway of glycolysis.
7. Anaerobic glycolysis in tissues. The enzymatic reactions of glycolysis anaerobic pathway. Stoichiometry of this process.
8. Regulation of glycolysis: hormonal regulation, mechanisms.
9. Energy balance of aerobic and anaerobic degradation of glucose.
10. Mechanisms for translocation of $NADH^+$ from cytosol into mitochondria matrix: α-glycerophosphate and malate-aspartate shuttle systems.

Self-testing questions

1. Write the structural features of β,D-glucopyranose, β,D-fructofuranose, N-acetyl-glucosamine.
2. Write the chemical structure of sucrose, lactose.
3. Write the linear disaccharides formed by D-glucose units linked through α-(1-4)-glycoside bonds.
4. Cellulose: its chemical structure and properties.
5. Write the exergonic reactions of glycolysis.
6. Write the endergonic reactions of glycolysis.
7. Why is the 1,3-biphosphoglycerate an energy-rich compound?
8. Name the enzymes of glycolysis: oxido-reductases, isomerases, lyases.
9. Write the reaction of alcoholic fermentation of lactate.
10. What is energy balance (ATP production) of glycolysis?

LESSON 14

Alcoholic fermentation. Glyconeogenesis. Oxidation of fructose and galactose. Pentose-phosphate pathway

Experiment 1*:* Inorganic phosphate consumption in alcoholic fermentation.

Method principle: Inorganic phosphate is used in the formation of 1,3-diphosphoglycerate during the alcoholic fermentation of glucose. Thus its content in the solution decreases.

Procedure:

Step 1. Use 4 test-tubes. Put 1 ml of tricloracetic acid, 10%, in each one, 1 g of yeast and 1 g of glucose (or sucrose) triturate, then add 5 ml of phosphate solution. Shake the mixture and immediatly transfer 1 ml of it into the 1st test-tube. Put the rest of the mixture into a bath with $t^oC=37^oC$. Every 30 min transfer 1

ml of mixture into the next numbered test-tube. These solutions filtrate and use for phosphate determination.

Step 2. Put 0,5 ml of each filtrate into numbered test-tubes. After that add 1 ml of molybdenic reagent, 0,5 ml solution of vitamin C and 8 ml H_2O dest. The intensity of the coloured solutions is compared in 15 min.

Conclusion: Explain the differences of colour.

Experiment 2: Determination of glucose in blood (O-toluidinic method).

Method principle: Glucose with solution of acetic acid at boiling composes the green-coloured complex. Between glucose concentration and intensity of coloured complex there is a direct correlation.

Procedure:

№	Reagents	Test-tubes	
		Test	Standard
1	Blood	0,1 ml	–
2	Standard (glucose, C=5 mM/l)	–	0,1 ml
3	TCA, 3%	0,9 ml	0,9 ml
	Centrifugation at 3 000 /min for 10 min		
4	Supernatant	0,5 ml	0,5 ml
5	O-toluidine solution	4,5 ml	4,5 ml
	Incubation: 8 min at t^o=100^o C		
	Determine the extinction (E) of the test and standard solutions using 5 mm cuvettes, red filter, water for comparison.		

Calculation: $C_t = C_{st}\ E_t/E_{st}$ = mmol/l.

Norma: 3,33 - 5,55 mmol/l.

Conclusion:

Clinico-diagnostical means: Hyperglycemia: diabetes mellitus, steroid diabetes, acute pancreatitis, etc. Hypoglycemia: hypofunction of thyroid gland, hypofunction of adrenal glands, deficiency of glucose absorbtion or reabsorbtion.

Self-preparing questions

1. Metabolism of fructose and galactose.
2. Alcoholic fermentation of glucose. Production of energy.
3. Pentose-phosphate pathway of glucose oxidation: location, biological role. Chemical reactions of apotomic cycle.
4. Gluconeogenesis: energetically irreversible chemical reactions (steps) of glycolysis, pathway, substrates, regulation.
5. Gluconeogenesis, usage of lactate for this process. Glucose-lactate cycle (Cori cycle) and glucose-alanine cycle, its biological role.
6. Amino acids as substrates for gluconeogenesis.

Self-testing questions

1. Name all vitamins of the pyruvate dehydrogenase complex (PDH-complex).
2. Why is malonate of the Krebs cycle inhibitor? Where is the site for its action?
3. Can you form the glycogen using:
   a) glycerol;
   b) lactate;
   c) pyruvate;
   d) succinate;
   e) acetyl-SCoA?
4. Calculate the number of ATP molecules produced during oxidation of one molecule of lactate in aerobic conditions.
5. How many molecules of ATP will be produced in aerobic conditions of glutamic acid oxidation?
6. Write the reaction which is catalized by glucose-6-phosphate dehydrogenase (G6PDH).
7. Why is the phosphogluconate pathway also called hexose monophosphate shunt?

LESSON 15
Regulation and pathology of carbohydrate metabolism

Self-preparing questions:

1. Carbohydrate metabolism regulation in cells, organs and the whole organism.
2. Assay of the glycoregulatory function of the organism (hyperglycemic tests and functional galactose test).
3. Disorders of the carbohydrate metabolism:
   a) hyperglycemia, hypoglycemia and glucosuria;
   b) diabetes mellitus (types I and II), metabolic disorders. Mechanism of insulin action.
   c) steroid diabetes.
   d) inborn deseases: fructose intolerance, galactosemia, lactose intolerance, glycogenoses and aglycogenoses.
   e) disorders of glucose metabolism in hypoxia.
4. Principle and methods of glucose determination in the blood.

Self-testing questions

1. Mechanism of insulin action (name the right answer):
   a) breakdown of glycogen in glucose;
   b) transport of glucose from the blood to tissues;
   c) activation of the Na^+,K^+-pump;
   d) insulin increases the cell membrane penetration for amino acids and fatty acids.
2. Processes leading to hypoglycemia:
   a) transport of glucose from blood to tissues;
   b) glycogen synthesis from glucose in the liver and skeletal muscles;
   c) absorption of glucose from the intestine;
   d) production of triacylglycerol from glucose in fat tis sue;

e) gluconeogenesis (in liver and kidney).

3. Which hormones do you know that are increased of glu cose content in the blood? (give the right answer).
   a) glucagon;
   b) insulin;
   c) somatostatin;
   d) adrenalin;
   e) cortisol.

LESSON 16
Colloqium: Carbohydrate metabolism

1. Carbohydrates: functions, classifications and nomenclature.
2. Chemical structures of monosaccharides, disaccharides (maltose, lactose, sucrose) and polysaccharides (starch, glycogen).
3. Glucosamine glycans (heteropolysaccharides): hyaluronic acid, chondroitin sulphates, heparin, structure and functions in organism.
4. Digestion and absorption of carbohydrates in the gastrointestinal tract.
5. Glycogen synthesis (glycogenogenesis): role, major tissue sites, reactions, enzymes, regulation.
6. Glycogen mobilization (glycogenolysis): role, reactions, enzymes and regulation.
7. Aerobic and anaerobic pathways of glycolysis: enzymatic reactions of glycolytic anaerobic pathway, regulation, substrate phosphorylation. Energy balance of glycolysis.
8. The aerobic pathway of glycolysis: partial reactions to pyruvate, end products, energy balance.
9. Mechanisms for translocation of H^+ and electrons from cytosol into mitochondria matrix: α-glycerophosphate and malate-aspartate shuttle systems. Glycolytic reactions of dehydrogenation, their link with the respiratory chain.

10. Gluconeogenesis: definition, functions, tissue location, partial reactions, energetic expenses. Gluconeogenesis (from pyruvate): reactions, enzymes, regulation.
11. The glucose-lactate cycle (Cori cycle) and glucose-alanine cycle, description and biologic role.
12. Neuro-hormonal regulation of the blood glucose concentration in the health organism: insulin, glucagon, glucocorticoid influence.
13. Disorders of carbohydrate metabolism: hyperglycemia, hypoglycemia, glucosuria. The glucose tolerance tests.
14. The pentose phosphate pathway: partial reactions, regulation, biological role.
15. The metabolism of fructose and galactose in liver and muscles.
16. Alcohol fermentation: reactions, energetic output, significance.
17. Diabetes mellitus, steroid diabetes: types, metabolic disorders and consequences. Mechanism of hyperglycemia, glucosuria, ketonemia and ketonuria in diabetes mellitus.

CHAPTER VI
Lipids chemistry and metabolism

Lipids are compounds which are insoluble in water and readily soluble in nonpolar solvents such as ether, chloroform, benzene, etc. Lipids are generally esters of fatty acids and alcohol. Lipids serve as storage and transport forms of metabolic fuel, provide structural components of cell membranes, have protective functions serving as a part of the outer coating between the body and the enviroment.

Lipids classifications are based on their chemical structure, physico-chemical properties and functions. There are monomers of lipids (fatty acids, superior alcohols and aminoalcohols, isoprene derivatives) and polycomponent lipids. The last class has

two subclasses: simple and complex polycomponent lipids. Simple polycomponent lipids are esters of alcohol and fatty acids (waxes, acylglycerols).

The complex polycomponent lipids have in their structure additional compounds: phosphoric acid (phosphoglycerides), sphingosine (sphingomyelin), mono- or oligosuccharides (glycolipids).

There are three main forms of lipids in the organism:

a) protoplasmic lipids are structural components of the cell membranes;
b) reserve lipids are concentrated in adipose tissue and serve as metabolic fuel;
c) plasmic lipoproteins are the forms of lipid transport in the blood.

Among the tissues of the organism, the structural lipids are distributed nonuniformly. The nerve tissue is the richest one in structural lipids (to 20-25%). In biomembranes of the cell the lipids make up about 40% (dry weight).

The daily requirement for lipids is 80 g, both of animal and vegetarian origin. An adequate diet includes fat-soluble vitamins (A, D, E, K) and essential fatty acids (linoleic and linolenic acids). Digestion and absorption of lipids are complex and require bile, pancreatic juice and lipolytic enzymes.

The bile acids and their salts:

a) act as detergents and contribuite to the emulsification of lipids,
b) activate lipid-specific enzymes,
c) participate in lipids absorption.

The $NaCO_3$ from pancreatic juice neutralizes the acid chyme, modifying pH to optimum values for the enzymes.

The most important lipolytic enzymes are pancreatic lipase, phospholipases, cholesteride esterase, sphingomyelinase and ceramidase. The enzymes hydrolyze the dietary fats to fatty acids, 2-monoacylglycerol, cholesterol, glycerol, sphingosine, which are absorbed by passive diffusion or micelles pinocitoza in jeju-

num and ileum. Two processes occur within the epithelial cells in the mucosa: re-esterification and formation of chylomicrones.

Lipids metabolism encompasses numerous catabolic and anabolic pathways. Catabolism of polycomponent lipids produces the fatty acids. β-Oxidation of fatty acids is the principal pathway for the fatty acids catabolism. It produces acetyl-CoA, $FADH_2$ and $NADH_2$, which are oxidized in the Krebs cycle and Respiratory chain and coupled with ATP production.

Biosynthesis of the polycomponent lipids requires a lot of fatty acids, that are synthesized in cytosol from acetyl-CoA and $NADPH_2$. $NADPH_2$ sources are the pentose phosphate pathway and citrate shuttle system. Acetyl-CoA and $NADPH_2$ are also required for biosynthesis of cholesterol and its derivatives (bile acids, vitamin D and steroid hormones). A small amount of acetyl-CoA is used for ketone bodies formation in physiological conditions. Synthesis is limited exept under conditions of high concentration of acetyl-CoA, when considerable production will occur. Accompanying ketonemia (high level of ketone bodies in the blood), there is high amount of ketone bodies in the urine (ketonuria).

Lipid metabolism disorders results in quantitative and/or qualitative changes in the blood or tissues lipids (dislipidemias and lipidoses). There are inborn familial dislipidemias (primary) and one (secondary), which is due to disease (diabetes mellitus, alcoholism, etc.). The most frequent lipidoses are obesity and cholelithiasis.

LESSON 17
Lipids: classification, structure, physico-chemical properties, role

Experiment 1: Total lipids assay in blood serum (nephelometric method).

Method principle: Lipids, that were extracted from serum with H_2SO_4 forming an emulsion. It optical density is proportional to the quantity of the lipids.

Procedure:

№	Reagents	Test-tubes	
		Test	**Control**
1	Blood serum	0,2 ml	–
2	Ethanol-ether mixture (3:1)	3,8 ml	–
3	Shake the solution, put it in a bath 50^o-70^o C and heat it to the boil point		
4	Cool the solution and filtrate it in measured test-tube (4 ml)		
5	Add ethanol-ether (3:1) solution until 4 ml		
6	Extract	2 ml	–
7	H_2SO_4, 1%	10 ml	10 ml
8	Ethanol-ether solution (3:1)	–	2 ml
9	Boil the solutions for 1 min		
10	Cool the solutions and incubate them for 60 min at room temperature		
11	Measure the optical density (extinction, E) of the test solution using the red filter, 10 mm cuvettes and control solution for comparison		
12	Determine lipids quantity using the calibrate graph		

Normal values: 4-8 g/l.
Calculation: E = C =
Conclusion:

Clinical-diagnostic significance: Lipids content in serum increases in diabetes mellitus, "steroid" diabetes, nephroses, biliary cirrhosis, obesity, atherosclerosis.

Self-preparing questions

1. Biological functions of lipids.
2. Classification of lipids.
3. Lipids storage – acylglycerols: representatives, structure,

physico-chemical properties, biological role.

4. Protoplasmatic lipids: phosphoglycerides, sphingolipids, glycolipids, cholesterol; structure, physico-chemical properties, biological role.
5. Plasma lipoproteins: types, composition, biological role.
6. Eicosanoids: prostaglandins, leucotrienens and thromboxane; structure and biorole.
7. Fat-soluble vitamins: A, D, E, K; structure and biological role.
8. Biological membranes:
   a) functions
   b) composition and structure - fluid mosaic model of S. J. Singer and G.L.Nicolson
   c) major features: fluidity, motion, selective permeability, asimmetry.
   d) diversity and specificity of the structure and functions of different membranes.

Self-testing questions

1. Which fatty acids are most frequently found in the human adipose tissue? Write their structure. Name the class of each one. What physico-chemical properties do they have? What are properties which confer to the polycomponent lipids?
2. The quantity of which lipids is constant: storage (reserve) and protoplasmatic ones?
   a) it changes a lot;
   b) it is practically constant in the human body. Explain, why?
3. Why lipids are the main energetic source in the organism?
4. Write the formulas of the next lipids and show the hydrophilic and the hydrophobic groups. Which of them have charge?
   a) Phosphatidyl choline;

b) phosphatidyl ethanolamine;
c) phosphatidyl serine;
d) sphingomyelin;
e) cerebroside;
f) ganglioside;
g) cholesterol.

5. What kind of chemical bonds are formed between membrane lipids and proteins?
   a) Hydrophobic;
   b) hydrogen;
   c) covalent;
   d) etheral.
6. What intracellular membranes is monolayer? How are influenced their properties by this structural particularity?
7. What are the differences between the plasmatic membrane of a normal and a tumor cell?

LESSON 18
Digestion, absorption, transport of lipids. Lipids catabolism in tissues

Experiment 1: **Phospholipase activity estimation.**

Method principle: Pancreatic phospholipases hydralyze phospholipids to glycerol, fatty acids, nitrogenous compound and phosphoric acid. Their activity is estimated by H_3PO_4 production. Phosphate is identified by molybdenum reaction: a yellow precipitate is formed when phosphoric acid is heated with ammonium molybdate.

Procedure:

№	Reagents	Test-tubes	
		Test	Control
1	Yolk solution	5 drops	5 drops
2	Pancreatin solution	2 drops	–
3	H_2O dist	–	2 drops
4	Incubate the solution for 30 min at 38° C		

5	Molybdenic reagent	5 drops	5 drops
6	Boil the solutions		
7	Precipitate (present or not)		

Conclusion:

Experiment 2: **Bile influence on lipase.**

Method principle: The rate of lipase activity is estimated by the fatty acids quantity that formed during lipids hydrolysis of milk. Lipase activity is expressed in number of ml of NaOH (0,05N) solution used for the titration of the fatty acids.

Note:

A. Pancreatic lipase cleaves triacylglycerols to the 2-acylmo noglycerol and two fatty acids.

B. Lipase is activated by bile acids.

Procedure: Prepare the initial solutions according to the table below.

№	Reagents	Flasks	
		I	II
1	Milk	50 ml	50 ml
2	Pancreatin solution, 5%	5 ml	5 ml
3	Bile solution	5 ml	–
4	H_2O dist	–	5 ml

Transfer 1 ml of each mixture into two test-tubes, add 1-2 drops of phenolphthalein and titrate them with 0,05N NaOH to rouse colour. The flasks with the mixtures incubate in a bath at 38^oC. Repeat the titration 4 -5 times in 10 min intervals.

Draw up a graph, using the scale below where:

V- volume of NaOH (0,05N) solution consumed for titration (ml)

T- time of incubation (min).

Conclusion:

Experiment 3: Identification of bile acids.

Method principle: When sucrose is treated with concentrated H_2SO_4 oxymethylfurfurol is formed. It reacts with bile acids to generate a red-violet complex.

Procedure:

Put into a test-tube 2 drops of bile, 2 drops of sucrose and shake the test-tube. Add 5-6 drops of concentrated H_2SO_4, wait 2-3 min for the result.

Conclusion:

Self-preparing questions

9. Dietary lipids and their importance.
10. Digestion and absorption of dietary lipids.
11. Bile acids: classification, structure and functions. Metabolism of bile acids (general conception).
12. Lipids re-esterification in the epithelial cells. Transport of lipids in the human organism, blood plasma lipoprotein metabolism.
13. Lipolysis of triacylglycerols in tissues: enzymatic steps, enzymes, regulation.
14. Glycerol oxidation: reactions, enzymes, coenzymes, energetic output (balance) of the anaerobic and aerobic pathways.
15. Fatty acid oxidation: enzymatic steps, regulation, energetic output (balance).
    a) oxidation of fatty acids with an even-number of carbon atoms
    b) oxidation of fatty acids with an odd-number of carbon atoms
    c) oxidation of unsaturated fatty acids
    d) oxidation of fatty acids in peroxisomes.
16. Oxidation of phosphoglycerides, sphingomyelins and glycolipids.
17. Ketone bodies metabolism. Nature of ketone bodies, production, oxidation and biological role.

Self-testing questions

1. Compare the number of ATP molecules that come out from complete oxidation (to CO_2 and H_2O) of 1 molecule of capronic acid and 1 molecule of glucose. Draw a conclusion?
2. The first three reactions of the β-oxidation of fatty acids are similar with the reactions from the Krebs cycle. Write these reactions.
3. How many molecules of ATP are formed during the complete oxidation (to CO_2 and H_2O) of 1 molecule of palmito-oleomarginate (C16, C18Δ9, C17). Explain it.
4. How many molecules of ATP are formed during the oxidation to CO_2 and H_2O of one molecule of β-hydroxybutiric acid?
5. Write the stoichiometric reaction of the glycerol oxidation to pyruvate. What enzymes are necessary except the glycolytic ones?
6. What is the consequence of carbohydrate absence on lipid oxidation?
7. Name and write the chemical structure of the essential fatty acids. What is the main dietary source of essential fatty acids?
8. Which of the following statements are correct regarding chylomicrons?
    a) They are formed in the duodenum and consist of lipid covalently bound with protein.
    b) They are formed in specialized hepatic cells and contain mainly cholesterol and protein.
    c) They are formed in blood from circulating lipoproteins and contained about 60% triacylglycerols.
    d) They are formed in the intestinal mucosal cells and contain about 85% triacylglycerols.
9. All statements regarding lipoprotein lipase are correct,

except:

a) it is localized in endothelial cell membranes
b) it also has phospholipase activity
c) it is activated by phospholipids and apoprotein CII
d) it hydrolizes the fatty acid at the 2nd carbon atom of the glycerol
e) it is absent or deficient in hyperlipidemia, type I.

LESSON 19

Biosynthesis of fatty acids, triglicerides, phospholipids, glycolipids and cholesterol

Experiment 1: Total lipid's assay in blood serum with phosphovanillinic reagent.

Method principle: Blood serum lipids are hydrolyzed with concentrated H_2SO_4. The final products react with phosphovanilinic reagent to form a red complex compound.

Procedure:

№	Reagents	Test-tubes		
		Test	Standard	Control
1.	Serum	0,02 ml	–	–
2.	Standard	–	0,02 ml	–
3.	Water dist	–	–	0,02 ml
4.	H_2SO_4, concentrated	1,5 ml	1,5 ml	1,5 ml
5.	Boil the solutions for 15 min			
6.	Cool the solutions			
7.	Hydrolysate	0,1 ml	0,1 ml	0,1 ml
8.	Phosphovanilinic reagent	1,5 ml	1,5 ml	1,5 ml
9.	Mix the solutions and incubate them 40 min at room temperature, protecting them from light			
10.	Measure the extinction (E) of the test and standard solutions using control solutions for comparison, 3 mm cuvettes and green filter (PEC)			

Calculation: C (g/l) = $(E_t/E_s) \cdot 8$ = where:

E_t - extinction of the test solution

E_s – extinction of the standard solution

8 – lipids content in the standard solution (g/l).

Normal values: 4-8 g/l.

Clinical-diagnostic significance: Lipids contents in serum increases in diabetes mellitus, "steroid" diabetes, nephroses, biliary cirrhosis, obesity, atherosclerosis.

Conclusion:

Experiment 2. Determination of cholesterol content in the blood serum.

Method principle: The addition of concentrated acetic acid, acetic anhydrid and sulfuric acid to cholesterol forms a green-coloured complex (Ilyk method).

Procedure:

Liberman-Burchard solution: 5 pat. acetic anhydrid, 1 pat. H_2SO_4, 1 pat. CH_3COOH.

N	Substance (solution)	Test	Control
1.	Liberman-Burchard solution	2,1 ml	2,2 ml
2.	Blood serum	0,1 ml	–
20 min incubation at t^o=25^o C			
Determine extinction (E) of test solution in comparison with control solution using the photoelectrocolorimeter (*l*=5 mm, λ=630 nm)			

Calculation: Use the calibrate curver. E = C =

Norma: 2,97 – 8,4 mmol/l.

Clinico-diagnostic means: Cholesterol content increases in patients with atherosclerosis, diabetes mellitus, "steroid" diabetes, hyperthyroidism.

Conclusion:

Self-preparing questions

5. Biosynthesis of fatty acids (site, enzymes, coenzymes, regulation):
   - saturated with even-number of carbon atoms

- unsaturated with even-number of carbon atoms
- saturated with odd-number of carbon atoms.

6. Production of triacylglycerols: substrates, enzymes, regulation.
7. Biosynthesis of phosphoglycerides: substrates, *de novo* and salvage pathways, enzymes and coenzymes, regulation. Lipotropic compounds, their role.
8. Sphingolipids and glicolipids biosynthesis: substrates, main reactions, enzymes and regulation.
9. Cholesterol metabolism. Cholesterol biosynthesis: substrates, stages, reactions of the 1^{st} stage, enzymes, coenzymes, regulation, utilization and elimination.
10. Eicosanoids. Cyclooxygenasic and lipooxygenasic path ways of their biosynthesis. Role of eicosanoids in the human organism, their inactivation.
11. Fat-soluble vitamins (A, D, E, K) metabolism: dietary sources, nutritional requirements, functions and metabolism.

Self-testing questions

1. Compare the following statements for oxidation and biosynthesis of the fatty acids:
   a) location (cite) in the cell
   b) "acyl"-residue transport through mitochondrial mem brane
   c) oxidative and reducing agents
   d) polyenzymatic systems.
2. What are the main two biochemical processes that formed $NADPH_2$ required for the fatty acids biosynthesis? Write the reactions in which are formed $NADPH_2$ directly.
3. Write the reactions of arachidonic acid biosynthesis. What are its functions in the organism?
4. Why ethanol may be transformed in fat and can not be transformed in carbohydrates? Write the succession of lipid synthesis from ethanol.

5. Explain the differences in cholesterol metabolism in case of its low and high diet content. What is the cause of this dif ference?
6. The following compounds are synthesized from cholesterol:
   a) chenodeoxycholic acid
   b) 1,2,5-hydroxycholecalciferol
   c) testosterone
   d) glycocholic acid
   e) cholecystokinin.
7. Which of the following statements about the synthesis of 1,2,5-hydroxycholecalciferol is correct? It is synthesized:
   a) from 7-dehydrocholestrol in the skin under the action of ultraviolet light
   b) in the liver from cholecalciferol
   c) in the kidhey from 25-hydroxycholecalciferol
   d) in the intestine from cholecalciferol
   e) it is not synthesized in mammals.
8. What compounds are activated in the biosynthesis?
   a) phosphatidylserine from serine
   b) phosphatidylethanolamine from ethanolamine
   c) ceramide from sphingosine
   d) sphingomyelin from ceramide
   e) cerebrosides from ceramides.
9. What of the following fatty acids are the precursors of the prostaglandins?
   a) linoleic acid ($C_{18}^{\Delta 9,12}$)
   b) linolenic acid ($C_{18}^{\Delta 9,12,15}$)
   c) Δ5,8,11-eicosotrienoic acid
   d) Δ8,11,14-eicosotrienoic acid.

LESSON 20
Regulation and disorders of lipids metabolism

<u>Experiment 1</u>*:* Identification of ketone bodies.

Method principle: Acetone and acetoacetate react with nitroprusside sodium in the presence of NaOH to form a permanganate colour.

Procedure:

Use only dry test-tubes!

Put in a test-tube 2 drops of urine, 2 drops of NaOH (10%) and 2 drops of nitroprusside sodium (10%). Shake the test-tube.

Clinical-diagnostic means: Ketonemia and ketonuria occurs in very high fat diets, in diabetes mellitus, "steroid" diabetes, alcoholism, both normal and toxic pregnancies.

Conclusion:

Experiment 2: Determination of β-lipoproteins content in blood serum (turbidimetric method).

Method principle: β-lipoproteins with $CaCl_2$ and heparin form of muddy complex. Turbidity of the solution correlates with amount of β-lipoproteins in the blood serum.

Procedure: Put into a test-tube 2 ml of 0,27% $CaCl_2$ solution and 0,2 ml of blood serum, mix. Determine the extinction coefficient (E_1) of mixture in cuvete (l=5 mm, λ=630 nm) using the photocolorimeter. Then add into cuvete 0,04 ml of 1% heparin, mix it. After 4 min of incubation at room temperature examine this sample, using the photocolorimeter (E_2).

Calculation: $C = (E_2 - E_1) \cdot 100$ UC

Norma: 35 – 55 UC

Conclusion:

Clinical-diagnostic means: β-lipoproteins amount increases in blood serum in patients with atherosclerosis, diabetes mellitus, "steroid" diabetes, obesity. Decrease of β-lipoproteins amount in the blood serum takes place in β_2-plasmacytome.

Self-preparing questions:

1. Regulation of lipid metabolism in the cell.

2. Hormonal regulation of lipid metabolism. Role of lipotropins, ACTH, thyroid hormones, insulin, glucagon, glucocorticoids and catecholamines.
3. Interrelationship between metabolism of the proteins, carbohydrates and lipids.
4. Disorders of lipids digestion and absorption. Pancreatic, hepatic and intestinal steatorhea.
5. Dislipemias:
   a) Hypolipemias: Tangier disease, α- and β-lipoproteinemia
   b) primary hyperlipemias
   c) secondary hyperlipemias in diabetes mellitus, alcoholism, endocrine diseases. Causes, mechanism of lipids metabolism disturbances, biochemical manifestations.
6. Tissular lipidoses:
   a) inborn diseases: Niemann-Pick, Tay-Sachs, Krabble, Gaucher's, Fabry's, metachromatic leukodystrophy, G_{M1} gangliosidosis
   b) secondary disturbanses: obesity, atherosclerosis, alcoholism. Causes and mechanism of lipid metabolism disorders, biochemical manifestations.
7. Hypo- and hypervitaminoses A, D, E, K, causes and manifestations.
8. Role of the eicosanoids in inflammatory and allergic reactions, regulation of blood fluidity in the human organism.

Self-testing questions

1. In patient with pancreatitis (disease of pancreatic gland):
   a) the digestion of lipids decreases, because the pH of duodenal juice decreases
   b) the absorption of lipids decreases, because the pH of duodenal juice increases
   c) the digestion and absorption of lipids decrease, because the secretion of lipolytic enzymes decreases

d) the absorption of the B group vitamins decreases.

2. All the statements regarding familial hypercholesterolemia (type I) are correct except:
   a) increased LDL content
   b) it is caused by LDL receptor deficiency
   c) the extrahepatic biosynthesis of cholesterol is disturbed
   d) a very high risk of atherosclerosis represents, especially in coronary artery
   e) it is a rare autosomal recessive disease.
3. What disorders of lipid metabolism load to liver steatosis in alcoholism?
4. What class of blood plasma lipoproteins is the "positive" factor for risk in atherosclerosis? Call the major enzyme of their metabolism and write the reaction that it is catalyzed.
5. Hypovitaminosis K: the reasons and the mechanism of the metabolic disorders.

LESSON 21
Colloqium: LIPIDS METABOLISM

1. Classifications of lipids. Biological functions of lipids in the human organism.
2. Protoplasmatic lipids: phosphoglycerides, sphingolipids, glycolipids, cholesterol, cholesterides: chemical structure, physico-chemical properties, biological role.
3. Biological membranes: composition and structure-fluid mosaic model of S.J. Singer and G.L. Nicolson. Major features: fluidity, motion, selective permeability, asimmetry. Diversity and specificity of the structure and functions of different membranes.
4. Storage lipids-acylglycerols: representatives, structure, physico-chemical properties, biological role.
5. Dietary lipids and their importance. Digestion and absor-

ption of dietary lipids in gastro-intestinal tract.

6. Bile acids: classification, chemical structure, functions. Metabolism of bile acids (general conception).
7. Lipids re-esterification in the enterocytes. Transport of lipids in the blood, plasma blood lipoproteins metabolism, types, composition, biological role.
8. Lipolysis of triacylglycerols in the tissues: enzymic steps, regulation.
9. Glycerol oxidation: reactions, enzymes, coenzymes, energetic output of the anaerobic and aerobic pathways.
10. Fatty acid oxidation: enzymic steps, regulation, energetic output (energy balance). Oxidation of fatty acid:
    - with an even number of carbon atoms;
    - with an odd number of carbon atoms;
    - an unsaturated;
    - in peroxisomes.
11. Biosynthesis of fatty acids (location, enzymes, coenzymes, regulation):
    - saturated with even number of carbon atoms;
    - unsaturated with even number of carbon atoms;
    - saturated with odd number of carbon atoms.
12. Synthesis of triacylglycerols: substrates, enzymes, regulation.
13. Biosynthesis of phospholipids: substrates, *de novo* and salvage pathways, enzymes and coenzymes, regulation. Lipotropic compounds, their role.
14. Sphingolipids and glycolipids biosynthesis: substrates, main reactions, enzymes and regulation.
15. Cholesterol metabolism. Cholesterol biosynthesis: location, substrates, stages, reactions of the 1^{st} stage, enzymes, coenzymes, regulation, utilization and elimination.
16. Ketone bodies metabolism: nature of ketone bodies, for mation, biological role.
17. Hormonal regulation of lipid metabolism: role of lipotro

pins, ACTH, thyroid hormones, insulin, glucagon, gluco cor-ticoids and catecholamines.

18. Disorders of lipids digestion and absorption.
19. Dislipidemias:
    - hypolipidemias: Tangier disease, α- and β-lipoprote-inemia;
    - primary hyperlipidemia;
    - secondary hyperlipidemias in: diabetes mellitus, alco holism, endocrine diseases. Causes, mechanism of lipid metabolism disturbances, biochemical manifestations.
20. Tissular lipidoses:
    - inborn diseases: Niemann-Pick, Tay-Sachs, Krabble, Gaucher, Fabry, metachromatic leukodystrophy, G_{MI} gangliosi-dosis;
    - secondary: obesity, atherosclerosis, alcoholism. Causes, mechanism of lipid metabolism disorders, biochemical manifestations.

CHAPTER VII
Metabolism of simple and complex proteins

Proteins and amino acids constitute the major source of nitrogen for mammalian organism, they are a linking chain in catabolism and anabolism of nitrogenous materials. In the adult human organism, about 400 g of protein per day are renewed. The renewal rate for different proteins varies from a few minutes to 10 and even more days. Certain proteins, such as collagen, are practically not subject to renewal. On the whole, the half-life of all the proteins in the human organism is about 80 days.

Proteolytic enzymes involved in the digestion of proteins and peptides are synthetized and secreted into the digestive tract cavity as proenzymes or zymogens. Proteolytic enzymes are activated in the intestinal lumen where they act on dietary proteins.

The activation of these enzymes is effected via partial proteolysis of their polypeptide chains. The human gastric juice contains two structurally related proteolytic enzymes, pepsin and gastricsin, which are derived from a common precursor. The proteolytic enzymes are excreted into the intestine from the pancreas as proenzymes: trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, proelastase. Polypeptide N-terminal amino acids undergo cleavage by the intestinal amino-polypeptidase. In the intestinal cells dipeptidases hydrolyze of dipeptides into two amino acids. The available variety of proteolytic enzymes provides for complete degradation of proteins to free amino acids.

Proteolysis, as it is effected in tissues, is important for renewal of proteins, for the disposal of defective protein molecules, and for the energetic mobilization of endogenic proteins (especially in starvation). The first step of protein renewal is proteolysis, which is effected by tissue proteinases or cathepsins.

The catabolic pathways by which amino acids degrade to the end-products may arbitrarily be divided into three groups: 1- catabolic pathways involving the conversion of NH_2 groups; 2- pathways for the breakdown of amino acid carbon frameworks; 3- decarboxylation of amino acid α-COOH groups. The third pathway is a version of the amino acid carbon framework conversion, employed in the production of biogenic amines. The cleavage of an amino group from an amino acid leads to the production of ammonia (NH_3). This process is called deamination. A direct or an indirect oxidative deamination may be in the human organism. Transamination, i.e. the transfer of an amino group from an amino acid to an α-keto-acid without the intermediary formation of ammonia. The aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are both organospecific enzymes for liver and heart.

Ammonia is a very toxic compound, especially for nervous system. In the organism tissues are operative mechanisms for neutralization of NH_3: 1- production of urea (liver); 2- reductive amination; 3- production of amino acid amides (asparagine and

glutamine); 4- production of ammonium salts (kidneys). The major route of ammonia detoxification is urea synthesis.

The carbon frameworks of protein amino acids ultimately convert into five products (oxaloacetate, acetyl-CoA, succinyl-CoA, α-keto glutarate, fumarate), that are involved in the Krebs cycle.

Conjugated (complex) macromolecules (nucleoproteins, hemoproteins) are hydrolyzed by proteases in the gastro-intestinal tract. The protein moiety is degraded into amino acids, and the nonprotein moiety, via special conversion routes. The hemoglobin of blood erythrocytes and of marrow cells accounts for a major portion (~83%) of hemoproteins in the human organism. The remainders are: myoglobin of skeletal muscles and heart (~17%) and cellular hemoproteins: cytochromes, catalase, peroxidases, etc. (~1%).

After hemolysis in the spleen, the first stage of hemoglobin degradation takes place, leading to bilirubin production. The second stage of hemoglobin conversion takes place inside the liver cells, conjugated forms of bilirubin, bilirubin glucuronides, are produced. The end product of bilirubin conversion, stercobilinogen, is chiefly excreted.

Glycine and succinyl-CoA are the starting compounds in heme synthesis. The enzymes involved in heme biosynthesis are found in the marrow, nucleated erythrocytes, liver, kidneys. The reactions leading to δ-aminolevulinic acid proceed in the mitochondria; the production of porphobilinogen and coproporphyrinogen III occurs in the cytoplasm, and the synthesis of heme from coprophorphyrinogen III, in mitochondria.

Mammals are not in need of dietary supply of nitrogenous bases or nucleotides despite the ability of the mammalian organism to assimilate directly these nutrients with food rich in nucleic acid. In the mammalian tissues the consumed purine and pyrimidine nucleotides are continually renewed via their synthesis from simple compounds (glycine, aspartate, glutamine, carbamoyl phosphate, etc.).

Nucleic acids undergo hydrolysis in the organism tissues through the assistance of nucleases: DNAses and RNAses. Polynucleotide chain is attacked by nucleases, it degrades into oligo- and mononucleotides. Mononucleotides break down into free bases, pentose and phosphate through the agency of nucleotidases and nucleoside hydrolases. Purine bases are oxidized to uric acid, and pyrimidine bases – to urea and β-alanine.

LESSON 22

Metabolism of simple proteins
Digestion of simple proteins in the gastro-intestinal tract
Bacterial putrefaction in the large intestine

Experiment 1. Examination of the of gastric juice acidity.

Method principle: The total acidity of the gastric juice includes: free hydrochloric acid, hydrochloric acid combined with peptides and proteins and other organic acids (lactic, butyric, acetic, and carbonic).

Acidity of gastric juice equals the number of millilitres 0,1N sodium hydroxide (NaOH) required for neutralization of 1000 ml of gastric juice:

a) titration of free HCl using Topfer reagent (0,5% alcoholic solution of DMAB, dimethylaminoasobenzene, pH=2,9-4,0);

b) titration of total acidity using phenolphtalein (pH=8,3-10,0).

Procedure: Examination of free HCl, combined HCl and total acidity made in one portion of gastric juice. 5 ml of juice put in a small retorta, add 1 drop of DMAB and 1 drop of phenolphthalein, mix it and titrate, using 0,1 N NaOH from a burrete until the last trace of orange colour disappears. Read the number of ml (point A, for free HCl). Continue titration until the lemon colour (point B), and then continue titration until the red colour of phenolphtalein appears, titrating to the point (C) at which further addition of alkali does not deepen the colour.

Calculation: Free HCl = A ml · 1000 · 0,1 / 5 ml = A· 20= (mmol/l);

***Norma* =** 20-40 mmol/l

Combined HCl = [(B+C)/2 – A] · 20= (mmol/l) ;

***Norma* =** 10-20 mmol/l

Total acidity = C · 20= (mmol/l);

Norm = 40-60 mmol/l. 20 - coefficient for calculation.

Conclusion:

Clinico-diagnostical means: In gastritis, gastric ulcer the acidity of the gastric juice may be: true achlorhydria, hypoacidity, normal or hyperacidity. The gastric juice without HCl and pepsin is called achylia.

Experiment 2. Identification of pathologic compounds in the gastric juice (lactic acid by Uffelman reaction).

Method principle: Lactic acid with ferric chloride solution and phenol form a characteristic greenish-yellow (canary yellow) coloured complex.

Procedure: Put in a test-tube 5 drops of pathologic gastric juice and Uffelman reagent (20 drops of 1% phenol solution + 2 drops of 1% solution $FeCl_3$), mix.

Conclusion:

Clinico-diagnostical means: Cancer, hypoacidity of gastric juice (gastritis).

Self-preparing questions:

1. Biological role of proteins. Adequate proteins intake. Nitrogenous balance.
2. Digestion mechanism of proteins in the gastro-intestinal tract: role of HCl (hydrochloric acid) for digestion of proteins, its neutralization in the intestinal tract.
3. Activation of proenzymes by partial proteolysis mechanism.

4. Endo- and exopeptidases, specificity of their action in proteins hydrolysis.

5. Mechanisms of amino acids absorption in the small intestine.

6. Bacterial putrefaction in the large intestine. Mechanisms of toxins production and detoxication in the liver.

7. Cathepsins (proteolytic enzymes): breakdown of tissue proteins to amino acids in the cells, pathologic autolysis of tissue proteins.

8. Chemical composition of the normal gastric juice (free HCl, combined HCl, total acidity) and its modification in pathology.

Self-testing questions:

1. What kind of people have the negative nitrogen balance?
2. What is the biological value (adequate) of proteins?
3. Which serine proteases do you know? Give examples.
4. What is indican ("animal indican")? Write down the chemical reactions of its formation. Clinico-diagnostic value of indican examination.
5. What are the mechanisms of the putrescin production and detoxication?
6. What are the "proteins canals" of the cell membranes? How many canals do you know for amino acids absorption into the small intestine?
7. Mechanism of lactic acid production in the stomach. Clinico-diagnostic means of lactic acid assay in the gastric juice.

LESSON 23
Catabolism of amino acids in tissues

<u>Experiment 1.</u> Urea determination in the urine with p-dimethylbenzaldehyde.

Method principle: Urea may be determined colorimetrically by its reaction with p-dimethylbenzaldehyde in acidic medium with production of yellow coloured complex. It' optical density is proportional to the quantity of urea in the urine.

Procedure: Put into a test-tube 0,2 ml of urine and 1,2 ml of p-dimethylamine benzaldehyde solution (2%), mix. Time for incubation of the test is 15 min at room temperature. After that, determine extinction (E) of the test sample against water using the photocolorimeter (green filter, 10 mm cuvettes).

Calculation: Urea quantity is determined using the standard curve. E= C=

Norm: 333-585 mM/daily.

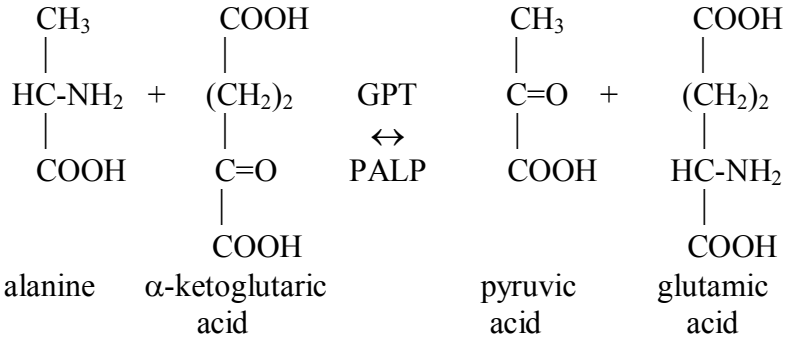
Conclusion:

Clinical-diagnostic means: The most common cause of increased urine urea nitrogen is anemia, increase breakdown of proteins and amino acids in tissues. Urine urea nitrogen decreases in nephritis, acidosis, uremia, diseases of liver (hepatitis, cancer).

Experiment 2. Determination of blood serum transaminases activity (GPT, GOT).

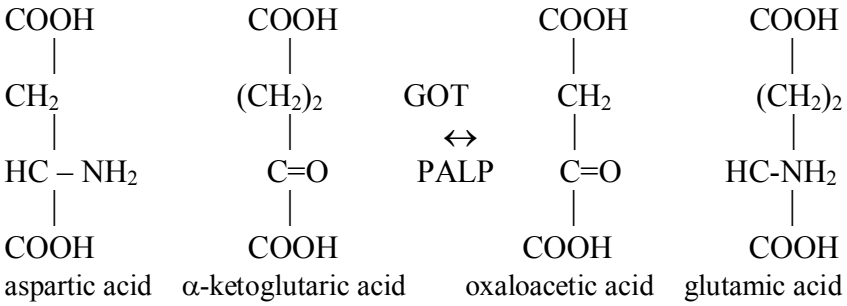
Method principle: The transaminases catalyze the interconversion of keto-acids and amino acids, the amino group is transferred from one molecule to another.

Serum glutamic pyruvic transaminase (GPT) catalyzes the following reaction:

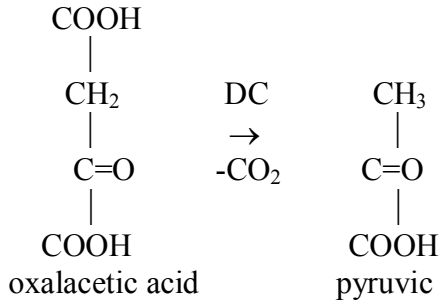


Serum glutamic oxalacetic transaminase (GOT) catalyzes the reaction:

1)



2) after that:



After incubation the resulting oxalacetic or pyruvic acid is measured colorimetrically by reaction with 2,4-dinitrophenyl-hydrasine (red-brown coloured complex).

Procedure:

№	Reagents	Test	Control
1.	Substrate (GPT) alanine	0,5 ml	0,5 ml
	$t^o = 37^o$ C	5 min	5 min
2.	Blood serum	0,1 ml	–
3.	0,9 % NaCl solution	–	0,1 ml
	$t^o = 37^o$ C	30 min	30 min
4.	2,4-DNPH solution	0,5 ml	0,5 ml
	$t^o = 20\text{-}25^o$ C	20 min	20 min

5.	0,4 N NaOH solution	5 ml	5 ml
	t^o = 20-25^o C	10 min	10 min
Read test sample against control sample in tube at λ = 540 *nm*, *l* = 10 mm			

Calculation: Use calibration curve. E= A=

***Norma*:** GPT= 0,16–0,68 mmol/ h·l (15-75 IU/L);
GOT= 0,10–0,45 mmol/ h·l (10-50 IU/L).

Conclusion:

Clinical-diagnostic means: Elevations in transaminases activity occur in myocardial infarction, infectious mononucleosis, infectious hepatitis. Enzymes are specific (organospecific) for tissues: GPT – liver; GOT – heart.

Self-preparing questions:

1. Transamination of amino acids: enzymes, coenzymes, clinical-diagnostic means.
2. Direct oxidative deamination of amino acids, its mechanism, role of glutamate dehydrogenase.
3. Indirect oxidative deamination (across transamination) of amino acids.
4. Mechanism of ammonia toxicity.
5. Mechanisms of ammonia detoxication in organism: glutamine and asparagine synthesis, carbamoyl phosphate formation, production of ammonia salts, reducing amination of α-ketoglutaric acid.
6. Urea production. Clinical-diagnostic means of urea determination in blood serum and urine in pathology.
7. Amino acid decarboxylation: enzymes, coenzymes. Biological role and inactivation of biogenic amines.
8. Fate of α-keto-acids, derived from amino acids, interrelation with pyruvate ehydrogenase complex and the Krebs cycle (CTA).

9. Possible mechanisms of amino acids synthesis in the human organism.

Self-testing questions:

1. Write the chemical reactions of synthesis:
   a) glutamic acid;
   b) alanine;
   c) aspartic acid;
   d) glutamine.
2. Pathways of glutamic acid catabolism:
   a) decarboxilation;
   b) transamination;
   c) oxidation;
   d) carboxylation.
3. Write the chemical reactions of alanine synthesis from glycerol.
4. Write the scheme of gluconeogenesis from glutamic acid and explain it.
5. Calculate the number of ATP molecules which are produced during aerobic oxidation (to CO_2 and H_2O) of the substances:
   a) alanine;
   b) glutamic acid;
   c) aspartic acid.
6. Is the direct oxidation of glutamic acid an anaplerotic reaction? Explain please.
7. Why are AlAT (GPT) and AsAT (GOT) called "organospecific enzymes"?
8. Which substances are the "transport form" of ammonia in the blood?
9. Why are kidney glutaminase activity and urine ammonia salts increased in acidosis?
10. Name the enzymes responsible for urea production in the liver. How can you classify them?
11. Is the urea cycle an amphibolic process? Explain it.

LESSON 24
Metabolism of individual amino acids. Regulation and pathology of protein metabolism

Experiment 1. Determination of creatinine in the urine (Folin method).

Method principle: Creatinine with picrinic acid form a coloured complex in basic medium. Its optical density is proportional to its quantity.

Procedure:

№	Reagents	Test	Control
1.	NaOH, 10%	0,1 ml	0,1 ml
2.	H_2O dist	–	0,1 ml
3.	Urine	0,1 ml	–
4.	Picrinic acid solution	0,15 ml	0,15 ml
Time for incubation at room temperature is 15 min			
After that H_2O enters into the test-tubes to the volume of 10 ml			
Measure the optical density of test sample (E_t) against control (E_c) in cuvettes(l=3 mm) using the green filter (540 nm).			

Calculation: Using the calibrate curve calculate the content of creatinine in daily urine.

E = C =

Norma: 8,8-17,7 mM (0,1-2,0 g) in male; 7,1-15,9 mM (0,8-1,8 g) in female.

Conclusion:

Clinico-diagnostical means: Hypercreatininuria: intensive muscle work, muscle atrophy. Hypocreatininuria: leukemia, kidney diseases.

Experiment 2. Identification of homogentisin (hydrochinoacetic) acid in the urine.

Phenylalanine and tyrosine intracellular breakdown into fumaric and acetoacetic acids. The intermediate of this process is homogentisin (hydrochinonacetic) acid, which is not present in norm in the human urine. In patient with molecular disease (alkaptonuria) takes place of homogentisin acid oxidation to its alkaptones.

Method principle: Homogentisin acid in presence of molibdenic reagent forms the blue coloured complex.

Procedure:

№	Reagents	Test	Control
1.	Urine (pathologic)	2 drops	–
2.	Urine (norma)	–	2 drops
3.	H_2O dist	10 drops	10 drops
4.	K_2HPO_4, solution (10%)	4 drops	4 drops
5.	Molibdenic reagent	4 drops	4 drops
Mix the solutions in the test-tubes and write the colour			

Conclusion:

Experiment 3. Identification of phenylpyruvic acid in the urine.

Method principle: A dark green colour that disappears after addition of a few drops of ferric chloride solution to phenylpyruvic acid.

Procedure: 8-10 drops of 10% ferric chloride solution add to 2 ml urine. The dark green colour disappears in 30-60 sec.

Conclusion:

Clinical-diagnostic means: Phenylketonuria is a disease inherited through a single autosomal recessive gene so that both parents of the patient must have the genetic defect.

Self-preparing questions:

1. Metabolism of phenylalanine and tyrosine. The main pathways and disorders of these amino acids.

2. Metabolism of glycine, serine and cysteine. The role of tetrahydrofolic acid (THFA) in their metabolism. The mechanism action of sulphanilamides.

3. Metabolism of methionine. The lipotropic factors.

4. Metabolism of triptophan. Production of vitamin PP, serotonine.

5. Metabolism of dicarboxylic amino acids, their utility as drugs.

6. Regulation and pathology of protein metabolism.

7. Interrelation of proteins, carbohydrates and lipids metabolism.

Self-testing questions:

1. Explain the necessity of glycine for the synthesis of:
   a) serine;
   b) heme;
   c) purine nucleotides;
   d) creatine and creatinine.
2. In the organism serine is utilized in the formation of:
   a) phospholipids;
   b) glycine;
   c) pyruvate.
3. Cycteine is a substrate for the production of:
   a) glutathione;
   b) taurine;
   c) cystine.
4. Glutamine is utilized in the formation of:
   a) purine and pyrimidine nitrogenous bases;
   b) hexosamines;
   c) urea;
   d) carbamoyl phosphate.
5. Aspartic acid is utilized in synthesis of:
   a) purine and pirimidine nitrogenous bases;
   b) urea;

c) oxaloacetic acid;
d) glucose.

6. Tyrosine is a substrate for production of:
a) DOPAmine;
b) noradrenalin and adrenalin;
c) thyroxine;
d) melanine.

7. The molecular pathologies of some amino acids metabolism are:
a) albinism;
b) alcaptonuria;
c) cystinuria;
d) phenylketonuria. Why may the mental development (dementia infantilis) occur in children?

CHAPTER VIII
Metabolism of nucleoproteins and chromoproteins

LESSON 25
Metabolism of nucleoproteins

Experiment 1. Determination of uric acid in the urine.

Method principle: Uric acid is determined by reducing the action upon a phosphotungaric acid solution, thus producing the blue colour. The intensity of coloured solution is proportional to the quantity of the uric acid.

$12\ Na_2WO_4 + 9\ H_3PO_4 \rightarrow P(W_2O_7)_6H_7 + 8\ Na_3PO_4 + 10\ H_2O$

Procedure: 1,5 ml of urine, 1 ml of 20% NaOH and 1 ml of phosphotungaric acid solution put into a test-tube. Mix and titrate it with 0,01N solution of $K_3Fe(CN)_6$ untill the blue colour appears.

Calculation: C_{ur}= 0,8·a· b / 1,5 mg per day (24 h)=

0,8 – content of uric acid, which is used for titration of 1 ml $K_3Fe(CN)_6$ (mg)

a - content of $K_3Fe(CN)_6$ is used for titration (ml)

b – daily urine (ml).

Norma: 1,6 - 3,54 mM (270 - 600 mg) daily.

Clinical-diagnostic means: The content of uric acid in the urine decreases in kidney diseases; it increases in intensive catabolism of nucleoproteins, gout, leukemia.

Conclusion:

Experiment 2. Determination of uric acid content in the blood serum.

Method principle: Uric acid is determined by its reducing action upon a phosphotungaric acid solution, producing a blue colour that is measured using the photometer.

Procedure:

Put 1 ml of the blood serum, 2 ml H_2O dist and 1 ml trichloracetic acid (CCl_3-COOH) into a centrifuge test-tube. Mix and incubate it for 5 min (t^o = 20-25^o C). After that centrifugate it for 5 min at 600 g (2500 – 3000 rpt/min). Then examine the supernatant:

№	Reagents	Test	Standard	Control
1.	Supernatant	2 ml	–	–
2.	Standard solution (C=5,95 mM), 100 mg%	–	2 ml	–
3.	H_2O dist	–	–	2 ml
4.	Na_2CO_3 solution	0,9 ml	0,9 ml	0,9 ml
5.	Phosphotungaric acid solution	0,1 ml	0,1 ml	0,1 ml

In 10 min read the test and standard samples in comparison with control sample, using the photocolorimeter at λ = 710 nm in l = 5 mm cuvettes.

Calculation:

$$C_t = \frac{E_t}{E_{st}} \cdot C_{st} \cdot a = \quad \text{(mM/l)}, \quad a \text{ – dilution of the test (4).}$$

Norma: 0,13 – 0,40 mM/l.

Clinical-diagnostic means: Increased (hyperuricemia) blood levels have been reported in the acute stages of infarction diseases, excessive exposure to X-rays, multiple myeloma, leukemia, gout. Blood uric acid decreases (hypouricemia) in anemia.

Experiment 3. Urea determination in the blood serum with diacetylmonooxime.

Method principle: Urea may be determined colorimetrically by its reaction with diacetylmonooxime, thiosemicarbozone and ferric chloride. This complex has red colour.

Procedure:

№	Reagents	Test	Standard	Control
1.	Blood serum	0,01 ml	0,01 ml	–
2.	Standard solution of urea (C=15 mmol/l)	–	0,01 ml	–
3.	H_2O dist	–	–	0,01 ml
4.	Colour solution	2,0 ml	2,0 ml	2,0 ml
Mix, time for incubation = 20 min (t^o=100^o C).				

Read test (E_t) and standard (E_{st}) samples in comparison with control sample using the photocolourimeter at λ=540 nm in cuvettes (*l*=3 mm).

Calculation: $C = C_{st} \cdot E_t / E_{st} =$ (mmol/l).

Norma: 2,50 - 8,33 mmol/l.

Clinical-diagnostic means: The most common cause of increased blood urea nitrogen is inadequate excretion usually due to kidney disease or urinary obstruction, malignancy. Blood urea nitrogen usually decreases in liver diseases.

Self-preparing questions:

1. Nucleoproteins. Digestion and absorption of nucleoproteins in the gastro-intestinal tract: enzymes, mechanisms.

2. Biosynthesis of purine nucleotides “de novo”: use of the nitrogenous bases (substrates, enzymes, coenzymes, its regulation).

3. Biosynthesis of pyrimidine nucleotides: substrates, enzymes, coenzymes, regulation. The role of tioredoxin in production of deoxyribonucleotides.

4. Catabolism of nucleoproteins in the tissues.

5. Catabolism of purine nucleotides, end products. Hyperuricemia, gout (podagra).

6. Catabolism of pyrimidine nucleotides (general overview), end products.

Self-testing questions:

1. Necessary substrates for synthesis of nitrogenous bases and nucleotides:
   a) glycine;
   b) aspartic acid;
   c) glutamine;
   d) carbamoyl phosphate;
   e) carbon dioxide (CO_2).
2. What are end substances in purine and pyrimidine catabolism? Explain it.
   a) urea;
   b) uric acid;
   c) CO_2 and NH_3;
   d) β-alanine.
3. What do you know about chemical mechanism action of F-uracil as cell growth inhibitor?

LESSON 26

Metabolism of chromoproteins (hemoproteins)

Experiment 1. Determination of bilirubin content in the blood serum.

Principle: Diazoreagent with direct (conjugated) bilirubin form a pink-coloured complex. The indirect (free) bilirubin can be transferred in a dissoluble condition by attachment to serum of caffeinic reagent, which increases the dissolubility of this pigment and allows determine it with the diazoreagent. The intensity of the coloured complex (azobilirubin) is proportional to the concentration of bilirubin and can be determined photometrically. The general contents of both bilirubin types in the blood serum are common bilirubin. Indirect bilirubin content is determined as difference between common and direct bilirubin.

Procedure:

№	Reagents	Direct bilirubin (I)	General (common) bilirubin (II)	Control (III)
1.	Blood serum	0,5 ml	0,5 ml	0,5 ml
2.	NaCl solution (0,9 %)	1,75 ml	–	–
3.	Diazoreagent	0,25 ml	0,25 ml	–
4.	Caffeinic reagent	–	1,75 ml	1,75 ml

The first sample (I) incubates during 10 min at room temperature; the second sample (II) – 20 min. Extinction of both samples and control determine against water at the green light filter (λ=530 nm) use photometer:

E_{db} , E_{cb} , E_c .

Calculation: Find the contents of a general (common, C_{cb}) and direct bilirubin (C_{db}) in calibration curve. Use these contents for calculation of indirect bilirubin (C_{ib}):

C_{db} = mcmol/L ; C_{cb}= mcmol/L

$C_{ib} = C_{cb} – C_{db}$ = mcmol/L

Norma: 1,7-20,5 mcmol/L – general (common) bilirubin;
1,86-4,3 mcmol/L – direct bilirubin;
1,7-17,1 mcmol/L – indirect bilirubin.

Conclusion:

Clinical-diagnostic means: The contents of the general (common) bilirubin exceed the norm (hyperbilirubinemia) in icteruses: mechanical (direct bilirubin), hemolytic (indirect), parenchymatous (direct and partially indirect). An increase of bilirubin is marked in a physiologically icteric newborn basically of indirect, that is connected to a functional hepatic insufficiency, in particular, insufficient activity of an enzyme UDP-glucuronyltransferase, necessary for formation of direct (conjugated) bilirubin in the liver.

Self-preparing questions:

1. Types of chromoproteins, their localization in the cell.
2. The main characteristics and biological role of chromoproteins (hemoproteins).
3. Digestion of chromoproteins in the gastrointestinal tract: enzymes, products of digestion in the intestine, their absorption.
4. Hemoglobin catabolism in the tissues: bilirubin formation and excretion. The interrelation between bile pigments of blood serum, urine and excrements (faeces).
5. Jaundices (icteruses). Differential biochemical diagnostics of jaundices (icteruses).
6. Heme biosynthesis. Biosynthesis of hemoglobin and its regulation. Pathology of this process.

Self-testing questions:

1. Write the formula of a heme.
2. What is destiny of an exogenic (nutritional) heme in the human organism?
3. What matters are hemolytic poisons?
4. Why are vitamins B_{12} and folic acid antianemic factors?
5. What do the hemoglobins of various kinds of organisms differ by? Explain their likeness.

6. Why is not bilirubin found in the urine of the patients with hemolytic icterus?

7. Icteruses (jaundices). Differential biochemical diagnostics of icteruses, explain it.

LESSON 27

Colloqium: Metabolism of simple proteins, nucleo- and chromoproteins

1. The biological means of proteins. Complete and incomeplete proteins.

2. Digestive mechanism of proteins. Mechanism activation of pepsinogen, and its specificity.

3. Mechanism activation of trypsinogen and chymotrypsinogen. Specifity of trypsinogen and chymotrypsinogen.

4. Mechanism activation and specificity of procarboxypeptidase, proaminopeptidase and prodipeptidase.

5. Mechanism activation and specificity of proelastase. Mechanism neutralization of HCl in the intestine (write the reaction of HCl neutralization).

6. Mechanism of digestion of nucleoproteins in the intestine. Absorption of products in the intestine.

7. Mechanism of absorption of protein hydrolyzates (amino acids). The role of Na^+,K^+- ATPase and γ-glutamyl transferase (GGT) in this process.

8. Pathology of proteins digestion and absorption of products.

9. Breakdown of proteins into amino acids in the tissues.

10. Direct oxidative deamination (of glutamic acid).

11. Indirect oxidative deamination (transdeamination).

12. Transdeamination. The role of GPT and GOT in the organism, write down the reactions of transamination.

13. Decarboxilation of amino acids. Production of histamine, serotonine and γ-aminobutyric acid (GABA). The biological role and mechanism of inactivation (detoxification) of biogenic amines.

14. Mechanism of ammonia toxication, write down the reaction, explain it.

15. Routes of metabolic detoxification of ammonia. Ammonium salt production.

16. Production of asparagine and glutamine.

17. Production of urea, write down the reactions.

18. Breakdown of nucleotides in the organism tissues (purines and pyrimidines). Production of uric acid (write down the reactions).

19. Biosynthesis of mononucleotides (purines and pyrimidines).

20. Hemoglobin catabolism in the tissues, bilirubin formation and excretion. The interrelation between bile pigments of blood serum, urine and excrements (faeces). Differential biochemical diagnostics of icteruses (jaundices).

21. Heme biosyntesis, its regulation and pathology.

22. Metabolism of phenylalanine and tyrosine. The main pathways and disorders of amino acids metabolism.

23. Metabolism of glycine, serine and cysteine. The role of tetrahydrofolic acid (THFA) in their metabolism. Mechanism action of sulphanilamides.

24. Metabolism of methionine and tryptophan. Lipotropic factors. Production of vitamin PP and serotonine.

25. Metabolism of dicarboxylic amino acids, their utility as drugs.

26. Interrelation of proteins, carbohydrates and lipids metabolism.

27. Bacterial putrefaction in the large intestine. Mechanisms of toxins production and their detoxification in the liver.

CHAPTER IX
Neuroendocrine control of metabolism

The neuroendocrine system includes special glands whose cells function is to secrete chemical regulators, commonly refe-

rred to as ***hormones,*** into the internal media of the organism, i.e. blood and lymph. The hormone-secreting glands are subdivided into the central glands (hypothalamus, pituitary gland) and the peripheral glands (thyroid, parathyroid, adrenal glands, etc.). By their chemical structure, hormones are subdivided into: protein-peptide hormones (insulin, calcitonin, vasopressin), amino acid derivatives (adrenalin), steroids (corticosteroids, sex hormones).

The required level of the hormone in the blood is maintained owing to the self-regulation mechanism. This mechanism is based on interhormonal relationships which are referred to as "***plus-minus***" *interactions,* or *mutually exclusive relationships.* Hormones, which are secreted by the glands into the blood, usually become bound to specific blood plasma transport proteins to be carried to the peripheral tissues, where they exert influence on metabolism and tissue function. The possible types of action for extracellular regulators (referred to as first messengers), including hormones, are: membrane, or local, action; membrane intracellular, or indirect, action; cytosolic, or direct, action.

For practical applications, hormones are produced by extraction from biological materials, by chemical synthesis, or by genetic engineering methods. Practically all the protein hormones can be prepared by laboratory synthesis. Insulin, somatostatin and related hormones have been prepared by the genetic engineering method in the laboratory.

In medical practice, hormones are used for substitution therapy and pathogenetic therapy. In the latter instance, certain specific properties of hormones (antiinflammatory, anabolic, etc.) are made use of, even though if the hormonal concentrations in the organism may be at anormal level.

Antihormones are compounds that exhibit antihormonal activity through binding to cytosolic receptors. The molecular mechanism of antihormonal action is based on the competition for binding sites with the corresponding cytosolic receptors. Natural antihormones include estrogens and androgens which compete with each other for binding with the receptors of opponent

hormone: estrogens block the androgenic receptors, and androgens, the estrogenic ones.

Prostaglandins are hormone-like compounds (hormonoids) derived from C_{20}-polyene fatty acids containing a cyclopentane ring. Prostaglandins are short-lived species that are synthetized at need in small amounts to exert a local biological effect at the site of their formation. An excessive production of prostaglandins or their deficiency may lead to pathologic processes such as inflammation, thrombosis, gastric ulcer, and others. Prostaglandens use in practice as drugs, for example, prostaglandin E_2 (dinoprostone and prostin E_2) is applied in arresting bronchial apasmodic attacks, hypertension, and peptic ulcer.

LESSON 28

Hormones: chemical structure, biorole, mechanism of action, synthesis. Hormones of hypothalamus, hypophysis, parathyroid glands

Experiment 1. Determination of calcium in the blood serum.

Method principle: The method is based on the titration of calcium iones (Ca^{2+}) in alkali medium by sodium salt of ethylendiamine tetraacetic acid (EDTA) in the presence of murexid as indicator.

Procedure: Put 50 ml of H_2O dist, 0,4 ml of 9N NaOH solution and a small amount of murexid, mix it (violet colour). The solution is divided into two portions and transferred into 2 retorts (muffles). One portion will be as the control, another one as the test. Add 1 ml of blood serum into the test-muffle. Serum Ca^{2+} and murexid produce a pink-violet complex. After that titrate the test with EDTA solution until the violet colour is obtained

(reverse). The end of titration is a relative colour of test and control samples.

Calculation: $C_t = 1{,}76 \cdot a =$ mmol/l; a – number of ml EDTA for titration of test.

Norma: 2,25 – 2,75 mmol/l blood serum.

Clinico-diagnostical means: Hypocalcemia: hypoparathyroidism, hyperthyroidism, uremia. Hypercalcemia: hypervitaminosis D, hyperparathyroidism, osteosarcoma.

Conclusion:

Self-preparing questions:

1. Hormones: definition, overview of the chemical nature, mechanisms of action, usage of the hormones as drugs.
2. Central regulation of the hormone functions: the role of hypothalamus and hypophysis.
3. Classification and properties of hormones.
4. Mechanisms of hormones action:
   membrane-cytosolic (indirect) mechanism of action, role of G-protein and second
   messengers in the transfer of the hormonal information;
   cytosolic (direct) mechanism of metabolic control.
5. Hormones of hypothalamus (liberins and statins).
6. Hormones of hypophysis. Chemical nature, mechanism of action, biological effect, regulation of secretion, utility as drugs in the therapy.
   corticotropin family hormones (ACTH, lipotropin);
   glycoprotein family hormones (FSH, TSH, LH and gonadotropin-chorionic-placentar);
   somatotropin family hormones (STH, prolactin and lactogenic placentar);
   vasopressin and oxytocin.
7. Hormones of parathyroid glands. Regulation of phosphate and calcium metabolism. Parathyrin and vitamin D interrelation. Disorders of parathyroid glands functions.

Self-testing questions:

1. What is the difference between hormones and hormonoids (parahormones)?
2. Name the hormones of central and peripheral glands.
3. What do you know about the extracellular regulators called the first messengers?
4. Write the chemical structure of the secondary messenger:
   a) 3',5'-cAMP;
   b) 3',5'-cGMP.
5. 3',5'-cAMP is the second messenger for the following hormones:
   a) adrenalin;
   b) glucagon;
   c) insulin;
   d) somatotropin.
6. Which is the mechanism action of parathyrin influence on phosphorus-calcium metabolism through vitamin D?

LESSON 29

Hormones of thyroid and pancreatic glands

Experiment 1. Determination of inorganic phosphate content in the blood serum.

Method principle: Phosphate ions along with ammonium molibdate form the ammonium phosphomolibdate in the acidic medium, which together with ascorbic acid form the blue-coloured complex. Its optical density is proportional to the quantity of phosphate.

Procedure:

№	Reagents	Test	Control
1.	Blood serum	1 ml	–
2.	H_2O dist	4 ml	2,5 ml
3.	TCA (trichloracetic acid), 10%	5 ml	2,5 ml

Centrifugation: 10 min at 600 g			
4.	Centrifugate (supernatant)	5 ml	–
5.	Ammonium molibdate solution	1 ml	1 ml
6.	Ascorbic acid solution, 1%	0,2 ml	0,2 ml
Incubation at room temperature: 20 min. After that use photocolorimeter (red filter) for determination of the optical density (E) of the test sample against control.			

Calculation: Use the calibrate curve. E = C =

Norma: 0,58 - 0,84 mmol/L (1,8 - 2,6 mg/100 ml).

Conclusion:

Clinical-diagnostic means: Phosphate content is decreased in patients with hyperparathyroidism, and increased in diseases of kidney, hypoparathyroidism, diabetes mellitus.

Experiment 2. Insulin identification.

Hormone insulin is a simple protein. Perform the following chemical reactions:

a) The biuret reaction (Piotrovskii): identification of the peptide chemical bond.

(see Lesson № 1, task 1).

Conclusion:

b) Identification of the sulfur-containing amino acids (Fol test).

(see Lesson 1, task 4).

Conclusion:

Self-preparing questions:

1. Hormones of the thyroid gland: iodothyronines and thyrocalcitonin, their chemical structure, mechanism of synthesis and action, regulation.
2. Hypo- and hyperfunction of the thyroid gland, pathologies, methods of their correction.
3. Hormones of the pancreatic gland: insulin, glucagon, somatotropin.

4. Insulin: chemical structure, mechanism of its synthesis, activation and inactivation. Mechanism of its action, metabolic role. Disorders of the insulin secretion: hyperinsulinemia, hypoinsulinemia. Pathology: diabetes mellitus (type I and II).

5. Glucagon: chemical structure, mechanism of its action, metabolic role.

6. Pharmaceutic drugs: laboratory synthesis of insulin.

Self-testing questions:

4. Mechanism of insulin action (choose the right answer):
   e) breakdown of glycogen into glucose;
   f) transport of glucose from blood to tissues;
   g) activation of Na^+,K^+-pump;
   h) insulin increases the cell membrane penetration for amino acids and fatty acids.
5. Processes leading to hypoglycemia:
   f) transport of glucose from blood to tissues;
   g) glycogen synthesis from glucose in the liver and skeletal muscles;
   h) absorption of glucose from the intestine;
   i) production of triacylglycerol from glucose in the fat tissue;
   j) gluconeogenesis (in the liver and kidney).
6. Which hormones do you know that increase the glucose content in the blood? (give the right answer).
   f) glucagon;
   g) insulin;
   h) somatostatin;
   i) adrenalin;
   j) cortisol.

LESSON 30
Adrenal glands hormones. Sex hormones. Hormonoides

Experiment 1. Identification of 17-ketosteroids in the urine.

Method principle: 17-ketosteroids with m-dinitrobenzen produce a red-violet complex in alkali medium.

Procedure: Put into the test-tube 20 drops of urine, 30 drops of m-dinitrobenzen solution 2% and 6 drops of 30% NaOH, mix it.

Conclusion:

Self-preparing questions:

1. Hormones of the adrenal glands: catecholamines and corticosteroids.
2. Catecholamines (adrenalin and noradrenalin): chemical structure, mechanism of their synthesis, mechanism of action, regulation of secretion.
3. Corticosteroids: glucocorticoids and mineralocorticoids. Chemical structure, mechanism of action, regulation of their secretion.
4. Aldosterone: chemical structure, metabolic role, pathology of its secretion.
5. Glucocorticoids (hydrocortisone, corticosterone): chemical structure, mechanism of action, metabolic role, pathology ("steroid" diabetes).
6. Sex hormones: androgens and estrogens. Regulation of their secretion.
   a) estrogens: chemical structure, physiological role and mechanism of action, pathology and its correction;
   b) androgens: chemical structure, biological role, mechanism of action, pathology and its correction;
   c) anabolic steroids (nerobolil, retabolil) as drugs.
7. Hormone-like compounds or hormonoids (local hormones, parahormones): histamine, serotonin, heparin, prost-aglandins, etc.
8. Antihormones, their utility in medicine.

Self-testing questions:

1. What is the precursor of the female and male hormones?
2. Usage of testosterone in medicine.
3. Is there any difference between patients with diabetes mellitus and diabetes “steroid”?

CHAPTER X
Biochemistry of blood

Blood is a fluid tissue composed of cells (formed elements of the blood) and an extracellular liquid medium***, plasma***. The blood cells account for about 45%, and the plasma, for about 55% of the blood volume. The clear liquid that separates from the blood when it is allowed to clot completely is called *blood serum*. The blood serum is the plasma from which fibrinogen has been removed in the process of clotting. Water accounts for about 83% of blood weight, and the dry residue of blood accounts for the rest. By its physico-chemical properties, blood is a viscous liquid of specific density 1.050-1.060. The blood pH value (7.36-7.44) is maintained by buffer systems at a constant level. Its variations do not exceed 0.05-0.10 pH unit.

Biochemical and physiological functions of the blood are defined by a cooperative participation of the blood cells and chemical components of the blood plasma in metabolism. The blood cells are: erythrocytes, leucocytes, lymphocytes, thrombocytes. Blood performs the following functions: transport, osmoregulatory, buffering, detoxifying, defensive, or immunologic, regulatory, or hormonal, hemostatic.

The transport function is the major function of the blood. A variety of materials are transported in the blood by mechanical transport: nutrients, gases (O_2 and CO_2), hormones, vitamins, etc. Blood maintains osmotic pressure inside the blood vessels. This function is carried out by blood plasma proteins, chiefly albumi-

ns, and by Na^+ cations. Inside the erythrocytes, the role of osmotic pressure regulation is assigned to hemoglobin and K^+ ions. The lowered blood plasma concentration of proteins, or *hypoproteinemia,* leads to a decrease in the oncotic pressure in blood capillaries and to oedematous disturbances.

The detoxifying function of the blood provides for detoxification and neutralization of materials supplied to the blood. Detoxification is effected by diluting the toxicants and their binding mainly to the albumins of the blood plasma. Passive neutralization of toxicants can be actively detoxified by enzymes found in the blood plasma and blood cells.

The blood cells and blood plasma are sources for production of various extracellular regulators involved in the control of metabolism and functions of tissues and organs. These regulators belong to local hormones, or hormonoids: serotonin, histamine, heparin, etc. The blood plasma proteins serve as substrates for the formation of biologically active polypeptides grouped under the common name *kinins*. They include bradykinin, kallidin, methionyl-lysyl-bradykinin. Under physiological conditions, the systems of production and inactivation of kinins are equilibrated. Pathologic alterations occur due to an excessive kinin generation, which is concomitant with the development of local inflammations and impaired blood circulation.

Hemostasis, or arrest of bleeding to prevent blood loss, is an important function of the blood. The blood coagulation system, thrombocytes, and vascular wall are involved in this process. Blood clotting is a multistage self-accelerating process in which plasmic and thrombocytic factors are involved. To date, 13 plasmic and 11 thrombocytic factors have been described. In the organism blood coagulation is controlled by agents that either accelerate the process (procoagulants) or decelerate it (anticoagulants). Natural anticoagulants include heparin, and procoagulants, vitamin K and Ca^{2+} ions.

Blood is used as raw material for producing a variety of medicinal preparations which, by their therapeutic applications, are

divided into four groups: systemic effect agents (albumin, protein, blood plasma); immunologically active preparations (γ-globulin, interferon, etc.); hemostatic preparations (thrombin, fibrinogen, fibrin film, fibrin sponge); antianemic and stimulating preparations (polyobolin, eryheme).

LESSON 31
Blood: metabolism of blood cells, chemical components of blood plasma

Experiment 1. Determination of total protein in the blood serum by refractometric method.

Principle: This method is based on the Tindal's effect. Light-ray that runs through a protein solution is refracted. There is a correlation between refracted light-corner and protein concentration.

Procedure: Put 1 drop of water on the surface of refracttometric set and close it. The line between the light semicircle and dark semicircle must be on the medium position of the cycle: $\alpha=1{,}336^{o}$ (water-dependent zero). After that put 1 drop of blood plasma on the surface of the set and close it. The line of the light and dark semicircles changes its position. Return it to medium position and determine the light-corner.

Calculation: Use the table data for determination of the protein concentration.

α= C=

***Norma*:** 65-85 g/l.

Conclusion:

Clinical-diagnostic means: The protein content decreases in nephritis, cirrhosis, starvation, hemorrhage, etc., this condition is named hypoproteinemia. Hyperprotein-emia is specific for myeloma, vomiting, diarrhea, dehydration, etc.

Experiment 2 Quantitative assay of the serum total protein (Biuret method).

Principle: The assay is based on Biuret reaction.

Procedure:

№	Reagents	Test	Control
1.	Blood serum	0,1 ml	–
2.	H_2O dist	–	0,1 ml
3.	Biuret reagent	2,9 ml	2,9 ml
Incubation at room temperature for 30 min			
Measure the extinction of the test sample in comparison with the control solution. Use the photocolorimeter: 10 mm cuvettes, green filter (λ = 540-560 nm).			

Calculation: Protein quantity is estimated using the calibration curve. E= C=

Normal values: 65 - 85 g/l.

Clinical-diagnostic means: see *Experiment 1.*

Conclusion:

Self-preparing questions:

1. Blood biological functions.
2. Blood cells: types, biorole, chemical components. Peculiarities of blood cells metabolism (erythrocytes, leucocytes, lymphocytes, thrombocytes).
3. Chemical components of blood plasma:

a) proteins of blood plasma (albumins, globulins, immunoglobulins, lipoproteins, interferon);

b) enzymes: characterictics, role of organospecific enzymes and isoenzymes (LDH, CPK, etc.) in diagnostics.

c) plasma non-proteic nitrogen compounds: fractions, clinico-diagnostical means in pathology. Hyperammonemia and hyperuremia, correction of these pathologies (hemodialysis, hemosorbtion).

4. Plasma non-nitrogenic organic compounds.

Self-testing questions:

1. Hemoglobin variants in the human organism.

2. What is the detoxifying function of the blood?

3. Write the chemical reaction catalyzing the carboanhydrase and explane it.

4. Explain the difference between metabolic and respiratory acidoses.

5. Organospecific isoenzymes are:

a) LDH_1 and LDH_2;

b) LDH_4 and LDH_5;

c) creatine phosphokinase (CPK): BB, MB and MM isoforms.

6. Why are vitamins B_{12} and foleic acid antianemic factors (drugs)?

LESSON 32

Physico-chemical properties of the blood plasma

Experiment 1. Determination of iron in biological liquids with rodanide (CNS^-).

Principle: Iron (Fe) interacts with rodanide producing a red-coloured complex. Its optical density is proportional to the iron amount.

Procedure: Blood serum and extracts of tissues may be used for the assay. Previously to the assay the samples are treated with sulfuric acid and hydrogen peroxide. After the filtration of this mixture, take 4 ml of the filtrate and put it into a test-tube. Also put 3 ml of H_2O dist and 1 ml of potassium rodanide (20%) into the test-tube, mix it. In 5 min read the test sample (E) against water in photometer using blue filter (490 nm) and 10 mm cuvettes.

Calculation: Use the graph. E= C=

***Norm*:** male: 16,1 - 21,1 mM/l; female: 14,3 - 21,5 mM/l.

Clinical-diagnostic means: Increased content of iron in the blood serum is detected in liver diseases, hemochromatosis.

Decreased one is revealed in diseases of the gastro-intestinal tract, disorder of iron absorption in the intestine.

Conclusion:

Self-preparing questions:

1. Water and mineral metabolism:
   a) water concentration and its disturbances in the organism;
   b) physico-chemical properties of water;
   c) water as solvent for natural substances and xenobiotics;
   d) biorole of the kidney in the metabolism of water and mineral salts;
   e) regulation of osmotic pressure and exracellular medium (hormonal and rennin-ngiotensinic).
2. Mineral components of blood plasma. Blood plasma ionogram:
   a) metabolism of macroelements (sodium, potassium, calcium, magnesium, iron, chloride, sulfate);
   b) mineral compounds, role in regulation of osmotic pressure (iso-, hypo- and hypertonic solutions);
   c) oligo- and microelements, role in metabolism (copper, zink, cobalt, selenium, etc).
3. The buffer systems of blood. Acid-base equilibrium, physiological and physico-chemical mechanism of buffering. Acid-base disorders (acidoses, alkaloses).

Self-testing questions:

1. Explain the difference between metabolic and respiratory acidoses.
2. Respiratory alkalosis:
   a) increases CO_2 concentration in the blood;
   b) decreases CO_2 concentration in the blood;
   c) increases the concentration of Cl-ions in the blood;

d) decreases the concentration of Cl-ions in the blood.

3. What is the concentration of Ca^{2+}- ions in the blood plasma?
4. Why does the human organism need Ca^{2+}-ions? Where is Ca^{2+}-stored in the human organism?
5. Where are Na^{+} and Cl^{-} ions stored in the human organism?

LESSON 33
Respiratory function of the blood. Blood clotting and anticoagulant mechanism

Experiment 1. Hemoglobin assay in the blood by cianmethemoglobinic method.

Principle: Potasium ferricianid oxidies hemoglobin in methemoglobin. In the presence of acetoncianhydrin it forms a red-coloured complex. Its optical density is proportional to the quantity of the hemoglobin.

Procedure:

№	Reagents	Test	Control
1.	Transformic reagent (solution)	5,0 ml	5,0 ml
2.	Blood	0,02 ml	–
Incubation at room temperature – 10 min. After that read the test sample (E) against transformic reagent in photometer using green filter and 10 mm cuvettes.			

Calculation: Hemoglobin quantity is determined using the standard curve. E= C=

Normal values: males: 132 - 164 g/l; females: 115 - 145 g/l.

Conclusion:

Clinical-diagnostic means: Hypohemoglobinemia: irondeficiency anemia, hemorragia. Hyperhemoglobinemia: erythremia, dehydration.

Self-preparing questions:

1. The respiratory function of the blood.

2. Oxygen transport: the role of hemoglobin in this process, cooperative mechanism and the sigmoidal curve of oxyhemoglobin formation. Regulation of hemoglobin affinity to oxygen (2,3-biphosphoglycerate, protons, temperature).

3. Disorders of oxygen transport: hypoxemia and hypoxia, types, causes.

4. Transport of CO_2 from the tissues to the lungs. Henderson cycle.

5. The biochemical mechanism of blood clotting. Modern theory of hemostasis. Factors and pathways of blood coagulation.

6. Anticoagulant factors, chemical nature, mechanisms of action:

a) natural anticoagulants;
b) fibrinolitic system;
c) synthetic anticoagulants.

Self-testing questions:

1. Vitamins K and D are procoagulants. Explain their role.
2. Explain the mechanism action of antivitamins K (dicoumarol, pelentan) on the blood clotting.
3. What is the difference between oxyhemoglobin A (Hb A) and oxyhemoglobin F (Hb F)?
4. What do you know about the disease “hemophylia”?
5. Write the chemical reaction catalyzing the carboanhydrase and explane it.

LESSON 34
Colloqium: Hormones. Blood

1. Hormones: chemical structure, classifications.
2. Mechanisms of hormones action:

a) membrane-cytosolic (indirect) mechanism of action, role of G-protein and second messengers in the transfer of the hormonal information;

b) cytosolic (direct) mechanism for metabolic control.

3. Central regulation of the hormone functions: role of hypothalamus and hypophysis.

4. Hormones of hypothalamus, chemical nature, biorole.

5. Hormones of hypophysis: chemical structure, mechanism of action, biological effect, regulation of secretion, utility in therapy.

6. Hormones of parathyroid glands: regulation of calcium and phosphate metabolism. Parathyrin and vitamin D interrelation. Disorders of parathyroid glands functions.

7. Hormones of thyroid gland: thyroxin, calcitonin. Biosynthesis, regulation, hypo- and hyperfunction of thyroid gland, pathologies, methods of their correction.

8. Hormones of pancreatic gland: insulin, glucagon, somatotropin. Their characteristics and metabolic role.

9. Insulin: chemical structure, mechanism of its synthesis, activation and inactivation. Mechanism of its action, metabolic role. Disorders of insulin secretion: hyperinsulinemia, hypoinsulinemia. Pathology: diabetes mellitus (type I and II).

10. Glucagon: chemical structure, mechanism of its action, metabolic role.

11. Hormones of adrenal glands: adrenalin, noradrenalin. Mechanism synthesis and action, metabolic role, regulation of secretion.

12. Corticosteroids (glucocorticoids): chemical structure, mechanism of action, metabolic role, regulation of secretion, disorders (“steroid” diabetes).

13. Aldosterone: chemical structure, metabolic role, pathology of its secretion.

14. Sex hormones: estrogens and androgens, chemical structure, mechanism of action, utility in medicine. Usage anabolic steroids as drugs.

15. Hormone-like compounds or hormonoids (local hormones, parahormones): histamine, serotonin, heparin, prostaglandins, etc. Antihormones, utility in medicine.

16. The blood biological functions and physico-chemical characteristics.

17. Blood cells: types, biorole, chemical components. Peculiarities of blood cells metabolism (leucocytes, erythrocytes, lymphocytes, thrombocytes, neutrophils, basophils).

18. Proteins of the blood plasma: fractions, clinico-diagnostical means in pathology.

19. Enzymes of the blood serum: characterictics, role of organospecific enzymes and isoenzymes (LDH, CPK, etc.) in diagnostics.

20. Non-proteic nitrogen compounds: fractions, clinico-diagnostical means in pathology.

21. Water-mineral metabolism: regulation of osmotic pressure and exracellular medium (hormonal and renin-angiotensinic).

22. Mineral components of the blood plasma. The blood plasma ionogram: metabolism of macroelements and microelements.

23. The buffer systems of blood. Acid-base equilibrium, physiological mechanism of buffering and its disorders (acidoses, alkaloses).

24. Respiratory function of blood: oxygen transport, role of hemoglobin in this process, cooperative mechanism, sigmoidal curve of oxyhemoglobin formation.

25. Regulation of hemoglobin affinity to oxygen (2,3-biphosphoglycerate, protons, temperature). Disorders of oxygen transport: hypoxemia and hypoxia, types, causes.

26. Transport of CO_2 in the blood (from tissues to the lungs). Henderson cycle.

27. Biochemical mechanism of blood clotting. Modern theory of hemostasis.

28. Factors and scheme of the blood coagulation.

29. Anticoagulants: chemical nature, mechanisms of action for natural and synthetic anticoagulants, fibrinolitic system.

30. Vitamins K and D, their interrelation with blood clotting (coagulation). Hypo- and hypervitaminoses K or D, pathologies and their correction.

CHAPTER XI. SPECIAL BIOCHEMISTRY
Biochemistry of connective, bone, tooth tissues. Saliva

LESSON 35
Biochemistry of connective tissue and bone

Experiment 1. The total content of proline and hydroxyproline assay in the urine.

Method principle: Proline is oxidized to hydroxyproline by lead peroxyde. Hydroxyproline is condensed with p-dimethyl-amino-benzaldehyde with formation of an orange-coloured complex. The intensity of the colour is proportional to the total quantity of proline and hydroxyproline.

Procedure:

№	Reagents	Test	Control
1.	Urine	2,5 ml	–
2.	H_2O dist	–	5,0 ml
3.	p-dimethyl-amino-benzaldehyde, 5%	0,5 ml	0,5 ml
4.	HCl, 2 N solution	1,0 ml	1,0 ml
Boil test-tubes for 1 min (100° C), then incubate it at room temperature for 10 min			
Read the extinction (E) of the test in comparison with the control use photocolourimeter: green filter (λ = 540 nm) in the cuvette (*l* = 10 mm)			

Calculation: For determination of the proline and hydroxyproline content use the calibrate curve. E= C=

Norma:

Clinical-diagnostic means: The total quantity of proline and hydroxyproline in the urine increases in rheumatism, trauma, pneumonia, etc.

Conclusion:

Experiment 2. **Determination of Ca concentration in the urine (Sulkovich method).**

Method principle: Calcium and oxalate in acidic medium form the turbidity complex. The intensity of the turbidity complex is directly proportional to Ca-ions quantity in the sample.

Procedure:

№	Reagents	Test	Control
1.	Urine	2,0 ml	–
2.	Sulkovich reagent	2,0 ml	4,0 ml
Incubation for 2 min at room temperature			
Read the extinction (E) of the test in comparison with the control using photocolorimeter: λ = 640 nm in the cuvette (*l* = 5 mm)			

Calculation: For determination of Ca content use the calibrated curve. E= C_1=

Use the obtained value (C_1) for determination of the daily excretion of calcium with urine: D= 1500 ml/24 h; $C=C_1 \cdot 1,5$= g/24 h; 1,5 – coefficient for calculation.

Norma: about 0,25g / 24 h.

Clinical-diagnostic means: The quantity of Ca excreted with urine increases essentially in hyperparathyroidism, hypervitaminosis D, renal pathologies, diabetes mellitus.

Conclusion:

Self-preparing questions:

1. The cells of connective tissue: the representatives, their biological role.
2. The extracellular matrix of the connective tissue:
   - proteins (collagen, elastin): the particularities of biochemical structure, biosynthesis and degradation, biological role.

- glucosamine glycans: representatives, their structure, physico-chemical properties, biological role, biosynthesis;
- proteoglycans: composition, structure, functions and metabolism.

3. Pathology of connective tissue metabolism:
   - age-dependent modifications;
   - acquired qualitative modifications (scurvy, diabetes);
   - genetic qualitative modifications: collagenoses, mucopolysaccharidoses, the causes of biochemical manifestation.

6. The cells of the bone tissue: the representatives, their biological role.

7. Mineral components of the bone tissue: the main anions and cations, their structural organization, minor ions and olygoelements, their role.

8. Organic compounds of the bone tissue: fibrilar and non-fibrilar proteins, proteoglycans, carbohydrates and lipids, their structural organization and biological role.

9. Formation and resorbtion of the bone: mechanisms, enzymes, regulation. Role of vitamins D, C and hormones (calcitonine, parathyrin) in these processes.

10. The pathology of bone tissue metabolism: age-dependent, osteoporosis, skeletal fluorosis.

LESSON 36
Biochemistry of dental tissues

Experiment 1. Determination of inorganic phosphate content in the urine.

Method principle: Phosphate ions along with ammonium molibdate form ammonium phosphomolibdate in acidic medium, which along with ascorbic acid form the blue-coloured complex. Its optical density is proportional to the quantity of phosphate.

Procedure:

№	Reagents	Test	Control

1.	Urine (diluted 1:100)	2,0 ml	–
2.	Molibdenic reagent	1,0 ml	1,0 ml
3.	Ascorbic acid solution, 1%	2,0 ml	2,0 ml
4.	H_2O dist	–	2,0 ml
Read the extinction (E) of the test in comparison with the control using the photocolorimeter: wavelength λ = 640 nm (red filter) in a cuvette l = 5 mm.			

Calculation: Determine of inorganic phosphate quantity using the calibration curve:

E_1 = C_1 =

Use the obtained value (C_1) for calculation of the daily excretion of phosphate with urine: D = 1500 ml/ 24 h C= $C_1 \cdot 1,5$ = g/24 h; 1,5 – coefficient.

Norma: about 1-5 g / 24 h.

Clinical-diagnostic means: The quantity of inorganic phophates excreted with urine increases essentially in leukaemia, infections, hyperparathyroidism. The quantity of phosphates decreases in hypovitaminosis D.

Conclusion:

Self-preparing questions:

Mineral components of dental tissue: representatives, forms, role.

Organic components of dental tissue: representatives, role.

Structural organization of the hard tissues of teeth (enamel and dentine).

Biochemical mechanism of the teeth mineralization.

Normal biochemical processes in the teeth.

Biochemical processes that take place in the development of dental caries.

Fluorosis of teeth: pathogenesis, biochemical disorders.

LESSON 37

Biochemistry of parodont. Biochemistry of saliva

Experiment 1. Identification of thiocyanate-ions (SCN^-) in the saliva.

Lactoperoxidase uses SCN-ions in presence of Cl-ions and H_2O_2 for production of OSCN-ions with stronger protective antimicrobial action. SCN-ion is also a product of CN-ion detoxication.

Method principle: Thiocyanate-ions combine with $FeCl_3$ with production of red coloured complex.

Procedure: Saliva may be used for assay. Put 5-10 drops of saliva and 1 ml of $FeCl_3$ into the test-tube, mix it.

Conclusion:

Clinical diagnognostic means: The content of thyocyanate increases in patients with gingivitis, parodontitis.

Experiment 2. Identification of salivary peroxidase.

Peroxidase is an important enzyme of saliva with protective antimicrobial and antioxidative properties.

Method principle: Peroxidase catalyzes benzidine in presence of H_2O_2 to p-chinondiimine, green coloured substance.

Procedure: Put 5-10 drops of saliva, 5-10 drops of 1% benzidine solution and 5-10 drops of H_2O_2 into the test-tube. Mix it.

Conclusion:

Clinical diagnostic means: Salivary peroxidase activity increases in patients with gigivitis, parodontitis.

Self-preparing questions:

1. Periodont. Main chemical compounds, their characteristics and biological role.
2. Physiological biochemical processes that take place in the periodont.

3. Biochemical processes that take place in the pathology of the paradont. The role of the inflammation mediators and immune system. Gingivitis, parodontitis.

4. Dental plaque, its chemical composition. Main biochemical processes in the plaque. Correlation with the caries formation and periodontal pathologies.

5. The role of vitamins in the metabolism of dental tissues and soft tissues of the mouth (vitamins A, D, C, etc).

6. Gingival liquid: causes of its appearance, chemical composition and its role.

7. Saliva: sources, chemical composition, physic-chemical properties and its role. Clinical diagnostic means of salivary components in medicine and dentistry.

LESSON 38

Colloqiu: Biochemistry of connective, tooth and bone tissues. Saliva

1. The cells of connective tissue: the representatives, their biological role.

2. The extracellular matrix of the connective tissue:

- proteins (collagen, elastin): the particularities of biochemical structure, biosynthesis and degradation, biological role.
- glucosamine glycans: representatives, their structure, physico-chemical properties, biological role, biosynthesis;
- proteoglycans: composition, structure, functions and metabolism.

3. Acquired qualitative modifications (scurvy, diabetes); genetic qualitative modifications: collagenoses, mucopolysaccharidoses, the causes of biochemical manifestation.

4. The cells of the bone tissue: the representatives, their biological role.

5. Mineral components of the bone tissue: the main anions and cations, their structural organization, minor ions and olygo-elements, their role.

6. Organic compounds of the bone tissue: fibrilar and non-fibrilar proteins, proteoglycans, carbohydrates and lipids, their structural organization and biological role.

7. Formation and resorbtion of the bone: mechanisms, enzymes, regulation. The role of vitamins D, C, and hormones (calcitonine, parathyrin) in these processes.

8. The pathology of bone tissue metabolism: age-dependent, osteoporosis, skeletal fluorosis.

9. Mineral components of dental tissue: representativies, forms, role.

10. Organic components of dental tissue: representativies, role.

11. Structural organization of the hard tissues of the teeth (enamel and dentine).

12. Biochemical mechanism of the teeth mineralization.

13. Normal biochemical processes in the teeth.

14. Biochemical processes that take place in development the dental caries.

15. Fluorosis of teeth: pathogenesis, biochemical disorders.

16. Periodont. Main chemical compounds, their characteristics and biological role.

17. Physiological biochemical processes that take place in the periodont.

18. Biochemical processes in the pathology of the paradont. Role of the inflammation mediators and immune system. Gingivitis, parodontitis.

19. Dental plaque, its chemical composition. Main biochemical processes in the plaque. Correlation with the caries formation and periodontal pathologies.

20. The role of vitamins in the metabolism of the dental and soft tissues of the mouth (vitamins A, D, C, etc).

21. Gingival liquid: causes of its appearance, chemical composition and its role.

22. Saliva: sources, chemical composition, physic-chemical properties and its role. Clinical diagnostic means of salivary components in medicine and dentistry.

BIOCHEMICAL INDICES

Chemical composition of blood plasma

I. Proteins

1. Total protein 65-85 g/L
2. Albumins 35-50 g/L
3. Globulins 25-35 g/L
4. Fibrinogen 2.0-7.0 g/L
5. Haptoglobin 0.28-1.90 g/L
6. Prothrombin 10-15 mg/dL
7. Plasminogen 1.4-2.8 μmol/L (20-40 mg/dL)
8. Transferrin 19.3-45.4 μmol/L (170-400 mg/dL)
9. Ceruloplasmin 1.52-3.31 μmol/L (23-50 mg/dL)
10. β-Lipoproteins 3.0-6.0 g/L (300-600 mg/dL)

HDL – high density lipoprotein (α-LP) 1.063-1.210 mmol/L (80-400 mg/dL)

LDL – low density lipoprotein (β-LP) 1.006-1.063 mmol/L (360-640 mg/dL)

II. Enzymes

1. Alanyl aminotransferase (ALT) 0.16-0.68 mmol/h ·L or (15-75 IU/L) (glutamate pyruvate transferase, GPT)
2. Aspartate aminotransferase (AST) 0.10-0.45 mmol/h · L or (10-50 IU/L) (glutamate oxaloacetate transferase, GOT)
3. Lactate dehydrogenase 0.8-4.0 mmol/h · L
4. Creatine kinase < 1.2 mmol/h · L or (< 90 IU/L)

5. Fructose-biphosphate aldolase (F-1,6-PA) 3.6-21.8 mmol/h · L
6. Acetylcholine esterase 160-340 mmol/ h · L
7. α-Amylase 15-30 g/h · L or (< 300 IU/L)
8. Alkaline phosphatase 30-150 IU/L
9. Acidic phosphatase < 62 nkat/L
10. γ-Glutamyl transferase (γGT or GGT) < 60 IU/L

III. Nonproteinic nitrogenous compounds

1. Nitrogen residual (nonproteinic) 19.5-30.0 mmol/L
2. Nitrogen of amino acids 3.5-5.5. mmol/L
3. Creatine 15-70 mmol/L
4. Creatinine 60-150 μmol/L
5. Urea 3.3-6.7 mmol/L
6. Uric acid 0.1-0.4 mmol/L
7. Bilirubin total 8-20 μmol/L
8. N-Acetylneiraminic acid 1.8-2.2 mmol/L
9. Histamine 7.99-71.94 nmol/L (0.2-0.8 μg/dL)
10. Adrenalin 1.91-2.46 nmol/L (0.35-0.45 μg/L)
11. Serotonine 0.3-1.7 μmol/L (5.0-30.0 μg/dL)
12. Thyroxine 64.36-141.59 nmol/L (5-11μg/dL)

IV. Carbohydrates and metabolites

1. Glucose 2.8-6.0 mmol/L
2. Lactate 0.5-2.0 mmol/L
3. Pyruvate < 0.1 mmol/l
4. Citric acid 88.5-156.1 μmol/L (1.7-3.0 mg/dL)

V. Lipids and metabolites

1. Total lipids 4.0-8.0 g/L
2. Triacylglycerides 0.5-2.1 mmol/L
3. Total phospholipids 2.0-3.5 mmol/L
4. Total cholesterol 4.0-8.6 mmol/L
5. Free fatty acids 0.3-0.8 mmol/L
6. Ketone bodies 100-600 μmol/L

VI. Mineral components

1. Sodium (Na^+) 135-155 mmol/L
2. Potassium (K^+) 3.6-5.0 mmol/L
3. Chlorides (Cl^-) 97-108 mmol/L
4. Calcium (Ca^{2+}) 2.25-2.75 mmol/L
5. Phosphate inorganic 0.8-1.4 mmol/L
6. Magnezium (Mg^{2+}) 0.7-1.0 mmol/l
7. Sulphates 0.4-0.6 mmol/L
8. Iron (Fe) 14-32 μmol/L (65-175 μg/dL)
9. Copper (Cu) 12-19 μmol/L
10. Zinc (Zn) 12-20 μmol/L
11. Ammonia 10-47 μmol/L

Indices of blood

1. Hemoglobin: males 130-180 g/L (13-18 g/dL)
   females 120-160 g/L (12-16 g/dL)
2. Hydrogen ion: arterial blood 35-46 nmol/L (pH= 7.36-7.44) (38^oC)
3. Oxygen (Po_2) in arterial blood: 11-15 kPa (85-105 mm Hg)
4. Bicarbonate total (CO_2) 22-30 mmol/L
5. Carbon dioxide (Pco_2) in arterial blood: 4.5-6.0 kPa (35-46 mm Hg)

Indices of urine

Density (ρ) 1.017-1.020
pH 5.7-6.5
Urea 220-609 mmol/24 h (20-35 g/24 h
Creatinine 7.1-17.7 mmol/24 h (0.8-2.0 g/24 h
Uric acid 1.60-3.54 mmol/24 h (270-600 mg/24 h)
Indican 46.0-56.4 mmol/24 h
Calcium (Ca^{2+}) 2.50-6.25 mmol/24 h (100-250 mg/24 h)
Cloride ion (Cl^-)
Ketone bodies: acetone 400-600 mg/L
acetoacetic acid 100-200 mg/L

Chondroitine sulphates	2.7-7.5 mg/24 h
Pyruvic acid	113.7-283.9 μmol/24 h (10-25 mg/24 h)
Uropepsinogen	150-300 IU/24 h (1.5-3.0 mg/24 h)
α-Amylase (diastase):	1.0-2.0 IU/ml (100-200 IU/dL)
Volgemut's method:	16-64 IU/L
Adrenalin	< 13.3 μg/24 h
Noradrenalin	< 79.8 μg/24 h
17-ketosteroids: males	42.48-46.75 μmol/24 h (12.83±0.8 mg/24 h)
female	34.78-39.16 μmol/24 h (10.61±0.66 mg/24 h)

Indices of saliva

Daily volume (V)	500-1500 ml (500-1500 ml/24 h)
pH	6.07-7.9
Density (ρ)	1.08-1.32

Nitrogen-contaning organic substances

Proteins	1.4-6.4 g/L
Mucine	0.8-6.0 g/L
Ammonia	0.01-0.12 g/L
Urea	0.14-0.75 g/L
Uric acid	0.005-0.029 g/L
Creatinine	0.005-0.750 g/L
Choline	0.005-0.036 g/L

Nitrogen-noncontaning organic substances

Glucose	0.10-0.30 g/L
Citric acid	< 0.020 g/L
Lactic acid	0.01-0.05 g/L
Cholesterol	0.025-0.500 g/L

Inorganic substances

Sodium (Na)	5.2-24.4 mmol/L
Potassium (K)	14-41 mmol/L
Calcium (Ca)	2.3-5.5 mmol/L

Chlorides (Cl^-)	15.1-31.6 mmol/L
Phosphate inorganic	0.080-0.217 g/L
Bicarbonate (HCO_3^-)	2.13-13.00 mmol/L
Fluor (F^-)	0.8-2.5 g/L
Brom (Br^-)	0.2-7.1 mg/L
Tiocianate (SCN^-)	0.12-0.33 g/L
Magnezium (Mg^{2+})	0.16-1.06 mmol/l
Sulphates	0.04-0.2 g/L
Copper (Cu)	0.5-7.6 mg/L
Zinc (Zn)	0.06-0.80 g/L

Vitamines

Thiamine (B_1)	0.7 μg/dL
Riboflavin (B_2)	5.0 μg/dL
Pyridoxine (B_6)	60 μg/dL
Nicotinic acid (PP)	3.0 μg/dL
Pantotenic acid	8.0 μg/L
Ascorbic acid (C)	0.58-3.78 mg/L
Biotin (H)	0.08 μg/dL
Phyllochinon (K)	1.5 μg/dL

LITERATURE

1. Bhagavan N.V. *Medical Biochemistry*. Fourth Edition, Academic Press, 2002.

2. Campbell P.N., Smith A.D. *Biochemistry illustrated*. International Edition, 2000.

3. Dati Francesco, Metzman Erwin. *Proteins. Laboratory testing and clinical use*. Includes index. Holzheim, Germany, 2005.

4. Dawson Rex M.C., Elliott Daphne C., Elliott William H., Jones Kenneth M. *Data for Biochemical Research*. Third Edition. Clarendon Press, Oxford, 1986.

5. Emery A.E.H., Mueller R.F. *Elements of Medical Genetics*. ELBS Longman Group UK Ltd., 1992.

6. Marshall William J. *Clinical Chemistry*. Fourth edition, 2000.

7. Metzler D.E. *Biochemistry. The chemical reactions of living cells*. Second Edition, vol. 1-2. Academic Press, 2003.

8. Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W. *Harper's illustrated Biochemistry*. Twenty-Sixth edition. International Edition, 2003.

9. Nelson D.L., Cox M.M. *Lehninger principles of Biochemistry*. 3-rd Edition p.cm. Includes index, 2000.

10. Russel P.J. *Genetics*, 3rd Edition. Harper Collins Publischers, New York, 1992.